Roberta Brayner
Fernand Fiévet
Thibaud Coradin *Editors*

Nanomaterials: A Danger or a Promise?

A Chemical and Biological Perspective

Springer

Nanomaterials: A Danger or a Promise?

Roberta Brayner · Fernand Fiévet
Thibaud Coradin
Editors

Nanomaterials: A Danger or a Promise?

A Chemical and Biological Perspective

Springer

Editors
Roberta Brayner
ITODYS
Université Paris Diderot
Paris Cedex 13
France

Thibaud Coradin
LCMCP
Université Pierre et Marie Curie/Collège
Paris Cedex 05
France

Fernand Fiévet
ITODYS
Université Paris Diderot
Paris Cedex 13
France

ISBN 978-1-4471-4212-6 ISBN 978-1-4471-4213-3 (eBook)
DOI 10.1007/978-1-4471-4213-3
Springer London Heidelberg New York Dordrecht

Library of Congress Control Number: 2012944379

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Popular arts constitute a fascinating and timely mirror of the perception of our societies toward technological and scientific advances. In 1962, during the Cold War, Marvel Comics published the first history of "The Incredible Hulk". This story is about a physicist working on a new type of nuclear bomb, who is accidentally irradiated by gamma rays. As a result of DNA damaging, the scientist can undergo a transformation into a giant, aggressive monster as a result of stress or anger. Forty years after, Ang Lee's movie "Hulk" tells a different story. The scientist is now a bionuclear researcher working on "nanomeds" for the US Army. He inherited modified DNA from his father, also a scientist working on genetic methods to improve tissue regeneration. Here, a combination of accidental exposition to both gamma rays and nanomeds turns him into Hulk.

This modification is revealing of the current concerns of the public toward nuclear energy, genetics and, at the beginning of this new century, nanoscience. It is interesting to emphasize that the positive impact of these technologies are not neglected (i.e. "nanomeds") but the message to us, as scientists, is clear: *take care*. Those of us who had the opportunity to take part in public discussion would probably agree that whatever the fascination for achievements and promises of nanotechnology is, we can no longer afford not to provide a more complete and critical picture of this fast-developing area.

The present volume was conceived while keeping this message in mind. We found important that the gathered contributions address the whole life cycle of nanomaterials, from synthesis to applications and from waste to environmental fate. By doing so, it was possible to present a balanced vision between the pros and the cons, with the hope to contribute to a better evaluation of the benefit-to-risk ratio of nanotechnology. Noticeably, this books closes with a number of contributions that emphasize the current efforts made worldwide to improve the acceptability of nanotechnology, both in terms of environmental and societal impact.

Should the readers find useful answers or unexpected questions in this book, we, as editors, together with all contributors who have heartedly agreed to share their expertise and vision, would feel rewarded for our common efforts and privileged to have such a unique opportunity to say: *we care*.

Roberta Brayner
Thibaud Coradin
Fernand Fiévet

Contents

Chapter 1
The Polyol Process

Fernand Fiévet and Roberta Brayner

Abstract Among the chemical, physical, or electrochemical processes generally used in particles production, the polyol-mediated synthesis of inorganic nanoparticles appears as an easy to carry out and versatile route. In this chapter, properties of polyols (α-diols and etherglycols) are first recalled in order to explain the versatility of this process. Guidelines which allow controlling the nucleation and growth steps in such media are then given in order to obtain particles with well-defined characteristics namely, a uniform shape, a mean size in the micron, submicron or nanometer range with a narrow size distribution, and a low degree of agglomeration. Examples of size tuning of ferromagnetic metals (Fe, Co, Ni, and their alloys) and noble metals are given as well as examples of shape control leading to 1D nanostructures with a particulate emphasis on the growth mechanism of silver nanorods or nanowires. Examples of polyol-mediated synthesis of oxide (spinel ferrites, Cu_2O, ZnO) nanoparticles through hydrolysis reaction are also given. Throughout this chapter it is pointed out how the polyol process allows tuning the size and shape-dependent magnetic properties of ferromagnetic metal or spinel ferrite particles which may be used as advanced functional materials in various fields: high permeability composite materials, high density recording media, high temperature permanent magnets, and in biomedical applications such as magnetic resonance imaging, cancer treatment by hyperthermia, or targeted drug delivery

F. Fiévet (✉) · R. Brayner
Laboratoire ITODYS, University of Paris Diderot,
Sorbonne Paris Cité, UMR 7086, 75205 Paris, France
e-mail: fievet@univ-paris-diderot.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_1,

1.1 Introduction

Over the recent years, metal, oxide, or semi-conducting nanoparticles have attracted considerable interest because of the size and shape dependence of their electronic, optical, magnetic, or catalytic properties and their potential use as tiny building blocks to make nanocomposite or bulk nanostructured materials. The fine-tuning of such properties for application in various fields such as electronics, photonics, information storage, and biomedicine, often requires the synthesis of non-agglomerated particles with a definite and uniform shape, a given mean size, and a narrow size distribution. Among the chemical, physical, or electrochemical processes generally used in particles production, the chemical ones, in particular those from solution, appear suitable for affording such fine particles with tailored morphological characteristics, if the precipitation, namely nucleation and growth steps, are conducted under kinetically controlled conditions. The polyol-mediated synthesis of inorganic nanoparticles, which was first studied by our group 20 years ago [1–3] and then extensively developed by many other groups as by ours, is able to fulfill these requirements in many cases as exemplified in this chapter for metals and oxides.

1.2 Description of the Polyol Process

1.2.1 Historical Review

The polyol process was described at first as a novel route for preparing finely divided metal powders of easily reducible metals such as copper [4], noble metals namely Au [5], Pd [6], Ag [7, 8], and their alloys [9, 10], or less reducible metals such as cobalt, nickel, iron, and their alloys [2, 3, 11–13] by reduction of inorganic precursors in liquid polyols. Polyols were either polyhydric alcohols namely α-diols such as 1,2-ethanediol (ethylene glycol), 1,2-propanediol (propylene glycol), or etherglycols namely di(ethylene) or tri(ethylene glycol). The solid precursor is suspended in the liquid polyol, which may be quite soluble (nitrate, chloride, acetate) or only slightly soluble (oxide, hydroxide). The solution or the suspension is stirred and heated to a given temperature which can reach the boiling point of the polyol for less reducible metals; conversely, for easily reducible metals (e.g. Pd) the reaction can be carried out at temperature as low as 0 °C. Polyols are interesting among non-aqueous solvents because like water and monoalcohols, they are hydrogen-bonded liquids with a high value of relative permittivity (Table 1.1); therefore they are able to dissolve, to some extent, ionic inorganic compounds. Moreover, polyols, as well as monoalcohols, are mild reducing agents but this reduction can be carried out in such solvents under atmospheric pressure up to 250 °C if necessary. Polyols, such as α-diols, owing to their chelating properties, are also coordinating solvents which can form

Table 1.1 Relative permittivity and boiling point under atmospheric pressure of some polyols comparison with water and monoalcohols

	Water	1,2-ethanediol	1,2-propanediol	Di(ethylene glycol)	Methanol	Ethanol	1-octanol
ε_r	78.5	38	32	32	33	24	10
T_b (°C)	100	198	189	245	65	78.5	194

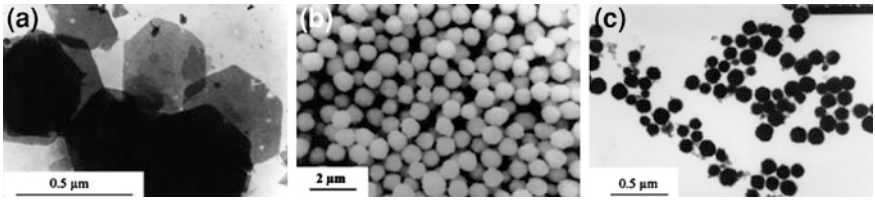


Fig. 1.1 TEM images of different inorganic compounds obtained from $Co(CH_3CO_2)_2{\cdot}4H_2O$: **a** cobalt hydroxyacetate (DEG, $h = 26$, T = 60 °C), **b** cobalt metal (EG/DEG, $h = 0$, T = 200 °C), **c** cobalt(II) oxide (DEG, $h = 4$, T = 185 °C) (*EG* ethylene glycol, *DEG* di(ethylene) glycol, *h* hydrolysis ratio) [19]

complexes with many metal cations. Therefore, they can form reactive intermediate species on one hand, and on the other adsorb onto the surface of the growing particles preventing aggregation.

Later, polyols have also been used to elaborate a large variety of oxides [14–19] and layered hydroxyl salts [20] through forced hydrolysis and inorganic polymerization. The final compound of these reactions depends mainly upon the hydrolysis ratio *h* defined as the ratio between the amount of water added in the polyol and the amount of metal involved as exemplified for cobalt (Fig. 1.1).

More recently, the polyol process has also been used with thiourea or sodium sulfide to prepare sulfide nanoparticles [21–23] or with sodium hydrogen phosphate to obtain phosphates [24, 25]. Finally, the polyol-mediated synthesis appears as a rather simple and convenient route to prepare various nanoscale functional materials [26] through different reactions.

1.2.2 A Versatile Process

Whatever the synthesis carried out in polyols, the reaction occurs via a progressive or total dissolution of the solid precursor rather than solid phase transformation. It is well known that the precipitation of a solid from a solution proceeds in two steps: nucleation and particle growth. During the nucleation step, nuclei are formed by a stepwise bimolecular addition of monomeric entities of the solute. In order to initiate the spontaneous growth of stable particles (growth step) the small aggregates which form the nuclei have to reach a critical size (nucleation step). Therefore, the

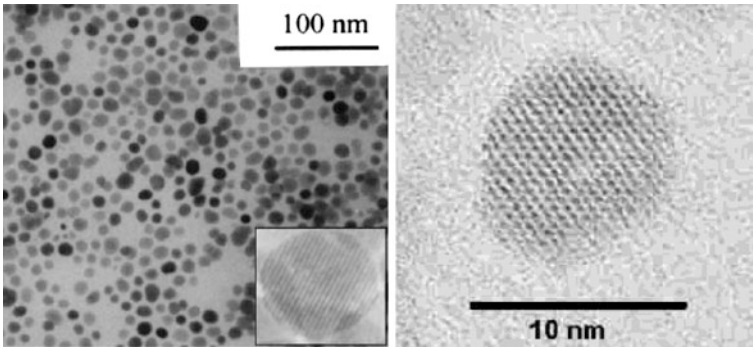


Fig. 1.2 TEM images of ferrite nanoparticles obtained in polyols showing their high crystallinity **a** Zn Fe_2O_4 [35], **b** Mn Fe_2O_4 [36]

morphological characteristics of the final particles, i.e., their size, shape, and degree of aggregation depend upon the relative rates of these two steps. In such a precipitation it is possible to tune these morphological characteristics by acting above numerous experimental parameters. To achieve this goal for a given system, one can act at first upon the reactants (nature and concentration of the solid precursor, nature of the polyol, pH of the solution) and the way they are brought together (progressive injection by a syringe pump to achieve a better control upon nucleation and growth for instance [27]). One can also act upon the heating conditions (temperature ramping rate and final temperature for usual heating, microwave heating) and the duration of reaction. If polyols are able to control the growth of the particles to some extent and to prevent their agglomeration by coordinating onto their surfaces, it may be useful in some cases, namely for the synthesis of noble metals, to add polymeric protective agents such as poly (vinylpyrrolidone) (PVP) for instance [7, 8, 27] or surfactants such as oleic or lauric acid [28]. For the preparation of metal nanoparticles the seeding of the reaction medium by foreign nuclei (heterogeneous nucleation) provides an efficient tool to steer the average size of spherical particles in a large size range [29]; it also allows in some particular cases to obtain nanoparticles of various shapes, [30] namely nanorods [31] or nanowires [32, 33].

For the polyol-mediated synthesis of oxides a defined amount of water has to be added into the polyol and the relative concentration of water versus the metal concentration, i.e., the hydrolysis ratio, is a critical parameter [16, 21] to tune the size of the nanoparticles obtained through spontaneous nucleation (homogeneous nucleation). One of the interesting characteristics of the so-obtained oxide nanoparticles is their surprisingly good crystallinity. For instance, of the various methods of fabricating ferrite nanoparticles, the forced hydrolysis of metal salts in polyol appears as an attractive soft chemistry route carried out under temperature close to hydrothermal conditions which then provides very well-crystallized nanoparticles showing enhanced magnetic characteristics (Fig. 1.2) [17, 34–36]. But, like other soft chemistry routes, it sometimes allows obtaining metastable phases such as for instance CoO or solid solutions ZnO–CoO with a high Co content [18].

Moreover, whatever the targeted nanoparticles, metals, oxides, or chalcogenides the polyol-mediated synthesis is rather easy to perform at laboratory scale. The reaction rate can be drastically increased under microwave heating as evidenced in the previous years [37]. It has also been shown that it is possible to scale up the production of the nanoparticles by replacing the conventional batch production by a continuous process, where the solvent and precursors are fed continuously [38, 39]. Altogether, the polyol process appears to be a versatile route suitable for the preparation of particles of a number of particulate inorganic compounds (elemental metals, oxides, chalcogenides) in a large size range and namely at the nanometer scale.

1.3 Control of the Size and Shape of the Particles in Polyols

1.3.1 Guidelines

Generally, metal, oxide, or chalcogenide particles obtained through precipitation in polyols show well-defined characteristics: a uniform shape often almost spherical, a rather narrow size distribution with a mean diameter either in the micron, submicron, or nanometer size range, and small degree of aggregation. The formation of bulk solids is always thermodynamically favored over the formation of small particles with a large surface area and many incompletely coordinated surface sites. Agglomeration of the particles is also thermodynamically favored for the same reason. Therefore, to obtain such well-defined characteristics two general conditions must be fulfilled: nucleation and growth must be two completely separated steps according to the model of LaMer and Dinegar [40] and coalescence of the particles must be prevented.

1.3.1.1 Control of the Nucleation Step

Metals, oxides, or chalcogenides are only sparingly soluble in polyols. The saturation level is low and spontaneous nucleation occurs when the concentration of the species generated by the reduction or hydrolysis reactions reaches a critical supersaturation level. If the final compound is generated slowly and the nucleation rate is high enough, then the sudden nucleation lowers almost immediately the concentration below this critical nucleation level. Under these conditions the nucleation step is very brief and is followed by the growth of particles from the original nuclei as long as the final compound is slowly generated as the concentration remains higher than the saturation one (Fig. 1.3a). Nucleation rate increases sharply versus concentration above the critical nucleation threshold, whereas growth rate increases slowly above the saturation concentration (Fig. 1.3b). To prevent further nucleation during the growth step supersaturation

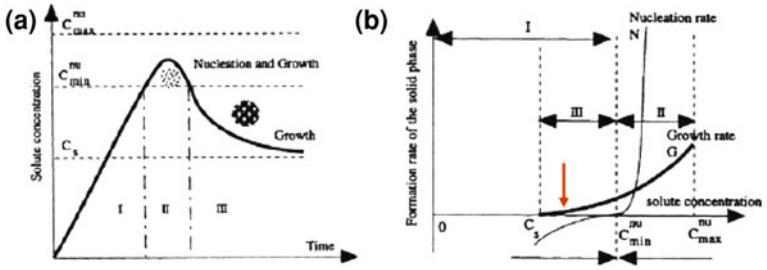


Fig. 1.3 **a** Nucleation and growth according to LaMer's model [40], **b** comparison of nucleation and growth rates versus concentration of the final compound in solution

must remain at a low level. This can be achieved by different methods. Starting from a very soluble precursor the reaction of formation of the final solid compound from the solution has to be carried out at a sufficiently low temperature. When various soluble precursors may be used one can select the most suitable one. In other cases it is possible to control the concentration of the precursor species in solution, these species being provided by the progressive dissolution of a sparingly solid phase (starting compound or intermediate solid phase) acting as a kind of reservoir. The dissolution equilibrium regulates the release of these species, controls the supersaturation ratio, and then allows having a very brief nucleation step. Nevertheless, it is possible to have an important yield at the end of the growth despite the low supersaturation ratio. In some cases, to achieve more easily the separation between the nucleation and growth steps and a better control of the average size of the final particles, homogeneous (spontaneous) nucleation can be replaced by heterogeneous nucleation by seeding the reactive medium with foreign nuclei obtained by adding a suitable nucleating agent.

1.3.1.2 Growth and Stabilization of Particles

Particles growth may proceed either by diffusion of the solute species toward the surface of the particles and stepwise addition of atoms or ions (Fig. 1.4a), or by coalescence of primary particles which form secondary larger particles (Fig. 1.4b).

Coalescence of primary particles usually results in uncontrolled growth leading to polydisperse secondary particles of various shapes. Thus, to obtain particles with well-defined morphological characteristics it is generally necessary to prevent the coalescence of the particles during their growth stage. This can be achieved by either steric or electrostatic stabilization. Steric stabilization can be provided by the polyol itself or by adding long-chain molecules. The adsorption of such protective agents can also be used to limit the growth if necessary in order to obtain nanoparticles. Electrostatic stabilization can be provided by carboxylate anions, namely acetate ions, when such metallic salts are used as starting compounds. Nevertheless, it must be pointed out that in some particular cases the formation of

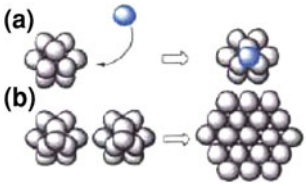


Fig. 1.4 **a** Growth by stepwise addition of atoms or ions; **b** growth by coalescence of primary particles

quasi-spherical secondary metal particles with a rather narrow size distribution through a coalescence mechanism has been evidenced [41]. It is also noteworthy that even when polydisperse nanoparticles are obtained in the early stages of the precipitation due to uncontrolled growth, it has been shown in some cases that final particles with a narrow size distribution can be obtained by a further growth of particles having larger sizes at the expense of smaller ones through Ostwald ripening [42].

A kinetic control of the nucleation and growth steps allows in certain systems to control the shape of the particles and to induce an anisotropic growth which generates 1D nanostructures such as nanorods or nanowires. Such syntheses have been carried out in polyols for metals which crystallize with a highly anisotropic crystal lattice such as *hcp* Co or Co–Ni alloys and also for metals which crystallize in the highly symmetric cubic lattice such as Ag (cf. Sects. 3.2.2 and 3.3.2).

1.3.2 Synthesis of Ferromagnetic Metal Nanoparticles

The magnetic properties of particulate ferro or ferrimagnetic materials are strongly dependent on elemental composition, particle shape and size, and species adsorbed onto the surface. This is especially true for micro and nanosized samples comparable to the domain length scale. The first attempts to achieve a polyol-mediated synthesis of metal particles with well-defined morphological characteristics were carried out with ferromagnetic metals Fe, Co, Ni, and their alloys [2, 3, 11–13]. As long as the nucleation is spontaneous, particles with a uniform quasi-spherical shape and a narrow size distribution were obtained in the micron and submicron range whatever their composition (Fig. 1.5).

Starting from cobalt or nickel acetates dissolved in polyols, the key for the formation of uniform particles was believed to be the formation upon heating of solid intermediate phases such as metallic hydroxyacetates or alkoxides (Fig. 1.6). Sodium hydroxide was added in the polyol in order to favor the formation of these phases which act as a reservoir for the metal solvated cations prior to their reduction into metal. Such a control of the metallic species concentrations allows making monodisperse metal particles through a control of the growth step of these particles which favors its separation from the nucleation one.

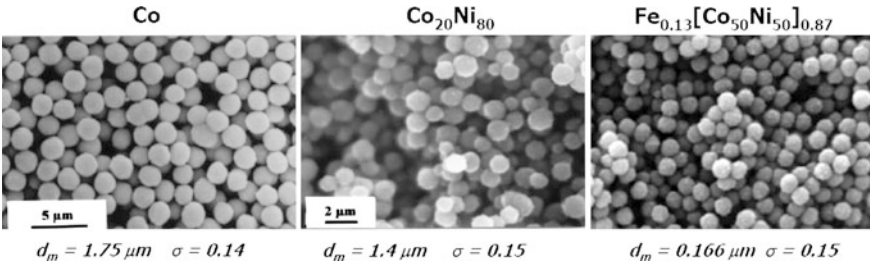


Fig. 1.5 SEM images of ferromagnetic metal particles obtained in polyols through spontaneous nucleation (d_m: mean diameter; σ: relative standard deviation)

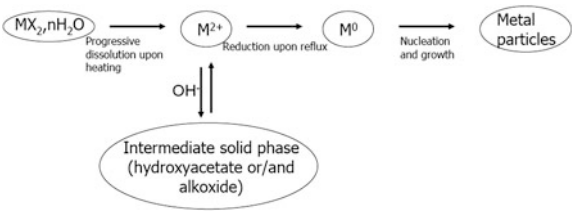


Fig. 1.6 Control of the growth of Co, Ni, and CoNi particles in polyols through the kinetics of dissolution of intermediate solid phases which act as a cation reservoir

1.3.2.1 Size Tuning

An accurate and reproducible control of the mean diameter of the particles can be achieved in the submicrometer and nanometer size ranges as well, by heterogeneous nucleation. As cations of noble metals such as Ag, Pt, or Ru are easily reduced by polyols, the seeding of the reaction medium was achieved using a soluble precursor such as $AgNO_3$, K_2PtCl_4, or $RuCl_3$ in order to generate in situ at low temperature numerous metal nuclei which then acted as suitable sites for the further growth of the ferromagnetic metal particles under reflux. Thus, the separation of nucleation and growth steps is easily achieved favoring the formation of monodisperse particles. An accurate and reliable control of their mean diameter is obtained by varying the ratio of the noble metal concentration to that of ferromagnetic metals in the range 10^{-2}–10^{-5} (Fig. 1.7).

Such samples have been used as model materials to study the evolution of the dynamic properties of fine particles with their size [13] and as components to make high permeability composite materials [11].

A few years ago, Jeyadevan's group succeeded in synthesizing submicron-sized Fe particles in a concentrated NaOH solution in ethylene glycol [43]. Moreover, they obtained recently through heterogeneous nucleation, using H_2PtCl_6 as nucleating agent, size-controlled Fe nanoparticles ranging between 90 and 10 nm with a cubic morphology above 25 nm, the smaller one being spherical and agglomerated (Fig. 1.8). The as-prepared 60 nm-sized particles are protected from oxidation in air by a biocompatible oxide layer. Their biocompatibility and their

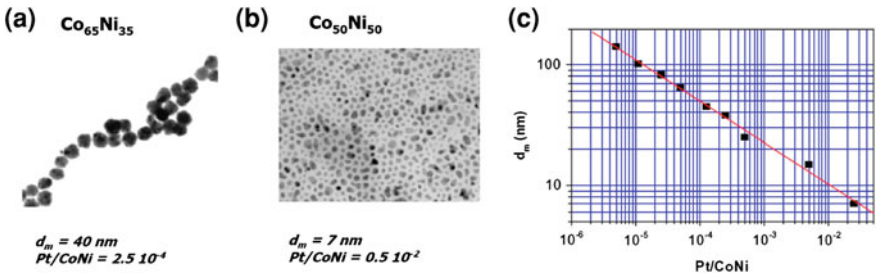


Fig. 1.7 **a**, **b** TEM images of CoNi alloys nanoparticles obtained in polyols through heterogeneous nucleation by generating in situ Pt nuclei, **c** accurate control of the mean size of CoNi particles by varying Pt concentration

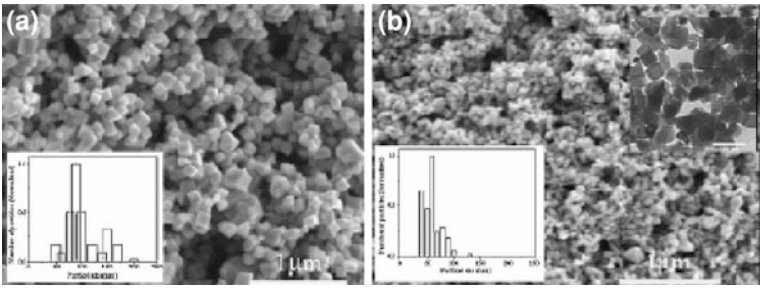


Fig. 1.8 SEM images of Fe nanocubes obtained in NaOH ethylene glycol solution from $FeCl_2.4H_2O$ through heterogeneous nucleation using H_2PtCl_6 as nucleating gent at different concentrations **a** 5×10^{-9} M, **b** 1×10^{-8} M. Particle size distribution in bottom insets, TEM image in top inset [44]

high saturation magnetization offer a potential advantage for biomedical applications such as targeted drug delivery [44]. Nanoparticles of Fe-based alloys such as FeCo [45] and Fe Pt [46] were synthesized by Jeyadevan's group as well. It must be pointed out that it is possible to synthesize by a modified polyol method equiatomic FePt nanoparticles with the $L1_0$ ordered structure without any subsequent annealing. Such nanoparticles whose diameters are 5–10 nm appear as promising candidates for ultra high density magnetic recording media due to their very large magnetocrystalline anisotropy.

1.3.2.2 Shape Control Toward 1D Nanostructure

Magnetic nanorods or nanowires have a high shape anisotropy which provides high coercivity. Despite a diameter in the nanometer range, ferromagnetic properties are observed at room temperature since the blocking temperature increases

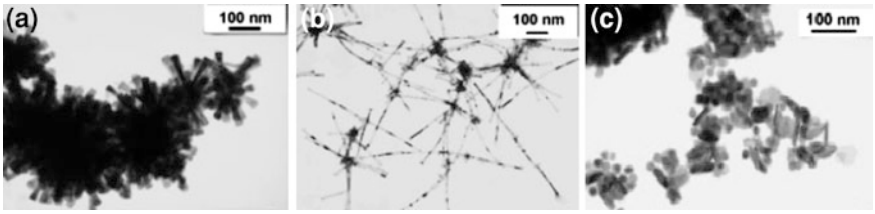


Fig. 1.9 TEM images of $Co_{80}Ni_{20}$ particles prepared by reduction of cobalt and nickel acetates in NaOH solution in 1,2-propanediol upon seeding with Ru with various hydroxide concentrations **a** 0.05 mol L^{-1}; **b** 0.15 mol L^{-1}; **c** 0.2 mol L^{-1} [32]

with the aspect ratio. Self-organized nanowires may find applications in high density magnetic recording [47]. Moreover, aligned nanowires or nanorods of hard magnetic materials were expected to provide an original "bottom up" fabrication method for macroscopic permanent magnets [31]. In this context several methods have been used for anisotropic growth of metal nanoparticles such as use of solid host templates [48] and templating effect of surfactant molecules through a self-organization process during the hydrogenation of organometallic compounds [47]. In the polyol-mediated synthesis of metals the first example of anisotropic growth was observed in the presence of poly(vinyl pyrrolidone) with silver particles obtained by heterogeneous nucleation upon seeding with platinum nuclei [7, 33]. Further, it was shown that anisotropic CoNi particles can be prepared by reduction of acetate in 1,2-propanediol with addition of various amounts of sodium hydroxide (Fig. 1.9). The key to obtain nanowires in a narrow hydroxide concentration range was the seeding with ruthenium nuclei with a high nucleating ratio [Ru]/[Co + Ni] = 2.5 mol% [32]. It was also observed that the particles' shape depends strongly upon the Co/Ni composition of the particles, wires being obtained only with cobalt content higher or equal to 50 at-%, whereas for nickel only platelets being obtained (Fig. 1.10) [49]. Increasing the sodium hydroxide concentration lowers the Co^{2+} and Ni^{2+} concentrations through the precipitation of intermediate solid phases which are alkoxide and hydroxy-acetate for Co and Ni respectively. These different solid phases suggest that the molecular complexes of Co(II) and Ni(II) in solution are distinct. These differences in coordination chemistry can explain the different behaviors of the two metals toward reduction. Different dissolution rates of the two solid phases and different reduction rates in solution for molecular species of a different nature are expected. The various shapes can be explained by nucleation and growth rates controlled both by the cobalt/nickel composition and sodium hydroxide concentration suggesting that the nanowires are obtained with a higher growth rate than the platelets.

In order to get optimized building blocks for the "bottom up" fabrication of macroscopic permanent magnets the synthesis of highly crystalline Co *hcp*-isolated nanorods with a high aspect ratio is required. In such materials the high magnetocrystalline anisotropy due to the highly anisotropic crystal lattice and the shape anisotropy would give high coercivity and remanent magnetization.

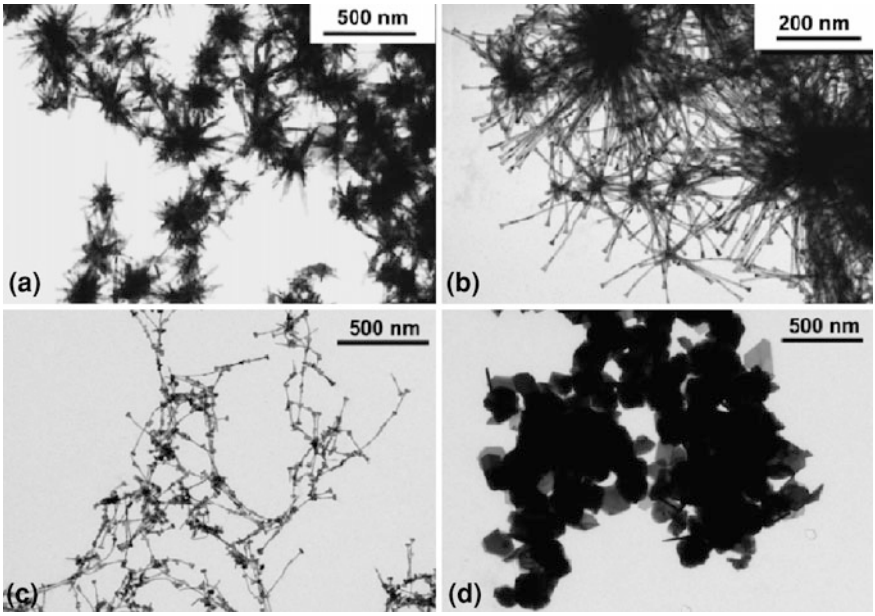


Fig. 1.10 TEM images of CoNi particles prepared by reduction of cobalt and nickel acetates in a 0.1 M NaOH solution in 1,2-propanediol: **a** Co wires; **b** $Co_{80}Ni_{20}$ wires; **c** $Co_{50}Ni_{50}$ wires; **d** Ni platelets [49]

Such a synthesis has been carried out by reduction of carboxylate salts of Co^{II} in 1,2-butanediol (BEG) [31]. By choosing the proper carboxylate counterions and varying the hydroxide concentration it is possible to change the nature of the intermediate solid phase, to adjust the concentration of the reducible Co^{II} species, to control the growth rate of the Co nanoparticles to favor an anisotropic growth, and finally to obtain rods with desired diameter and aspect ratio (Figs. 1.11, 1.12, and 1.13). Pressed powders and magnetically oriented samples made up with such nanorods exhibit a high coercivity and have the suitable properties to realize "high temperature magnets" competitive with AlNiCo or SmCo permanent magnets. They could also be used as recording media for high density magnetic recording [50].

1.3.3 Synthesis of Noble Metal Nanoparticles

Noble metal nanoparticles can be synthesized by many chemical methods using different reducing agents and solvents. Long ago, shape control has received considerable attention for silver and gold nanoparticles which have numerous applications in surface plasmons, surface enhanced Raman spectroscopy (SERS),

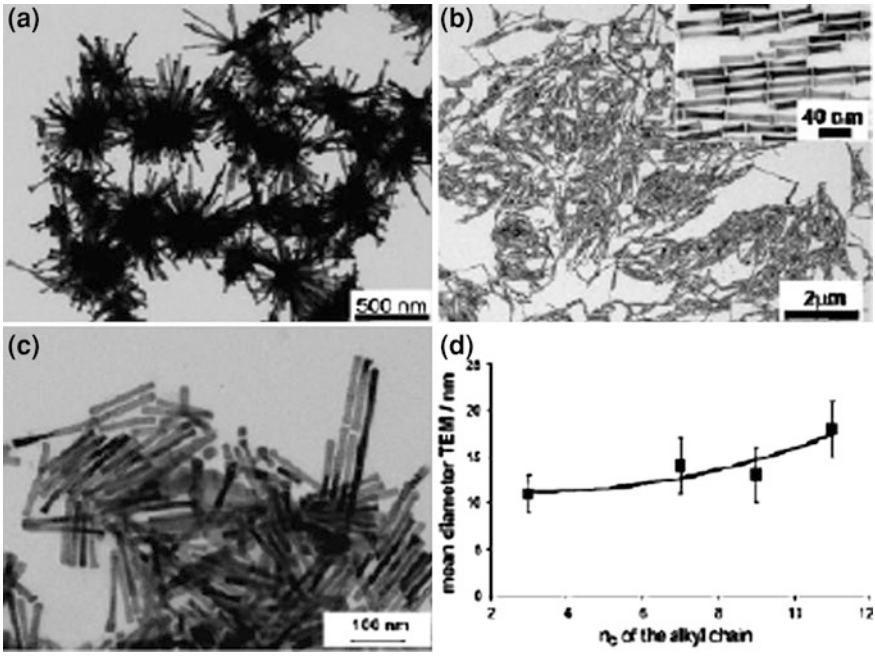


Fig. 1.11 TEM images of Co particles obtained by reduction of different carboxylates in BEG **a** sea-urchin-like particles from Co acetate, **b** nanorods ($L_m = 99$ nm, $\sigma_{Lm}/L_m = 17$ %; $d_m = 15$ nm, $\sigma_{dm}/d_m = 9$ %) from laurate, **c** nanorods from cobalt caproate ($n_c = 5$). **d** Influence of the length of the carboxylate carbon chain on d_m of the nanorods [31]

or as biological sensors. It was shown early that polyols can be used both as mild reducing agents and solvents to obtain particles of noble metals (Au, Pd, Ag) or alloys (AgPd) [5–10]. In contrast to the synthesis of ferromagnetic metal particles, this can be carried out at temperature lower than the boiling point, without formation of solid intermediate phases but with addition of a protective agent such as PVP to prevent the strong tendency of fine metal particles to coalesce. In these first studies it was shown that nanoparticles with a uniform spherical shape, a few nanometers in size, and a narrow size distribution can be obtained with Ag [8] and Pt-group metals as well [51]. It was possible to gain a first insight into the reaction mechanism for the synthesis of silver nanoparticles, the size homogenization of the particles resulting from Ostwald ripening [52]. Moreover, it was evidenced that silver rods particles can be obtained through heterogeneous nucleation with platinum nuclei formed in situ [7]. During the last decade good progress has been made in the control of size, shape, and surface capping in the synthesis of noble metal nanoparticles and in the understanding of the growth mechanism as exemplified in the following two sections.

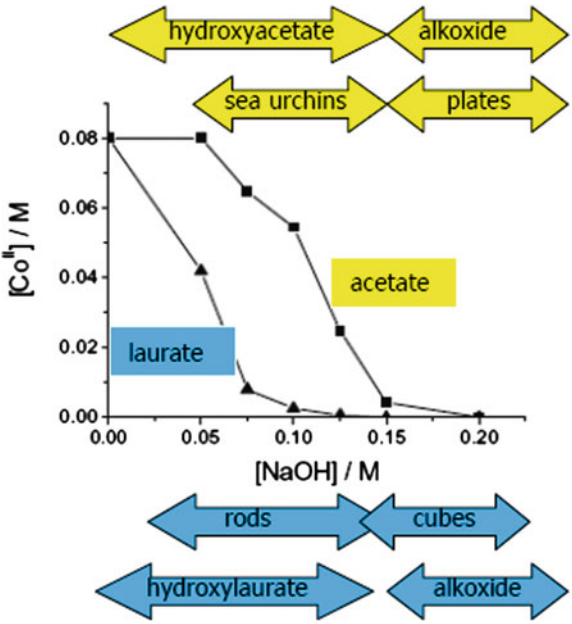


Fig. 1.12 [Co^{II}] in basic solution of BEG immediately before reduction, nature of the intermediate solid phase, and shape of the final particles versus [NaOH] using the acetate and laurate precursors

1.3.3.1 Ruthenium Nanoparticles: Size, Shape, and Self-assemblies

Monodisperse ruthenium nanoparticles were obtained from $RuCl_3$ from acetate solution in polyols [53]. Due to their strong affinity for such particles acetate ions added in sufficient amount are found to be the best way to prevent agglomeration through electrostatic stabilization. The mean diameter of the particles is tuned reproducibly in the 1.5–6 nm range by varying the synthesis temperature and the acetate concentration, a very narrow size distribution being observed in the whole sample without any further size selection processes (Fig. 1.14a, b and Table 1.2). The higher the temperature the higher the nucleation rate, and for a given concentration of metal, the lower the mean particle size. Nucleation and growth rates depend upon [AcO-]/[Ru] ratio because acetate ions can act as ligands for both the metal particles and the Ru(III) and Ru(II) species in solution. Among a majority of nearly isotropic particles, a significant number of platelets with an aspect ratio as low as ¼ is observed.

For samples with the lowest size standard deviation self-organization of particles coated with dodecane thiol can be observed on the carbon membrane of the TEM grid. Depending upon thiol concentration columnar units made of edgewise stacked platelets or hexagonal arrays of isotropic particles juxtaposed with a mean interparticle distance of 2 nm are evidenced (Fig. 1.14c–d). Close-packed or non compact stacking of two hexagonal layers are observed depending on the evaporation rate of the solvent.

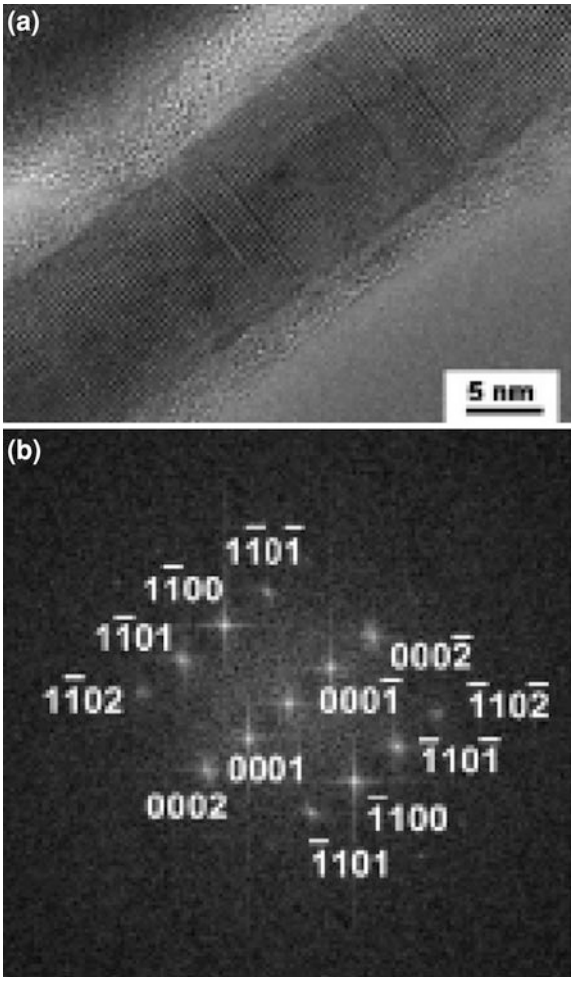


Fig. 1.13 **a** HRTEM image of a single *hcp* cobalt rod with the *c* axis parallel to the rod axis, **b** the corresponding numerical diffraction pattern. [31]

1.3.3.2 Growth Mechanism and Shape Control of Silver Nanoparticles

During the last decade Xia's group made several modifications to the early protocol used in the polyol-mediated synthesis of silver particles in order to achieve a better control over the nucleation and growth steps [27]. This allows for the reproducible preparation of nanoparticles of different well-defined and controllable shapes such as nanocubes, nanowires, or nanospheres by simply varying the concentration of the starting compound ($AgNO_3$) and the relative amount of PVP used as capping agent.

A growth mechanism which accounts for these different morphologies has been proposed. Formation of metal clusters and seeds of different crystallinities have been evidenced [54, 55]: (i) single crystal (SC) cuboctahedra with {111} and

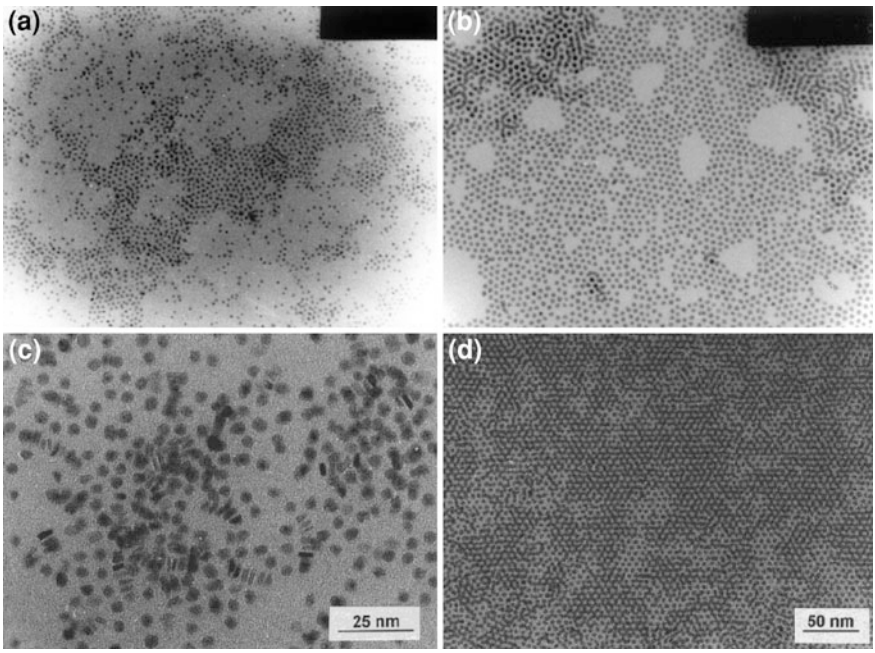


Fig. 1.14 TEM images of ruthenium nanoparticles prepared in sodium acetate solution in 1,2-propanediol **a** $d_m = 2{,}5$ nm $\sigma/d_m = 0.11$, **b** $d_m = 3.95$ nm $\sigma/d_m = 0.066$, **c** lines of a few 4-nm sized platelets particles, **d** mono and bi layer showing compact stacking [53]

Table 1.2 Dependence of the mean diameter and size distribution of Ru nanoparticles obtained in 1,2-propanediol on temperature and acetate concentration [53]

Reaction time (min)	T (°C)	Acetate (mol L^{-1})	d_m (nm)	σ (nm)	σ/d_m
10	165	$1.1\ 10^{-2}$	1.6	0.24	0.15
10	165	$2.2\ 10^{-2}$	1.4	0.18	0.13
10	165	$4.4\ 10^{-2}$	1.8	0.18	0.10
10	150	$1.0\ 10^{-2}$	3.95	0.26	0.066
10	150	$2.0\ 10^{-2}$	1.7	0.21	0.12
10	150	$4.0\ 10^{-2}$	2.0	0.25	0.125
10	150	$8.8\ 10^{-2}$	2.5	0.28	0.11
30	140	$1.0\ 10^{-2}$	6		
30	140	$2.0\ 10^{-2}$	2.2		

{100} facets, (ii) multiply-twinned particles (MTP) often in the decahedral shape with {111} facets only, (iii) quasi-spherical multiply-twinned particles. The control of the shape of the final particles is tentatively explained as follows. MTP decahedra seeds are the most thermodynamically stable ones since they are bond only by {111} facets which have the lowest energy. Nevertheless, if nucleation and growth are fast enough the available time for twin defects to form is short and the

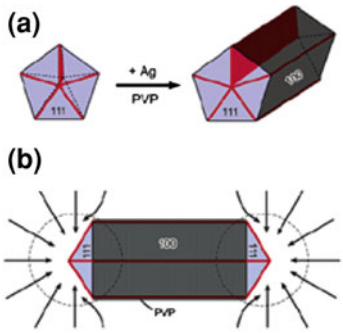


Fig. 1.15 Growth mechanism of pentagonal silver nanowires: **a** growth of a nanorod from an MTP decahedral seed with limited access of silver atoms to {100} facets due to selective adsorption of PVP, **b** diffusion of silver atoms the two ends of a nanorod [54]

formation of SC cuboctahedra seeds is kinetically favored. By varying the [PVP]/[$AgNO_3$] ratio the thickness of the PVP layer and the location of the polymer chains could be modified due to a preferential adsorption onto the {100} facets. Thus, the growth rates of the different facets can be tuned and nanoparticles with distinct shapes can be obtained. Nanocubes are the result of a kinetically controlled growth from SC cuboctahedra; pentagonal nanowires the result of a twin-induced anisotropic growth from MTP decahedra (Fig. 1.15), and nanospheres the result of an isotropic growth of MTP seeds coated with a thick PVP layer on each facet. This mechanism is consistent with many experimental features evidenced by Xia's group, namely the influence upon morphology of trace amounts of inorganic species such as chloride ion and oxygen which promote the formation of single crystal truncated cubes and tetrahedron [55] or CuCl or $CuCl_2$ which favor the rapid synthesis of nanowires [56]. Moreover, according to the same guidelines shape control of nanoparticles has been achieved with platinum and palladium as well [57, 58].

1.3.4 Synthesis of Oxide Nanoparticles

The first report on the elaboration of oxide particles in polyol deals with the synthesis of zinc oxide from acetate dihydrate in di(ethylene glycol) [14, 59]. Single nanocrystals or monodisperse submicrometer polycrystalline particles formed by aggregation of small crystallites were obtained depending upon the precursor concentration. A plausible mechanism was proposed where acetate ions play two main roles. They deprotonate partially the polyol leading to alkoxy groups and they act as a complexing agent toward the cation with formation of an alkoxyacetate complex, such an alkoxyacetate complex being indeed isolated and characterized [60]. Then the forced hydrolysis occurs upon reflux with the progressive development of ZnO nuclei and crystal growth via olation and oxolation reactions. Such a mechanism is similar to that occurring in the well-known sol–gel process [61], where the alkoxyacetate is formed by addition of acetic acid to an

alcohol solution containing the alkoxide instead to be formed in situ in the described polyol-mediated synthesis.

Since this pioneer work Feldmann's group have extended the polyol route to the synthesis of various oxides to obtain nanoscale functional materials such as luminescent materials (phosphor host lattices: e.g. Y_2O_3), color pigments ($CoAl_2O_4$, Cr_2O_3, $ZnCo_2O_4$, $Ti_{0.85}Ni_{0.05}Nb_{0.10}O_2$), transparent conductive oxide ($ZnO:In^{3+}$), and catalytically active oxides (CeO_2, Mn_3O_4, V_2O_5) [26]. Stable colloidal suspensions of almost non-agglomerated nanoscale particles 30–200 nm in size were obtained from a suitable metal precursor (acetate, alcoholate, halogenide) in di(ethylene glycol). The solid content can be up to 20 %.

Simultaneously, during the past decade Ammar's group used the polyol process to synthesize nanoparticles of spinel-like oxides such as Fe_3O_4, γFe_2O_3, and ferrites MFe_2O_4 (M = Co, Ni, Zn, Mn) in order to tune and optimize their magnetic properties for different applications such as high density magnetic recording [17, 62, 63], high frequency applications [34, 35, 64], biomedical ones (targeted drug delivery, magnetic resonance imaging [65], and cancer treatment by magnetic hyperthermia [66, 67]. Nanoparticles of a few nanometers' size are currently obtained; they have a good crystallinity and a high saturation magnetization with a distribution of the cations on the two sites of the spinel structure which may differ from that of corresponding bulk materials and varies with the particle size [68]. Not very long ago intensive research has focused on the polyol-mediated synthesis of cuprous oxide with the aim to control particles size and shape in order to optimize the electrochemical performance of this material as a lithium-ion battery anode [69–72]. Whatever the nanoparticulate oxide synthesized, the polyol acts as a solvent, a complexing and capping agent which limits particle growth and prevents agglomeration almost always without addition of other additives. Furthermore, the used polyols are weak stabilizers which can be removed easily through surface chemistry exchange induced by specific functional groups. In particular cases the polyol acts also as a reducing agent, for instance when Fe_3O_4 and Cu_2O particles are obtained from Fe(III) and Cu(II) precursors respectively.

Conversely to metal nanoparticles synthesis, oxide nanoparticles are always obtained through spontaneous nucleation without seeding with foreign nuclei. Many experimental factors can modify the size, shape, or crystallinity of the particles. A tuned precursor has to be selected but for a given precursor the characteristics of the particles may depend upon the nature of the polyol as exemplified in Fig. 1.16. The relative amount of water added defined by the hydrolysis ratio can affect the nature of the final compound (Sect. 2.1) but for a given oxide it can affect the particle size as well by acting upon the reflux temperature (Fig. 1.17). An increase in the temperature leads to larger particles but at a defined temperature the particle diameter can be steered by adjusting the duration of the reaction. In particular cases, varying the basicity of the polyol solution by adding sodium hydroxide or sodium acetate can lead to particles of different shapes [73]. The potentiality of the polyol process to synthesize monodisperse oxide nanoparticles with an accurate and reliable mean size and with controlled shapes will be exemplified by some examples in the following sections.

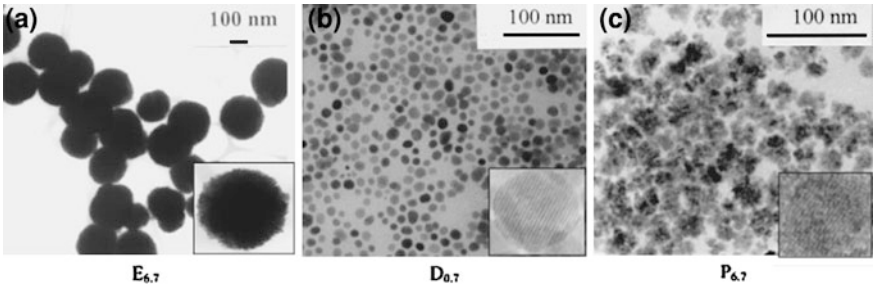


Fig. 1.16 TEM images of $ZnFe_2O_4$ particles obtained from $Zn(CH_3CO_2)_2.2H_2O$ in different polyols under reflux **a** ethylene glycol, $h = 6.7$, **b** di(ethylene glycol), $h = 0.7$, **c** propylene glycol, $h = 6.7$, in inset the HRTEM image of **a** an isolated polycrystalline particle, **b**, **c** a single crystal [35]

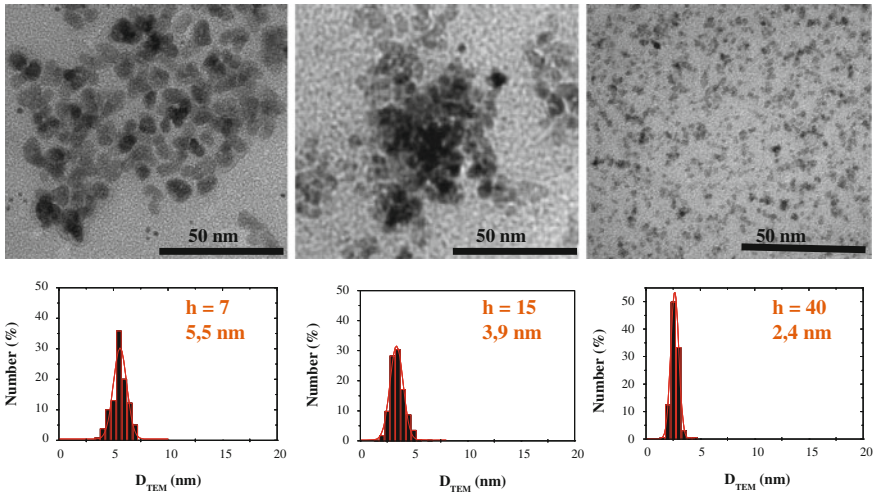


Fig. 1.17 TEM images of $CoFe_2O_4$ samples obtained in DEG under reflux with different amount of water added. The mean diameter decreases when the hydrolysis ratio h increases since the reflux temperature is lowered

1.3.4.1 Size Control of Magnetic Particles for Biological Applications

Colloids of superparmagnetic nanoparticles are promising materials for various biomedical applications, namely magnetic resonance imaging (MRI) and cancer treatment by hyperthermia. Such applications require monodisperse, highly crystalline, chemically stable particles to provide high and reliable magnetization values. Moreover, they must be water soluble and have a low toxicity to ensure good biocompatibility. Currently, superparamagnetic iron oxide nanoparticles (*SPIONs*), Fe_3O_4 (magnetite) and γ-Fe_2O_3 (maghemite) used in biomedicine are obtained by

coprecipitation of iron salts in aqueous solution. Such a method does not ensure a simultaneous control over size and crystallinity and therefore the control of the magnetic properties needs post-size selection process or/and thermal treatment. Alternatively, thermal decomposition of metal organic precursors in high boiling organic solvents with stabilizing ligands leads to highly crystalline, size-controlled monodisperse particles, but requires a post treatment to remove the stabilizing ligands. Recently, *SPIONs* with suitable characteristics have been obtained by the polyol process without the drawbacks of these previous methods. The synthesis of magnetite nanoparticles was carried out by Wan et al. by reacting iron (III) acetylacetonate in tri(ethylene glycol) at elevated temperature without any surfactants [74]. The particles (8 ± 1.1 nm) are superparamagnetic at room temperature. The high reaction temperature favors a high crystallinity and a high magnetization, the particles being stable in aqueous solution or in physiological buffer due to their surface coating by the polyol. Magnetite and maghemite nanoparticles have been obtained by Basti et al. [65] by refluxing Fe(II) acetate solution in di(ethylene glycol) with a hydrolysis ratio equal to 1 and 11 respectively. In order to stabilize the particles in a physiological environment the as-prepared particles were transferred to an aqueous medium through ligand exchange chemistry of the adsorbed polyol species with the dopamine or the catechaldehyde. As expected from the characteristics of these particles (mean size, hydrodynamic radius of coated particles, magnetic characteristics), in vitro resonance imaging essays have shown that they are clearly strong negative contrast agents.

To be used as potential agents for hyperthermia, superparamagnetic nanoparticles must be able to induce selective death of tumoral cells upon heating in the temperature range $42\ °C < T < 47\ °C$. Their efficiency depends strongly upon the average size of the particles and increases sharply when the standard deviation decreases. Therefore, monodisperse particles with a given optimal size and a high magnetization at the body temperature are required. Moreover, a Curie temperature just higher than the therapeutic temperature range would ensure a self-regulated temperature control in an alternating field.

For such an application stoichiometric $Mn_{0,2}Zn_{0,8}Fe_2O_4$ monodisperse nanoparticles were obtained by Ammar's group [67] by forced hydrolysis in di(ethylene glycol) (Fig. 1.18). As Zn-substituted MFe_2O_4 (M : Ni, Co, Fe, Mn) nanoparticles have interesting temperature-sensitive magnetic properties that can be tuned through their chemical composition and cation distribution, a systematic study was then undertaken on $M_{1-x}Zn_xFe_2O_4$ samples varying the cation M, the composition x, and the nature of the polyol with two targeted mean particle sizes (4 and 10 nm) [66]. Table 1.3 exemplifies the potentiality of the polyol process to synthesize monodisperse nanoparticles with a finely tuned size. In some particular cases the polyol process also allows to obtain particles of different shapes as exemplified in the following section.

1.3.4.2 Oxide Particles of Various Shapes

In the past years several groups have focused on the shape control of cuprous oxide polyol-mediated nanoparticles. Park et al. reported on the gram scale synthesis of Cu_2O nanocubes from copper (II)acetylacetonate in 1,5 pentanediol with addition

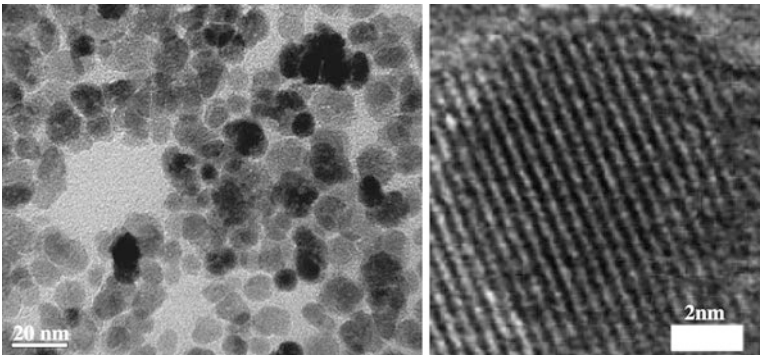


Fig. 1.18 TEM image (*left*) and HRTEM image (*right*) of $Mn_{0,2}Zn_{0,8}Fe_2O_4$ nanoparticles obtained from iron chloride, manganese, and zinc acetates in di(ethylene glycol) under reflux [67]

Table 1.3 Mean particle size and standard deviation inferred from TEM image analysis for various samples prepared in di(ethylene glycol) or tetra(ethylene glycol) with a target size of 4 nm (samples labeled P) or a target size of 10 nm (samples labeled G) [66]

Sample	M	Composition	<D > /nm
PNi1	Ni	$Ni0.1Zn0_{.9}Fe_2O_4$	3.5 ± 0.4
G_{Ni1}			9.5 ± 1.6
P_{Ni2}		$Ni_{0.2}Zn_{0.8}Fe_2O_4$	3.6 ± 0.3
G_{Ni2}			8.3 ± 1.1
P_{Co1}	Co	$Co_{0.1}Zn_{0.9}Fe_2O_4$	4.1 ± 0.5
G_{Co1}			10.9 ± 1.5
P_{Co2}		$Co_{0.2}Zn_{0.8}Fe_2O_4$	4.3 ± 0.5
G_{Co2}			10.3 ± 1.4
P_{Fe1}	Fe	$Fe_{0.1}Zn_{0.9}Fe_2O_4$	4.4 ± 0.5
G_{Fe1}			11.5 ± 1.7
P_{Fe2}		$Fe_{0.2}Zn_{0.8}Fe_2O_4$	4.3 ± 0.5
G_{Fe2}			12.5 ± 1.5
P_{Mn1}	Mn	$Mn_{0.1}Zn_{0.9}Fe_2O_4$	4.1 ± 0.4
G_{Mn1}			10.7 ± 1.1
P_{Mn2}		$Mn_xZn_{1-x}Fe_2O_4$	4.0 ± 0.4
G_{Mn2}		$0.1 < x < 0.2$	11.7 ± 1.8

of PVP [72]. The as-obtained highly monodisperse cubes with an average edge size of 53 ± 3 nm ($\sigma = 6$ %) are single crystals. Their controlled oxidation leads to CuO hollow cubes, hollow spheres, and urchin-like particles through a sequential dissolution–precipitation process, the latter having the best electrochemical performance for lithium-ion batteries.

Orel et al. synthesized Cu_2O nanowires from $Cu(CH_3COO)_2.H_2O$ in di(ethylene glycol) without the addition of surfactant or stabilizer [70]. The nanowires are built with tiny single crystals embedded in an amorphous matrix (Fig. 1.19).

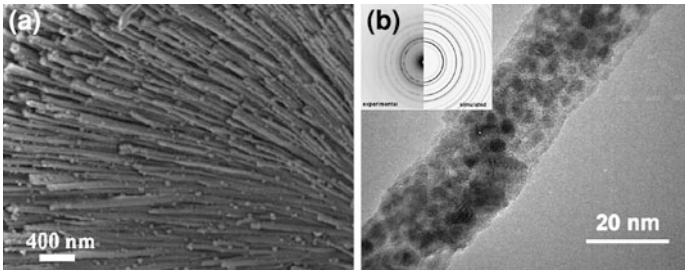


Fig. 1.19 **a** SEM images of Cu_2O nanowires obtained from $Cu(CH_3COO)_2.H_2O$ in di(ethylene glycol) without any additives **b** HRTEM image of Cu_2O nanowire with 2–5 nm crystallites embedded in an amorphous matrix. Inset of b) experimental and simulated SAED patterns [70]

Such a morphology is obtained in a narrow concentration range of the starting acetate under controlled heating and is tentatively explained by the formation of a fibrous glycolate complex which decomposes upon heating leading to Cu_2O retaining the wire-like morphology. Huang et al. controlled the shape of Cu_2O particles by reducing $Cu(CH_3COO)_2.H_2O$ by ethylene glycol with different concentrations of PVP [71]. Nanoparticles prepared without PVP are nanoboxes with an average edge size of 100 nm and a wall thickness of about 20 nm. Nanocubes and nanospheres are obtained at PVP concentrations of 1.0 and 2.0 mM respectively. Whatever their morphology the particles grow by aggregation of smaller single nanocrystals. Previous studies exemplify the potentiality of the polyol process to get nanoparticles of different shapes by using various starting compounds or polyols and by adding a stabilizer at different concentrations.

Several authors reported on modification of shape of zinc oxide nanoparticles. In order to study biocidal effects and cellular internalization of ZnO nanoparticles on *Escherichia coli* bacteria ZnO nanoparticles were synthesized in di(ethylene glycol), particle size and shape being controlled by varying the hydrolysis ratio and/or by addition of small molecules and macromolecules such as tri-n-octylphosphine oxide (TOPO), sodium dodecyl sulfate (SDS), polyoxyethylene stearyl ether (Brij-76), and bovine serum albumin (BSA) [75]. For ZnO prepared without addition of small molecules or macromolecules (Fig. 1.20a), spherical nanoparticles with a broad size distribution were observed. On the other hand, a unique kind of one-dimensionally organized ZnO nanoparticles was obtained by electrostatic stabilization with BSA as templating material (Fig. 1.20b). A very narrow size distribution of ZnO spherical nanoparticles was obtained with addition of TOPO as a template (Fig. 1.20c). In this case, TOPO acts as a template and also as a linker in the self-assembly of ZnO nanoparticles. For ZnO-TOPO synthesis, when hydrolysis ratio increased from $h = 10$ to $h = 30$ and TOPO concentration decreased from 10^{-1} to 10^{-2} M, ZnO nanorods formation was observed (Fig. 1.20d). Finally, ZnO anisotropic morphologies were obtained by coalescence of spherical and cubic nanoparticles in the presence of SDS and

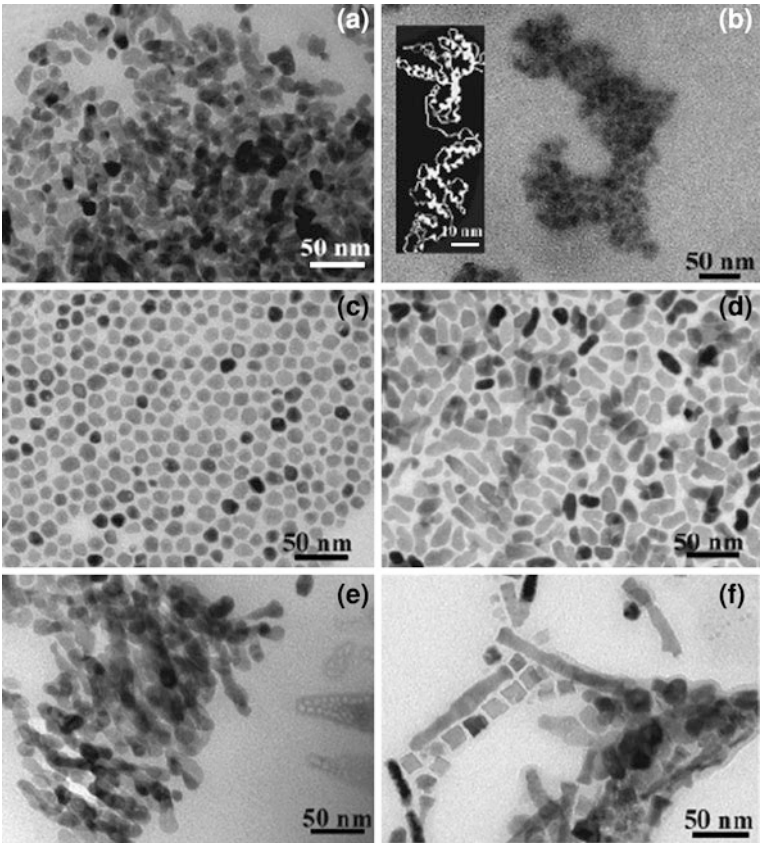


Fig. 1.20 TEM micrographs of ZnO nanoparticles synthesized in DEG medium: **a** ZnO without addition of macromolecules; **b** ZnO-BSA (*top left* schematic BSA conformation); **c** ZnO-TOPO ($h = 10$); **d** ZnO-TOPO ($h = 30$); **e** ZnO-SDS; and **f** ZnO-Brij-76 [75]

Brij-76 macromolecules, respectively (Fig. 1.20e–f). In the case of Brij-76, used as a structure director, assemblies of ZnO nanocubes were observed. These nanocubes have a coalescence tendency that allows formation of ZnO nanobelts (Fig. 1.20f).

By acting upon other synthesis parameters, namely the basicity of the reaction medium, Dakhlaoui et al. synthesized ZnO nanoparticles of various shapes as well. They were obtained from $Zn(CH_3COO)_2.2H_2O$ in di(ethylene glycol) where various amounts of sodium hydroxide were added. When the alkaline ratio ($b = n_{sodium\ \ hydroxide}/n_{metal}$) increases the particles' morphology evolves from anisotropic shape (conical, nanorod-like) to a spherical one [73]. A growth mechanism is proposed on the basis of a selective adsorption of polyol molecules on the non-polar facets and of OH^- ions on the polar {001} ones.

1.4 Conclusion

This chapter provides an overview of an easy to carry out and versatile route to synthesize particulate inorganic materials, the so-called polyol process. It is shown how it is possible to take advantages of the properties of polyols (α-diols and etherglycols), namely their rather high permittivity, their mild reducing power, and their chelating properties, to obtain in such reacting media metal or oxide particles from various inorganic metallic salts and complexes through reduction or hydrolysis reactions. A kinetic control of the nucleation and growth steps allows getting non-agglomerated monodisperse well-crystallized particles. As exemplified by many examples, an accurate and reliable control of the average particle size can be achieved in the nanometer range by modifying experimental parameters or through seeding by foreign nuclei. It is also shown that such a kinetic control allows in certain systems to steer the shape of the particles and namely to induce an anisotropic growth which generates 1D nanostructures such as nanorods or nanowires. This chapter exemplifies the potentiality of the polyol process to tune the size and shape-dependent magnetic properties of ferromagnetic metal or spinel ferrite particles used as advanced functional materials in various fields: high permeability composite materials, high density recording media, high temperature permanent magnets, and biomedical applications such as magnetic resonance imaging, cancer treatment by hyperthermia, or targeted drug delivery.

References

1. US Patent : 4 539 041 (1985) Europe Patent : 0 113 281, 1987
2. Fiévet F, Lagier J-P, Figlarz M (1989) MRS Bull 14:29–34
3. Fiévet F, Lagier J-P, Blin B, Beaudoin B, Figlarz M (1989) Solid State Ion 32(33):l98–205
4. Fiévet F, Fiévet-Vincent F, Lagier J-P, Dumont B, Figlarz M (1993) J Mater Chem 3:627–632
5. Silvert P-Y, Tekaia-Elhsissen K (1995) Solid State Ion 82:53–60
6. Ducamp-Sanguesa C, Herrera-Urbina R, Figlarz M (1993) Solid State Ion 63–65:25–30
7. Ducamp-Sanguesa C, Herrera-Urbina R, Figlarz M (1992) J Solid State Chem 100:272–280
8. Silvert P-Y, Herrera-Urbina R, Duvauchelle N, Vijayakrishnan V, Tekaia-Elhsissen K (1996) J Mater Chem 6:573–577
9. Silvert P-Y, Vijayakrishnan V, Vibert P, Herrera-Urbina R, Tekaia-Elhsissen K (1996) Nanostruct Mater 7:611–618
10. Tekaia-Elhsissen K, Bonet F, Silvert P-Y, Herrera-Urbina R (1999) J Alloy Compd 292:96–99
11. Toneguzzo P, Viau G, Acher O, Fiévet-Vincent F, Fiévet F (1998) Adv Mater 10:1032–1035
12. Viau G, Fiévet-Vincent F, Fiévet F (1996) J Mater Chem 6:1047–1053
13. Toneguzzo P, Viau G, Acher O, Guillet F, Bruneton E, Fiévet-Vincent F, Fiévet F (2000) J Mater Sci 35:3767–3784
14. Jézéquel D, Guenot J, Jouini N, Fiévet F (1995) J Mater Res 10:77–83
15. Feldmann C, Merikhi J (2000) J Colloid Interface Sci 223:229–234
16. Feldmann C, Jungk HO (2001) Angew Chem Int Ed 40:359–362
17. Ammar S, Helfen A, Jouini N, Fiévet F, Villain F, Rosenman I, Danot M, Molinié Ph (2001) J Mater Chem 10:186–192
18. Poul L, Ammar S, Jouini N, Fiévet F, Villain F (2001) Solid State Sci 3:31–42

19. Poul L, Ammar S, Jouini N, Fiévet F, Villain F (2003) J. Sol-Gel Sci Tech 26:261–265
20. Poul L, Jouini N, Fiévet F (2000) Chem Mater 12:3123–3132
21. Feldmann C, Metzmacher C (2001) J Mater Chem 11:2603–2606
22. Antoun T, Brayner R, Al Terary S, Fiévet F, Chehimi M, Yassar A (2007) Eur J Inorg Chem 1275–1284
23. Al Terary S, Mangeney C, Brayner R, Antoun T, Fiévet F, Yassar A (2008) Sens Lett 6:511–517
24. Feldmann C, Jungk H-O (2002) J Mater Sci 37:3251–3254
25. Roming M, Feldmann C (2008) J Mater Sci 43:5504–5507
26. Feldmann C (2003) Adv Funct Mater 13:101–107
27. Wiley B, Sun Y, Mayers B, Xia Y (2005) Chem Eur J 11:454–463
28. Luna C, Morales MP, Serna CJ, Vazquez M (2003) Mater Sci Eng C Biomimetic and Supramol Sys C23(6–8):1129–1132
29. Viau G, Toneguzzo P, Pierrard A, Acher O, Fiévet-Vincent F, Fiévet F (2001) Scripta Mater 44:2263–2267
30. Ung D, Viau G, Fiévet-Vincent F, Herbst F, Richard V, Fiévet F (2005) Prog Solid State Chem 33:137–145
31. Soumare Y, Garcia C, Maurer T, Chaboussant G, Ott F, Fiévet F, Piquemal J-Y, Viau G (2009) Adv Funct Mat 19(12):1971–1977
32. Ung D, Viau G, Ricolleau C, Warmont F, Gredin P, Fiévet F (2005) Adv Mater 17:338–344
33. Sun Y, Gates B, Mayers B, Xia Y (2002) Nano Lett 2:165–168
34. Chkoundali S, Ammar S, Jouini N, Fiévet F, Molinié P, Danot M, Villain F, Grenèche J-M (2004) J Phys Condens Matter 16:4357–4372
35. Ammar S, Jouini N, Fiévet F, Stephan O, Marhic C, Richard M, Villain F, Cartier dit Moulin Ch, Brice S, Sainctavit P (2004) J Non-Crystal Solids 345 and 346:658–662
36. Caruntu D, Remond Y, Chou NH, Jun M-J, Caruntu G, He J, Goloverda G, O'Connor C, Kolesnichenko V (2002) Inorg Chem 41:6137–6146
37. Wang W–W (2008) Mater Chem Phys (2008) 108:227–231
38. Gold SH, Bruce RW, Fliflet AW, Lewis D, Kurihara LK, Imam MA (2007) Rev Sci Instrum 78:023901/1-023901/6
39. Nishioka M, Miyakawa M, Kataoka H, Koda H, Sato K, Suzuki TM (2011) Nanoscale 3:2621–2626
40. LaMer VK, Dinegar RH (1950) J Am Chem Soc 72:4847–4854
41. Viau G, Fiévet-Vincent F, Fiévet F (1996) Solid State Ion 84:259–270
42. Silvert P-Y, Herrera-Urbina R, Tekaia-Elhsissen K (1997) J Mater Chem 7:293–299
43. Joseyphus RJ, Kodama D, Matsumoto T, Sato Y, Jeyadevan B, Tohji K (2007) J Magn Magn Mater 310:2393–2395
44. Joseyphus RJ, Shinoda K, Kodama D, Jeyadevan B (2010) Mater Chem Phys 123:487–493
45. Kodama D, Shinoda K, Sato K, Konno Y, Joseyphus RJ, Motomiya K, Takahashi H, Matsumoto T, Sato Y, Tohji K, Jeyadevan B (2006) Adv Mater 18:3154–3159
46. Takahashi M, Ogawa T, Hasegawa D, Jeyadevan B (2005) J Appl Phys 97:10J307-1-6
47. Dumestre F, Chaudret B, Amiens C, Respaud M, Fejes P, Renaud P, Zurcher P (2003) Angew Chem Int Ed 42:5213–5216
48. Zang Z, Dai S, Blom D, Shen J (2002) Chem Mater 14:965–968
49. Ung D, Soumare Y, Chakroune N, Viau G, Vaulay M-J, Richard V, Fiévet F (2007) Chem Mater 19:2084–2094
50. Maurer T, Ott F, Chaboussant G, Soumare Y, Piquemal J-Y, Viau G (2007) Appl Phys Lett 91:172501
51. Bonet F, Delmas V, Grugeon S, Herrera Urbina R, Silvert P-Y, Tekaia-Elhsissen K (1999) Nanostruct Mater 11:1277–1284
52. Silvert P-Y, Herrera-Urbina R, Tekaia-Elhsissen K (1997) J Mater Chem 7:293–299
53. Viau G, Brayner R, Poul L, Chakroune N, Lacaze E, Fiévet-Vincent F, Fiévet F (2003) Chem Mater 15:486–494
54. Sun Y, Mayers B, Herricks T, Xia Y (2003) Nano Lett 3:955–960
55. Wiley B, Herricks T, Sun Y, Xia Y (2004) Nano Lett 4:1733–1739

56. Korte KE, Skrabalak SE, Xia Y (2008) J Mater Chem 18:437–441
57. Chen J, Herricks T, Geissler M, Xia Y (2004) J Am Chem Soc 126:10854–10855
58. Xiong Y, Chen J, Wiley B, Xia Y (2005) J Am Chem Soc 127:7332–7333
59. Poul L (2000) PhD-Thesis. University Pierre et Marie Curie, Paris, France
60. Jouini N, Poul L, Robert F, Fiévet F (1995) Eur J Solid State Inorg Chem 32:1129–1136
61. Sanchez C, Livage J (1990) New J Chem 14:513–521
62. Ben Tahar L, Smiri LS, Artus M, Joudrier A-L, Herbst F, Vaulay M-J, Ammar S, Fiévet F (2007) Mater Res Bull 42:1888–1896
63. Artus M, Ammar S, Sicard L, Piquemal J-Y, Herbst F, Vaulay M-J, Fiévet F, Richard V (2008) Chem Mater 20:4861–4872
64. Beji Z, Ben Chaabane T, Smiri LS, Ammar S, Fiévet F, Jouini N, Grenèche JM (2006) Phys Stat sol (a) 203:504–512
65. Basti H, Ben Tahar L, Smiri LS, Herbst F, Vaulay M-J, Chau F, Ammar S, Benderbous S (2010) J Colloid Interface Sci 341:248–254
66. Basti H (2010) PhD-Thesis. University Paris Diderot, Paris, France
67. Beji Z, Hanini A, Smiri LS, Gavard J, Kacem K, Villain F, Grenèche J-M, Chau F, Ammar S (2010) Chem Mater 22:5420–5429
68. Ammar S, Jouini N, Fiévet F, Beji Z, Smiri L, Molinié P, Danot M, Grenèche JM (2006) J Phys Condens Mater 18:9055–9069
69. Grugeon S, Laruelle S, Herrera-Urbina R, Dupont L, Poizot P, Tarascon J-M (2001) J Electrochem Soc 148:A285–A292
70. Orel ZC, Anzlovar A, Drazic G, Zigon M (2007) Cryst Growth Des 7:453–458
71. Huang L, Peng F, Yu H, Wang H (2008) Mater Res Bull 43:3047–3053
72. Park JC, Kim J, Kwon H, Song H (2009) Adv Mater 21:803–807
73. Dakhlaoui A, Jendoubi M, Smiri LS, Kanaev A, Jouini N (2009) J Cryst Growth 311: 3989–3996
74. Wan J, Cai W, Meng X, Liu E (2007) Chem Commun 47:5004–5006
75. Brayner R, Ferrari-Iliou R, Brivois N, Djediat S, Benedetti M, Fiévet F (2006) Nano Lett 6:866–870

Chapter 2
Synthesis of Organic and Bioorganic Nanoparticles: An Overview of the Preparation Methods

Joachim Allouche

Abstract Since the emergence of Nanotechnology in the past decades, the development and design of organic and bioorganic nanomaterials has become an important field of research. Such materials find many applications in a wide range of domains such as electronic, photonic, or biotechnology, which contribute to impact our society and our way of life. The improvement of properties and the discovery of new functionalities are key goals that cannot be obtained without a well controlled and a better understanding of the preparation methods which constitute the starting point of the design of a specific organic material. In this context, this chapter gives a general but non-exhaustive overview of the methods of preparation of organic and bioorganic nanoparticles. Some general definitions about organic nanoparticles and description of organic compounds are given before describing the most common methods used divided into two families, the two-step and one-step procedures. The major part of the two-step procedures is based on an emulsification step followed by generation of nanoparticles through different mechanisms such as precipitation, gelation, or polymerization. The one-step procedures are founded on generation of nanoparticles through different techniques such as nanoprecipitation, desolvation, or drying processes without preliminary emulsification step. For each method, the description is supported by several examples and focused on the explanation of the general mechanisms and of the major key parameters involved in the control

J. Allouche (✉)
IPREM-ECP, Université de Pau et des Pays de l'Adour (UPPA),
Technopôle Hélioparc, 2 avenue du Président Angot, 64000 Pau, France
e-mail: joachim.allouche@univ-pau.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_2,

of the nanoparticles formation. In addition, since emergence and improvement of syntheses are often associated to development of experimental setups, technological aspects are also mentioned.

2.1 Introduction

Organic nanoparticles can be commonly described as solid particles composed of organic compounds (mainly lipids or polymeric) ranging in diameter from 10 nm to 1 μm [1, 2]. Over the past decades, this type of nanoparticles has met a great expansion and intensive investigations due to their high potentialities in a wide spectrum of industrial areas ranging from electronic to photonic, conducting materials to sensors, medicine to biotechnology, and so forth [3–13]. Therefore, the choice of the synthetic route is central to optimize the final properties of nanoparticles designed for a specific application. This choice has to be guided by a series of factors such as physico-chemical parameters of the organic compound, chemical composition, nanoparticles diameter, structure, morphology, or environmental considerations which constitute definitely an increasingly incontrovertible criterion. Consequently, a suitable preparation method cannot be dissociated from a real compromise chosen in function of the different constraints which have to be overcome to design well-controlled organic nanomaterials.

This chapter focuses on the description of the most used preparation methods reported in the literature for the preparation of organic nanoparticles. It starts by giving some general definitions on the different types of nanoparticles and features of organic compounds. The second part is devoted to the description of the preparation methods highlighting the general principles and mechanisms involved and the parameters governing the particles formation and their properties.

2.2 Definition and Description of the Different Types of Particles

2.2.1 Structure and Morphology

Nanoparticles can be divided into main two groups: nanospheres and nanocapsules (Fig. 2.1). Nanospheres are considered as matrix particles whose entire mass are solid whereas nanocapsules are composed of a liquid or empty core surrounded by an organic solid shell. Nanospheres and nanocapsules are generally spherical but non-spherical shape can be encountered. The obtaining of the different types of nanoparticles depends evidently on the methods selected for the preparation.

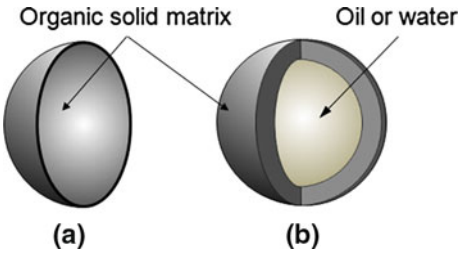


Fig. 2.1 The different types of nanoparticles. **a** Nanospheres, **b** nanocapsules

2.2.2 Organic Materials Composing the Particles

2.2.2.1 Polymeric Nanoparticles

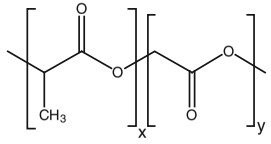
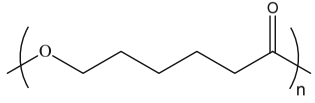
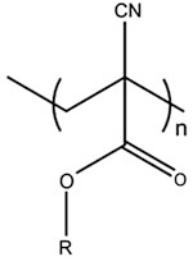
Polymeric nanoparticles constitute by far the most studied organic particles in the literature [14–18]. Although polymeric nanoparticles can be designed for a wide spectrum of applications, two major families can be distinguished. The first one is related to nanoparticles elaborated for drug delivery and/or for biomedical purposes [19–22]. In this case, the macromolecules require biodegradable or biocompatible properties. Number of synthetic or natural polymers can be used and the most widely used are reported in Table 2.1. Despite the great potential of polymer chemistry today, one can observe that only a limited number of molecules can be used as constituents of drug delivery nanocarriers. This is particularly due to the drastic constraints and requirements which characterized in vivo applications in terms of toxicity and biocompatibility.

The second family of polymeric particles is constituted of conjugated polymeric nanoparticles that exhibit electronic or opto-electronic properties [15, 23]. Among these conjugated polymers; polyaniline, polypyrrole, polyacetylene, and their derivatives have been widely studied for their intrinsic conductivity [24–30], while polythiophenes, polyfluorenes, poly(p-phenylenevinylene)s, and poly(p-phenyleneethynylene)s derivatives [31–38] have rather been studied for their electro-optical and photoluminescence behaviors.

2.2.2.2 Solid Lipid Nanoparticles

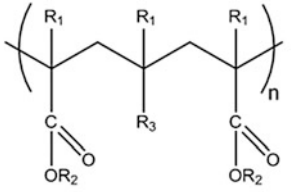
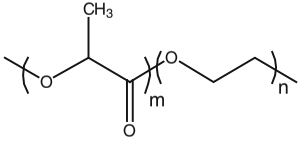
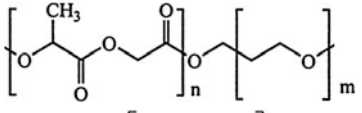
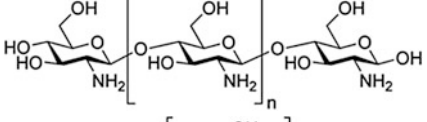
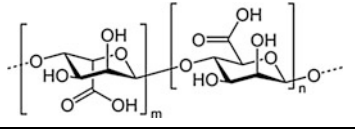
Solid lipid nanoparticles (SLNs) are composed of lipid matrices derived generally from glycerol esters of fatty acids [39–41]. The lipid compound is characterized by a melting temperature above 37 °C in order to ensure solidification at physiological temperature. Due their high stability (several years), good biocompatibility and low toxicity, SLNs are considered as promising drug delivery systems and a good alternative to polymeric nanoparticles especially for parenteral method. But the limit of this type of particles lies in the fact that the majority of drugs have a poor solubility in lipids [42]. Ongoing investigations are conducted to increase encapsulations rates using nanostructured lipids matrices or lipid-drug conjugates [43, 44].

Table 2.1 Description of the most widely used polymers in the synthesis of organic nanoparticles

Materials	Full name	Abbreviation or commercial name	Molecular structure
Synthetic Homopolymers	Poly(lactide)	PLA	
	Poly(lactide-co-glycolide)	PLGA	
	Poly(epsilon-caprolactone)	PCL	
	Poly(isobutylcyanoacrylate)	PICBA	
	Poly(isohexylcyanoacrylate)	PIHCA	
	Poly(n-butylcyanoacrylate)	PBCA	

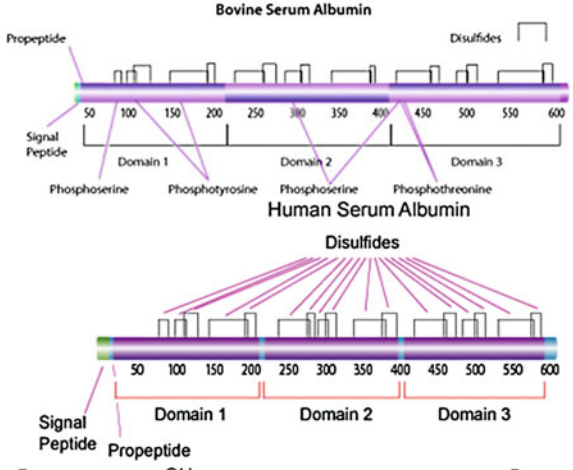
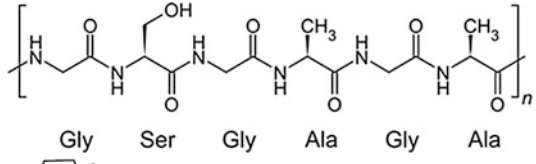
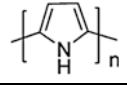
(Continued)

Table 2.1 (continued)

Materials	Full name	Abbreviation or commercial name	Molecular structure
	Poly(acrylate) and poly(methacrylate)	Eudragit®	 $R_1 = CH_3$; H $R_2 = CH_3$; CH_3CH_2- R_3 = COOH (Eudragit® L and S) $R_3 = COOCH_2CH_2N^+(CH_3)_3Cl^-$ (Eudragit® RL and LS)
Copolymers	Poly(lactide)-poly(ethylene glycol)	PLA-PEG	
	Poly(lactide-co-glycolide)-poly(ethylene glycol)	PLGA-PEG	
Natural polymers and biopolymers	Chitosan		
	Alginate		

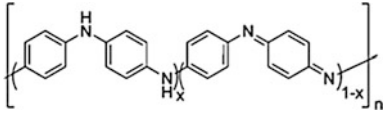
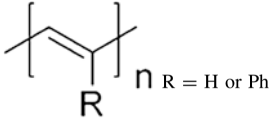
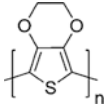
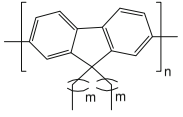
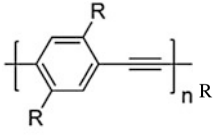
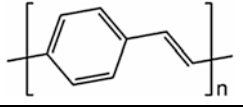
(Continued)

Table 2.1 (continued)

Materials	Full name	Abbreviation or commercial name	Molecular structure
	Gelatin[a]		-Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-
	Albumin[b]		
	Fibroin[a]		
Conjugated polymers	Polypyrrole	PPy	

(Continued)

Table 2.1 (continued)

Materials	Full name	Abbreviation or commercial name	Molecular structure
	Polyaniline	PANI	
	Polyacetylene		 R = H or Ph
	Poly[3,4-(ethylenedioxy)thiophene]	PEDOT	R = H or Ph 
	Poly(dialkylfluorene)		
	Poly(p-phenyleneethynylene)	PPE	 R = alkyl chain
	Poly(p-phenylenevinylene)	PPV	R = alkyl chain 

[a] Typical amino acids sequence
[b] Structures taken from the Sigma-Aldrich® website

2.3 Methods of Preparation of Nanoparticles

The preparation of organic and bioorganic nanoparticles is divided into two main methods [14, 16, 17]. The first approach is based on a two-step procedure involving generally the preparation of an emulsification system during the first step carried out to generate nanodroplets of definite sizes wherein organic compounds (polymer, monomer, lipid) are previously solubilized. The strategies of emulsification developed in the literature differ from their high- or low-energy stirring procedures. The nanoparticles are formed in the second step of the process by various mechanisms such as precipitation, gelation, or polymerization.

The second approach consists in conduction of one-step procedures where emulsification is not required prior to the formation of nanoparticles. The methods are generally based on the precipitation of organic compounds in solution occuring through different routes including nanoprecipitation by solvent displacement or self-assembly mechanisms induced by ionic gelation or by the formation of polyelectrolyte complexes. A few other methods have also been reported recently based on strategies involving spray-drying [45, 46], supercritical fluid technologies [14, 47, 48], or piezoelectrical ways [49].

2.3.1 Two-Step Procedures Based on Emulsification

2.3.1.1 Emulsions and Methods of Emulsification

The term emulsion is defined basically as a mixing of two or more totally or partially immiscible liquids obtained in the presence or absence of a surface active agent. Generally, depending on the type of dispersed phase and of the dispersion medium, o/w (oil in water) direct emulsion or w/o (water in oil) inverse emulsion can be formed but more complex systems such as O/O (oil in oil) or multiple emulsions of different kinds (W/O/W, O/W/O, W/O/O) can also be obtained (Fig. 2.2). Depending on the sizes of droplets, the emulsion formed can be classified into three main categories: a microemulsion type which is characterized by a thermodynamically stable behavior with droplet diameters ranging from 10 to 100 nm and a miniemulsion or a macroemulsion systems that are both thermodynamically unstable with drop sizes comprised between 100 nm and 1 μm and up to 1 μm, respectively [50–52].

Over the past decade, methods to prepare suitable emulsions with nanoscaled droplets to design organic nanoparticles have been considerably evolved due to the technological development of emulsification devices and due to the expansion of low-energy stirring routes in constant progress, thanks to environmental constraints. Indeed, low- and high-energy emulsification techniques constitute two strategies to obtain nanodroplets and consequently nanoparticles.

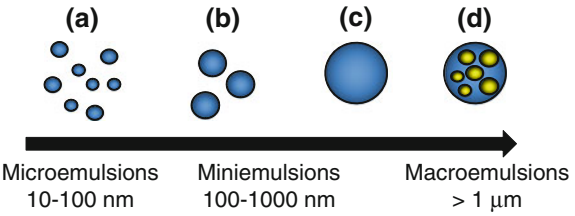


Fig. 2.2 Different types of emulsions. **a** Microemulsion, **b** nanoemulsion, **c** simple macroemulsion, **d** multiple emulsion

Low-energy Emulsification Methods

Nanoemulsions can be generated by low-energy emulsification techniques classified into two groups in the literature. The first one is the so-called spontaneous emulsification [50, 51, 53–55] obtained by the rapid diffusion of a water-soluble solvent, solubilized first in the oily phase, moving toward the aqueous one when the two phases are mixed. This phenomenon, presented as a good alternative of high-energy methods, has been described in several works as a solvent displacement method [56–61] (also called the "Ouzo effect") where nanoemulsion is obtained by a rapid diffusion of an organic solvent generally acetone or ethanol from the oily phase to the aqueous phase. Direct O/W emulsion as well as inverse W/O emulsion can be produced by this way. Spontaneous emulsification mechanism originates from interfacial turbulence related to surface tension gradient produced by the diffusion of solutes between two phases [54]. It is assumed that drops are created by interfacial corrugations caused by a Marangoni effect causing severe interfacial fluctuations. In the case of the presence of surfactants, fluctuations of the interfacial amphiphile concentration create local supersaturation of the surfactant at interface resulting in the nucleation and growth of drops [62–64]. The study of the basic mechanism involved can be illustrated by a simple ternary water/alcohol/oil ternary system through the determination of a phase diagram which is essential to describe the diffusion path and to target the spontaneous emulsification domain [54]. In such diagram, as illustrated in Fig. 2.3, a two-phase equilibrium region occurs corresponding to the spontaneous emulsification (SE) domain. Upon dilution, the diffusion path of the water phase crosses the SE region inducing spontaneous emulsification and the formation of nanoemulsion.

In the field of nanoparticles synthesis, more complex systems are involved and the ternary diagram has to be adjusted to take into account the potential influences of additional components such as monomers, polymers, or surface active agents on the modification of the diffusion pathway. Among all key parameters modifying the droplet sizes and stability of nanoemulsions (pH, water and oil proportions, solvent type and content, etc.), temperature strongly affects the solubility of the organic solvent in the water and the oil phase and modifies consequently the diffusion process.

Another route to produce spontaneous emulsification is the so-called emulsion inversion point method (EIP) carried out at constant temperature. The method is

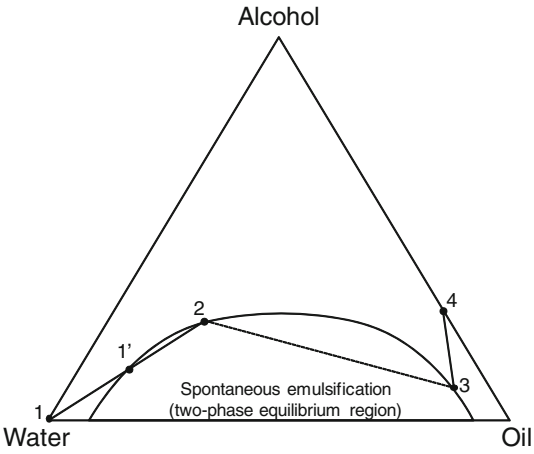


Fig. 2.3 Diffusion path in a water/alcohol/oil system. Segment (1–2): diffusion path of the aqueous phase. Segment (2–3): interfacial equilibrium. Segment (3-4): diffusion path of the oily phase. Segment (1′-2): crossing of the two-phase equilibrium region induces spontaneous emulsification

based on a progressive dilution with water or oil of a microemulsion or liquid crystals leading kinetically stable nanoemulsions [65–73]. Indeed, by changing the water and oil proportion in microemulsion network, interfacial instabilities are occurring resulting in the destabilization of the thermodynamically stable microemulsion structure into nanoemulsions. Keeping in mind that the best conditions have to be found to obtain the smallest drop sizes, determination of phase diagrams is also required in this case and have to be adjusted carefully in function of the formulation used for the design of nanoparticles (type of monomers, polymers, initiators, drug encapsulated, etc.).

The third group of low-energy emulsification methods is the so-called phase inversion temperature (PIT) method [74, 75] offering the main advantages to obtain nanoemulsions with a potentially low amount of surfactant (typically less than 5 wt %), a reduced toxicity since no organic solvent is needed, and a relatively easy handling. It makes the method suitable for biotechnology applications (nanomedecine, pharmaceutical science or cosmetics) since degradation of drug to be encapsulated is avoided. This versatile way uses the ability of polyethylene oxide (PEO)-based surfactants to change their affinity for water and oil in function of temperature leading to a so-called "transitional phase inversion" of emulsions. Typically, when temperature increases, the PEO blocks undergo dehydration which modify the amphiphilic character of surfactants toward higher lipophilic behavior. Consequently, an O/W emulsion produced at low temperature inverts into a W/O one upon a temperature rising. Likewise, in the transitional region at temperatures for which the surfactant exhibits similar affinity for the two immiscible phases, ultralow interfacial tension and low curvature create bicontinuous microemulsion nanostructures. Therefore, the principle of the PIT method is to suddenly breakup such structures maintained at the PIT temperature by rapid cooling or dilution generating immediately kinetically stable nanoemulsions [76–81]. Once again, phase diagram has to be established to determine the transitional inversion region in function of the formulation parameters (temperature,

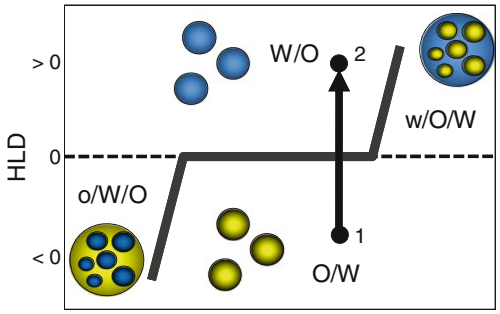


Fig. 2.4 Typical 'formulation-composition' map for a given water/surfactant/oil system showing the emulsion inversion zones and the different types of emulsions. Path (1–2) illustrates a possible transitional inversion from O/W emulsion to nano-W/O emulsion inducing by the crossing of the ultra-low interfacial tension region HLD = 0

oil type, salinity, etc.) and of the water to oil ratio (WOR). This is suitable to target the inversion path which has to be followed to produce nanoemulsions. The group of Salager et al. has developed an empirical "Hydrophilic Lipophilic Deviation" (HLD) expression (Eq. 2.1) based on the difference of the chemical potential of surfactants in the two phases [82–86].

$$\mathrm{HLD} = \alpha - \mathrm{EON} + \mathrm{b}S - k\mathrm{ACN} + t\Delta T + \mathrm{aA} \tag{2.1}$$

where EON is the number of ethylene oxide groups for surfactants, S is the weight percentage of electrolytes in the aqueous phase, ACN the amount of carbon numbers of the n-alcane composing the oily phase, ΔT the temperature difference from the reference temperature (25 °C), A the weight percentage of alcohol potentially added, α, k, t the parameters in function of the used surfactant, a, a constant function of the types of alcohol and surfactants, and finally b a constant function of the nature of the added electrolytes. It comes that HLD can be calculated and its value depends on the different formulation parameters of the system representing by the terms of the equation. HLD = 0 corresponds to the optimum formulation where the surfactant has equal affinity to water and oil whereas HLD > 0 and HLD < 0 represent higher lipophilic or hydrophilic behaviors, respectively. Moreover, formulation-composition maps can be constructed as illustrated in Fig. 2.4 [83, 87–91]. For a given system at constant stirring conditions and for a fixed surfactant concentration, zones of macroemulsion, multiple emulsions, and transitional region appear. It allows the identification of the best path conditions (especially the temperature range) to produce transitional inversion of emulsion [91–96]. Although transitional inversion presents many advantages for the preparation of nanoparticles, this method is still anecdotal and very recently developed in the literature for the production of lipid nanocarriers and nanocapsules or polymer nanoparticles compared to high-energy emulsification techniques.

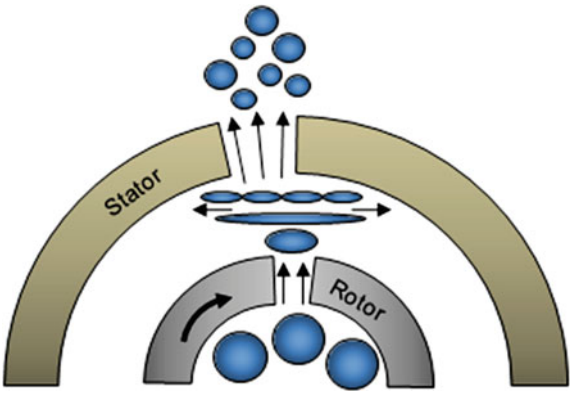


Fig. 2.5 Scheme of the principle of emulsification with a rotor–stator device

High-Energy Emulsification Methods

Most of methods of preparation of nanoemulsions are based on mechanical processes related to high-energy stirring techniques. In this field, the most common device consists in a rotor–stator apparatus in which a shear stress is applied to induce deformation of pre-emulsion droplets leading to their breaking into smaller ones of uniform size (Fig. 2.5). The final diameter of the daughter droplets is mainly determined by the applied stress and only weakly depends on the viscosity ratio between the dispersed and the continuous phase [97–102].

Sonication is also a process widely used in the literature for nanoemulsification [50, 103–114] and for the generation of polymer or lipid nanoparticles [115–127]. This process of ultrasound emulsification is performed under high frequency where large drops are generated by the instability of interfacial waves [104, 105]. The drops are subsequently broken into smaller ones through a cavitation mechanism. Some authors have shown that optimal conditions for sizes reduction and better nanoemulsion stability are obtained using high-power setting for short exposure times [103]. Indeed, longer exposure times produce degradation of surfactant by radicals which form during the thermal decomposition of water [128, 129].

In the past few years, others machines have been designed to obtain droplets with more reproducible calibrated sizes and well-defined characteristics in the view of large-scale production. These machines have been related to microfluidic techniques that constitute at the moment an intense research area in progress [130–137]. The principle is based on an extrusion mechanism where the dispersed phase is forced to permeate through a microfiltration device to calibrate droplets in the continuous phase (Fig. 2.6). The microfiltration units differ from their technological design and can be engineered as porous membranes, flow-focusing, Y-shaped or T-type microchannels [138]. Polymeric particles of PLGA–PEG [139], PCL [140], or alginate [141] have for example already been produced by this technique.

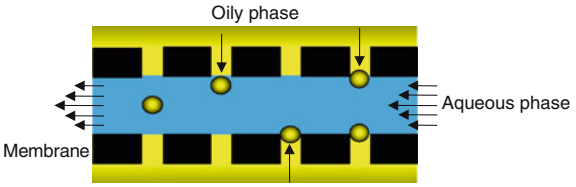


Fig. 2.6 Scheme of the principle of emulsification using a microfiltration device based on a porous membrane

2.3.1.2 Generation of Nanoparticles from Emulsion

Precipitation Induced by Solvent Removal

Macromolecules dissolved in the dispersed phase (mainly the oily one) of the emulsion can undergo precipitation upon removal of the organic, often volatile solvent. To perform this solvent extraction, several methods such as solvent evaporation, solvent diffusion, or salting-out procedures have been developed and constitute by far the most famous routes carried out in the field of organic nanoparticles formation from emulsified systems. It comes from the versatility character of this way that can be applied to a wide range of organic compounds including synthetic polymers and natural bioorganic macromolecules such as chitosan, polysaccharides, alginate, or gelatin.

Solvent Evaporation

The method consists in the preparation of nanoemulsion formulated with a polymer dissolved in a volatile solvent solution [142]. Dichloromethane and chloroform are the most widely used solvents but are often replaced by ethyl acetate, less toxic and hence much more adapted to the synthesis of controlled release systems where drug encapsulation is generally involved. In this method performed under vacuum, suspension is produced by evaporation of the polymer solvent from emulsion droplets which is allowed to diffuse through the continuous phase [19]. This slow procedure involves first a fast evaporation period during which at least 90 % of the polymer solvent is evacuated followed by a slow evaporation period where the few percent of the remaining solvent is extracted. During the first step, droplets sizes dramatically decrease to reach a minimum value due to the high solvent lost. In contrary, the second step is characterized by a significant increase of the droplet diameters in reason to coalescence. This coalescence process can be accentuated in the case of a polymer having interfacial adsorption properties whereas for polymers characterized by poor surface active properties, the coalescence is reduced. In addition, partially miscible solvents in the preparation of emulsion can be used and change the conditions of evaporation. The volatile solvent removal can be in this case realized by distillation [143].

Numerous examples of nanoparticles preparations by solvent evaporation can be found in the literature with various polymers such as PLGA [144–147], PLA [148, 149], or PCL [150] (Fig. 2.7). Also, amphiphilic copolymers (PEG-PLA

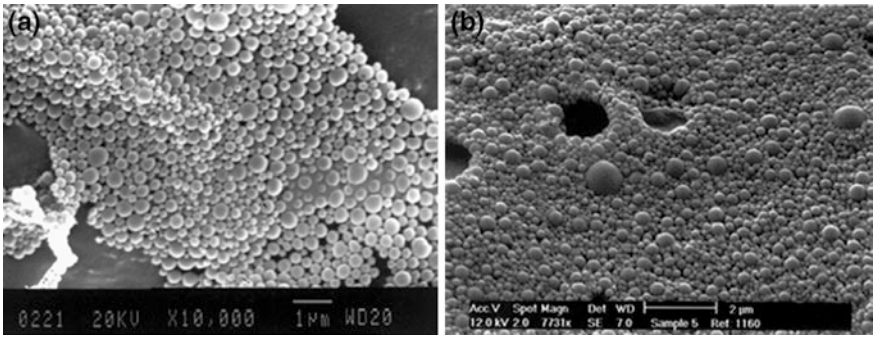


Fig. 2.7 Examples of nanoparticles produced by the emulsion–solvent evaporation method. **a** PLGA nanoparticles (adapted with permission from [145]). **b** PCL nanoparticles (adapted with permission from [150])

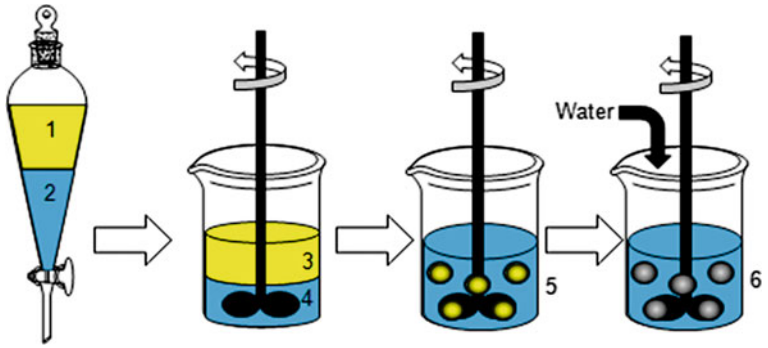


Fig. 2.8 Scheme of preparation of nanoparticles by the emulsion–diffusion procedure. (*1*) Partially water miscible solvent saturated with water. (*2*) Water saturated with solvent. (*3*) Solvent saturated with water + dissolved polymer. (*4*) Water saturated with solvent + surfactant. (*5*) Emulsification. (*6*) Dilution with water and formation of polymeric nanoparticles from emulsion

[151, 152], PEG–PLGA [153], polysaccharides-PCL [154, 155]) nanospheres can be produced by this method with no need of surfactant to ensure the emulsion formation and the stability of the final nanoparticles suspension.

Solvent Diffusion

The solvent diffusion method also called solvent displacement method (Fig. 2.8) requires a polymer solvent partially soluble in water [156–158]. The preparation of emulsion involves two immiscible phases, but where the oil dispersed one is composed of water saturated with the polymer solvent and where the continuous one is composed of oil saturated with water. This can be obtained by mixing the polymer solvent and water and waiting the decantation to get a two-phase system. At the bottom and at the top of the resulting system, the solvent saturated water and the

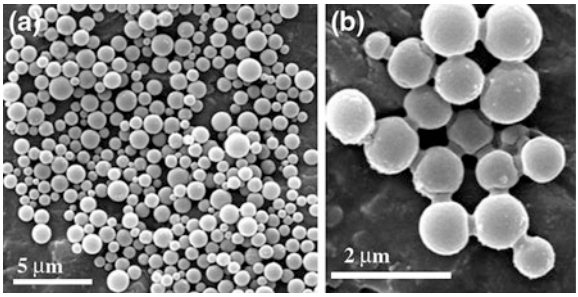


Fig. 2.9 Scanning Electron Microscopy images of PLA nanoparticles obtained with the solvent diffusion method (adapted with permission from [161])

water saturated solvent can be collected, respectively. Oil-in-water emulsion is prepared with the previous two immiscible phases and subsequently diluted with a high quantity of water. This leads to the precipitation of the polymer induced by a rapid diffusion of the organic solvent from the oil droplets to the continuous phase. Several polymer solvents can be used such as ethyl acetate [143], isopropyl acetate [159], benzyl alcohol, propylene carbonate [160], and surfactants such as pluronic F68® or Polyvinyl alcohol (PVA) is often employed to play a role in the stabilization and sizes of the emulsion droplets. PLA [161] (Fig. 2.9), PLGA [162–164], and PCL [157] are the most common and suitable polymers used but gelatin or chitosan nanoparticles [165–167] have also been synthesized by this method.

In addition, this method involves a pure diffusion mechanism where the droplet sizes drop suddenly in a millisecond time scale during solvent extraction and polymeric nanoparticles formation. Among all factors impacting the reduction of the particle diameters, one can cite the increase of the miscibility of water with the organic solvent or of the stirring rate and the use and concentration of stabilizing agents added in the emulsion. On the contrary, the rise of the polymer concentration leads to significant increase of the particle sizes and polydispersity. Generally, nanospheres are produced by this technique but adding a small amount of oil in the organic phase results in the generation of nanocapsules.

Salting-Out

Very close to the solvent-diffusion method, this process involves emulsification with a polymer solvent generally acetone that is normally totally miscible with water. Actually, the artifice used to emulsify water and acetone is to dissolve high contents of salt or sucrose in the aqueous phase to provoke a strong salting-out effect modifying the solubility of water with the solvent [168]. The emulsion can hence be formed with a polymer dissolved in the solvent droplets. Particles precipitation is induced, as in the solvent-diffusion process, by diluting the emulsion and adding a large amount of water in the continuous phase to drop the salt concentration and to cause extraction of the solvent out of the droplets.

The suitable electrolytes for this process are generally magnesium chloride [169–171] or calcium chloride [172] but salting-out can also be produced by saturation of the aqueous phase by PVA [173] which acts in addition as a

viscosity-increasing agent and emulsion stabilizer. Poly(ethylene oxide) [169], PLGA [172, 174], or poly(trimethylene carbonate) [175] particles, for instance, have been synthesized by this method with diameters in a 100-500 nm range. The stirring energy required for this method is also reduced which provides lower particle diameter and less pronounced influence of the polymer concentration and of the stirring speed on the emulsion droplets in comparison to more conventional emulsification routes.

Gelation of the Emulsion Droplets

Another method to obtain nanoparticles after nanoemulsification is to gelify polymer or crystalize lipid dissolved in the droplets [39, 176]. For instance, in the case of agarose [177] or gelatin [127], the preparation of the nanoemulsion can be performed at moderate high temperature above the melting point and subsequent cooling down induces gelation of the emulsion droplets and their conversion into nanoparticles. The same procedure can be applied for the production of solid lipid nanoparticles by crystallization of the lipid under the melting temperature [39]. Gelation can be produced by other physicochemical factors such as pH or by adding components like divalent cation (generally calcium) to induce ionic gelation [178]. Polysaccharides biopolymers such as alginate or pectin are particularly adapted since their chemical compositions based on uronic acids functions are responsive to pH or to complexation with cations. In this kind of gelation, two emulsions are generally prepared, the first one containing the dissolved biopolymer and the second one containing the pH controlling agent or the cation. The two emulsions are mixed under strong agitation to provoke droplets collision which is essential to induce gelation and hence formation of nanoparticles.

Polymerization in Emulsion

Among all techniques used for the generation of nanoparticles from emulsions, polymerization is the subject of the abundant literature since well-defined and desired nanoparticles properties for a particular application can be attained through this process [179–184]. In this case, instead of the previously described techniques for which a solution of a preformed polymer is prepared, macromolecules form through polymerization of monomers. The major emulsion polymerization techniques can be classified into different methods such as conventional emulsion polymerization, surfactant-free emulsion polymerization, as well as mini- (or nanoemulsions) and microemulsions polymerizations which differ from the kinetically and thermodynamically different emulsion behaviors. In addition, we can cite interfacial polymerization, a very useful method for the preparation of nanocapsules and living/controlled radical polymerization process that offers at this moment a much better control of the polymer characteristics in comparison to older conventional polymerization techniques.

Conventional Emulsion Polymerization

This method can be considered as the traditional way to generate nanospheres from emulsion polymerization and is still widely used nowadays. Generally, the components are water, a monomer of low water solubility, a water-soluble initiator which may be an ion or a free-radical and a surfactant. Polymerization starts in this case when a monomer molecule collides with an initiator molecule. Another way consist in initiating radical from the monomer itself using UV irradiation, ultrasonication, or γ-radiation. Before or after the termination of the polymerization, the solid particles can be formed. Various types of polymeric nanoparticles could be produced by this technique such as poly(vinylcarbazole) [185], poly(methylmethacrylate) [186, 187], polystyrene [188–195], or poly(alkylcyanoacrylate) [196–201] nanoparticles. In the latter case, anionic polymerization for which initiation occurs by any nucleophilic groups like hydroxyl groups of water is the most common way. In addition, performing anionic polymerization in acidic conditions slows down the rate of reaction and hence favors the formation of nanospheres instead of polymer aggregates. The particles sizes depend on the surfactant used and can be obtained generally in a 50–300 nm range.

Surfactant-Free Emulsion Polymerization

In contrary to conventional emulsion polymerization, surfactant-free emulsion polymerization is performed without emulsifier offering the major advantage to obtain nanoparticles without any step of surfactant removal [202–208]. This could be environmentally, energetically, and time-consuming advantageous especially for high-scale productions of nanoparticles. The ingredients in such emulsifier-free system are water, a water-soluble initiatior (like potassium persulfate), and monomers which are generally vinyl or acrylic. The stabilization of nanoparticles in formation is ensured in this case by the use of ionizable initiators or ionic co-monomers. Two mechanisms of polymerization are involved, micellar-like nucleation [209, 210] and homogeneous nucleation [211–216] that differs from the aqueous solubility of the monomer. PMMA nanoparticles have been obtained by this technique using microwave irradiation [217, 218], redox initiation [219], or laponite clays as stabilizing agent for the emulsion [220]. The general trend is that monomer concentration is a key parameter influencing the particle sizes as illustrated in Fig. 2.10 where the increase of the monomer concentration increases the particle size.

Polyacrylate nanospheres were also obtained and some authors have shown that ultrasonic irradiation or the concentration of a stabilizing agent (4-styrene sulfonic acid) altered the particle sizes and distribution [221]. Polythiophene nanoparticles have been equally successfully prepared by Fe^{3+} oxidative polymerization [222] where the difference in the polymerization rates of monomers and the electrostatic attraction between sulfonate and Fe^{3+} ions results in the production of core–shell morphology with a size distribution ranging from 300 to 800 nm.

Although surfactant-free emulsion polymerization is considered as a simple and "green" way for polymeric nanoparticles preparation, improvements have to be

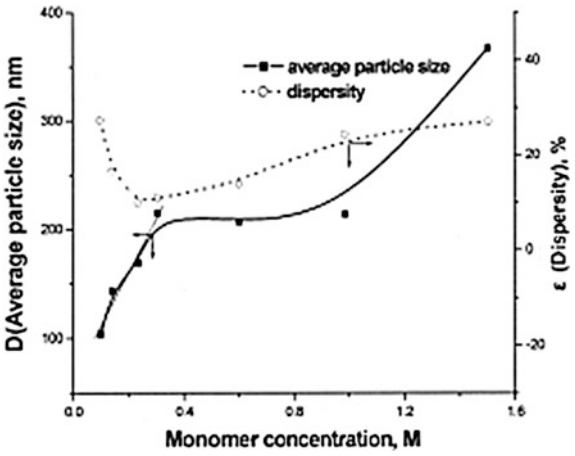


Fig. 2.10 Influence of monomer concentration on dispersity and average particle size of PMMA nanoparticles obtained through surfactant-free emulsion polymerization (reprinted with permission from [217])

conducted in the future to obtain monodisperse and more precisely controlled particle sizes.

Miniemulsion Polymerization

This method can be distinguished from conventional and surfactant-free emulsion polymerization by the generation of nanoemulsions using high-energy methods (Fig. 2.11) and generally a low molecular mass compound as co-stabilizer prior initiating polymerization [223–231]. The typical formulation consists in water, monomer mixture, co-stabilizer, surfactant, and initiator. The droplets of the nanoemulsion are generally composed of a pure monomer phase stabilized by a suitable adsorbed surfactant. The polymerization mechanism widely employed is radical polymerization which is initiated in the emulsions droplets via the incorporation, in most cases, of the initiator in the continuous phase. The general mechanism assumed in the literature is the droplet nucleation mechanism suggesting that radicals are generating in each monomer droplet taken as individual reaction site [180, 232]. The number and size of particles do not consequently vary during the polymerization process. The choice of the initiator and its solubility has evidently a great influence on the final particles properties especially the particle size. Even if inverse nanoemulsion polymerization is possible using hydrophilic ingredients [233–235], in most cases, the hydrophobic character of the dispersed monomer phase requires an oil-soluble initiator which is more suitable to obtain well-defined nanoparticles.

Although radical polymerization is often selected for the generation of nanoparticles in miniemulsion polymerization, non-radical polymerization methods such as polyaddition [236, 237], anionic polymerization [238], or metal-catalyzed reactions [239] are less aggressive for drug encapsulation applications and can also be used. Some authors [236, 237] have demonstrated for instance the preparation

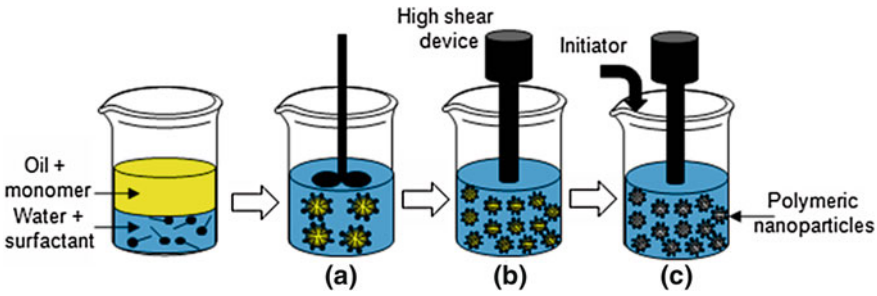


Fig. 2.11 Scheme of nanoparticles preparation from a typical miniemulsion polymerization method. **a** Pre-emulsification **b** nanoemulsification using a high shear device **c** formation of polymeric nanoparticles upon addition of initiator

of polyurethane latex nanospheres using reaction between diisocyanate and activated diol in nanoemulsion droplets. The low water solubility of reactants and the slower polymerization kinetics than emulsification are the main key factors inducing the successful synthesis.

Microemulsion Polymerization

The main difference between microemulsion and miniemulsion polymerization methods relies on the kinetic character of the dispersed phase produced in the emulsified system. Indeed, in contrary to miniemulsion, microemulsion is a thermodynamically and spontaneous stable system prepared with a high quantity of surfactant and characterized by an interfacial tension at the oil/water interface close to zero [52, 64]. The generation of nanoparticles through microemulsion polymerization generally results in smaller particle size (typically less than 80 nm) than in miniemulsion polymerization processes. A water-soluble initiator is introduced in the aqueous phase initiating the polymerization in only some swollen micelles of the microemulsion containing the monomer. As time elapses, the osmotic and elastic influence of the polymeric chains in formation destabilizes the microemulsions leading to an increase in the particle size, to the formation of empty micelles, and to a secondary nucleation [232, 240].

In the literature, various formulations have been investigated to prepare for instance nanoparticles of polyvinyl acetate [241, 242], polyaniline [243–246], polyacrylamide, or polypyrrole [247]. The studies highlighted the critical factors that influence the final particle properties as the type of initiator and concentration, the surfactant, the concentration of monomer, and the reaction temperature.

Microemulsion polymerization has a great potential in many applications but retains important drawbacks relating to its high dilute formulation and its high amount of surfactant which limit in a large extent the commercial use of this technique.

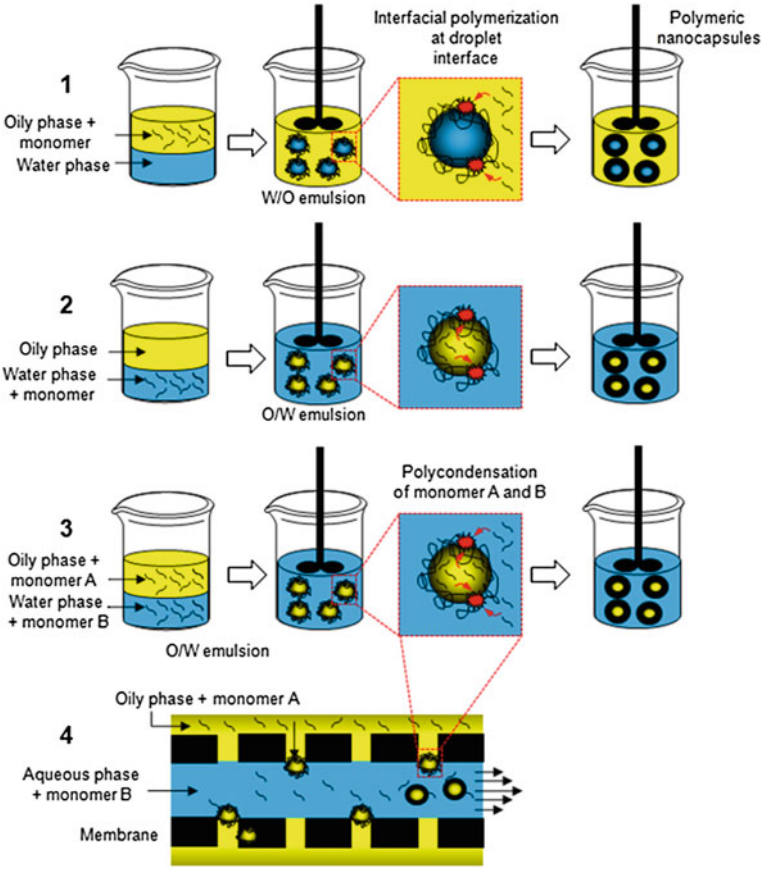


Fig. 2.12 Scheme of the different strategies of interfacial polymerization. (1) Introduction of the monomer in the oily phase. (2) Introduction of monomer in the aqueous phase. (3) Introduction of monomers in the oily and aqueous phase. (4) Polymerization using a membrane reactor device

Interfacial Polymerization

Interfacial polymerization is characterized by the polycondensation of monomers at the droplet interface leading to the generation of mainly nanocapsules instead of nanospheres. Different strategies have been developed (Fig. 2.12) which depend on the formulation selected and the choice of monomers introduced either in the continuous and/or in the dispersed phase.

The first way consists in introducing the monomer in the continuous phase of the emulsified system. The monomer can subsequently react with the emulsion droplets to form nanocapsules. Due to the hydrophobic character of the majority of monomers used, the emulsions prepared in this case are generally of the W/O type. Thus, a good solubility of the monomer for the external phase and its sufficient

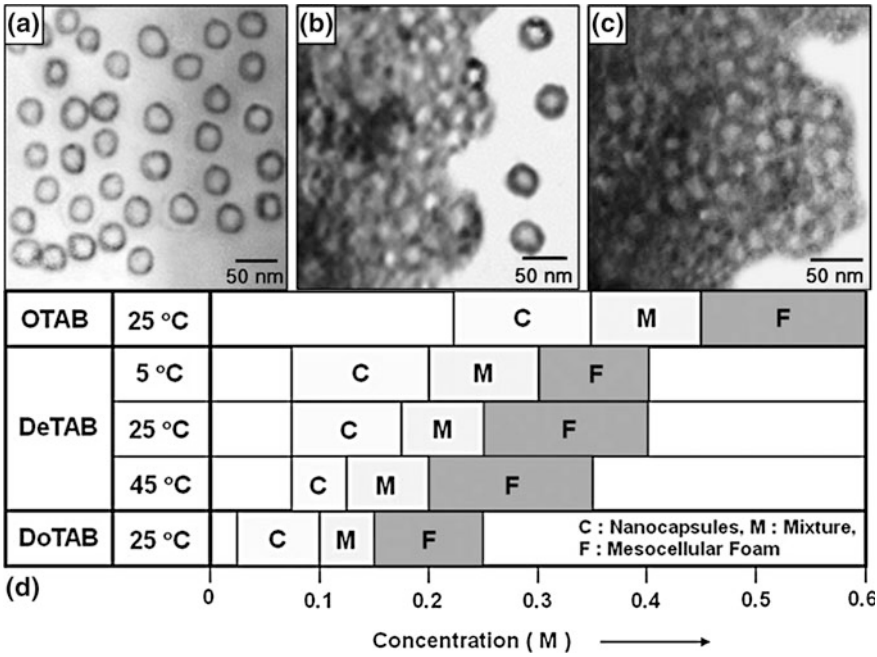


Fig. 2.13 TEM images of PEDOT nanocapsules and mesocellular foams. **a** Nanocapsules prepared using 0.15 M of DeTAB. **b** Mixture of nanocapsules and mesocellular foams obtained using 0.20 M of DeTab. **c** Mesocellular foams prepared using 0.30 M of DeTab. **d** The concentration ranges for formation of PEDOT nanomaterials as a function of the surfactant hydrocarbon length and polymerization temperature. *DeTab* Decyltrimethylammonium bromide, *OTAB* Octadecytrimethylammonium bromide, *DoTAB* Dodecyltrimethylammonium bromide. (adapted with permission from [257])

reactivity toward the aqueous phase are critical factors that influence the success of the procedure. For instance, in the case of the polymerization of alkylcyanoacrylate such as isobutyl-cyanoacrylate, hydroxyl ions catalyze the reaction and w/o nanocapsules can be generated [248–255]. Lipophilic diisocyanate can also be used due to its high hydrolyzable properties which induce conversion of the isocyanate functions into amine [256]. The amine function can react subsequently with another monomer molecule leading to polymerization at droplet interface. Another interesting work showed by Jang et al. [257] is the possibility to obtain from this first strategy different architectures of poly(3,4-ethylenedioxythiophene) (PEDOT) nanomaterials. The method is based on a so-called "Surfactant Mediating Interfacial Polymerization (SMIP)" method allowing selective synthesis of either nanocapsules or mesocellular foams in function of the concentration of quaternary ammonium-based surfactants (Fig. 2.13).

In the second strategy, the monomer is introduced in the droplet phase and polymerization occurs by reaction with the continuous phase or by adding initiator in the external phase [258, 259]. Initiator can also be added in the dispersed phase

and polymerization is initiated by temperature. In this case, as polymerization proceeds; gradual segregation of the polymer in formation toward the water/oil interface creates nanocapsules. Another interesting procedure that could be possible is the simultaneous generation of interfacial polymerization and nanoemulsion using solvent diffusion. For instance, alkyl cyanoacrylate monomers can be introduced in the oil dispersed phase containing a water miscible organic solvent [62, 260–263]. Polymerization can thus be initiated along with the rapid solvent diffusion to the aqueous continuous phase. Nanocapsules are hence formed at the same time than the nanoemulsion.

Finally, the third route involves two reactive species of different solubilities introduced, respectively, in the continuous and dispersed phases. The reaction takes place at the interface of the two liquids. This method is the most commonly used for the generation of nanocapsules. Evidently, the type of monomers added defines the nature of the final polymer shell. For instance, polyamides [264], polyurea [265], polyurethanes [57] nanocapsules can be produced by this technique using generally the reaction of isocyanate functions with activated diol. Some authors have shown that the thickness of the polymer wall is independent of the concentration of the lipophilic monomer but varies with the amount of the hydrophilic monomer added [264].

From the strategies described above, different methods have been developed to design nanoparticles by interfacial polymerization but a main problem inherent to the well control of the particle size remains. In the recent past years, the use of membrane reactors has been developed to overcome this problem since a better controlled addition of one reactant to another reactant can be achieved [266, 267]. Indeed, this versatile technique allows the preparation of either nanospheres or nanocapsules and offers the possibility to target the nanoparticle size by a suitable choice of the membrane parameters (membrane pore radius, cross-flow velocity, shape of the pore opening, transmembrane pressure, etc.) and the formulation factors (viscosity of the dispersed and continuous phase, type of surfactant, etc.) [268, 269]. However, nanoparticles preparation by membrane reactors is often considered as an expensive process due to a complicated technological development which constitutes a major drawback to its widespread utilization.

Controlled/Living Radical Polymerization

Main limitations caused by fast radical–radical terminations are inherent to radical polymerization including the control of the molar mass and mass distribution, the end-functionalities and the macromolecular architecture. To obtain a better control of such parameters, the controlled/living radical polymerization [270–272] has emerged as a new field in the recent past years helped by the industrial production of hydrophilic polymeric nanoparticles designed specifically for biomedical applications and by environmental concern with the development of the so-called “green chemistry”. The principal methods of controlled/living radical polymerization are nitroxide-mediated polymerization (NMP) [273–277], atom transfer radical polymerization (ATRP) [278–284], and reversible addition and fragmentation transfer

chain polymerization (RAFT) [285–287]. Suitable properties of nanoparticles can be obtained by optimizing different parameters such as the nature and concentration of the monomer, surfactant, initiator, and the type of emulsion but above all the type and concentration of the mediating (control) agent. Several kinds of polymeric nanoparticles have been synthesized by this technique such as for instance poly(butyl acrylate) [275, 276, 288], poly(styrene) [277, 286], or poly(methyl methacrylate) [282, 284] nanoparticles with typical particle size in a 30–400 nm range, using either NMP, ATRP, or RAFT approaches and different formulations. The presence of residual control agent is at this moment the major problem of controlled/living radical polymerization. For environmental purpose, the removal of control agent needs to be performed which caused additional difficulties and cost for this process.

2.3.2 One-Step Procedures

2.3.2.1 Nanoprecipitation

Nanoprecipitation method also called solvent displacement method was developed by Fessi et al. [63] in the end of 1980s. It is one of the easiest, most economic, and reproducible routes to produce nanospheres using preformed polymers instead of monomers. This method, very close to the previous described spontaneous emulsification technique, is based on the interfacial deposition of a polymer after displacement of a semipolar solvent, miscible with water, from a lipophilic solution. Three ingredients are required to achieve the process: the polymer, the polymer solvent, and the non-solvent of the polymer. The polymer can be synthetic, semisynthetic, or natural and the most frequently used polymer solvents are ethanol, acetone, hexane, methylene chloride, or dioxane. The choice of the polymer solvent is guided by two factors: a high solubility in water and an easy removal by evaporation. To satisfy these conditions, acetone is often selected [63, 289, 290] but a binary blend of solvent like acetone with a small amount of water or blends of acetone and ethanol [291–293] or methanol [294] can be used. The non-solvent phase is composed of one or a mixture of nonsolvent of the polymer with eventually the addition of surfactants. The nanoparticles are generated by a rapid diffusion of the polymer solvent in the non-solvent phase by mixing the polymer solution with the latter one. This results in a drop of the interfacial tension between the two phases causing an increase of the surface area and the instantaneous precipitation of polymeric nanoparticles. The lipophilic polymer solution is generally added slowly to the non-polymer solvent but the reverse order also produces nanoparticles. Smaller, more well-defined and narrower distribution of the nanoparticle sizes, typically in a 75–900 nm range, can be obtained than those obtained by emulsification solvent evaporation technique. Many parameters are conditioning the final nanoparticle properties such as the organic phase injection rate, the agitation during addition of the polymer solution, the miscibility of the organic solvent with the non-solvent phase, or the nature of

the polymer/solvent interactions. The type and concentration of added surface active agents also influence the nanoprecipitation process [157, 295] since surfactants help in the stabilization of the nanoparticles prevented from aggregation which is especially useful for long storage periods of suspensions.

The method of nanoprecipitation can be performed with various formulation including a wide range of polymers such as poly(ε-caprolactone) [295–299], polylactide [300, 301], poly(lactide-co-glycolide) [302, 303], poly(hydroxyl butyrate) [304], or even peptides [305]. Moreover, the process can be applied to non-polymeric compounds such as cyclodextrin [306] and drug [307].

Finally, nanoprecipitation is a method widely used for the preparation of polymeric or non-polymeric nanospheres due its simplicity, rapidity, and reproducibility even though the low polymer concentration required limits the recovering yield of nanoparticles.

2.3.2.2 Dialysis

Very close to the above described method, dialysis is based on a solvent displacement mechanism but includes, in contrary to the conventional nanoprecipitation technique, additional tools such as dialysis tubes or semi-permeable membranes with suitable molecular weight cutoff which serve as a physical barrier for the polymer [308–310]. Thus, dialysis is performed against a nonsolvent of the polymer miscible with the polymer solvent. The displacement of the polymer solvent through the membrane induces a progressive loss of solubility of the polymer leading to the formation of homogeneous suspensions of nanoparticles. According to the solvents used, the morphology and size of the particles can be affected [311]. Various formulations have been investigated and nanoparticles of different types of polymers such as poly(lactide)-b-poly(ethylene oxide) [312], polystyrene [313], poly(L-lactic acid)-b-poly(ethylene glycol) [314], poly(γ-glutamic acid) [311], or cellulose-derived polymers [315] can be obtained by dialysis.

2.3.2.3 Desolvation

The desolvation method is based on a slow addition of a desolvation factor such as salts, alcohols, or solvents in a solution of macromolecules to provoke the precipitation of the polymer [316, 317]. This method, rather similar than other nanoprecipitation methods based on a loss of solubilization of the polymer, is however often associated to the generation of biopolymeric nanoparticles. Indeed, desolvation process is generally employed for the production of nanoparticles of different types of proteins (Fig. 2.14) such as human serum albumin [318–323], bovine serum albumin [324, 325], gliadin [326–328], or gelatin [318, 329–335]. The desolvation is often followed by a cross-linking step performed with the addition of a certain amount of aldehyde (typically glutaraldehyde) to stabilize the formed nanoparticles. In the case of gelatin (type A), a two-step desolvation route

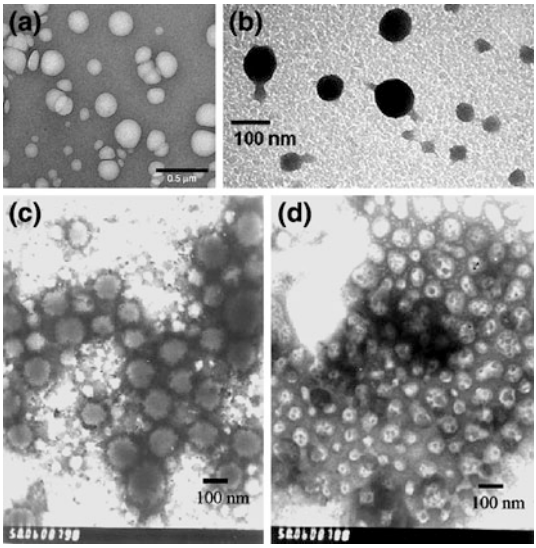


Fig. 2.14 TEM images of Gelatin (**a**), Human serum albumin (**b**), and Bovine serum albumin (**c**, **d**; high and low magnification, respectively) nanoparticles prepared by the desolvation method (reprinted with permission from [323, 324, 334] respectively)

has been developed by Coester et al. [329, 330, 336]. In the first step, the low molecular gelatin fractions present in the supernatant is removed by decanting. The sediment is then redissolved and desolvated in a second step at pH 2.5. This two-step process can also be applied to type B gelatin but the pH is adjusted at 12. Polysaccharide particles of chitosan [337] or hyaluronic acid [338] can also be obtained by desolvation but using sodium sulfate as desolvating agent in these cases.

2.3.2.4 Self-Assembly and Gelation

Polyelectrolytes Complexation

In this case, the organic nanoparticles are obtained based on the association of oppositely charged macromolecules forming, when mixed in specific conditions, polyelectrolyte complexes. Such particles are widely used and developed as in vivo drug delivery carrier of nucleic acids [339, 340]. In this case, nucleic acids play the role of drug as well as a component of the drug delivery system. The polycations, typically poly(ethylenimine), poly(lysine) or chitosan, are generally employed as the opposite (positive) charged compound able to interact with the negative charges of the nucleic acid phosphate groups [340]. One of the key parameters to optimize the nanoparticles formation is the ratio N/P of the positive amine groups noted "N" (as nitrogen) to the nucleic acid negative phosphate groups noted "P". Thus, a value of N/P above one means a polyelectrolyte complex positively charged where the internal polyelectrolyte chains of the system are able to be swollen by water.

Other types of complexes can be form based on the association of alginate, a negatively charged polysaccharide, and poly(lysine), a positively charged peptide [341]. Dextran sulfate and chitosan can also interact to form complexes [342–344].

An interesting feature of polyelectrolyte complexes is the possibility to add one polyelectrolyte in excess compared to the other in order to modulate the net charge of the nanoparticles and to induce colloidal stabilization. Indeed, the excess of component is segregated at the outer shell of the complex which produces a core–shell structure where the surface charge of the nanosphere is conferred by the polyelectrolyte in excess. In addition, the nanoparticle size is influenced by the chain length ratio of the macromolecules which conditions the mutual role of each electrolyte in the complex. Indeed, the highest molecular weighted compound serves as host for the lowest weighted one which is defined as the guest [342].

"Lock and Key" Nanogels

Supramolecular nanoassemblies based on a "lock and key" concept lead nanospheres formed by neutral association of a certain type of macromolecules that have been developed recently [345, 346]. These polymers are composed of dextran modified by the grafting of alkyl chains and a poly(beta-cyclodextrin). The assembly occurs spontaneously in aqueous medium forming a hydrogel since the alkyl chains of the modified dextran are incorporated as a guest in the hydrophobic cavities of the host poly(beta-cyclodextrin). One of the main advantages of this system is the efficiency of the association phenomena which can lead 95 % of incorporation of the guest in the nanogel as well as a nondependence of the protocol followed (order of introduction of the polymer solutions, method of mixing, temperature, etc.). However, the final properties of nanogels depend on various factors such as the polymer concentrations, the weight ratio between the host and the guest, the number of carbons in the alkyl chains, or the percentage of substitution of the glucose units of dextran by the alkyl chains. Finally, these nanogels used as drug delivery devices of hydrophobic drugs such as benzophenone and tamoxifen exhibit excellent loading efficiencies (at least 90 %) and a well- controlled release of the drugs over a period of 16 days.

Ionic Gelation

Synthesis of nanoparticles by ionic gelation is commonly performed with biopolymers especially charged polysaccharides in aqueous medium in very dilute solution (Fig. 2.15). Indeed, the polymer is dissolved in water with a concentration below the gel point and can react with small ions of the opposite charges to form clusters. These clusters can be stabilized subsequently using oppositely charged polyelectrolytes. For instance, using alginate, gelation is typically carried out in the presence of calcium ions leading to a pre-gel phase which is then stabilized

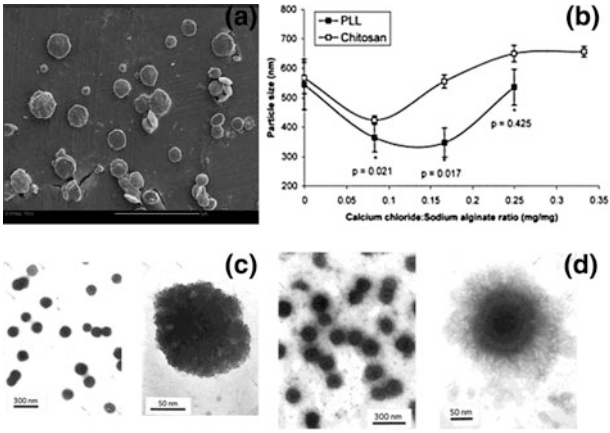


Fig. 2.15 Examples of polysaccharides-based nanoparticles prepared by ionic gelation. **a** Alginate/chitosan nanoparticles (adapted with permission from [357]). **b** Influence of calcium chloride/sodium alginate ratio on alginate/polylysine and alginate/chitosan nanoparticles (Reprinted with permission from [348]). **c** Chitosan nanoparticles. **d** Chitosan-coated PEO–PPO diblock copolymer (adapted with permission from [359])

with polycations such as polylysine [341, 347–350] or chitosan [348, 351–357]. It is noted that alginate can react with polylysine without addition of cations to form a simple polyelectrolyte complex but a pre-gel phase ensures a more compact structure of the nanogel. Furthermore, the size of the nanoparticles obtained greatly depends on the concentration of the biopolymers and is also influenced by the molecular weight of the opposite charged macromolecule.

Chitosan nanoparticles can also be produced via ionic gelation. In contrary to alginate, chitosan is positively charged at neutral pH and in consequence, can form nanogels with anionic ions like tri-polyphosphates (TPP). Thus, the pre-gel phase is induced, as for alginate, in diluted solution with the addition of a small amount of TPP. The nanoparticles can be stabilized by copolymers such as pluronic® and their sizes depend on the concentration of chitosan but are not influenced by the TPP concentration. Calvo et al. [358, 359] have demonstrated the possibility to design chitosan nanoparticles and chitosan coated with a diblock PEO-PPO copolymer using this method (Fig. 2.15). The authors have shown the modification of sizes and zeta potential in function of the amount of the copolymer. An interesting feature of chitosan nanoparticles obtained from this method is their ability to swell and shrink upon ionic strength or pH variations. Indeed, an increase of the pH from acidic to basic causes deprotonation of the chitosan glucosamine units and, as a consequence, a gel shrinking due to the reduction of the intramolecular electric repulsions inside the particles [360]. In addition, variation of the ionic strength by increasing the concentration of salt (typically KCl) in the medium drops the chitosan–TPP interactions which favor the particles swelling or even

their complete restructuration. Thus, due to their triggered swelling response upon pH or ionic strength variations, chitosan nanogels are evidently investigated as drug delivery nanocarriers [361–365].

Finally, ionic gelation is a method that offers the main advantages of a solvent-free and a relative simple preparation of organic nanoparticles but suffers from the high diluted conditions which limit the yield of production of particles.

2.3.2.5 Organic Nanoparticles Prepared by Drying Processes

In the past few years, environmental considerations have motivated research on the development of methods of nanoparticles synthesis avoiding the utilization of organic solvents. In this aim, supercritical or spray drying processes offer the possibility to design and prepare nanoparticles without the main drawbacks of the traditional methods [47, 48, 366–370].

Supercritical Drying

Two procedures have been developed for the production of nanoparticles using supercritical fluid. The first one is based on a rapid expansion of a supercritical solution and the second one is founded on a rapid expansion of a supercritical solution into liquid solvent.

Rapid Expansion of Supercritical Solution

In this method, organic macromolecules are solubilized in a supercritical fluid solution which subsequently undergoes a rapid expansion through a nozzle into ambient air. Well-dispersed particles can form resulting from a homogeneous nucleation imposed by the high supersaturation conditions combined with the rapid pressure reduction [371]. Generally, CO_2 is the supercritical fluid used in the majority of studies. A typical experimental apparatus is composed of three units: a high pressure stainless steel mixing cell, a syringe pump, and a pre-expansion unit. The polymer is dissolved in a CO_2 solution at ambient temperature in the mixing cell. The solution moves in the pre-expansion unit with the help of the syringe pump and is heated isobarically to the pre-expansion temperature until it expands through the nozzle at ambient pressure. Poly(heptadecafluorodecyl acrylate) [372] or poly(L-lactic acid) [373] nanoparticles were yet prepared by this technique and it appears that various factors impact the properties of the particles formed such as the concentration and degree of saturation of the polymer, the processing conditions, the molecular mass and the melting point of the polymer, etc. Although the method is performed without organic solvents and produces a majority of nano-sized particles, the main drawback is the generation of microsized particles or agglomerates. This is due to a coalescence mechanism involved in the free jet. To overcome this problem, another technology has been developed.

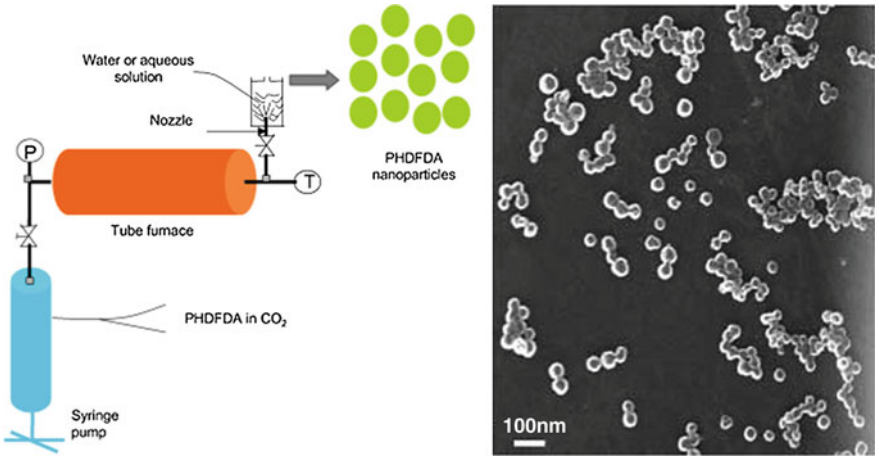


Fig. 2.16 *Left*: Scheme showing the experimental setup of the rapid expansion of supercritical fluid solution into liquid solvent process. *Right*: SEM images of PHDFDA nanoparticles obtained in presence of NaCl and about 5 min after the rapid expansion process. (adapted with permission from [375])

Rapid Expansion of Supercritical Solution into Liquid Solvent

In contrary to the above method, the supercritical solution expands in this case into a liquid solvent instead of ambient air [374] (Fig. 2.16). The primary nanosized particles are not allowed to grow in the expansion jet due to the presence of the liquid solvent. For instance, poly(heptadecafluorodecylacrylate) (PHDFDA) [375] particles were produced using water as the solvent in which were expanded the supercritical solution and precipitated the polymer. It was shown that the particle formation results from the aggregation of initially formed nanoparticles. In addition, the presence of NaCl in the water phase helps to a better stabilization of the nanoparticles due to an increase in the ionic strength.

Poly(methyl methacrylate) and poly(L-lactic acid) nanomaterials were also synthesized by this method using a CO_2-cosolvent as the supercritical fluid. The cosolvent allows a better solubilization of the polymers in the supercritical solution and the presence of NaCl in the water solution generates only nanosized particles [376].

However, in spite of the wide spectrum of fluids available (carbon dioxide, n-pentane, ammonia, etc.), the poor solubility of polymers in these supercritical fluids remains a main drawback of this technology.

Spray-Drying

Spray-drying process has been used in the past few years for the production of microsized organic particles or to convert nanoparticle suspensions in dry powder mainly for biomedical and pharmaceutical applications especially in drug

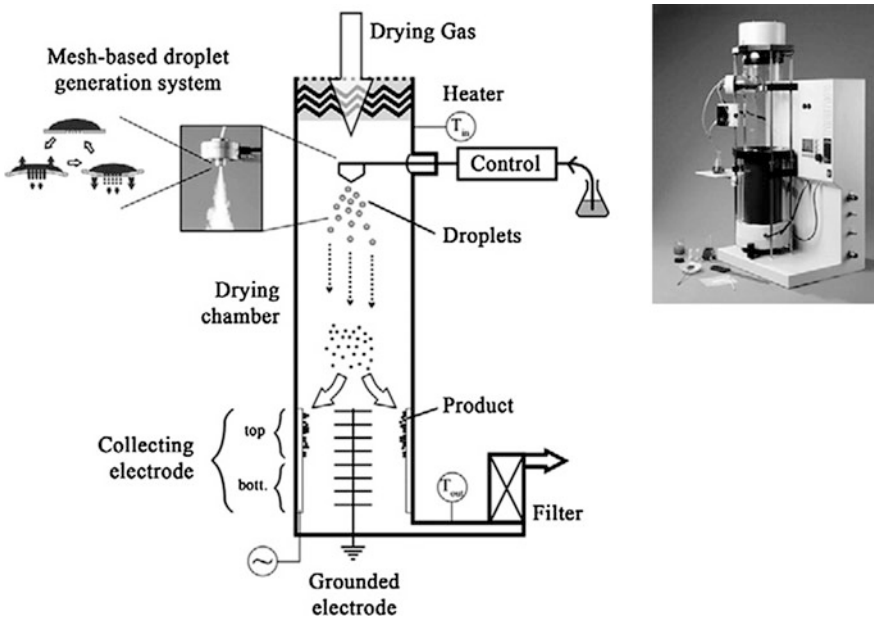


Fig. 2.17 Scheme of the nano Spray-Dryer B-90 (reprinted with permission from [45])

delivery [377–379]. A typical spray-drying process consists in the atomization of a liquid into a spray of fine droplets brought subsequently in contact with a hot drying gas to evaporate the moisture and to form the solid product which is finally recovered via generally a cyclone unit. Spray-drying technology has undergone constant evolution in the past years and the synthesis of polymeric nanosized particles obtained in a one-step procedure by spray-drying a polymer solution has emerged recently. For instance, Li et al. [45] described the preparation of different types of polymeric nanoparticles such as Arabic gum, whey protein, polyvinyl alcohol, modified starch, and maltodextrin based on a "nano spray dryer", an innovative new spray-drying technology developed by Büchi® (Fig. 2.17).

Very recently, the group of Lee et al. [46] has used the same technology to produce bovine serum albumin nanoparticles. In contrary to conventional spray dryer, the "nano spray dryer" is characterized by a vibration mesh spray technology creating tiny droplets in a range of a smaller order of magnitude than the conventional apparatus. The generation of droplets is based on a piezoelectric actuator driven at an ultrasonic frequency (i.e., 60 kHz) ensuring vibration of a thin perforated membrane with micron-sized holes which can vary from 4 to 7 μm in diameter. The membrane vibration causes ejection of millions of nanodroplets per second with a very narrow size distribution. The final sizes and standard deviation of the nanoparticles obtained depend on several parameters such as the nature and concentration of the polymer, the spray mesh size, the operating conditions (drying temperature, feed rate, drying gas flow rate, etc.), or the concentration of surfactant, if present in the formulation. Finally, another advantage of this novel technology is the high yield production of particles that can be in 70–95 % range.

2.4 Conclusion

This chapter provides an overview of the main synthesis methods of organic and bioorganic nanoparticles reported in the literature. Two approaches are highlighted based on either one- or two-step procedures. In the case of two-step procedures, a nanoemulsification step is required prior to conversion of nanodroplets into nanoparticles. It constitutes an important part of the challenge to obtain materials with well-defined structures and morphologies. High-energy emulsifications are by far the most widely used methods but low-energy emulsifications, still few reported, are undergoing a great expansion due to their main advantage in terms of environmental impact. Conversion of nanoemulsions into nanoparticles can be done subsequently in the second step through several ways including nanogelation, solvent removal, salting out, or polymerization.

In the case of one-step procedures, no nanoemulsification is required and the nanoparticles can be generated via different mechanisms such as nanoprecipitation, desolvation, self-assembly, nanogelation, or using more technological ways such as supercritical drying or nanospray-drying methods.

In the field of organic nanoparticles, according to the synthesis methods described above, the control of size, morphology, and structure of particles is still submitted to a number of difficulties that have to be overcome to develop new functional nanomaterials based on organic nanoparticles in the future. A better fundamental knowledge of the processes and mechanisms controlling the particles synthesis should be the subject of an intensive research in the next decades. Both technological aspects and precipitation techniques in solution should be developed and improved simultaneously to ensure a wide spectrum of preparation methods easily adapted to a large and increasing range of organic materials available.

References

1. Kreuter J (1994) Nanoparticles. In: Kreuter J (ed) Colloidal drug delivery systems. Marcel Dekker Inc, New York, pp 219–342
2. Couvreur P (1988) Polyalkylcyanoacrylates as colloidal drug carriers. Crit Rev Ther Drug Carr Syst 5:1–20
3. Schmid G (2004) Nanoparticles: from theory to application. Wiley-VCH Publisher, Weinheim
4. Geckeler KE, Nishide H (2010) Advanced nanomaterials. Wiley-VCH Publishers, Weinheim
5. Geckeler KE, Rosenberg E (2006) Functional nanomaterials. American Scientific Publishers, Valencia
6. Hosokawa M, Nogi K, Naito M, Yokoyama T (2007) Nanoparticle technology handbook. Elsevier, Amsterdam
7. Grimsdale AC, Chan KL, Martin RE, Jokisz PG, Holmes AB (2009) Synthesis of light-emitting conjugated polymers for applications in electroluminescent devices. Chem Rev 109:897–1091
8. Müllen K, Scherf U (2006) Organic light-emitting devices. Wiley-VCH Publisher, Weinheim
9. Nalwa HS, Rohwer LS (eds) (2003) Handbook of luminescence, display materials, and devices. American Scientific Publishers, Stevenson Ranch

10. Hadziioannou G, Malliaras GG (eds) (2000) Semiconducting polymers. Wiley-VCH Publisher, Weinheim
11. Couvreur P, Vauthier C (2006) Nanotechnology: intelligent design to treat complex disease. Pharm Res 23:1417–1450
12. Pinto Reis C, Neufeld RJ, Ribeiro AJ, Veiga F (2006) Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomed Nanotechnol Biol Med 2: 8–21
13. Pinto Reis C, Neufeld R J, Ribeiro A J, Veiga F (2006) Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. Nanomed Nanotechnol Biol Med 2:53–65
14. Rao JP, Geckeler KE (2011) Polymer nanoparticles: preparation techniques and size-control parameters. Prog Polym Sci 36:887–913
15. Pecher J, Mecking S (2010) Nanoparticles of conjugated polymers. Chem Rev 110: 6260–6279
16. Anton N, Benoit J-P, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates–A review. J Controlled Release 128:185–199
17. Vauthier C, Bouchemal K (2009) Methods for the preparation and manufacture of polymeric nanoparticles. Pharm Res 26:1025–1058
18. Landfester K, Musyanovych A, Mailänder V (2010) From polymeric particles to multifunctional nanocapsules for biomedical applications using the miniemulsion process. J Polym Sci A Polym Chem 48:493–515
19. Allemann E, Gurny R, Doelker E (1993) Drug-loaded nanoparticles—Preparation methods and drug targeting issues. Eur J Pharm Biopharm 39:173–191
20. Quintanar-Guerrero D, Alle?mann E, Fessi H, Doelker E (1998) Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug Dev Ind Pharm 24:1113–1128
21. De Jaeghere F, Doelker E, Gurny R (1999) Nanoparticles, In: Mathiowitz E (ed) Encyclopedia of controlled drug delivery, vol 2. Wiley-VCH, New York, pp 641–664
22. Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C (2002) Nanocapsule technology: a review. Crit Rev Ther Drug 19:99–134
23. Tuncel D, Demir HV (2010) Conjugated polymer nanoparticles. Nanoscale 2:484–494
24. Gangopadhyay R, Conducting Polymer Nanostructures, In: Nalwa H S (ed) (2004) Encyclopedia of nanoscience and nanotechnology, vol 2. American Scientific Publishers, Stevenson Ranch, pp 105–131
25. Wallace GG, Innis PC (2002) Inherently conducting polymer nanostructures. J Nanosci Nanotechnol 2:441–451
26. Stejskal J (2001) Colloidal dispersions of conducting polymers. J Polym Mater 18:225–258
27. Vincent B (1995) Electrically conducting polymer colloids and composites. Polym Adv Technol 6:356–361
28. Armes SP (1995) Electrically conducting polymer colloids. Polym News 20:233–237
29. Aldissi M, Armes SP (1991) Colloidal dispersions of conducting polymers. Prog Org Coat 19:21–58
30. Armes SP, Vincent B (1988) Post-doping of sterically-stabilized polyacetylene latexes. Synth Met 25:171–179
31. Groenendaal L, Jonas F, Freitag D, Pielartzik H, Reynolds JR (2000) Poly(3,4-ethylenedioxythiophene) and its derivatives: past, present, and future. Adv Mater 12:481–494
32. Huyal IO, Ozel T, Tuncel D, Demir HV (2008) Quantum efficiency enhancement in film by making nanoparticles of polyfluorene. Opt Express 16:13391–13397
33. Ozel IO, Ozel T, Demir HV, Tuncel D (2010) Non-radiative resonance energy transfer in bi-polymer nanoparticles of fluorescent conjugated polymers. Opt Express 18:670–684
34. Grigalevicius S, Forster M, Ellinger S, Landfester K, Scherf U (2006) Excitation energy transfer from semi-conducting polymer nanoparticles to surface-bound fluorescent dyes. Macromol Rapid Commun 27:200–202
35. Pecher J, Mecking S (2007) Nanoparticles from step-growth coordination polymerization. Macromolecules 40:7733–7735

36. Pecher J, Mecking S (2008) Poly(p-phenylene vinylene) nanoparticles by acyclic diene metathesis (ADMET) polycondensation in aqueous emulsion. Polymer Preprints (American Chemical Society, division of Polymer Chemistry), pp 363–364
37. Rahim NAA, McDaniel W, Bardon K, Srinivasan S, Vickerman V, So PTC, Moon JH (2009) Conjugated polymer nanoparticles for two-photon imaging of endothelial cells in a tissue model. Adv Mater 21:3492–3496
38. Hittinger E, Kokil A, Weder C (2004) Synthesis and characterization of cross-linked conjugated polymer milli-, micro-, and nanoparticles. Angew Chem Int Ed 43:1808–1811
39. Müller RH, Mäder K, Gohla S (2000) Solid lipid nanoparticles (SLN) for controlled drug delivery—A review of the state of the art. Eur J Pharm Biopharm 50:161–177
40. Pragati S, Kuldeep S, Ashok S, Satheesh M (2009) Solid lipid nanoparticles: a promising drug delivery technology. Int J Pharm Sci Nanotechnol 2:509–516
41. Basu B, Garala K, Bhalodia R, Joshi B, Mehta K (2010) Solid lipid nanoparticles:a promising tool for drug delivery system. J Pharm Res 3:84–92
42. Freitas C, Müller RH (1999) Correlation between long-term stability of solid lipid nanoparticles (SLN(TM)) and crystallinity of the lipid phase. Eur J Pharm Biopharm 47:125–132
43. Müller RH, Radtke M, Wissing SA (2002) Nanostructured lipid matrices for improved microencapsulation of drugs. Int J Pharm 242:121–128
44. Olbrich C, Gessner A, Kayser O, Müller RH (2002) Lipid-drug-conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediaceturate. J Drug Targeting 10:387–396
45. Li X, Anton N, Arpagaus C, Belleteix F, Vandamme TF (2010) Nanoparticles by spray drying using innovative new technology: the Büchi Nano spray dryer B-90. J Controlled Release 147:304–310
46. Lee SH, Heng D, Ng WK, Chan H-K, Tan RBH (2011) Nano spray drying: a novel method for preparing protein nanoparticles for protein therapy. Int J Pharm 403:192–200
47. York P (1999) Strategies for particle design using supercritical fluid technologies. Pharm Sci Technol Today 2:430–440
48. Shariati A, Peters CJ (2003) Recent developments in particle design using supercritical fluids. Curr Opin Solid State Mater Sci 7:371–383
49. Wright IK, Higginbotham A, Baker SM, Donnelly TD (2010) Generation of nanoparticles of controlled size using ultrasonic piezoelectric oscillators in solution. ACS Appl Mater Interfaces 2:2360–2364
50. Becher P (1965) Emulsions: theory and practice. Reinhold Pub Corp, New York
51. Becher P (1985) Encyclopedia of emulsion technology. Marcel Dekker Inc, New York
52. Mittal KL, Lindman B (eds) (1984) Surfactants in solution. Plenum, New York
53. Ruschak KJ, Miller CA (1972) Spontaneous emulsification in ternary systems with mass transfer. Ind Eng Chem Fundam 11:534–540
54. Miller CA (1988) Spontaneous emulsification produced by diffusion—a review. Colloids Surf 29:89–102
55. El-Aasser MS, Lack CD, Vanderhoff JW, Fowkes FM (1988) The miniemulsification process—different form of spontaneous emulsification. Colloids Surf 29:103–118
56. Ganachaud F, Katz JL (2005) Nanoparticles and nanocapsules created using the Ouzo effect: spontaneous emulsification as an alternative to ultrasonic and high-shear devices. ChemPhysChem 6:209–216
57. Bouchemal K, Briançon S, Perrier E, Fessi H (2004) Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. Int J Pharm 280:241–251
58. Kawashima Y, Yamamoto H, Takeuchi H, Hino T, Niwa T (1998) Properties of a peptide containing DL-lactide/glycolide copolymer nanospheres prepared by novel emulsion solvent diffusion methods. Eur J Pharm Biopharm 45:41–48
59. Quintanar-Guerrero D, Allémann E, Doelker E, Fessi H (1997) A mechanistic study of the formation of polymer nanoparticles by the emulsification-diffusion technique. Colloid Polym Sci 275:640–647

60. Quintanar-Guerrero D, Allémann E, Fessi H, Doelker E (1999) Pseudolatex preparation using a novel emulsion-diffusion process involving direct displacement of partially water-miscible solvents by distillation. Int J Pharm 188:155–164
61. Quintanar-Guerrero D, Allémann E, Doelker E, Fessi H (1998) Preparation and characterization of nanocapsnles from preformed polymers by a new process based on emulsification-diffusion technique. Pharm Res 15:1056–1062
62. Gallardo M, Couarraze G, Denizot B, Treupel L, Couvreur P, Puisieux F (1993) Study of the mechanisms of formation of nanoparticles and nanocapsules of polyisobutyl-2-cyanoacrylate. Int J Pharm 100:55–64
63. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S (1989) Nanocapsule formation by interfacial polymer deposition following solvent deplacement. Int J Pharm 55:25–28
64. Ostrovsky MV, Good RJ (1984) Mechanism of microemulsion formation in systems with low interfacial tension: occurrence, properties, and behavior of microemulsions. J Colloid Interface Sci 102:206–226
65. Marszall L, Shick MJ (eds) (1987) Nonionic surfactants, surfactant sciences series, vol 23. Marcel Dekker Inc, New York
66. Taylor P, Ottewill RH (1994) The formation and ageing rates of oil-in-water miniemulsions. Colloids Surf A 88:303–316
67. Taylor P, Ottewill RH (1994) Ostwald ripening in O/W miniemulsions formed by the dilution of O/W microemulsions. Prog Colloid Polym Sci 97:199–203
68. Forgiarini A, Esquena J, González C, Solans C (2001) Formation of nano-emulsions by low-energy emulsification methods at constant temperature. Langmuir 17:2076–2083
69. Wu H, Ramachandran C, Weiner ND, Roessler BJ (2001) Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int J Pharm 220:63–75
70. Porras M, Solans C, González C, Martínez A, Guinart A, Gutiérrez JM (2004) Studies of formation of W/O nano-emulsions. Colloids Surf A 249:115–118
71. Usón N, Garcia MJ, Solans C (2004) Formation of water-in-oil (W/O) nano-emulsions in a water/mixed non-ionic surfactant/oil systems prepared by a low-energy emulsification method. Colloids Surf A 250:415–421
72. Solè I, Maestro A, Pey CM, González C, Solans C, Gutiérrez JM (2006) Nano-emulsions preparation by low energy methods in an ionic surfactant system. Colloids Surf A 288:138–143
73. Solè I, Maestro A, González C, Solans C, Gutiérrez JM (2006) Optimization of nano-emulsion preparation by low-energy methods in an ionic surfactant system. Langmuir 22:8326–8332
74. Shinoda K, Saito H (1968) The effect of temperature on the phase equilibria and the types of dispersions of the ternary system composed of water, cyclohexane, and nonionic surfactant. J Colloid Interface Sci 26:70–74
75. Shinoda K, Saito H (1969) The stability of O/W type emulsions as functions of temperature and the HLB of emulsifiers: the emulsification by PIT-method. J Colloid Interface Sci 30:258–263
76. Izquierdo P, Esquena J, Tadros TF, Dederen C, Garcia MJ, Azemar N, Solans C (2002) Formation and stability of nano-emulsions prepared using the phase inversion temperature method. Langmuir 18:26–30
77. Izquierdo P, Esquena J, Tadros TF, Dederen JC, Feng J, Garcia-Celma MJ, Azemar N, Solans C (2004) Phase behavior and nano-emulsion formation by the phase inversion temperature method. Langmuir 20:6594–6598
78. Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma MJ (2005) Nano-emulsions. Curr Opin Colloid Interface Sci 10:102–110
79. Förster T, Von Rybinski W, Wadle A (1995) Influence of microemulsion phases on the preparation of fine-disperse emulsions. Adv Colloid Interface Sci 58:119–149
80. Pons R, Carrera I, Caelles J, Rouch J, Panizza P (2003) Formation and properties of miniemulsions formed by microemulsions dilution. Adv Colloid Interface Sci 106:129–146
81. Anton N, Gayet P, Benoit J-P, Saulnier P (2007) Nano-emulsions and nanocapsules by the PIT method: an investigation on the role of the temperature cycling on the emulsion phase inversion. Int J Pharm 344:44–52

82. Salager J-L, Marquez N, Graciaa A, Lachaise J (2000) Partitioning of ethoxylated octylphenol surfactants in microemulsion–oil–water systems: influence of temperature and relation between partitioning coefficient and physicochemical formulation. Langmuir 16:5534–5539
83. Salager JL, Antón RE, Andérez JM, Aubry JM (2001) Formulation des micro-émulsions par la méthode HLD, Techniques De L'Ingénieur, Génie Des Procédés J2: 1–20
84. Salager JL (1999) Microemulsions, handbook of detergents—part A: properties. Marcel Dekker Inc, New York, pp 253–302
85. Salager JL (2000) Pharmaceutical emulsions and suspensions, formulation concepts for the emulsion maker. Marcel Dekker Inc, New York, pp 19–72
86. Bourrel M, Salager JL, Schechter RS, Wade WH (1980) A correlation for phase behavior of nonionic surfactants. J Colloid Interface Sci 75:451–461
87. Rondón-González M, Sadtler V, Choplin L, Salager J-L (2006) Emulsion inversion from abnormal to normal morphology by continuous stirring without internal phase addition. Effect of surfactant mixture fractionation at extreme water-oil ratio. Colloids Surf A 288:151–157
88. Rondón-Gonzaléz M, Sadtler V, Choplin L, Salager J-L (2006) Emulsion catastrophic inversion from abnormal to normal morphology. 5. Effect of the water-to-oil ratio and surfactant concentration on the inversion produced by continuous stirring. Ind Eng Chem Res 45:3074–3080
89. Tyrode E, Allouche J, Choplin L, Salager J-L (2005) Emulsion catastrophic inversion from abnormal to normal morphology. 4. Following the emulsion viscosity during three inversion protocols and extending the critical dispersed-phase concept. Ind Eng Chem Res 44:67–74
90. Tyrode E, Mira I, Zambrano N, Márquez L, Rondón-Gonzalez M, Salager J-L (2003) Emulsion catastrophic inversion from abnormal to normal morphology. 3. Conditions for triggering the dynamic inversion and application to industrial processes. Ind Eng Chem Res 42:4311–4318
91. Salager J-L, Forgiarini A, Márquez L, Peña A, Pizzino A, Rodriguez MP, Rondón-González M (2004) Using emulsion inversion in industrial processes. Adv Colloid Interface Sci 108–109:259–272
92. Allouche J, Tyrode E, Sadtler V, Choplin L, Salager J-L (2004) Simultaneous conductivity and viscosity measurements as a technique to track emulsion inversion by the phase-inversion-temperature method. Langmuir 20:2134–2140
93. Salager J-L, Marquez L, Pena AA, Rondon M, Silva F, Tyrode E (2000) Current phenomenological know-how and modeling of emulsion inversion. Ind Eng Chem Res 39:2665–2676
94. Salager J-L (1996) Guidelines for the formulation, composition and stirring to attain desired emulsion properties (type, droplet size, viscosity and stability), Surfactant Science Series, pp 261–295
95. Marquez L, Graciaa A, Lachaise J, Salager J-L, Zambrano N (2003) Hysteresis behavior in temperature-induced emulsion inversion. Polym Int 52:590–593
96. Pizzino A, Rodriguez MP, Xuereb C, Catte M, Van HE, Aubry J-M, Salager J-L (2007) Light backscattering as an indirect method for detecting emulsion inversion. Langmuir 23:5286–5288
97. Stork M, Tousain RL, Wieringa JA, Bosgra OH (2003) A MILP approach to the optimization of the operation procedure of a fed-batch emulsification process in a stirred vessel. Comput Chem Eng 27:1681–1691
98. Mabille C, Leal-Calderon F, Bibette J, Schmitt V (2003) Monodisperse fragmentation in emulsions: mechanisms and kinetics. Europhys Lett 61:708–714
99. Mabille C, Schmitt V, Gorria P, Calderon FL, Faye V, Deminiere B, Bibette J (2000) Rheological and shearing conditions for the preparation of monodisperse emulsions. Langmuir 16:422–429
100. Trotta M, Pattarino F, Ignoni T (2002) Stability of drug-carrier emulsions containing phosphatidylcholine mixtures. Eur J Pharm Biopharm 53:203–208

101. Lizarraga MS, Pan LG, Anon MC, Santiago LG (2008) Stability of concentrated emulsions measured by optical and rheological methods. Effect of processing conditions-I. Whey protein concentrate. Food Hydrocoll 22:868–878
102. Bengoechea C, Cordobes F, Puppo MC, Guerrero A (2007) Linear viscoelasticity and droplet size distribution of O/W emulsions stabilized by plant proteins. Afinidad 64:696–704
103. Higgins DM, Skauen DM (1972) Influence of power on quality of emulsions prepared by ultrasound. J Pharm Sci 61:1567–1570
104. Li MK, Fogler HS (1978) Acoustic emulsification—1. The instability of the oil–water interface to form the initial droplets. J Fluid Mech 88:499–511
105. Li MK, Fogler HS (1978) Acoustic emulsification—2 breakup of the large primary oil droplets in water medium. J Fluid Mech 88:513–528
106. Griffin WC (1979) Emulsions. In: Kirk-Othmer (ed) Encyclopedia of Chemical Technology, vol 8. Wiley, Weinheim, pp 900–930
107. Johnson JC (1979) Emulsifiers and emulsifying techniques. Noyes Data Corp, Park Ridge
108. Reddy SR, Fogler HS (1980) Emulsion stability of acoustically formed emulsions. J Phys Chem 84:1570–1575
109. Eberth K, Merry J (1983) A comparative study of emulsions prepared by ultrasound and by a conventional method. Droplet size measurements by means of a Coulter Counter and microscopy. Int J Pharm 14:349–353
110. Walstra P (1993) Principles of emulsion formation. Chem Eng Sci 48:333–349
111. Abismail B, Canselier JP, Wilhelm AM, Delmas H, Gourdon C (1999) Emulsification by ultrasound: drop size distribution and stability. Ultrason Sonochem 6:75–83
112. Mongenot N, Charrier S, Chalier P (2000) Effect of ultrasound emulsification on cheese aroma encapsulation by carbohydrates. J Agric Food Chem 48:861–867
113. Corzo-Martinez M, Soria AC, Villamiel M, Olano A, Harte FM, Moreno FJ (2011) Effect of glycation on sodium caseinate-stabilized emulsions obtained by ultrasound. J Dairy Sci 94:51–58
114. Wood RW, Loomis AL (1927) The physical and biological effects of high frequency sound waves of great intensity. Philos Mag 4:417–436
115. Akiyoshi K, Kang E-C, Kurumada S, Sunamoto J, Principi T, Winnik FM (2000) Controlled association of amphiphilic polymers in water: thermosensitive nanoparticles formed by self-assembly of hydrophobically modified pullulans and poly(n-isopropylacrylamides). Macromolecules 33:3244–3249
116. Xia H, Wang Q (2001) Synthesis and characterization of conductive polyaniline nanoparticles through ultrasonic assisted inverse microemulsion polymerization. J Nanopart Res 3:401–411
117. Xia H, Zhang C, Wang Q (2001) Study on ultrasonic induced encapsulating emulsion polymerization in the presence of nanoparticles. J Appl Polym Sci 80:1130–1139
118. Hou D, Xie C, Huang K, Zhu C (2003) The production and characteristics of solid lipid nanoparticles (SLNs). Biomaterials 24:1781–1785
119. Li Y, Dong L, Jia A, Chang X, Xue H (2006) Preparation and characterization of solid lipid nanoparticles loaded traditional chinese medicine. Int J Biol Macromol 38:296–299
120. Luo Y, Chen D, Ren L, Zhao X, Qin J (2006) Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. J Controlled Release 114:53–59
121. Wong HL, Bendayan R, Rauth AM, Xue HY, Babakhanian K, Wu XY (2006) A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer–lipid hybrid nanoparticle system. J Pharmacol Exp Ther 317:1372–1381
122. Yegin BA, Benoit J-P, Lamprecht A (2006) Paclitaxel-loaded lipid nanoparticles prepared by solvent injection or ultrasound emulsification. Drug Dev Ind Pharm 32:1089–1094
123. Sharma P, Ganta S, Denny WA, Garg S (2009) Formulation and pharmacokinetics of lipid nanoparticles of a chemically sensitive nitrogen mustard derivative: chlorambucil. Int J Pharm 367:187–194
124. Lee KJ, Oh W-K, Song J, Kim S, Lee J, Jang J (2010) Photoluminescent polymer nanoparticles for label-free cellular imaging. Chem Commun 46:5229–5231

125. Aji A, Chacko AJ, Jose S, Souto EB (2011) Lopinavir loaded solid lipid nanoparticles (SLN) for intestinal lymphatic targeting. Eur J Pharm Sci 42:11–18
126. Das S, Ng WK, Kanaujia P, Kim S, Tan RBH (2011) Formulation design, preparation and physicochemical characterizations of solid lipid nanoparticles containing a hydrophobic drug: effects of process variables. Colloids Surf B 88:483–489
127. Allouche J, Boissiere M, Helary C, Livage J, Coradin T (2006) Biomimetic core-shell gelatine/silica nanoparticles: a new example of biopolymer-based nanocomposites. J Mater Chem 16:3120–3125
128. Makino K, Mossoba MM, Riesz P (1982) Chemical effects of ultrasound on aqueous solutions. Evidence for hydroxyl and hydrogen free radicals (.cntdot.OH and.cntdot.H) by spin trapping. J Am Chem Soc 104:3537–3539
129. Makino K, Mossoba MM, Riesz P (1983) Chemical effects of ultrasound on aqueous solutions. Formation of hydroxyl radicals and hydrogen atoms. J Phys Chem 87:1369–1377
130. Seo M, Nie Z, Xu S, Mok M, Lewis PC, Graham R, Kumacheva E (2005) Continuous microfluidic reactors for polymer particles. Langmuir 21:11614–11622
131. Atencia J, Beebe DJ (2005) Controlled microfluidic interfaces. Nature 437:648–655
132. Nisisako T, Okushima S, Torii T (2005) Controlled formulation of monodisperse double emulsions in a multiple-phase microfluidic system. Soft Matter 1:23–27
133. Jahn A, Reiner JE, Vreeland WN, DeVoe DL, Locascio LE, Gaitan M (2008) Preparation of nanoparticles by continuous-flow microfluidics. J Nanopart Res 10:925–934
134. Engl W, Backov R, Panizza P (2008) Controlled production of emulsions and particles by milli- and microfluidic techniques. Curr Opin Colloid Interface Sci 13:206–216
135. Kumacheva E, Zhang H, Nie Z (2009) Polymerization in microfluidic reactors. In: Microchemical engineering in practice , pp. 361–383 John Wiley and Sons Inc., Weinheim, Germany
136. Schaerli Y, Hollfelder F (2009) The potential of microfluidic water-in-oil droplets in experimental biology. Mol BioSyst 5:1392–1404
137. Zhao C-X, Middelberg APJ (2011) Two-phase microfluidic flows. Chem Eng Sci 66: 1394–1411
138. Zhao C-X, He L, Qiao SZ, Middelberg APJ (2011) Nanoparticle synthesis in microreactors. Chem Eng Sci 66:1463–1479
139. Karnik R, Gu F, Basto P, Cannizzaro C, Dean L, Kyei-Manu W, Langer R, Farokhzad OC (2008) Microfluidic platform for controlled synthesis of polymeric nanoparticles. Nano Lett 8:2906–2912
140. Charcosset C, Fessi H (2005) Preparation of nanoparticles with a membrane contactor. J Membr Sci 266:115–120
141. Rondeau E, Cooper-White JJ (2008) Biopolymer microparticle and nanoparticle formation within a microfluidic device. Langmuir 24:6937–6945
142. Gurny R, Peppas NA, Harrington DD, Banker GS (1981) Development of biodegradable and injectable latices for controlled release of potent drugs. Drug Dev Ind Pharm 7:1–25
143. Quintanar-Guerrero D, Allémann E, Fessi H, Doelker E (1999) Pseudolatex preparation using a novel emulsion-diffusion process involving direct displacement of partially water-miscible solvents by distillation. Int J Pharm 188:155–164
144. Choonara YE, Pillay V, Ndesendo VMK, du Toit LC, Kumar P, Khan RA, Murphy CS, Jarvis D-L (2011) Polymeric emulsion and crosslink-mediated synthesis of super-stable nanoparticles as sustained-release anti-tuberculosis drug carriers. Colloids Surf B 87:243–254
145. Lee W-K, Park J-Y, Jung S, Chul WY, Kim W-U, Kim H-Y, Park J-H, Park J-S (2005) Preparation and characterization of biodegradable nanoparticles entrapping immunodominant peptide conjugated with PEG for oral tolerance induction. J Controlled Release 105:77–88
146. Venier-Julienne MC, Benoît JP (1996) Preparation, purification and morphology of polymeric nanoparticles as drug carriers. Pharm Acta Helv 71:121–128
147. Song CX, Labhasetwar V, Murphy H, Qu X, Humphrey WR, Shebuski RJ, Levy RJ (1997) Formulation and characterization of biodegradable nanoparticles for intravascular local drug delivery. J Controlled Release 43:197–212

148. Zambaux MF, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso MJ, Labrude P, Vigneron C (1998) Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. J Controlled Release 50:31–40
149. Bazile DV, Ropert C, Huve P, Verrecchia T, Marland M, Frydman A, Veillard M, Spenleuhauer G (1992) Body distribution of fully biodegradable [14C]-poly(lactic acid) nanoparticles coated with albumin after parenteral administration to rats. Biomaterials 13:1093–1102
150. Singh J, Pandit S, Bramwell VW, Alpar HO (2006) Diphtheria toxoid loaded poly-([epsilon]-caprolactone) nanoparticles as mucosal vaccine delivery systems. Methods 38:96–105
151. Quellec P, Gref R, Dellacherie E, Sommer F, Tran MD, Alonso MJ (1999) Protein encapsulation within poly(ethylene glycol)-coated nanospheres. II. Controlled release properties. J Biomed Mater Res 47:388–395
152. Bazile D, Prud'Homme C, Bassoullet M-T, Marlard M, Spenlehauer G, Veillard M (1995) Stealth Me.PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. J Pharm Sci 84:493–498
153. Luo G, Yu X, Jin C, Yang F, Fu D, Long J, Xu J, Zhan C, Lu W (2010) LyP-1-conjugated nanoparticles for targeting drug delivery to lymphatic metastatic tumors. Int J Pharm 385:150–156
154. Lemarchand C, Couvreur P, Vauthier C, Costantini D, Gref R (2003) Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. Int J Pharm 254:77–82
155. Lemarchand C, Couvreur P, Besnard M, Costantini D, Gref R (2003) Novel polyester-polysaccharide nanoparticles. Pharm Res 20:1284–1292
156. Moinard-Checot D, Chevalier Y, Briançon S, Fessi H, Guinebretière S (2006) Nanoparticles for drug delivery: review of the formulation and process difficulties illustrated by the emulsion–diffusion process. J Nanosci Nanotechnol 6:2664–2681
157. Moinard-Chécot D, Chevalier Y, Briançon S, Beney L, Fessi H (2008) Mechanism of nanocapsules formation by the emulsion–diffusion process. J Colloid Interface Sci 317: 458–468
158. Leroux J-C, Allemann E, Doelker E, Gurny R (1995) New approach for the preparation of nanoparticles by an emulsification-diffusion method. Eur J Pharm Biopharm 41:14–18
159. Quintanar-Guerrero D, Tamayo-Esquivel D, Ganem-Quintanar A, Allémann E, Doelker E (2005) Adaptation and optimization of the emulsification-diffusion technique to prepare lipidic nanospheres. Eur J Pham Sci 26:211–218
160. Quintanar-Guerrero D, Fessi H, Allémann E, Doelker E (1996) Influence of stabilizing agents and preparative variables on the formation of poly(D,L-lactic acid) nanoparticles by an emulsification-diffusion technique. Int J Pharm 143:133–141
161. Trimaille T, Pichot C, Ela A, Fessi H, Briançon S, Delair T (2003) Poly(D,L-lactic acid) nanoparticle preparation and colloidal characterization. Colloid Polym Sci 281:1184–1190
162. Choi S-W, Kwon H-Y, Kim W-S, Kim J-H (2002) Thermodynamic parameters on poly (d,l-lactide-co-glycolide) particle size in emulsification-diffusion process. Colloids Surf A 201:283–289
163. Kwon H-Y, Lee J-Y, Choi S-W, Jang Y, Kim J-H (2001) Preparation of PLGA nanoparticles containing estrogen by emulsification-diffusion method. Colloids Surf A 182:123–130
164. Swarnakar NK, Jain AK, Singh RP, Godugu C, Das M, Jain S (2011) Oral bioavailability, therapeutic efficacy and reactive oxygen species scavenging properties of coenzyme Q10-loaded polymeric nanoparticles. Biomaterials 32:6860–6874
165. El-Shabouri MH (2002) Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. Int J Pharm 249:101–108
166. Yan C, Chen D, Gu J, Qin J (2006) Nanoparticles of 5-fluorouracil (5-FU) loaded N-succinyl-chitosan (Suc-Chi) for cancer chemotherapy: preparation, characterization—in vitro drug release and anti-tumour activity. J Pharm Pharmacol 58:1177–1181
167. Yan C, Gu J, Yan C, Zhan H, Chen D (2010) In vivo biodistribution for tumor targeting of 5-fluorouracil (5-FU) loaded N-succinyl-chitosan (Suc-Chi) nanoparticles. BioChem Indian J 4:21–25

168. Ibrahim H, Bindschaedler C, Doelker E, Buri P, Gurny R (1992) Aqueous nanodispersions prepared by a salting-out process. Int J Pharm 87:239–246
169. De Jaeghere F, Allémann E, Leroux J-C, Stevels W, Feijen J, Doelker E, Gurny R (1999) Formulation and lyoprotection of poly(Lactic acid-co-ethylene oxide) nanoparticles: influence on physical stability and in vitro cell uptake. Pharm Res 16:859–866
170. Nguyen CA, Allémann E, Schwach G, Doelker E, Gurny R (2003) Synthesis of a novel fluorescent poly(D,L-lactide) end-capped with 1-pyrenebutanol used for the preparation of nanoparticles. Eur J Pham Sci 20:217–222
171. Zweers MLT, Engbers GHM, Grijpma DW, Feijen J (2004) In vitro degradation of nanoparticles prepared from polymers based on DL-lactide, glycolide and poly(ethylene oxide). J Controlled Release 100:347–356
172. Perugini P, Simeoni S, Scalia S, Genta I, Modena T, Conti B, Pavanetto F (2002) Effect of nanoparticle encapsulation on the photostability of the sunscreen agent, 2-ethylhexyl-p-methoxycinnamate. Int J Pharm 246:37–45
173. Allemann E, Gurny R, Doelker E (1992) Preparation of aqueous polymeric nanodispersions by a reversible salting-out process: Influence of process parameters on particle size. Int J Pharm 87:247–253
174. Konan YN, Gurny R, Allémann E (2002) Preparation and characterization of sterile and freeze-dried sub-200 nm nanoparticles. Int J Pharm 233:239–252
175. Galindo-Rodriguez S, Allémann E, Fessi H, Doelker E (2004) Physicochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion, and nanoprecipitation methods. Pharm Res 21:1428–1439
176. Reis CP, Neufeld RJ, Vilela S, Ribeiro AJ, Veiga F (2006) Review and current status of emulsion/dispersion technology using an internal gelation process for the design of alginate particles. J Microencapsul 23:245–257
177. Wang N, Wu XS (1997) Preparation and characterization of agarose hydrogel nanoparticles for protein and peptide drug delivery. Pharm Dev Technol 2:135–142
178. Reis CP, Neufeld RJ, Ribeiro AJ, Veiga F (2006) Design of insulin-loaded alginate nanoparticles: influence of the calcium ion on polymer gel matrix properties. Chem Ind Chem Eng Q 12:47–52
179. Arshady R (1988) Preparation of polymer nano- and microspheres by vinyl polymerization techniques. J Microencapsul 5:101–114
180. Asua JM (2002) Miniemulsion polymerization. Prog Polym Sci 27:1283–1346
181. Landfester K (2006) Encapsulation through (mini)emulsion polymerization. Functional coating Wiley-VCH, Weinheim, Germany
182. Chern CS (2006) Emulsion polymerization mechanisms and kinetics. Prog Polym Sci 31:443–486
183. Thickett SC, Gilbert RG (2007) Emulsion polymerization: state of the art in kinetics and mechanisms. Polymer 48:6965–6991
184. Landfester K (2009) Miniemulsion polymerization and the structure of polymer and hybrid nanoparticles. Angew Chem Int Ed 48:4488–4507
185. Yoon S-J, Chun H, Lee M-S, Kim N (2009) Preparation of poly(N-vinylcarbazole) (PVK) nanoparticles by emulsion polymerization and PVK hollow particles. Synth Met 159:518–522
186. Costa C, Santos AF, Fortuny M, Araújo PHH, Sayer C (2009) Kinetic advantages of using microwaves in the emulsion polymerization of MMA. Mater Sci Eng C 29:415–419
187. Cheng X, Chen M, Zhou S, Wu L (2006) Preparation of SiO2/PMMA composite particles via conventional emulsion polymerization. J Polym Sci Part A Polym Chem 44:3807–3816
188. Muñoz-Bonilla A, Van Herk AM, Heuts JPA (2010) Preparation of hairy particles and antifouling films using brush-type amphiphilic block copolymer surfactants in emulsion polymerization. Macromolecules 43:2721–2731
189. Garay-Jimenez JC, Gergeres D, Young A, Lim DV, Turos E (2009) Physical properties and biological activity of poly(butyl acrylate-styrene) nanoparticle emulsions prepared with conventional and polymerizable surfactants. Nanomed Nanotechnol Biol Med 5: 443–451

190. Lu S, Qu R, Forcada J (2009) Preparation of magnetic polymeric composite nanoparticles by seeded emulsion polymerization. Mater Lett 63:770–772
191. Gao J, Wu C (2005) Modified structural model for predicting particle size in the microemulsion and emulsion polymerization of styrene under microwave irradiation. Langmuir 21:782–785
192. Zhang J, Cao Y, He Y (2004) Ultrasonically irradiated emulsion polymerization of styrene in the presence of a polymeric surfactant. J Appl Polym Sci 94:763–768
193. Chang Y-H, Lee Y-D, Karlsson OJ, Sundberg DC (2001) Particle nucleation mechanism for the emulsion polymerization of styrene with a novel polyester emulsifier. J Appl Polym Sci 82:1061–1070
194. Thickett SC, Gaborieau M, Gilbert RG (2007) Extended mechanistic description of particle growth in electrosterically stabilized emulsion polymerization systems. Macromolecules 40:4710–4720
195. Mock EB, De Bruyn H, Hawkett BS, Gilbert RG, Zukoski CF (2006) Synthesis of anisotropic nanoparticles by seeded emulsion polymerization. Langmuir 22:4037–4043
196. El-Samaligy MS, Rohdewald P, Mahmoud HA (1986) Polyalkyl cyanoacrylate nanocapsules. J Pharm Pharmacol 38:216–218
197. Couvreur P, Kante B, Roland M (1979) Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties. J Pharm Pharmacol 31:331–332
198. Vauthier C, Labarre D, Ponchel G (2007) Design aspects of poly(alkylcyanoacrylate) nanoparticles for drug delivery. J Drug Target 15:641–663
199. Nicolas J, Couvreur P (2009) Synthesis of poly(alkyl cyanoacrylate)-based colloidal nanomedicines. Wiley interdisciplinary reviews. Nanomed Nanobiotechnol 1:111–127
200. Bertholon I, Lesieur S, Labarre D, Besnard M, Vauthier C (2006) Characterization of dextran-poly(isobutylcyanoacrylate) copolymers obtained by redox radical and anionic emulsion polymerization. Macromolecules 39:3559–3567
201. Bertholon I, Ponchel G, Labarre D, Couvreur P, Vauthier C (2006) Bioadhesive properties of poly(alkylcyanoacrylate) nanoparticles coated with polysaccharide. J Nanosci Nanotechnol 6:3102–3109
202. Hearn J, Wilkinson MC, Goodall AR, Chainey M (1985) Kinetics of the surfactant-free emulsion polymerization of styrene: the post nucleation stage. J Polym Sci Part A Polym Chem 23:1869–1883
203. Song Z, Poehlein GW (1990) Kinetics of emulsifier-free emulsion polymerization of styrene. J Polym Sci Part A Polym Chem 28:2359–2392
204. Zou D, Ma S, Guan R, Park M, Sun L, Aklonis JJ, Salovey R (1992) Model filled polymers. V. Synthesis of crosslinked monodisperse polymethacrylate beads. J Polym Sci Part A Polym Chem 30:137–144
205. Shouldice GTD, Vandezande GA, Rudin A (1994) Practical aspects of the emulsifier-free emulsion polymerization of styrene. Eur Polym J 30:179–183
206. Pang S-W, Park H-Y, Jang Y-S, Kim W-S, Kim J-H (2002) Effects of charge density and particle size of poly(styrene/(dimethylamino)ethyl methacrylate) nanoparticle for gene delivery in 293 cells. Colloids Surf B 26:213–222
207. Akgöl S, Öztürk N, Denizli A (2010) New generation polymeric nanospheres for lysozyme adsorption. J Appl Polym Sci 115:1608–1615
208. Liu G, Liu P (2010) Synthesis of monodispersed crosslinked nanoparticles decorated with surface carboxyl groups via soapless emulsion polymerization. Colloids Surf A 354:377–381
209. Goodall AR, Wilkinson MC, Hearn J (1977) Mechanism of emulsion polymerization of styrene in soap-free systems. J Polym Sci Polym Chem Ed 15:2193–2218
210. Landfester K (2001) Polyreactions in miniemulsions. Macromol Rapid Commun 22:896–936
211. Fitch R M, Tsai C H (1971) Particle formation in polymer colloids. III. Prediction of the number of particles by a homogeneous nucleation theory. Polymer Colloids. 73–102

212. Hansen FK, Ugelstad J (1979) Particle nucleation in emulsion polymerization—3 nucleation in systems with anionic emulsifier investigated by seeded and unseeded polymerization. J Polym Sci Part A Polym Chem 17:3047–3067
213. Hansen FK, Ugelstad J (1982) Particle formation mechanisms, In: Piirma I (ed) Emulsion polymerization. Academic press, New York, pp 51–92
214. Choi YT, El-Aasser MS, Sudol ED, Vanderhoff JW (1985) Polymerization of styrene miniemulsion. J Polym Sci Part A Polym Chem 23:2973–2987
215. Chern CS, Liou YC, Chen TJ (1998) Particle nucleation loci in styrene miniemulsion polymerization using alkyl methacrylates as the reactive cosurfactant. Macromol Chem Phys 199:1315–1322
216. Chern CS, Chen TJ, Liou YC (1998) Miniemulsion polymerization of styrene in the presence of a water-insoluble blue dye. Polymer 39:3767–3777
217. Bao J, Zhang A (2004) Poly(methyl methacrylate) nanoparticles prepared through microwave emulsion polymerization. J Appl Polym Sci 93:2815–2820
218. An Z, Tang W, Hawker CJ, Stucky GD (2006) One-step microwave preparation of well-defined and functionalized polymeric nanoparticles. J Am Chem Soc 128:15054–15055
219. Chiu T-P, Don T-M (2008) Synthesis and characterization of poly(methyl methacrylate) nanoparticles by emulsifier-free emulsion polymerization with a redox-initiated system. J Appl Polym Sci 109:3622–3630
220. Fang FF, Kim JH, Choi HJ, Kim CA (2009) Synthesis and electrorheological response of nano-sized laponite stabilized poly(methyl methacrylate) spheres. Colloid Polym Sci 287:745–749
221. Cui X, Zhong S, Wang H (2007) Emulsifier-free core-shell polyacrylate latex nanoparticles containing fluorine and silicon in shell. Polymer 48:7241–7248
222. Lee JM, Lee SJ, Jung YJ, Kim JH (2008) Fabrication of nano-structured polythiophene nanoparticles in aqueous dispersion. Curr Appl Phys 8:659–663
223. Wang S, Wang X, Zhang Z (2007) Preparation of polystyrene particles with narrow particle size distribution by gamma-ray initiated miniemulsion polymerization stabilized by polymeric surfactant. Eur Polym J 43:178–184
224. Bardajee GR, Vancaeyzeele C, Haley JC, Li AY, Winnik MA (2007) Synthesis, characterization, and energy transfer studies of dye-labeled poly(butyl methacrylate) latex particles prepared by miniemulsion polymerization. Polymer 48:5839–5849
225. Rotureau E, Raynaud J, Choquenet B, Marie E, Nouvel C, Six J-L, Dellacherie E, Durand A (2008) Application of amphiphilic polysaccharides as stabilizers in direct and inverse free-radical miniemulsion polymerization. Colloids Surf A 331:84–90
226. Yildiz U, Landfester K (2008) Miniemulsion polymerization of styrene in the presence of macromonomeric initiators. Polymer 49:4930–4934
227. Ethirajan A, Ziener U, Landfester K (2009) Surface-functionalized polymeric nanoparticles as templates for biomimetic mineralization of hydroxyapatite. Chem Mater 21:2218–2225
228. Crespy D, Landfester K (2009) Synthesis of polyvinylpyrrolidone/silver nanoparticles hybrid latex in non-aqueous miniemulsion at high temperature. Polymer 50:1616–1620
229. Wu M, Dellacherie E, Durand A, Marie E (2009) Poly(n-butyl cyanoacrylate) nanoparticles via miniemulsion polymerization (1): Dextran-based surfactants. Colloids Surf B 69:141–146
230. Baruch-Sharon S, Margel S (2010) Synthesis and characterization of polychloromethylstyrene nanoparticles of narrow size distribution by emulsion and miniemulsion polymerization processes. Colloid Polym Sci 288:869–877
231. Jiang X, Dausend J, Hafner M, Musyanovych A, Röcker C, Landfester K, Mailänder V, Ulrich Nienhaus G (2010) Specific effects of surface amines on polystyrene nanoparticles in their interactions with mesenchymal stem cells. Biomacromolecules 11:748–753
232. Antonietti M, Landfester K (2002) Polyreactions in miniemulsions. Prog Polym Sci 27: 689–757
233. Landfester K, Willert M, Antonietti M (2000) Preparation of polymer particles in nonaqueous direct and inverse miniemulsions. Macromolecules 33:2370–2376

234. Wormuth K (2001) Superparamagnetic latex via inverse emulsion polymerization. J Colloid Interface Sci 241:366–377
235. Oh JK, Tang C, Gao H, Tsarevsky NV, Matyjaszewski K (2006) Inverse miniemulsion ATRP: a new method for synthesis and functionalization of well-defined water-soluble/cross-linked polymeric particles. J Am Chem Soc 128:5578–5584
236. Landfester K, Tiarks F, Hentze H-P, Antonietti M (2000) Polyaddition in miniemulsions: a new route to polymer dispersions. Macromol Chem Phys 201:1–5
237. Li C-YU, Chiu W-Y, Don T-M (2005) Preparation of polyurethane dispersions by miniemulsion polymerization. J Polym Sci Part A Polym Chem 43:4870–4881
238. Maitre C, Ganachaud F, Ferreira O, Lutz JF, Paintoux Y, Hémery P (2000) Anionic polymerization of phenyl glycidyl ether in miniemulsion. Macromolecules 33:7730–7736
239. Tomov A, Broyer J-P, Spitz R (2000) Emulsion polymerization of ethylene in water medium catalysed by organotransition metal complexes. Macromol Symp 150:53–58
240. Puig JE (1996) Microemulsion polymerization, In: Salamone JC (ed) Polymeric materials encyclopedia, vol 6. CRC Press, New York, pp 4333–4341
241. Sosa N, Peralta RD, López RG, Ramos LF, Katime I, Cesteros C, Mendizábal E, Puig JE (2001) A comparison of the characteristics of poly(vinyl acetate) latex with high solid content made by emulsion and semi-continuous microemulsion polymerization. Polymer 42:6923–6928
242. Sosa N, Zaragoza EA, López RG, Peralta RD, Katime I, Becerra F, Mendizábal E, Puig JE (2000) Unusual free radical polymerization of vinyl acetate in anionic microemulsion media. Langmuir 16:3612–3619
243. Kim B-J, Oh S-G, Han M-G, Im S–S (2000) Preparation of polyaniline nanoparticles in micellar solutions as polymerization medium. Langmuir 16:5841–5845
244. Choi JW, Han MG, Kim SY, Oh SG, Im SS (2004) Poly(3,4-ethylenedioxythiophene) nanoparticles prepared in aqueous DBSA solutions. Synth Met 141:293–299
245. Han MG, Cho SK, Oh SG, Im SS (2002) Preparation and characterization of polyaniline nanoparticles synthesized from DBSA micellar solution. Synth Met 126:53–60
246. Li X-G, Huang M-R, Zeng J-F, Zhu M-F (2004) The preparation of polyaniline waterborne latex nanoparticles and their films with anti-corrosivity and semi-conductivity. Colloids Surf A 248:111–120
247. Jang J, Oh JH, Stucky GD (2002) Fabrication of ultrafine conducting polymer and graphite nanoparticles. Angew Chem Int Ed 41:4016–4019
248. Lambert G, Bertrand JR, Fattal E, Subra F, Pinto-Alphandary H, Malvy C, Auclair C, Couvreur P (2000) EWS Fli-1 antisense nanocapsules inhibits Ewing sarcoma-related tumor in mice. Biochem Biophys Res Commun 279:401–406
249. Lambert G, Fattal E, Pinto-Alphandary H, Gulik A, Couvreur P (2000) Polyisobutylcyanoacrylate nanocapsules containing an aqueous core as a novel colloidal carrier for the delivery of oligonucleotides. Pharm Res 17:707–714
250. Lambert G, Fattal E, Pinto-Alphandary H, Gulik A, Couvreur P (2001) Polyisobutylcyanoacrylate nanocapsules containing an aqueous core for the delivery of oligonucleotides. Int J Pharm 214:13–16
251. Lambert G, Fattal E, Couvreur P (2001) Nanoparticulate systems for the delivery of antisense oligonucleotides. Adv Drug Delivery Rev 47:99–112
252. Toub N, Angiari C, Eboué D, Fattal E, Tenu J-P, Le Doan T, Couvreur P (2005) Cellular fate of oligonucleotides when delivered by nanocapsules of poly(isobutylcyanoacrylate). J Controlled Release 106:209–213
253. Toub N, Bertrand J-R, Tamaddon A, Elhamess H, Hillaireau H, Maksimenko A, Maccario J, Malvy C, Fattal E, Couvreur P (2006) Efficacy of siRNA nanocapsules targeted against the EWS-Fli1 oncogene in Ewing sarcoma. Pharm Res 23:892–900
254. Hillaireau H, Le Doan T, Besnard M, Chacun H, Janin J, Couvreur P (2006) Encapsulation of antiviral nucleotide analogues azidothymidine-triphosphate and cidofovir in poly(isobutylcyanoacrylate) nanocapsules. Int J Pharm 324:37–42

255. Hillaireau H, Le Doan T, Chacun H, Janin J, Couvreur P (2007) Encapsulation of mono- and oligo-nucleotides into aqueous-core nanocapsules in presence of various water-soluble polymers. Int J Pharm 331:148–152
256. Anton N, Saulnier P, Gaillard C, Porcher E, Vrignaud S, Benoit J-P (2009) Aqueous-core lipid nanocapsules for encapsulating fragile hydrophilic and/or lipophilic molecules. Langmuir 25:11413–11419
257. Jang J, Bae J, Park E (2006) Selective fabrication of poly(3,4-ethylenedioxythiophene) nanocapsules and mesocellular foams using surfactant-mediated interfacial polymerization. Adv Mater 18:354–358
258. Landfester K (2001) The generation of nanoparticles in miniemulsions. Adv Mater 13: 765–768
259. Tiarks F, Landfester K, Antonietti M (2001) Preparation of polymeric nanocapsules by miniemulsion polymerization. Langmuir 17:908–918
260. Al Khouri Fallouh N, Roblot-Treupel L, Fessi H (1986) Development of a new process for the manufacture of polyisobutylcyanoacrylate nanocapsules. Int J Pharm 28:125–132
261. Al Khouri N, Fessi H, Roblot-Treupel L (1986) Original procedure for preparation of nanocapsules of polyalkyl cyanoacrylates by interfacial polymerization. Pharm Acta Helv 61:274–281
262. Rollot JM, Couvreur P, Roblo-Treupel L, Puisieux F (1986) Physicochemical and morphological characterization of polyisobutyl cyanoacrylate nanocapsules. J Pharm Sci 75:361–364
263. Chouinard F, Kan FWK, Leroux J-C, Foucher C, Lenaerts V (1991) Preparation and purification of polyisohexylcyanoacrylate nanocapsules. Int J Pharm 72:211–217
264. Bouchemal K, Couenne F, Briançon S, Fessi H, Tayakout M (2006) Polyamides nanocapsules: modelling and wall thickness estimation. AiChe 52:1–10
265. Takasu M, Kawaguchi H (2005) Preparation of colored latex with polyurea shell by miniemulsion polymerization. Colloid Polym Sci 283:805–811
266. Sirkar KK, Shanbhag PV, Kovvali AS (1999) Membrane in a reactor: a functional perspective. Ind Eng Chem Res 38:3715–3737
267. Drioli E, Criscuoli A, Curcio E (2003) Membrane contactors and catalytic membrane reactors in process intensification. Chem Eng Technol 26:975–981
268. Charcosset C, Fessi H (2006) A membrane contactor for the preparation of nanoparticles. Desalination 200:568–569
269. Yanagishita T, Fujimura R, Nishio K, Masuda H (2010) Fabrication of monodisperse polymer nanoparticles by membrane emulsification using ordered anodic porous alumina. Langmuir 26:1516–1519
270. Matyjaszewski K, Xia J (2001) Atom transfer radical polymerization. Chem Rev 101: 2921–2990
271. Zetterlund PB, Kagawa Y, Okubo M (2008) Controlled/living radical polymerization in dispersed systems. Chem Rev 108:3747–3794
272. Zetterlund PB, Nakamura T, Okubo M (2007) Mechanistic investigation of particle size effects in TEMPO-mediated radical polymerization of styrene in aqueous miniemulsion. Macromolecules 40:8663–8672
273. Nicolas J, Ruzette A-V, Farcet C, Gérard P, Magnet S, Charleux B (2007) Nanostructured latex particles synthesized by nitroxide-mediated controlled/living free-radical polymerization in emulsion. Polymer 48:7029–7040
274. Dire C, Magnet S, Couvreur L, Charleux B (2009) Nitroxide-mediated controlled/living free-radical surfactant-free emulsion polymerization of methyl methacrylate using a poly(methacrylie acid)-based macroalkoxyamine initiator. Macromolecules 42:95–103
275. Farcet C, Nicolas J, Charleux B (2002) Kinetic study of the nitroxide-mediated controlled free-radical polymerization of n-butyl acrylate in aqueous miniemulsions. J Polym Sci Part A Polym Chem 40:4410–4420
276. Farcet C, Charleux B, Pirri R (2001) Poly(n-butyl acrylate) homopolymer and poly[n-butyl acrylate-b-(n-butyl acrylate-co-styrene)] block copolymer prepared via nitroxide-mediated

living/controlled radical polymerization in miniemulsion [6]. Macromolecules 34: 3823–3826
277. Farcet C, Lansalot M, Charleux B, Pirri R, Vairon JP (2000) Mechanistic aspects of nitroxide-mediated controlled radical polymerization of styrene in miniemulsion, using a water-soluble radical initiator. Macromolecules 33:8559–8570
278. Li W, Matyjaszewski K, Albrecht K, Möller M (2009) Reactive surfactants for polymeric nanocapsules via interfacially confined miniemulsion ATRP. Macromolecules 42: 8228–8233
279. Siegwart DJ, Srinivasan A, Bencherif SA, Karunanidhi A, Jung KO, Vaidya S, Jin R, Hollinger JO, Matyjaszewski K (2009) Cellular uptake of functional nanogels prepared by inverse miniemulsion ATRP with encapsulated proteins, carbohydrates, and gold nanoparticles. Biomacromolecules 10:2300–2309
280. Oh JK, Perineau F, Charleux B, Matyjaszewski K (2009) AGET ATRP in water and inverse minlemulsion: A facile route for preparation of high-molecular-weight biocompatible brush-like polymers. J Polym Sci Part A Polym Chem 47:1771–1781
281. Li W, Min K, Matyjaszewski K, Stoffelbach F, Charleux B (2008) PEO-based block copolymers and homopolymers as reactive surfactants for AGET ATRP of butyl acrylate in miniemulsion. Macromolecules 41:6387–6392
282. Min K, Gao H, Yoon JA, Wu W, Kowalewski T, Matyjaszewski K (2009) One-pot synthesis of hairy nanoparticles by emulsion ATRP. Macromolecules 42:1597–1603
283. Min K, Gao H, Matyjaszewski K (2006) Development of an ab initio emulsion atom transfer radical polymerization: from microemulsion to emulsion. J Am Chem Soc 128:10521–10526
284. Min K, Matyjaszewski K (2005) Atom transfer radical polymerization in microemulsion. Macromolecules 38:8131–8134
285. Rieger J, Zhang W, Stoffelbach F, Charleux B (2010) Surfactant-free RAFT emulsion polymerization using poly(N,N -dimethylacrylamide) trithiocarbonate macromolecular chain transfer agents. Macromolecules 43:6302–6310
286. Manguian M, Save M, Charleux B (2006) Batch emulsion polymerization of styrene stabilized by a hydrophilic macro-RAFT agenta. Macromol Rapid Commun 27:399–404
287. Zhou X, Ni P, Yu Z (2007) Comparison of RAFT polymerization of methyl methacrylate in conventional emulsion and miniemulsion systems. Polymer 48:6262–6271
288. Nicolas J, Charleux B, Guerret O, Magnet S (2005) Nitroxide-mediated controlled free-radical emulsion polymerization using a difunctional water-soluble alkoxyamine initiator. Toward the control of particle size, particle size distribution, and the synthesis of triblock copolymers. Macromolecules 38:9963–9973
289. Dalpiaz A, Vighi E, Pavan B, Leo E (2009) Fabrication via a nonaqueous nanoprecipitation method, characterization and in vitro biological behavior of N6-cyclopentyladenosine-loaded nanoparticles. J Pharm Sci 98:4272–4284
290. Cheng F-Y, Wang SP-H, Su C-H, Tsai T-L, Wu P-C, Shieh D-B, Chen J-H, Hsieh PC-H, Yeh C-S (2008) Stabilizer-free poly(lactide-co-glycolide) nanoparticles for multimodal biomedical probes. Biomaterials 29:2104–2112
291. Murakami H, Kobayashi M, Takeuchi H, Kawashima Y (1999) Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. Int J Pharm 187:143–152
292. Chang J, Jallouli Y, Kroubi M, Yuan X-b, Feng W, Kang C-s, Pu P-y, Betbeder D (2009) Characterization of endocytosis of transferrin-coated PLGA nanoparticles by the blood-brain barrier. Int J Pharm 379: 285–292
293. Nassar T, Rom A, Nyska A, Benita S (2009) Novel double coated nanocapsules for intestinal delivery and enhanced oral bioavailability of tacrolimus, a P-gp substrate drug. J Controlled Release 133:77–84
294. de Assis DN, Mosqueira VCF, Vilela JMC, Andrade MS, Cardoso VN (2008) Release profiles and morphological characterization by atomic force microscopy and photon correlation spectroscopy of 99mTechnetium-fluconazole nanocapsules. Int J Pharm 349:152–160

295. Limayem Blouza I, Charcosset C, Sfar S, Fessi H (2006) Preparation and characterization of spironolactone-loaded nanocapsules for paediatric use. Int J Pharm 325:124–131
296. Moinard-Chécot D, Chevalier Y, Briançon S, Beney L, Fessi H (2008) Mechanism of nanocapsules formation by the emulsion-diffusion process. J Colloid Interface Sci 317: 458–468
297. Zili Z, Sfar S, Fessi H (2005) Preparation and characterization of poly-epsilon-caprolactone nanoparticles containing griseofulvin. Int J Pharm 294:261–267
298. Kim E, Yang J, Choi J, Suh J-S, Huh Y-M, Haam S (2009) Synthesis of gold nanorod-embedded polymeric nanoparticles by a nanoprecipitation method for use as photothermal agents. Nanotechnology. 20: 365602 (p 7)
299. Ferranti V, Marchais H, Chabenat C, Orecchioni AM, Lafont O (1999) Primidone-loaded poly-epsilon-caprolactone nanocapsules: incorporation efficiency and in vitro release profiles. Int J Pharm 193:107–111
300. Seyler I, Appel M, Devissaguet J-P, Legrand P, Barratt G (1999) Macrophage activation by a lipophilic derivative of muramyldipeptide within nanocapsules: Investigation of the mechanism of drug delivery. J Nanopart Res 1:91–97
301. Legrand P, Lesieur S, Bochot A, Gref R, Raatjes W, Barratt G, Vauthier C (2007) Influence of polymer behaviour in organic solution on the production of polylactide nanoparticles by nanoprecipitation. Int J Pharm 344:33–43
302. Nehilla BJ, Bergkvist M, Popat KC, Desai TA (2008) Purified and surfactant-free coenzyme Q10-loaded biodegradable nanoparticles. Int J Pharm 348:107–114
303. Yallapu MM, Gupta BK, Jaggi M, Chauhan SC (2010) Fabrication of curcumin encapsulated PLGA nanoparticles for improved therapeutic effects in metastatic cancer cells. J Colloid Interface Sci 351:19–29
304. Deepak V, Ram Kumar Pandian SB, Kalishwaralal K, Gurunathan S (2009) Purification, immobilization, and characterization of nattokinase on PHB nanoparticles. Bioresour Technol 100: 6644–6646
305. Duclairoir C, Nakache E, Marchais H, Orecchioni A-M (1998) Formation of gliadin nanoparticles: Influence of the solubility parameter of the protein solvent. Colloid Polym Sci 276:321–327
306. Skiba M, Wouessidjewe D, Puisieux F, Duchêne D, Gulik A (1996) Characterization of amphiphilic beta-cyclodextrin nanospheres. Int J Pharm 142:121–124
307. Lannibois H, Hasmy A, Botet R, Chariol OA, Cabane B (1997) Surfactant limited aggregation of hydrophobic molecules in water. J Phys I 7:319–342
308. Jeong Y-I, Cho C-S, Kim S-H, Ko K-S, Kim S-I, Shim Y-H, Nah J-W (2001) Preparation of poly(DL-lactide-co-glycolide) nanoparticles without surfactant. J Appl Polym Sci 80: 2228–2236
309. Kostag M, Köhler S, Liebert T, Heinze T (2010) Pure cellulose nanoparticles from trimethylsilyl cellulose. Macromol Symp 294:96–106
310. Jeon H-J, Jeong Y-I, Jang M-K, Park Y-H, Nah J-W (2000) Effect of solvent on the preparation of surfactant-free poly(DL-lactide-co-glycolide) nanoparticles and norfloxacin release characteristics. Int J Pharm 207:99–108
311. Akagi T, Kaneko T, Kida T, Akashi M (2005) Preparation and characterization of biodegradable nanoparticles based on poly(gamma-glutamic acid) with L-phenylalanine as a protein carrier. J Controlled Release 108:226–236
312. Lee J, Cho EC, Cho K (2004) Incorporation and release behavior of hydrophobic drug in functionalized poly(D,L-lactide)-block-poly(ethylene oxide) micelles. J Controlled Release 94:323–335
313. Chronopoulou L, Fratoddi I, Palocci C, Venditti I, Russo MV (2009) Osmosis based method drives the self-assembly of polymeric chains into micro-and nanostructures. Langmuir 25:11940–11946
314. Na K, Lee KH, Lee DH, Bae YH (2006) Biodegradable thermo-sensitive nanoparticles from poly(L-lactic acid)/poly(ethylene glycol) alternating multi-block copolymer for potential anti-cancer drug carrier. Eur J Pham Sci 27:115–122

315. Hornig S, Heinze T (2008) Efficient approach to design stable water-dispersible nanoparticles of hydrophobic cellulose esters. Biomacromolecules 9:1487–1492
316. Marty JJ, Oppenheim RC, Speiser P (1978) Nanoparticles–a new colloidal drug delivery system. Pharm Acta Helv 53:17–23
317. Kommareddy S, Amiji MM (2007) Protein nanospheres for gene delivery: preparation and in vitro transfection studies with gelatin nanoparticles. Gene transfer. Cold Spring Harbor Laboratory Press, New York, pp 527–540
318. Weber C, Coester C, Kreuter J, Langer K (2000) Desolvation process and surface characterisation of protein nanoparticles. Int J Pharm 194:91–102
319. Weber C, Kreuter J, Langer K (2000) Desolvation process and surface characteristics of HSA-nanoparticles. Int J Pharm 196:197–200
320. Langer K, Anhorn MG, Steinhauser I, Dreis S, Celebi D, Schrickel N, Faust S, Vogel V (2008) Human serum albumin (HSA) nanoparticles: reproducibility of preparation process and kinetics of enzymatic degradation. Int J Pharm 347:109–117
321. Langer K, Balthasar S, Vogel V, Dinauer N, von B, Schubert D (2003) Optimization of the preparation process for human serum albumin (HSA) nanoparticles. Int J Pharm 257: 169–180
322. Wartlick H, Spankuch-Schmitt B, Strebhardt K, Kreuter J, Langer K (2004) Tumour cell delivery of antisense oligonucleotides by human serum albumin nanoparticles. J Controlled Release 96:483–495
323. Mo Y, Barnett ME, Takemoto D, Davidson H, Kompella UB (2007) Human serum albumin nanoparticles for efficient delivery of Cu, Zn superoxide dismutase gene. Mol Vis 13: 746–757
324. Li F-Q, Su H, Wang J, Liu J-Y, Zhu Q-G, Fei Y-B, Pan Y-H, Hu J-H (2008) Preparation and characterization of sodium ferulate entrapped bovine serum albumin nanoparticles for liver targeting. Int J Pharm 349:274–282
325. Zhao D, Zhao X, Zu Y, Li J, Zhang Y, Jiang R, Zhang Z (2010) Preparation, characterization, and in vitro targeted delivery of folate-decorated paclitaxel-loaded bovine serum albumin nanoparticles. Int J Nanomed 5:669–677
326. Duclairoir C, Irache JM, Nakache E, Orecchioni A-M, Chabenat C, Popineau Y (1999) Gliadin nanoparticles: formation, all-trans-retinoic acid entrapment and release, size optimization. Polym Int 48:327–333
327. Umamaheshwari RB, Jain NK (2003) Receptor mediated targeting of lectin conjugated gliadin nanoparticles in the treatment of *Helicobacter pylori*. J Drug Target 11:415–424
328. Ramteke S, Jain NK (2008) Clarithromycin- and omeprazole-containing gliadin nanoparticles for the treatment of Helicobacter pylori. J Drug Target 16:65–72
329. Coester CJ, Langer K, van Briesen H, Kreuter J (2000) Gelatin nanoparticles by two step desolvation a new preparation method, surface modifications and cell uptake. J Microencapsul 17:187–193
330. Coester C, Kreuter J, von B, Langer K (2000) Preparation of avidin-labeled gelatin nanoparticles as carriers for biotinylated peptide nucleic acid (PNA). Int J Pharm 196:147–149
331. Vandervoort J, Ludwig A (2004) Preparation and evaluation of drug-loaded gelatin nanoparticles for topical ophthalmic use. Eur J Pharm Biopharm 57:251–261
332. Balthasar S, Michaelis K, Dinauer N, von B, Kreuter J, Langer K (2005) Preparation and characterization of antibody modified gelatin nanoparticles as drug carrier system for uptake in lymphocytes. Biomaterials 26:2723–2732
333. Azarmi S, Huang Y, Chen H, McQuarrie S, Abrams D, Roa W, Finlay WH, Miller GG, Lobenberg R (2006) Optimization of a two-step desolvation method for preparing gelatin nanoparticles and cell uptake studies in 143B osteosarcoma cancer cells. J Pharm Pharm Sci 9:124–132
334. Saraogi GK, Gupta P, Gupta UD, Jain NK, Agrawal GP (2010) Gelatin nanocarriers as potential vectors for effective management of tuberculosis. Int J Pharm 385:143–149
335. Taheri Qazvini N, Zinatloo S (2011) Synthesis and characterization of gelatin nanoparticles using CDI/NHS as a non-toxic cross-linking system. J Mater Sci Mater Med 22:63–69

336. Zwiorek K, Kloeckner J, Wagner E, Coester C (2004) Gelatin nanoparticles as a new and simple gene delivery system. J Pharm Pharm Sci 7:22–28
337. Agnihotri SA, Aminabhavi TM (2007) Chitosan nanoparticles for prolonged delivery of timolol maleate. Drug Dev Ind Pharm 33:1254–1262
338. Al-Ghananeem AM, Malkawi AH, Muammer YM, Balko JM, Black EP, Mourad W, Romond E (2009) Intratumoral delivery of paclitaxel in solid tumor from biodegradable hyaluronan nanoparticle formulations. AAPS PharmSciTech 10:410–417
339. De Martimprey H, Vauthier C, Malvy C, Couvreur P (2009) Polymer nanocarriers for the delivery of small fragments of nucleic acids: oligonucleotides and siRNA. Eur J Pharm Biopharm 71:490–504
340. Howard KA, Kjems J (2007) Polycation-based nanoparticle delivery for improved RNA interference therapeutics. Expert Opin Biol Ther 7:1811–1822
341. Rajaonarivony M, Vauthier C, Couarraze G, Puisieux F, Couvreur P (1993) Development of a new drug carrier made from alginate. J Pharm Sci 82:912–917
342. Schatz C, Domard A, Viton C, Pichot C, Delair T (2004) Versatile and efficient formation of colloids of biopolymer-based polyelectrolyte complexes. Biomacromolecules 5:1882–1892
343. Drogoz A, David L, Rochas C, Domard A, Delair T (2007) Polyelectrolyte complexes from polysaccharides: formation and stoichiometry monitoring. Langmuir 23:10950–10958
344. Drogoz A, Munier S, Verrier B, David L, Domard A, Delair T (2008) Towards biocompatible vaccine delivery systems: Interactions of colloidal PECs based on polysaccharides with HIV-1 p24 antigen. Biomacromolecules 9:583–591
345. Daoud-Mahammed S, Ringard-Lefebvre C, Razzouq N, Rosilio V, Gillet B, Couvreur P, Amiel C, Gref R (2007) Spontaneous association of hydrophobized dextran and poly-beta-cyclodextrin into nanoassemblies. Formation and interaction with a hydrophobic drug. J Colloid Interface Sci 307:83–93
346. Gref R, Amiel C, Molinard K, Daoud-Mahammed S, Sébille B, Gillet B, Beloeil J-C, Ringard C, Rosilio V, Poupaert J, Couvreur P (2006) New self-assembled nanogels based on host-guest interactions: characterization and drug loading. J Controlled Release 111:316–324
347. Boissière M, Meadows PJ, Brayner R, Hélary C, Livage J, Coradin T (2006) Turning biopolymer particles into hybrid capsules: the example of silica/alginate nanocomposites. J Mater Chem 16:1178–1182
348. De S, Robinson D (2003) Polymer relationships during preparation of chitosan-alginate and poly-L-lysine-alginate nanospheres. J Controlled Release 89:101–112
349. Aynie I, Vauthier C, Chacun H, Fattal E, Couvreur P (1999) Spongelike alginate nanoparticles as a new potential system for the delivery of antisense oligonucleotides. Antisense Nucleic Acid Drug Dev 9:301–312
350. González Ferreiro M, Tillman L, Hardee G, Bodmeier R (2002) Characterization of alginate/poly-L-lysine particles as antisense oligonucleotide carriers. Int J Pharm 239:47–59
351. Douglas KL, Tabrizian M (2005) Effect of experimental parameters on the formation of alginate-chitosan nanoparticles and evaluation of their potential application as DNA carrier. J Biomater Sci Polym Ed 16:43–56
352. Sarmento B, Ribeiro AJ, Veiga F, Ferreira DC, Neufeld RJ (2007) Insulin-loaded nanoparticles are prepared by alginate lonotropic pre-gelation followed by chitosan polyelectrolyte complexation. J Nanosci Nanotechnol 7:2833–2841
353. Das RK, Kasoju N, Bora U (2010) Encapsulation of curcumin in alginate-chitosan-pluronic composite nanoparticles for delivery to cancer cells. Nanomedicine 6:153–160
354. Li P, Dai Y-N, Zhang J-P, Wang A-Q, Wei Q (2008) Chitosan-alginate nanoparticles as a novel drug delivery system for nifedipine. Int J Biomed Sci 4:221–228
355. Sarmento B, Ferreira DC, Jorgensen L, van de Weert M (2007) Probing insulin's secondary structure after entrapment into alginate/chitosan nanoparticles. Eur J Pharm Biopharm 65:10–17
356. Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D (2007) Alginate/chitosan nanoparticles are effective for oral insulin delivery. Pharm Res 24:2198–2206

357. Sarmento B, Ferreira D, Veiga F, Ribeiro A (2006) Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. Carbohydr Polym 66:1–7
358. Calvo P, Remuñan-López C, Vila-Jato JL, Alonso MJ (1997) Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res 14:1431–1436
359. Calvo P, Remuñán-López C, Vila-Jato JL, Alonso MJ (1997) Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. J Appl Polym Sci 63:125–132
360. López-León T, Carvalho ELS, Seijo B, Ortega-Vinuesa JL, Bastos-González D (2005) Physicochemical characterization of chitosan nanoparticles: electrokinetic and stability behavior. J Colloid Interface Sci 283:344–351
361. Fernández-Urrusuno R, Calvo P, Remuñán-López C, Vila-Jato JL, Alonso MJ (1999) Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharm Res 16:1576–1581
362. Cetin M, Aktas Y, Vural I, Capan Y, Dogan LA, Duman M, Dalkara T (2007) Preparation and in vitro evaluation of bFGF-loaded chitosan nanoparticles. Drug Deliv 14:525–529
363. Janes KA, Calvo P, Alonso MJ (2001) Polysaccharide colloidal particles as delivery systems for macromolecules. Adv Drug Delivery Rev 47:83–97
364. Katas H, Alpar HO (2006) Development and characterisation of chitosan nanoparticles for siRNA delivery. J Controlled Release 115:216–225
365. Dung TH, Lee S-R, Han S-D, Kim S-J, Ju Y-M, Kim M-S, Yoo H (2007) Chitosan-TPP nanoparticle as a release system of antisense oligonucleotide in the oral environment. J Nanosci Nanotechnol 7:3695–3699
366. Reverchon E (1999) Supercritical antisolvent precipitation of micro- and nano-particles. J Supercrit Fluids 15:1–21
367. Jung J, Perrut M (2001) Particle design using supercritical fluids: literature and patent survey. J Supercrit Fluids 20:179–219
368. Vemavarapu C, Mollan MJ, Lodaya M, Needham TE (2005) Design and process aspects of laboratory scale SCF particle formation systems. Int J Pharm 292:1–16
369. Mishima K (2008) Biodegradable particle formation for drug and gene delivery using supercritical fluid and dense gas. Adv Drug Deliv Rev 60:411–432
370. Kawashima Y (2001) Panoparticulate systems for improved drug delivery. Adv Drug Deliv Rev 47:1–2
371. Weber M, Thies MC (2002) Understanding the RESS process. In: Sun Y-P (ed) Supercritical fluid technology in materials science and engineering: synthesis, properties, and applications. CRC press, New York, pp 387–437
372. Blasig A, Shi C, Enick RM, Thies MC (2002) Effect of concentration and degree of saturation on RESS of a CO_2-soluble fluoropolymer. Ind Eng Chem Res 41:4976–4983
373. Sane A, Thies MC (2007) Effect of material properties and processing conditions on RESS of poly(l-lactide). J Supercrit Fluids 40:134–143
374. Sun Y-P, Rollins HW, Jayasundera B, Meziani MJ, Bunker CE (2002) Preparation and processing of nanoscale materials by supercritical fluid technology. In: Sun Y-P (ed) Supercritical fluid technology in materials science and engineering: synthesis, properties, and applications. CRC Press, New York, pp 491–576
375. Meziani MJ, Pathak P, Hurezeanu R, Thies MC, Enick RM, Sun Y-P (2004) Supercritical-fluid processing technique for nanoscale polymer particles. Angew Chem Int Ed 43:704–707
376. Meziani MJ, Pathak P, Wang W, Desai T, Patil A, Sun Y-P (2005) Polymeric nanofibers from rapid expansion of supercritical solution. Ind Eng Chem Res 44:4594–4598
377. Vehring R (2008) Pharmaceutical particle engineering via spray drying. Pharm Res 25: 999–1022
378. Schuck P, Dolivet A, Méjean S, Zhu P, Blanchard E, Jeantet R (2009) Drying by desorption: a tool to determine spray drying parameters. J Food Eng 94:199–204
379. Arpagaus C, Schafroth N (2009) Laboratory scale spray drying of biodegradable polymers. Respir Drug Deliv Eur 2:269–274

Chapter 3
Quantum Dots as Biomarker

Michel Boissiere

Abstract Quantum dots (QDs) are semiconductor nanocrystals with unique optical and electronic properties. They have distinct advantages over traditional fluorescent organic dyes in chemical and biological studies in terms of tunable emission spectra, signal brightness, photostability, and can be conjugated to a wide range of biological targets, including proteins, antibodies, and nucleic acid probes. Currently, the major type of QDs is the heavy metal containing II-VI, IV–IV, or III-V QDs. The new generations of QDs, have far-reaching potential for the study of intracellular processes at the single-molecule level, high resolution cellular imaging, long-term in vivo observation of cell trafficking, tumor targeting, and diagnostics. However, with respect to medical applications, caution must be exercised with QDs due to their toxic components. Development of suitable health and safety regulations is necessary for commercialization. Despite of these difficulties, QDs appear to be too valuable to nanomedecine to dismiss, and will eventually come essential into practical use.

3.1 Introduction

Among various nanomaterials, quantum dots (QDs) distinguish themselves in their far-reaching possibilities in many avenues of biomedicine. QDs are nanometer-sized semiconductor crystals with unique photochemical and photophysical properties that are not available from either isolated molecules or bulk solids.

M. Boissiere (✉)
ERRMECe EA1391, Institut des Matériaux,
Université de Cergy-Pontoise,
95302 Pontoise, France
e-mail: michel.boissiere@u-cergy.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_3, © Springer-Verlag London 2013

QD research started in 1982 with the realization that the optical and electric properties of small semiconductor particles were heavily dependent on particle size due to quantum confinement of the charge carriers in small spaces. During the next two decades, extensive research was carried out for potential applications in optoelectronic devices, QD lasers and high-density memory. In 1998, two seminal reports simultaneously demonstrated that QDs could be made water soluble and could be conjugated to biological molecules, providing the first glimpse of the vast potential of QDs as probes for studying biological systems [1, 2]. In comparison with organic dyes and fluorescent proteins, QDs have the advantages of improved brightness, resistance against photobleaching, and multicolor fluorescent emission. These properties could improve the sensitivity of biological detection and imaging by at least one to two orders of magnitude. Significant improvements have been made in the synthesis, surface modification, and biofunctionalization of QDs in the following years, and indeed the current literature is rife with examples of QDs used in various biomedical applications. It can, now, be said with confidence that QDs have completed the transition from a once curious demonstration of quantum confinement in semiconductors to ubiquitous fluorophores providing unique insights into biological investigations [3].

In this chapter, an attempt will be made to provide a comprehensive, state-of-the-art overview of QD applications in biology imaging and diagnostic. Following, a brief introduction describes the photophysics and chemistry properties of QDs and provides a clear understanding of the merits of using QDs in bio-imaging and diagnostic, as well as the requirements and challenges in the synthesis, surface modification, and bioconjugation of QDs in order to make them amenable to bioapplications. The following section describes QDs cytotoxicity because to assess their biomedical application promising, it is important to characterize their behavior in vivo. Next, some recent advances in the use of QDs in various biological applications for detection and diagnosis of different diseases are detailed, both in vivo and in vitro. The literature cited in this chapter is confined to reports, representative, and which are innovative studies.

3.2 Optical and Spectroscopic Properties of Quantum Dots

QDs are colloidal semiconductor nanocrystals that exhibit unique electro-chemiluminescent properties, strong light absorbance, bright fluorescence, size-tunable (2–10 nm) narrow emission spectra, and provide excellent fluorescence quantum yields.

QDs are composed of elements from groups II–VI, III–V, or IV–IV of the periodic table. In comparison with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties such as size and composition-tunable light emission, improved signal brightness, resistance to photobleaching which makes them useful for continuous monitoring of biological phenomena, and simultaneous excitation of multiple fluorescence colors (Fig. 3.1).

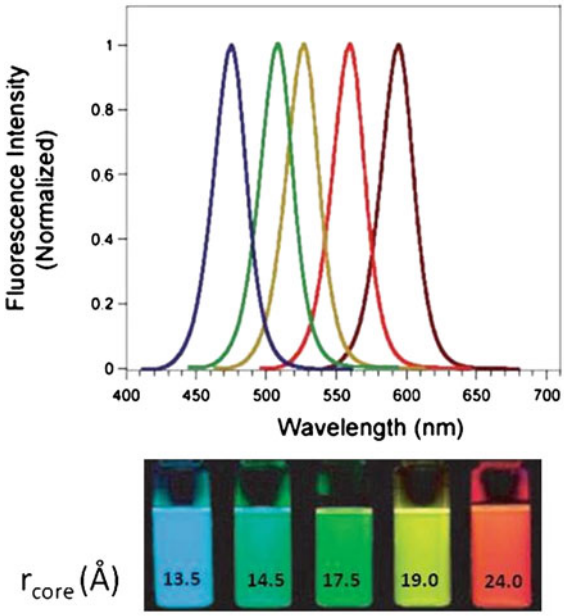


Fig. 3.1 Illustration of size-tunable (CdSe)ZnS QDs and their fluorescence spectra

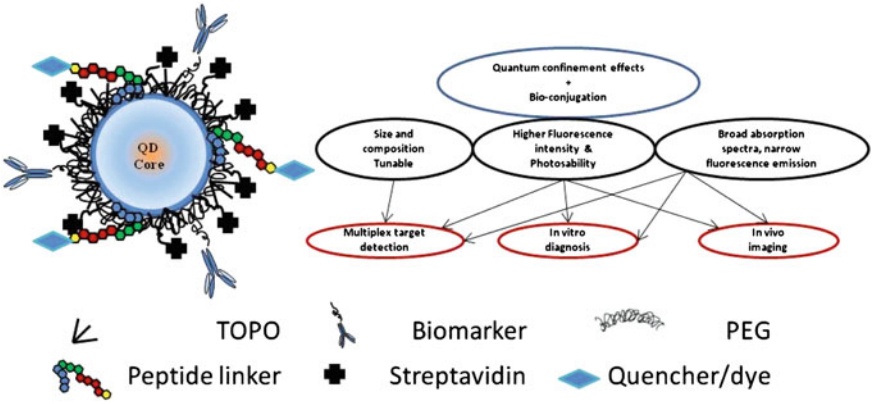


Fig. 3.2 Properties of decorated quantum dots

Also, the long luminescent lifetime (30–100 ns) of QDs diminishes interference, from background autofluorescence in live cell imaging. In addition, different colors of QDs can be simultaneously excited with a single light source, with minimal spectral overlapping, which provides significant advantages for multiplexed detection of target molecules [4–9] (Fig. 3.2). Usually, the core of QDs may be composed of cadmium selenide (CdSe), cadmium telluride (CdTe), or

Table 3.1 Application of different bioconjugated QDs for cancer diagnosis

Cancer type	Biomarker detected	Detection limit	Description
Proteolytic activity for some cancer types [72]	Proteolytic enzymes	200 nM	Nanoscale sensing assemblies (FRET) consisting of QD–peptide conjugates that are capable of specifically detecting the activity of proteolytic enzymes.
Epithelial cancer [13]	Mucin 1	250 nM	Aptamer-based Quantitative detection system with QD labeling
Breast carcinoma cells [73]	Cancer stem cell (CSC) markers CD44v6 + and CD24-	nc	QD conjugated antibodies
Human ovarian cancer [74]	HER2/neu oncomarker	nc	QD conjugated anti-HER2/neu4D5
Lung carcinoma cells (A549) [75]	Folate receptors	nc	Folate-conjugated QDs
J4656-FR mouse lymphoma cells [76]	Folate receptors	nc	Folate-conjugated QDs-8
Human nasopharyngeal epidermal carcinoma cell line (KB) and a human lung carcinoma cell line (A549) [42]	Folate receptors	nc	Folate-conjugated InP-ZnS QDs
Mouse myeloma cells and human cancer cell lines: breast MCF7, prostate LNCaP, lymphoma SKW 6 [77]	Small bivalent antibody fragments, cys-diabodies	nc	Bioconjugated CdSe/ZnS Qdots
Hepatoma detection in vivo [78]	AFP an important marker for hepatocellular carcinoma cell lines	nc	Bioconjugated quantum dots to AFP (alpha-fetoprotein)
In vitro dual color fluorescence imaging some cancer cell lines [79]	Aptamer (nucleolin), integrin $\alpha_v\beta_3$)	nc	QDs conjugated by the AS1411 and arg–gly–asp acid
Microfluidic protein chip for an ultrasensitive and multiplexed assay of cancer biomarkers [80]	Cancer biomarker CEA and AFP (serum)	250 fM	Multicolor imaging and multiplexed bioassay using bioconjugated secondary antibodies (goat anti-mouse IgG) QDs

(continued)

Table 3.1 (continued)

Cancer type	Biomarker detected	Detection limit	Description
Detection of protein lung cancer biomarker [81]	Nitrated ceruloplasmin	8 ng/mL	Using a portable fluorescence biosensor based on quantum dots antinitrotyrosine conjugate and a lateral flow test strip
Determinations of cancer biomarkers [69]	CEA, cancer antigen 125, and HER-2/Neu	0.02 ng/mL in serum and saliva	QD labeled antibodies
Prostate cancer biomarker [82]	PSA	nc	PSA antibodies conjugated QDs
Protein microarrays and quantum dot probes for early cancer detection [83]	Detect six differents cytokines TNF-α, IL-8, IL-6, MIP-1β, IL-13 and IL-1β	pM	Two different models of quantum dot probes: antibody specific to the selected marker—IL-10, and the second by use of streptavidin coated quantum dots and biotinylated detector antibody
Breast cancer [84]	Human epidermal growth factor receptor 2 (HER2)	nc	QDs linked to immunoglobulin G (IgG) and streptavidin
Breast cancer invasion [85]	Human epidermal growth factor receptor 2 (HER2)	nc	Conjugated QDs two primary antibodies from two species (e.g. mouse and rabbit)
Immunosensor for the detection of prostate cancer biomarker [86]	PSA	nc	QD–functionalized graphene sheets (GS) as labels for the secondary antibody (Ab2) GS as labels for signal amplification

indium phosphide (InP), among other combinations. The most common QDs used in biomedical applications are those with a CdSe core surrounded by a semiconductor shell, e.g., zinc sulfide (ZnS) epitaxial grown around the core [10–14]. The function of the ZnS shell is to reduce the oxidation of the core and leaching of metal ions from the core. By passivating the core, the shell also increases the photoluminescence quantum yield [15]. However, as QDs are hydrophobic by nature, it is necessary to solubilize QDs before application by surface modification with biofunctional molecules [16], because QDs have large surface areas for the attachment of such molecules. When conjugated with diagnostic (e.g., optical) and therapeutic (e.g., anticancer) agents, QDs can be used for cancer diagnosis and therapy with high specificity [17–19]. Significant research efforts have been

focused on early cancer diagnosis with QDs [3]. As early as 2002, after overcoming the limitation in obtaining biocompatible nanocrystals, Dubertret [20] showed the potential to revolutionize biological imaging. In case of imaging probes, active targeting of cancer antigens (molecular imaging) has become an area of tremendous interest because of the potential to detect early stage cancers and their metastases [21–23]. Major recent developments in this regard are summarized in Table 3.1.

3.3 Cytotoxicity

In spite of the attractive properties and successful applications, it should be realized that the emerging labeling approaches using QDs have limitations as well. Experimental barrier for the large-scale application of the QDs includes the lack of consensus methods for labeling biomolecules with QDs. To this end, organic dyes are advantageous due to the availability of well established labeling protocols, purification, and characterization techniques for dye bioconjugates [24]. Other technical limitations include the lack of reproducibility, limited knowledge on their clearance in living systems, reduced luminescence activity due to their relatively large surface areas and sensitivity to oxidation, and photolysis [25]. Moreover, the cytotoxicity of QDs has been observed in a large number of in vitro studies [26–31], affecting cell growth, and viability [32]. The extent of cytotoxicity has been found to be dependent upon a number of factors including size, capping materials, color, dose of QDs, surface chemistry, coating bioactivity, and processing parameters [31, 33, 34]. Even if not inducing significant alterations in cell physiology, QDs can produce subtle alterations of function which may affect the quality of data derived from their use [30, 35, 36]. The toxic nature of QDs due to the release of free cadmium ions during their degradation poses environmental concerns and generates serious hurdle for diagnostic applications [34, 37, 38]. Examination of QD toxicity in a hepatocyte culture model showed that exposure of core CdSe to an oxidative environment causes decomposition and desorption of Cd^{2+}ions. Such exposure during synthesis and processing played an important role in subsequent toxicity [39]. In addition to the effects of the QD core, ligands added to render the probe biologically active may have toxic effects on cells. Mercaptopropionic acid (MPA) and mercaptoacetic acid, which are commonly used for solubilization, have both been shown to be mildly cytotoxic [28]. 11-mercaptoundecanoic acid (MUA), cysteamine and TOPO have all been shown to have the ability to damage DNA in the absence of the QD core [40]. PEGylated QDs have been shown to have reduced cytotoxicity, but modification of these to produce PEG-amine for biological activity renders them cytotoxic once again [41].

In order to overcome the nanotoxicity and biocompatibility issues of QDs different innovative approaches have been explored. Introduction of functionalized layers, encapsulating shell and capping materials can reduce the sensitivity to oxidative, photolytic, and mechanical degradation that, in turn, abate the QD

toxicity [37]. Groups III–V QDs may provide a more stable alternative to groups II–VI QDs due to the presence of a covalent, rather than an ionic, bond, and have been reported to have lower cytotoxicity [42]. However, these QDs are difficult to prepare on a competitive time scale, and tend to have much lower quantum efficiencies, meaning uptake has been slow.

Combination of semiconductor QDs with plasmonic materials has been found to be effective for partially resolving the nanotoxicity and biocompatibility issues of QDs [43]. Concurrent with the exploration of new effects, further sophisticated applications of quantum particles in medical research are being explored and new avenues for early diagnosis and treatment might soon open up in the imminent future. The multiplexed detection capability and subpicomolar sensitivity can make QDs a good choice for the medical diagnosis once the technical limitations and issues associated with toxicity are surmounted successfully [21, 44, 45]. Recently, in the demand of using biocompatible and nontoxic QDs as nanoprobes, rare earth (RE) elements are used to fabricate a new type of QDs, such as Gd-doped, ZnO QDs. RE-doped QDs have distinct advantages over heavy metal-containing QDs, not only because of avoiding the increase of particle size by polymer or silica coating in synthesis procedure, but also providing a simple, green synthesis method. Liu et al. reported the development of Gd-doped ZnO QDs with enhanced yellow fluorescence, and these QDs can be used as nanoprobes for quick cell detection with very low toxicity [46].

3.4 Biological Applications of QDs

3.4.1 QDs in Molecular Imaging and Cancer Medicine

3.4.1.1 Bioconjugated QDs for In Vitro and In Vivo Imaging

One of the most advancing applications of QDs is in vitro imaging of cancer cells. Soon after the introduction of biocompatible QDs for cell imaging by Chan and Nie [2] and Bruchez et al. [1], many research groups applied QDs for imaging of cancer cells. QDs conjugated with cancer specific ligands/antibodies/peptides were found to be effective for detecting and imaging human cancer cells derived from prostate cancer [47], breast cancer, pancreatic cancer [44], metastatic tumor [48], glioblastoma [49] and cancers of the bone marrow [50], and tongue [51].

Compared to the study of living cells in culture, different challenges arise with the increase in complexity to a multicellular organism, and with the accompanying increase in size. Unlike monolayers of cultured cells and thin tissue sections, tissue thickness becomes a major concern because biological tissue attenuates most signals used for imaging. Optical imaging, especially fluorescence imaging, has been used in living animal models, but it is still limited by the poor transmission of visible light through biological tissue. It has been suggested that there is a near-infrared optical window in most biological tissue that is the key to deep tissue optical imaging [52].

In vivo application of QDs was first tested by Akerman et al. in 2002 [53]. They injected CdSe/ZnS QDs coated with peptides into the tail vein in mouse, and found that the injected QDs preferentially distribute in endothelial cells in the lung blood vessels. Also, based on ex vivo fluorescence microscopic imaging of tissue sections, they found that the QD-peptide conjugates were preferentially bound to tumors.

One of the most immediately successful applications of QDs in vivo has been their use as contrast agents for the two major circulatory systems of mammals, the cardiovascular system, and the lymphatic system. In 2003, Larson et al. demonstrated that green-light emitting QDs remained fluorescent and detectable in capillaries of adipose tissue and skin of a living mouse following intravenous injection [54]. This work was aided by the use of near-infrared two-photon excitation for deeper penetration of excitation light, and by the extremely large two-photon cross-sections of QDs [55]. In other work, Lim et al. used near-infrared QDs to image the coronary vasculature of a rat heart [56], and Smith et al. imaged the blood vessels of chicken embryos with a variety of near-infrared and visible QDs [57]. The later report showed that QDs could be detected with higher sensitivity than traditionally used fluorescein–dextran conjugates, and resulted in a higher uniformity in image contrast across vessel lumena. Jayagopal et al. [58], recently, demonstrated the potential for QDs to serve as molecular imaging agents for vascular imaging. Spectrally distinct QDs were conjugated to three different cell adhesion molecules (CAMs), and intravenously injected in a diabetic rat model. Fluorescence angiography of the retinal vasculature revealed CAM-specific increases in fluorescence, and allowed imaging of the inflammation-specific behavior of individual leukocytes, as they freely floated in the vessels, rolled along the endothelium, and underwent leukostasis. The unique spectral properties of QDs allowed the authors to simultaneously image up to four spectrally distinct QD tags.

With the development of QDs in recent years, many studies explored the potential of QDs in wider fields. Kobayashi reported fluorescence lymphangiography by injecting five QDs with different emission spectra [59]. Through simultaneous injection of five QDs into different sites in the middle of phalanges, the upper extremity, the ears, and the chin, different parts of the mouse body can be identified by certain fluorescence color. This is the first demonstration of simultaneous imaging of trafficking lymph nodes with QDs having different emission spectra in vitro cells imaging. Another study was reported by Kim et al. who used near-infrared QDs for sentinel node mapping in cancer surgery in animals. QDs were injected intradermally in distal extremities and imaging used to track their movement along lymphatic channels, with identification of the sentinel node. Furthermore, these experiments demonstrated high contrast between auto fluorescence and emission signal, allowing minimal surgical incision for removal of positive sentinel node [60]. Other teams [61, 62] have, recently, undertook fluorescent tracking of solubilized near-infrared QDs injected subcutaneously in the anterior paw in mice demonstrating accumulation in regional lymph nodes within 5 min of injection and with a maximum concentration at 4 h which then gradually fell over the next 10 days, with resultant low-level uptake in other organs. Tracking using fluorescent imaging was compared with inductively coupled plasma mass spectroscopy, demonstrating viability of fluorescent imaging (Fig. 3.3).

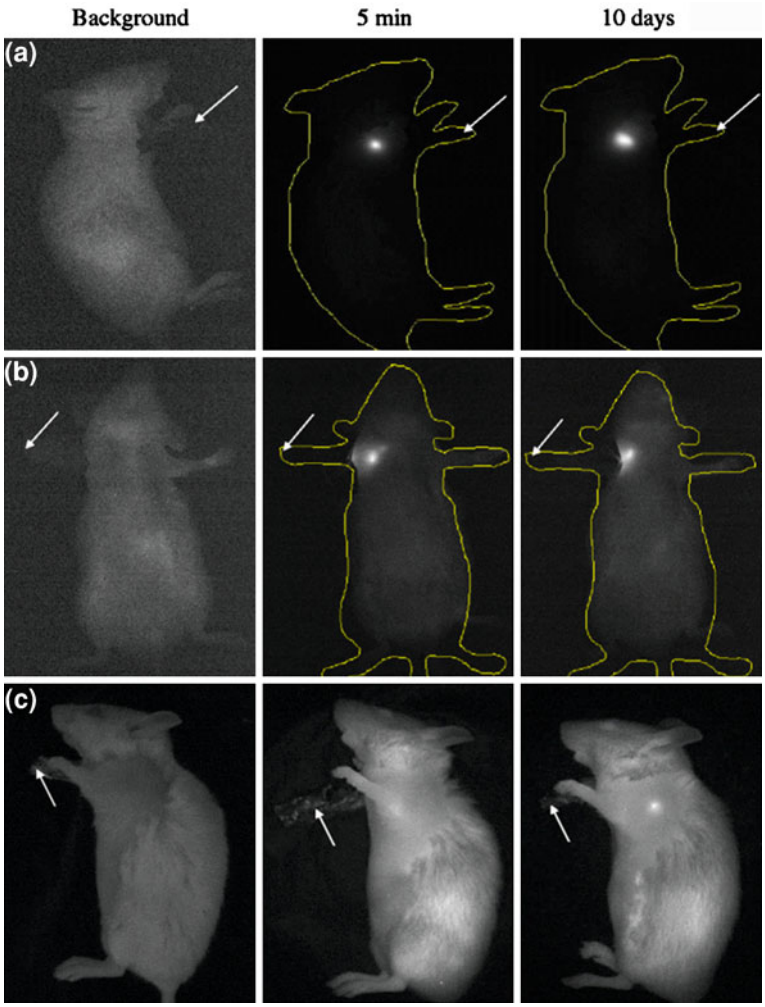


Fig. 3.3 In vivo fluorescence imaging of mice after s.c. injection of 20 pmol of QDs. **a** Images of the *right flank* (visualization of RALN). **b** Images on dorsal decubitus (observation of RLTLN). **c** Images of *left flank* (visualization of LALN). Left column corresponds to background signal, middle and right columns to images at 5 min and 10 days post-injection, respectively. For **a** and **b** images, the exposure time is 10 ms, and for **c** images, the exposure time is 100 ms. The *white arrow* indicates the injection point. With kind permission from Springer Science in Molecular imaging and biology [61]

In another field of application of imaging, QDs have been developed for multimodal imaging. Magnetic resonance imaging (MRI), radiography, and fluorescence imaging are powerful biomedical imaging modalities. Each imaging modality has its merits and demerits and hence cannot achieve comprehensive imaging. Quality imaging requires high spatial and temporal resolutions, 3-D tomography, excellent

signal-to-noise ratio, and noninvasiveness. Individual modalities lack one or more of these qualities and therefore, multimodality has been sought as active imaging technology in basic research and biomedical applications. Independent implementation of imaging probes for different modalities cannot be an ideal solution to achieve multimodal imaging, because different probes often differ in their biodistribution and other pharmacodynamic properties. Thus, grouping the properties for different imaging modalities in the same chemical entity have been sought after. Multimodal imaging probes have components that function synergistically, complementing, and enhancing the functionality of each other. Notably, QDs are promising multimodal probes as it is possible to combine multiple probe characteristics in QDs. For example, fluorescence imaging using QDs can be combined with MRI and radiography imaging if interfaced with molecules/materials having paramagnetism and radioactivity on the surface of QDs [63, 64].

In vivo studies, in particular for cancer imaging and therapy, have been limited owing to the poor stability or short systemic circulation times in living animals. Aiming to this problem, Park et al. [65] described tumor targeting, long circulating, micellar hybrid NPs (MHNs) that contain Magnetic Nanoparticles (MNs), QDs, and the anti-cancer drug Dox within a single poly(ethylene glycol)-phospholipid micelle modified with F3 peptide, and provide the first example of simultaneous targeted drug delivery and dual-mode NIR fluorescence imaging and MRI of diseased tissue in vitro and in vivo. The PEG coating of micelles prevented them from recognition and endocytosis by reticuloendothelial system, and prolonged the circulation and targeting time, which was a key factor for the successful application in vivo.

3.4.1.2 Bioconjugated QDs for Cancer Diagnosis

Biomarker assays may be useful for the screening, diagnosis and prognosis of disease, monitoring the effect of treatment and detecting cancer if a set of molecular markers can be quantified and statistically differentiated between cancerous cells and healthy cells. In early stage of cancer, biomarkers are often present at very low concentrations, so methods capable of low detection limits are required. QDs are emerging as promising probes for ultrasensitive detection of cancer biomarkers. QDs attached to antibodies, aptamers, oligonucleotides, proteins, or peptides can be used to target cancer markers. Their fluorescent properties have enabled QDs to be used as labels for in vitro assays to quantify cancer biomarkers, and they have been investigated as in vivo imaging agents [47, 66–68]. Antibodies are proteins that are capable of specific recognition of an antigen, and have been used in QD assays to detect carcinoembryonic antigen (CEA) [69] and prostate-specific antigen (PSA) [70]. Aptamers are synthetic single-stranded DNA or RNA that are selected for their high binding affinity from a random library of 10^{13} to 10^{15} oligonucleotides in an in vitro process termed “systematic evolution of ligands by exponential enrichment” (SELEX) [71].

These nanometer-sized semiconductor particles bind to target biomolecules through electrostatic interaction, covalent cross linking, or via the implication of specific tagging molecules. QDs have been used as biological probes for the simultaneous detection of multiple biomarkers directly from physiological components [25]. During the past two decades several groups have reported use of QDs for detection of different types of cancers (Table 3.1).

Wu et al. [84] explored a new technology to label HER2 (human epidermal growth factor receptor 2) on breast cancer cell membrane, which is known as c-erbB-2/HER2/neu and overexpressed in approximately 25–30 % invasive breast cancer and plays an important role in breast cancer prognosis and treatment selection. This study reported the multiplexed detection of breast cancer markers using semiconductor QDs as immunofluorescent probes. Simultaneous detection of HER2 and other cellular targets was performed using QDs with different emission spectra conjugated to IgG and streptavidin. This study testified the efficiency of QDs for simultaneous labeling of multiple molecular targets at a subcellular level. After that, several studies on the detection of HER2 for breast cancer diagnosis with QDs have completed [85, 87, 88]. Yezhelyev et al. [89] reported the use of multicolor QDs for quantitative and simultaneous profiling of multiple biomarkers using intact breast cancer cells and clinical specimens and the comparison between the new QDs-based molecular profiling technology with standard western blotting and Fluorescence In Situ Hybridization (FISH). The multicolor bioconjugates were used for simultaneous detection of the five clinically significant tumor markers, including HER2 (QD-HER2), Estrogen Receptor (QD-ER), Progesterone Receptor PR (QD-PR), Epithelial Growth Factor Receptor (QD-EGFR), and mammalian Target Of Rapamycin (QD-mTOR), in breast cancer cells MCF-7 and BT474.

Multiplexed detection of cancer biomarkers, CEA and alpha-fetoprotein (AFP), directly from crude serum using a QD-based microfluidic protein chip was recently reported [78]. In this study, they designed a versatile fluorescent probe by conjugating secondary antibodies (goat anti-mouse IgG), QDs, and found that the QD-based protein chip could rapidly detect CEA and AFP with high sensitivity and selectivity, even in human serum and in the format of both sandwich immunoassay, and reverse phase immunoassay. Multicolor imaging and multiplexed bioassay using QDs directly prepared in aqueous phase CdTe/CdS with different emission wavelengths were also developed. QD-antibody conjugates are also well suited for the multiplexed detection of low abundance cancer biomarkers directly on human tissue biopsies [90, 91]. Use of multicolor and multiplexing capabilities of semiconductor QDs, enabled the authors to detect four protein biomarkers. First is CD15 a transmembrane protein expressed in malignant Hodgkin and Reed-Sternberg (HRS) cells and certain types of epithelial cells. And the last three are CD30 cytokine receptor belonging to the tumor necrosis factor (TNF), CD45, and Pax5. This last used to detect the malignant HRS cells from infiltrating immune cells such as T and B lymphocytes of Hodgkin's lymphoma from lymphoma tissues (Fig. 3.4 [90]).

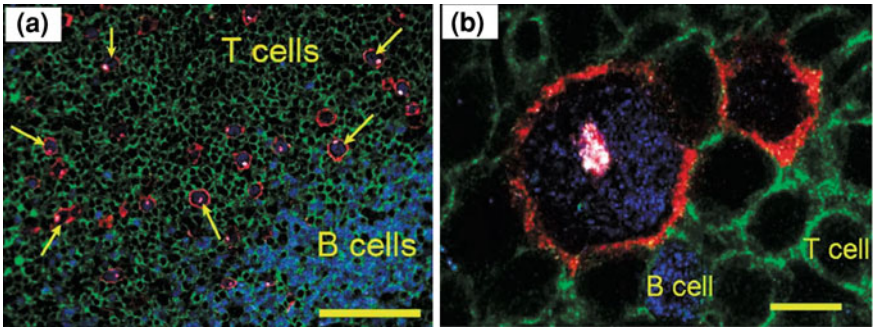


Fig. 3.4 Multiplexed QD staining images of HRS malignant cells and infiltrating immune cells on lymph node tissue specimens of a Hodgkin's lymphoma patient. **a** Malignant HRS cells (*red* membrane, *blue* nuclear, and *red/whitish* Golgi) are identified by a unique multiplexed staining pattern of CD30 positive (membrane staining), CD15 positive (Golgi staining), Pax5 positive (nuclear staining), and CD45 negative. They are differentiated from infiltrating B cells (*blue* nuclear staining) and T cells (*green* membrane staining). A few prominent HRS cells are indicated with arrows. Scale bar: 100 μm. **b** Detailed view showing the distinct staining patterns of HRS cells, B cells, and T cells. Scale bar: 10 μm. Reprinted with permission from Analytical Chemistry, Vol. 82, N°14, july 15, 2010. Copyright (2011) American chemical Society [90]

Simultaneous visualization of multiple biomarkers using multiplexed QD staining was beneficial for the selective identification of rare HRS cells, a primary diagnostic target for Hodgkin's disease, which was not achievable using traditional immunohistochemistry (IHC) assays.

In another studies, Shi et al. [92] showed the superior quality of QDs, in comparison to IHC, for the detection of androgen receptor (AR) and PSA in prostate cancer cells. With QDs probes conjugated to a PSMA monoclonal antibody (Ab), another marker for prostate cancer diagnosis and therapy, Gao et al. [47] have achieved sensitive and multicolor fluorescence imaging of cancer cells under in vivo conditions (Fig. 3.5). Both of those two studies, showing the potential ability of QDs as a diagnosis technology, are good examples to demonstrate why QDs are promising nanoparticles for diagnostic applications. In a second study, Gao et al. [93] demonstrated the potential of QDs as a new diagnosis technology for metastasis prostate cancer.

Usually, antibodies conjugated to QDs are full-length antibody, which leads to dramatically reduced binding activities. Recently, a study demonstrated that the use of single-chain antibody fragments (scFvs) conjugated with QDs appears to have a number of advantages, in terms of solubility, activity, ease of preparation and ease of structure-based genetic engineering, which were approved by detecting prostate cancer cells [94]. In another study, CdSe/CdS/ZnS QDs were used for improved photoluminescence efficiency and stability as optical agent for imaging pancreatic cancer cells using transferrin and anti-Claudin-4. Pancreatic cancer specific uptake is also demonstrated using the monoclonal antibody anti-Claudin-4. This targeted QDs platform will be further modified to develop early detection imaging tool for pancreatic cancer [95]. One of the greatest challenges in preparing highly efficient

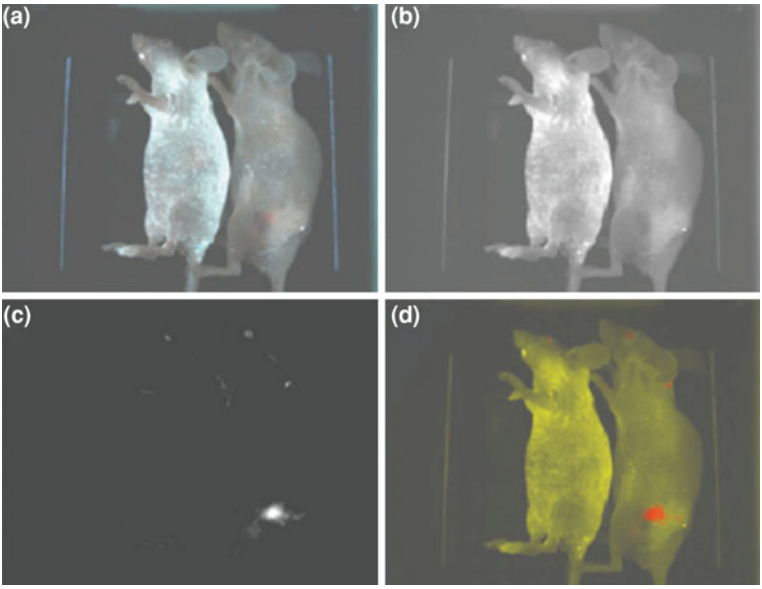


Fig. 3.5 Spectral imaging of QD-PSMA Ab conjugates in live animals harboring C4-2 tumor xenografts. *Orange-red* fluorescence signals indicate a prostate tumor growing in a live mouse (*right*). Control studies using a healthy mouse (no tumor) and the same amount of QD injection showed no localized fluorescence signals (*left*). **a** Original image; **b** Unmixed autofluorescence image; **c** Unmixed QD image; and **d** Super-imposed image. After in vivo imaging, histological and immunocytochemical examinations confirmed that the QD signals came from an underlying tumor. Note that QDs in deep organs such as liver and spleen were not detected because of the limited penetration depth of visible light. REPRINTED BY PERMISSION FROM Macmillan Publishers Ltd on behalf of Cancer Research UK: [Nature biotechnologies] [47], copyright (2004)

QDs involves the selection of QDs core, biocompatible, and nontoxic. For imaging live pancreatic cancer cells Yong et al. [96] used noncadmium-based InP/ZnS QDs conjugated with pancreatic cancer specific monoclonal antibodies, such as anti-Claudin-4 which allow specific in vitro targeting of pancreatic cancer cell line. The receptor mediated delivery of the bioconjugates was further confirmed by the observation of poor in vitro targeting in nonpancreatic cancer cell lines without Claudin-4 receptor. These observations suggest the immense potential of InP/ZnS QDs as noncadmium based safe and efficient optical imaging nanoprobes in diagnostic imaging.

AFP is an important tumor marker for hepatocellular carcinoma (HCC). In a prior study, Liang Chen [97] used CdSe/ZnS QDs with emission wavelength of 590 nm (QDs 590) linked to AFP monoclonal antibody (Ab) as a probe for fluorescence spectral analysis of HCC. In another study [98], they tested the biocompatibility, hemodynamics, tissues distribution of the QDs-AFP-Ab probes, and studied the imaging of HCC and its metastasis in vitro and in vivo. Their results indicate that such QDs-based probes have good stability, specificity, and biocompatibility for ultrasensitive fluorescence imaging of molecular targets in their liver cancer model system.

There are evidences supporting the application of QD-conjugated protein microarrays for the detection of cancer biomarkers [82]. Use of QD-conjugated PSA anti-bodies for fabrication of protein biochip allowed selective detection of the target protein PSA and effectively minimized nonspecific binding. High specificity, reduction of nonspecific binding, and elimination of need of any blocking or additional biotin–streptavidin interactions have been achieved by QD-conjugated protein microarray, which are improvement over the conventional microarray approaches.

Apart from classical QDs, rod shaped nanocrystals known as quantum rods (QRs) are also attractive probes for cancer detection and imaging [44]. Authors have employed CdSe/CdS/ZnS QRs bioconjugated with lysine and transferrin (Tf) for specific targeted bioimaging. The internalization of QR-Tf bioconjugates in human cancer cell line (HeLa) which overexpress the transferrin receptor (TfR) was demonstrated by confocal and two-photon imaging. The application of QRs as biological probes has started recently and few initial studies have suggested that they can act as brighter single molecule probes than QDs and exhibit good potential for biomedical applications [99].

In summary, the use of QDs in cancer investigations has increased dramatically due to their unique size-dependent optical properties. Trends in the applications of bioconjugated QDs in cancer research clearly show that QDs can provide powerful tools for cancer management. There is still scope for improvement in terms of developing QD probes with improved target specificity, signal intensity, multimodality, and therapeutic potentials. However, toxicological and pharmacological issues remain in the advancement of QD technology towards the diagnosis and therapy of cancer and other diseases. This advancement is setback due to mismatch between rapid developments of QD bioprobes and fewer studies on the toxicology [100].

3.4.2 Bioconjugated QDs Used as Biosensors

Fluorescence resonance energy transfer (FRET) involves the transfer of fluorescence energy from a donor particle to an acceptor particle whenever the distance between the donor and the acceptor is smaller than a critical radius, known as the Förster radius [101]. This leads to a reduction in the donor's emission and excited state lifetime, and an increase in the acceptor's emission intensity. FRET is suited to measuring changes in distance, rather than absolute distances, making it appropriate for measuring protein conformational changes [102], monitoring protein interactions [103], and assaying of enzyme activity [104]. Several groups have attempted to use QDs in FRET technologies [105], particularly when conjugated to biological molecules [106], including antibodies [107], for use in immunoassays. QDs can be used as donors in assays involving FRET [13, 14, 72, 108, 109], or as acceptors in bioluminescence resonance energy transfer (BRET) [110, 111]. In a recent study, quantitative maltose sensing has provided an example of how QDs might play a role in enzyme assays. In this study, QDs

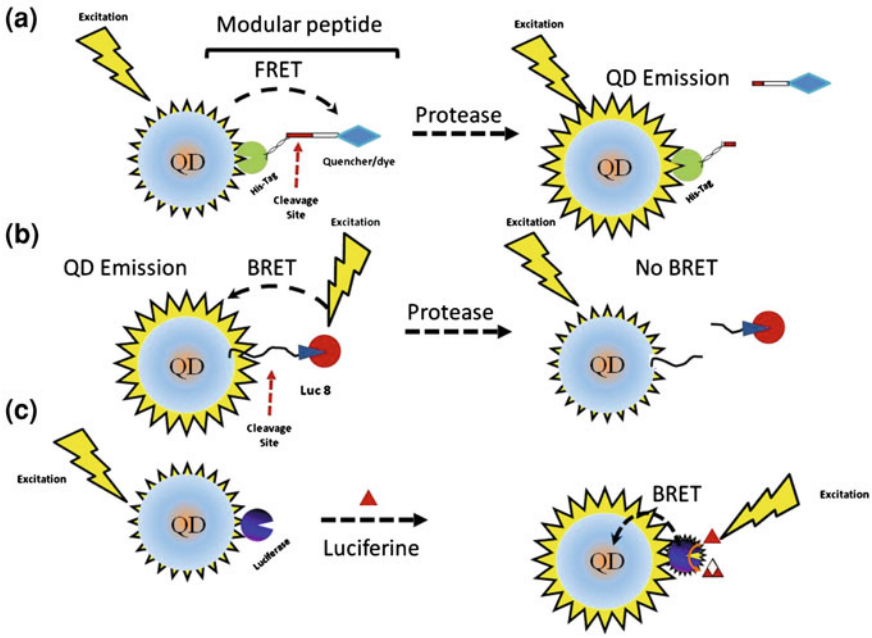


Fig. 3.6 Schematics diagrams showing biosensing for enzymes. **a** QD base protease activity sensors. Quencher conjugated protease substrate peptides bind the QD through a His-tag. Where QD fluorescence is quenched through FRET. Upon proteases mediated substrate cleavage the QD fluorescence is recovered [14, 72]. **b** A BRET strategy for analysis of protease, in the absence of protease, the peptide linker holds Luc8 in close proximity to the QD, and transfer of bioluminescence energy to the QD occurs. In presence of an active protease, the peptide is cleaved, and BRET is disrupted. Reproduced from [110] with permission of ACS. **c** QD is covalently coupled to a BRET donor, Luc8. The bioluminescence energy of Luc8-catalysed oxidation of Luciferine is transferred to QD, resulting in quantum dot emission [111]

conjugated to maltose binding protein (MBP) allowed binding of either maltose or a quenching molecule [11]. The quenching molecule, with a binding affinity similar to that of maltose, was readily displaced on addition of maltose, and a concentration-dependent increase in luminescence was observed. Several studies have exploited QD-FRET for imaging activity of proteases [112–115]. For this application, a QD-probe conjugate is bound to a quencher probe by a peptide sequence which is recognized by a protease, in which state the fluorophore is quenched. On cleavage of the two molecules by a protease, emission is restored, allowing its activity to be visualized (Fig. 3.6a). QDs gave an increased luminescence compared to previous results using organic fluorophores [116, 117]. In addition to FRET, QDs can be involved in another nonradiative energy transfer process known as BRET [118, 119]. The mechanism is similar, but the source of energy does not come from an external light source [119]. The BRET process involves a donor with intrinsic fluorescent properties, for example a light-emitting protein, and QDs are energy acceptors. The method is advantageous because the

possibility of direct excitation of the acceptor molecule by external light is eliminated. Therefore, the background signal is lower [110]. A BRET-based sensor has been developed to detect the activity of matrix metalloproteinases MMP-2, MMP-7, and urokinase-type plasminogen activator (uPA) [110]. Luciferase-catalyzed substrate oxidation produced luminescence, which was the source of energy in the system. The QD and luciferase were held in close proximity by a peptide linker, and cleavage by a specific protease disrupted BRET (Fig. 3.6b). The protease concentration was determined from the change in BRET ratio, as an increase in protease concentration caused a decreased BRET ratio (QD emission decreased, and luciferase bioluminescence increased). The reliability of the assay is enhanced because rather than the change in acceptor emission, the change in the ratio of emission from donor and acceptor is measured [110]. Another representative BRET study using QD acceptors was reported by Rao et al. [111]. They selected an optimized eight-mutation variant of luciferase (Luc8) with improved catalytic efficiency to facilitate BRET; an average of six copies of Luc8 were coupled, via EDC condensation, to carboxyl-modified 655 nm emitting QDs. Upon addition of coelenterazine substrate to the complex, a strong emission peak from the QDs was detected in addition to the 480 nm Luc8 donor emission (Fig. 3.6c). To estimate the efficiency of these interactions, a BRET ratio (similar to FRET efficiency) defined as QD acceptor (A) emission-to-Luc8/donor (D) emission was used. By using different emission/colors of QDs and varying the center-to-center separation distance, the authors found that the BRET ratio was sensitive to both changes in D-A separation distance and the overall "spectral overlap" between Luc8 emission and QD absorption, with redder emitting QDs providing more efficient energy transfer. Furthermore, the authors coupled Luc8 to QDs with emissions ranging from 605 to 800 nm and observed distinct emissions from each or all when mixed together in a multiplex format. Testing these conjugates in cell lines and in vivo within mice tissues, they showed that after addition of the substrate complex, luminescence spectra characteristic of the QD combination used could be collected and deconvolution of each QD color can be performed. Additionally, enhanced sensitivity and high signal to background ratios were measured when performing in vivo imaging in mice for comparatively small amounts of QD Luc8 conjugates.

3.5 Conclusion

QDs have drawn interests due to their unique and advantageous optical properties, which include broad absorption, narrow size-tunable photoluminescence spectra, and superior resistance to photobleaching [120]. They have appeared as a new promising class of fluorescent probes for biomolecular detection, cell-based application, and in vivo animal imaging. QDs' size-tunable properties enable them to solve the problems of spectral overlap in conventional organic labels. Moreover, QDs can be immobilized on solid surfaces or embedded in microbeads to realize multiplexed detection. At the same time, QDs optical properties such as

photostability and narrow emission spectra also make them robust labels in cell-based applications. Besides these regular advantages, QDs exceed fluorescent dyes in in vivo imaging because of their fluorescence in NIR region, which offers low tissue absorption. However, the use of QDs in biological systems, especially in in vivo application, suffers from their toxicity. Due to their chemical composition of toxic metal atoms (e.g. Cd, Hg, Pb, As), hindrance exists when QDs are applied in living cells and animals [121]. Although metal ions like divalent cadmium today are covered with inert zinc sulfide and encapsulated within a stable polymer, they might still be toxic in living bodies. Moreover, QDs could aggregate or bind nonspecifically to cellular membranes and intracellular proteins [121]. It has been reported that concentration of cadmium in the liver and kidneys could gradually increase after intravenous administration of cadmium-based QDs [122]. Former studies have shown that QDs' size, shape, and surface coating all could affect their toxicity [121]. Overall, the unique optical properties of QDs and the modulation of those properties have provided researchers versatile toolkit for bioanalysis [123]. Importantly, multiplexed detection is possible as a new type of simple, flexible method for novel diagnostic technologies, and intracellular probes [123]. Both quantum efficiency and sensitivity should be increased in the future [124]. As for in vivo applications, new types of QDs exempt from heavy metal atoms should be developed [125, 126]. Moreover, nanoparticle distribution, excretion, metabolism, pharmacokinetics, and pharmacodynamics should be included in nanotoxicology studies in animal models in vivo [121]. The next challenge associated with QD-based medical applications is the commercialization of the products and development of the appropriate regulations. Undoubtedly, biologists will catch on to these exciting developments and will find as yet unforeseen applications for this new toolkit, thus enhancing and complementing their existing arsenal of bioimaging tools.

References

1. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos P (1998) Semiconductor nanocrystals as fluorescent biological labels. Science 281:2013
2. Chan WC, Nie S (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281:2016–2018
3. Medintz IL, Mattousi H, Clapp AR (2008) Potential clinical applications of quantum dots. Int J Nanomed 3(2):151–161
4. Michalet X, Pinaud FF, Bentolila LA et al (2005) Quantum dots for live cells, in vivo imaging, and diagnostics. Science 307:538–544
5. Manninen SM, Reimann M (2002) Electronic structure of quantum dots. Rev Mod Phys 74:1283–1342
6. Gokarna A, Jin LH, Hwang JS, Cho YH, Lim YT, Chung BH et al (2008) Quantum dot-based protein micro- and nanoarrays for detection of prostate cancer biomarkers. Proteomics 8:1809–18
7. Marchal F, Pic E, Pons T, Dubertret B, Bolotine L, Guillemin F (2008) Quantum dots in oncological surgery: the future for surgical margin status? Bull Cancer 95:1149–1153

8. Weng KC, Noble CO, Papahadjopoulos-Sternberg B et al (2008) Targeted tumor cell internalization and imaging of multifunctional quantum dot-conjugated immunoliposomes in vitro and in vivo. Nano Lett 8:2851
9. Zhou I, Ghosh M (2007) Quantum dots and peptides: a bright future together. Biopolymers 88:325–339
10. Zhang CY, Yeh HC, Kuroki MT et al (2005) Single-quantumdot-based DNA nanosensor. Nat Mater 4:826
11. Medintz IL, Clapp AR, Mattoussi H et al (2003) Self-assembled nanoscale biosensors based on quantum dot FRET donors. Nat Mater 2:630
12. Bagalkot V, Zhang L, Levy-Nissenbaum E et al (2007) Quantum dot—aptamer conjugates for synchronous cancer imaging, therapy, and sensing of drug delivery based on Bi-fluorescence resonance energy transfer. Nano Lett 7:3065
13. Cheng AK, Su H, Wang YA, Yu HZ (2009) Aptamer-based detection of epithelial tumor marker mucin 1 with quantum dot-based fluorescence readout. Anal Chem 81:6130–6139
14. Boeneman K, Mei BC, Dennis AM et al (2009) Sensing caspase 3 activity with quantum dot-fluorescent protein assemblies. J Am Chem Soc 131:3828
15. Dabbousi BO, RodriguezViejo J, Mikulec FV et al (1997) (CdSe) ZnS core-shell quantum dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. J Phys Chem B 101:9463
16. Erogbogbo F, Yong KT, Roy I, Xu GX, Prasad PN, Swihart MT (2008) Biocompatible luminescent silicon quantum dots for imaging of cancer cells. ACS Nano 2:873–878
17. Ben-Ari ET (2003) Nanoscale quantum dots hold promise for cancer applications. J Natl Cancer Inst 95(7):502–504
18. Hezinger AFE, Tessmar J, Gopferich A (2008) Polymer coating of quantum dots—a powerful tool toward diagnostics and sensorics. Eur J Pharm Biopharm 68:138–152
19. Yong KT, Roy I, Swihart MT, Prasad PN (2009) Multifunctional nanoparticles as biocompatible targeted probes for human cancer diagnosis and therapy. J Mater Chem 19(27):4655–4672
20. Dubertret B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A (2002) In vivo imaging of quantum dots encapsulated in phospholipid micelles. Science 298(5599):1759–1762
21. Smith AM, Dave S, Nie S, True L, Gao X (2006) Multicolor quantum dots for molecular diagnostics of cancer. Expert Rev Mol Diagn 6:231–244
22. Zhang H, Yee D, Wang C (2008) Quantum dots for cancer diagnosis and therapy: biological and clinical perspectives. Nanomedicine 3(1):83–91
23. Smith AM, Duan H, Mohs AM, Nie S (2008) Bioconjugated quantum dots for in vivo molecular and cellular imaging. Adv Drug Deliv Rev 60:1226–1240
24. Resch-Genger U, Grabolle M, Cavaliere-Jaricot S, Nitschke R, Nann T (2008) Quantum dots versus organic dyes as fluorescent labels. Nat Methods 5:763–775
25. Azzazy HM, Mansour MM, Kazmierczak SC (2007) From diagnostics to therapy: prospects of quantum dots. Clin Biochem 40:917–927
26. Medintz IL, Uyeda HT, Goldman ER, Mattoussi H (2005) Quantum dot bioconjugates for imaging, labelling and sensing. Nat Mater 4:435–446
27. Derfus AM, Chan WCW, Bhatia SN (2004) Probing the cytotoxicity of semiconductor quantum dots. Nano Lett 4:11–18
28. Kirchner C, Liedl T, Kudera S, Pellegrino T, Javier AM, Gaub HE et al (2005) Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. Nano Lett 5:331–338
29. Clarke SJ, Hollmann CA, Zhang Z, Suffern D, Bradforth SE, Dimitrijevic NM et al (2006) Photophysics of dopamine-modified quantum dots and effects on biological systems. Nat Mater 5:409–417
30. Hsieh SC, Wang FF, Hung SC, Chen Y, Wang YJ (2006) The internalized CdSe/ZnS quantum dots impair the chondrogenesis of bone marrow mesenchymal stem cells. J Biomed Mater Res Part B-Appl Biomater 79B:95–101
31. Lovric J, Bazzi HS, Cuie Y, Fortin GRA, Winnik FM, Maysinger D (2005) Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. J Mol Med 83:377–385

32. Chen FQ, Gerion D (2004) Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. Nano Lett 4:1827–1832
33. Shiohara A, Hoshino A, Hanaki K, Suzuki K, Yamamoto K (2004) On the cyto-toxicity caused by quantum dots. Microbiol Immunol 48:669–675
34. Hardman R (2006) A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ Health Perspect 114:165–172
35. Zhang TT, Stilwell JL, Gerion D, Ding LH, Elboudwarej O, Cooke PA et al (2006) Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics mea-surements. Nano Lett 6:800–808
36. Mansson A, Sundberg M, Balaz M, Bunk R, Nicholls IA, Omling P et al (2004) In vitro sliding of actin filaments labelled with single quantum dots. Biochem Biophys Res Commun 314:529–534
37. Rzigalinski BA, Strobl JS (2009) Cadmium-containing nanoparticles: perspectives on pharmacology and toxicology of quantum dots. Toxicol Appl Pharmacol 238:280–288
38. Pelley JL, Daar AS, Saner MA (2009) State of academic knowledge on toxicity and biological fate of quantum dots. Toxicol Sci 112:276–296
39. Selvan ST, Tan TT, Ying JY (2005) Robust, non-cytotoxic, silica-coated CdSe quantum dots with efficient photoluminescence. Adv Mater 17:1620–1625
40. Hoshino A, Fujioka K, Oku T, Suga M, Sasaki YF, Ohta T et al (2004) Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. Nano Lett 4:2163–2169
41. Ryman-Rasmussen JP, Riviere JE, Monteiro-Riviere NA (2007) Surface coatings determine cytotoxicity and irritation potential of quantum dot nanoparticles in epidermal keratinocytes. J Investig Dermatol 127:143–153
42. Bharali DJ, Lucey DW, Jayakumar H, Pudavar HE, Prasad PN (2005) Folate-receptor-mediated delivery of InP quantum dots for bioimaging using confocal and two-photon microscopy. J Am Chem Soc 127:11364–11371
43. Jin Y, Gao X (2009) Plasmonic fluorescent quantum dots. Nat Nanotechnol 4:571–576
44. Yong KT, Qian J, Roy I, Lee HH, Bergey EJ, Tramposch KM et al (2007) Quantum rod bioconjugates as targeted probes for confocal and two-photon fluorescence imaging of cancer cells. Nano Lett 7:761–765
45. Soman CP, Giorgio TD (2008) Quantum dot self-assembly for protein detection with sub picomolar sensitivity. Langmuir 24:4399–4404
46. Liu A, Peng S, Soo JC, Kuang M, Chen P, Duan H (2011) Quantum dots with phenylboronic acid tags for specific labeling of sialic acids on living cells. Anal Chem 83(3):1124–1130
47. Gao XH, Cui YY, Levenson RM et al (2004) In vivo cancer targeting and imaging with semiconductor quantum dots. Nat Biotechnol 22:969
48. Voura E, Jaiswal J, Mattoussi H et al (2004) Tracking metastatic tumor cell extra-vasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. Nat Med 10:993–998
49. Cai W, Rao J, Gambhir SS, Chen X (2006) How molecular imaging is speeding up anti-angiogenic drug development. Mol Cancer Ther 5:2624–2633
50. Stroh M, Zimmer JP, Duda DG, Levchenko TS, Cohen KS, Brown EB, Scadden DT, Torchilin VP, Bawendi MG, Fukumura D et al (2005) Quantum dots spectrally distinguish multiple species within the tumor milieu in vivo. Nat Med 11:678–682
51. Li Z, Wang K, Tan W, Li J, Fu Z, Ma C et al (2006) Immunofluorescent labeling of cancer cells with quantum dots synthesized in aqueous solution. Anal Biochem 354:169–174
52. Weissleder R (2001) A clearer vision for in vivo imaging. Nat Biotechnol 19:316–317
53. Akerman ME, Chan WCW, Laakkonen P, Bhatia SN, Ruoslahti E (2002) Nanocrystal targeting in vivo. Proc Natl Acad Sci 99:12617–12621
54. Larson DR, Zipfel WR, Williams RM, Clark SW, Bruchez MP, Wise FW, Webb WW (2003) Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. Science 300:1434–1436

55. Rubart M (2004) Two-photon microscopy of cells and tissue. Circ Res 95:1154–1166
56. Lim YT, Kim S, Nakayama A, Stott NE, Bawendi MG, Frangioni JV (2003) Selection of quantum dot wavelengths for biomedical assays and imaging. Mol Imaging 2:50–64
57. Smith JD, Fisher GW, Waggoner AS, Campbell PG (2007) The use of quantum dots for analysis of chick CAM vasculature. Microvasc Res 73:75–83
58. Jayagopal A, Russ PK, Haselton FR (2007) Surface engineering of quantum dots for in vivo vascular imaging. Bioconjug Chem 18:1424–1433
59. Kobayashi H, Hama Y, Koyama Y, Barrett T, Regino CA, Urano Y, Choyke PL (2007) Simultaneous multicolor imaging of five different lymphatic basins using quantum dots. Nano Lett jun 7(6):1711–1716
60. Kim S, Lim YT, Soltesz EG, De Grand AM, Lee J, Nakayama A, Parker JA, Mihaljevic T, Laurence RG, Dor DM, Cohn LH, Bawendi MG, Frangioni JV (2004) Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. Nat Biotechnol 22:93–97
61. Pic E, Pons T, Bezdetnaya L, Leroux A, Guillemin F, Dubertret B, Marchal F (2010) Fluorescence imaging and whole-body biodistribution of near-infrared-emitting quantum dots after subcutaneous injection for regional lymph node mapping in mice. Mol Imaging Biol 12:394–405
62. Cassette E, Pons T, Bouet C et al (2010) Synthesis and characterization of near-infrared Cu-In-Se/ZnS core/shell quantum dots for in vivo imaging. Chem Mater 22(22):6117–6124
63. Cheon J, Lee J-H (2008) Synergistically integrated nanoparticles as multimodal probes for nanobiotechnolgy. Acc Chem Res 41:1630–1640
64. Jennings LE, Long NJ (2009) Two is better than one's probes for dual-modality molecular imaging. Chem Commun pp 3511–3524
65. Park JH, von Maltzahn G, Ruoslahti E, Bhatia SN, Sailor MJ (2008) Micellar hybrid nanoparticles for simultaneous magnetofluorescent imaging and drug delivery. Angew Chem Int Ed Engl 47:7284–7288
66. Weng KC, Noble CO, Papahadjopoulos-Sternberg B et al (2008) Targeted tumor cell internalization and imaging of multifunctional quantum dot-conjugated immunoliposomes in vitro and in vivo. Nano Lett 8:2851
67. Smith BR, Cheng Z, De A et al (2008) Real-time intravital imaging of RGD-quantum dot binding to luminal endothelium in mouse tumor neovasculature. Nano Lett 8:2599
68. Choi HS, Liu W, Liu F et al (2010) Design considerations for tumour-targeted nanoparticles. Nat Nano 5:42
69. Jokerst JV, Raamanathan A, Christodoulides N, Floriano PN, Pollard AA, Simmons GW et al (2009) Nano-bio-chips for high performance multiplexed protein detection: determinations of cancer biomarkers in serum and saliva using quantum dot bioconjugate labels. Biosens Bioelectron 24:3622–9
70. Wang J, Liu G, Wu H et al (2008) Quantum-dot-based electrochemical immunoassay for high-throughput screening of the prostate-specific antigen. Small 4:82
71. Hamula CLA, Guthrie JW, Zhang H et al (2006) Selection and analytical applications of aptamers. Trends Anal Chem 25(7):681–691
72. Medintz IL, Clapp AR, Brunel FM et al (2006) Proteolytic activity monitored by fluorescence resonance energy transfer through quantum-dot-peptide conjugates. Nat Mater 5:581–589
73. Snyder EL, Bailey D, Shipitsin M, Polyak K, Loda M (2009) Identification of CD44v6(+)/CD24? breast carcinoma cells in primary human tumors by quantum dot-conjugated antibodies. Lab Invest 89:857–66
74. Zdobnova TA, Dorofeev SG, Tananaev PN, Zlomanov VP, Stremovskiy OA, Lebedenko EN, Balalaeva IV, Deyev SM, Petrov RV (2005) Imaging of human ovarian cancer SKOV-3 cells. Dokl Biochem Biophys 430(1):41–44
75. Song EQ, Zhang ZL, Luo QY, Lu W, Shi YB, Pang DW (2009) Tumor cell targeting using folate-conjugated fluorescent quantum dots andrecep-tor-mediated endocytosis. Clin Chem 955–963

76. Schroeder JE, Shweky I, Shmeeda H, Banin U, Gabizon A (2007) Folate-mediated tumor cell uptake of quantum dots entrapped in lipid nanoparticle. J Controlled Release 124:28–34
77. Barat B, Sirk SJ, McCabe KE, Li J, Lepin EJ, Remenyi R et al (2009) Cys-diabody quantum dot conjugates (ImmunoQdots) for cancer marker detection. Bioconjug Chem 20: 1474–1481
78. Hu M, Yan J, He Y, Lu H, Weng L, Song S et al (2010) Ultrasensitive, multiplexed detection of cancer biomarkers directly in serum by using a quantum dot-based microfluidic protein chip. ACS Nano 4:488–494
79. Ko MH, Kim S, Kang WJ, Lee JH, Kang H, Moon SH et al (2009) In vitro derby imaging of cancer biomarkers using quantum dots. Small 5:1207–1212
80. Yu X, Chen L, Li K, Li Y, Xiao S, Luo X et al (2007) Immunofluorescence detection with quantum dot bioconjugates for hepatoma in vivo. J Biomed Opt 12:14008
81. Li Z, Wang Y, Wang J, Tang Z, Pounds JG, Lin Y (2010) Rapid and sensitive detection of protein biomarker using a portable fluorescence biosensor based on quantum dots and a lateral flow test strip. Anal Chem 82:7008–7014
82. Gokarna A, Jin LH, Hwang JS, Cho YH, Lim YT, Chung BH et al (2008) Quantum dot-based protein micro- and nanoarrays for detection of prostate cancer biomarkers. Proteomics 8:1809–1818
83. Zajac A, Song D, Qian W, Zhukov T (2007) Protein microarrays and quantum dot probes for early cancer detection. Colloids Surf B 58:309–314
84. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP et al (2003) Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. Nat Biotechnol 21:41–46
85. Liu X-L, Peng C-W, Chen C, Yang X-Q, Ming-Bai H, Xia H-S, Liu S-P, Pang D-W, Li Y (2011) Quantum dots-based double-color imaging of HER2 positive breast cancer invasion. Biochem Biophys Res Commun 409(3):577–582
86. Yang M, Javadi A, Gong S (2011) Sensitive electrochemical immunosensor for the detection of cancer biomarker using quantum dot functionalized graphene sheets as labels. Sens Actuators B Chem 155(1):357–360
87. Li-Shishido S, Watanabe TM, Tada H, Higuchi H, Ohuchi N (2006) Reduction in non fluorescence state of quantum dots on an immunofluorescence staining. Biochem Biophys Res Commun 351(1):7–13
88. Xiao Y, Gao X, Gannot G et al (2008) Quantitation of HER2 and telomerase biomarkers in solid tumors with IgY antibodies and nanocrystal detection. Int J Cancer 122(10): 2178–2186
89. Yezhelyev MV, Al-Hajj A, Morris C et al (2007) In situ molecular profiling of breast cancer biomarkers with multicolor quantum dots. Adv Mater 19(20):3146–3151
90. Liu J, Lau SK, Varma VA, Kairdolf BA, Nie S (2010) Multiplexed detection and characterization of rare tumor cells in Hodgkin's lymphoma with multicolor quantum dots. Anal Chem 82:6237–6243
91. Xing Y et al (2007) Bioconjugated quantum dots for multiplexed and quantitative immunohistochemistry. Nat Protoc 2:1152–1165
92. Shi C, Zhou G, Zhu Y et al (2008) Quantum dots-based multiplexed immunohistochemistry of protein expression in human prostate cancer cells. Eur J Histochem 52(2):127–134
93. Gao X, Chung LWK, Nie S (2007) Quantum dots for in vivo molecular and cellular imaging. Methods Mol Biol 374:135–145
94. Wang Y, Dossey AM, Froude JW II et al (2008) PSA fluoroimmunoassays using anti-PSA ScFv and quantum-dot conjugates. Nanomedicine 3(4):475–483
95. Qian J, Yong K-T, Roy I et al (2007) Imaging pancreatic cancer using surface-functionalized quantum dots. J Phys Chem B 111(25):6969–6972
96. Yong KT, Ding H, Roy I et al (2009) Imaging pancreatic cancer using bioconjugated InP quantum dots. ACS Nano 3(3):502–510
97. Yu X, Chen L, Li K et al (2007) Immunofluorescence detection with quantum dot bioconjugates for hepatoma in vivo. J Biomedical Opt 12(1):014008

98. Chen L-D, Liu J, Yu X-F et al (2008) The biocompatibility of quantum dot probes used for the targeted imaging of hepatocellular carcinoma metastasis. Biomaterials 29(31):4170–4176
99. Fu A, Gu W, Boussert B, Koski K, Gerion D, Manna L, et al (2007) Semiconductor quantum rods as single molecule fluorescent biological labels. Nano Lett 7:179–182
100. Vasudevanpillai B, Sathish M, Ramakrishnapillai VO, Abdulaziz A, Mitsuru I (2011) Bioconjugated quantum dots for cancer research: Present status, prospects and remaining issues. Biotechnol Adv 29(2):259–260
101. Riegler J, Nann T (2004) Application of luminescent nanocrystals as labels for biological molecules. Anal Bioanal Chem 379:913–919
102. Heyduk T (2002) Measuring protein conformational changes by FRET/LRET. Curr Opin Biotechnol 13:292–296
103. Day RN, Periasamy A, Schaufele F (2001) Fluorescence resonance energy transfer microscopy of localized protein interactions in the living cell nucleus. Methods 25:4–18
104. Li JJ, Bugg TDH (2004) A fluorescent analogue of UDP-N-acetylglucosamine: application for FRET assay of peptidoglycan translocase II (MurG). Chem Commun pp 182–183
105. Kagan CR, Murray CB, Nirmal M, Bawendi MG (1996) Electronic energy transfer in CdSe quantum dot solids. Phys Rev Lett 76:1517–1520
106. Willard DM, Carillo LL, Jung J, Van Orden A (2001) CdSe-ZnS quantum dots as resonance energy transfer donors in a model protein–protein binding assay. Nano Lett 1:469–474
107. Wang SP, Mamedova N, Kotov NA, Chen W, Studer J (2002) Antigen/antibody immunocomplex from CdTe nanoparticle bioconjugates. Nano Lett 2:817–822
108. Bailey VJ, Easwaran H, Zhang Y et al (2009) MS-qFRET: a quantum dot-based method for analysis of DNA methylation. Genome Res 19:1455
109. Kim Y, Oh Y, Oh E et al (2008) Energy transfer-based multiplexed assay of proteases by using gold nanoparticle and quantum dot conjugates on a surface. Anal Chem 80:4634
110. Xia Z, Xing Y, So M et al (2008) Multiplex detection of protease activity with quantum dot nanosensors prepared by inteinmediated specific bioconjugation. Anal Chem 80:8649
111. So MK, Xu CJ, Loening AM, Gambhir SS, Rao JH (2006) Self-illuminating quantum dot conjugates for in vivo imaging. Nat Biotechnol 24:339–343
112. Chang E, Miller JS, Sun JT, Yu WW, Colvin VL, Drezek R et al (2005) Protease-activated quantum dot probes. Biochem Biophys Res Commun 334:1317–1321
113. Xu CJ, Xing BG, Rao HH (2006) A self-assembled quantum dot probe for detecting beta-lactamase activity. Biochem Biophys Res Commun 344:931–935
114. Medintz IL, Clapp AR, Brunel FM, Tiefenbrunn T, Uyeda HT, Chang EL et al (2006) Proteolytic activity monitored by fluorescence resonance energy transfer through quantum-dot-peptide conjugates. Nat Mater 5:581–589
115. Shi LF, De Paoli V, Rosenzweig N, Rosenzweig Z (2006) Synthesis and application of quantum dots FRET-based protease sensors. J Am Chem Soc 103:10378–10379
116. Messerli SM, Prabhakar S, Tang Y, Shah K, Cortes ML, Murthy V et al (2004) A novel method for imaging apoptosis using a caspase-1 nearinfrared fluorescent probe. Neoplasia 6:95–105
117. Pham W, Choi YD, Weissleder R, Tung CH (2004) Developing a peptidebased near-infrared molecular probe for protease sensing. Bioconjugate Chem 15:1403–1407
118. Xu Y, Piston DW, Johnson CH (1999) A bioluminescence resonance energy transfer (BRET) system: application to interacting circadian clock proteins. Proc Natl Acad Sci USA 96:151
119. Xia Z, Rao J (2009) Biosensing and imaging based on bioluminescence resonance energy transfer. Curr Opin Biotechnol 20:37
120. Russ Algar W, Krull UJ (2008) Quantum dots as donors in fluorescence resonance energy transfer for the bioanalysis of nucleic acids, proteins, and other biological molecules. Anal Bioanal Chem 391:1609–1618
121. Smith AM, Duan H, Mohs AM, Nie S (2008) Bioconjugated quantum dots for in vivo molecular and cellular imaging. Adv Drug Deliv Rev 60:1226–1240
122. Yang RH, Chang LW, Wu JP, Tsai MH, Wang HJ, Kuo YC, Yeh TK, Yang CS, Lin P (2007) Persistent tissue kinetics and redistribution of nanoparticles, quantum dot 705, in mice: ICP-MS quantitative assessment. Environ Health Perspect 115:1339–1343

123. Russ Algar W, Krull UJ (2010) New opportunities in multiplexed optical bioanalyses using quantum dots and donor-acceptor interactions. Anal Bioanal Chem 398:2439–2449
124. Mazumder S, Dey R, Mitra MK, Mukherjee S, Das GC (2009) Biofunctionalized quantum dots in biology and medicine. J Nanomater 1–17:815734–815741
125. Pradhan N, Battaglia DM, Liu YC, Peng XG (2007) Efficient, stable, small, and water-soluble doped ZnSe nanocrystal emitters as non-cadmium biomedical labels. Nano Lett 7:312–317
126. Pradhan N, Peng XG (2007) Efficient and color-tunable Mn-doped ZnSe nanocrystal emitters: control of optical performance via greener synthetic chemistry. J Am Chem Soc 129:3339–3347

Chapter 4
Magnetic Nanoparticles for Magnetic Resonance Imaging and Hyperthermia Applications

Emil Pollert, Graziella Goglio, Stéphane Mornet and Etienne Duguet

Abstract Medicine provides an increasing interest for magnetic nanoparticles, thanks not only to the growing control of their chemical and morphological design and colloidal stabilization, but also to the increasing tendency to use magnetic fields in diverse medical areas such as radiology, neurosurgery, or oncology. This contribution focuses on their potential usefulness as contrast agents for magnetic resonance imaging (MRI) and colloidal mediators for magnetic fluid hyperthermia (MFH). A physical background of these nanoparticles magnetism is first considered discussing their behavior either under static magnetic field or an alternating one. The design and preparation of magnetic fluids is then described from the synthesis of nanoparticles up to their colloidal stabilization in physiological media. Requirements with regard to in vivo administration are subsequently presented, i.e., the factors affecting their biocompatibility, their biodistribution, the solutions envisaged for enhancing their half-life in the blood compartment, and the active targeting of tumor cells. Finally, magnetic nanoparticles are considered as contrast agents for MRI and mediators for MFH, highlighting the involved problems and the current and future possibilities for solving them.

E. Pollert
Institute of Physics ASCR, Praha, Czech Republic

G. Goglio · S. Mornet · E. Duguet (✉)
CNRS, University of Bordeaux, ICMCB, Pessac, France
e-mail: duguet@icmcb-bordeaux.cnrs.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_4,

4.1 Introduction

Magnetism and medicine have been sharing a fascinating common history for several centuries [1]. Some of the earlier applications of magnetic materials include removal of metallic objects from the bodies of animals and humans and the use of magnetite grains as antidote for the accidental swallowing of rust. In ayurvedic therapies, various kinds of iron oxides are still used as iron supplements for humans, especially children and pregnant women. More recently, miniaturization of electromagnets, development of superconducting electromagnets, and introduction of strong permanent magnets (Sm–Co and Nd–Fe–B) have stimulated the medical use of magnets in fields as diverse as radiology, dentistry, cardiology, neurosurgery, oncology, and immunoassays.

Moreover, well-calibrated magnetic nanoparticles (MNPs) in the size range 1–100 nm are currently produced in large quantities thanks to reproducible synthetic protocols. Several chemical routes allow modification of their surface for ensuring their colloidal stability in physiological media, protecting them from corrosion/dissolution, and making them less cytotoxic and derivatizable with biorelated molecules.

There are several reasons why MNPs are of particular interest in medicine. First, they are smaller than cells (10–100 m) and comparable to viruses (20–450 nm), proteins (5–50 nm), or genes (2 nm wide and 20–450 nm long). Therefore, they are able to circulate in the bloodstream (even in the thin lung capillaries), to cross-biological membranes, and to interact closely with biomolecules. Second, when their diameter is lower than about 10–15 nm, MNPs exhibit in the static regime at body temperature superparamagnetic behavior, i.e., without coercivity and remanent magnetization. Therefore, they are movable in magnetic field gradients and capable to create local magnetic fields. Consequently, they can be remotely manipulated in the "magnetically transparent" living tissues. Further, they may shorten the relaxation times of surrounding protons and generate heat due to magnetic losses when they are subjected to AC magnetic fields. Therefore, MNPs in a form of aqueous suspensions became recently inescapable tools for biologists, pharmacologists, and physicians. They are now routinely used as contrast agents in magnetic resonance imaging (MRI) and very soon clinical applications are expected in the field of cancer thermal treatment, namely magnetic fluid hyperthermia (MFH).

Even if some publications deal with oral MRI contrast agents for the gastrointestinal tract [2], the majority of published studies concern the intravenous administration route. The use of MNPs in the blood compartment depends on specific requirements concerning their plasma half-life and final biodistribution. The problem of the non-natural stealthiness of the nanoparticles toward the immune system, and the possibilities for resolving it, have been widely studied in the field of drug delivery from liposomes and polymeric nanoparticles [3]. Indeed, retention of drugs in circulation is a key step in the design of drug delivery carriers. The reason is obvious: even the most active compound in vitro is useless if in vivo it does not reside in the blood long enough to reach its target, while managing to

avoid, to some extent, premature metabolism, immunological reactions, toxicity, rapid excretion, and captation by undesired tissues [4]. Presently, many pieces of information are available concerning the immune system mechanisms, the factors affecting the biodistribution of nanoparticles, such as their size and shape, the hydrophobic/hydrophilic balance of their surface, their surface charge, and the solutions envisaged for targeting specific organs, tissues, or cells.

This review presents simultaneously the basic knowledge and an updated survey of the potential of MNPs for MRI and MFH applications through an understanding of the problems involved from the viewpoint of their overall requirements. One main intention of this contribution is to impart information about both the state of the art as well as the need for further progress and clinical development. Only typical examples among the most promising ones will be reported and discussed and a special emphasis will be made on cancer diagnosis and therapy. Only general references are provided for the reader to turn to for more information.

4.2 Magnetism of MNPs

4.2.1 Behavior in Static Magnetic Fields

4.2.1.1 Materials Magnetism Background

The magnetism of a solid is originated from the contributions of the constituting electrons, thanks to their quantum properties, that is, their spin angular moment and their orbital angular moment, which contribute to their magnetic moment. These electrons also determine the strength of the interaction between atoms in a solid, making the basis of the different macroscopic behaviors observed in nature. Massive materials can thus be classified into diamagnetic, paramagnetic, ferromagnetic, ferrimagnetic, and antiferromagnetic according to the arrangement of their magnetic dipoles in the absence and presence of an external magnetic field [5, 6].

If a magnetic material is placed in a magnetic field of strength H, the individual atomic moments in the material contribute to its overall response, the magnetic induction B:

$$B = \mu_0(H + M) \tag{4.1}$$

where μ_0 is the vacuum permeability, and the magnetization $M = m/V$ is the magnetic moment per unit volume, where m is the magnetic moment on a volume V of the material [7]. All materials are magnetic to some extent, with their response depending on their atomic structure and temperature. They may be conveniently classified in terms of their volumetric magnetic susceptibility χ, where

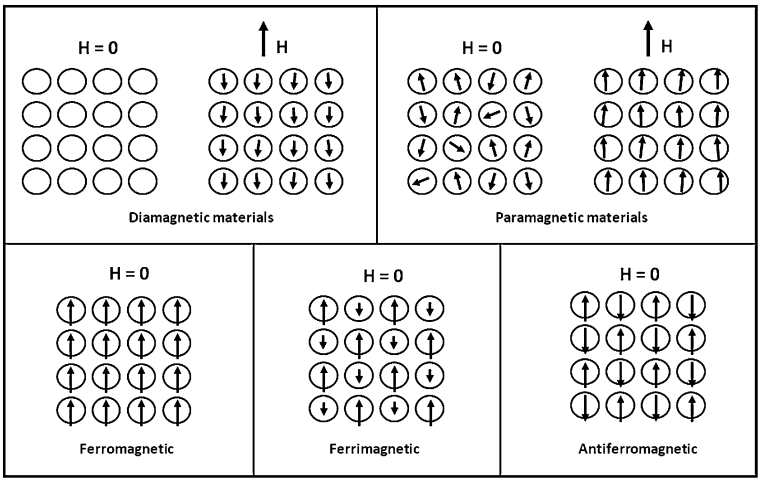


Fig. 4.1 Scheme illustrating the arrangements of magnetic dipoles for five different types of materials in the absence or presence of an external magnetic field (H). Redrawn from Ref. [8]

$$M = \chi H \tag{4.2}$$

describes the magnetization induced in a material by H.

Figure 4.1 shows schematic diagrams of these five different situations. If a material does not have magnetic dipoles in the absence of an external field and has weak induced dipoles in the presence of a field, the material is referred to as diamagnetic. The magnetization of a diamagnet responds in the opposite direction to the external field, within the range -10^{-6}–-10^{-3}. If a material has randomly oriented dipoles that can be aligned in an external field, it is paramagnetic. The magnetization of a paramagnet responds in the same direction as the external field ($10^{-6} < \chi < 10^{-1}$). The magnetic interactions derived from the above two types of materials are very weak because the thermal agitation at room temperature can make the magnetic moments to flip over continuously.

Some materials, however, exhibit ordered magnetic states and are magnetic even without an applied field. For a ferromagnetic material, the magnetic dipoles always exist in the absence and presence of an external field and exhibit long-range order (Fig. 4.1). Macroscopically, such a material displays a permanent magnetic moment and M is typically 10^4 times larger than it would appear otherwise. The difference in the source of the net magnetic moment can also be used to distinguish ferromagnetism from both ferrimagnetism and antiferromagnetism. In a ferrimagnetic material, there are always weaker magnetic dipoles aligned antiparallel to the adjacent, stronger dipoles in the absence of an external magnetic field. For an antiferromagnetic material, the adjacent dipoles are antiparallel in the absence of an external field and cancel each other.

That is why magnetic materials are referred to those characterized by either ferro- or ferrimagnetic features. Both ferro and ferrimagnetic materials can be

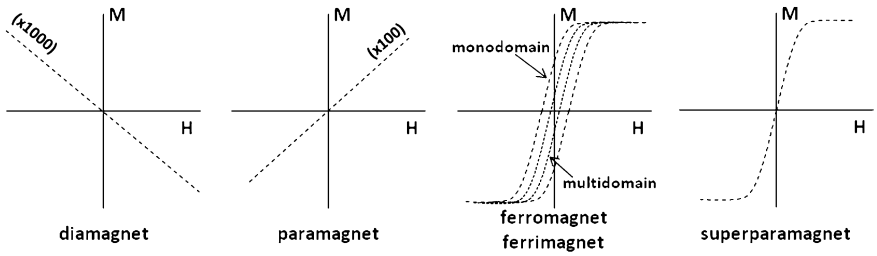


Fig. 4.2 Typical magnetization curves for the different types of magnetic materials

described using a number of basic parameters derived from the magnetization curve, where the magnetization M or flux density B is plotted against H (Fig. 4.2). Although a ferromagnetic material should have all its magnetic moments pointing in the same direction, a macroscopic piece of material cannot have this configuration because the amount of magnetostatic energy stored should be huge [9]. The way in which a solid can reduce this otherwise huge magnetostatic energy is to break itself up into regions called magnetic domains, or Weiss domains. Within a single domain, all magnetic moments remain parallel, but the individual domains are randomly oriented so that the net magnetic moment of the sample is nearly canceled (Fig. 4.3a). This situation generates interfaces between domains called domain walls or Bloch walls, where adjacent magnetic moments are in a nonfavorable configuration, so that these domain walls are highly energetic. Even though some energy is stored inside domain walls, the overall decrease in the total magnetic energy favors the multidomain configuration. Being formed by a competition between magnetostatic and exchange energies, domain walls have a finite width, determined by the ratio between these energies.

The susceptibility in ferro- and ferrimagnetic materials depends not only on temperature, but also on H, which gives rise to the characteristic sigmoidal shape of the magnetization curve (M–H curve), with M approaching a saturation value at large values of H (Fig. 4.2). Furthermore, one often sees hysteresis: it characterizes the irreversibility in the magnetization and demagnetization processes that are related to the pinning of magnetic domain walls at defects, dislocations, or vacancies within the material, as well as to intrinsic effects such as the magnetic anisotropy of the crystalline lattice. This gives rise to open M–H curves, called hysteresis loops. Actually, domain walls can move in response to an applied field: creation, growth, and extinction of domains can be induced by an external magnetic field because the external field imposes a preferred direction for the magnetic moments. For the spins in a given domain to change their orientation, it is required that the walls of that domain move. However, the magnetic field required to eliminate all domain walls (i.e., to align all magnetic moments in the same direction) has a definite value for a given sample, and is reproducible.

The saturation magnetization M_S (the maximum value of M), the remanence magnetization M_R (the residual magnetization at zero applied field strength), and

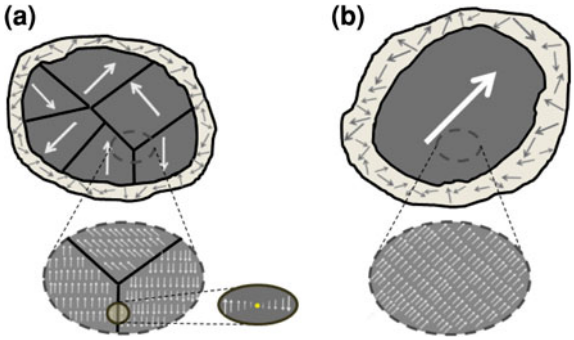


Fig. 4.3 **a** Schematic view of magnetic domains in a multidomain ferromagnetic particle having size larger than the critical diameter ($D > D_C$). For this particle, the whole material breaks down into randomly oriented magnetic regions. At the interface between domains, magnetic moments are twisted to fit the orientation at both sides of the domain walls. **b** Schematic view of a monodomain ferromagnetic particle having size smaller than the critical diameter ($D < D_C$). The spin disorder at the particle surface is represented by an annular region in both cases. Redrawn from Ref. [9]

the coercivity H_C (the external field required to reduce the magnetization back to zero) are deduced from the magnetization curve. A material is called a soft magnet if it can be magnetized readily in a weak field of $\sim$10 Oe (1 Oe = 1,000/4π Am^{-1}). On the other hand, hard magnets have very strong H_C and require large external fields applied in the opposite direction in order to be demagnetized.

Lastly, as the temperature increases, thermal motion competes with the tendency for the spins to align. When the temperature rises beyond a certain point, called the Curie temperature T_C, there is a second-order phase transition and the system can no longer maintain a spontaneous magnetization, although it still responds paramagnetically to an external field.

4.2.1.2 Magnetism of Nanoparticles

Magnetism is highly volume and temperature dependent because this property arises from the collective interaction of atomic magnetic dipoles [8]. When the size of a ferro- or ferrimagnet decreases to a certain critical value D_C, MNPs change from a state with multiple magnetic domains to one with a single domain (Fig. 4.3b). In that transition situation, H_C is maximal and the hysteresis loop is broad (Fig. 4.2). This critical size is achieved when the magnetostatic energy, which increases in proportion to the volume of the materials, becomes equal to the domain wall energy, which varies proportionally with the interfacial area between domains. For spherical and noninteracting MNPs, the critical size can be given as

$$D_C \approx \frac{18\sqrt{AK_{\text{eff}}}}{\mu_0 M_S^2} \tag{4.3}$$

where A is the exchange constant and K_{eff} is the effective anisotropy constant, i.e., a function of magnetocrystalline, shape and surface anisotropies. This critical size, which is generally of a few nanometers to a few tens of nanometers, varies from material to material.

The resulting magnetic behavior of an MNP with a size corresponding to a single magnetic domain depends on a competition between two energy contributions, i.e., on the terms $K_{\text{eff}}V$ and k_BT and the magnetic moment of the particle relaxes to its equilibrium position with a relaxation time τ_N given by the relation [10, 11]

$$\tau_N = \tau_0 \exp\left(\frac{K_{\text{eff}}V}{k_BT}\right) \tag{4.4}$$

where V is the particle volume, k_B is the Boltzman's constant, T is the absolute temperature, and τ_0 is a constant in the range of 10^{-9}–10^{-12} s.

Evidently due to a competition of the respective energetic contributions, two different situations have to be distinguished. Namely of $K_{\text{eff}}V \gg k_BT$, when the relaxation time $\tau_N \gg \tau_m$: the magnetic moment is stable and aligned parallel to the easy axis of magnetization and the MNP exhibits ferromagnetic or ferrimagnetic ordering. On the other hand, for a sufficiently small volume of the MNP, we have $k_BT \gg K_{\text{eff}}V$ and the relaxation time $\tau_N \ll \tau_m$. Then spontaneous fluctuations of the direction of magnetic moment between the two orientations along the (uniaxial) easy axis appear. It leads under an applied field to anhysteretic but still sigmoidal, M–H curves (Fig. 4.2) and the state of the system is called superparamagnetic.

The superparamagnetic state is displayed solely below the Curie temperature where the usual paramagnetic behavior starts. The border between the magnetically ordered and superparamagnetic state is, for a given particle volume, defined by the so-called blocking temperature T_B, evaluated from the zero field (ZFC) and field cooled experiments (FC) as the maximum in the temperature dependence of $d(\chi_\rho \text{ ZFC} - \chi_\rho FC)/\text{d}T$ [12].

4.2.1.3 Surface Effects

As the particles size decreases, a large percentage of all atoms in a nanoparticle are surface atoms, which implies that surface and interface effects become more important. Then an arising spin disorder and a reduced coordination of the broken exchange bonds lead to a spin-glass-like behavior of the surface spins or presence of canted spins on the outer surface layer of MNP (Fig. 4.3). Hence, a so-called magnetic dead layer arises and a decrease of the magnetization is observed. Another surface-driven effect is an enhancement of the magnetic anisotropy (K_{eff}) with decreasing particle size. Lastly, a magnetically inert surface coating, for example, silica or organic polymer, may modify the extent of the dipolar coupling between MNPs because its thickness controls the minimum distance between them. Overall, it must be concluded that the magnetic response of a system to an

inert coating is rather complex and system specific, so that no firm correlations can be established at present.

4.2.2 Behavior in AC Magnetic Fields

The situation in the static magnetic field, discussed in the preceding section, however changes under acting of an alternating field as it can be seen from the following relation deduced from the expression (4.4) for the relaxation time introduced above:

$$T_B = \frac{K_{\text{eff}} V}{C k_B} \tag{4.5}$$

The constant $C = ln(\tau_m/\tau_0)$ shows the sensitivity of the blocking temperature on the time window. It is equal to 27.6 for the DC measurements ($\tau_m = 100$ s) and decreases in the AC regime, e.g., down to the value of 10.8 for 480 kHz which is a frequency currently used in the magnetic fluid hyperthermia experiments. Therefore, the blocking temperature is shifted to higher temperatures and the field of the magnetically ordered state is extended in a corresponding way markedly towards higher temperatures. Evidently, it has an important influence on the magnetic losses decisive for the use of synthesized materials in magnetic fluid hyperthermia.

Returning to the hysteresis which gives rise to the open M–H curves seen for ferro- or ferrimagnets, it is clear that energy is needed to overcome the barrier to domain wall motion imposed by the intrinsic anisotropy and the pinning of magnetic domain walls in the material. This energy is delivered by the applied field and can be characterized by the area enclosed by the hysteresis loop. This leads to the concept that if a time-varying magnetic field is applied to a ferro- or ferrimagnetic material, one can establish a situation in which there is a constant flow of energy into that material, which is perforce transferred into thermal energy. A similar argument regarding energy transfer can be made for superparamagnets, where the energy is needed to coherently align the particle moments to achieve the saturated state [13].

4.2.2.1 Loss Mechanisms

Generally the power dissipation originating from the cyclic increase of internal energy produced by the magnetic work on the system is given by the relation

$$P = m_0 v \oint_H M \cdot \mathrm{d}H \tag{4.6}$$

where μ_0 is the permeability of free space and v the frequency of the AC field. Three different mechanisms can then be distinguished, namely hysteresis power losses, originating in the irreversibility of the magnetization process, Néel relaxation, conditioned by the rotation of the magnetic moments of the particles, and friction losses due to the Brownian rotation of the magnetic particles as a whole.

Hysteresis Losses

They are connected with the existence of irreversible magnetization loops and the power losses correspond to the relation derived from the general expression (4.6)

$$P = \mu_0 \pi \chi''(v) v H^2 \tag{4.7}$$

where χ'', the imaginary component of the susceptibility, is for polydisperse suspensions in a good approximation frequency-independent in the range of 100 kHz–1 MHz, currently used in MFH systems [14, 15].

Néel and Brownian Losses

Gradual decrease of the particle size leads to a transition to superparamagnetic state, where there is no more irreversible behavior characterized by the hysteresis loop and where the magnetic energy is converted to the thermal one by the rotation of the particle moment between two metastable antiparallel orientations, effectuated by two different ways, namely by the rotation of magnetic moments themselves (Néel rotation) and rotation of particles (Brownian rotation). The power dissipation can be described by similar functions as

$$P = \frac{\mu_0 \pi \chi_0 v^2 H^2 \tau_i}{[1 + (v \tau_i)^2]} \tag{4.8}$$

It is worth mentioning that the Néel relaxation time $\tau_i = \tau_N$ depends according to the relation (4.4) exponentially on the core volume (V), while the Brown relaxation time $\tau_i = \tau_B$ given by the relation (4.9)

$$\tau_B = \frac{3 \eta V_h}{kT} \tag{4.9}$$

involves the hydrodynamic volume of the particles (V_H) that may differ from that of the crystalline cores due to the presence of a stabilizing shell or due to agglomeration [16]. The resulting mean dependence of the corresponsive relaxation times is presented in Fig. 4.4. In realistic systems, the faster process dominates the overall remagnetization process, and for similar timescales, both mechanisms contribute as (4.10):

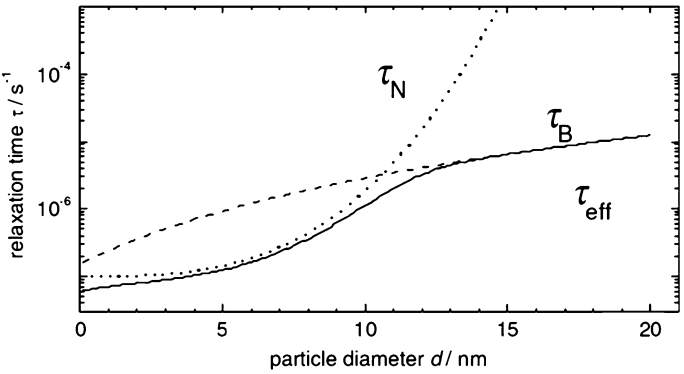


Fig. 4.4 Theoretical Néel (τ_N) and Brown (τ_B) relaxation times and effective relaxation time (τ_{eff}) for Fe_3O_4 magnetic nanoparticles versus particle diameter. Reprinted with permission from Ref. [16]. Copyright © 2007 Springer Science

$$\tau_{\text{eff}} = \frac{\tau_N \tau_B}{\tau_N + \tau_B} \tag{4.10}$$

It is then observed that below a critical size of the particles, the Néel loss mechanism is more effective while above this the Brownian loss mechanism plays a major role. Near to the critical size, both types of losses act equivalently.

The Brownian losses generally depend upon hydrodynamic size of the particles, but not on the size of the magnetic cores. Therefore, the type and thickness of coating over the magnetic materials can greatly influence heating. Lastly, in some situations, MFs based on ferro- and ferrimagnetic particles may also be concerned by Brownian losses.

4.2.2.2 Specific Absorption Rate

The heating ability of Magnetic Fluids (MFs) in the presence of AC magnetic fields can be estimated by colorimetric method and it is termed as specific absorption rate (SAR). SAR is estimated on the basis of direct observation of temperature rise and can be defined as the amount of heat generated in a second by one gram of magnetic element:

$$\text{SAR}_T = C \frac{m_S}{m_M} . \frac{\text{d}T}{\text{d}t} \tag{4.11}$$

where C is the specific heat capacity of the MF (or water in the case of diluted dispersions), dT/dt is the slope of temperature versus time graph at the considered temperature T, m_S is the mass of MF ,and m_M is the mass of the magnetic element in MF.

Concerning the electromagnetic devices used for colorimetry experiments, the technology of an AC magnetic field is still under development. Most experiments

were done with laboratory-made generators in the frequency range of 50 kHz to 1 MHz, with magnetic field amplitudes up to a few tens of kAm^{-1}. These parameters depended more on the technical availability of the generators used rather than on theoretical predictions for optimized SAR. Indeed, a frequency scan is technically tricky in this broad frequency range because of frequency-dependent skin effects and resistance of magnetic applicators. The majority of hyperthermia experiments were performed in an induction coil or in the air-gap of a magnetic inductor, cooled by water or air.

For clinical purposes, the heat generation, and hence, the SAR value is very crucial, the higher the value, the lower the injected dose. However, the SAR values measured by various scientists differed because of their strong dependence on factors such as the physical and chemical properties of the carrier fluid, the frequency and amplitude of the applied field, and the size, shape, surface coating, and chemical composition of the MNPs [17]. Therefore, in order to facilitate a comparison of various materials, two approaches were recently proposed and introduced.

The first approach is based on the use of the intrinsic loss parameter (ILP) independent of the frequency and amplitude of the AC magnetic field defined as

$$\mathrm{ILP} = \frac{\mathrm{SAR}}{H^2\, v} \tag{4.12}$$

Further, it is an effort to minimize the losses of heat arising due to the temperature gradient between the sample and its environment. Its elimination is achieved by a specific adiabatic shield heated continuously to the temperature equal to that of the sample [18, 19].

A simpler possibility to deal with the problem is based on the independent determination of the thermal losses [20] according to the relation

$$\mathrm{SAR}^{T}_{\mathrm{corr}} = \frac{1}{m_{\mathrm{magn}}}\left(-\frac{\mathrm{d}Q}{\mathrm{d}t}\right)^{T} + \mathrm{SAR}^{T}_{\mathrm{meas}} \tag{4.13}$$

where m_{magn} is the mass of the magnetically active matter and $\mathrm{d}Q/\mathrm{d}t$ are the thermal losses, i.e., the heat flow out of the sample. Nevertheless, let us note that the actual condition in the application of MFH may be even more complicated when the particles are placed in a moving medium, e.g., blood flow.

4.3 MF Design and Preparation

4.3.1 Synthesis of MNPs

MNPs can be synthesized by a wide variety of techniques ranging from mechanical (top-down approach) to chemical processes (bottom-up approach). For

in vivo applications, chemical processes are generally preferred, because they allow a better control of the purity, crystallinity, and size distribution. These processes include aqueous coprecipitation, thermal decomposition, sonochemistry, microemulsion, sol–gel, self-combustion, microwave refluxing, etc., for the synthesis of maghemite, magnetite, substituted iron oxides ($\gamma-M_xFe_{2-x}O_3$ or $M_xFe_{3-x}O_4$, where M = Al, Mn, Mg, Zn, Ni, Co, and Cu), metallic iron or cobalt and alloys such as FePt. For a detailed description of these synthetic routes, the reader will report on general review papers [8, 21–23] or review papers more specifically dedicated to iron oxide MNPs for biomedical applications [24–27].

These synthesis processes have their own benefits, but the achieved features of the MNPs depend strongly on the control of the experimental conditions. For instance, coprecipitation is a simple technique, but the type of salts (e.g., chlorides, sulfates, and nitrates), the cation oxidation states, the reaction temperature, the pH value, and ionic strength of the media greatly influence the size, shape, and composition of the final particles. Moreover, the morphology and size of the nanoparticles synthesized by thermal decomposition process greatly depend upon the ratios of the organometallic precursor, solvent, and surfactant, the temperature, and the reaction duration.

4.3.2 Colloidal Stabilization in Physiological Medium

Like other dispersions, aqueous MNPs dispersions are subjected to a number of destabilizing forces that can lead to phase separation due to flocculation and sedimentation [16].

One of the main causes of phase separation is the Van der Waals interactions between the high-surface particles that result in the formation of agglomerated material. In contrast to this, the sedimentation of single particles by gravitational force is prevented by thermal motion in the size range up to 20 nm. However, formation of small agglomerates due to the Van der Waals interactions significantly reduces thermal motion, and sedimentation may play an important role in the destabilization of a magnetic colloid.

In addition, the magnetic dipolar particles possess magnetic interactions that may be further enhanced by alignment of the dipoles in relation to their relative distance in outer magnetic field gradients [28]. Therefore, the colloids have to be stabilized against agglomeration by creating a protecting shell for each particle that prevents the particles from coming too close in contact with each other. This is possible using classical concepts from colloid chemistry, including electrostatic stabilization and steric stabilization.

As an example of electrostatically stabilized MFs, maghemite-based cationic fluids are easily prepared according to the method described by Massart [29]. In this process, magnetite nanoparticles are initially prepared by alkaline coprecipitation of iron (II) and iron (III) precursors in aqueous solution with an excess of concentrated ammonium hydroxide. Magnetite is oxidized into maghemite by heating in the

presence of HNO_3 and iron (III) nitrate. Maghemite particles are peptized in dilute HNO_3 solution to create positive surface charges on the particles. Washing and decantation with acetone using a magnet can remove the acidic precipitates. Simply by dispersing these particles into water produces aqueous MFs.

For in vivo applications, surface-charged nanoparticles are not advised and steric stabilization is generally preferred to electrostatic stabilization for physiological reasons (see Sect. 4.4.2). That is why the MFs are often prepared into aqueous solution of hydrophilic macromolecules or such macromolecules are grafted onto MNPs in a second step.

4.4 Size/Surface Requirements with Regard to i.v. Administration

4.4.1 Biocompatibility

Similar to other biomaterials, the biocompatibility of MNPs is assessed successively in vitro with animal or human cells, followed by in vivo tests with animals, before human clinical trials [30, 31].

For in vitro cytotoxic assays, the animal or human cells are generally grown on the culture plates. Well-grown cells are then treated with bare or surface-modified MNPs or MFs for several hours (up to 96 h). The effect of the nanoparticles on the cells is then estimated by the comparative study of treated and untreated cells (control) by visual counting of the viable cells. More sophisticated cell viability assays are generally performed. MTT assay is based on the reduction of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan by succinate dehydrogenase. The formazan product is then quantified spectrofluorometrically, and hence, directly correlated to the activity of mitochondrial reductase enzymes. In sulforhodamine-B (SRB) assay, SRB binds to the basic amino acids of the cellular proteins. Thus, colorimetric measurement of the bound dye provides an estimate of the total protein mass that is related to the cell number. The distribution of MNPs on the cells can also be estimated by staining the treated or untreated cells with Pearl's Prussian blue (which stains mainly Fe^{3+}) or with Turnbull's reagent (which stains Fe^{2+} ions).

Animals such as mice, rabbits, dogs, and sheep are often chosen for experimental in vivo studies. The animals are grown under the prescribed guidelines of the animal ethics committee. The required dose of MF is then injected into the body of the animal and all the clinical changes are observed very carefully. For various time periods, blood samples are collected for hematological and serum biochemical analysis. During hematological analysis, the amount of hemoglobin, platelets, erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes, basophiles, eosinophils, and hematocrit are generally compared with that of the untreated animal. In serum biochemical analysis, serum level of total protein,

bilirubin, albumin, glucose, cholesterol, alanine transaminase, aspartate transaminase, triglyceride, blood urea nitrogen, phosphorus, creatinine, calcium, and lactic acid dehydrogenase are compared with those of normal untreated animals.

It seems difficult to assert today which nanoparticles are toxic and which ones are not. MNPs absorption, distribution, metabolism, excretion, and toxicity depend on multiple factors derived from inherent physicochemical properties such as concentration, size, surface charge, nature of the surface chemical groups, and redox activity. First, the toxicity of particles that may dissolve in physiological conditions even at a very low rate has to be considered also from the viewpoint of the toxicity of the released metal cations. In such situations, endogenous chemical elements such as iron are preferred because the soluble iron becomes part of the normal iron pool (e.g., ferritin, hemosiderin, transferrin, hemoglobin). The lethal dose LD_{50} of pristine iron oxide nanoparticles is 300–600 mg_{Fe} per kilogram of body mass. A barrier coating may be engineered around each nanoparticle in the case of toxic metal elements. Second, in the case of insoluble particles, surfaces matter more than chemical composition, and efforts are in progress for embedding MNPs in biocompatible coating, often made of hydrophilic macromolecules and/or silica.

It is obvious that the MNPs toxicity is directly related to the concentration required for the diagnostic or therapeutic effect. Once the active targeting strategies (see Sect. 4.4.3) become efficient, and hence, allow concentrating MNPs essentially in the targeted organs, MNPs nontoxicity will become a less acute requirement [41].

4.4.2 Biodistribution and Passive Targeting Strategies

As soon as bare particles are, intentionally or otherwise, injected into the blood compartment, they are subjected to the action of the mononuclear phagocyte system (MPS) [32, 33]. It is defined as the cell family comprising bone marrow progenitors, blood monocytes, and tissue macrophages (such as Kupffer cells in liver). These macrophages are widely distributed and strategically placed in many tissues of the body to recognize and clear senescent cells, invading microorganisms or particles. The first step of the clearance mechanism is the opsonization process. Opsonins are circulating plasma proteins (various subclasses of immunoglobulins, complement proteins, fibronectin, etc.), that adsorb themselves spontaneously onto the surface of any invading entity. They are capable of interacting with the specialized plasma membrane receptors on monocytes and macrophages, thus promoting particle recognition by these cells. The second step consists of the endocytosis/phagocytosis of the particles by the circulating monocytes or the fixed macrophages, leading to their elimination from circulation and their simultaneous concentration in organs with high phagocytic activity. Therefore, after *i.v.* administration, particles are cleared up within minutes from the bloodstream; their typical final biodistribution is 80–90 % in the liver, 5–8 % in the spleen, and 1–2 % in the bone marrow [4]. Consequently, the remarkable

organization of the immune system is not compatible with long circulation times of any invading nanoparticle and MPS-mediated clearance is a major factor in determining their biodistribution. Nevertheless, it has provided an opportunity for the efficient delivery of therapeutic agents to these phagocytic cells, and therefore, to the related organs and more generally to any area where the macrophage activity is high, for example, infection/inflammation regions. Such an MPS-mediated targeting is called passive targeting.

If monocytes and macrophages are not the desired target, minimizing or delaying the nanoparticle uptake by the MPS is mandatory. The most satisfactory strategy consists of designing macrophage-evading nanoparticles (stealth nanoparticles) with plasma half-life as long as possible, in order to increase the probability to attain the desired target. The more efficient route is to prevent the opsonin adsorption and therefore to avoid the macrophage recognition. Among the physicochemical factors which are known to affect the opsonization process, the size, the surface charge density, and the hydrophilicity/hydrophobicity balance have been widely studied [32]. Therefore, the smaller the more neutral and the more hydrophilic the carrier surface the longer its plasma half-life.

In particular for hydrophobic particles, many studies concerned the development of core–corona structures where the corona is made of hydrophilic macromolecules for creating polymer brushes, acting as a steric surface barrier and reducing opsonin adsorption. Among the natural or artificial macromolecules, linear dextrans and derivatives are widely used. Other biological macromolecules have been investigated, for example, poly (sialic acid), heparin, and heparin-like polysaccharides complement regulatory proteins, etc., but because of their high cost and/or the possible immunological consequences associated with bacterial-made macromolecules, efforts have been directed to design synthetic hydrophilic macromolecules. In particular, great efforts have dealt with the covalent anchorage of poly (ethylene glycol) (PEG) macromolecules onto the particle surface. This process is so widely used that it is called 'PEGylation.' In fact, PEG is a flexible polyether, hydrophilic (but also soluble in some organic media), not biodegradable, but easily excreted from living organisms. Its functional end-groups are available for derivatization leading to numerous routes for covalent attachment onto preformed functional surfaces or anchoring during the synthesis of particles. PEG has been shown to be the most effective polymer for suppressing protein adsorption, the optimal molecular weight varying between 2,000 and 5,000 $g.mol^{-1}$ [32]. These values are still in debate, and in particular, the surface density appears to be as important as the molecular weight [33].

An escape of the macrophage-evading particles from the circulation is normally restricted to sites where the capillaries have opened fenestrations as in the sinus endothelium of the liver or when the integrity of the endothelial barrier is perturbed by inflammatory processes (e.g., rheumatoid arthritis, infarction, infections) or by some types of tumors. Therefore, the idea of exploiting such vascular abnormalities for extravasating and accumulating nanoparticles in these inflammatory sites or tumors is also particularly attractive. This strategy is also considered as a passive targeting one, but independently of the MPS mediation.

4.4.3 Active Targeting Strategies

In order to increase the probability to redirect long-circulating particles to the desired target, their surface has to be labeled with ligands that specifically bind to surface epitopes or receptors on the target sites (molecular recognition processes such as antibody–antigen interactions). Such a strategy should open the possibility of targeting specific cell types or subsets of cells within the vasculature and even elements of vascular emboli and thrombi [33–36]. In the case of cancer therapy, active targeting could allow the selective destruction of the cancer cells, even if they have escaped the tumor mass and disseminated as metastatic cells. These ligands must be coupled to the surface of stealth carriers. They include oligosaccharides, oligopeptides, folic acid, antibodies, and their fragments. As an example of tumor targeting, folic acid (vitamin B essential for cell division processes) may be used to take advantage of the frequent overexpression of folate receptors onto the surface of human cancer cells [37].

4.4.4 Surface Activation and Bioconjugation

In particular for the active targeting purpose, the surface of MNPs shall be derivatized. The process of proper linkage of biomolecules with nanoparticles under mild conditions is commonly called bioconjugation. Two main approaches to attach biomolecules on the surface of MNPs are physisorption and chemisorption. In physisorption, biomolecules directly attach to the surface of particles owing to weak interaction (electrostatic or Van der Waals forces), whereas, in chemisorption, biomolecules attach by the formation of covalent chemical bonds.

Oxide or metal nanoparticles themselves may not be capable of covalent attachment to biomolecules owing to their poor surface chemical reactivity. Hence, surface activation is required to create the chemical functions needed for coupling biomolecules to MNPs. The direct surface modification of metal oxides generally uses carboxylic acids, alkyltrialkoxysilanes, or alkyl phosphonates. Reactive function such as amino, thiol, aldehyde, or carboxylic group are generally present on the alkyl fragment and become the main surface groups. A coupling agent is generally required for linking this surface function with biomolecules (Table 4.1).

In numerous situations, the direct coupling with the inorganic surface is not preferred and one alternative strategy consists in taking advantage of the intrinsic reactivity of the hydrophilic macromolecules used for embedding and sterically stabilizing MNPs.

Table 4.1 Non-exhaustive list of coupling strategies of biomolecules on the activated surface of MNPs

Surface reactive function available on MNPs	Reactive function available on biomolecules	Possible coupling agent
-COOH	$-NH_2$	sulfo-N-hydroxysuccimide
$-NH_2$	-COOH	sulfo-N-hydroxysuccimide
$-NH_2$	$-NH_2$	glutaraldehyde
$-NH_2$	-SH	sulfo-SMCC[a]
-SH	$-NH_2$	sulfo-SMCC[a]
-OH	$-NH_2$	carbonyl diimidazole

[a] *SMCC* succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate

4.5 MNPs as Contrast Agents for MRI

4.5.1 MRI Background

Medical applications of MRI have steadily widened over the past decade and MRI is now one of the preferred cross-sectional imaging modalities [7, 38]. The principle of MRI is based on the behavior of the hydrogen protons present in a tissue under an applied transverse radiofrequency pulse perturbing their spins from the ordered, aligned state established initially by the applied magnetic field, B_0. The subsequent return of the spins to the original state is denoted as the relaxation phenomenon. In fact, it consists of two independent processes, namely longitudinal relaxation which is the return of longitudinal magnetization in alignment with B_0 and is termed T_1-recovery and transverse relaxation which is the vanishing of transverse magnetization and is termed T_2-decay. As a time constant, T_1 is the time which is required for 63 % of the longitudinal magnetization to recover in the tissue. In contrast, T_2 decay is not a process of dissipation or absorption of energy into tissue. During the *rf*-pulse, hydrogen nuclei are spinning in phase with each other. After the *rf*-pulse, the magnetic fields of all the nuclei interact with each other; energy is exchanged between those nuclei. The nuclei lose their phase coherence and spins become arranged randomly. Time constant T_2 is the time which is required to decrease the transverse magnetization to 37 % of its initial value.

In order to correlate the signal to its spatial origin, at least one of the two fields (i.e., B_0 or the *rf*-field) has to vary over space. Relaxation data are collected by a computer which applies a two-dimensional (2D) Fourier transform to give the amplitudes of NMR signals and permits reconstruction of the 3D images. Thanks to sequence parameters such as the repetition time *TR* (elapsed time between successive *rf*-excitation pulses) and the delay time *TE* (time interval between the *rf*-pulse and the measurement of the first signal), the operator obtains the desired type of image contrast. Basically, short *TRs* increase T_1 effects, whereas long *TRs* allow tissues to reach complete longitudinal magnetization, reducing T_1 effects. Short *TEs* minimize T_2 effects of tissues, whereas long *TEs* allow the loss of

transverse signal, enhancing T_2 effects. Therefore, T_1-weighted imaging is obtained by utilizing a short *TR* and a short *TE*, allowing full recovery of tissues with a short T_1 (e.g., fat) while allowing only partial recovery of tissues with long T_1 (e.g., cerebrospinal fluid). On the other hand, for T_2-weighted imaging, long *TRs* and long *TEs* are used. Fluids have a very long T_2 and they are frequently associated with pathologies (e.g., internal injuries, cancer lesions), so T_2-weigted images are generally preferred for such diagnostics.

4.5.2 MR Contrast Agents

In actual practice, tissues may be differentiated on MR images because T_1 and T_2 depend on proton environment. However, in many clinical situations, these intrinsic differences are small and exogenous contrast media are sometimes required for a better delineation of tissues. An ability to increase the relaxation rates of surrounding proton spins is called relaxivity $r_{1,\,2}$ and defined as:

$$\left(1/T_{1,2}\right)_{\text{in presence of contrast agent}} = \left(1/T_{1,2}\right)_{\text{without contrast agent}} + r_{1,2}[\text{contrast agent}]$$

where *[contrast agent]* is the contrast agent concentration in millimolars.

The first generation of these contrast agents consisted of high spin paramagnetic ions, usually Gd^{3+} in very stable nontoxic chelate form obtained through complexation by low molecular weight chelating molecules, such as diethylenetriaminepentaacetic acid [39]. Gd-chelates have an extracellular distribution before their excretion by the kidneys. Hydrogen atoms of water in proximity to such chelates experience a faster T_1-relaxation ($r_1 = 3.7\ \text{mM}^{-1}\text{s}^{-1}$). Consequently, differences in agent concentration result in contrast enhancement on T_1-weighted images ('positive' contrast). They have to be administered in concentrations of about 0.1 mmol per kilogram of body mass to produce visible effects on the images. Gd-chelates are routinely used for distribution into the intravascular and interstitial space to enhance signal of fluid compartments or lesions (renal function, status of the blood–brain barrier, etc.). Their main disadvantages are their short plasma half-life and their relatively low relaxivity.

Superparamagnetic MNPs have also been developed as MR contrast agents because of their larger magnetic moment in the presence of B_0 (susceptibility agents) [2, 40]. Therefore, their relaxivity is significantly higher ($r_1 = 10$–$15\ \text{mM}^{-1}\text{s}^{-1}$ and $r_2 = 40$–$190\ \text{mM}^{-1}\text{s}^{-1}$) than that of Gd-chelates. In most situations, they are used for their significant capacity to produce predominantly T_2 relaxation effects, which result in signal reduction on T_2-weighted images ('negative' contrast). Basically, the phenomenon may be described by the large magnetic field heterogeneity around the nanoparticle through which water molecules diffuse. This predominant effect on the T_2 relaxation time does not prevent their use on the T_1 relaxation time on the condition of low concentrations and appropriate imaging sequences.

This new generation of contrast agents is often called (U)SPIO for (Ultrasmall) SuperParamagnetic Iron Oxide. They consist of nonstoichiometric iron oxide cores (3–10 nm in diameter) whose chemical composition varies continuously from Fe_3O_4 to γ-Fe_2O_3. For *i.v.* administration, they are generally synthesized in a one-step process by alkaline coprecipitation or iron (II) and iron (III) precursors in aqueous solutions of hydrophilic macromolecules, essentially dextran or carboxy-dextran. The role of this macromolecular corona is the limitation of the magnetic core growth during the synthesis, their steric stabilization in water (and later in physiological medium) and in vivo the reduction of the opsonization process. Interactions between magnetic cores and macromolecules are weak (essentially Van der Waals and hydrogen interactions) and generally prevent any efficient derivatization of dextran corona without macromolecule depletion.

(U)SPIO pharmacokinetics, biodistribution, and toxicity properties were studied and allowed to define the potential uses of these contrast agents. The lethal dose LD_{50} of a dextran–iron oxide complex was found to be 2,000–6,000 mg_{Fe} per kilogram of body mass, whereas it is 300–600 mg_{Fe} per kilogram for pristine iron oxide. (U)SPIO have in common their specific uptake by the MPS, explaining why, if they are not entirely captured by the liver and the spleen, they are currently widely evaluated as MRI contrast agents for the diagnosis of inflammatory and degenerative disorders associated with a high macrophage phagocytic activity. The nanoparticles are metabolized in lysosomes and it may be noticed that the iron load resulting from administration of the clinical dose ($\sim$1 mg_{Fe} per kilogram of body) is low compared with the total store in the human body (about 3,500 mg).

4.5.3 Liver MR Imaging

SPIO, with a hydrodynamic diameter over 50 nm (up to 150 nm), are efficiently accumulated in MPS-organs ($\sim$80 % of the injected dose in liver and 5–10 % in the spleen with plasma half-life lower than 10 min.). Therefore, SPIO decrease liver and spleen signal within several minutes after *i.v.* administration. Malignant tumors or metastases as small as 2–3 mm, which are typically devoid of a substantial number of phagocytic Kupffer cells, appear as hyperintense (bright) lesions contrasted against the hypointense (black) liver on T_2-weighted sequences. The first SPIO, Endorem® (Guerbet, France), was marketed in Europe more than 15 years ago.

4.5.4 Metastatic Lymph Node MR Imaging (MR Lymphography)

Nodal disease is an independent adverse prognostic factor in many types of cancer. Thanks to their smaller size (lower than 40 nm) and the hydrophilicity of their

dextran corona, USPIO may act as stealth particles; their plasma half-life is higher than 20 h and some particles leak to the interstitium, where they are cleared by the macrophages of the lymphatic system or drained via the lymphatic system and subsequently accumulated in the lymph nodes. Hence, they allow diagnosing hyperplastic and tumorous lymph node by MR lymphography. A decrease in signal intensity indicates active uptake of particles into macrophages in normally functioning nodes, whereas metastatic nodes remain isointense with the precontrast image (Fig. 4.5). Clinical trials have reached phase III using Sinerem® (Guerbet, France) for the detection of lymph node metastases (head and neck and pelvis regions) [2].

4.5.5 Further Clinical Applications in Progress

Because USPIO are taken up by the liver and spleen more slowly than SPIO, they remain in the blood circulation for a longer time. On the other hand, USPIO have a more favorable T_1/T_2 ratio that facilitates T_1-weighted MR techniques at low B_0 (lower than 0.5 T). Thus, USPIO significantly shorten the T_1 of blood during the first hours after *i.v.* injection and may be used as T_1-agent. Thanks to these combined features, Sinerem® has been developed as MR blood pool agents for indications such as evaluation of cerebral perfusion, myocardial or renal perfusion, angiography or detection of hepatic vascular lesions [2].

Animal experiments and clinical studies are currently dedicated to USPIO-tagged macrophage imaging in several inflammatory and degenerative diseases. This modality could be used not only primarily for disease detection, but also for prediction and follow-up of the therapeutic efficacy [2]. Encouraging results were obtained in the case of cerebral ischemic lesions and brain inflammations, atheromatous plaques prone to rupture, multiple sclerosis, kidney disease, arthritis, infection caused by Staphylococcus aureus, and kidney and cardiac graft rejection. Moreover, USPIO are now reputed to be useful to evaluate (i) areas of blood–brain barrier dysfunction related to tumors and other neuroinflammatory pathologies, (ii) the cerebrovasculature using perfusion-weighted MRI sequences, and (iii) in vivo cellular tracking in central nervous system disease or injury [43].

Molecular MR imaging is one of the main MRI developments in progress, because clinicians dream about a contrast agent that would accumulate highly and specifically in malignant tumors, allowing an accurate diagnosis at a stage when the disease would be still treatable. Such tools would also be of a great interest for cardiovascular, inflammatory, and degenerative diseases. It concerns tissue-specific MR contrast agents [39, 44, 45]. Ligand-labeled (U)SPIO were designed (e.g., human polyclonal IgG, anti carcinoembryonic antigen, anti-glioma, L6 antibody, anti-epidermal growth factor receptor) and investigations carried out in small animals revealed the possibility to achieve a distinct concentration of the magnetic label at the target. However, the required dose of the labeled antibody is still too high to make a commercial development realistic. That is why the use of small

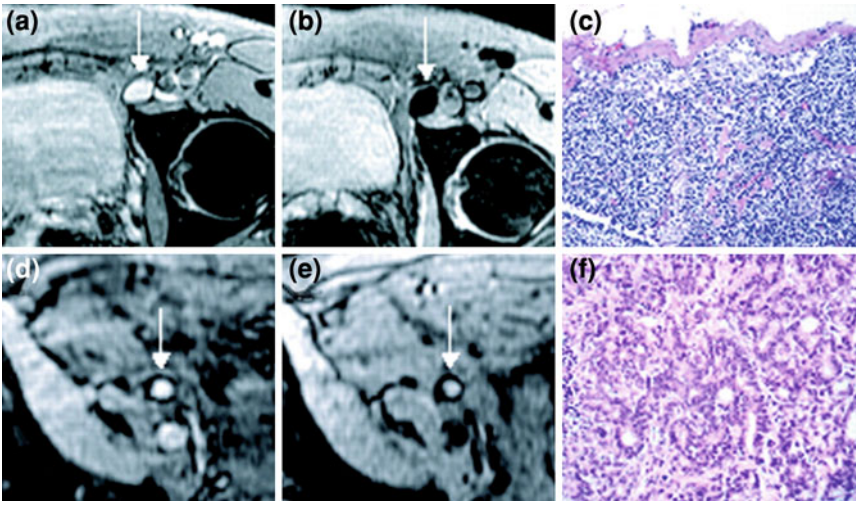


Fig. 4.5 Magnetic resonance imaging nodal abnormalities in two patients with prostate cancer. As compared with conventional MRI (Panel **A**), MRI obtained 24 h after the administration of USPIO (Panel **B**) shows a homogeneous decrease in signal intensity due to the accumulation of lymphotropic superparamagnetic nanoparticles in a normal lymph node in the left iliac region (*arrow*). Panel **C** shows the corresponding histologic findings (hematoxylin and eosin, x125). Conventional MRI shows high signal intensity in an unenlarged iliac lymph node completely replaced by tumor (*arrow* in Panel **D**). Nodal signal intensity remains high (arrow in Panel **E**). Panel **F** shows the corresponding histologic findings (hematoxylin and eosin, x200). Reprinted with permission from Ref. [42]. Copyright © 2003 Massachusetts Medical Society. All rights reserved

ligands, in particular, folate-mediation, appears more realistic for tumor MRI diagnostic. Lastly, spatial resolution is the key parameter for the detection of low concentrations of ligand-labeled (U)SPIO and the development of high magnetic field (up to 7 T) would allow achieving a high spatial resolution while maintaining a satisfactory signal-to-noise ratio.

The development of stem cell-based therapies requires quantitative and qualitative assessment of stem cell distribution to target organs (in the case of systemic administration), differentiation outcome, and engraftment. If labeled with MR contrast agents, they could be visualized and tracked by MR imaging. In the reported experiments, stem cells are generally labeled in vivo by using eventually a transfection agent (e.g., protamine sulfate, polylysine, cationic liposome, or cationic dendrimers) for USPIO, which are less efficiently internalized than larger SPIO. Labeled cells showed a gradual decline of intracellular iron oxide particles with increasing time after incubation. This effect can be attributed to cell division and exocytosis or release of iron from nonviable cells. Several experiments on animal models were reported and recently reviewed [2].

4.5.6 Next Developments

Even if (U)SPIO contrast agents are now routinely used in hospitals, no precise structure/activity relationship has been described to fully predict their stability, as well as their pharmacokinetics and safety [25]. An extended physicochemical characterization is required but remains difficult owing to their small dimensions, reaching the analytical resolution limits of many commercial instruments.

In addition to the clinical developments presented in the previous sections, current efforts are concerning the improvement of the (U)SPIO platforms. In particular, their chemical stability is insufficient, if dextran macromolecules must be derivatized for bioconjugation (see Sects. 4.5.2 and 4.5.5). Improvement may be achieved when dextran macromolecules are cross-linked in a second step for enhancing the mechanical entrapment of magnetic cores [46] or when they are chemically bonded to the magnetic cores through the use of coupling agents [47].

In order to improve the MR contrast and/or decrease the magnetic core size, metal MNPs are currently investigated. Indeed, because of their higher saturation magnetization, larger effects on proton relaxation are expected. Moreover, below $\sim$8 nm, inorganic nanoparticles can be readily excreted from the body by renal clearance [48]. For instance, cobalt, iron or FePt MNPs were recently obtained and evaluated as T_1 or T_2 contrast agents. Preliminary results were promising, nevertheless, chemical stability against oxidation, surface chemistry for bioconjugation, and toxicity/pharmacokinetics concerns shall be reconsidered in comparison to the now well-known (U)SPIO. Enhanced MRI sensitivity was also achieved with $MnFe_2O_4$ MNPs that displayed the highest magnetic susceptibility and the strongest r_2 relaxivity value of 358 $mM^{-1}s^{-1}$ (to compare to 40–190 $mM^{-1}s^{-1}$ for conventional (U)SPIO). They would enable the MR detection of tumors as small as 50 mg ($2 \times 5 \times 5$ mm^3). More recently, thermal decomposition method allowed to prepare a series of 15 nm sized Zn^{2+} doped nanoparticles of $(Zn_xMn_{1-x})Fe_2O_4$ [49]. It was shown that for $x = 0.4$ the r_2 value of ferrite was 13.8 and 7.8 times larger than those of conventional iron oxide-based contrast agents, respectively.

Lastly, a significant development for the future of magnetic imaging in the human body concerns a new imaging modality, magnetic particle imaging (MPI), initially developed by Philips Research [48]. The technique takes advantage of the nonlinear magnetization curve of small magnetic particles to generate harmonic responses to time-varying fields that can be detected using standard lock-in methods to a high degree of precision, and with a very low background signal to contend with. The imaging capability is the result of the following concept: in the presence of a large enough DC magnetic field, the magnetization curve is flat, and as such, the harmonic signals disappear. The corollary of this is that if one applies a DC field to all but a small 'field-free point' on the sample, the only harmonic signal received comes from that field-free point, and all other signals are damped out. MPI has great potential for medical applications such as vascular or small intestine imaging, where fast dynamic information is required, and the targets are

located relatively deep below the skin, the latter because the MPI signal is virtually unattenuated by intervening tissue.

4.6 MNPs as Mediators for Magnetic Hyperthermia

4.6.1 Hyperthermia Modalities

The choice of therapies against cancer such as surgery, radiation therapy, chemotherapy, hormone therapy, or biological therapy depends to a great extent upon the type and location of the cancer, the stage of the disease, age, and general health of the patient. Each has its own limitations, as they cause severe side effects to the surrounding tissues in the case of the localized therapy and to the whole body in the case of systemic therapy. Heat can be applied locally or to the whole body according to the requirements and exhibit low side effects. Further, both in vitro and in vivo studies and clinical experiments showed improved results when hyperthermia is applied in combination with other therapies such as chemo or radiotherapy [50].

As the name indicates, the hyperthermia treatment is generally carried out in the temperature range of 42–46 °C, which is above physiological growth temperature (for humans, it is above 37 °C). This is termed as mild hyperthermia. In this temperature range, tumor hypoxic (poorly oxygenated) cells are more heat-sensitive compared to the normal euoxic (well oxygenated) cells. Hence, the normal cells survive, while the cancerous cells get killed; this creates an interesting way for the therapy of cancer with minimal side effects. In addition, the cancerous tissues have inadequate blood supply, and thus, are not able to dissipate heat energy at the same rate as that of normal ones. Hyperthermia is applied sometimes above 46 °C and it is termed as thermoablation. The effect of hyperthermia exclusively depends upon the treatment temperature and on the exposure time at that temperature. Interestingly, it has been observed that a temperature decrease of 1 °C in the temperature range of 42.5–47 °C required doubling of the exposition time, whereas below 42.5 °C, the heat exposure time has to be prolonged by four times to get the same effect.

Hyperthermia during in vitro studies can cause several morphological changes in the cells and can detach them from the culture flask and finally kill them [51]. The mechanism of heat-induced cell death causes numerous cellular changes such as in the cell membrane, pH, nuclear and cytoskeleton structures, cellular metabolism, macromolecular synthesis, intracellular signal transduction in hormone-receptor interactions, expression of the heat shock proteins, chromatin organization, and synthesis of DNA and RNA. The degeneration of mitochondria, enzymes, and various kinds of proteins also get induced by hyperthermia.

The cell death due to hyperthermia could be classified either by apoptosis or necrosis. Apoptosis is an orderly process of programmed cell death and can be

characterized by distinct morphological features such as nuclear condensation, membrane blebbing, blisters, and echinoid. On the other hand, thermoablation treatment induces necrotic cell death, which is a pathologic phenomenon; it usually occurs when the cells find it difficult to survive following a major external damage. Necrotic cells have swollen nuclei without chromatin condensation, and can be characterized by a gradual dissolution of cell structure and lysis of the plasma membrane.

The selection of hyperthermia modalities that can be applied locally or to the whole body generally depends upon the heating source and on the types of cancer. On the basis of types of heating source, it can be divided into three groups, namely, heat applied from external source (e.g., hot bath, air, wax, blanket), contactless sources (e.g., radiofrequency, ultrasound, microwave, and infrared devices), and heat source inserted into body (e.g., probes, antenna, laser fibers, and mediators) [47].

The advanced hyperthermia devices such as ultrasound or electromagnetic radiation have their own limitations as the heat energy generated by them cannot be delivered to deeply situated cancer tissues without destroying the surrounding normal one. However, this could be overcome by inserting the heating source mediators inside the body where the electromagnetic energy applied on these deep situated mediators by an external electrical or magnetic field gets transformed into heat.

The inserted mediators can be either of macro, micro or nanometric size and they can be stimulated either by capacitive applicators using the electrical component of electromagnetic fields (*E*-field), or by inductive applicators using magnetic component (*H*-field). Macroscopic mediators must be inserted within the body by surgical intervention, whereas micro or nanoscale mediators can be injected as particle dispersion. However, during capacitive heating, *E*-field may cause heating of the surrounding tissues due to their conducting nature (eddy currents). Further, the difference in the dielectric permeabilities of different tissues leads to inhomogeneous temperature rise. On the other hand, in inductive mediator heating, this problem could be overcome as body tissues do not produce any heat in the presence of AC magnetic field. However, to suppress the effect of *E*-field completely, the optimized value of the product of $H \times f$ (where H is the amplitude and f the frequency of the AC magnetic field) is restricted to 4.85×10^8 $\mathrm{Am^{-1}s^{-1}}$ for a patient treated for one hour [17]. Depending on the diameter of the exposed body region, on the duration of the treatment, and on the seriousness of the illness, this critical product may be exceeded. Moreover, to avoid neuromuscular electrostimulation, f should be higher than 50 kHz, and it should be lower than 10 MHz for appropriate penetration of the *rf*-field.

4.6.2 Magnetic Fluid Hyperthermia

Even by stressful intervention, the macroscopic inductive heating mediators such as ferromagnetic rods or seeds (1 mm diameter and 1–7 cm of length) cannot be accessible to all tumors. Nevertheless, lack of uniform temperature may also cause

potential thermal underdosage to some critical regions. Hence, the magnetic fluid hyperthermia (MFH), in which colloidal dispersion of inductive heating mediators of micro or nanoscale is used, appears as the most promising therapy. In this case, the particles could be homogeneously distributed due to their ultramicron size, and hence, could produce uniform temperature all around the tissue. It has already been discussed that the cells uptake MNPs by endocytosis and the uptake by the cancerous tissue is even higher owing to their leaky vasculature, which is further helpful during cancer therapy. There is the benefit of stable deposits of MNPs surrounding the tumor tissues in the sense that once the particles are applied, then repeated thermotherapy can be performed without any further addition of particles. Even the daughter cells from a particle containing parent cell should contain up to 50 % of the particle amount of the parent cell, and thus, the descendants would still be cured by future MFH sessions.

The concept of using magnetic hyperthermia as cancer therapy was initiated by Gilchrist and coll. in the late fifties [52]. After a considerable gap, investigations were renewed in vitro, and now, in vivo. The first studies used magnetite nanoparticles surrounded by a dextran corona similar to the (U)SPIO contrast agents developed for MRI. In vitro experiments showed that these nanoparticles were internalized by cancer cells. Applying an alternating magnetic field (520 kHz, 7–13 kAm^{-1}) caused the tumor to diminish in the same way as it would in a bath of hot water. Subsequently, dextran was abandoned because it would appear to degrade too soon. It was then shown that direct injection of particles into the tumor has an advantage (still not fully understood) called the thermal bystander effect: even though the particles are concentrated at the deposition points before applying the alternating magnetic field, they distribute themselves much more uniformly after the first application of the AC magnetic field [53].

Jordan, Maier-Hauff and their collaborators at Clinique Charité in Berlin are now the most famous clinical research team, because they developed a full-sized human magnetic field applicator (MFHW 300 F, Hyperthermiesystem GmbH, Berlin at a frequency: 100 kHz, variable field strength: 2.5–18 kAm^{-1}) and a biocompatible MF made now of surface-aminated magnetite nanoparticles (MagForce Applications GmBH, Berlin, and MagForce Nanotechnologies AG, Berlin, Germany). They performed the first-ever clinical studies based on MFH [54]. The MNPs dispersion is injected within the tumor under general anesthesia. To solve the problem of the temperature distribution, numerical simulations based on tomographic images of the tumor allow optimizing the position of the MNPs deposits (Fig. 4.6). The expected heat distribution within the tumor is estimated by the Nano-PlanW software planning system (MagForce Nanotechnologies AG, Berlin, Germany).

Phase I clinical trials (feasibility studies) were performed to patients having prostate carcinoma [56], as well as glioblastoma multiforme [57]. This last study involved 14 patients receiving treatment via a combination of fractionated external beam radiotherapy and several sessions of thermotherapy. Aminated-MNPs were injected into multiple sites throughout each tumor. The choice of injection sites was based on data from a comprehensive series of MRI scans of the cranium

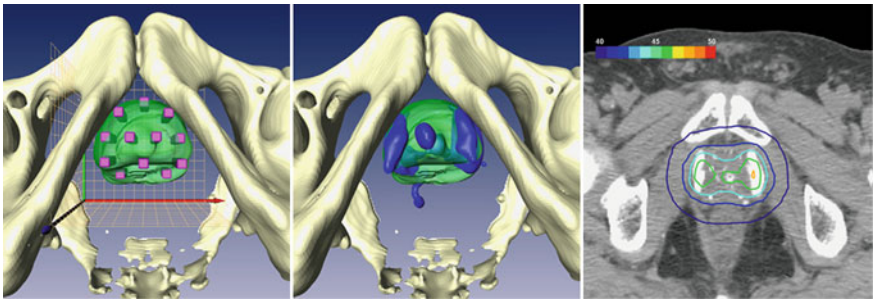


Fig. 4.6 *Left* Tomography of a prostate (*green region*), indicating the path of needles used to deposit 0.5–1 mL (per path) of the particle dispersion. *Center* Tomography after injecting the nanoparticles (*blue*). *Right* Image showing the nanoparticle deposits (*white* because denser than the prostate tissue) and calculated isotherms. Reprinted with permission from Ref. [55]. Copyright © 2007 Elsevier

coupled with the NanoPlanW software. The iron oxide nanoparticles (core size 15 nm) were dispersed in water at a concentration of 112 $mg_{Fe}mL^{-1}$. Each tumor was injected with 0.1–0.7 mL of the MF per milliliter of tumor and then exposed to a magnetic field of 3.8–13.5 kAm^{-1} alternating at 100 kHz. The study successfully demonstrated that this form of thermotherapy using MNPs could be safely applied to the treatment of brain tumors and that hyperthermic temperatures could be achieved. Follow-up computed tomography scans and reproducible temperature measurements confirmed that these deposits were stable over several weeks. Patient survival and local tumor control were not considered primary endpoints of this study; however, clinical outcomes were observed to be promising with the therapy being well tolerated by all patients. More complete evaluation of clinical outcomes is to be assessed in a phase II study (efficacy study) on 65 patients with recurrent glioblastoma multiform. Phase I clinical trials concerning pancreatic and esophageal cancers and local recurrences of residual tumors (e.g., cervical and prostate cancers, sarcomas) are also in progress.

The rather long period of gestation from first in vitro studies to eventual clinical application reflects the considerable technological and regulatory difficulties to be overcome in any attempt to develop a clinically acceptable and useful therapy of this type [48]. It is not merely enough to develop MNPs that heat upon exposure to an AC magnetic field, although that is clearly an important prerequisite. It is also important to know how to appropriately administer sufficient amounts of the particles to the intended target tissue and to be able to generate enough heat from them by exposure to a tolerable level of AC magnetic field that does not itself cause any undesirable side effects. The methodology developed by the MagForce group seems to successfully address each of these issues.

4.6.3 Next Developments

It is clear that if magnetically induced hyperthermia is to achieve the desired results, a certain number of aspects need to be improved. This includes not only the synthesis and properties of the MNPs-based mediators but also a better understanding of the physics involved in the loss phenomena, modeling of the in vivo temperature distribution, and development of a safe, efficient, and reasonably priced active targeting strategy.

4.6.3.1 Mediators Improvement

Improving SAR values remains a challenging task, because the higher the SAR of the MNPs dispersion, the lower the dose to inject to the patient. Among the improvement routes, that which consists in the preparation of narrow size distribution MNPs is particularly pertinent for superparamagnetic mediators. Indeed, in conventional synthetic routes, the MNPs are far from being monodisperse in size, resulting in a distribution of relaxation times in the MNPs ensemble. As a consequence, for a specific frequency of the AC magnetic field, only a small fraction of MNPs heat efficiently (those whose relaxation times are fitting). Therefore, narrowing the size distribution could greatly improve the SAR values, because all the MNPs will heat efficiently at the same frequency.

However, very efficient mediators should not forget that the temperature in vivo must be tuned very precisely (see Sect. 4.6.1) because, on the one hand, heat conduction and energy adsorption are widely unknown in vivo, and on the other hand, local overheating may damage safe tissue. An original route could exploit the temperature dependence of magnetic properties of ferro-/ferrimagnets. So, it would consist in designing MNPs with a T_C adjusted to just above the temperature that should not be exceeded in vivo. In this way, should that temperature be reached, each nanoparticle mediator would function as its own fuse by losing its magnetic properties, and hence, also its heating capability. Such a strategy has already been developed for alloy seeds (macroscopic mediators) in order to prevent local tissue overheating and reduce the need for invasive thermometry. For MNPs, manganites ($La_{1-x}Sr_xMnO_3$) are promising materials whose T_C can be tuned in the range of 32–60 °C by varying the amount of Sr. The stabilization of temperature near T_C was observed for such MNPs when they were kept under AC magnetic fields [58]. Their synthesis in the form of narrow size distribution MNPs, their biocompatibility, and surface chemistry are currently under investigation by several groups.

Some of these constraints pull in opposite directions, for example, under physiological conditions, maximal SAR values are attained for superparamagnetic compounds, while in principle, only ferromagnetic or ferrimagnetic compounds allow self-controlled regulation. The aim here will thus be to find the best possible compromise. In addition, it does seem somewhat risky to depend only on

Brownian relaxation to reach sufficiently high values of the SAR from the therapeutic point of view, because unfortunately, once in the target tissues, or target cells, it is not obvious that the particles will be able to turn round owing to a high local viscosity or strong interactions with the cell membranes.

4.6.3.2 Physics of Magnetic Loss Phenomena

If the heating capacities of the mediators are to be improved, the models used up to now will need to be refined and extended, to increase their predictive power and to cover the whole range of sizes and magnetic behaviors. They must be systematically validated by experiment and the difficulties inherent in this approach will not be overcome without close collaboration between chemists and physicists, both in theory and practice. In particular, it is probably crucial to understand the influence of local clustering of MNPs in order to fully optimize the heating from real samples [48]. Moreover, SAR measurements by colorimetry must be performed in strict adiabatic conditions, that is, considering all the probable heat losses by convection, conduction, and radiation [18].

Lastly, modeling the temperature distribution in vivo remains a priority for determining the optimal ways of depositing energy, for example, continuously or in pulses.

4.6.3.3 Targeting Strategies and Limits

One of the last challenges will be to develop the intracellular hyperthermia route, which is based on *i.v.* administered stealth MNPs designed for selective uptake by tumor cells and which would be the optimal method allowing to selectively overheat tumor cells even in disseminated metastases in any region of the body. However, certain problems still exist as mentioned later.

The first one is the ligand-mediated targeting to improve the colloidal mediator uptake by cancer cells. Among several in vitro studies, a single group worked in vivo, with the use of ^{111}In-chimeric L6 monoclonal antibodies grafted onto dextran-coated iron oxide cores for human breast cancer in mice [59]. They reported a mean concentration of MNPs per gram of tumor of about 14 % of the injected dose, equivalent to around 0.315 mg per gram of tumor (315 mgmL^{-1}). This is an exceedingly small amount of MNPs being used to heat the tumor mass compared, for example, with the intratumoral concentrations obtained by the direct injection method of Jordan et al., which were greater than 10 mgmL^{-1} of tumor.

The second one is the monitoring of the tissue distribution prior to the heating sequence. MRI appears to be the most suited method, and therefore, a great interest exists to investigate a unique and versatile magnetic device that will be able to reveal tumors and metastases and treat them by hyperthermia.

The last one concerns the debate about the expected higher efficacy of intracellular thermotherapy, which is obtained with magnetic particles internalized

within cells, with regard to extracellular thermotherapy. According to a theoretical model, it would seem that there is no thermotherapeutic effect on the nanoscale (the scale of the MNPs) or the microscale (the scale of the cells). In fact, thermotherapy operates on the millimeter scale (the scale of the tumor), since the thermal insulation behavior of the cell membrane is negligible [60]. Any difference that may be observed between intracellular and extracellular thermotherapy would thus appear to be due to a mechanical effect of rotation or vibration of the particles in the cell.

Extending this work to highlight the difficulties of using currently available MNPs to heat anything smaller than a 10 mm diameter tumor, an excellent analysis of the various opportunities and limitations was recently published [61]. The issue is essentially one of heat loss into the surrounding tissue. If one wishes to generate and sustain a large temperature imbalance within a tumor, the heat flow into that tumor has to be so large as to overcome the heat flow out. Roughly speaking, the bigger the tumor, the smaller the surface area to volume ratio, the less important the outward heat flow, and the easier it is to heat. Following this argument, the authors concluded that the MNPs SAR must be unrealistically high (certainly several orders of magnitude greater than the best currently reported) to heat a 3 mm cluster of cells, even with concentrations of iron in the cellular mass of 10–50 mgmL^{-1}. These figures are relevant given that $\sim$3 mm is the size of a subclinical metastasis that is undetectable by normal imaging techniques, and 10 mgmL^{-1} is substantially more than was used in vivo by DeNardo et al., but in the realm of that used by Jordan et al. The situation becomes even worse if the aim is to heat individual cells [48].

4.7 Conclusion

In this chapter, recent advances in biomedical applications of MNPs are reviewed. Iron oxide MNPs are now routinely used as contrast agents in MRI and very next clinical applications are expected in the field of cancer thermal treatment. These devices will be nanomaterials of higher and higher added value because they are expected to be multifunctional, smart, and versatile. Therefore, their chemical composition shall be more and more hybrid combining inorganic, organic, and biologic components. Nevertheless, optimization efforts remain for improving the repeatability of surface functionalization, diagnostic or therapeutic efficacy, biodistribution, and in vivo stability and safety. But the readers shall be convinced that targeting remains the main challenge, for which concentrated research efforts are still required.

Such developments of medicine-directed nanotechnology are probably among the best examples where physicians, pharmacologists, biologists, chemists, and physicists are working together. In this day, when the public opinion worries rightly about the possible nanotechnology consequences on mankind and its environment, it is important to mention that the single aim of a large number of these research efforts on nanoparticles is the humanity survival and comfort.

References

1. Häfeli U (2007) In Magnetism in Medicine: A Handbook. Andrä W, Nowak H (eds). Wiley, Weinheim pp 3–25
2. Corot C, Robert P, Idée JM, Port M (2006) Adv Drug Deliv Rev 58:1471–1504
3. Moghimi SM, Szebeni J (2003) Prog Lipid Res 42:463–478
4. Monfardini C, Veronese FM (1998) Bioconjug Chem 9:418–450
5. O'Handley RC (2000) Modern magnetic materials: principles and applications. Wiley, New York
6. Spaldin N (2003) Magnetic materials: fundamentals and device applications. Cambridge University Press, Cambridge
7. Pankhurst QA, Connolly J, Jones SK, Dobson JJ (2003) Phys D Appl Phys 36:R167–R181
8. Jeong U, Teng X, Wang Y, Yang H, Xia Y (2007) Adv Mater 19:33–60
9. Goya GF, Grazú V, Ibarra MR (2008) Curr Nanosci 4:1–16
10. Néel L (1949) Ann Géophys 5:99–136
11. Brown WF (1963) Jr Phys Rev 130:1677–1686
12. Lu JJ, Deng HY, Huang HL (2000) J Magn Magn Mater 209:37–41
13. Hergt R, Dutz S, Müller R, Zeisberger MJ (2006) Phys Condens Matter 18:S2919–S2934
14. Rosensweig REJ (2002) Magn Magn Mater 201:370–374
15. Kallumadil M, Tada M, Nakagawa T, Masanori A, Southern P, Pankhurst QA (2009) J Magn Magn Mater 321:1509–1513
16. Schmidt AM (2007) Colloid Polym Sci 285:953–966
17. Hergt R, Andrä W (2007) In magnetism in medicine: a handbook. In: Andrä W, Nowak H (eds) Wiley, Berlin pp 550–570
18. Natividad E, Castro M, Mediano A (2008) Appl Phys Lett 92:093116
19. Natividad E, Castro M, Mediano AJ (2009) Magn Magn Mater 321:1497–500
20. Kaman O, Veverka P, Jirák Z, Maryško M, Knížek K, Veverka M, Kašpar P, Burian M, Šepelák V, Pollert EJ (2011) Nanopart Res 13:1237–1252
21. Lu AH, Salabas EL, Schüth F (2007) Angew Chem Int Ed 46:1224–1244
22. Duran JDG, Arias JL, Gallardo V, Delgado AVJ (2008) Pharm Sci 97:2948–2983
23. Roca AG, Costo R, Rebolledo AF, Veintemillas-Verdaguer S, Tartaj P, Gonzalez-Carreno T, Morales MP, Serna CJJ (2009) Phys D Appl Phys 42:224002–224011
24. Dave SR, Gao X (2009) Wiley Interdiscip Rev Nanomed Nanobiotechnol 1:583–609
25. Di Marco M, Sadun C, Port M, Guilbert I, Couvreur P, Dubernet C (2007) Int J Nanomed 2:609–622
26. Lin MM, Kim DK, El Haj AJ, Dobson J (2008) IEEE Trans Nanobiosci 7:298–305
27. Majewski P, Thierry B (2007) Crit Rev Solid State Mater Sci 32:203–215
28. Jech TJ, Odenbach S, Odenbach S (2003) Ferrofluids: magnetically controllable fluids and their applications. Springer, New York
29. Massart R (1981) IEEE Trans Magn 17:1247–1248
30. Neuberger T, Schöpf B, Hofmann H, Hofmann M, von Rechenberg BJ (2005) Magn Magn Mater 293:483–496
31. Shubayev VI, Pisanic TR, Jin S (2009) Adv Drug Deliv Rev 61:467–477
32. Moghimi SM, Hunter AC, Murray JC (2001) Pharmacol Rev 53:283–318
33. Vonarbourg A, Passirani C, Saulnier P, Benoit JP (2006) Biomaterials 27:4356–4373
34. Arruebo M, Valladares M, Gonzalez-Fernandez A (2009) J Nanomater 24; article ID 439389
35. Peng XH, Qian X, Mao H et al (2008) Int J Nanomed 3:311–321
36. Thierry B (2009) Curr Drug Deliv 6:391–403
37. Stella B, Arpicco S, Peracchia MT et al (2000) J Pharm Sci 89:1452–1464
38. Brown MA, Semelka RC (2003) MRI: basic principles and applications, 3rd edn. Wiley, New York
39. Weinmann HJ, Ebert W, Misselwitz B, Schmitt-Willich H (2003) Eur J Radiol 46:33–44
40. Bonnemain BJ (1998) Drug Target 6:167–174

41. Wallis F, Gilbert FJJR (1999) Coll Surg Edinb 44:117–125
42. Harisinghani MG, Barentsz J, Hahn PF et al (2003) New Engl J Med 348:2491–2499
43. Weinstein JS, Varallyay CG, Dosa E et al (2010) J Cereb Blood Flow Metab 30:15–35
44. Bulte JWM, Kraitchman DL (2004) NMR Biomed 17:484–499
45. McCarthy JR, Weissleder R (2008) Adv Drug Deliv Rev 60:1241–1251
46. Högemann D, Josephson L, Weissleder R, Basilion JP (2000) Bioconjug Chem 11:941–946
47. Mornet S, Vasseur S, Grasset F, Duguet EJ (2004) Mater. Chem. 14:2161–2175
48. Pankhurst QA, Thanh NKT, Jones SK, Dobson JJ (2009) Phys D Appl Phys 42:224001–224015
49. Jang JT, Nah H, Lee JH, Moon SH, Kim MG, Cheon J (2009) Angew Chem Int Ed 48:1234–1238
50. Sneed P K, Stea B (1996) In thermoradiotherapy and thermochemotherapy. In: Seegenschmiedt MH, Fessenden P, Vernon CC (eds) Springer, Berlin, vol 2, pp 159–173
51. Luchetti F, Mannello F, Canonico B et al (2004) Apoptosis 9:635–648
52. Gilchrist RK, Medal R, Shorey WD et al (1957) Ann Surg 146:596–606
53. Jordan A, Scholz R, Wust P et al (1997) Int J Hyperther 13:587–605
54. MagForce nanotechnologies AG, www.Magforce.de
55. Johannsen M, Gneveckow U, Thiesen B et al (2007) Eur Urol 52:1653–1662
56. Johannsen M, Gneveckow U, Eckelt L et al (2005) Int J Hyperther 21:637–647
57. Maier-Hauff K, Rothe R, Scholz R et al (2007) J Neurooncol 81:53–60
58. Pollert E, Knizek K, Marysko M, Kaspar P, Vasseur S, Duguet EJ (2007) Magn Magn Mater 316:122–125
59. DeNardo SJ, DeNardo GL, Natarajan A et al (2007) J Nucl Med 48:437–444
60. Rabin Y (2002) Int J Hyperther 18:194–202
61. Hergt R, Dutz SJ (2007) Magn Magn Mater 311:187–192

Chapter 5
Nanomaterials: Applications in Drug Delivery

Christine Vauthier, Patrick Couvreur and Elias Fattal

Abstract Soon after the discovery of liposomes by Bangham in 1966 (Bangham et al. 1969), nanomaterials were introduced in medicine and the development of the first polymer nanoparticles for oral administration was achieved by Speiser in the late 1960s (Khanna and Speiser 1969). In the early 1970s, these objects were considered as the possible "magic bullet" that was a concept proposed 60 years before by the Nobel Prize laureate in Medicine Paul Ehrlich. The aim of the magic bullet is to improve treatments by targeting drugs to diseased tissues cells and subcellular compartments (Kreuter 2007). The introduction of nanomaterials in drug formulation strategies became sources of major innovations in drug delivery over the last 40 years (Couvreur and Vauthier 2006; Kreuter 2007; Bosch and Rosich 2008).

5.1 Introduction

Soon after the discovery of liposomes by Bangham in 1966 [12], nanomaterials were introduced in medicine and the development of the first polymer nanoparticles for oral administration was achieved by Speiser in the late 1960s [62]. In the early 1970s, these objects were considered as the possible "magic bullet" that was a concept proposed 60 years before by the Nobel Prize laureate in Medicine Paul Ehrlich. The aim of the magic bullet is to improve treatments by targeting drugs to diseased tissues cells and subcellular compartments [64]. The introduction of nanomaterials in drug formulation strategies became sources of major innovations in drug delivery over the

C. Vauthier (✉) · P. Couvreur · E. Fattal
Université Paris Sud, Physico-chimie, Pharmacotechnie et Biopharmacie,
UMR CNRS 8612, Faculté de Pharmacie, 92296 Chatenay-Malabry Cedex, France
e-mail: christine.vauthier@u-psud.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_5,

last 40 years [18, 28, 64]. Liposomes [50] and a few types of biodegradable polymer nanoparticles [26] were on the basis of key achievements in the early ages of the nanomedicine leading to major progresses and the marketing of a new generation of drug formulations [2, 53, 78, 83, 93, 101, 116, 119]. At present, a myriad of nanomaterials are considered as suitable nanomedicines [1, 36, 93]. They have emerged from polymer chemistry and from a better understanding of mechanisms governing molecules self-assembling properties which allowed the development of many types of nano-objects according to a bottom-up approach. Most advanced developments are intended to design multifunctional nanomaterials helping the diagnostic and at the same time, if necessary, the delivery of the right amount of drug on demand and at the right place [36, 118].

It is noteworthy that any developments that concern medicines applied to human and animals are submitted to a strong regulation. Risks are taken into consideration at the root of developments and many projects are aborted in their earlier age because of safety problems. During development of pharmaceutics, the benefice to risk balance needs to be proved and must satisfy stringent requirements imposed by rules of agencies in charge of authorizations for marketing of the new drug products. Obviously, developments of new medicines using nanomaterials are concerned by these rules. However, in the absence of sufficient knowledge on nanomaterials regarding their safety and protocols of evaluation, drugs formulated in nanotechnologies are currently still developed on the basis of regulations in use for conventional drugs. Regulatory authorities from different agencies are discussing to improve the current framework to make relevant adaptations to be applied to the specific case of nanomedicines [3, 4, 14, 25, 43, 46, 47, 57, 83, 103, 114].

It can be expected that frameworks will evolve as soon as the different parameters to be integrated in the file will clearly be identified as well as relevant methods for their evaluation [57, 83]. As emphasized by the different agencies, specific regulations recommend the pharmaceutical companies to run pharmacokinetic and toxicokinetic studies particularly on the drug carrier itself, as well as toxicity studies particularly on the target tissue where the nanoparticles tend to distribute. Finally, immunotoxicity, reproduction toxicity, genotoxicity as well as the potential for carcinogenic effect should be explored. Recent advances have demonstrated that despite a strong interaction and internalization of biodegradable nanoparticles by lung cells no inflammation was observed even with high amount of nanomaterials [84, 85].

This chapter has considered both the promise and the danger of nanomaterials and aims to point out the great potentialities brought by the use of nanomaterials in medicine to improve treatments of several diseases and to develop new therapeutic methods for patient's benefit. The first part of this chapter gives a brief description of the different nanomaterials proposed so far as drug delivery systems for in vivo applications. The second part will discuss the use of nanomaterials in drug delivery applications to improve clinical applications in terms of successes and difficulties. It will also highlight upcoming challenges. The last part of this chapter is aimed to present another facet of the application of nanomaterials in medicine which can be used to design vaccines aiming to protect populations from infectious diseases.

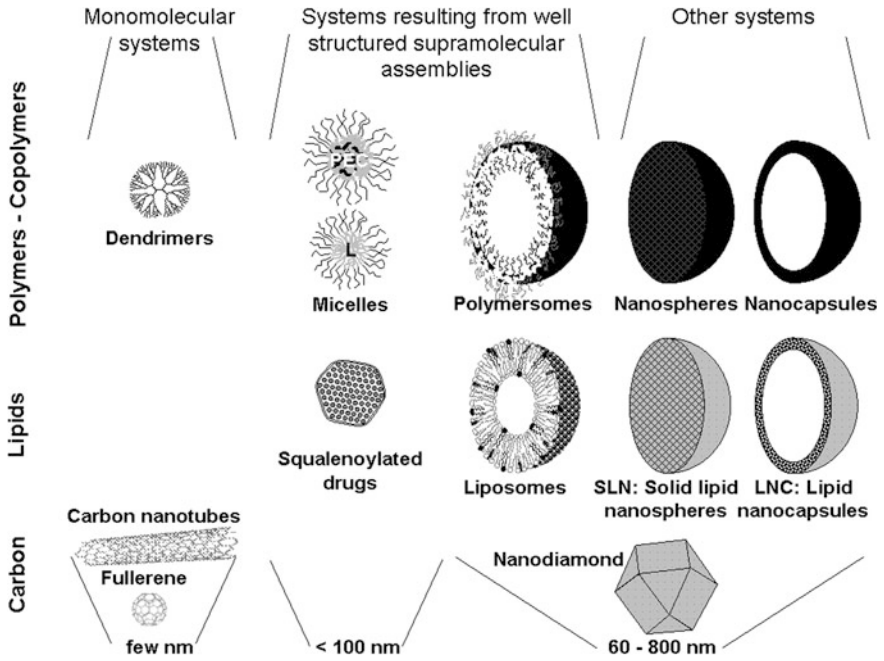


Fig. 5.1 Scheme of the different types of nanomaterials developed for the in vivo delivery of drugs

5.2 Nanomaterials Developed as Drug Delivery Systems

Nanomaterials designed to be used as drug delivery systems can occur in various types of particles whose size are all comprised in the nanometers size range [36, 81, 83, 93, 99, 118, 119]. Typically, their size varies from a few nanometres to 250–300 nm. The rationale behind the design of different types of nanomaterials is that characteristics of drug carriers need to be tuned according to the nature of the active drug to be carried in vivo. It also needs to suit with the drug delivery mission, which includes protection of the drug against degradation, transport across biological barriers, and drug targeting. Top-down and bottom-up approaches can be used to prepare the different types of nanomaterials [11, 36, 93, 115]. Most of nanomaterials developed as drug delivery systems are made of lipids or synthetic or natural polymers or even metal-based components. Generally, the shape of a single particle is spherical and the structure is a matrix composed of lipids or of polymers or corresponds to a vesicle in which the envelope is formed by either lipids or polymers (Fig. 5.1). Polymer-based nanomaterials with non-spherical shapes are also currently investigated [38, 77, 118]. Carbon-based materials were recently introduced as potential nanomaterials to improve the delivery of drugs in vivo. They include fullerenes, carbon nanotubes, and nanodiamonds [7, 52, 69] (Fig. 5.1). Recently, metal organic frameworks were used to construct highly porous nanocarriers which can provide a solution for the loading

of drugs difficult to encapsulate, since their structures and porosities are tuneable [59]. So far, the number of works on these systems remains limited while questions regarding their safety as drug delivery applications are still open.

The design of nanomaterials may greatly influence the drug's in vivo fate. Nanomaterials therefore need to be engineered according to their biological fate and associated therapeutic challenges. In general, morphology, shape, structure, and size of nanomaterials are set during their preparation [36, 77, 115]. Drug loading occurs preferentially during preparation but in some cases, the drug can only be associated on preformed nanomaterials [33, 36, 115]. For many nanoparticulate delivery systems, drug loading remains a challenge as the drug payloads do not exceed a few percent of the mass of the nanomaterial. In comparison, high payloads, around 50 % or more of the mass of the formulation, were achieved by synthesizing squalenoylated-drug derivatives that self-assemble as nanoparticles when they are dissolved in aqueous media [27, 35]. Surface of nanomaterials have important roles. First, it needs to ensure that individual particles remain stable and do not aggregate with others included in the biological media. Second, the decoration with specific molecules is needed to control the nanomaterial fate in biological systems. For instance, surface of nanomaterials designed to be administered by the intravenous route should fulfil several criteria such as (i) remain stable, (ii) reach the target place, and once there (iii) find the right molecular signature expressed on the target cells. This last equipment is expected to make possible specific recognition between the drug carrier and the diseased cells. Some reports from the literature have pointed out that a very precise engineering of nanomaterial surface is required to achieve this goal [81]. In practice, nanomaterial surfaces can be decorated with various chemical functionalities and/or macromolecules which are expected to promote the required interactions between nanomaterials and components of the biological outside world [6, 93, 109, 110]. As shown in Table 5.1, strategies followed to modify surface properties of nanomaterials greatly depend on routes of in vivo administration which in turn defines types of interactions that control in vivo fate [81, 110, 114, 119]. Besides the few strategy roadmaps given in Table 5.1, there is still a need for comprehensive studies to improve our understanding on interactions between nanomaterials and biomolecules or cells [72, 82]. These would help rationalizing our approaches in designing new nanomaterial platforms with predefined drug delivery goals.

5.3 Nanomaterials to Improve Drug Delivery

5.3.1 Present Situation for Patients

Introduction of nanomaterials as new tools in drug formulation strategies gave opportunities to bring safer and more efficient treatments to patients, thanks to a better control of the drug distribution toward diseased cells, especially in treatments given by intravenous injections. Although extremely attractive, turning this

Table 5.1 Strategies of surface modifications to improve drug delivery properties of nanomaterials

Route of administration	Expected benefit	Properties that need to be optimized	Type of materials suggested to be grafted on the nanomaterial surface to obtain the required surface properties
Intravenous	Control of the tissue distribution of the drug to achieve delivery in the targeted tissue	Stealthiness to escape massive uptake by defence mechanisms of organisms	Hydrophilic polymers such as PEG and polysaccharides. Grafting on the end-on position was found mandatory with polysaccharides
		Targeting to improve specific recognition of the target tissue and cells	Ligands that are complementary to receptors found on the surface of target cells. Examples of ligands are folate residues to target cancer cells overexpressing the folate receptor, antibodies specific to membrane proteins expressed on the cell surface
Mucosal	Slow down clearance of drug from mucosa to prolong duration of contact and to increase concentration of the drug near absorption sites	Mucoadhesion	Mucoadhesive polymers such as chitosan, thiomers, polyacrylates, and PEG

Table 5.2 Medicines included nanomaterials used in clinics for the treatments of cancer and severe infections. (adapted from [119, 114])

Platforms	Trade name	Approved date	Drug	Routes of administration	Indication
Liposomes	AmBisome®	8/11/1997	Amphotericin B	Intravenous	Aspergillosis Candidiasis Cryptococcal meningitis Fungal infections Visceral Leishmaniosis Histoplasmosis
	Amphotec®	11/22/1996	Amphotericin B	Intravenous	Fungal infections
	DaunoXome®	4/8/1996	Daunorubicin	Intravenous	Advanced HIV associated Kaposi's sarcoma
	Doxil® (USA) Caelix® (Europe, Canada)	11/17/1995	Doxorubicin	Intravenous	AIDS related Kaposi's sarcoma Ovarian cancer Multiple myeloma
	DepoCyt®	4/1/1999	Citarabine	Intrathecal through lumbar puncture	Lymphomatous meningitis
Nanoparticles	Abraxane®	1/7/2005	Paclitaxel	Intravenous	Metastatic breast cancer
Nanotubes	Somatulin depot®	8/30/2007	Lanreotide acetate	Subcutaneous injection	Acromegaly

concept from theory to product is a difficult task. There are many biological barriers to overcome in order to achieve a perfect control of the in vivo fate of nanomaterials allowing the delivery of their drug payload specifically to the biological target. These barriers are physical obstacles such as epithelium, endothelium, cell membranes, and biochemical including enzymes and proteins found in biological fluids. They can either compromise or potentiate the drug delivery mission of nanomaterials [1, 81]. Many of the mechanisms behind these obstacles are still not fully elucidated and this hampered the efficient design of nanomaterials for which the in vivo fate can be fully anticipated with a high precision of targeting. Nevertheless, it is well known that nanomaterials can be used to target drugs to macrophages of the mononuclear phagocyte system (MPS), mainly the Kupffer cells in the liver and spleen macrophages, leading to some possible therapeutic or imaging applications [38, 98, 101]. These cells are parts of the defence systems in charge of the clearance of all undesirable material circulating in the blood which normally comprises pathogenic agents, i.e., bacteria, viruses, parasites, fungus, and senescent cells. Nanomaterials being artificial, naturally concentrate in these cells after intravenous administration where they can exert a specific pharmacological activity. This natural targeting was used to deliver Amphotericin B to infected macrophages with liposomes which were marketed in 1997. Although very active against many severe pathogenic agents including fungus, parasites and candida, the use of amphotericin B in conventional formulation was, indeed, limited by a significant nephrotoxicity. The higher tolerance obtained with the liposomal formulation allowed increasing the dose of Amphotericin B (Ambisome®) administered to patient with a comfortable therapeutic margin. Thus, the efficacy of the treatment could be improved as a consequence of a higher dose, better delivered to infected cells [2, 87, 117]. This treatment is recommended for various AIDS-associated serious infections as indicated in Table 5.2 [80]. Doxorubicin Transdrug (Livatag®) is another illustration of a successful nanomedicine approach resulting from a liver targeting. Transdrug is a doxorubicin-loaded poly(isohexyl cyanoacrylate) nanoparticle formulation that showed considerable antitumor activity against multidrug-resistant protein overexpressing hepatocellular carcinoma in vivo [13]. Similarly, a formulation of paclitaxel-loaded thermosensitive liposomes showed encouraging potential in treatment of hepatocellular carcinoma too [99]. These formulations are currently investigated in clinical trials to bring novel treatments to patients suffering from hepatocellular carcinoma which is a terminal liver cancer [16].

Delivery of drugs apart from Kupffer cells and spleen macrophages requires manipulations of nanomaterial surface to reduce recognition and massive uptake by such scavenging cells of the defence barrier. A method consists in camouflaging the original nanomaterial surface with a polymer that modifies opsonization by serum proteins. The corresponding drug carriers are commonly named "stealth®" in reference to their invisibility by defence systems of the organism. The commercial liposomal formulation of doxorubicin (Doxil® in the USA, Caelix® in the Europe) includes stealth liposomes. The stealthiness is conferred by the coverage of the liposome surface with poly(ethylene glycol) chains. The small

size of these liposomes and their long circulation time in the blood are favorable factors for tumor targeting since they can accumulate passively into the tumoral tissue, thanks to the leaky endothelium of tumor vasculature and to the retention effect resulting from a reduced lymphatic drainage (so-called "enhanced permeability and retention effect") [23, 73]. Another interesting marketed drug delivery technology consists in albumin nanoparticles. This carrier was recently introduced in the market under the trade name Abraxane® to deliver paclitaxel for the treatment of metastatic breast cancers. In this nanomaterial, albumin stabilizes nanosized particles of paclitaxel, which is a drug insoluble in aqueous media. The albumin targets paclitaxel to the tumour, thanks to its binding to a specific receptor expressed on the surface of endothelial cells which signals the internalization followed by the transcytosis of the whole nanoparticles across the endothelium. The nanoparticles and their cargos are then delivered to the tumoral tissue [5, 78, 97]. An increased antitumoral activity can be accessed as higher doses of paclitaxel can reach the tumour. This methodology resolved problems found with conventional-based formulations of paclitaxel which included severe side effects and poor effectiveness. It also allowed the administration of higher drug doses which is another benefit for the success of treatments.

In summary, the therapeutic benefits are several including an increased efficacy, an improvement of the safety profile, and a better quality of life of the patients [83, 96]. In addition, it appears from pharmacoeconomic analysis that treatments with nanomedicines are less expensive than those based on the use of the conventional formulations of the corresponding drug [15, 49]. This can be explained by taking the overall cost of the treatment into consideration. Although the drug price is high, improvement of patients' tolerability considerably reduces the costs related to side effects and toxicity. In some cases, the number of administrations required is lower which also contributes to cost reductions.

5.3.2 Ongoing Research and Discussions on the Use of Nanomaterials to Deliver Drugs by Intravenous Injections

Only a few pharmaceutics based on the use of nanomaterials were so far marketed with clinical indications for the treatments of severe diseases such as cancer and severe infections (Table 5.2) [119, 114]. A few more are under clinical trials [30]. Besides identified applications, nanomaterials were found useful to resolve several problems related to in vivo drug delivery. Their primary interest is that they can improve precision of the delivery of the drug to the biological target. They are also efficient to make possible the delivery of potential drugs for which developments are hampered due to specific delivery problems. In therapeutics, nanomaterials can be considered as potential drug-targeting devices in treatments for various diseases [28, 71, 74, 93, 107].

Cancers continue to stimulate an enormous number of works. Various strategies are developed to improve treatments in which nanomaterials are partners to achieve the delivery of the biologically active compound in a controlled manner. Formulations of well-known anticancer agents in nanomaterials are the foundations of these researches. While works on this direction are still continuing, other therapeutic strategies were identified [70]. For instance, development of treatments based on the use of nucleic acids to correct genetic disorders responsible for the occurrence of cancer is of great interest as it is expected to provide very specific therapeutic methods avoiding side effects. Other strategies followed in anticancer treatments require the administration of peptides which are other molecules that need protection against degradation and transport down to their target site. They include stimulation of macrophages that are infiltrated tumours so the macrophages will be more efficient to eliminate cancer cells [42]. Although under debate at present, another strategy in cancer treatment is based on the targeting of antiangiogenic agents to reduce neovascularization of tumors [31]. In all these new therapeutic strategies, the active molecules are protein-based inhibitors and nucleic acids (siRNA or microRNA) [45]. These compounds are degraded within minutes in biological fluids and are unable to cross biological barriers to reach their intracellular biological target site by themselves [36]. Their biological activity is extremely specific and they are active at very low concentration reducing risks of side effects. However, the only chance to convert these molecules into usable medicines is to associate them with a carrier. In this aim, nanomaterials hold promise to turn these active molecules into usable medicines by protecting and transporting them in a safe way from the administration site to the biological target [33, 36, 40, 100, 113]. In general, the reduction of the tumour growth is spectacular while the injection of the free molecule shows no activity at all. Efforts of having suitable formulations for their in vivo delivery are still needed to avoid depriving patients of these potential new drugs.

Serious infections are a second type of diseases motivating intense research as many new potential molecules proposed against infectious agents are poorly soluble in aqueous media and quite toxic [71, 74, 93, 107]. Thus, for these molecules, nanomaterials are an option to develop efficient treatments integrating a targeting potential. Several strategies including treatments of viral infections with nucleic acids are also investigated due to potentials brought by nanomaterials [20].

During the last 10 years, researches were intensified considering nanomaterials as potent tools to deliver drugs to the brain. Indeed, blood vessels composing the blood–brain barrier (BBB) are generally impermeable to drugs, except the small molecules with a pronounced lipophilic character [10, 89, 90]. This is a serious obstacle when drugs need to be delivered in the brain through the intact BBB. Several major health threats are concerned by such drug delivery problems, for instance fatal brain cancers such as glioblastoma at their earlier stage, diseases associated with a degeneration of the brain tissue (incl. Alzheimer and Parkinson diseases), and brain ischemic episodes. Novel potential treatments of these diseases involving peptides will not be provided to patients without a suitable delivery method. Indeed, in addition to their poor stability in biological fluids, peptides do not cross the BBB and they need a carrier to reach tissues of the central nervous system [61].

In general, treatments by intravenous administration have the advantage to give accessibility to almost all organs and tissues of the organisms as far as the drug can diffuse out of the blood compartment. The situation is the same with nanomaterials if they need to carry the drug toward cells located outside the blood compartment. As crossing the endothelium might be an obstacle, few nanoparticle-based strategies have considered the possibility to target endothelial cells that can express specific markers of different pathologies [63]. When the integrity of blood vessels is dramatically affected by the pathological process and the endothelium becomes leaky because of the appearance of fenestrations, nanomaterials of small enough size can diffuse out of the compartment hence in the tissue to reach target cells. Although the theory is quite clear, to turn it into reality is a challenging task. As recently pointed out by Adair et al. [1], there are numerous requirements to integrate in a single object to build up efficient drug delivering nanomaterials. Those which reached the market already proved that there is a benefit of developing further drug-targeting strategies and that nanomaterial can help in this task. However, the task is difficult and even marketed compounds are still not ideals. Although they deliver drugs in a controlled manner to define organs, they are not equipped with antennas making possible very precise recognition of the specific target cells. Additionally, it appeared through large-scale clinical use that they are potentially recognized by the immune system through a very unique reaction which generally occurred during the first treatment [111]. This reaction was described as a complement activation-related pseudoallergy (CARPA). While in general this syndrome could be controlled and circumvented, it could become fatal for a few patients. This is a major issue that makes treatments with nanomaterials still limited in numbers. It also slows down developments as risks caused by the CARPA phenomenon are not tolerated for non-terminal diseases. Studies to fully understand the mechanisms behind this reaction are still ongoing [9, 111]. It is expected that results from these researches will provide enough information to design a new generation of nanomaterials that will avoid the occurrence of the CARPA phenomenon [111]. Already, there is a consensus that the risk of CARPA syndrome may be significantly decreased if not suppressed by: (i) avoiding treating patients with an allergic history, (ii) giving to the patient a corticoid-based premedication, and (iii) by administering the nanomedicine as a slow infusion. From this discussion, it appears that the promises brought by nanomaterials to propose new treatments to patients are currently hampered by difficulties of designing efficient nanomaterials which are safe. At present, developments are only considered for terminal diseases for which the benefice to risk balance is in favour of an enormous benefice to the patient and for which the use of formulations based on nanomaterials is the only live-saving treatment that can be proposed to the patients. Despite current serious restrictions, promises brought by nanomedicines for intravenous treatments are still present, especially in the urge to find solutions for the administration of peptide and nucleic acid drugs. According to Szebeni et al. [111], safety problems caused by CARPA will be solved in the future by finding appropriate prevention methods.

5.3.3 Improving Drug Delivery With Nanomaterials by Other Routes of Administrations, Challenges and Therapeutic Potentials

Although, as already explained before, drug delivery methods with nanomaterials were extensively developed for intravenous injection, the use of nanomaterials to administer drugs by other routes was also explored [21, 24, 28, 41, 58, 74, 116]. Promises were expected for the administration of peptides by mucosal routes which are far less invasive compared to parenteral routes. For instance, nanodevices were proposed to improve methods of administration for the treatment of metabolic diseases that concerns millions of patients around the world suffering from diabetes and osteoporosis. The oral route which is the most accepted by patients and the safer for the administration of drugs has generated a large part of the research. It is challenging for the delivery of peptides because these drugs cannot survive the hash conditions of the gastrointestinal tract and are additionally poorly absorbed intact by the intestinal mucosa. In general, the biological activity of peptides was found to be well preserved in the gastrointestinal conditions after association with appropriate nanomaterials [24]. Consistently, several papers have reported biological activity of insulin and calcitonin formulated in nanomaterials after oral administration to laboratory animals [22]. Delivery of insulin by the oral route by means of nanomaterials is a very active and competitive area of research. Although new applications for clinical investigations in human are constantly approved by FDA over many years, data from clinical investigations are still rare in the literature [55]. This situation may be related to the fact that finding oral treatment with insulin for diabetics is a very hot topic for the pharmaceutical industry with high potential economic refunds. However, it can be expected that more clinical data will be published in the future as now results from clinical investigations must be made available to the scientific community. From another point of view, a lot of research is still needed as many questions remain open including those related to safety [22, 24, 48].

Apart from peptides, association of poorly soluble anticancer and antiviral drugs with nanomaterials represents an alternative to unsuccessful oral delivery with conventional formulations of drugs [36]. For poorly soluble drugs, a nanosized formulation is expected to accelerate solubilization, thanks to the enormous area of the interface between the solid drug and the biological surrounding environment. For some of the molecules, the poor solubility is only part of the deleterious factors compromising oral administration. Others may interfere with absorption mechanisms when the drug is on its way through the gut epithelium. For instance, the low oral bioavailability of paclitaxel (below 10 % of the administered dose [94]) is due to the combination of three deleterious parameters: (i) the poor solubility of this compound as explained before, (ii) as substrate of the multidrug efflux transporter P-gp it does not penetrate in the absorption cells of the gut, and (iii) it is recognized and metabolized by the cytochrome P-450 [121]. Besides improvement of solubility, nanomaterials can also confer protection of the

drug during transportation through the gut epithelium. Although it is expected that nanomaterials may help drug transport through the gastrointestinal tract epithelium, more data from fundamental research are still needed to improve design of nanomaterials with a high capacity to go through the gut epithelium to deliver their cargo to the systemic circulation [24, 66].

Another mucosal route of administration that received attention with nanomaterials is the pulmonary route. Bronchial cells as well as pulmonary alveoli can be reached by nanomaterials delivered in aerosols [58, 67, 84, 104]. This route of administration is interested to deliver drugs for local activity including treatments of lung cancers [54, 67, 76, 120]. Another potential interest is that lung alveoli provide with an easy access to the blood circulation which is attractive to achieve systemic drug delivery. Therefore, administration of drugs by the pulmonary route was identified as an alternative to injections when oral delivery is not possible. Benefits expected in terms of drug delivery are the control of the distribution of the drug deposition in the different parts of the lungs and the control of the amount of drug delivered in time, thanks to the releasing properties of the nanomaterial drug delivery device. Although potentials are clearly identified, safety issues are still disputable especially for long-term treatments [58]. Despite the risks clearly demonstrated for lung exposition to nanoparticles [86], the use of biodegradable nanoparticles was shown to be safe in vitro on bronchial cells [85] and in vivo [29]. Administration of drugs by the nasal route is believed to favor drug distribution to the brain tissue [8, 79]. Researches on nanomaterials designed to improve drug delivery to the brain by the nasal route are ongoing. These investigations are dedicated to find efficient treatments for diseases resulting from disorders in the brain including epilepsies and depressions [75]. Treatments of pain are also relevant candidates for which improvements are expected using a drug formulated in nanomaterials administered by the nasal route [60].

Although less explored, the buccal mucosa is another route of administration that can be considered for drug delivery [21, 55, 91]. For example, formulations with nanomaterials have been considered for local activity of anaesthetics in dentistry. Buccal administration with the aim of reaching the systemic circulation is investigated as an alternative method to oral administration for the delivery of various drugs. The main drug candidates for this route of administration are the poorly soluble drugs in aqueous media and the peptides [55]. In this approach, nanomaterials are used to concentrate the drug on the mucosa and to prolong time of contact. The combination of the two effects is expected to create favourable conditions to achieve a controlled release delivery of the drug to the systemic circulation. In general, nanomaterials designed to improve drug delivery by the buccal route are bioadhesives. They also include absorption enhancing promoters.

Still works are needed to understand how nanomaterials are delivering drugs by mucosal routes. As already explained, several studies have demonstrated that they can cross the mucosa to reach the systemic circulation but the extend of absorption remains a disputable question [44, 102]. Besides this, it is still unclear whether or not it is mandatory that the carrier needs to cross the epithelium of the mucosa as an intact object to deliver its payload in the systemic circulation. The answer to this question may depend on the nature of the drug to be delivered. For instance,

drugs diffusing easily through epithelia without being metabolized may simply need to be concentrated near the brush border of the epithelial cells. In contrast, hydrophilic macromolecules like peptides and nucleic acids may require that the carrier is also absorbed. This is a point that is urged to elucidate as it will greatly influence the orientation of further studies designed to evaluate the safety issues of treatments with nanomaterials administered by a mucosal route.

Nanomaterials were also proposed to improve treatments of eye diseases refractory to local and/or systemic treatments. There are numerous evidences that this is actually the case [32, 68]. Nanomedicines for ocular delivery of drugs were found to enhance potential of drugs in local treatments as well as those of the posterior parts of the eye which are the most difficult to achieve in this organ. They were also found suitable for the administration of nucleic acids including si-RNA and antisense oligonucleotides [39]. With these molecules, new therapeutic approaches can be designed to treat eye diseases in which the reduction of the expression of a well-defined protein can reduce the impact of ongoing pathological processes [39, 51]. The use of nanomaterials to deliver drugs to treat eye diseases is also expected to improve the life quality of patients who suffer from deleterious pathologies by becoming progressively blind. Additionally, these treatments are more easy and comfortable to administer to patients especially those requiring injections in the vitreous and in the posterior segment of the eye [37, 41]. Indeed, injections can be achieved with a small gauge needle which is an incredibly more comfortable alternative method of administration of drugs to the eye compared with those of implantable formulations that require invasive surgeries. The number of injection can also be reduced by formulating nanomaterials with a slow release of the drug.

5.4 Nanomaterials as Delivery Systems of Vaccines

Vaccines are used as common methods of prevention for the occurrence of infectious diseases. They are extremely efficient to control the spreading of pathogens in populations and they even have the power to completely eradicate the incidence of infectious disease. They have tremendously improved human life giving the body the capacity to defend itself against infectious agents. It is admitted that they are parts of the few major progress in medicines that considerably reduced mortality and contributed to population growth [19, 88, 92].

Early vaccines dedicated to prevent infectious diseases still exist today but concepts to design new vaccines have greatly evolved following progresses in immunology and in biotechnologies. In general, the molecules to deliver in vaccines are antigens which are proteins or peptides. In very few cases including therapeutic vaccines, it can be the gene that encodes for the antigenic peptide [17, 65]. In both cases, the target cells are cells from the immune system. The type of target cells and the way immune cells are stimulated at the moment they receive the antigen are extremely important because it influences the type of the induced immune response [17, 19, 65, 105].

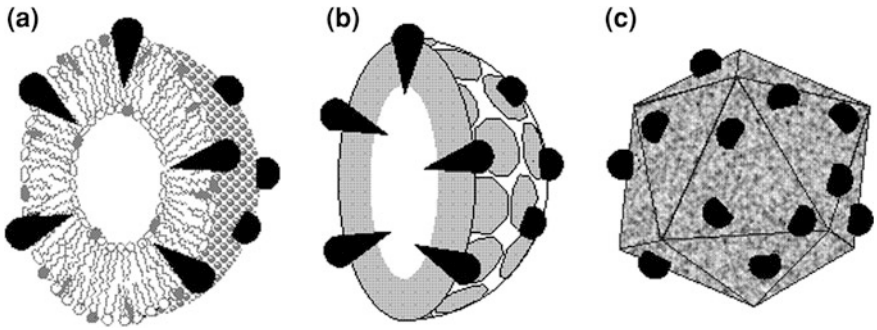


Fig. 5.2 Nanomaterials suggested as antigen-presenting devices in new generations of vaccine formulations. **a** Virosomes are phospholipid vesicles in which the antigen (*black* component on the schemes) and immunostimulating peptides are included. **b** Virus-like Particles (VLP) are artificial viral capsides obtained from recombinant viral proteins in which antigens are included. **c** Immuno Stimulating Complex (ISCOM) is aggregates of saponins, cholesterol, and phospholipids in which the antigen is included

It has been found that efficient vaccines cannot be conceived with soluble proteic or petidic antigens [95]. In Mother Nature, invading pathogens carrying antigens are particles. Thus, it is not surprising that early adjuvants used in vaccines occurred as particles although they were developed purely on empiric basis. Thus, nanomaterials may appear as interesting potential adjuvants for vaccine developments. Compared with adjuvants used in formulation of the early age vaccines, adjuvants derived from nanomaterials are, indeed, very well defined in terms of their composition, purity, characteristics, reproducibility of production, and safety. Their mechanisms of interactions with the immune system are also better understood which help to trigger a predefined immune response [105].

Today, a few adjuvants derived from nanomaterials are approved by the health authorities and are used in marketed vaccines [34, 56, 88, 92, 106]. They include virosomes and an oil in water emulsion of squalene oil (adjuvant MF59) adjuvant which were accepted as safe vaccines to protect populations of human against flu and hepatitis at a large scale [56, 88]. Several vaccines for veterinary applications were developed with the adjuvant platform based on the ISCOM (Immuno Stimulating Complex) technology [106]. This is very encouraging for the future of these materials, as the development of new adjuvants for vaccination is demanding in terms of safety. Indeed, vaccines designed to trigger protection against infections are administered to large populations of healthy individuals and no side effects would be tolerated. It seems that nanomaterials now have their place this challenge. They also hold promise for providing vaccines for major infection threats including infections by HIV. One of the reasons is that they are able to deliver antigens produced by biotechnologies in an efficient manner to the immune cells. These antigens which are recombinant proteins are very safe compared with vaccines of the earlier generations prepared from living materials or inactivated pathogens. However, they generally display poor immunogenicity which needs to

be counterbalanced by associating them with suitable (nano) adjuvants able to present the antigen to immune competent cells. It is noteworthy that some of the nanomaterials designed as vaccines show very similar structure to viral particles although they are made from completely artificial components [92, 108, 112] (Fig. 5.2).

5.5 Conclusions and Perspectives

The few marketed medicines based on the use of nanomaterials to deliver drugs in vivo have paved the road to the era of nanomedicine. They improved precision of drug delivery, reducing incidences of side effects considering drugs having a bad initial toxicological profile. This has been made possible through a better control of the in vivo fate of the drug molecules. Another potential of these materials is to turn molecules that have a bad profile of "drugability" into usable medicines. These include molecules from biotechnologies such as therapeutic peptides and nucleic acids as well as compounds showing unfavourable solubility properties. Results coming out from researches are extremely exiting, but a lot of efforts still deserve to be spent in the design of safe nanomaterial platforms able to achieve perfectly controlled drug delivery missions. A way toward this aim is to focus future investigations on fundamental research to elucidate the influence of structures and physico-chemical properties of nanomaterials on their in vivo pharmacological activity. Safety is another issue considering not only the nanomaterials but also the drugs as their new biodistribution profile (given by the delivery system) may reveal unknown side effects. To permit further progresses, developments of appropriate and well-validated methods of characterization are also needed [83]. These lacks of information can explain why only few nanomedicines have been marketed so far. It can be expected that developments of nanomaterials designed for applications in controlling drug delivery will be further intensified in the future. Economic indicators showed that it became the activity sector in pharmacy that generated the largest growth of investments over the past few years, considering from one side the drying of the new chemical entities in the pipe of the pharmaceutical industry and from the other side the diversity of the therapeutic applications for nanotechnologies [14, 83]. This indicates that it is now well established that nanomedicine may represent a real option in the therapeutic arsenal: as treatments for deadly diseases or to improve patient's comfort in terms of administration methods and living standards. In the on going perspectives, nanomaterials used as drug targeting systems will gain in sophistications integrating several functionalities in one single nanomaterial. Nanodevices that combine diagnosis and therapeutic (i.e., "Theragnostics") functions are already proposed at the preclinical stage. It is also anticipated that nanomaterials can become an important part of the tool box in the development of personalized medicine. In this concept, the therapy is chosen according to a fine diagnostic of the clinical profile of the patient which aims to determine the stage of the disease

and allows to anticipate the response to a given treatment [60, 103]. In this therapeutic approach, high precision of both diagnostic and drug delivery are keys to success in which nanomaterials will be a key player.

References

1. Adair JH, Parette MP, Altinoğlu EI, Kester M (2010) Nanoparticulate alternatives for drug delivery. ACS Nano 4:4967–4970
2. Adler-Moore JP, Proffin RT (1993) Development, characterization, efficacy and mode of action of ambisome, A unilamellar liposomal formulation of amphotericin B. J Liposome Res 3:429–450
3. AFSSAPS (2011a) http://www.afssaps.fr/var/afssaps_site/storage/original/application/2551c1616b5fced270dbc1d789557fb3.pdf. NanoDM Report: Biological assessment of medical devices containing nanomaterials, scientific report from 18/08/2011. Accessed 14 Oct 2011
4. AFSSAPS (2011b) http://www.afssaps.fr/var/afssaps_site/storage/original/application/2968a90b774b563b03405379b7d4f4e6.pdf. Recommendations for toxicological evaluation of nanoparticle medicinal products, version 2 from 04/10/2011. Accessed 07 Feb 2012
5. Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE (2009) Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Adv Drug Deliv Rev 61:428–437
6. Aktaş Y, Yemisci M, Andrieux K, Gürsoy RN, Alonso MJ, Fernandez-Megia E, Novoa-Carballal R, Quiñoá E, Riguera R, Sargon MF, Celik HH, Demir AS, Hincal AA, Dalkara T, Capan Y, Couvreur P (2005) Development and brain delivery of chitosan-PEG nanoparticles functionalized with the monoclonal antibody OX26. Bioconjug Chem 16:1503–1511
7. Alhaddad A, Adam MP, Botsoa J, Dantelle G, Perruchas S, Gacoin T, Mansuy C, Lavielle S, Malvy C, Treussart F, Bertrand JR (2011) Nanodiamond as a vector for siRNA delivery to Ewing sarcoma cells. Small 7:3087–3095
8. Ali J, Ali M, Baboota S, Sahani JK, Ramassamy C, Dao L, Bhavna (2010) Potential of nanoparticulate drug delivery systems by intranasal administration. Curr Pharm Des 16:1644–1653
9. Andersen AJ, Hashemi SH, Andresen TL, Hunter AC, Moghimi SM (2009) Complement: alive and kicking nanomedicines. J Biomed Nanotechnol 5:364–372
10. Andrieux K, Couvreur P (2009) Polyalkylcyanoacrylate nanoparticles for delivery of drugs across the blood-brain barrier. Nanomed Nanobiotechnol 1:463–474
11. Anton N, Benoit JP, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates—a review. J Control Release 128:185–199
12. Bangham AD, Standish MM, Weissmann G (1965) The action of steroids and streptolysin S on the permeability of phospholipid structures to cations. J Mol Biol 13:253–259
13. Barraud L, Merle P, Soma E, Lefrançois L, Guerret S, Chevallier M, Dubernet C, Couvreur P, Trépo C, Vitvitski L (2005) Increase of doxorubicin sensitivity by doxorubicin-loading into nanoparticles for hepatocellular carcinoma cells in vitro and in vivo. J Hepatol 42:736–743
14. Bawa R (2009) NanoBiotech 2008: exploring global advances in nanomedicine. Nanomed Nanotech Biol Med 5:5–7
15. Bennett CL, Calhoun EA (2004) Pharmacoeconomics of liposomal anthracycline therapy. Semin Oncol 31:191–195
16. BioAlliance Pharma (2011) http://www.bioalliancepharma.com/fre/R-D/Projets. Accessed 19 Nov 2011
17. Bolhassani A, Safaiyan S, Rafati S (2011) Improvement of different vaccine delivery systems for cancer therapy. Mol Cancer 7:10–13

18. Bosch F, Rosich L (2008) The contributions of Paul Ehrlich to pharmacology: a tribute on the occasion of the centenary of his Nobel Prize. Pharmacology 82:171–179
19. Bramwell VW, Perrie Y (2005) The rational design of vaccines. Drug Discov Today 10:1527–1534
20. Buxton DB (2009) Nanomedicine for the management of lung and blood diseases. Nanomedicine 4:331–339
21. Campisi G, Paderni C, Saccone R, Di Fede O, Wolff A, Giannola LI (2010) Human buccal mucosa as an innovative site of drug delivery. Curr Pharm Des 16:641–652
22. Card JW, Magnuson BA (2011) A review of the efficacy and safety of nanoparticle-based oral insulin delivery systems. Am J Physiol Gastrointest Liver Physiol (E-Pub ahead Sept 15) 301(6):G956–G967
23. Ceh B, Winterhalter B, Frederik PM, Vallner JJ, Lasic DD (1997) Stealth® liposomes: from theory to product. Adv Drug Deliv Rev 24:165–177
24. Chen MC, Sonaje K, Chen KJ, Sung HW (2011) A review of the prospects for polymeric nanoparticle platforms in oral insulin delivery. Biomaterials 32:9826–9838
25. Claude JR, Members of Afssaps Working Party (2009) Recommendations for toxicological evaluation of nanoparticle medicinal products . In: Cartwrightand AC, Matthews BR (eds) International pharmaceutical product registration (Chap 44), Informa Healthcare USA, Inc., New York, pp 755–761
26. Couvreur P, Kante B, Roland M, Guiot P, Bauduin P, Speiser P (1979) Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties. J Pharm Pharmacol 31:331–332
27. Couvreur P, Stella B, Reddy LH, Hillaireau H, Dubernet C, Desmaële D, Lepêtre-Mouelhi S, Rocco F, Dereuddre-Bosquet N, Clayette P, Rosilio V, Marsaud V, Renoir JM, Cattel L (2006) Squalenoyl nanomedicines as potential therapeutics. Nano Lett 6:2544–2548
28. Couvreur P, Vauthier C (2006) Nanotechnology: intelligent design to treat complex disease. Pharm Res 23:1417–1450
29. Dailey LA, Jekel N, Fink L, Gessler T, Schmehl T, Wittmar M, Kissel T, Seeger W (2006) Investigation of the proinflammatory potential of biodegradable nanoparticle drug delivery systems in the lung. Toxicol Appl Pharmacol 215:100–108
30. Danhier F, Feron O, Préat V (2010) To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release 148:135–146
31. De Bock K, Mazzone M, Carmeliet P (2011) Antiangiogenic therapy, hypoxia, and metastasis: risky liaisons, or not? Nat Rev Clin Oncol 8:393–404
32. de Kozak Y, Andrieux K, Villarroya H, Klein C, Thillaye-Goldenberg B, Naud MC, Garcia E, Couvreur P (2004) Intraocular injection of tamoxifen-loaded nanoparticles: a new treatment of experimental autoimmune uveoretinitis. Eur J Immunol 34:3702–3712
33. de Martimprey H, Vauthier C, Malvy C, Couvreur P (2009) Polymer nanocarriers for the delivery of small fragments of nucleic acids: oligonucleotides and siRNA. Eur J Pharm Biopharm 71:490–504
34. De Temmerman ML, Rejman J, Demeester J, Irvine DJ, Gander B, De Smedt SC (2011) Particulate vaccines: on the quest for optimal delivery and immune response. Drug Discov Today 16:569–582
35. Desmaële D, Gref R, Couvreur P (in press) Squalenoylation: a generic platform for nanoparticular drug delivery. J Control Release (in press)
36. Devalapally H, Chakilam A, Amiji MM (2007) Role of nanotechnology in pharmaceutical product development. J Pharm Sci 96:2547–2565
37. Diebold Y, Calonge M (2010) Applications of nanoparticles in ophthalmology. Prog Retin Eye Res 29:596–609
38. Doshi N, Matragotri S (2010) Macrophages recognize size and shape of their targets. PLoS ONE 4:e10051
39. Fattal E, Bochot A (2006) Ocular delivery of nucleic acids: antisense oligonucleitodes, aptamers and siRNA. Adv Drug Deliv Rev 58:1203–1223

40. Fattal E, Bochot A (2008) State of the art and perspectives for the delivery of antisense oligonucleotides and siRNA by polymeric nanocarriers. Int J Pharm 364:237–248
41. Fattal E, Bochot A (2012) Liposomes for intravitreal drug delivery: a state of the art. J Control Release (in press)
42. Fattal E, Barratt G (2009) Nanotechnologies and controlled release systems for the delivery of antisense oligonucleotides and small interfering RNA. Br J Pharmacol 157:179–194
43. FDA (2002) Guidance for industry, liposome drug products chemistry, manufacturing, and controls; human, pharmacokinetics and bioavailability; and labeling documentation, J:\!GUIDANC \2191dft.doc, 07/29/02. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070570.pdf. Accessed 07 Feb 2012
44. Florence AT (2007) The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. Pharm Res 14:259–266
45. Gardlik R, Celec P, Bernadic M (2011) Targeting angiogenesis for cancer (gene) therapy. Bratisl Lek Listy 112:428–434
46. Gaspar R (2007) Regulatory issues surrounding nanomedicines: setting the scene for the next generation of nanopharmaceuticals. Nanomedicine (London) 2:143–147
47. Gaspar R, Duncan R (2009) Polymeric carriers: preclinical safety and the regulatory implications for design and development of polymer therapeutics. Adv Drug Deliv Rev 61:1220–1231
48. Geho WB, Geho HC, Lau JR, Gana TJ (2009) Hepatic-directed vesicle insulin: a review of formulation development and preclinical evaluation. J Diabetes Sci Technol 3:1451–1459
49. Gibbs WJ, Drew RH, Perfect JR (2005) Liposomal amphotericin B: clinical experience and perspectives. Expert Rev Anti Infect Ther 3:167–181
50. Gregoriadis G (1973) Drug entrapment in liposomes. FEBS Lett 36:292–296
51. Gomes Dos Santos AL, Bochot A, Doyle A, Tsapis N, Siepmann J, Siepmann F, Schmaller J, Besnard M, Behar-Cohen F, Fattal E (2006) Sustained release of nanosized complexes of polyethylenimine and anti-TGF-β2 oligonucleotide improves the outcome of glaucoma surgery. J Control Release 112:369–381
52. Gomez-Gualdrón DA, Burgos JC, Yu J, Balbuena PB (2011) Carbon nanotubes engineering biomedical applications. Prog Mol Biol Transl Sci 104:175–245
53. Haley B, Frenkel E (2008) Nanoparticles for drug delivery in cancer treatment. Urol Oncol Sem Orig Invest 26:57–64
54. Hassan MS, Lau R (2010) Effect of particle formulation on dry powder inhalation efficiency. Curr Pharm Des 16:2377–2387
55. Heinemann L, Jacques Y (2009) Oral insulin and buccal insulin: a critical reappraisal. J Diabetes Sci Technol 3:568–584
56. Herzog C, Hartmann K, Künzi V, Kürsteiner O, Mischler R, Lazar H, Glück R (2009) Eleven years of Inflexal® V—a virosomal adjuvanted influenza vaccine. Vaccine 27:4381–4387
57. Hock SC, Ying YM, Wah CL (2011) A review of the current scientific and regulatory status of nanomedicines and the challenges ahead. PDA J Pharm Sci Technol 65:177–195
58. Hohenegger M (2010) Novel and current treatment concepts using pulmonary drug delivery. Curr Pharm Des 16(22):2484–2492
59. Horcajada P, Chalati T, Serre C, Gillet B, Sebrie C, Baati T, Eubank JF, Heurtaux D, Clayette P, Kreuz C, Chang JS, Hwang YK, Marsaud V, Bories PN, Cynober L, Gil S, Férey G, Couvreur P, Gref R (2010) Porous metal-organic-framework nanoscale carriers as a potential platform for drug delivery and imaging. Nat Mater 9:172–178
60. Jain KK (2009) Role of nanobiotechnologies in the development of personalized medicine. Nanomedicine 4:249–252
61. Karatas H, Aktas Y, Gursoy-Ozdemir Y, Bodur E, Yemisci M, Caban S, Vural A, Pinarbasli O, Capan Y, Fernandez-Megia E, Novoa-Carballal R, Riguera R, Andrieux K, Couvreur P, Dalkara T (2009) A nanomedicine transports a peptide caspase-3 inhibitor across the blood-brain barrier and provides neuroprotection. J Neurosci 29:13761–13769
62. Khanna SC, Speiser P (1969) Epoxiresin beads as a pharmaceutical dosage form I: methods of preparation. J Pharm Sci 58:1114–1117

63. Koren E, Torchilin VP (2011) Drug carriers for vascular drug delivery. IUBMB Life 63:586–595
64. Kreuter J (2007) Nanoparticles—a historical perspective. Int J Pharm 331:1–10
65. Krishnamachari Y, Geary SM, Lemke CD, Salem AK (2011) Nanoparticle delivery systems in cancer vaccines. Pharm Res 28:215–236
66. Kruijtzer CM, Beijnen JH, Schellens JH (2002) Improvement of oral drug treatment by temporary inhibition of drug transporters and/or cytochrome P450 in the gastrointestinal tract and liver: an overview. Oncologist 7:516–530
67. Kurmi BD, Kayat J, Gajbhiye V, Tekade RK, Jain NK (2010) Micro- and nanocarrier-mediated lung targeting. Expert Opin Drug Deliv 7:781–794
68. Lajavardi L, Bochot A, Camelo S, Goldenberg B, Naud MC, Behar-Cohen F, Fattal E, De Kozak Y (2007) Down-regulation of endotoxin-induced uveitis by intravitreal injection of vasoactive intestinal peptide encapsulated in liposomes. IOVS 48:3230–3238
69. Lam R, Ho D (2009) Nanodiamonds as vehicles for systemic and localized drug delivery. Expert Opin Drug Deliv 6:883–895
70. Lee PY, Wong KK (2011) Nanomedicine: a new frontier in cancer therapeutics. Curr Drug Deliv 8:245–253
71. Lembo D, Cavalli R (2010) Nanoparticulate delivery systems for antiviral drugs. Antivir Chem Chemother 21:53–70
72. Lynch I, Cedervall T, Lundqvist M, Cabaleiro-Lago C, Linse S, Dawson KA (2007) The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. Adv Colloid Interface Sci 134–135:167–174
73. Maeda H (2001) The enhanced permeability and retention (E.P.R.) effect in tumor vasculature: the key role of tumor-semective macromolecular drug targeting. Adv Enzyme Regul 41:189–207
74. Malam Y, Loizidou M, Seifalian AM (2009) Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci 30:592–599
75. Malam Y, Lim EJ, Seifalian AM (2011) Current trends in the application of nanoparticles in drug delivery. Curr Med Chem 18:1067–1078
76. Mansour HM, Rhee YS, Wu X (2009) Nanomedicine in pulmonary delivery. Int J Nanomed 4:299–319
77. Martínez-Barbosa ME, Cammas-Marion S, Bouteiller L, Vauthier C, Ponchel G (2009) PEGylated degradable composite nanoparticles based on mixtures of PEG-b-poly(γ-benzyl L-glutamate) and poly(γ-benzyl L-glutamate). Bioconjug Chem 20:1490–1496
78. Miele E, Spinelli GP, Miele E, Tomao F, Tomao S (2009) Albumin-bound formulation of paclitaxel (Abraxane ABI-007) in the treatment of breast cancer. Int J Nanomed 4:99–105
79. Mistry A, Stolnik S, Illum L (2009) Nanoparticles for direct nose-to-brain delivery of drugs. Int J Pharm 379:146–157
80. Moen MD, Lyseng-Williamson KA, Scott LJ (2009) Liposomal amphotericin B: a review of its use as empirical therapy in febrile neutropenia and in the treatment of invasive fungal infections. Drugs 69:361–392
81. Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. FASEB J 19:311–330
82. Moyano DF, Rotello VM (2011) Nano meets biology: structure and function at the nanoparticle interface. Langmuir 27:10376–10385
83. Murday JS, Siegel RW, Stein J, Wright JF (2009) Translational nanomedicine: status assessment and opportunities. Nanomedicine 5:251–273
84. Mura S, Hillaireau H, Nicolas J, Kerdine-Römer S, Le Droumaguet B, Deloménie C, Nicolas V, Pallardy M, Tsapis N, Fattal E (2011) Biodegradable nanoparticles meet the bronchial airway barrier: how surface properties affect their interaction with mucus and epithelial cells. Biomacromolecules 12:4136–4143
85. Mura S, Hillaireau H, Nicolas J, Le Droumaguet B, Gueutin C, Zanna S, Tsapis N, Fattal E (2011) Influence of surface charge on the potential toxicity of PLGA nanoparticles towards Calu-3 cells. Int J Nanomed 2011:2591–2605

86. Nemmar A, Hoylaerts MF, Hoet PH, Vermylen J, Nemery B (2003) Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis. Toxicol Appl Pharmacol 186:38–45
87. Ng AWK, Wasan KM, Lopez-Berestein G (2003) Development of liposomal polyene antibiotics: a historical perspective. J Pharm Pharm Sci 6:67–83
88. O'Hagan DT (2007) New generation of vaccine adjuvants. In: Encyclopedia of life sciences, Wiley. www.els.net doi: 10.1002/9780470015902.a0020177
89. Orringer DA, Koo YE, Chen T, Kopelman R, Sagher O, Philbert MA (2009) Small solutions for big problems: the application of nanoparticles to brain tumor diagnosis and therapy. Clin Pharmacol Ther 85:531–534
90. Pardridge WM (2010) Biopharmaceutical drug targeting to the brain. J Drug Target 18:157–167
91. Patel VF, Liu F, Brown MB (2011) Advances in oral transmucosal drug delivery. J Control Release 153:106–116
92. Peek LJ, Middaugh CR, Berkland C (2008) Nanotechnology in vaccine delivery. Adv Drug Deliv Rev 60:915–928
93. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R (2007) Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2:751–760
94. Peltier S, Oger JM, Lagarce F, Couet W, Benoît JP (2006) Enhanced oral paclitaxel bioavailability after administration of paclitaxel-loaded lipid nanocapsules. Pharm Res 23:1243–1250
95. Perrie Y, Mohammed AR, Kirby DJ, McNeil SE, Bramwell VW (2008) Vaccine adjuvant systems: enhancing the efficacy of sub-unit protein antigens. Int J Pharm 364:271–280
96. Petre CE, Dittmer DP (2007) Liposomal daunorubicin as treatment for Kaposi's sarcoma. Int J Nanomed 2:277–288
97. Petrelli F, Borgonovo K, Barni S (2010) Targeted delivery for breast cancer therapy: the history of nanoparticle-albumin-bound paclitaxel. Expert Opin Pharmacother 11:1413–1432
98. Pisani E, Tsapis N, Galaz B, Santin M, Berti R, Taulier N, Kurtisovski E, Lucidarme O, Ourevitch M, Thuy Doan B, Beloeil J-C, Gillet B, Urbach W, Bridal L, Fattal E (2008) Perfluorooctyl bromide polymeric capsules as dual contrast agents for ultrasonography and magnetic resonance imaging. Adv Functional Mater 18:2963–2971
99. Puri L, Loomis K, Smith B, Lee JH, Yavlovich A, Heldman E, Blumenthal R (2009) Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinic. Crit Rev Ther Drug Carrier Syst 26:523–580
100. Raouane M, Desmaele D, Gilbert-Sirieix M, Gueutin C, Zouhiri F, Bourgaux C, Lepeltier E, Gref R, Ben Salah R, Clayman G, Massaad-Massade L, Couvreur P (2011) Synthesis, characterization, and in vivo delivery of siRNA-squalene nanoparticles targeting fusion oncogene in papillary thyroid carcinoma. J Med Chem 54:4067–4076
101. Reddy LH, Couvreur P (2011) Nanotechnology for therapy and imaging of liver diseases. J Hepatol 55:1461–1466
102. Ruenraroengsak P, Cook JM, Florence AT (2010) Nanosystem drug targeting: facing up to complex realities. J Control Release 141:265–276
103. Sakamoto JH, van de Ven AL, Godin B, Blanco E, Serda RE, Grattoni A, Ziemys A, Bouamrani A, Hu T, Ranganathan SI, De Rosa E, Martinez JO, Smid CA, Buchanan RM, Lee SY, Srinivasan S, Landry M, Meyn A, Tasciotti E, Liu X, Decuzzi P, Ferrari M (2010) Enabling individualized therapy through nanotechnology. Pharmacol Res 62:57–89
104. Salama R, Traini D, Chan HK, Young PM (2009) Recent advances in controlled release pulmonary therapy. Curr Drug Deliv 6:404–414
105. Salvador A, Igartua M, Hernández RM, Pedraz JL (2011) An overview on the field of micro- and nanotechnologies for synthetic Peptide-based vaccines. J Drug Deliv 2011:181646
106. Sanders MT, Brown LE, Deliyannis G, Pearse MJ (2005) ISCOM TM-based vaccines: the second decade. Immunol Cell Biol 83:119–128
107. Santos-Maghalaes NS, Mosqueira VCF (2010) Nanotechnologies applied to the treatment of malaria. Adv Drug Deliv Rev 62:560–575

108. Skwarczynski M, Toth I (2011) Peptide-based subunit nanovaccines. Curr Drug Deliv 8:282–289
109. Stella B, Arpicco S, Peracchia MT, Desmaële D, Hoebeke J, Renoir M, D'Angelo J, Cattel L, Couvreur P (2000) Design of folic acid-conjugated nanoparticles for drug targeting. J Pharm Sci 89:1452–1464
110. Stella S, Marsaud V, Arpicco S, Géraud G, Cattel L, Couvreur P, Renoir JM (2007) Biological characterization of folic acid-conjugated poly(H2NPEGCA-co-HDCA) nanoparticles in cellular models. J Drug Target 15:146–153
111. Szebeni J, Muggia F, Gabizon A, Barenholz Y (2011) Activation of complement by therapeutic liposomes and other lipid excipient-based therapeutic products: prediction and prevention. Adv Drug Deliv Rev 63:1020–1030
112. Tiwari S, Agrawal GP, Vyas SP (2010) Molecular basis of the mucosal immune system: from fundamental concepts to advances in liposome-based vaccines. Nanomedicine (Lond) 5:1617–1640
113. Toub N, Malvy C, Fattal E, Couvreur P (2006) Innovative nanotechnologies for the delivery of oligonucleotides and siRNA. Biomed Pharmacother 60:607–620
114. Tyner K (2011) Nanomedicine and the regulatory path, FDA FDA/CDER/DDSR May 17, 2011. http://www.nanobiotech.org/uploads/files/Tyner_COIN.pdf, Accessed 9 October 2011
115. Vauthier C, Bouchemal K (2009) Methods for the preparation and manufacture of polymeric nanoparticles. Pharm Res 26:1025–1058
116. Vauthier C, Couvreur P (2007) Nanomedicines: a new approach for treatment of serious diseases. J Biomed Nanotechnol 3:1–12
117. Viviani MA, Rizzardini G, Tortorano AM, Fasan M, Capetti A, Roverselli AM, Gringeri A, Suter F (1994) Lipid-based amphotericin B in the treatment of cryptococcosis. Infections 22:137–142
118. Yoo JW, Doshi N, Matragotri S (2011) Adaptive micro and nanoparticles: temporal control over carrier properties to facilitate drug delivery. Adv Drug Deliv Rev (in press)
119. Zhang I, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC (2008) Nanoparticles in medicine: therapeutic applications and development. Clin Pharmacol Ther 83:761–769
120. Zhang J, Wu L, Chan HK, Watanabe W (2011) Formation, characterization, and fate of inhaled drug nanoparticles. Adv Drug Deliv Rev 63:441–455
121. Zhang Y, Benet LZ (2001) The gut as a barrier to drug absorption: combined role of cytochrome P450 3A and P-glycoprotein. Clin Pharmacokinet 40:159–168

Chapter 6
Titanium Dioxide in Photocatalysis

S. Cassaignon, C. Colbeau-Justin and O. Durupthy

Abstract TiO_2-based heterogeneous photocatalysis is a process that develops rapidly in environmental engineering and it is now employed in several industrial domains, including water treatment, air purification, and self-cleaning surfaces. Photocatalysis is a natural phenomenon in which the TiO_2 accelerates a chemical reaction through the action of light, without being altered. The illuminated TiO_2 induces the formation of reactive species, able to decompose by oxidation and/or reduction reactions organic or inorganic substances. The major part of the applications of photocatalysis corresponds to organic oxidation, and it is now considered as one of the Advanced Oxidation Technologies (AOTs), gathering the reactions mainly based on hydroxyl radical ($HO^{\bullet}$) chemistry. The development of a system based on photocatalysis requires gathering knowledge of numerous and various scientific domains: physical-chemistry, materials science, catalysis, environmental chemistry, biology, and engineering science. This chapter is therefore designed to give a detailed survey of the different scientific fields concerning TiO_2-

S. Cassaignon (✉) · O. Durupthy
Chimie de la Matière Condensée de Paris, UMR 7574, Collège de France, UPMC Univ Paris 06, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, France
e-mail: sophie.cassaignon@upmc.fr

S. Cassaignon · O. Durupthy
CNRS, Chimie de la Matière Condensée de Paris, UMR 7574 , Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, France

S. Cassaignon · O. Durupthy
Chimie de la Matière Condensée de Paris, Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05, France

C. Colbeau-Justin
Laboratoire de Chimie Physique, UMR8000, Univ Paris-Sud, 91405 Orsay, France

C. Colbeau-Justin
CNRS, Laboratoire de Chimie Physique, UMR8000, Univ Paris-Sud, 91405 Orsay, France

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_6,

based photocatalysis. Various aspects are developed: materials (synthesis, crystal chemistry, electronic and optical properties of TiO_2), physical-chemistry (photon absorption, charge-carrier dynamics, surface adsorption, and photooxidation mechanisms), environmental chemistry (dyes, pesticides, bacteria, and antibiotic photodegradation, real industrial wastewater treatment), and engineering (photocatalytic reactor design and simulation).

6.1 Introduction

Today more than ever, the human activity and modern life style are responsible for the worsening environmental pollution. Sources of pollution are becoming more numerous and diverse (industry, automobile, petroleum, waste plastics and computer, consumer products). The accumulation of gaseous or liquid exhausts provokes the pollution of the atmosphere as well as water resources. But air pollution is not limited to the outside, it includes also indoor where we spend about 90 % of our time.

Heterogeneous photocatalysis is a process that develops rapidly in environmental engineering and it is now employed in several industrial domains, including systems for (i) water depollution: water purification, treatment of industrial effluents in order to limit the release of toxic compounds, (ii) air depollution: destruction of bacteria that cause odor nuisance or that are present in hospital, reducing air pollution in an urban environment (conversion of NO_x to NO_3^-, then trapped in water), and also (iii) self-cleaning surfaces: increasing duration between cleaning of a surface. The main advantages of the photocatalysis are: low cost, ease of initiation and stopping the reaction, the low energy consumption, the variety of degradable pollutants, and a high efficiency in pollutants mineralization (conversion of organics to H_2O, CO_2 and NO_3^-, PO_4^{3-}, halide ions, etc.)

Photocatalysis is a natural phenomenon (thermodynamically favored) in which a substance called photocatalyst accelerates a chemical reaction through the action of light (natural or artificial), without being altered. Using light energy, photocatalysts induce the formation of reactive species, able to decompose by oxidation and/or reduction reactions organic or inorganic substances. The mechanism of photocatalysis consists of four stages. (1) The photocatalysts are semiconductors, which can be excited by light with higher energy than the bandgap ($h\nu > Eg$), and (2) energy-rich electron–hole pairs are formed which dissociate into free photoelectrons in the conduction band (CB) and photo-holes in the valence band (VB). Then (3), there is migration of the charge carriers toward the surface of material and simultaneously, in the presence of a fluid phase (gas or liquid), adsorption occurs spontaneously. (4) According to redox potential (or energy level) of each adsorbate, an electron transfer occurs to the molecules with acceptor character while the positive photo-holes are transferred to the molecules with donor

character. During the reaction of photodegradation, one or more reactive species of water or air may be implied ($HO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, etc.). The photocatalytic activity is controlled by several factors intrinsic to the material: (i) its coefficient and its range of optical absorption; (ii) the speed of reduction or oxidation on the surface by the electron and the hole; (iii) the rate of recombination of the electron–hole pair.

Several photocatalytic reactions have direct environmental applications. Such applications have played an important role in the development of photocatalysis, both as scientific discipline and industrial market.

The major part of those applications corresponds to organic oxidation, and photocatalysis is now considered as one of the Advanced Oxidation Technologies (AOTs). The AOTs are gathering the reactions mainly based on hydroxyl radical ($HO^{\bullet}$) chemistry, which is the major reactive intermediate responsible for organic substrate oxidation. With photocatalysis, the AOTs also include ozonation, photo-Fenton, and H_2O_2 reactions [1]. The complete list, given by Bhatkhande et al. [2], of organic molecules photooxidized by TiO_2 is very large and confirms photocatalysis as a very successful AOT.

However, photocatalysis can also be used in environmental chemistry for metal reduction, for example, in the case of Cr^{6+} reduction to Cr^{3+}, As^{5+}, and As^{3+} reduction to As^0, Hg^{2+}, and Hg^0 [3], or NO_x and SO_x reduction.

Titanium dioxide is one of the most important semiconductors in the family of transition metal oxides. It is mainly used as a white pigment (in paint, plastics, papers, foods, pharmaceuticals) and as UV absorber in sunscreens. It has been widely investigated because of its attractive application in photovoltaic and photocatalysis. The process of photocatalysis used for purification of air and water has mainly developed around the TiO_2 due to significant advantages presented by this compound: chemical stability, non-toxicity, low cost, abundant natural resources, and ability to degrade a wide range of both gaseous and liquid pollutants. Indeed, the energy levels of TiO_2 (VB and CB) are located in an adequate way compared to the redox potential of many organic species and those of water and oxygen. However, its large bandgap (around 3.2 eV), corresponding to an onset of the optical absorption band at about 380 nm, means that the photoactivity can be observed only under UV light excitation.

Numerous excellent reviews have been written in the field especially on the topic of photocatalysis for pollutant degradation [4–7]. This article is focused on the oxidative properties of TiO_2. The first part of this chapter is devoted to introduction of TiO_2 and its photo-induced processes (Sects. 6.1, 6.2 and 6.3), after which we treat photocatalytic reaction and mechanisms (Sect. 6.4) in detail. The next two parts describe the benefit of the photocatalysis in environmental chemistry (Sect. 6.5) and examples of reactors used for wastewater treatment are described for illustrative purposes (Sect. 6.6) and finally, conclusions are given in the last part (Sect. 6.7).

Table 6.1 Crystallographic data of different TiO_2 polymorphs

	Rutile	Anatase	Brookite
Crystallographic structure	Tetragonal	Tetragoanl	Orthorhombic
Space group	$P4_2/mnm$	$I4_1/amd$	Pcab
Cell parameters (A)	a = 4,5933 b = – c = 2,9592	a = 3,7852 b = – c = 9,5139	a = 9,1819 b = 4,4558 c = 5,1429
Z (molecules/cell)	2	4	8
Structure			
Ti coordinance	6	6	6
Density	4,24	3,83	4,17
Ti–O distances (A)	2 at 1,946 4 at 1,984	2 at 1,937 4 at 1,964	2 at 1,993 1 at 1,865 1 at 1,919 1 at 1,945 1 at 2,040

6.2 General Aspects

6.2.1 Crystalline Structure

Three polymorphs of titanium are mainly observed, rutile (thermodynamic phase), anatase, and brookite (metastable phases). All structures are built-up of TiO_6 octahedra but differ in their stacking. Rutile crystallizes in the tetragonal system (space group $P4_2/mnm$) [8]. The structure can be described as arrays of linear chains of edge-sharing octahedra along the *z*-direction and the connection between these channels is through the sharing of vertices in both directions *x* and *y*. Anatase crystallizes in the tetragonal system (space group $I4_1/amd$) [8]. The structure can be described also as arrays of zigzag chains of octahedra linked by edges and each octahedron shares four edges. Brookite crystallizes in the orthorhombic system (space group Pcab) [9]. Its structure can be described as arrays of zigzag chains of octahedra sharing three edges. These chains are plans in the *x* and *z* and the connection between these plans is done along the *y* direction by sharing vertices. Their structures are shown in Table 6.1.

Brookite possesses a more distorted structure than anatase or rutile as it has only two Ti–O bonds of identical length. These dissimilarities may involve differences in reactivity at the particle surface as well as in the redox level. Similarly, rutile possesses a more compact structure than the other two, which can also have consequences on its reactivity.

6.2.2 Electronic and Optical Properties of TiO_2

Being the thermodynamic phase, rutile is stable under thermal treatment while anatase and brookite undergo a non-reversible phase transition into rutile when heated at high temperature. At nanoscale, it was reported that the transformation sequence among the three TiO_2 phases is dependent on size of the particle (d) and pH. Indeed, the energies of the three phases are sufficiently close to one another to be reversed by small differences in surface energy. Anatase is thermodynamically most stable when $d < 11$ nm, brookite is the most stable phase when for $11 < d < 35$ nm, and while $d > 35$ nm, rutile is the most stable phase [10]. However, rutile is stabilized relative to anatase in very acidic solutions, whereas in very alkaline solutions anatase is stabilized relative to rutile and brookite [11].

The electronic properties of the TiO_2 vary according to the studied polymorph as describe in detail later. Detailed studies on rutile at low temperatures showed that the bandgap near the edge is dominated by direct transitions at 3.06 eV (405 nm, $T = 1.6$ K) [12–15]. In the case of anatase there is a consensus that the absorption edge is around 3.2 eV (384 nm), associated with indirect transitions [16, 17]. Brookite has bandgap energies ranging from 3.1 to 3.4 eV (365–400 nm), both smaller and larger than the anatase one, and there appears to be no consensus on whether direct or indirect transitions dominate the optical response [18–21]. These values give transitions in the ultraviolet domain. Due to lower size the bandgap of TiO_2 particles at the nanoscale are larger than the bandgap of bulk TiO_2 [18, 22–25]. For example, the lower edge of the CB for nanosheet is approximately 0.1 V higher, while the upper edge of the VB is 0.5 V lower than bulk anatase [23].

6.2.3 Synthesis of TiO_2

The synthesis of nanostructured TiO_2 (nanoparticles, thin films, nanoporous materials) is available, thanks to the variety of preparation methods: mechanical, chemical, or physical; in liquid or gas medium.

Rutile is mainly prepared by hydrothermal approach (relatively high temperature and pressure). Indeed, the rutile phase is thermodynamically stable and its formation needs "hard" conditions such as acidic media, high temperatures, and/or long aging time [26–30]. Nanorutile can, nonetheless, be obtained by soft chemistry [31–34]. Synthesis routes of anatase are numerous and varied because of its metastability. The hydrothermal syntheses are frequently employed in the literature, but the hydrolysis of Ti(IV) precursors under mild conditions is the most widely used method, sometimes through the intermediary of a gel [35, 36]. The precipitation in aqueous solution is also reported [34]. Some works refer to other ways of syntheses [37–39]. Brookite phase is still an exotic phase and few works report the synthesis of pure brookite especially at the nanoscale. Indeed, the most common synthesis routes are hydrothermal ways but they lead essentially to micrometer-sized particles [19, 40–45]. The

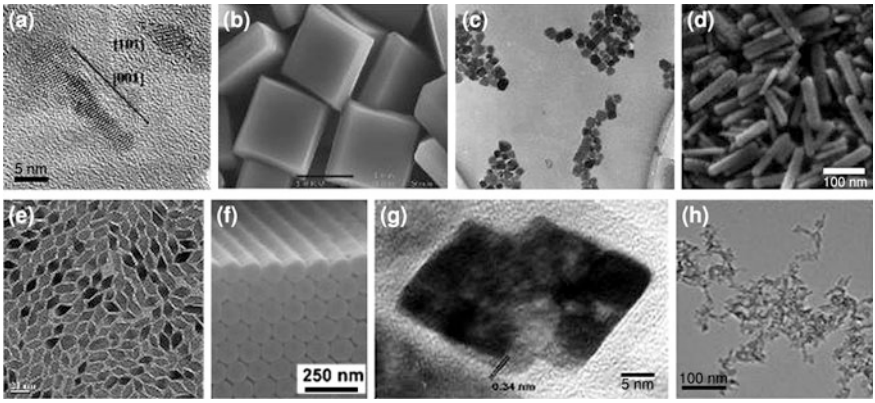


Fig. 6.1 Particle morphologies of nanosized TiO_2 for **a**–**f** Anatase, **g** Brookite, and **h** Rutile. For synthesis methods and details: **a** [49], **b** [50], **c** [36], **d** [51], **e** [52], **f** [53], **g** [48], **h** [31]

nanometric brookite can be synthesized by thermohydrolysis of $TiCl_4$ in aqueous media [20, 32, 46–48]. Depending on synthesis conditions the observed morphologies are highly various, even for a same crystalline structure (Fig. 6.1).

6.2.3.1 Sol–Gel Synthesis

It is a versatile synthesis and a low-cost process. In the case of TiO_2, it consists of hydrolysis of a titanium precursor [Ti(IV) alkoxide or a salt] followed by condensation at low temperature leading to the inorganic framework. In aqueous solution, a colloidal suspension is then formed (the sol) and depending on the synthesis conditions (pH, concentration, acidity, temperature, etc.), anatase, rutile, or brookite phases can be synthesized with a fine control of size and morphology of the particles. In alcoholic medium, one can observe a transition to gel after loss of solvent and total polymerization. By adjusting the reaction conditions (pH, solvent, additives), it is possible to design the nanoparticles obtained.

Many nanostructures can be formed by sol–gel methods: size and shape controlled nanoparticles [54, 55], nanocubes [36, 56], nanorods [57], and nanowires [58]. Thin films can also be obtained by coating the sol onto various substrates by dip-coating or spin-coating. Porous materials (meso- or nanoporous) are widely studied since the specific area of the materials is considerably increased. The use of surfactant is often described, which allows, in some cases, the formation of a template during the synthesis [59, 60].

The advantages of this method are the purity of products, the homogeneity, the flexibility, the ease of implementation, the possibility of introducing dopants in high concentration, and its easy use to make thin films on surfaces.

The methods of micelles and reverse micelles can also be cited for the formation of well-controlled nanosized particles [61, 62].

6.2.3.2 Hydro- and Solvothermal Synthesis

These methods involve chemical reactions of a titanium precursor in aqueous (hydrothermal method) or organic solvent (solvothermal method), at controlled temperature and pressure. The temperature may approach the boiling water temperature in hydrothermal method and thus the saturation vapor pressure, or may be much higher for the solvothermal method where a solvent with high boiling point is used. These techniques allow obtaining nanoparticles, small size distribution, and controlled crystallinity by adjusting the experimental conditions. Nanowires, nanotubes, or nanorods can also be synthesized by these methods [63–65]. Finally, other synthesis approaches, not really in liquid media but rather in a supercritical fluid, have been studied [66].

6.2.3.3 Direct Oxidation and Electrodeposition

The direct oxidation of titanium consists of chemical or anodic oxidation of titanium metal [53, 67, 68] and leads to the formation of TiO_2 nanorods or nanotubes.

As for electrodeposition, it is a technique used to produce surface coatings. The substrate plays the role of cathode and is immersed in a solution of precursor salts of the material to be deposited. By adjusting the parameters such as the electrolyte, the working potential, the current density, the temperature, and the pH, it is possible to control the structure and morphology of the deposit. TiO_2 nanoparticles could be deposited on various substrates such as carbon nanotubes [69].

6.2.3.4 Chemical Vapor Deposition

It involves a chemical reaction during which a precursor in vapor phase is condensed to form a solid-phase material. This process that can be used in a flow production is employed to form coatings on many substrates, films, and fibers or to develop composite materials by infiltration [70]. Thus thin films of TiO_2 with controlled grain size, nanoparticles, or nanorods have been synthesized [71, 72].

6.2.3.5 Mechanosynthesis

This technique consists of grinding the micrometric powders by action of ceramic balls under strong agitation. The material is then crushed until a nanosized powder is obtained. The mechanochemical synthesis, where the nanopowder is formed by a chemical reaction induced by mechanical grinding [73] is also used.

6.2.4 *Influence of Some Parameters on Photocatalytic Efficiency*

Photocatalytic activity is dependent on the surface and structure properties of the semiconductor such as crystal structure, surface area, particles size, porosity, bandgap, and surface hydroxyl density. Despite a huge number of publications in which TiO_2 nanoparticles with different crystalline phase, size, and morphology were studied in terms of photocatalytic activity, the relationships between morphological and structural properties and the catalytic behavior still remain unclear.

6.2.4.1 Crystalline Structure Effect

Anatase, brookite, and rutile TiO_2 have a photocatalytic activity [74]. It was shown that anatase was the most active [75, 76]. Indeed, the potential of the CB is more negative for anatase and for rutile, which promotes the reduction of oxygen and thus reducing recombination, making more efficient anatase form [77]. It also seems that other parameters are involved, as the mobility of charges created in the matrix of the semiconductor TiO_2 under the effect of photons. Work on the comparison of the photoconductivity of the anatase and rutile has shown that the lifetime of charge carriers is higher for anatase than for rutile [78, 79]. In addition, the rate of recombination is significantly higher for rutile. This recombination slows down the photodegradation of pollutants because it prevents the formation of oxidizing species, mandatory for the mineralization of organic matter adsorbed on the surface of grains.

Mainly because of the difficulties encountered in its synthesis, phase-pure brookite has been significantly less characterized as compared to anatase or rutile but good photoactivity is reported in the literature [44, 80–82]. For example, brookite nanoplates exhibit higher efficiency than rutile and anatase in the bleaching of methyl orange solution under UV irradiation [51, 83, 84].

Although the anatase phase usually has a better photocatalytic activity than brookite and rutile, the mixture of anatase and rutile in the *Evonik* P25 material ($\sim$80 % of anatase and 20 % of rutile) demonstrated extraordinary photocatalytic activity. It has become a reference for photocatalytic tests. The series of PCX anatase from *Cristal Global* (X refers to the specific surface, it decreases with the increasing sintering temperature) and *Hombikat* UV-100 from *Sachtleben* are also frequently used and compared with various synthesized materials. The characteristics of the reference powders are given in Table 6.2

The good photocatalytic activity of *Evonik* P25 was reported to be due to slowed down recombination between electrons and holes. It was postulated that the smaller bandgap of rutile absorbs the photons and generates electron–hole pairs. The electron transfer takes place from the rutile CB to electron traps in the anatase phase [85]. Concerning the *Hombikat* UV-100 it was reported that the high photoactivity is due to the fast interfacial electron transfer rate [86].

Table 6.2 Characteristics of the PCX series P25 and UV100

Sample	Phase composition (%)	Crystallite size (nm)	S_{BET} (m^2 g^{-1})	Mean pore diameter (nm)
PC10	Anatase 100	65–75	10	24.1
PC50	Anatase 100	20–30	54	20.1
PC105	Anatase 100	15–25	85–95	15.3
PC500	Anatase 100	5–10	317	6.1
P25	Anatase 79 Rutile 21	22	51	31.5
UV100	Anatase 100	13	289	<50

6.2.4.2 Size Effect

The size of the particles of the catalyst is an important parameter for photocatalytic efficiency since higher initial surface sites is expected with the decrease in size [87]. However, the predominant pathway for electron–hole recombination may be different depending on the particles size, especially at the nanoscale, where the surface/volume ratio is very large. In 1984, Tsai and Chung [88] showed that the decomposition of CO increased as the radius of the particles decreased. According to the theoretical and experimental studies found in the literature it seems that there is an optimal value of the particles size around 10–20 nm. For example, Zhang et al. [89] have shown that a size of 10 nm was the most appropriate for the photodegradation of chloroform. This is actually a compromise between surface reaction and recombination of electron–hole pairs. Tomkiewicz [90] has written a review on the influence of particle size in photocatalysis. However, the optimal size rather depends on the photocatalyst used, the pollutant studied, as well as the reaction conditions.

Another parameter involved in the particle size effect is the crystallinity of the particles. Indeed, the quality of the semiconductor crystals free of defects will help to avoid the recombination of electron–hole pairs. It has been reported that the photocatalytic activity of amorphous TiO_2 is negligible showing the importance of crystallinity [91, 92]. Several studies have shown that the crystallinity is improved with the calcination of the particles leading to increased photocatalytic degradation efficiency; nonetheless, a prolonged calcination results in a reduced efficiency since the surface area of the catalyst decreases.

The surface area is also a key parameter, since photocatalysis occurs through a surface transfer and consequently, the higher the surface, the higher the reaction sites number. However, the surface represents a zone of defects, which are traps for the electron–hole pairs, if the surface area increases too much, increasing the number of defects can involve a too high rate of recombination. The relationship between physical properties and photocatalytic activity is complex. Structure and size of TiO_2 nanoparticles are significantly dependent on the calcination temperature [93]. Indeed, the temperature promotes phase transformation and a growth of the crystallites. Agrios and Pichat [94] have studied the effect of the sintering

temperature of the PCX photocatalysts on photodecompostion of phenol, anisole, and pyridine in water. They have shown that the sintering has two important effects: (i) decreasing the concentration of crystal defects and (ii) increasing the average particle size, i.e., lowering the surface area. This results in a decreased rate of recombination of photogenerated electrons and holes, and also in a sintering of small crystallites into larger agglomerates leading to a decrease in surface area. In the case of phenol, reaction rates increase in the order PC500 < PC105 < PC50 < PC10, that is, in order of increasing heating although PC10 has a 30-fold less surface area than PC500. These results show that, for removal of phenol (without adsorption on photocatalyst), the decrease in the electron–hole recombination rate outweighs the decrease in surface area. Pyridine degradation follows the opposite tendency; since pyridine mainly reacts on the TiO_2 surface, the importance of surface area is essential.

6.2.4.3 Surfaces and Exposed Faces Effect

The nature of the surface of the nanoparticles of TiO_2 plays an important role in the photodegradation due to the presence of surface hydroxyl groups. Indeed, the OH groups can directly trap the photogenerated holes producing very reactive surface $HO^{\bullet}$ [4]. In addition, the surface charge, depending on the protonation/deprotonation equilibrium of the OH groups, induces a modification of not only the adsorption of the pollutant but also the amount of $HO^{\bullet}$ formed. That is why the pH of the reaction dramatically influences the efficiency of the photodegradation [95]. The isoelectric point (IEP) of TiO_2 is about 6 (6,25 for P25 for instance [96]), therefore interactions with cationic electron donors and acceptors will be favored at pH > IEP, while interactions with anionic electron donors and acceptors will be favored at pH < IEP. However, the difference in IEP values of various TiO_2 can affect the reaction mechanism; moreover specific interactions may occur between certain pollutants and selected surface sites.

In addition the pH value can modify the state of chemical species in solution, which is closely related not only to the dissociation constant but also to the adsorption mode of pollutants. It is thus important to study the nature of the TiO_2 particles and the pollutants to be degraded and determine the optimum pH.

The morphology of the photocatalysts is also reported to be an important parameter [97]. Under equilibrium conditions, anatase crystals are mainly octahedral bipyramids with {101} facets, which have the lowest energy rather than the more reactive {001} facets [52]. Recently, numerous studies have reported the preparation of anatase TiO_2 nanoparticles with higher energy surfaces [50, 98]. They successfully synthesized high-quality anatase TiO_2 crystals with exposed {001} facets. Their higher efficiency in the photocatalytic degradation of organic compounds is related to the predominance of {001} surfaces [99–103].

It has also been reported that the photocatalytic activity of TiO_2 nanotubes (synthesized from anodization of Ti) can be much higher than for TiO_2 nanoparticles [68]. The higher efficiency of the tubes (in spite of their lower surface

area) compared with P25 can be ascribed to (i) a more optimized geometry with significantly shorter carrier-diffusion paths in the tube walls (10–15 nm), including less trapping and recombination of electron–hole pairs (ii) a shorter diffusion path for pollutant molecules from the solution to the active surface area.

6.2.5 Improvements

The optical absorption of TiO_2 is unable to use efficiently the whole solar light spectrum for photocatalysis since less than 5 % of the solar light energy can be absorbed by TiO_2 while the visible part of the spectrum represents ~43 % of total energy received. This explains the continuos interest in increasing the photoactivity under visible light. Three ways are studied: (i) the doping of TiO_2 with metallic ions [104] or heteroelements [105] to change the gap by inserting new states in the bandgap, (ii) the sensitization by organic or inorganic dyes, which can improve optical activity in the visible region (not presented here) [106, 107], (iii) a third possibility is to modify the surface of TiO_2 by pretreatment or by coating with other semiconductors [108] or metals to improve the charge transfer between TiO_2 and the overall system.

6.2.5.1 Doping

The syntheses are realized by wet chemistry (usually involving hydrolysis of a titanium precursor in a mixture of water and other reagents, followed by heating), high-temperature treatment, or ion implantation into TiO_2 nanomaterials.

Cation doping: Different metals have been used to dope TiO_2 by methods of wet chemistry [104], treatment at high temperature, or ion implantation [109]. In the literature doping with metal ions V, Cr, Mn, Fe, Co, Ni, Cu, Zr, Sn, W, etc., lanthanides La, Ce, Nd, etc., [110] or with alkali metals Li, Na, K [111] are found.

Anion doping: TiO_2 has been doped with several hetero atoms: B, C, N, O, F, S, Cl, Br, by the three different methods. For example, hydrolysis of titanium tetra isopropoxide (TTIP) in a water/amine medium followed by treatment with amines [112] or high-temperature annealing of TiO_2 under ammonia [113] or ion implantation under nitrogen [114] leads to a nitrogen-doped TiO_2.

6.2.5.2 Surface Modification

Surface treatment: The surface can be modified by pretreatments such as sulfation, reduction with hydrogen, or halogenation. For example, TiO_2 has been treated by SO_4^{2-} to form superacid solid, which shows an increased photoactivity in various organic reactions [115]. Sulfation of the catalyst leads to an increase of the surface

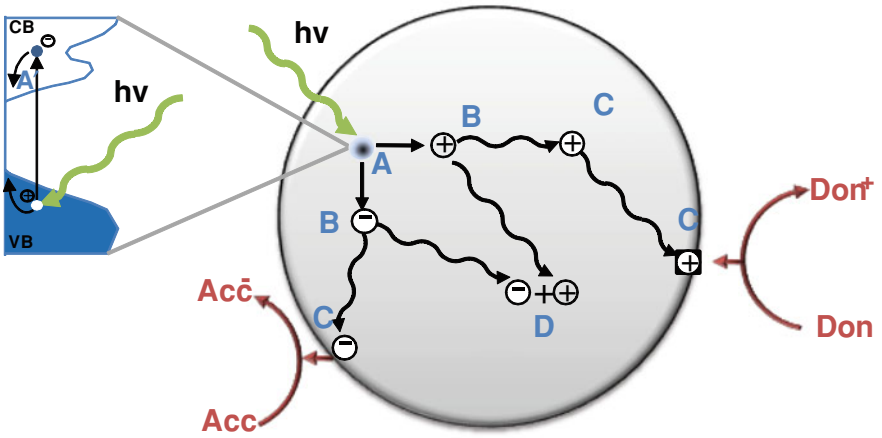


Fig. 6.2 Schematized description of different steps of the photocatalytic process

acidity [116] and an increase of adsorption strength and therefore to an improvement of the adsorption coverage of the substrates.

Any materials with a narrower bandgap or absorption in the visible or infrared domain can be used to coat TiO_2 materials.

Sensitization by semiconductors: The synthesis method is usually the sol–gel method and the sensitizers are numerous. We can find studies on CdS, PbS, Ag_2S, Sb_2S_3, and Bi_2S_3, AgI, CdSe, InP, WO_3, Fe_2O_3, ZnO, and SnO_2 [117–119].

Metal coating: Metal deposition on the TiO_2 surface enhances photocatalytic reactions by accelerating the transfer of electrons to dissolved oxygen molecules. There is an optimum loading value above which metal deposition has a detrimental effect on the photocatalytic activity. Metals such as Pd, Pt, Ag, Cu have been deposited on TiO_2 particles, by sol–gel methods [120], mechanical mixing [121], chemical deposition, precipitation–reduction [122], and photodeposition.

6.3 Fundamental Aspects

6.3.1 Introduction

The entire photocatalysis process may be decomposed into several single steps occurring successively or simultaneously in the material, onto the surface or close to this surface. These different fundamental physical and chemical phenomena are summarized in the scheme displayed in Fig. 6.2.

The first step corresponds to the promotion of an electron from the VB to the empty CB (and thus the creation of a positive hole in the VB) induced by the uptake of a photon by the semiconductor. The generated charges (electron and

hole) are effectively separated and may then migrate, be trapped, or recombine. The charges that migrate or are trapped close to the surface may either be transferred to the electronic levels of the targeted surface adsorbate or generate radicals that allow remote photocatalysis reactions. Some of those events proceed in the time scale of the femtosecond hence requiring time-resolved experiments with high accuracy. Others happen more slowly but may not easily be studied, as no physical parameter is directly accessible experimentally. Consequently, these fundamental issues were only addressed in the last 30 years, especially in the last decade. The aim of this section is not to give a precise description of the state of the art in these fundamental domains, several recent reviews are available [123–128] and among them the very detailed review of the professor Henderson [129], but rather to draw an overview of the knowledge in the domain and the issues still in debate. The different aspects will be discussed in the order they occur in the whole catalytic process and the focus is put on the two main TiO_2 structures anatase and rutile, the brookite phase being too scarcely obtained as pure materials to be extensively studied.

6.3.2 Photon Adsorption

The optical properties of the TiO_2 structures are now experimentally well known and confirmed by theoretical calculations [130–132]. The lowest photon energies needed to promote an electron from the valence band to the conduction band are 3.2 and 3.0 eV for anatase and rutile, respectively, with an indirect bandgap in the former case and a direct one in the latter [130, 132]. However, when considering the energy levels in both bands displaying the highest density of states (i.e., the highest probability of transition) the transition is closer to 4 eV in energy. The light absorption coefficient increases exponentially with photon energy in the case of anatase while it is rather linear for rutile. When the photon energy used to promote the electron from the VB to the CB is much higher than the bandgap, the result is an electron in the CB significantly higher in energy than the bottom of the band or a hole generated not just below the top of the VB (cf Fig. 6.2) [133]. The bandgap values given for pure bulk materials at room temperature depend on various parameters listed and discussed below. Several of these parameters are used to tune the optical absorption domain of the materials and its efficiency.

First, a temperature increase was shown to decrease the optical absorption edge [133]. Moreover, the relative orientation of the anatase crystal toward the polarization direction of incident light was said to impact on the adsorption efficiency [133, 134]. However, this issue is still a matter of debate and the physical backgrounds are not clearly established. More important questions are raised by the use of nanoscale materials with a high surface/volume ratio that may alter the adsorptive properties. A geometric distortion of the TiO_2 unit cells for very small particles may also have consequences. Indeed, a blueshift is observed for particles

smaller than 10 nm in size. This effect is even more pronounced in particles under 3 nm as confirmed by calculations [135, 136].

Surface modification by adsorbates and bulk doping are the two most used strategies to decrease the optical bandgap. The surface additives may be either organic such as dyes [137] and surfactants [138] or inorganic such as metallic ions and clusters [139, 140] or metal oxides [141]. For bulk doping of TiO_2, the most studied route is an *n*-type self-doping associated to the presence of Ti^{3+} ions in the lattice. Thus, the bandgap is decreased of about 0.8 eV but the question whether the transition occurs only between localized states is still controversial [142, 143]. A variety of cation-doped TiO_2 samples were prepared and tested in photocatalysis. Indeed, depending on the nature of the cation, the dopant creates additional energy states either at the bottom of the VB (with Fe) or at the top of the CB (with V) [144]. However, the presence of the cations in the lattice is not benign since some of the additives increase the charge carriers' lifetime while others decrease it [145]. The doping with anions was much more studied since the first demonstration of the photocatalytic activity of nitrogen-doped TiO_2 under visible light in 2001 [105], and the majority of the studies are focused on this element. The influence of the preparation mode and of the localization of nitrogen in the structure on the photocatalytic activity was extensively discussed [146]. The doping significantly decreases the UV activity of TiO_2 in comparison with the pure material because it favors recombination of charge carriers but it allows photocatalysis by visible light [147].

6.3.3 Charge Carriers Migration and Trapping

Once the electron is promoted into the CB it is still physically in the vicinity of the created hole and the charge carriers' pair is called an exciton. The stability of the charge pair is due to the mutual attractive electrostatic interaction, which may be hampered by the high dielectric constant of TiO_2. Its destiny is almost always seen as the evolution (migration, trapping) of separated holes and electrons. Indeed, very few is known about the way the excitonic state may be trapped or migrate while it is known that the charge separation may not occur immediately [130, 133].

When electrons and holes are not trapped in an excitonic state or do not recombine (cf. below) they may migrate in the solid. In parallel to that migration, the charge carriers will 'thermalize', that is to say reach their lowest energy state at the edges of the bandgap. This thermalization is quite fast for 'hot' electrons, in the range of the 100 fs time scale [148] and longer for 'hot' holes (up to 100 ps) so those carriers may reach the surface without being totally thermalized [149]. As for charge transport in a semiconductor, mobility may be seen for both carriers as a succession of hopping from one lattice site to another [150, 151]. Some sites (especially those close to the surface) that correspond to deeper surface potential wells are able to stop the charge migration for a certain duration and are consequently considered as trapping sites. Electron transport is strongly promoted by

electron–phonon coupling, and as a consequence, is said to be anisotropically efficient [150–152]. The Marcus theory for polaron hopping was then efficiently used for bulk anatase and rutile systems. Differences between the two structures and between two different directions in the same structure were observed [153]. The hole transport phenomenon is less understood as the interactions with phonons seem to be less effective.

An important issue in the lifetime of charge carriers concerns their trapping in specific sites. This trapping may be seen as an undesired event if it prevents the charges to reach the surface but may also be considered as favorable if it facilitates charge separation or if the charges are trapped close enough to the surface to react. Most of the electrons are believed to be trapped close to the surface but the precise location is matter of debate for some studies propose an electron trapping on subsurface Ti sites while others studies locate it on under coordinated Ti located at the surface [154, 155]. However, the exact localization is not easy to determine for no experimental probe is able to address this issue. The amount of trapped electron is generally in the range of one trapped electron per nanoparticle [156]. The trapping of electron is very fast (shorter than 100 fs) while the trapping lifetime is at least in the picoseconds scale and may last several months if no electron scavenger is present [156, 157].

The hole trapping is much more difficult to study than the electron one and few quantitative results are available. EPR studies indicate that trapped holes are localized on negatively charged under coordinated surface oxygen sites [158]. Holes may be trapped as fast as electrons and the trapping lifetime may be equally long.

6.3.4 Charges Recombination or Transfer on a Surface Adsorbate

The charge recombination is the undesired fate of e−/h+ pairs since the photoconverted energy is lost either through a radiative or more often a non-radiative way. Consequently, the measurement of the amount of recombination is of paramount importance to determine the efficiency of a photocatalyst. Some studies used the amount of mobile charge carriers to estimate the recombination rate [159, 160] while others used the consequences of the recombination such as the emitted photons or the heat generated [161, 162]. The photoluminescence due to radiative recombination also gives information about the trapping sites and the carrier thermalization. The charge recombination kinetic and intensity strongly depend on materials characteristics and especially its crystallinity [163]. Photons are mainly absorbed in the bulk and recombination most often happens between ‘free’ holes and electrons trapped at the surface. It was shown that for 2 nm anatase particles 78 % of the excitation events led to recombination after 20 ps while only 17 % in 27 nm particles [164]. More generally, half of the excitons are believed to be recombined in TiO_2 in the tenths of nanoseconds time scale.

In order to play its role the charge must be transferred on a surface acceptor (*A*) or donor (*D*) that may be the final target compound of the photocatalysis, or may be simply involved in the catalytic process or only serve as a charge scavenger. Formally, the acceptor or donor may also be another solid with a band structure.

The electrons generated in TiO_2 may be transferred in an empty energy state of the acceptor on the condition that the targeted state is below that of the edge of the CB or more precisely below the energy level of the trapped electrons. This electron transfer which corresponds to the starting point of a photoreduction mechanism demands a strong coupling between the energy levels in order to avoid electron back-transfer. Studies on the electron transfer on methyl viologen by UV-Visible time-resolved spectroscopy indicate that the electron is transferred in the pico-seconds to nanosecond time scales [165]. In order to improve the quality of TiO_2 for photooxidation, it is very important to improve the electron transfer to a scavenger that will prevent them to recombine with the photogenerated holes.

The photooxidation itself requires the electron transfer from a donor to TiO_2 VB hole. Again, the relative position of the energy level of the donor and that of the edge of the VB is of peculiar importance. The good coupling between the two levels is also important. Indeed, a study showed that adsorbed alcohols (methanol and isopropanol) trap the hole in their vicinity to promote the charge recombination rather than transferring an electron into the VB [166]. Kinetic studies using SCN^- probe as a model adsorbate for hole transfer demonstrated that the transfer is done in the same time scale as that of electron one from the CB [167]. A paradox is raised here since a hole transfer process is said to be quite fast and efficient while photooxidation is usually said to be slow and inefficient. This last assertion is explained by the fact that the photooxidation of a molecule often involves multiple hole transfers with adsorbate that display different energy states and configuration. Hence all the steps are not equally fast and the global process is slow.

The charge transfer is the final step of the photocatalytic process with the TiO_2 material and the next steps may be seen as the photodegradation mechanisms with a molecular approach. However, these mechanisms are truly photocatalyst dependent since the efficiency of the transferred electrons and holes depends on their pristine energy levels. In the case of radical-mediated mechanisms (as described below) the nature and amount of these radicals strongly depends on the used semiconductor. That is why the next section focuses first on the generation of radicals in the reactive medium and then on the direct photooxidation of organic molecules on the TiO_2 surface.

6.4 Mechanisms

6.4.1 Generalities on Photooxidation on TiO_2

A large amount of studies try to elucidate the whole degradation mechanism of various organic and inorganic pollutants but the goal of this section is more focused on the mono-electronic events in which the TiO_2 phase plays a role.

Besides the direct transfer of charges between the semiconductor and adsorbed targeted molecules, titanium dioxide may activate the solvent in order to generate radicals in solution such as $OH^{\bullet}$ radicals. In aqueous solution and in a solid–gas reaction intermediate molecules such as oxygen may also create active radicals. These generated radicals are likely to react far from the photocatalyst and yet their nature and amount may be specific to the material used. Indeed, TiO_2 is able to generate O_2^- ions while it is not the case for photocatalysts such as Bi_2WO_6. The observed degradation mechanisms are also dependent on the ability of the material to photoadsorb and photodesorb species that promote or inhibit the process [168]. For instance, the photodesorption of a product is interesting while the photoadsorption of an inert molecule may consume a useful charge carrier and block a reactive site at the surface of the material.

This section is mostly devoted to photooxidation of organic molecules but in non-photoelectrochemical settings the associated photoreduction process must happen in close vicinity. Consequently, the presence of electron scavengers are required to obtain good efficient photocatalysis conditions and knowledge about them is summarized below. Oxygen is the most used and studied electron scavenger but it plays different roles that are not yet fully understood. This molecule may be simply physisorbed on regular and fully oxidized surfaces while O_2 dissociative adsorption proceeds on defective surfaces (oxygen vacancies) or on regular surface sites where electron transfer may easily occur via subsurface Ti^{3+} [169]. The species generated from O_2 adsorption and charge transfer are more diverse when performed on hydroxylated surfaces and in the presence of water molecules: HO_2 and H_2O_2 species could be the observed near to the surface. In addition to thermal adsorption, O_2 may also be photoadsorbed forming directly O_2^- species that rapidly evolve into O_3^- or O_2^{2-} in addition to the already quoted $HO_2^{\bullet}$ and H_2O_2 species. The electron transfer process on O_2 often regulates the kinetics of the whole reaction process. Two additional electron scavengers proposed in the literature are Fe^{3+} ions in aqueous solution [170] and NO in the gas phase [171] but even if their role of scavenger is similar, the so-generated species do not play as an important role in the photodegradation mechanism as oxygen-derived species.

6.4.2 Photooxidation of Alcohols, Aldehydes, and Ketones

The photodegradation of alcohols was extensively studied and especially that of methanol and ethanol for they are model molecules for alcohols with longer chains and constitute intermediate in other photodegradation mechanisms. The photooxidation of methanol may follow both direct and indirect processes depending on the amount of water and oxygen present. Formate and formaldehyde intermediates were detected but the attribution of an intermediate to one or the other process is still controversial [172, 173]. Additional intermediates could be detected with low temperature (1.9 K) EPR study such as $CH_2OH^{\bullet}$ and $CHO^{\bullet}$ [174]. The temporal scheme of the different mechanistic steps is up to now not well established.

Similar to methanol, intermediate species observed with ethanol are acetaldehyde and acetate but formate is also detected [175]. The question of reactants/products surface coverage also has its importance since formed carboxylates are able to block the access of O_2 to the surface and consequently the photodegradation of ethanol. Accordingly, other studies indicated that the first step of ethanol degradation involves O_2^- ions [176].

Another compound frequently used in photodegradation tests is phenol while relatively few studies are devoted to mechanistic details [177]. The first oxidation steps correspond to a hydroxylation on the aromatic ring involving $OHHO^\bullet$ radical rather than a direct hole-mediated oxidation. However, the adsorption mode of phenol prior to oxidation remains unknown.

Among aldehydes, acetaldehyde was the most studied and was shown to transform into acetate. EPR studies of the reaction revealed a $CH3C(O)OOHO^\bullet$ radical intermediate indicating the attack of O_2^- on the adsorbed aldehyde [178]. Formaldehyde degradation mechanism is hard to study since it is rapidly converted into CO_2.

For ketones degradation, mechanistic studies mostly involved acetone which mainly adsorb on oxide surfaces via an oxygen lone pair. A low temperature EPR study detected the formation of $CH_3COCH_2OOHO^\bullet$ during the first reaction steps and the authors proposed that its formation occurs by the reaction of O_2 on an unstable $CH_3COCH_2HO^\bullet$ radical generated by a first hole-mediated reaction [179]. The intermediates observed in another study are mainly acetate, formate, acetaldehyde, and formic acid corresponding to a C–C bond cleavage [180].

6.4.3 Photooxidation of Carboxylic Acids

Carboxylic acids photodegradation was widely studied because these molecules are initially often present in the pollutants composition or they may be obtained as photodegradation products of other organic molecules (alcohols, aldehydes, etc.). Carboxylates generally strongly adsorb on TiO_2 surfaces. Their stability on the oxide surface and the good knowledge of their different adsorption modes make them very interesting systems to understand the photo-induced reactions. Formic acid was widely studied; nevertheless, the mechanism is still controversial. The question remains between a direct hole-mediated pathway also called photo-Kolbe reaction (1) or an indirect pathway implying a $HOHO^\bullet$ radical attack (2) [181].

$$HCOO^-_{(ads)} + h^+ \rightarrow CO_{2(g)} + H_{(ads)} \tag{6.1}$$

$$HCOO^-_{(ads)} + HO \rightarrow CO^-_{2(a)} + H_2O_{(ads)} \tag{6.2}$$

In any cases, no intermediate could be detected before the formation of CO_2 [175]. The addition of water or oxygen in a solid–gas phase system significantly increases the degradation rate [175].

Another model molecule is acetic acid that was studied both on single crystals [182] and on high surface area systems [183]. Similar to formate, acetate may be degraded via the direct and indirect pathways but additionally, $HO^{\bullet}$ radicals may attack the C–H bond of the methyl group leading to the formation of $^{\bullet}CH_2COOH$ radicals. The photo-Kolbe reaction on acetic acid (3) also yields $^{\bullet}CH_3$ radicals and consequently by-products such as CH_4 and C_2H_6. Carbon isotopic labeling of acetate revealed that carboxylate carbon is first released as CO_2 while the release of the other carbon is delayed and proceed through the chain methoxy, formaldehyde, and formate [183]. A detailed study on different rutile single crystals demonstrated that the amount of the obtained products significantly vary with the nature of the surface sites [184]. For other linear carboxylic acids the photodecomposition is said to proceed through a cascade of photo-Kolbe reactions that shortens the carbon chain of one unit each time up to formate. The Kolbe reaction is often observed in the degradation pathway of the carboxylic acids, except for phenyl-containing systems such as benzoic acid where an OH addition to the phenyl ring is favored [185].

6.4.4 Photooxidation of Alkanes, Alkenes, and Halo Hydrocarbons

The main point of the photooxidation of alkanes and alkenes is their relative inertness toward the oxide surface and consequently the first reaction step creates intermediates that are more likely to bind to the surface. The next two examples of alkane degradation well show the diversity of possible intermediates and products. Cyclohexane leads in a first photocatalytic step to cyclohexanol and cyclohexanone with no ring opening or double bond formation [186]. The same authors proposed that cyclohexanol is formed from the cyclohexyl radical (obtained by a hole-mediated reaction on cyclohexane) while the cyclohexanone formation requires the attack of O_2^{-} anion. In the case of methane, different studies have shown either the formation of methanol, formate, and even ethane through the coupling of methyl radicals [187, 188].

Aromatics represent somehow a particular class of alkenes and their reactivity is very interesting even though not fully understood. Benzene and polyaromatics first undergo a $HO^{\bullet}$ attack on the aromatic cycle prior to any ring-opening event [189]. However, in the case of toluene, the oxidation of the methyl group is favored in comparison with that of the aromatic ring leading to benzyl alcohol and benzaldehyde as reaction intermediates. According to an EPR study, this selectivity for the methyl group is attributed to the attack of an O_2^{-} leading to the formation of the C_6H_5-CH_2-$OO^{\bullet}$ radical [190].

In comparison to the reactivity of alkanes that of halocarbons is complicated because the C–X bond may easily be broken to generate $X^{\bullet}$ radicals and the free radicals may create mechanistic pathways in parallel to those involving the charge

carriers. That is well exemplified by the family of C_1 chlorocarbons $CH_{4-x}Cl_x$ for which numerous intermediates and products were observed such as carbon monoxide and dioxide, non-chlorinated C_1 compounds, chlorinated C_1, and species with more than one carbon. The formation of more chlorinated species is a good indicator of the presence of radical chain reactions [191]. An additional specificity of halocarbons is the ability of the halogens to replace in certain conditions the surface OH group and consequently to poison the surface or to prevent the total mineralization of reactants by creating more halogenated species. Consequently, the addition of water may help to displace the surface OH/X equilibrium and thus favors the complete photodegradation process.

6.4.5 *Photooxidation of Nitrogen-Containing Molecules and Miscellaneous*

As reported in this review [123], the N-containing molecules are likely to be at the same time subject to photooxidation and photoreduction. The alkylamines are more reactive under their neutral form as the lone pair of nitrogen is said to react with the $HO^{\bullet}$ radical leading to the formation of an aldehyde and a less substituted alkylamine. The fate of the nitrogen of those molecules is generally nitrites, nitrates, and ammonia whereas the generation of N_2 results mainly of a non-photocatalytic reaction between NO_x and $NH_2^{\bullet}$ [192].

In the phosphorous-containing molecules the P–C bond seems to be more stable against oxidation than the P–O–C links and the oxidized products (usually a phosphate) poison the catalyst surface. This can be avoided if the reaction is carried out in presence of water in order to hydrate the adsorbed species and help to remove them from the surface. The same behavior of surface poisoning may be observed for organosulfides that yield sulfate surface groups.

The multi-functional molecules such as polysubstituted aromatics, or amino acids and halogenated acids were also studied, the two main questions tackled being the priority order in the degradation of the different organic functions and the efficiency of a cooperative effect between the different functions. For instance, a study led on chlorophenol showed that, depending on the wave length of the incident light, the molecule could react by OH addition without Cl^- removal to form chlorocatechol or with Cl^- removal to form hydroquinone [193]. In the halogenated acids family, the carboxylate group interacts with TiO_2 and a Kolbe reaction is first observed in most of the cases [194].

The present overview of the mechanistic aspects of photooxidation of organic species on TiO_2 clearly demonstrates that various parameters impact on the kinetic pathway: the functional groups, the presence of oxygen, and the nature solvent that may interact with the catalyst surface. Moreover, the nature of the exposed TiO_2 surface and the presence of surface defects may affect the reaction mechanism as well.

6.5 Photocatalysis in Environmental Chemistry

The environmental applications of photocatalysis with TiO_2 are gathered in three categories: air treatment, water treatment, and self-cleaning surfaces. The three categories include several domains:

- Air treatment: indoor and outdoor air decontamination, Volatile Organic Compounds (VOCs) and Polycyclic Aromatic Hydrocarbons (PAHs) elimination, sterilization, deodorization, etc.
- Water treatment: industrial and municipal wastewater treatment, discoloration, decontamination and disinfection, recalcitrant Chemical Oxygen Demand (COD) degradation, etc.
- Self-cleaning surfaces: self-cleaning and self-sterilizing, windows, buildings, tiles, lighting, walls, paintings, and pavements.

All these applications are using photoactive TiO_2, mainly under anatase form, considered as the most active phase. The photocatalyst can be used as free or supported powder, or in thin film, depending on the application. The set photocatalyst/support will be called photocatalytic media.

From now on, the described systems will mainly focus on organic oxidation in water. It should be noted that the problems are rather similar in the cases of inorganic reduction in water (in spite of different photocatalytic mechanisms) or organic oxidation in air (in spite of different reactor design). The category of self-cleaning surfaces is more distant and will not be developed here.

To be used in an environment application like wastewater treatment, the photocatalytic media should be active enough to realize the reactions in a reasonable time. It means that its activity should be previously estimated. Today, standardized tests for photocatalytic media are still in debate, and there is no established and indisputable molecule to evaluate the photoactivity.

In water, colored molecules such as rhodamine B or methylene blue (MB) have been widely used because the kinetic of the photoreaction is relatively high and because of the visual effect procured by the discoloration. However, their use is now contested because the bleaching may be ambiguous and not only due to the photodegradation of the molecule. In the case of MB, in the absence of oxygen and in the presence of a sacrificial electron acceptor (SED), MB is photoreduced to its colorless leucoform, LMB, by the TiO_2 photocatalyst. While prolonged irradiation leads to the eventual complete mineralization of the dye, the initial observed photobleaching of the dye is not necessarily due to the dye oxidation, especially if the reaction is carried out under conditions that favor the formation of LMB [195].

Phenol is one of the most employed test molecule. It has been proposed by Serpone et al. [196], in 1996, as standard test molecule, and presents some advantages:

- It does not undergo degradation by photolysis or catalysis.
- It presents an absorption band at 269 nm detectable by UV-Visible spectroscopy.

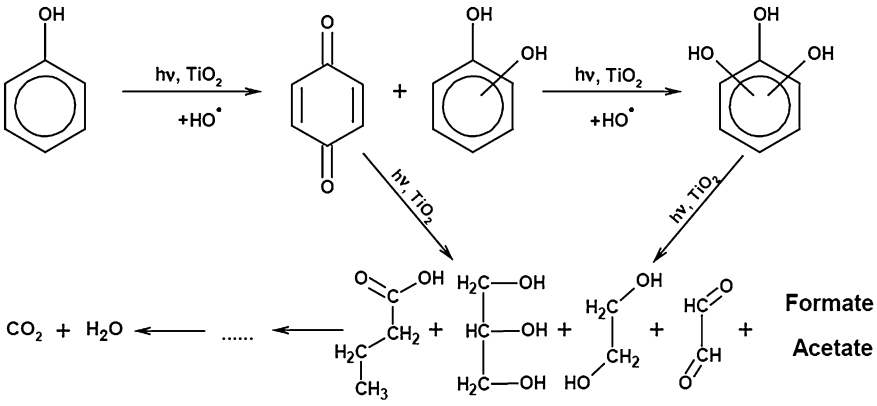


Fig. 6.3 Proposed mechanism for phenol photodegradation on TiO_2 [177]

- Its degradation mechanism is quite identified. The principal intermediates formed are benzoquinone, hydroquinone, and catechol. A mechanism is proposed in Fig. 6.3 [177].
- It follows a complete mineralization to CO_2 and H_2O.
- It adsorbs very weakly at the surface of TiO_2.
- It is a real pollutant of water.

Other used test molecules are, for example, formic acid (mineralization without intermediates), stearic acid (model molecule for fat acids), 4-chlorophenol, trichloroethylene (model molecules for chlorinated compounds), and oxalic acid (molecule with adsorption on TiO_2).

The test molecules are especially necessary for the setup of the reactor and for the elaboration of the photocatalyst, but to evaluate the performance of photocatalysis in water treatment, numerous studies are realized with real or model industrial wastewaters. It is quite difficult to compare these works with deeply different reactor design and photocatalytic media, but some interesting results may be pointed out. In most of these works, the problem is to evidence the degradation of the pollutant by photocatalysis, follow its kinetics, and analyze the eventual intermediates formation (intermediates may be more pollutant than the initial compounds). Photocatalysis is often presented as part of a general treatment process.

The next section presents recent obtained results of photocatalysis treatments on real industrial wastewaters, dyes, pesticides, antibiotics, and bacteria.

6.5.1 Real Industrial Wastewaters

The general objective of the study of Pichat et al. [197] was to evaluate under real conditions, in a parcel of vineyard, the practicability for the wine grower and the efficiency of a solar photocatalytic prototype device to treat rinse waters from tractor cisterns which are used to spray various mixtures of pesticides and

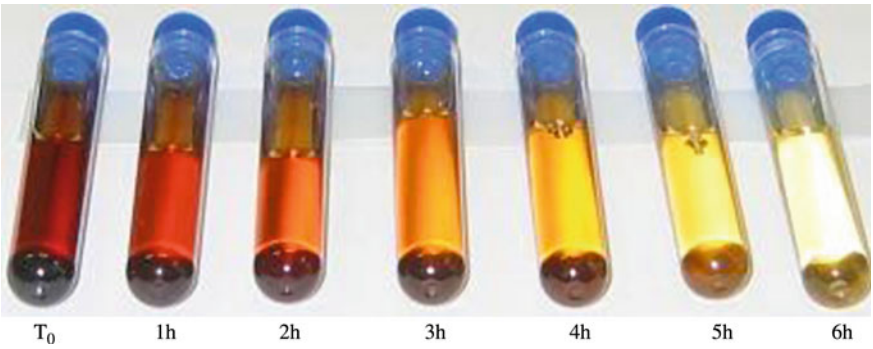


Fig. 6.4 Discoloration of margins obtained by photocatalysis with TiO_2 (photo Marjorie Mirau)

additives. This specific application of solar TiO_2 photocatalysis shows promising results, although more on-site experimentations are required regarding its viability. Moreover, toxicity linked to inorganic ions remains a problem.

The photocatalytic treatment of an effluent from black table olive processing over TiO_2 suspensions was investigated by Chatzisimeon et al. [198]. The study focused on the effect of various operating parameters on the treatment efficiency including initial organic load, catalyst type, concentration and reuse, and addition of hydrogen peroxide. Depending on the conditions employed, nearly complete discoloration was obtained, while mineralization never exceeded 50 %. Tests with non-acclimated activated sludge showed that both the original and photocatalyzed effluents were degradable aerobically. The biodegradation rate of the original effluent was three times greater than the oxidized one. On the other hand, photocatalytic oxidation of the original effluent was at least two orders of magnitude faster than its biological oxidation to achieve comparable levels of degradation.

Figure 6.4 is an illustration of the discoloration of margins (olive production wastewater) obtained by photocatalysis with TiO_2.

Rodrigues et al. [199] have investigated the combined treatment of post-bleaching effluent from cellulose and paper industry. The effluent was first submitted to a coagulation–flocculation treatment applying $FeCl_3$ as coagulating agent and chitosan as auxiliary. The aqueous soluble phase obtained from the first treatment was then submitted to a $UV/TiO_2/H_2O_2$ system. This combined method resulted in a high biodegradability index, transparency, and absence of color and odor in the treated water, suggesting good water quality.

In the work of Alinsafi et al. [200], photocatalysis with TiO_2 particles immobilized on a glass slide or on a non-woven glass fiber fabric has been applied to pure reactive dyes (azo and metal phthalocyanines) solutions as well as textile wastewater containing the same dyes under UV and solar irradiation. Discoloration of textile wastewater was in the range 21–74 % under solar irradiation, with good COD removal rate. These values are strongly dependent upon the fine chemical structure of the dyes and the global composition of the wastewater. The results are encouraging for textile

wastewater remediation. The increase of biodegradability is an additional positive factor, as it would improve the efficiency of a biological downstream treatment.

An alternative method for municipal wastewater treatment has been developed by Antoniadis et al. [201]. It is based on solar photocatalytic oxidation and natural processes. The system combines the synergetic action of homogeneous photocatalytic oxidation with surface flow constructed wetlands in order to utilize the high solar irradiation and the ability of the constructed wetlands to improve water quality through natural processes. The authors have presented the design, the development, and an experimental evaluation of the combined system. Experiments were conducted at laboratory scale using artificial as well as solar irradiation, for the treatment of both synthetic and cesspool wastewater. The data evaluation revealed that the combined system may effectively reduce the organic load and nutrients of wastewater, even in cases of great inflow variability, in terms of hydraulic and organic load, and thus may be a promising, competitive, and environmental friendly solution for wastewater treatment in the near future.

6.5.2 Dyes

The photocatalytic degradation of five various dyes has been investigated in TiO_2/UV aqueous suspensions by Lachheb et al. [202]. They have tried to determine the feasibility of such a degradation by varying the chemical structures like anthraquinonic (Alizarin S), or azoic (Crocein Orange G, Methyl Red, Congo Red), or heteropolyaromatic (Methylene Blue). In addition to a prompt removal of the colors, TiO_2/UV-based photocatalysis was simultaneously able to fully oxidize the dyes, with a complete mineralization of carbon into CO_2. Sulfur heteroatoms were converted into innocuous SO_4^{2-} ions. The mineralization of nitrogen was more complex. The results suggest that TiO_2/UV photocatalysis may be envisaged as a method for treatment of diluted colored wastewaters not only for discoloration, but also for detoxification.

Sauer et al. [203] proposed a detailed investigation of the adsorption and photocatalytic degradation of the Safira HEXL dye, an anionic azo dye of reactive class. The dye is easily degraded by a TiO_2-assisted method in aqueous dispersions under irradiation by UV light. The adsorption of the dye is a prerequisite for the degradation. Both adsorption and photodegradation occur more extensively when the pH is near the PZC.

Among all the works on dyes photodegradation, Basic Red 46 [204] and C.I. Reactive Orange 4 [205] have also been studied and a rapid discoloration of the compounds is evidenced.

6.5.3 Pesticides

Herrmann and Guillard [206] have studied the influence of the basic photocatalytic parameters on the catalyst activity as well as the identification of reaction intermediate products and degradation pathways in model and real solution,

encountered in agricultural areas (rinsing waters). Various pesticides intensively used in agriculture such as herbicides [2,4-D(dichloro-phenoxy-acetic acid)] or insecticides (tetrachlorvinphos, fenitrothion, pirimiphos-methyl, fenamiphos) were successfully degraded, either as pure active agents or as commercial formulated reactants.

Devipriya and Yesodharan [207] realized a complete and quite exhaustive review on pesticides degradation by photocatalysis, with special reference to the mechanism of the process involved, the nature of the reactive intermediates, and the final products. Pesticides, insecticides, fungicides, herbicides, and additives were taken into account.

6.5.4 Bacteria

Guillard et al. [208] presented a fundamental research on the efficiency of photocatalysis in water disinfection. Two model strains of *Escherichia coli* were selected and a comparison of the efficiencies of TiO_2 Evonik P25 versus TiO_2 Cristal Global PC500 was estimated. This work clearly pointed out the important difference with the photocatalytic removal of organic molecules. One has to take into account the size of the microorganisms compared to those of semiconductor and of organic molecules. The efficiencies of TiO_2 Evonik P25 on the inactivation of *E. coli* strains were comparable, whereas a more important inactivation of one of the strain of *E. coli* was obtained on TiO_2 Cristal Global PC500.

More recently, Pigeot-Rémy et al. [209] also studied the effect of Evonik P25 on *E. coli*.

In the study of Rizzo [210] the potential application of TiO_2 photocatalysis as primary disinfection system of drinking water was investigated in terms of coliform bacteria inactivation and injury. As model water, the effluent of biological denitrification unit for nitrate removal from groundwater, which is characterized by high organic matter and bacteria release, was used. Photocatalysis was effective in coliform bacteria inactivation, although no total removal was observed after 60 min irradiation time. Photocatalysis process did not result in any irreversible injury under investigated conditions, thus a bacteria regrowth may take place under optimum environment conditions if any final disinfection process (e.g., chlorine or chlorine dioxide) is not used.

6.5.5 Antibiotics

In the study realized by Rizzo et al. [211], the degradation kinetics and mineralization of diclofenac (DCF) by photocatalysis on TiO_2 were investigated in terms of UV absorbance and COD measurements for a wide range of initial DCF concentrations and photocatalyst loadings in a batch reactor system.

TiO_2 photocatalysis was found to be very efficient for the mineralization, degradation, and detoxicification of DCF, using low TiO_2 doses at achievable half-life time as well as using a lower potential lamp than previous studies.

In the work presented by Giraldo et al. [212], a photocatalytic system using titanium *Evonik* P25 in suspension was used to evaluate the degradation of antibiotic oxolinic acid (OA). The effects of catalyst load and pH were evaluated and optimized. The results indicated that TiO_2 photocatalysis allows a rapid and efficient removal of OA; transforming the initial substrate into by-products with no antimicrobial activity and lower toxicity, which could then be degraded in a subsequent biological step.

Degradation of amoxicillin, ampicillin, and cloxacillin antibiotics in aqueous solution by TiO_2 photocatalysis under UVA (365 nm) irradiation have been studied by Elmolla and Chaudhuri [213]. Enhancement of photocatalysis by addition of H_2O_2 was also evaluated. The influence of pH on antibiotic degradation is evidenced. Kinetics of degradation was characterized.

6.6 Photocatalytic Reactors

The photocatalytic properties of TiO_2 described previously allow the development of wastewater treatment processes. For this purpose, a photocatalytic reactor must be designed. The photocatalytic reactors are different from classical ones (thermal or thermocatalytic) because of the irradiation, which activates the catalyst. The role of the reactor is to place simultaneously in contact the wastewater, oxygen, the UV illumination, and the photocatalyst. The photocatalytic reactor design implies taking into account parameters like the immobilization and the irradiation of the photocatalyst, the flow and the concentration of the wastewater and the oxygen, the cooling and the protection of the lamp, and the electric circuit. The final proposed design of the photocatalytic reactor will depend on those parameters.

In the photoreactor, the photocatalytic powder should be in close contact with the effluent. To avoid filtration difficulties, an immobilization of the photocatalyst is necessary. Depending on the design, it can be realized on glass beads or plates, sand, steel, or structuring porous materials (silica, zeolites, active carbon, carbon nanotubes, and nanorods). As demonstrated in the previous section, the sol–gel method allows the synthesis of a photocatalytic film or deposit [214]. Besides, the Ahlstrom Company produces a paper with deposited TiO_2 usable as flexible photocatalytic media [215]. It must be noted that, whatever is the support, the immobilization lowers the photoactivity because part of the solid is no more reachable to the UV photons.

The illumination producing photocatalysis may be provided artificially by a UV lamp or naturally by the sun.

In the case of the sun illumination, because of the weak intensity of the UV usable radiation, the illuminated surface and the photocatalyst/water contact surface must be high. The main questions are getting around photocatalyst

Fig. 6.5 Compound Parabolic Collecting Reactor (CPCR) at the Plataforma Solar de Almería (PSA) [218]

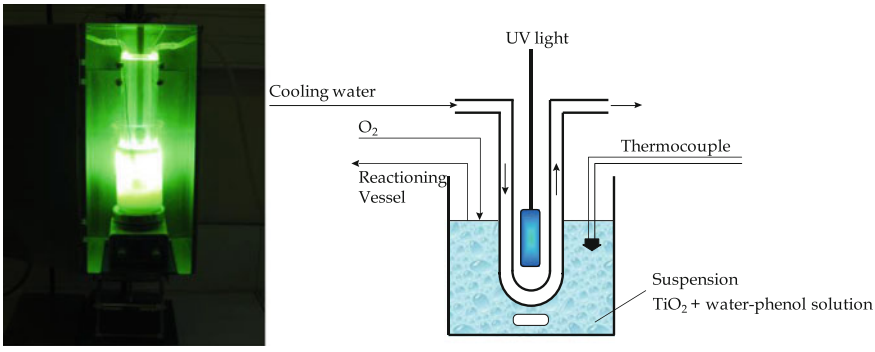


Fig. 6.6 Batch reactor for photodegradation of phenol in water by TiO_2

immobilization and sunlight concentration [216]. Different photoreactors have been developed Parabolic Trough Reactor (PTR), Thin Film Fixed Bed Reactor, Compound Parabolic Collecting Reactor (CPCR), Double Skin Sheet Reactor [216, 217]. Their design is different if they use solar concentrator and if the TiO_2 is deposited or in suspension. PTR and CPCR are developed at the Platform a Solar of Almería located in the south of Spain, the place receiving the maximal sunshine of Europe (Fig. 6.5 [218]).

With artificial light, generally provided by one or several mercury lamp, the design is less restrained. Numerous examples are proposed [219]. The simplest model is the Batch Reactor allowing the illumination of a TiO_2 suspension [4]. It is represented on Fig. 6.6. This kind of reactor, without immobilization of the

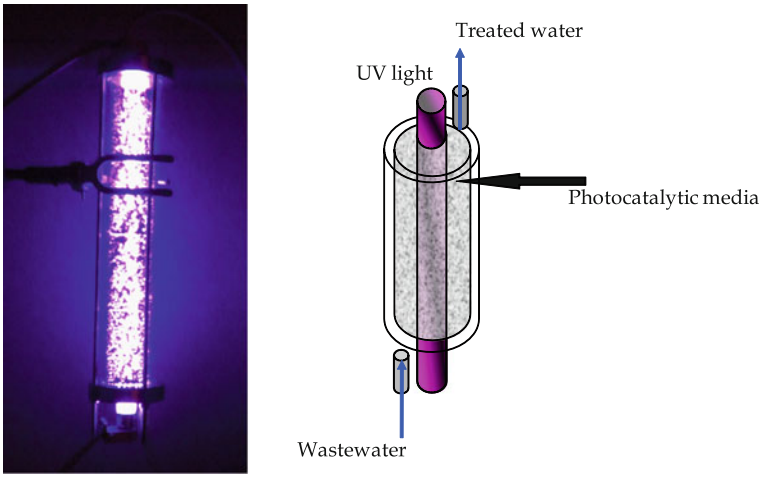


Fig. 6.7 Annular reactor for wastewater treatment by photocatalysis

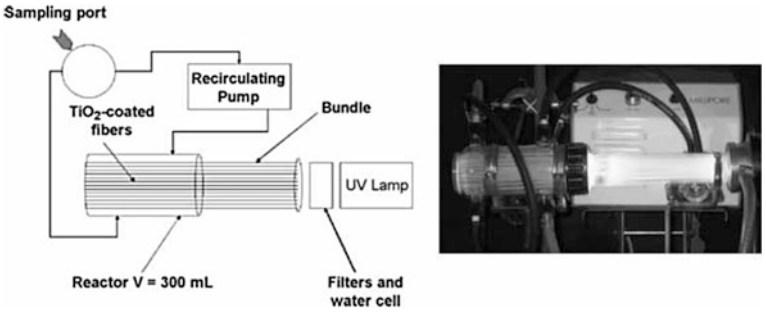


Fig. 6.8 Photocatalytic reactor using optic fibers [226]

photocatalyst is mainly useful in the framework of photocatalytic tests for materials.

To avoid the filtration step, fluidized bed and fixed bed reactors are proposed. In the last case, an annular geometry represented in Fig. 6.7, with external or internal illumination, is often preferred [216].

Reactors with planar geometry are also described. More rarely, less classical systems are adopted (Rotating Drum Reactor [220], Intermittent Flow Reactor [219], Fiber Optic Cable Reactor [221, 222], Photocatalytic Membrane Reactor [223], Falling Film Photoreactor [224], Pulsed Baffled Tube Photoreactor [225], etc.).

Figure 6.8 from Danion et al. [226] shows a reactor using TiO_2 deposited by sol–gel method on optical fibers.

Some processes use TiO_2 monolith rather than an immobilized titanium dioxide but. Some systems are sometimes combining chemical, physical, or biological activity to the photoactivity [219].

The theoretical aspects of photocatalytic reactors have been mainly described by Cassano et al. [216, 227–229]. In this case, kinetic models of photodegradation including the simulation of the radiative field in the reactor are proposed for several geometries.

The radiation field inside photocatalytic reactors can be predicted by solving the radiative transfer equation (RTE). From the solution of the RTE, the local volumetric rate of photon absorption (LVRPA) can be obtained. This LVRPA is an important parameter in photocatalytic reactor design, energy efficiency assessments, and kinetic studies of photocatalytic reactions. However, to solve the RTE, optical parameters are needed: the absorption and scattering coefficients and the phase function. Solving the RTE may be arduous depending on the reactor geometry or the photocatalytic media.

In recent studies, the LVRPA has been evaluated in slurry using Monte Carlo techniques. In such simulated photocatalytic reactor, a photon is emitted from the lamp, travels a distance l and then is, according to a determined probability, either absorbed or scattered within the reacting medium [230].

6.7 Conclusions

The fact that an exponentially increasing number of articles are devoted to the synthesis of titanium dioxide nanoparticles and its application in photocatalysis is not surprising. It is indeed the reference material for that specific application and lots of authors evoke the possibility to improve the photoactivity to motivate their research. Moreover, various research domains still bring their contributions to the elaboration of the best photocatalyst possible for the whole process is quite complex and consequently very rich and diverse.

Indeed, a fruitful exchange occurred between physicists and material science chemists. While the former focus more on the fundamental aspects of the photocatalytic process from the photon interaction with the semiconductor up to the charge transfer to the reacting molecule, chemists are now tailoring very finely the nanoparticles in terms of composition, size, shape, and structure. A better understanding of the influence of each characteristic of a TiO_2 powder on its photocatalytic efficiency is now possible but many fundamental issues are still under discussion.

A burning issue that involves both scientific communities is the question of how to use a higher amount of solar light to do photocatalysis. Each of the proposed solutions, such as bulk doping with nitrogen or heterostructures, presents drawbacks and hardly reaches the efficiency observed under UV light.

The capacity of TiO_2 to photodegrade organic molecules via photooxidation was demonstrated from the simplest functional molecule of formic acid up to the scale of the bacteria. The understanding of each degradation step for the smaller molecules

may help in the future both to explain degradation of more complex ones and provide an interesting feedback to the elaboration of better TiO_2 photocatalyst.

A detailed study of the bibliography on degradation mechanisms of model organic molecules shows that certain experimental conditions such as the solvent, the bubbling of specific gas, or even the dispersion of the photocatalyst may favor the degradation of a molecule and block that of another. Consequently, the question of the definition of a good photcatalyst is very important and however very difficult to define. The need of normalized tests may emerge in a close future.

An important work is also devoted to preparation of reactors tuned to the desired decontamination process. The relative position of the light source, the photocatalyst, and the medium to be decontaminated must be carefully thought. Moreover, the global system must be scaled to reach the requirements of the decontamination efficiency and rate.

Finally, in every system where populations may use more or less directly the decontaminated fluids (air or water) a particular attention must be paid to the release of TiO_2 nanoparticles during the photodecontamination. Toxicology of TiO_2 nanoparticles is still under debate and numerous research projects are devoted to its toxicity as aerosols or in effluents. This is an especially important issue for all the people in laboratories and industries will have to handle this material that will surely be studied and used for the next decades.

References

1. Gogate PR, Pandit AB (2004) Adv Environ Res 8(3–4):501–551
2. Bhatkhande D, Pangarkar VG, Beenackers AACM (2001) J Chem Technol Biotechnol 77(1):102–116
3. Litter MI (1999) Appl Catal B 23(2–3):89–114
4. Hoffmann MR, Martin ST, Choi W, Bahnemann DW (1995) Chem Rev 95:69–96
5. Carp O, Huisman CL, Reller A (2004) Prog Solid State Chem 32(1–2):33–177
6. Chen X, Mao SS (2007) Chem Rev 107(7):2891–2959
7. Fujishima A, Zhang X, Tryk DA (2008) Surf Sci Rep 63(12):515–582
8. Burdett JK, Hughbanks T, Miller GJ, Richardson JW, Smith JV (1987) J Am Chem Soc 109(12):3639–3646
9. Baur WH (1961) Acta Crystallogr 14(3):214–216
10. Zhang HZ, Banfield JF (1998) J Mater Chem 8(9):2073–2076
11. Finnegan MP, Zhang H, Banfield JF (2007) J Phys Chem C 111(5):1962–1968
12. Amtout A, Leonelli R (1995) Phys Rev B Condens Matter 51(11):6842–6851
13. Boschloo GK, Goossens A, Schoonman J (1997) J Electrochem Soc 144(4):1311–1317
14. Choi WY, Termin A, Hoffmann MR (1994) Angew Chem Int Ed 33(10):1091–1092
15. Pascual J, Camassel J, Mathieu H (1978) Phys Rev B Condens Matter 18(10):5606–5614
16. Asahi R, Taga Y, Mannstadt W, Freeman AJ (2000) Phys Rev B Condens Matter 61(11):7459–7465
17. Kavan L, Gratzel M, Gilbert SE, Klemenz C, Scheel HJ (1996) J Am Chem Soc 118(28):6716–6723
18. Koelsch M, Cassaignon S, Guillemoles JF et al (2002) Thin Solid Films 403:312–319
19. Li J-G, Ishigaki T, Sun X (2007) J Phys Chem C 111(13):4969–4976
20. Mattsson A, Osterlund L (2010) J Phys Chem C 114(33):14121–14132

21. Mo SD, Ching WY (1995) Phys Rev B Condens Matter 51(19):13023–13032
22. Monticone S, Tufeu R, Kanaev AV, Scolan E, Sanchez C (2000) Appl Surf Sci 162: 565–570
23. Sakai N, Ebina Y, Takada K, Sasaki T (2004) J Am Chem Soc 126(18):5851–5858
24. Sakai N, Fukuda K, Shibata T, Ebina Y, Takada K, Sasaki T (2006) J Phys Chem B 110(12):6198–6203
25. Shibata T, Sakai N, Fukuda K, Ebina Y, Sasaki T (2007) Phys Chem Chem Phys 9(19):2413–2420
26. Aruna ST, Tirosh S, Zaban A (2000) J Mater Chem 10(10):2388–2391
27. Kaper H, Endres F, Djerdj I, Antonietti M, Smarsly BM, Maier J, Hu Y-S (2007) Small 3(10):1753–1763
28. Li YL, Ishigaki T (2002) Thin Solid Films 407(1–2):79–85
29. Wang CC, Ying JY (1999) Chem Mater 11(11):3113–3120
30. Shen XJ, Zhang JL, Tian BZ (2011) J Hazard Mater 192(2):651–657
31. Cassaignon S, Koelsch M, Jolivet J-P (2007) J Phys Chem Solids 68(5–6):695–700
32. Cassaignon S, Koelsch M, Jolivet J-P (2007) J Mater Sci 42(16):6689–6695
33. Kao L-H, Hsu T-C, Lu H-Y (2007) J Colloid Interface Sci 316(1):160–167
34. Pottier AS, Cassaignon S, Chaneac C, Villain F, Tronc E, Jolivet J-P (2003) J Mater Chem 13(4):877–882
35. Sugimoto T (2004) Chem Commun 2004(14):1584–1585
36. Sugimoto T, Zhou X, Muramatsu A (2003) J Colloid Interface Sci 259(1):53–61
37. Niederberger M, Garnweitner G, Krumeich F, Nesper R, Colfen H, Antonietti M (2004) Chem Mater 16(7):1202–1208
38. Trentler TJ, Denler TE, Bertone JF, Agrawal A, Colvin VL (1999) J Am Chem Soc 121(7):1613–1614
39. Yang GX, Zhuang HR, Biswas P (1996) Nanostruct Mater 7(6):675–689
40. Buonsanti R, Grillo V, Carlino E, Giannini C, Kipp T, Cingolani R, Cozzoli PD (2008) J Am Chem Soc 130(33):11223–11233
41. Deng Q, Wei M, Hong Z, Ding X, Jiang L, Wei K (2010) Curr Nanosci 6(5):479–482
42. Keesmann I, Anorg Z (1966) Allg Chem 346(1–2):30–43
43. Kobayashi M, Tomita K, Petrykin V, Yoshimura M, Kakihana M (2008) J Mater Sci 43(7):2158–2162
44. Kominami H, Ishii Y, Kohno M, Konishi S, Kera Y, Ohtani B (2003) Catal Lett 91 (1–2):41–47
45. Kominami H, Kohno M, Kera Y (2000) J Mater Chem 10(5):1151–1156
46. Bhave RC, Lee BI (2007) Mater Sci Eng A 467(1–2):146–149
47. Inada M, Iwamoto K, Enomoto N, Hojo J (2011) J Ceram Soc Jpn 119(1390):451–455
48. Pottier A, Chaneac C, Tronc E, Mazerolles L, Jolivet JP (2001) J Mater Chem 11(4):1116–1121
49. Durupthy O, Bill J, Aldinger F (2007) Cryst Growth Des 7(12):2696–2704
50. Yang HG, Sun CH, Qiao SZ, Zou J, Liu G, Smith SC, Cheng HM, Lu GQ (2008) Nature 453(7195):638–642
51. Kandiel TA, Feldhoff A, Robben L, Dillert R, Bahnemann DW (2010) Chem Mater 22(6):2050–2060
52. Wu B, Guo C, Zheng N, Xie Z, Stucky GD (2008) J Am Chem Soc 130(51):17563–17567
53. Roy P, Berger S, Schmuki P (2011) Angew Chem Int Ed 50(13):2904–2939
54. Chemseddine A, Moritz T (1999) Eur J Inorg Chem 2:235–245
55. Moritz T, Reiss J, Diesner K, Su D, Chemseddine A (1997) J Phys Chem B 101(41): 8052–8053
56. Kanie K, Sugimoto T (2004) Chem Commun 14:1584–1585
57. Miao L, Tanemura S, Toh S, Kaneko K, Tanemura M (2004) J Cryst Growth 264(1–3): 246–252
58. Lin Y, Wu GS, Yuan XY, Xie T, Zhang LD (2003) J Phys Condens Matter 15(17): 2917–2922

59. Crepaldi EL, Soler-Illia G, Grosso D, Cagnol F, Ribot F, Sanchez C (2003) J Am Chem Soc 125(32):9770–9786
60. Sakatani Y, Grosso D, Nicole L, Boissiere C, Soler-Illia G, Sanchez C (2006) J Mater Chem 16(1):77–82
61. Andersson M, Osterlund L, Ljungstrom S, Palmqvist A (2002) J Phys Chem B 106(41):10674–10679
62. Yener HB, Sarkaya S, Helvaci SS (2010) Trends Colloid Interface Sci Xxiii 137:23–28
63. Kim CS, Moon BK, Park JH, Choi BC, Seo HJ (2003) J Cryst Growth 257(3–4):309–315
64. Yao BD, Chan YF, Zhang XY, Zhang WF, Yang ZY, Wang N (2003) Appl Phys Lett 82(2):281–283
65. Zhang YX, Li GH, Jin YX, Zhang Y, Zhang J, Zhang LD (2002) Chem Phys Lett 365 (3–4):300–304
66. Desmoulins-Krawiec S, Aymonier C, Loppinet-Serani A, Weill F, Gorsse S, Etourneau J, Cansell F (2004) J Mater Chem 14(2):228–232
67. Dong X, Tao J, Li Y, Zhu H (2010) Appl Surf Sci 256(8):2532–2538
68. Macak JM, Zlamal M, Krysa J, Schmuki P (2007) Small 3(2):300–304
69. Jiang L-C, Zhang W-D (2009) Electroanalysis 21(8):988–993
70. El-Sheikh AH, Newman AP, Al-Daffaee H, Phull S, Cresswell N, York S (2004) Surf Coat Technol 187(2–3):284–292
71. Pradhan SK, Reucroft PJ, Yang FQ, Dozier A (2003) J Cryst Growth 256(1–2):83–88
72. Seifried S, Winterer M, Hahn H (2000) Chem Vap Depos 6(5):239–244
73. Anuradha TV, Ranganathan S (2007) Bull Mater Sci 30(3):263–269
74. Ding Z, Lu GQ, Greenfield PF (2000) J Phys Chem B 104(19):4815–4820
75. Fujishima A, Hashimoto K, Watanabe T (1999) TiO2 photocatalysis: fundamentals and applications. BKC Inc., Tokyo
76. Schindler KM, Kunst M (1990) J Phys Chem 94(21):8222–8226
77. Tanaka K, Capule MFV, Hisanaga T (1991) Chem Phys Lett 187(1–2):73–76
78. Gratzel M, Rotzinger FP (1985) Chem Phys Lett 118(5):474–477
79. Tang H, Prasad K, Sanjines R, Schmid PE, Levy F (1994) J Appl Phys 75(4):2042–2047
80. Di Paola A, Addamo M, Bellardita M, Cazzanelli E, Palmisano L (2007) Thin Solid Films 515(7–8):3527–3529
81. Di Paola A, Cufalo G, Addamo M, Ellardita MB, Campostrini R, Ischia M, Ceccato R, Palmisano L (2008) Colloids Surf A 317(1–3):366–376
82. Ohtani B, Handa J, Nishimoto S, Kagiya T (1985) Chem Phys Lett 120(3):292–294
83. Iskandar F, Nandiyanto ABD, Yun KM, Hogan CJ Jr, Okuyama K, Biswas P (2007) Adv Mater 19(10):1408–1412
84. Li FB, Li XZ, Hou MF (2004) Appl Catal B 48(3):185–194
85. Hurum DC, Agrios AG, Gray KA, Rajh T, Thurnauer MC (2003) J Phys Chem B 107(19):4545–4549
86. Evgenidou E, Bizani E, Christophoridis C, Fytianos K (2007) Chemosphere 68(10): 1877–1882
87. Gutfraind R, Sheintuch M, Avnir D (1991) J Chem Phys 95(8):6100–6111
88. Tsai SC, Chung YW (1984) J Catal 86(1):231–234
89. Zhang ZB, Wang CC, Zakaria R, Ying JY (1998) J Phys Chem B 102(52):10871–10878
90. Tomkiewicz M (2000) Catal Today 58(2–3):115–123
91. Maeda M, Watanabe T (2007) Surf Coat Technol 201(22–23):9309–9312
92. Tian S, Yang H, Cui M, Shi R, Zhao H, Wang X, Wang X, Zhang L (2011) Appl Phys A 104(1):149–158
93. Luis AM, Neves MC, Mendonca MH, Monteiro OC (2011) Mater Chem Phys 125(1–2): 20–25
94. Agrios AG, Pichat P, Photochem J (2006) Photobiology A 180(1–2):130–135
95. Watts RJ, Kong S, Orr MP, Miller GC, Henry BE (1995) Water Res 29(1):95–100
96. Zhu XL, Yuan CW, Bao YC, Yang JH, Wu YZ (2005) J Mol Catal A Chem 229(1–2): 95–105

97. Mogyorosi K, Balazs N, Sranko DF, Tombacz E, Dekany I, Oszko A, Sipos P, Dombi A (2010) Appl Catal B 96(3–4):577–585
98. Yang XH, Li Z, Sun C, Yang HG, Li C (2011) Chem Mater 23(15):3486–3494
99. Gong XQ, Selloni A (2005) J Phys Chem B 109(42):19560–19562
100. Liu S, Yu J, Jaroniec M (2010) J Am Chem Soc 132(34):11914–11916
101. Liu S, Yu J, Jaroniec M (2011) Chem Mater 23(18):4085–4093
102. Tachikawa T, Yamashita S, Majima T (2011) J Am Chem Soc 133(18):7197–7204
103. Zhao X, Jin W, Cai J, Ye J, Li Z, Ma Y, Xie J, Qi L (2011) Adv Funct Mater 21(18): 3554–3563
104. Choi WY, Termin A, Hoffmann MR (1994) J Phys Chem 98(51):13669–13679
105. Asahi R, Morikawa T, Ohwaki T, Aoki K, Taga Y (2001) Science 293(5528):269–271
106. Hagfeldt A, Gratzel M (1995) Chem Rev 95(1):49–68
107. Oregan B, Gratzel M (1991) Nature 353(6346):737–740
108. Rajeshwar K, de Tacconi NR, Chenthamarakshan CR (2001) Chem Mater 13(9):2765–2782
109. Takeuchi M, Yamashita H, Matsuoka M, Anpo M, Hirao T, Itoh N, Iwamoto N (2000) Catal Lett 67(2–4):135–137
110. Wang YQ, Cheng HM, Hao YZ, Ma JM, Li WH, Cai SM (1999) Thin Solid Films 349 (1–2):120–125
111. Bessekhouad Y, Robert D, Weber JV, Chaoui N, Photochem J (2004) Photobiology A 167(1):49–57
112. Burda C, Lou YB, Chen XB, Samia ACS, Stout J, Gole JL (2003) Nano Lett 3(8): 1049–1051
113. Nakamura R, Tanaka T, Nakato Y (2004) J Phys Chem B 108(30):10617–10620
114. Diwald O, Thompson TL, Goralski EG, Walck SD, Yates JT (2004) J Phys Chem B 108(1):52–57
115. Jin TS, Ma YR, Sun X, Liang D, Li TS (2000) J Chem Res Synop 2:96–97
116. Corma A, Martinez A, Martinez C (1996) Appl Catal A 144(1–2):249–268
117. Zaban A, Micic OI, Gregg BA, Nozik AJ (1998) Langmuir 14(12):3153–3156
118. Vogel R, Hoyer P, Weller H (1994) J Phys Chem 98(12):3183–3188
119. Vinodgopal K, Kamat PV (1995) Environ Sci Technol 29(3):841–845
120. Ranjit KT, Viswanathan B (1997) J Photochem Photobiol A 108(1):79–84
121. Ohtani B, Zhang SW, Ogita T, Nishimoto S, Kagiya T (1993) J Photochem Photobiol A 71(2):195–198
122. Vorontsov AV, Savinov EN, Jin ZS (1999) J Photochem Photobiol A 125(1–3):113–117
123. Calza P, Pelizzetti E, Minero C (2005) J Appl Electrochem 35(7–8):665–673
124. Emeline AV, Ryabchuk VK, Serpone N (2005) J Phys Chem B 109(39):18515–18521
125. Fujishima A, Zhang X, Tryk DA (2008) Surf Sci Rep 63(12):515–582
126. Tachikawa T, Fujitsuka M, Majima T (2007) J Phys Chem C 111(14):5259–5275
127. Thompson TL, Yates JT Jr (2006) Chem Rev 106(10):4428–4453
128. Yates JT (2009) Surf Sci 603(10–12):1605–1612
129. Henderson MA (2011) Surf Sci Rep 66(6–7):185–297
130. Murakami M, Matsumoto Y, Nakajima K, Makino T, Segawa Y, Chikyow T, Ahmet P, Kawasaki M, Koinuma H (2001) Appl Phys Lett 78(18):2664–2666
131. Leytner S, Hupp JT (2000) Chem Phys Lett 330(3, 4):231–236
132. Wang Z, Helmersson U, Kall P-O (2002) Thin Solid Films 405(1–2):50–54
133. Tang H, Berger H, Schmid PE, Levy F (1994) Solid State Commun 92(3):267–271
134. Asahi R, Taga Y, Mannstadt W, Freeman AJ (2000) Phys Rev B Condens Matter Mater Phys 61(11):7459–7465
135. Qu Z-W, Kroes G-J (2006) J Phys Chem B 110(18):8998–9007
136. Satoh N, Nakashima T, Kamikura K, Yamamoto K (2008) Nat Nanotechnol 3(2):106–111
137. Szarko JM, Neubauer A, Bartelt A, Socaciu-Siebert L, Birkner F, Schwarzburg K, Hannappel T, Eichberger R (2008) J Phys Chem C 112(28):10542–10552
138. Zou B, Xiao L, Li T, Zhao J, Lai Z, Gu S (1991) Appl Phys Lett 59(15):1826–1828

139. Du Y, Deskins NA, Zhang Z, Dohnalek Z, Dupuis M, Lyubinetsky I (2009) J Phys Chem C 113(2):666–671
140. Lee HS, Woo CS, Youn BK, Kim SY, Oh ST, Sung YE, Lee HI (2005) Top Catal 35 (3–4):255–260Park JH, Park OO, Kim S (2006) Appl Phys Lett 89(16):163106–163108
142. Khomenko VM, Langer K, Rager H, Fett A (1998) Phys Chem Miner 25(5):338–346
143. Komaguchi K, Maruoka T, Nakano H, Imae I, Ooyama Y, Harima Y (2010) J Phys Chem C 114(2):1240–1245
144. Umebayashi T, Yamaki T, Itoh H, Asai K (2002) J Phys Chem Solids 63(10):1909–1920
145. Choi J, Park H, Hoffmann MR (2010) J Phys Chem C 114(2):783–792
146. Maeda M, Watanabe T (2006) J Electrochem Soc 153(3):C186–C189
147. Balcerski W, Ryu SY, Hoffmann MR (2007) J Phys Chem C 111(42):15357–15362
148. Turner GM, Beard MC, Schmuttenmaer CA (2002) J Phys Chem B 106(45):11716–11719
149. Shen Q, Katayama K, Sawada T, Yamaguchi M, Kumagai Y, Toyoda T (2006) Chem Phys Lett 419(4–6):464–468
150. Abuabara SG, Rego LGC, Batista VS (2005) J Am Chem Soc 127(51):18234–18242
151. Rego LGC, Batista VS (2003) J Am Chem Soc 125(26):7989–7997Hendry E, Wang F, Shan J, Heinz TF, Bonn M (2004) Phys Rev B 69(8):081101(R)–081104(R)
153. Deskins NA, Dupuis M (2009) J Phys Chem C 113(1):346–358
154. Henderson MA, White JM, Uetsuka H, Onishi H (2003) J Am Chem Soc 125(49): 14974–14975
155. Deskins NA, Rousseau R, Dupuis M (2009) J Phys Chem C 113(33):14583–14586
156. Ikeda S, Sugiyama N, Murakami S-Y, Kominami H, Kera Y, Noguchi H, Uosaki K, Torimoto T, Ohtani B (2003) Phys Chem Chem Phys 5(4):778–783
157. Kuznetsov AI, Kameneva O, Alexandrov A, Bityurin N, Marteau P, Chhor K, Sanchez C, Kanaev A (2005) Phys Rev E Stat Nonlin Soft Matter Phys 71(2 Pt 1):021403
158. Hurum DC, Gray KA, Rajh T, Thurnauer MC (2005) J Phys Chem B 109(2):977–980
159. Martin ST, Herrmann H, Choi W, Hoffmann MR (1994) J Chem Soc Faraday Trans 90(21):3315–3322
160. Colbeau-Justin C, Kunst M, Huguenin D (2003) J Mater Sci 38(11):2429–2437
161. Tang H, Prasad K, Sanilines R, Schmid PE, Levy F (1994) J Appl Phys 75(4):2042–2047
162. Yamakata A, Ishibashi T-A, Onishi H (2007) Chem Phys 339(1–3):133–137
163. Katoh R, Huijser A, Hara K, Savenije TJ, Siebbeles LDA (2007) J Phys Chem C 111(28):10741–10746
164. Serpone N, Lawless D, Khairutdinov R, Pelizzetti E (1995) J Phys Chem 99(45): 16655–16661
165. Asahi T, Furube A, Masuhara H (1997) Chem Phys Lett 275(3, 4):234–238
166. Rabani J, Yamashita K, Ushida K, Stark J, Kira A (1998) J Phys Chem B 102(10): 1689–1695
167. Furube A, Asahi T, Masuhara H, Yamashita H, Anpo M (2001) Res Chem Intermed 27(1–2):177–187
168. Ryabchuk V (2004) Int J Photoenergy 6(3):95–113
169. Wendt S, Sprunger PT, Lira E, Madsen GKH, Li Z, Hansen JO, Matthiesen J, Blekinge-Rasmussen A, Laegsgaard E, Hammer B, Besenbacher F (2008) Science 320(5884): 1755–1759
170. Babay PA, Emilio CA, Ferreyra RE, Gautier EA, Gettar RT, Litter MI (2001) Water Sci Technol 44(5):179–185
171. Lisachenko AA, Mikhailov RV, Basov LL, Shelimov BN, Che M (2007) J Phys Chem C 111(39):14440–14447
172. Wu W-C, Chuang C-C, Lin J-L (2000) J Phys Chem B 104(36):8719–8724
173. Arana J, Dona-Rodriguez JM, Cabo CGI, Gonzalez-Diaz O, Herrera-Melian JA, Perez-Pena J (2004) Appl Catal B 53(4):221–232
174. Micic OI, Zhang Y, Cromack KR, Trifunac AD, Thurnauer MC (1993) J Phys Chem 97(50):13284–13288
175. Liao L-F, Wu W-C, Chen C-Y, Lin J-L (2001) J Phys Chem B 105(32):7678–7685

176. Jayaweera PM, Quah EL, Idriss H (2007) J Phys Chem C 111(4):1764–1769
177. Sobczynski A, Duczmal L, Zmudzinski W (2004) J Mol Catal A Chem 213(2):225–230
178. Jenkins CA, Murphy DM (1999) J Phys Chem B 103(6):1019–1026
179. Carter E, Carley AF, Murphy DM (2007) ChemPhysChem 8(1):113–123
180. Coronado JM, Kataoka S, Tejedor-Tejedor I, Anderson MA (2003) J Catal 219(1):219–230
181. Kraeutler B, Bard AJ (1978) J Am Chem Soc 100(7):2239–2240
182. Henderson MA (2005) J Phys Chem B 109(24):12062–12070
183. Blount MC, Kim DH, Falconer JL (2001) Environ Sci Technol 35(14):2988–2994
184. Wilson JN, Idriss H (2002) J Am Chem Soc 124(38):11284–11285
185. Ajmera AA, Sawant SB, Pangarkar VG, Beenackers AACM (2002) Chem Eng Technol 25(2):173–180
186. Boarini P, Carassiti V, Maldotti A, Amadelli R (1998) Langmuir 14(8):2080–2085
187. Gondal MA, Hameed A, Yamani ZH, Arfaj A (2004) Chem Phys Lett 392(4–6):372–377
188. Shi D, Feng Y, Zhong S (2004) Catal Today 98(4):505–509
189. Ohno T, Tokieda K, Higashida S, Matsumura M (2003) Appl Catal A 244(2):383–391
190. Coronado JM, Soria J (2007) Catal Today 123(1–4):37–41
191. Stark J, Rabani J (1999) J Phys Chem B 103(40):8524–8531
192. Yamazoe S, Okumura T, Hitomi Y, Shishido T, Tanaka T (2007) J Phys Chem C 111(29):11077–11085
193. Emeline AV, Serpone N (2002) J Phys Chem B 106(47):12221–12226
194. Lukaski AC, Muggli DS (2003) Catal Lett 89(1–2):129–138
195. Mills A, Wang J (1999) J Photochem Photobiol A 127(1–3):123–134
196. Serpone N, Sauve G, Koch R, Tahiri H, Pichat P, Piccinini P, Pelizzetti E, Hidaka H (1996) J Photochem Photobiol A 94(2–3):191–203
197. Pichat P, Vannier S, Dussaud J, Rubis J-P (2004) Sol Energy 77(5):533–542
198. Chatzisymeon E, Stypas E, Bousios S, Xekoukoulotakis NP, Mantzavinos D (2008) J Hazard Mater 154(1–3):1090–1097
199. Rodrigues AC, Boroski M, Shimada NS, Garcia JC, Nozaki J, Hioka N (2008) J Photochem Photobiol A 194(1):1–10
200. Alinsafi A, Evenou F, Abdulkarim EM, Pons MN, Zahraa O, Benhammou A, Yaacoubi A, Nejmeddine A (2007) Dyes Pigm 74(2):439–445
201. Antoniadis A, Takavakoglou V, Zalidis G, Poulios I (2007) Catal Today 124(3–4):260–265
202. Lachheb H, Puzenat E, Houas A, Ksibi M, Elaloui E, Guillard C, Herrmann J-M (2002) Appl Catal B 39(1):75–90
203. Sauer T, Cesconeto Neto G, José HJ, Moreira RFPM (2002) J Photochem Photobiol A 149(1–3):147–154
204. Mounir B, Pons MN, Zahraa O, Yaacoubi A, Benhammou A (2007) J Hazard Mater 148(3):513–520
205. Gonçalves MST, Pinto EMS, Nkeonye P, Oliveira-Campos AMF (2005) Dyes Pigm 64(2):135–139
206. Herrmann J-M, Guillard C (2000) Comptes Rendus de l'Académie des Sciences—Series IIC—Chemistry 3(6):417–422
207. Devipriya S, Yesodharan S (2005) Sol Energy Mater Sol Cells 86(3):309–348
208. Guillard C, Bui T-H, Felix C, Moules V, Lina B, Lejeune P (2008) C R Chim 11(1–2): 107–113
209. Pigeot-Rémy S, Simonet F, Errazuriz-Cerda E, Lazzaroni JC, Atlan D, Guillard C (2011) Appl Catal B 104(3–4):390–398
210. Rizzo L (2009) J Hazard Mater 165(1–3):48–51
211. Rizzo L, Meric S, Kassinos D, Guida M, Russo F, Belgiorno V (2009) Water Res 43(4):979–988
212. Giraldo AL, Peñuela GA, Torres-Palma RA, Pino NJ, Palominos RA, Mansilla HD (2010) Water Res 44(18):5158–5167
213. Elmolla ES, Chaudhuri M (2010) Desalination 252(1–3):46–52

214. Guillard C, Beaugiraud B, Dutriez C, Herrmann J-M, Jaffrezic H, Jaffrezic-Renault N, Lacroix M (2002) Appl Catal B 39(4):331–342
215. Guillard C, Disdier J, Monnet C, Dussaud J, Malato S, Blanco J, Maldonado MI, Herrmann J-M (2003) Appl Catal B 46(2):319–332
216. Alfano OM, Bahnemann D, Cassano AE, Dillert R, Goslich R (2000) Catal Today 58(2–3):199–230
217. Bahnemann D (2004) Sol Energy 77(5):445–459
218. Malato S, Blanco J, Vidal A, Richter C (2002) Appl Catal B Environ 37(1):1–15
219. Blake DM (2001) Bibliography of work on photocatalytic removal of hazardous compounds from water and air, NREL/TP-510-31319, National Renewable Energy Laboratory, Golden, Colorado
220. Zhang L, Anderson WA, Zhang ZJ (2006) Chem Eng J (Lausanne) 121(2–3):125–134
221. Miller LW, Anderson MA (1998) J Adv Oxid Technol 3(3):238–242
222. Danion A, Disdier J, Guillard C, Abdelmalek F, Jaffrezic-Renault N (2004) Appl Catal B 52(3):213–223
223. Molinari R, Mungari M, Drioli E, Di Paola A, Loddo V, Palmisano L, Schiavello M (2000) Catal Today 55(1–2):71–78
224. Sclafani A, Sciascia A, Rizzuti L (1999) J Adv Oxid Technol 4(1):91–96
225. Fabiyi ME, Skelton RL (1999) J Photochem Photobiol A 129(1–2):17–24
226. Danion A, Disdier J, Guillard C, Jaffrezic-Renault N (2007) J Photochem Photobiol A 190(1):135–140
227. Cassano AE, Alfano OM (2000) Catal Today 58(2–3):167–197
228. Brandi RJ, Rintoul G, Alfano OM, Cassano AE (2002) Catal Today 76(2–4):161–175
229. Brandi RJ, Citroni MA, Alfano OM, Cassano AE (2003) Chem Eng Sci 58(3–6):979–985
230. Moreira J, Serrano B, Ortiz A, de Lasa H (2010) Ind Eng Chem Res 49(21):10524–10534

Chapter 7
Nanotechnology Assets in Biosensors Design for Environmental Monitoring

Claude Durrieu, Florence Lagarde and Nicole Jaffrezic-Renault

Abstract In the last decade an intensive research effort has been performed in the field of biosensors design. These tools are very promising to detect chemical pollutants in the environment because they can provide rapid, sensitive, simple, and low-cost on-field detection. Nowadays the use of nanomaterials for the construction of biosensing devices constitutes one of the most exciting approaches. The extremely promising prospects of these devices accrue from the unique properties of nanomaterials. Different nanostructures can be employed and the assets of this new technology in biosensors design are reviewed in this chapter. The properties related to these nanomaterials used in the different transduction modes are presented at first, and then we discuss the interest of nanotechnologies to provide a stable immobilization of biomolecules in retaining their bioactivity. Enzymes-based biosensors, immunosensors, and cell-based biosensors are finally considered separately in their use for environmental monitoring application. The main advantages of the different nanosensing devices are discussed.

7.1 Introduction

Nowadays, biosensors for continuous detection and one-site monitoring are in great demand in ecosystems management. Indeed, intensive industrialization and farming associated to domestic uses of a growing number of chemicals have led to the release

C. Durrieu (✉)
University of Lyon, ENTPE, UMR CNRS 5023, Rue Maurice Audin,
69518 Vaulx-en-Velin Cédex, France

F. Lagarde · N. Jaffrezic-Renault
Institute of Analytical Sciences, UMR CNRS 5280, University of Lyon, Claude Bernard
University Lyon 1, 43 Boulevard 11 November 1918, 69622 Villeurbanne Cédex, France

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_7,

of many toxic compounds in the environment, causing an important pollution of aquatic ecosystems. So, a range of initiatives and legislative requirements force governments worldwide to investigate environmental processes and monitor inputs and temporal trends as well as subsequent future and impact of releases.

Examples include:

- the Water Framework Directive WFD 2000/60/EC lays down the monitoring of a large number of substances, the so-called "priority substances", with the objective of restoring a good chemical and ecological status of all water bodies by 2015 [3].
- Nitrates from agriculture Directive (Directive 91/676/EEC) in relation to impact of diffuse agricultural input
- Urban Waste Water Treatment Directive (Directive 91/271/EEC). United Nations Environment Program (UNEP), global monitoring programme for persistent organic pollutants (POPs)
- OSPAR Stategy to Combat eutophication in relation to the quality of the marine environment
- Urban Waste Water Treatment Directive (Directive 91/271/EEC).

To implement effective monitoring and treatment programs, complementary analytical methods are required:

– low cost and high throughput screening methods for semi-quantitative determination of families of compounds and/or prediction of their harmful biological effects (overall toxicity, genotoxicity, estrogenicity),
– conventional methods based on chromatographic separation techniques (LC/MS, LC/MS/MS, GC/MS, or ICP-MS), which are more time consuming, costful and require trained operators. These methods do not provide informations on water toxicity but allow the rescan of positive samples for more accurate analytes identification [131].

Biological techniques, such as bioassays and biosensors, constitute the first category of methods. Many works in the past have been focused on the development of bioassays and have led to the commercialization of bacterial bioassays and immunoassays [3, 42]. In recent years, biosensors have received particular attention owing to their high sensitivity, low cost and possible easy adaptation for on-line measurements [8]. These biosensors constitute a detection system for signaling a potential damage in the environment. Early recognition will prevent eventual damage to the environment matrices. Ideally early warning signals in ecosystems using biosensors would not only tell us the initial levels of damage, but these signals will also provide us with answers for the development of control strategies and precautionary measures [44].

Hereafter, the biosensors are defined as a device that consists of two fundamental components connected in series: a biological recognition system, often called "bioreceptor", and a transducer. The interaction of the analyte with the bioreceptor is designed to produce an effect measurable with a specific transducer (electrical, optical, mechanical), which converts the physical parameter into an electrical signal.

Different types of bioreceptors (enzymes, receptors, antibodies, DNA, or microorganisms) combined with electrochemical, optical, or mechanical transductions have been used for the elaboration of a large spectrum of biosensors that enable the monitoring of pollutants. Such biosensors could be designed for different applications in environmental analysis as monitoring concentration of pollutants, monitoring general toxicity, or detection of target bacteria in soil and water [5, 132]. To answer to the ever increasing requirements of environment monitoring legislation, not only in terms of amount and reliability of informations provided, but also in terms of rapidity of response, selectivity, sensitivity and cost, tremendous efforts have been devoted in the last few years to improve the different elements contributing to the overall response of the biosensors, i.e., bioreceptor, transducer, and bioreceptor/transducer interface. The main purpose of the recognition system is to provide the sensor with a high degree of selectivity for the analyte or group of analytes to which the biosensing elements binds. (Turner et al. [152], [158]).

Currently, advances in nanotechnology have recently led to a new generation of nanotools. Nanomaterials can be used to improve the performance of biosensors due to their specifical properties. Until now, various nanomaterials have been employed to design biosensors with huge success. Lately, graphene has drawn wide concern as an interesting material due to its unique properties [95, 163], such as high surface area, high electrical conductivity, and exceptional thermal and mechanical properties [138, 144]. In this chapter, we focused on the properties that characterise nanotransducers and give them their particular behaviour. With particular focus on applications in the environmental field, we discuss the main types of nanosensors developed to date and highlight the relationship between the property monitored and the type of nanomaterial used. All these aspects will be addressed in the present chapter. New advances recorded in the field during the last 5 years will be more particularly emphasized. In a first time, the different transduction modes in the design of biosensors are described and the asset of nanotransducers is emphasized.

7.2 Transduction Modes

7.2.1 Electrochemical Transduction

Electrochemical sensors are classified according to their transduction mode, which may be potentiometric, amperometric, conductimetric, or impedimetric. In a general way, electrochemical transducers measure the electron transfers occurring between electroactive species (molecules or ions) present in a solution and an electrode, in well defined analytical conditions [50]. Over the past 10 years, electrochemical transduction technology has evolved significantly. Novel electrode materials such as boron doped diamond (BDD) have emerged as possible

alternative materials to conventional gold, platinum, or carbon [103]. Owing to the recent advances in microfabrication techniques, it is also now possible to prepare microelectrodes of various sizes and geometries as well as to construct parallel arrays of microsensors on a same chip [164]. Such systems are powerful tools able to answer to most of the environmental monitoring requirements such as rapidity of response, sensitivity, and parallel analysis of a large number of parameters and/or samples. Moreover, the small size is useful for the design of portable biosensors intended for on-field applications.

7.2.1.1 Potentiometric Transduction

The two classical types of potentiometric transducers are ion selective electrodes (ISEs) and semiconductor-based field-effect devices (FEDs). The inherent miniaturization of ISEs and FEDs and their compatibility with advanced microfabrication technology make them very attractive for the integration into sensing arrays and microfluidic platforms and thus, the creation of miniaturized analytical systems suitable for environmental monitoring [6, 11].

ISEs involve ion exchange equilibria at the interface between the solution and a membrane made of an ionic conducting material (inorganic solid electrolyte or organic liquid membrane). The nature of the membrane depends on exchanged ions, special glasses being typically used for H^+, ionic solid for halide ions, polymers including specific ionophores for other ions. Practically, potentiometric biosensors measure the difference of potential *Ep* between the selective electrode on which the bioreceptor is immobilized and a reference electrode when no significant current flows between them. *Ep* can be expressed by Nernst equation:

$$Ep = E^0 + RT/nF \ln a_A n+ \tag{7.1}$$

where E^0 is the selective electrode constant, $a_A n+$is the activity of A^{n+} ion

Significant efforts have been made during the past decade to improve the robustness of conventional ISEs, widen the range of ions detected and miniaturize the electrodes [6, 153].

FEDs belong to the second class of potentiometric transducers and include ion-sensitive field-effect transistors (ISFETs) and light-addressable potentiometric sensors (LAPSs). At present, only ISFET sensors measuring H^+ ions are commercially available. By deposition of enzymes or bacteria, it is possible to monitor enzymatic and metabolic reactions generating H^+. LAPS devices are also extensively used to monitor cellular acidification in response to pollutants. Several recent reviews document the main features of these devices and their application to biosensing [87, 120, 136].

7.2.1.2 Conductometric Transduction

Conductometric biosensors rely on the direct measurement of conductance variations in electrolytic media containing mobile electric charges. For that, an alternating

voltage is applied between the working electrode, on which the bioreceptor is immobilized, and a reference electrode. The frequency value is chosen in order to minimize polarization effects. The conductance can be expressed by the following equation:

$$G = \gamma \frac{S}{l} \tag{7.2}$$

where γ (S. cm^{-1}) is the specific conductance or conductivity, characteristics of the medium; S (cm^2) is the working electrode surface; l (cm) is the distance between the electrodes.

Recent advances in the field have led to the production of miniaturized interdigitated electrodes that have been used to the elaboration of enzyme-based and cell-based biosensors for water monitoring [24, 63, 69]. Enzymatic reactions between the pollutant and the bioreceptor induce a local change of conductivity due to the production of charged species.

These last years different works have showed that nanoparticles can provide electronic conductivity, while the organic matrix furnishes the selective binding sites on which the adsorption of analyte molecules takes place [76]. An attractive feature of this approach is the ability to control the sensor properties by molecular design. Different works have reported the tenability of the selectivity of sensor nanoparticles films by introducing chemical functionality into an organic ligand shell [39, 71] showed the detection of CO and NH3 in the 300 ppb to 5,000 ppm range by cross-linking gold and platinum nanoparticles with nonanedithiol.

7.2.1.3 Impedimetric Transduction

Impedimetric transduction measures charge transfer processes occurring at electrode/electrolyte interfaces. Practically, measurement is performed using three electrodes, a working electrode modified by the bioreceptor, a reference electrode and an auxiliary electrode. A small amplitude sinusoidal voltage is imposed between reference and working electrodes and the resulting current generated between working and counter electrodes is measured. The applied voltage over measured current intensity ratio defines the impedance of the electrochemical system. Impedimetric data can be modeled by an equivalent electrical circuit from which electrical parameters that define charge transfer processes can be deduced [74]. Impedimetric transduction is particularly wellsuited to investigate reactions based on molecular affinity such as antigen–antibody or receptor–target interactions. Cell adhesion to the electrode surface is also expected to increase impedance value due to the insulative properties of the cell membrane. In the presence of cytotoxicants, morphological changes or functional alterations, and even death of the cells are also observed, inducing impedance variations. Therefore, these properties have been extensively exploited for water pollutants biosensing and toxicity assessment using electrodes modified with antibodies, receptors, or cells.

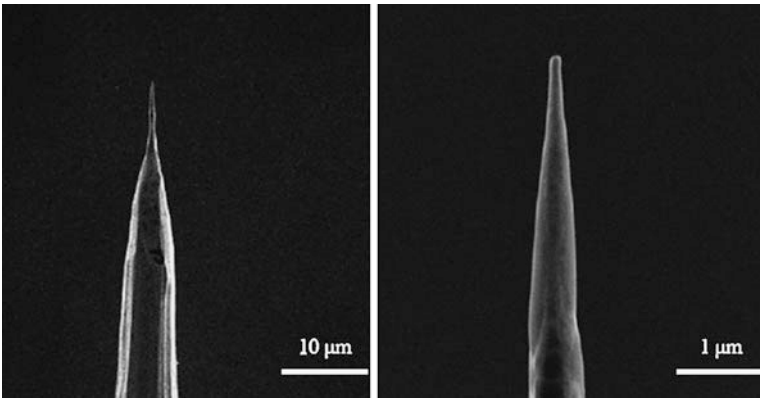


Fig. 7.1 Iridium oxide nano pH sensor (from [114])

7.2.1.4 Nanoscale Electrodes

There are several benefits of using nanoscale electrodes in electrochemical sensors. First, since current (i) is proportional to the electrode area, nanoelectrodes will further reduce the Ohmic (iR) drop distortion and can be used to detect electrochemical reactions in poorly conducting media, even in the absence of a supporting electrolyte. Second, double layer capacitances are proportional to electrode area, and so are greatly reduced for nanoelectrodes which have small surface area, resulting in electrochemical cells with small RC (R: resistance, C: capacitance) time constants. Such nanoelectrode electrochemical cells enable high speed voltammetric experiments that can probe the kinetics of very fast electron transfer and coupling reactions or the dynamics of processes. Third, the rate of mass transport to and from the electrode (and the related current density) increases as the electrode size decreases. Models also predict substantially higher mass transfer rates for nanoelectrodes due to radial (nonplanar) diffusion, which would enable ultrafast electrochemical measurements, compared to measurements using bulk electrodes that operate via planar diffusion. As a consequence of the increase in mass transport rates and the reduced charging currents, nanoelectrodes will exhibit excellent signal to background noise (S/N) ratio in comparison to their macroscopic counterparts. Small sensors have high concentration gradients and do not significantly deplete the solution, which also benefits many kinetic studies.

Besides the use of individual nanoelectrodes, where device miniaturization provides benefits, the use of nanoscale components in large scale devices has advantages. Single sensors are normally used to measure a single analyte. An example is the novel iridium oxide nano pH sensor designed to work in the range of pH 3–14 (see Fig. 7.1) [114].

Devices that combine multiple individual sensors can either provide a multi-analyte measurement capability (sensor arrays) or be used to obtain spatial

distribution measurements for a single analyte (nanoelectrode ensembles). Since the signal is the same but the background is several orders of magnitude lower, the S/N for nanoelectrode ensembles is significantly higher than for conventional electrodes [180]. The design of biomolecular sensors for ultrahigh sensitivity applications has to take into account the limits imposed by analyte transport in fluidic systems. The detection limit reported to date for nanoscale biosensors is in the range of femtomolar concentrations which is very likely limited by analyte transport rather than signal transduction.

7.2.1.5 Nanowires

Silicon nanowire (SiNW) biosensors along with nanotubes and conducting-polymer nanowires are promising label-free electronic biosensors. The most important and powerful advantage of SiNW sensors is the possibility of multiplexed, real-time detection. The progress in nanofabrication techniques allows us to make an array of identical structures, which leads to massively parallel measurements. As most nanofabrication techniques originated in microelectronics, they can be easily scaled up and transferred to a mass production line with high reliability. The underlying mechanism of nanowire sensors is based on the principle of field-effect transistors (FETs). In the case of a p-type semiconductor, a positive gate voltage depletes carriers and reduces the conductance, while a negative gate voltage leads to an accumulation of carriers and an increase in the conductance.

For biosensors, binding of a charged species on the surface of the SiNWs is analogous to applying a gate voltage. By monitoring the conductance change, the binding of targets to probe molecules can be detected on the Si surface. Several research groups have already demonstrated the successful solution-phase SiNW sensing of DNA [14], viruses [118], small molecules [162], and proteins [178]. The current detection sensitivity is about fM range, which is several orders of magnitude more sensitive than a conventional ELISA assay. For most of those experiments, however, low salt buffer solutions were used to avoid the screening effect associated with solution counter ions. The charge of target molecules is screened by the counter ions in solution and effective only on the scale of the Debye length. The Debye length for a 0.1 M solution is about 1 nm and biologically relevant media is typically a 0.14 M electrolyte. Since the salt concentration and pH are important factors for the binding between biomolecules, it is necessary to find an alternative way of overcoming the charge screening to perform an ideal sensing measurement with NW-FETs. For DNA sensing, the charge screening can be overcome by electrostatically immobilizing ssDNA on the SiNW surface [14]. With antibodies, however, the biomolecular recognition event occurs *10 nm away from the wire due to the antibody's large size. In biological media, the binding event usually takes place farther away than the Debye screening length, so alternative small capture agents are required to bring the binding event closer to the nanowire. Thus, finding small molecules that have the same specificity for proteins as antibodies and that can distinguish between slightly different proteins is

critical. Due to their small scale, high sensitivity, and real-time detection capability, nanowire based sensors could be used to study single cells. Gold nanoparticles can provide the ultrasensitive detection of nucleic acids and proteins. When Si nanowires were coated with them, the nanocomplex exhibits high sensitivity for pesticide detection when used as an electrochemical nanosensor [145].

Nanopores

Biological nanopores have existed for a long time, but with the development of nanolithography techniques, it has been possible to create engineered nanoscale pores and holes. Researchers have also found that nanopores can be used for single molecule detection, especially with oligonucleotides and protein molecules. The first demonstration of this technique was to use α- hemolysin (HL), a biologically based nanopore, to identify a DNA sequence [30]. In comparison to solid state nanopores, a biological nanopore is usually cheaper, but it is difficult to generate precise pore sizes using biological techniques, which leads to a non-uniform signal due to the lack of stability. Another disadvantage is that biological nanopore works in a limited range of pHs and temperatures. Thus, a solid-state nanopore is more stable, flexible, and robust for identifying nucleic acid and proteins. Solid-state nanopore has been developed by using forced-ion-beams, micromolds, e-beam, and TEM techniques on SiO_2 or Si_3N_4 thin layers. The pore size of the biological nanopore in α-HL is fixed at 1.5 nm, but, a wide range of pore sizes (1–80 nm) are possible using these fabrication techniques. The working principle of the nanopore technique as a sensor is to measure the current when molecules pass through the nanopores. As a molecule passes through a nanopore, the current is temporarily blocked. The current pulse profile is then recorded to determine when a molecular-translocation event has occurred. To increase the sensitivity and selectivity of the nanopore sensor, the surface of nanopores can be modified by a specific protein or nucleic acid. For example, the surface of a solid-state nanopore was functionalized with a protein binding molecular recognition agent (MRA) and an oligonucleotide binding MRA to increase the selectivity of the nanopore [107]. In another case, hairpin loop DNA was immobilized on the nanopore wall to increase the selectivity of single stranded DNA.

7.2.2 Optical Transduction

Optical transducers are based on various technologies of optical phenomena, which are the results of interactions between analytes and biological entities used as bioreceptors. The various types of optical transducers exploit properties as simple light absorption, fluorescence/phosphorescence, bio/chemiluminescence, reflectance, Raman scattering, and refractive index Apart from speed, sensitivity, and robustness, other attractive features of optical sensors include their suitability for component minutiarization [44].

Over the past decades, the increasing market of telecommunications has supported new optical materials research which can be subdivised according to the type of optical properties which have been applied in sensing:

- absorbance measured in transparent medium.
- Reflectance measured in non transparent media, usually using an immobilized indicator.
- Luminescence based on the measurement of the intensity of light emitted by a chemical reaction in the receptor system.
- Fluorescence, measured as the positive emission effect caused by irradiation; Also, selective quenching of fluorescence may be the basis of such devices.
- Refractive index, measured as the result of a change in solution composition. This may include also a surface Plasmon resonance effect (SPR)
- Optothermal effect, based on a measurement of the thermal effect caused by light absorption;
- Light scattering, based on effects caused by particles of definite size present in the sample.

In general, two different protocols can be implemented in optical biosensing. The first one requires a preliminary functionalization of the bioreceptor or the target analyte with an optically active tag (labeling). Although this process produces highly sensitive biosensors, it is time-consuming and may interfere with the function of a biomolecule. In contrast, in the second protocol, target molecules are not labeled or altered, and are detected in their natural forms. This type of detection is relatively easy and cheap to perform [41]. The most recent innovations in optical transduction applied to environmental biosensing are related to the development of new solid-state devices, microarrays, and microfluidic systems for continuous monitoring [96].

7.2.2.1 Optical Fiber Sensors

Optical fiber-based biosensors employ an optical fiber or optical fiber bundle as a platform for the biological recognition element, and as a conduit for excitation light and/or the resultant signal [112].

An optical fiber is a waveguide that classically consists of a silica core (optical index: $\boldsymbol{n_1}$) surrounded with a cladding of index $\boldsymbol{n_2}$, slightly lower than $\boldsymbol{n_1}$. The fiber is placed in a medium of index $\boldsymbol{n_0}$. The light-guiding conditions are defined by:

$$n_0^2 \sin^2\theta_0 = n_1^2 - n_2^2 \tag{7.3}$$

where θ_0 represents the numerical aperture of the fiber or the limit injection angle of the incident beam.

Fiber optical biosensors are all of extrinsic type. In some of them, called punctual biosensors, the physical or chemical effect is measured at the tip of the fiber on which the bioreceptor is deposited. The biosensor operates in reflection mode. In the so-called

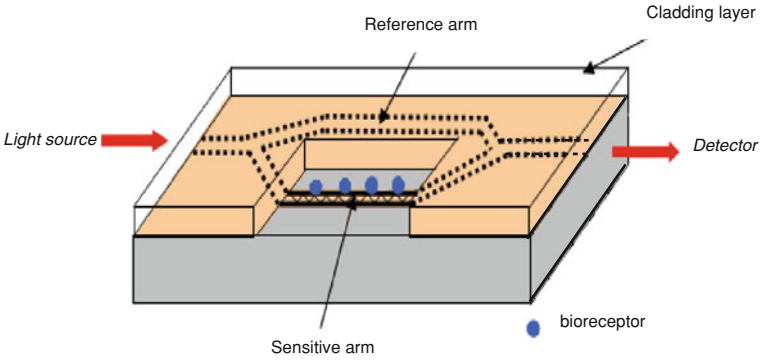


Fig. 7.2 Design of a Mach–Zehnder interferometer

continuous biosensors, measurements are performed on a well defined length of the fiber where cladding is removed. The bioreceptor layer is directly deposited on the core. This system can operate in reflection or in transmission modes. Optical fiber biosensors are classified by the nature of recognition element used for sensing: enzyme, antibody/antigen, nucleic acid, whole cellwhich are generally immobilized by adsorption or covalent attachment to a membrane, or are covered with a semipermeable membrane. The most conventional fiber biosensors are based on absorption, fluorescence, or luminescence detection [48]. Physical properties of the evanescent wave, which corresponds to the light power lost at the core-cladding interface, can also be exploited. These biosensors, for which a stripping of the fiber is required, are more fragile than the massive optical systems based on the same principles and described in the following sections.

7.2.2.2 Mach-Zender Interferometers

This type of sensor relies on the perturbation of the light propagating in one arm of an optical waveguide. In a typical Mach–Zehnder interferometer configuration, the light guide is divided into two branches via a Y-junction. A branch, functionalised with the biosensing element, is used as the sensitive arm, while the other is the reference branch (Fig. 7.1). The two branches recombine at the output, resulting in an interference, and a photodetector measures the intensity. A change in the refractive index at the surface of the functionalised arm results in an optical phase change and a subsequent variation in the light intensity measured at the photodetector. The intensity is proportional to cos $(\Delta n_2 k_o L)$, where Δn_2 represents the refractive index change, k_o the amplitude of the wave vector and L the length of the sensitive region. These structures are made in glass or silicon and may be easily integrated into lab-on-chip laboratories [137] (Fig. 7.2).

7.2.2.3 Surface Plasmon Resonance (SPR) Sensors

These sensors are based on the physical principle of surface plasmon resonance [64]. The bioreceptor is deposited on a metal surface covering a glass support attached to the base of a prism (Kretschmann configuration). Interaction between the target and biorecognition molecules can be investigated in real time, with high precision and sensitivity, without specific labeling, through the measurement of the variations of refractive index near the interface. These sensors have been extensively used for the study of affinity interactions (e.g, antigen–antibody). SPRi systems allowing real-time and simultaneous imaging of several spots functionalised with different affinity systems are currently in full expansion [135]. In order to attain even higher sensitivity and lower limit of detection, novel SPR structures and approaches have been intensively investigated worldwide. One of the most promising novel structures in SPR sensors is an optical multilayer structure supporting long range surface plasmons (LRSPs); Long range surface plasmons represent a special surface plasmon mode, which can be generated when a thinner metal film, typically 20–25 nm, is sandwiched between two dielectric media of refractive indices. These SPR sensors employing LRSPs are expected to offer better performance compared to sensors with conventional surface plasmons. Indeed, optical field enhancement due to LRSPs has been shown to be an order of magnitude larger than that of conventional surface plasmons [165]. Compared to conventional surface plasmons LRSPs also have lower propagation loss and longer field penetration.

7.2.2.4 Localized Surface Plasmon Resonance (LSPR) Sensors

In noble metals, nanostructures of smaller size than the Broglie wavelength for electrons lead to an intense absorption in the visible/near-UV region that is absent in the spectrum of the bulk material. The conduction electrons are then trapped in these “metal boxes” and show a characteristic collective oscillation that leads to the surface plasmon band (SPB) observed near 530 nm for nanoparticles in the 5–20 nm range. This extinction band arises when the incident photon frequency is resonant with the collective oscillation of the conduction electrons and is known as the Localised Surface Plasmon Resonance (LSPR). The LSPR spectrum depends on the NP itself (i.e., its size, material, and shape), but also on the external properties of the NP environment. This makes noble metal NPs extremely valuable from the sensing point of view.

LSPR nanosensors can be implemented using small light, robust, extremely simple, and inexpensive equipment for unpolarized UV–Vis extinction spectroscopy in transmission mode. The glass containing the arrays of NPs is inside a flow cell that is coupled to a source of white light and a miniature spectrometer through an optical fiber. The cell is also linked to a reservoir containing the analyte to be detected [56, 128].

7.2.2.5 Optical Waveguide Light Mode Spectroscopy (OWLS)

This is a new detection technique based on evanescent field for in situ and label free investigation of surface processes at molecular level. It is based on accurate measurement of the resonance angle of a linearly polarized laser light, diffracted by a grating, and coupled in a thin layer of the waveguide. Resonance coupling occurs at a specific angle characteristic of the refractive index of the medium covering the waveguide surface. The light is guided by total internal reflection on the edges where the detection is performed via photodiodes. By varying the light incidence angle, a spectrum is obtained, which allows the calculation of effective indices for both the electric and magnetic fields [104].

7.2.2.6 Total Internal Reflection Fluorescence (TIRF)

This technique has been used with planar waveguides and optical fibers as optical transducers in many biosensors. The light propagates along the waveguide, generating an evanescent wave on the surface of the optically denser part of the waveguide (quartz) as well as in the adjacent less dense medium (aqueous medium). The evanescent wave amplitude decreases exponentially with distance in the lower refractive index medium. The fluorescence of a fluorophore excited by the evanescent field can then be detected. Only fluorophores bound to the surface are excited. Real time kinetics of interaction of bioanalytes with molecules immobilized on the surface of the waveguide can be measured using Total Internal Reflection Fluorescence (TIRF). This is a rapid, nondestructive, and sensitive technique used for the development of automated detection systems for environment monitoring [150].

7.2.3 Mechanical Transduction

Various mechanical methods have been used as detection in biosensors. These transducers have become increasingly popular over the years.

7.2.3.1 Transducers Based on Piezoelectric Effect

A quartz crystal, to which a sinusoidal electric field is imposed, undergoes mechanical deformation due to the electrical potential appearing at its surface (piezoelectric effect). The crystal oscillates at its resonance frequency that depends on its structure (orientation, thickness). Any change in mass (Δm) occurring at the crystal surface causes a proportional decrease in its resonance frequency (ΔF). This linear relationship is expressed quantitatively by the Sauerbrey equation:

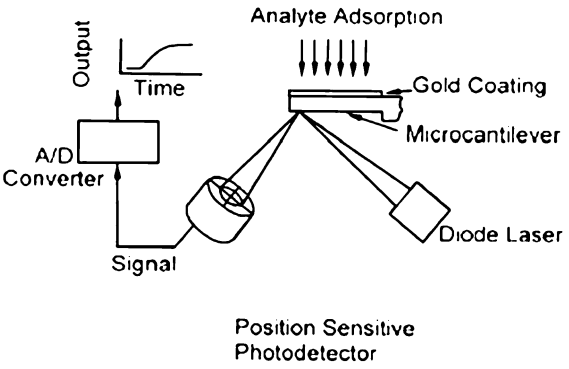


Fig. 7.3 Experimental arrangement used to determine absorption-induced bending of microcantilever exposed to analyte

$$\Delta F = -\frac{2F_0^2}{\sqrt{\mu_Q \cdot \rho_Q}} \cdot \frac{\Delta m}{A} \tag{7.4}$$

where F_o is fundamental frequency; A the geometric surface; μ_Q the shearing mode; ρ_Q the density of piezoelectric crystal

The Sauerbrey equation applies only for thin and rigid layers, excluding viscoelastic films, e.g., polymer or polyelectrolyte films. The most common transducer based on piezoelectric effect is the quartz crystal microbalance (QCM).

7.2.3.2 Cantilever-Based Biosensors

Micro/nano-cantilevers act as a force transducer for biosensing applications and function by detecting changes in cantilever bending or vibration frequency [179] when molecules bind to the surface of the cantilever (Fig. 7.3). Characterization of the forces and dynamics of biomolecular interactions with cantilever-based sensors offers an opportunity for the development of highly sensitive, miniaturized, parallel, and label-free biological sensors.

Advancement in microelectromechanical systems (MEMS) technology over the past decade has led to cost-effective, miniaturized cantilevers with low spring constants with a high sensitivity to applied forces, or high resonance frequencies for faster response times [58]. The classic perception that smaller sensors are more sensitive (sensitivity $\sim -0.5\ \omega_r/m_c$, where ω_r is the resonant frequency and m_c is the mass of the cantilever) has motivated scaling of biosensors to nanoscale dimension [54]. Accordingly, efforts have been made to develop smaller cantilevers with higher frequencies and better mass resolution. The recent development of nanoscale cantilevers with frequencies in the MHz range and an integrated electronic displacement transducer has scaled down the technology further and increased the capability for rapid, ultrasensitive, and selective detection of captured biomolecules. For example, a cantilever sensor array with a pitch of 250 nm and individual cantilevers of 500 nm × 100 nm × 20–500 nm dimension operating a 2–4 kHz allowed for real-time monitoring of lipid bilayer formation on the

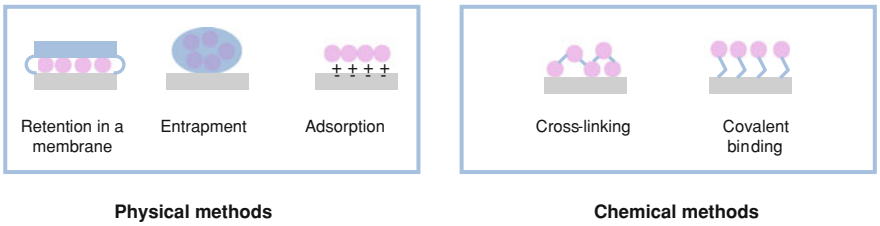


Fig. 7.4 The different methods for bioreceptor immobilization

cantilever and fast microorganism detection [94]. An oligonucleotide detection system with an ability to identify for label-free genes within a complete genome or an unlabeled gene in total RNA was also created [174]. Further advancement to a high degree of parallelization with nanomechanical biosensors may eventually allow for high-throughput screening for disease diagnosis and drug discovery. Several challenges still remain before cantilever array sensors can be used as one of the primary, next-generation diagnostic tools. For example, efficient immobilization techniques are required to functionalize the large number of cantilevers in an array. Damping, viscosity, and thermal fluctuations also combine to make precise measurements in fluids difficult. Advances in integration of electric, mechanical, and fluidic designs are required to accelerate the design of fully integrated cantilever-biosensor devices.

7.3 Bioreceptor Immobilization

Nowadays, it is well established that the performance of biosensors depends greatly on the influence imposed on biomolecules by immobilization. Apart from preserving the functionality of the biomaterial, the immobilization method must ensure the accessibility of the cells toward target analytes as well as a close proximity between the bioreceptor and the transducer. The selection of an appropriate immobilization method depends on the nature of the biological element and of the transducer, the physicochemical properties of the analyte, and the operating conditions of the biosensor. Several methods have been proposed in the literature, including chemical and physical methods (Fig. 7.4) [32].

Physical methods include adsorption, retention into a membrane, or entrapment within a polymeric network. Adsorption is based on the establishment of low energy interactions between the functional groups of the bioreceptor and of the substrate surface. This type of immobilization offers the advantage of preserving bio receptor properties but results in the formation of weak bondings that favors its desorption. To avoid leakage processes, biological elements can be covered with a thin polymer membrane that allows diffusion of the target molecule or can be entrapped in a chemical or biological polymeric matrix. Sol–gel silica or hydrogels

are typically used for that purpose. These polymers can efficiently protect the bioreceptor from external aggressions but may form a diffusion barrier that restricts the accessibility to the substrate and/or decrease light and electronic transfers to the transducer. The swelling properties of hydrogels may also limit their practical application in some cases.

In chemical methods, biosensing elements may be attached directly to the transducer through covalent bindings or to an inert and biocompatible matrix through crosslinking using a bifunctional reagent. Proteinic supports, e.g., bovine serum albumin or gelatine are typically used to constitute the network with glutaraldehyde (GA) as cross-linking agent. This second technique is primarily used to attach enzymes or antibodies to the transducer, and more recently for whole cell immobilization [23, 24]. Indeed, cross-linking involves the formation of covalent bindings between the functional groups located on the outer membrane of cells and GA. Generally this mode of immobilization is consequently not suited when cell viability is absolutely required or enzymes involved in the detection are expressed at the cell surface but it can be used in some cases without alteration of cell activity [24, 53]. Finally, covalent grafting is based on the reaction between functional groups of the biological element and previously activated groups of the transducer. Functional groups of the bioreceptor are typically ε-amines of lysine, carboxyl groups of aspartate or glutamate, sulfhydryl groups of cysteine, and hydroxyphenolic groups of tyrosine, which belong to the side chains of amino acids in proteins (enzymes, antibodies, or external cellular proteins). To ensure the formation of covalent bondings with the transducer, this latter has to be functionalised first. Metal surfaces such as gold and silver can be functionalised with amine, hydroxyl, or carboxyl groups through reaction with aminoalkanethiols, hydroxyalcanethiols, and carboxyalcanethiols, respectively. Oxide surfaces are functionalised with organosilanes. More recently, metal electrodes on which films of functionalised conducting polymer (polypyrrole, polythiophene, polyaniline) are deposited electrochemically, have been used to immobilize active biomolecules through covalent bonding formation [148].

7.3.1 Nanotechnology Assets in Immobilization Techniques

More recently, particular attention has been also paid to the use of nanotechnologies for immobilization step as sol–gel technique, gold nanoparticles, magnetic beads, carbon nanotubes (CNTs), or quantum dots (QDs) [168, 176, 181]. Their particular chemical and physical properties make them very attractive to improve bioreceptor stability as well as biosensor sensitivity.

7.3.1.1 Sol–gel

This technique allows the obtention of an integrated network from a colloidal suspension.

Contrary to conventional glass-making processes requiring reaction at elevated temperatures which are not compatible with entrapment of fragile biomolecules, the sol–gel process involves the transition at low temperature of a system from a molecular precursor state through the formation of nano-sized bricks into a solid gel phase and finally transitions into a dried ceramic material. Silica matrixes are relatively inexpensive to synthesize and have interesting properties including optical transparency, biocompatibility and chemical inertness. The design flexibility of sol–gel technique and simplicity of fabrication can fulfill to create the surfaces with structural and chemical features that could be compatible with biomaterials. Immobilization of chemicals in such inorganic structures is not restricted to organic molecules. Since the sol–gel process can be carried out in moderate conditions, the structural elements of the nano-sized sol bricks are preserved and enable the encapsulation of biomolecules [34] and even bacteria [67]. Biomolecules entrapped in sol–gel matrixes typically exhibit improved resistance to thermal and chemical denaturation, and increased storage and operational stability. This technique has been applied to immobilize various biological molecules (proteins, enzymes, antibodies) using the alkoxide route with tetraethyl orthosilicate (TEOS) or tetramethyl orthosilicate (TEMOS) as precursors.

7.3.1.2 Nanoparticles

The ability of nanoparticles to provide a stable immobilization of biomolecules retaining their bioactivity is a major advantage for the preparation of biosensors. Furthermore, nanoparticles permit direct electron transfer between redox proteins and bulk electrode materials, thus allowing electrochemical sensing to be performed with no need for electron transfer mediators. Characteristics of nanoparticles such as high surface to volume ratio, high surface energy, ability to decrease proteins–metal particles distance, and the functioning as electron-conducting pathways between prosthetic groups and the electrode surface have been claimed as reasons to facilitate electron transfer between redox proteins and electrode surfaces [99, 170]. nanoparticles have also demonstrate to constitute useful interfaces for the electrocatalysis of redox processes of molecules such as H_2O_2, O_2 or NADH involved in many significant biochemical reactions [120].

Much of the research on biosensors involving nanoparticles has been devoted to enzyme electrodes. Methodology consists on the direct deposition of nanoparticles onto the electrode surface which has been modified with self-assembled monolayers (SAMs) of thiols. nanoparticles and enzymes can further be immobilized on functionalized sites [27].

Nanoparticles can also be used in immunosensors design. They play a crucial role both in the enhancement of the electrochemical signal transducing the binding reaction of antigens at antibody-immobilized surfaces and in the ability of increasing the amount of immobilized immunoreagents in a stable mode [92]. nanoparticles play also an important role in DNA immobilization on electrode surfaces and constitute suitable labels to improve detection of hybridization events [176, 181].

All the work carried out recently on the development of nanoparticles-based biosensors show clearly the potentialities and advantageous features of this approach to construct biosensors with improved performances compared to the previous model. The unique properties of nanoparticles concerning immobilization of biomolecules retaining their biological activity and as efficient conducting interfaces with electrocatalytic ability make them a powerful tool to modify electrode materials and to construct robust and sensitive biosensors which can find applications in environmental monitoring.

7.3.1.3 Carbon Nanotubes

The unique chemical and physical properties of CNT have paved the way to new and improved sensing devices, in general, and electrochemical biosensors, in particular. CNT-based electrochemical transducers offer substantial improvements in the performance of amperometric enzyme electrodes, immunosensors, and nucleic-acid sensing devices. The greatly enhanced electrochemical reactivity of hydrogen peroxide and NADH at CNT-modified electrodes makes these nanomaterials extremely attractive for numerous oxidase and dehydrogenase based amperometric biosensors. Aligned CNT "forests" can act as molecular wires to allow efficient electron transfer between the underlying electrode and the redox centers of enzymes. Bioaffinity devices utilizing enzyme tags can greatly benefit from the enhanced response of the biocatalytic reaction product at the CNT transducer and from CNT amplification platforms carrying multiple tags [159].

7.4 Biosensors for Environmental Monitoring

The applications of biosensors to environmental monitoring deal with the detection of specific (or a group) of pollutants or more generally allow the overall toxicity assessment toward the recognition element.

7.4.1 Application to the Determination of Specific (Groups of) Pollutants

7.4.1.1 Enzyme-Based Biosensors

A large number of enzymes have been used in the construction of water pollution biosensors. They may be classified into different families corresponding to the type of reaction they catalyze, typically oxidation, reduction, and hydrolysis. The enzyme is immobilized on a transducer that detects the consumption of a co-factor,

e.g. oxygen in the case of oxidase enzymes, or the appearance of a product following enzymatic reaction. Hydrolase enzymes are generally associated with optical fibers, potentiometric or conductometric transducers to detect local changes in pH or in conductivity. For their part, reductase or oxidase enzymes are generally immobilized on an amperometric or conductometric transducer to record electronic transfers toward the electrode. These electronic transfers may be promoted by the use of redox mediators that allow the application of lower potentials and limit interferences from other electroactive species.

Tyrosinase is a copper monooxygenase that catalyzes the hydroxylation of monophenols and the oxidation of diphenols into reactive quinones. This reaction has been extensively exploited for the determination of phenolic compounds using tyrosinase-based amperometric and optical biosensors. In electrochemical systems, substances produced by the reduction of quinones at the electrode can be detected at a low potential value, in the absence of mediator. Various electrode materials, such as gold [160], platinum [171], carbon paste [29, 110], glassy carbon [17, 19, 60, 78, 80, 88, 160, 161], or BDD [115, 177, 182] have been used for that purpose. Very recently, Yuan et al. [172] developed an amperometric biosensor using a carbon fiber paper (CFP) electrode. This biosensor exhibited short response times (10–20s) and very high sensitivities to phenolic compounds such as catechol, phenol, bisphenolA, and 3-aminophenol, corresponding detection limits being 2, 5, 5, and 12 nM, respectively. A seen in Table 7.1, these values are much better than other figures reported in the literature and are 4–10 times lower than the values obtained in the same experimental conditions, by the same authors, using a commercial screen-printed carbon electrode (SCPE). In this work, tyrosinase was immobilized in photoreticulated polyvinylalcohool (PVA-SbQ) matrix. Many other modes of immobilization have been proposed to stabilize tyrosinase on the transducer, including, among others entrapment into titania sol–gel [78], polyacrylamide microgel [60], Fe_3O_4- or multiwalled carbon nanotubes (MWNT)-chitosane composites [80, 161], MWNT-epoxy resin [119], physical adsorption on ZnO nanorods [177], or covalent binding [160, 182]. Optical tyrosinase-based biosensors have been also reported [70]. Table 7.1 presents some recent biosensors based on tyrosinase enzyme, with the type of transducer and immobilization used, as well as the detection limits obtained for typical phenolic contaminants.

Another enzyme, organophosphate hydrolase (OPH), has been also commonly used for the development of electrochemical, optical, and mechanical biosensors for organophosphorous pesticides detection, while nitrate reductase, nitrite reductase, maltose phosphorylase, pyruvate oxidase have been employed for the determination of trophic pollutants such as nitrates, nitrites, or phosphates (Table 7.1).

7.4.1.2 Immunosensors

Immunosensors are based on highly selective antibody (Ab)—antigen (Ag) reactions. The immobilized sensing element can be either an Ab or an Ag which can be chemically modified (hapten). In the first case, analyte binding is measured

Table 7.1 Examples of enzymatic biosensors for the detection of chemical pollutants

Polluant	Enzyme	Transduction	Detection limit (μM)	References
Trophic pollutants				
Nitrates	Nitrate reductase	Amperometry		
		Pt electrode/Ppy	10	[41]
		Conductometry		
		Au electrode/GA/Nafion	5	[169]
Nitrites	Nitrite reductase	Amperometry		
		GC electrode/[ZrCr-AQS]-LDH/GA	0.004	[18]
		GC electrode/modified MV	0.06	[125]
		Conductometric		
		Au electrode/Nafion	0.05	[181]
Phosphates	Maltose phosphorylase	Conductometric		
		Au electrode/GA	1	[181]
	Pyruvate oxidase	Amperometry		
		Pt electrode/Nafion/PCS hydrogel	3.6	[84]
		SPC electrode/acetate cellulose	<300	[46]
Organic Matter (proteic fraction)	Protéinase K + pronase	Conductometric	0.583 μg/L for TOC	[79]
Phenolic compounds				
17β-estradiol	Tyrosinase	Amperometry		
		BDD electrode	10	[115]
Bisphenol A	Tyrosinase	Amperometry		
		BDD electrode	1	[115]
		CP electrode/SWCNT	0.02	[110]
		Carbon fiber paper/PVA	0.005	[172]
2,4-dichlorophenol	Tyrosinase	Amperometry		
		GC electrode/MWNT+chitosane	0.06	[80]

(continued)

Table 7.1 (continued)

Polluant	Enzyme	Transduction	Detection limit (μM)	References
Catechol	Tyrosinase	Amperometry		
		GC electrode/MWNT/TiO_2/Nafion	0.087	[82]
		MWNT/epoxy resin	10	[119]
		GC electrode/MWNT/TiO_2/Nafion	0.09	[78]
		GC/Teflon/Au nanoparticles	0.003	[17]
		GC/polyacrylamide microgel	0.3	[60]
		Pt electrode/EDP	0.01	[171]
		GC electrode/TiO_2	0.09	[78]
		CP electrode/CoPc	0.25	[29]
		GrC-acethylcellulose/CoPc	0.45	[29]
		Carbon fiber paper/PVA	0.002	[172]
		SPCelectrode/PVA	20	[172]
		Optical		
		Glass/SiO_2/Nafion	2.1	[1]
Phenol	Tyrosinase	Amperometry		
		GC electrode/MWNT/TiO_2/Nafion	0.095	[88]
		Functionalized Au electrode	0.1	[160]
		GC electrode/palygorskite	0.05	[19]
		Functionalized BDD electrode	0.2	[182]
		GC/polyacrylamide microgel	1.4	[60]
		Pt/EDP	0.1	[171]
		GC electrode/TiO_2	0.13	[78]
		BDD electrode/ZnO nanorods	0.5	[177]
		Carbon fiber paper/PVA	0.005	[172]
		SPCE/PVA	20	[172]
		Optical		
		Glass microarray/PEG-DA/QD	1	[70]
		Glass/SiO_2/Nafion	1.9	[1]

(continued)

Table 7.1 (continued)

Polluant	Enzyme	Transduction	Detection limit (μM)	References
4-chlorophenol	Tyrosinase	Amperometric		
		GC electrode/MWNT/TiO_2/Nafion	0.11	[88]
		GCelectrode+Teflon+Au nanoparticles	0.02	[17]
		Functionalized BDD electrode	0.1	[182]
		GC/polyacrylamide microgel	0.03	[60
		GC electrode/TiO_2	0.17	[78]
		BDD electrode/ZnO nanorods	0.4	[83]
Organophosphorous pesticides				
Dichlorvos	Tyrosinase (inhibition)	Amperometric		
		GC electrode/NQ/Nafion	0.17	[78]
		GC electrode/NQ/o-PPD	0.19	
		GC electrode/Nafion	0.07	
		SiliconNanowire	0.04	[177]
Methyl parathion	OPH	Amperometric		
		GC electrode/MWCNT/Au/QD	0.004	[155]
		GC electrode/MWCNT	0.8	[145]
Demeton-S	OPH	Amperometric		
		CSP electrode/MWCNT	1	[33]
Paraoxon		Amperometric		
		GC electrode/MWCNT	0.15	[31]
		GC electrode/mesoporous C/C black	0.12	[89]
		Piezoelectric		
		Microcantilever/LbL	0.1	[73]
		SPR		
		Au/SiO_2	20	[102]
		OWLS		
		Glass/TiO_2 array	2.5	[127]
		PMMA/sol gel	0.004	[183]

AQS anthraquinone sulfonate, *BDD* boron doped diamond, *CoPc* cobalt phthalocyanine, *CP* carbon paste, *CSP* carbon screen-printed, *EDP* electrodeposition polymer, *GA* glutaraldehyde, *GC* glassy carbon, *GrC* graphite carbon, *Ppy* polypyrroll, *LbL* layer by layer, *LDH* Layered double hydroxide, *MV* methylviologen, *MWCNT* multi-walled carbon nanotubes, *NQ* naphthoquinone, *PCS* poly(carbamoyl) sulfonate, *PVA* polyvinylalcohol, *QD* quantum dot, *SPC* screen-printed carbon, *SWCNT* single-walled carbon nanotubes

directly. In the second case, the method is based on the competition between immobilized Ag, the analyte (Ag), and a fixed amount of Ab. All types of immunosensors can either be run as nonlabeled or labeled immunosensors. Label free immunosensors rely on the direct detection of antigen—antibody complex formation by measuring variations in electrical properties using electrochemical impedance spectroscopy (EIS), or changes in optical properties using SPR. The second type of immunosensors use signal-generating labels which allow more sensitive and versatile detection modes. Peroxidase, glucose oxidase, alkaline phosphatase, catalase enzymes, and electroactive compounds such as ferrocene are the most common labels used for electrochemical detection, while fluorescent labels (rhodamine, fluorescein, Cy5, etc.) are employed for optical detection. Some recent examples are presented in Table 7.2. Over the two past decades, a large number of immunosensors targeting individual pollutants or groups of pollutants and based on these different configurations have been reported. Recent developments have been focused on label free immunosensors using EIS and SPR detection [111, 124] as well as on improvements in antibody design [25]. Direct immunosensors based on quartz crystal microbalance (QCM), surface plasmon resonance (SPR), and impedimetric devices have been reported to detect direct binding of the analyte with the antibody [100].

7.4.1.3 Cell-Based Biosensors

These biosensors examine the effects of an analyte on an intact microorganism. Many works have been focused on the development of cell-based biosensors. Bacteria, algae, and yeasts are the main organisms used for that purpose. Various types of strains have been exploited, from commercial and well-characterized cells harboring a broad range of substrates to genetically engineered organisms specially constructed to detect specific molecules or groups of molecules, passing through environmental cells isolated from polluted sites offering higher robustness and more specific enzymatic properties. In some cases cell membranes can be permeabilized in order to improve accessibility to internal enzyme, in other cases reactions occur on cell surface and permeabilization is not necessary. It has been shown that for *Chlorella vulgaris* microalgae, some alkaline phosphatases [35] and esterases [36] belong to the cell wall, their activities can then be monitored rapidly.

Compared to their individual components (enzymes, antibodies, or DNA), cells are easier to produce in large quantities and are more tolerant to pH, ionic strength, and temperature variations. Owing to the large number of enzymes and cofactors that the cells contain, a large variety of biosensors has been proposed for the detection of specific (groups of) analytes. Several reviews have been published on the topics [91], some of them being more specifically dedicated to yeast-based sensors [9], genetically modified bacteria sensors [28, 47, 59, 154] or electrochemical cell biosensor [85].

Table 7.2 Examples of immunologic biosensors for the detection of chemical pollutants

Pollutant	Detection mode	Transduction	Detection limit (ng L^{-1})	Reference
Pesticides				
Isoproturon	Competitive/Cy5.5 fluorescence labeling	TIRF	20	[150]
Chlorpyrifos	Competitive/no labeling	SPR	55	[108]
DDT and derivated products	Competitive/no labeling	SPR	15	[109]
Atrazine	Direct	EIS	10	[62]
	Competitive/Cy5.5 fluorescence labeling	TIRF	10	[150]
	Competitive	SPR	500	[43]
Picloram	Competitive HRP labeling	Amperometric	<1	[20]
EDCs				
Testosterone	Competitive/labeling	TIRF	1.7	[151]
	Competitive/no labeling	Amperometric	170	[37]
Estradiol	Competitive	SPR	300	
Bisphenol A	Direct	EIS	400	[126]
	Competitive/labeling	TIRF	8	[106] [150]
Nonylphenols	Direct/HRP labeling	Amperometric	10,000	[40]
2,4-dichlorophenoxyacetic acid	Competitive/Cy5.5 fluorescence labeling	TIRF	90	[101]
	Competitive/no labeling	SPR	100	[77]
	Competitive (signal amplification)	SPR	8	[77]
Toxins				
Microcystin-LR	Competitive/Cy5.5 fluorescence labeling	TIRF	30	[101]

EDC endocrine disrupting compound

Electrochemical Biosensors

Amperometry is the most common electrochemical transduction mode used in whole cell biosensors. It allows detecting oxygen consumption or production during respiration/photosynthesis processes, consumption or production of specific compounds in course of analyte metabolization, or induction of a specific enzyme activity by genetically modified microorganisms [85]. Different microbial strains exhibiting a wide range of substrates have been used for the determination of biological demand of oxygen (BOD), an index of the amount of degradable organic compounds present in the sample [113, 122]. Oxygen consumption during biological respiration is generally detected by means of conventional Clark type electrodes, but miniaturized systems based on small-size carbon screen-printed electrodes (SPEs) have been also proposed in recent years. In the same way, biosensors able to detect surfactants, phenolic derivatives, alcohols, or organophosphorous pesticides have been constructed by immobilizing bacteria degrading specifically these groups of pollutants on classical oxygen, graphite carbon, or carbon paste electrodes, more recently on SPEs [85].

A conductometric biosensor using immobilized *Chlorella vulgaris* microalgae as bioreceptors was used as a bi-enzymatic biosensor. Algae were immobilized inside bovine serum albumin membranes reticulated with glutaraldehyde vapors deposited on interdigitated conductometric electrodes. Local conductivity variations caused by algae alkaline phosphatase and acetylcholinesterase activities have been detected. The results have shown that this sensor is quite sensitive to heavy metals and organophosphorous (OP) pesticides. Alkaline phosphatase was inhibited by heavy metal while acetylcholinesterase was inhibited by OP pesticides [24].

Hnaien et al. [63] developed an original approach by designing a miniaturized bacterial conductometric biosensor for trichloroethylene (TCE) detection. *Pseudomonas putida* cells were immobilized at the surface of gold interdigitated microelectrodes through a three dimensional alkanethiol self-assembly monolayer carbon nanotube architecture functionalised with pseudomonas antibodies; This biosensor was successfully applied to the determination of TCE in spiked ground water samples collected in an urban industrial site contaminated with TCE. It was the first time that the resulting combination of SAM and CNT was conjugated to bacteria for the detection of a specific analyte.

Table 7.3 presents the most recent examples of electrochemical biosensors developed for BOD measurement and for the determination of specific (groups of) analytes. To modify cell resistance and sensitivity toward toxic compounds, microorganisms may be genetically modified. Buonasera et al. recently combined on a single biosensing platform amperometric and optical modes of transduction as well as several genetically modified algal strains harboring various degrees of sensitivity and resistance toward pesticides. The system allowed detecting different subclasses of pesticides in the 0.1–10 nM range [13]. To enhance selectivity, genetical modification of the cells is also possible by fusing a natural regulatory circuit existing in the microorganism with a promotorless gene encoding for an easily measurable protein expressed only when the analyte(s) is present. The most common gene used for electrochemical detection is *lacZ* encoding β-galactosidase Activation of

Table 7.3 Some recent examples of cell based biosensors for the detection of specific (groups of) pollutants

Target	Microorganism	Transduction	Detection limit	Reference
BOD	*Saccharomyces cerevisiae*	Amperometry	6.6 mg L^{-1}	[113]
	Microbial consortium (BODSEED)	Amperometry	< 5 mg L^{-1}	[98]
	Escherichia coli DH5α	Potentiometry	1 mg L^{-1}	[21]
	Photobacterium phosphoreum IFO 13896	Luminescence	1 mg L^{-1}	[133]
	B. licheniformis, D. maris, M. marinus	Optical fiber	0.2 mg L^{-1}	[97]
Phenolic compounds				
Phenol	*Pseudomonas putida DSM 50026*	Amperometry	500 μM	[149]
p-nitrophenol	*Pseudomonas* sp.	Amperometry	< 10 μM	[7]
Organophosphorous pesticides				
Paraoxon, parathion	Modified *P. putida JS444*	Amperometry	0.001 μM	[90]
Fenithron, EPN			0.005 μM	[92]
Heavy metals				
Cu	*S. cerevisiae* 19.3C/YEp352 CUP*:lacZ*	Amperometry	0.1 μM	[146]
	S. cerevisiae SEY6210/YEp352 CUP*:lacZ*		33 μM	
Endocrine disrupting compounds	*S. cerevisiae* Y190 medER ::lacZ	Amperometry	–	[68]
Antibiotics				
Cephalosporins	*P. aeruginosa* MTCC 647	Potentiometry	100 μM	[82]
Organic solvents				
Benzene	*P.putida* ML2	Amperometry	10 μM	[86]
Trichlorethylene	*P. aeruginosa* JI104	Potentiometry	0.22 μM	[57]
	P. putida F1	Conductometry	0.07 μM	[63]

β-galactosidase is generally followed through the increase of its enzymatic activity using *p*-aminophenyl β-D-galactopyranoside (PAPG) as substrate. PAPG is transformed into *p*-aminophenol oxidized at the amperometric electrode. Tag et al. [146] proposed another method of detection using lactose as deputy substrate.

Potentiometric and conductometric biosensors have been also developed for the determination of specific pollutants. For example, a biosensor based on *P. aeruginosa* JI104 immobilized on a chloride ions-selective solid-state electrode has been reported for trichloroethylene detection in waters. More recently, [63] proposed a fast, sensitive, and miniaturized whole cell conductometric biosensor for the determination of the same pollutant. The biosensor assembly was prepared by immobilizing *P. putida* F1 bacteria at the surface of gold microelectrodes through a three dimensional alkanethiol self-assembly monolayer/carbon nanotubes architecture functionalised with *Pseudomonas* antibodies. pH electrodes have been also widely used to detect H^+ ions produced through enzymatic reactions [82].

Optical Biosensors

The majority of recent whole cell optical fiber based biosensing publications target toxic materials as analytes [112]. Optical biosensors rely on the modulation of cell optical properties (UV–Visible absorption and biochemiluminescence, reflectance, fluorescence) following interaction with compounds present in the sample. Most of the optical biosensors proposed are based on bioluminescence or fluorescence detection. The so-called "light-off" systems measure a decrease in the cellular light emission following exposure to the pollutant(s). They are mainly used for water toxicity assessment. The detection of specific analytes or groups of analytes is rather performed using "light-on" type biosensors, where the interaction causes an increase of light signal proportional to the analyte concentration. "Light-on" microorganisms are produced naturally or via genetic engineering. The most frequently used genes are *lux* gene encoding for luminescent luciferase and *gfp* gene encoding for fluorescent GFP (green fluorescent protein). A variety of well-characterized promoters are available for genetic manipulations and has been used to construct new organisms able to sense specifically different classes of pollutants, including metals (copper, mercury, lead, cadmium, arsenic), hydrocarbons, and organic solvents or pesticides. Many examples have been reported in the literature [28, 91]. Naturally emitting bacteria have been also used for BOD determination [97, 133]. Gu et al. [51] developed a multi-channel whole cell optical fiber-based toxicity monitoring system for continuous analysis of aqueous samples. Their system employed four parallel two-stage bioreactors with four different bioluminescent strains of *E. coli* to assist in toxin classification. [155] developed a whole cell optical fiber-based biosensor for quantifying aqueous herbicides using *Chlorella vulgaris* microalgae, a sensor cell, and a bifurcated optical fiber probe for light transmission. Algae were immobilized on quartz microfiber filters, which were placed in a five filter cassette for analysis via the fiber optic probe. Chlorophyll fluorescence was monitored in response to the addition of the herbicides.

The chlorophyll exhibited an increase in fluorescence response and reversibility for the pesticides tested. However, this sensor provided an adequate measure of overall toxicity, it does not allow differentiating various herbicides from one another. A recent study highlights the action of two herbicides commonly found in the environment (diuron and glyphosate) on two specific metabolic activities of unicellular algae (esterase activity and chlorophyll fluorescence) [36].

7.4.2 Application of Biosensors to the Assessment of Aquatic Toxicity

Most of biosensors developed for toxicity assessment exploit toxic effects of the pollutants, including enzyme inhibition, such as acethylcholinesterase inhibition by neurotoxic compounds, interaction with a specific receptor (androgenicity, estrogenicity), interaction with and damage of DNA or RNA (genotoxicity). The detection of some biomarkers of toxicity may be also used.

7.4.2.1 Enzyme Biosensors

A major contribution of enzyme biosensors to ecotoxicological studies concerns aquatic neurotoxicity assessment. This latter may be due to organophosphate and carbamate pesticides, heavy metals, or detergents that inhibit esterase enzymes. Neurotoxicity biosensors proposed in the literature are mostly based on two enzymes belonging to this family, acethylcholinesterase (AChE) and butylcholinesterase. Many works and two reviews have been published on this type of biosensor [69, 100]. Current developments aim to improve enzymatic activity, either by genetic modification [12] or by a better immobilization on the transducer. The use of new materials based on gold, silver, or iron nanoparticles [33, 45, 49, 140, 177] or on carbon nanotubes [157] also allows significant increase of the sensitivity of electrochemical and optical biosensors. A portable system using a potentiometric transduction and AChE as bioreceptor has been recently validated on different samples of water [61]. Cortina et al. [26] proposed an enzyme-based array that used three AChE enzymes: the wild type and two different genetically modified enzymes. Multianalyte devices combining informations from several different types of enzymes have been also reported. For example, Soldatkin et al. recently developed an amperometric multibiosensor using the inhibition of AChE, butyryl- cholinesterase, urease, glucose oxidase, and three-enzyme system (invertase, mutarotase, glucose oxidase) for water toxicity assessment [142].

7.4.2.2 Estrogen Receptor-Based Biosensors

Endocrine disruptors (EDCs) are chemical substances that cause hormonal imbalances and impair endocrine or nervous systems. Some of these compounds

affect the synthesis of endogenous hormones or that of their receptors. Others are structurally similar to estrogens and bind to their receptors, leading to their inactivation or to abnormal behaviours. Many molecules, such as synthetic hormones or chemical substances such as phthalates, surfactants, PCBs, alkylphenols, parabens, PAHs, dioxins, and some pesticides, are EDCs. To date, 320 priority substances suspected to disrupt endocrine system have been identified by the European Community. Some of them (nonylphenol, di-2-ethylhexylphthalate, and polybrominated diphenyl ethers) have been included in the list of priority substances of the Water Framework Directive. A review has addressed the use of biosensors for environmental EDCs monitoring [129].

Toxicity biosensors rely on EDCs binding on estrogen receptors immobilized on the surface of a transducer. The estrogen receptor of human origin (ER-α) is the most often used. Different transduction modes such as fluorescence, cyclic voltammetry, SPR, electrophoretic mobility, have been proposed. Portable systems, mainly based on SPR detection have also been developed [55]. Recently, a biosensor containing carbon nanotubes functionalised with the α-type human estrogen receptor and using a FET as transducer has been reported. The response time was extremely rapid (2 min) [134]. Another biosensor, using impedance as transduction mode, was fabricated by immobilizing ER-α in a supported bilayer lipid membrane modified with Au nanoparticles (Xia et al. 2010). The results indicated that the biosensor was able to detect the natural estrogen 17β-estradiol with an acceptable linear correlation ranging from 5 to 150 ng/L and a detection limit of 1 ng/L. The biosensor could also detect bisphenol A and 4-nonylphenol. Im et al. (Im et al. 2010) propose to bind ER-α receptor covalently on a gold electrode for impedimetric detection of 17β-estradiol. The detection limit was 1 μM.

7.4.2.3 Immunosensors

As seen in Sect. 2.2.2 a large number of applications of immunosensors relate to the determination of pollutants or groups of target pollutants. It is also possible to exploit them for the detection of substances, called biomarkers that are produced by an organism following exposure to specific pollutants. Vitellogenin, for example, is a phospholipo-serum glycoprotein secreted in large quantities by fish exposed to EDCs. Its presence is suitable for identifying estrogenotoxic effects of natural or anthropogenic substances. Vitellogenin may be detected using electrochemical, optical, or piezoelectric biosensors based on carp anti-vitellogenin antibody [13].

7.4.2.4 DNA Biosensors

DNA structure is extremely sensitive to the influence of environmental pollutants such as heavy metals, polycyclic aromatic compounds, and PCBs. These substances possess high affinity for DNA, at the origin of mutagenic and carcinogenic effects. Biosensors, measuring the interactions between these substances and single or

double strand DNA molecules immobilized on a transducer, have been developed and used for water genotoxicity assessment. Electrochemical transduction is the most commonly used [116]. The compounds bound to DNA are detected, either directly when electroactive species are involved, or through the modification of DNA electrochemical signal. Toxicity biosensors based on the detection of DNA bases oxidation (mainly guanine, but also guanosine and adenosine) or on the degradation of the strands using an electrochemical probe, have been developed and applied to the analysis of water samples containing different types of genotoxic aquatic contaminants (metals, pesticides, PCBs, aromatic amines). Some of these biosensors were favorably compared to commercial genotoxic assays.

Other types of biosensors using either optical (SPR, fluorescence) or mechanical transduction have been also proposed [117].

7.4.2.5 Biosensors Based on Whole Cells

Bacteria, yeasts, algae, and fish cells have been also used for the development of toxicity biosensors [9, 28, 47, 59, 85, 91, 154, 166]. The biosensor response may be due to a change in cell metabolism (inhibition of enzyme activity, respiration, or photosynthesis), cell alteration, death, or change in the expression of certain genes (modified organisms). Many examples of electrochemical and optical biosensors proposed for toxicity assessment may be found in the different reviews cited above. The most recent ones are given in Table 7.4. Optical biosensors are mainly based on luminescent modified bacteria, using mainly the *recA, uvrA, NrdA* promoters for DNA damage detection, the *grpE* and *dnaKp* promoters for protein damage detection, and the *fab A* promoter for cell membrane damage [166].

Microbes have a number of advantages as biological sensing materials in the biosensor design: they are present ubiquitously and are able to metabolize a wide range of chemical compounds. Microorganisms have a great capacity to adapt to adverse conditions and to develop the ability to degrade new molecules with time. Compared to biosensors using purified enzyme, whole-cell biosensors are more resistant to the activity loss because their enzymes and cofactors are hosted in an environment optimized by nature. Therefore, these biosensors are more suitable to meet all the requirements for environmental surveillance [35, 155] they can identify in situ the presence of a toxic compound as soon as it is released in aquatic environment.

Numerous whole cell biosensors were constructed from genetically modified cells [21]. However, those techniques may improve the biosensor sensitivity and selectivity but are no longer able to reflect the ecosystem operating conditions.

Table 7.4 Some recent examples of toxicity cell-based biosensors

Toxicity mechanism	Pollutants	Microorganisms	Transduction	Reference
Inhibition of AChE activity	Cd, Zn	*C. vulgaris*	Conductometry	[24]
Inhibition of AP activity	OPs	*C. vulgaris*	Conductometry	[24]
	Cd	*C. vulgaris*	Conductometry	[22, 53]
	Cd, Zn	*C. vulgaris*	Amperometry	
Inhibition of respiratory activity	Antibiotics	*E.coli* JM 105	Amperometry	[105]
	Hg, Cu, Zn, Ni, phenolic compounds	*E.coli*	Amperometry	[161]
	KCN, As_2O_3, Hg^{2+}	*E.coli* DHα	Amperometry	[100]
Inhibition of photosynthetic activity	Atrazine, DCMU	*C. vulgaris*	Amperometry	[139]
	Formaldehyde	*C. vulgaris* /*P. subcapitata/C.reinhardtii*	Amperometry	[147]
Inhibition of luminol peroxidase activity	Pb^{2+}, Hg^{2+}, Cu^{2+}	*Vibrio fischeri*	Luminescence	[79]
Genotoxicity	Nalidixic acid, mitomycin C, H_2O_2	*E.coli nrdA :: luxCDABE*	Luminescence (induction)	[65]
	Mitomycin C, nalidixic acid, MNNG, 4-NQQ	*E. coli* DPD2794 (recA ::*lux*), BBT (nrdA::*lux*, dinI::*lux,* sbmC ::*lux*, recN::*lux*), EB (sulA::*lux,* alk::*lux*)	Luminescence (induction)	[2] Ben-Yoav et al.
	Nalidixic acid			
	IQ	*E. coli* RFM443/pBR2TTS *sulA ::phoA*	Amperometry (induction of AP)	[10]
	Mitomycin C, ethidium bromide,H_2O_2, toluene, pyrene, benzo[a]pyrene, MMS	*S. typhimurium* TA1535 pSK1002		[143]
		Acinetobacter baylyi ADP1 *recA::luxCDABE*	Luminescence (induction)	[38] [81]
	Mitomycin, H_2O_2	*E. coli* MG1655 pColD:*luxCDABE* and pRecA:*luxCDABE*	Luminescence (induction)	
	Mitomicin C, pentachlorophenol, H_2O_2	DPD2794 recA ::luxCDABE	Luminescence (induction)	
Protein damage	Phenol	*E. coli DnakA:lacZ*	Amperometry (induction of β–galactosidase)	[123]
		E. coli grpE:lacZ		

(continued)

Table 7.4 (continued)

Toxicity mechanism	Pollutants	Microorganisms	Transduction	Reference
Membrane damage	Phenol	*E. coli fabA:lacZ*	Amperometry (induction of β–galactosidase)	[123]
Heat shock	Mitomicin C, pentachlorophenol, H_2O_2	*E. coli* grpE :luxCDABE *E. coli* pIbpA:luxCDABE	Luminescence (induction)	[81]
Oxidative stress	Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}	*E. coli* DH5α	Fluorescence (induction)	[4]
	H_2O_2, menadione, selenite, arsenite, triphenyltin naphthalene	*pRSET::roGFP2* E.coli *pKatG ::lux*CDABE and *pSoxS::lux*CDABE	Luminescence (induction)	[81] [88]
	Mitomycin C, pentachlorophenol, H_2O_2	Various strains and promoters		
	Paraquats and derivatives, H_2O_2		Luminescence (induction)	

AChE acetylcholinesterase, *AP* alkaline phosphatase; *DCMU* 3-(3,4-dichlorophenyl)-1,1-diethylurea, *IQ* 2-amino-3-methylimidazo[4,5-f]quinoline, *MNNG* 1-methyl-1-nitroso-N-methylguanidine, *MMS* Methyl methanesulfonate, *4-NQQ* 4-nitroquinoline N-oxide

7.5 Conclusion

Despite the large number of works carried out on the field of biosensors for water analysis, and although they have many benefits, very few systems have so far been marketed, unlike bioassays. Most commercial biosensors are versatile and suitable for applications in various fields such as environment, biological analysis or medical [130].

Significant efforts still have to be done to obtain selective, robust, rapid and sensitive tools usable in the field. The limitations of the proposed systems come for one part to the biological elements. Current developments include enhancement of their sensitivity, selectivity, and their stability by genetic engineering [16, 25, 47]. Recent progress in this area allows glimpsing the possibility of constructing arrays of microorganisms or of enzymes arranged on a single detection platform for simultaneous multi-pollutants. In parallel, the development of new biomimetic receptors such that molecularly imprinted polymers (MIP) or aptamers (synthetic oligonucleotides) is expanding to overcome the fragility of natural bioreceptors [52, 163]. Efficient methods for bioreceptors immobilization will also have to be developed to improve biosensor robustness and sensitivity. The exploration of new materials, including gold nanoparticles, carbon nanotubes, or quantum dots is an extremely promising route to achieve this goal.

Essential progress has also been made in recent years in the miniaturization of transducers (nanoelectrodes, nanowaveguides, BioMEMS) and will contribute to reduce significantly the amount of biological element required, but also to improve the integration of the systems into lab-on-chip laboratories [96, 164]. Nanotechnolgy can be expected to be an effective approach to develop more sophisticated multianalyte detection systems with low cost. Now, progress in data numerisation and transmission as well as processing allow the realization of microarrays for multipollutant detection.

In summary, nanobiosensors have shown great promise in the field of environmental monitoring. We can expect that commercial nanotechnology products will be utilized as early warning system for detection of pollutants in field.

References

1. Abdullah J, Musa A, Lee YH, Nadarajah K, Hamidah S (2006) Stacked films immobilization of MBTH in nafion/sol-gel silicate and horseradish peroxidase in chitosan for the determination of phenolic compounds. Anal Bioanal Chem 386:1285–1292
2. Ahn JM, Hwang ET, Youn CH, Banub DL, Kim BC, Niazi JH, Gu MB (2009) Prediction and classification of the modes of genotoxic actions using bacterial biosensors specific for DNA damages. Biosens Bioelectron 25(4):767–772
3. Allan IJ, Vrana B, Greenwood R, Mills GA, Roig B, Gonzalez C (2006) A “toolbox” for biological and chemical monitoring requirements for the European Union’s Water Framework Directive. Talanta 69(2):302–322
4. Arias-Barreiro CR, Okazaki K, Koutsaftis A, Inayat-Hussain SH, Tani A, Katsuhara M, Kimbara K, Mor IC (2010) A bacterial biosensor for oxidative stress using the constitutively expressed redox-sensitive protein roGFP2. Sensors 10(7):6290–6306

5. Badihi-Mossberg M, Buchner V, Rishpon J (2007) Electrochemical biosensors for pollutants in the environment. Electroanalysis 19(19–20):2015–2028
6. Bakker E, Pretsch E (2008) Nanoscale potentiometry. Trends Anal Chem 27(7):612–618
7. Banik RM, Mayank Prakash R, Upadhyay SN (2008) Microbial biosensor based on whole cell of Pseudomonas sp. for online measurement of p-nitrophenol. Sens Actuators B 131(1):295–300
8. Barcelo D, Hansen PD (eds) (2009) Handbook of environmental chemistry, vol 5J. Biosensors for environmental monitoring of aquatic systems, Springer, Berlin
9. Baronian KHR (2004) The use of yeast and moulds as sensing elements in biosensors. Biosens Bioelectron 19(9):953–962
10. Ben-Yoav H, Biran A, Pedahzur R, Belkin S, Buchinger S, Reifferscheid G, Shacham-Diamand Y (2009) A whole cell electrochemical biosensor for water genotoxicity bio-detection. Electrochim Acta 54:6113–6118
11. Bratov A, Abramova N, Ipatov A (2010) Recent trends in potentiometric sensor arrays-a review. Anal Chim Acta 678(2):149–159
12. Bucur B, Fournier D, Danet A, Marty JL (2006) Biosensors based on highly sensitive acetylcholinesterases for enhanced carbamate insecticides detection. Anal Chim Acta 562(1):115–121
13. Bulukin E, Meucci V, Minunni M, Pretti C, Intorre L, Soldani G, Mascini M (2007) An optical immunosensor for rapid vitellogenin detection in plasma from carp (Cyprinus carpio). Talanta 72(2):785–790
14. Bunimovich YL, Shin YS, Yeo WS, Amori M, Kwong G, Heath JR (2006) Quantitative real-time measurements of DNA hybridization with alkylated nonoxidized silicon nanowires in electrolyte solution. J Am Chem Soc 128:16323–16331
15. Buonasera K, Pezzotti G, Scognamigli V, Tibuzzi A, Giardi MT (2010) New platform of biosensors for prescreening of pesticide residues to support laboratory analyses. J Agric Food Chem 58(10):5982–5990
16. Campas M, Prieto-Simon B, Marty JL (2009) A review of the use of genetically engineered enzymes in electrochemical biosensors. Semin Cell Dev Biol 20(1):3–9
17. Carralero V, Melena ML, Gonzalez-Cortes A, Yanez-Sedeno P, Pingarron JM (2006) Development of a high analytical performance-tyrosinase biosensor based on a composite graphite–Teflon electrode modified with gold nanoparticles. Biosens Bioelectron 22(5):730–736
18. Chen H, Mousty C, Cosnier S, Silveira CM, Moura JJG, Almeida MG (2007) Highly sensitive nitrite biosensor based on the electrical wiring of nitrite reductase by [ZnCr-AQS] LDH. Electrochem Commun 9(9):2240–2245
19. Chen J, Jin Y (2010) Sensitive phenol determination based on co-modifying tyrosinase and palygorskite on glassy carbon electrode. Microchim Acta 169(3–4):249–254
20. Chen L, Zeng G, Zhang Y, Tang L, Huang D, Liu C, Pang Y, Luo J (2010) Trace detection of picloram using an electrochemical immunosensor based on three-dimensional gold nanoclusters. Anal Biochem 407(2):172–179
21. Chiappini SA, Kormes DJ, Bonetto MC, Sacco N, Cortona E (2010) A new microbial biosensor for organic water pollution based on measurement of carbon dioxide production. Sens Actuators B 148(1):103–109
22. Chong KF, Loh KP, Ang K, Ting YP (2008) Whole cell environmental biosensor on diamond. Analyst 133(6):739–743
23. Chouteau C, Dzyadevych S, Chovelon JM, Durrieu C (2004) Development of novel conductometric biosensors based on immobilized whole cell Chlorella vulgaris microalgae. Biosens Bioelectron 19:1089–1096
24. Chouteau C, Dzyadevych S, Durrieu C, Chovelon JM (2005) A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples. Biosens Bioelectron 21:273–281
25. Conroy PJ, Hearty S, Leonard P, O'Kennedy RJ (2009) Antibody production, design and use for biosensor-based applications. Semin Cell Dev Biol 20(1):10–26

26. Cortina M, Del Valle M, Marty JL (2008) Electronic tongue using an enzyme inhibition biosensor array for the resolution of pesticide mixtures. Electroanalysis 20:54–60
27. Crespilho FN, Ghica ME, Florescuc M, Nart FC, Oliveira ON, Brett CMA (2006) A strategy for enzyme immobilization on layer-by-layer dendrimer gold nanoparticle electrocatalytic membrane incorporating redox mediator. Electrochem Commun 8:1665–1670
28. Daunert S, Barrett G, Feliciano JS, Shetty RS, Shrestha S, Smith-Spencer W (2000) Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes. Chem Rev 100(7):2705–2738
29. De Albuquerque YDT, Ferreira LF (2007) Amperometric biosensing of carbamate and organophosphate pesticides utilizing screen-printed tyrosinase-modified electrodes. Anal Chim Acta 596:210–221
30. Dekker C (2007) Solid-state nanopores. Nat Nanotechnol 2:209–215
31. Deo RP, Wang J, Block I, Mulchandani A, Joshi KA, Trojanowicz M, Scholz F, Chen W, Lin Y (2005) Determination of organophosphate pesticides at a carbon nanotube/organophosphorus hydrolase electrochemical biosensor. Anal Chim Acta 530(2):185–189
32. D'Souza SF (2001) Microbial biosensors. Biosens Bioelectron 16:337–353
33. Du D, Chen W, Zhang W, Liu D, Li H, Lin Y (2010) Covalent coupling of organophosphorus hydrolase loaded quantum dots to carbon nanotube/Au nanocomposite for enhanced detection of methyl parathion. Biosens Bioelectron 25(6):1370–1375
34. Dunn B, Miller JM, Dave BC, Valentine JS, Zink JI (1998) Strategies for encapsulating biomolecules in sol-gel Acta materiala 46(3):737–741
35. Durrieu C, Tran-Minh C (2002) Optical algal biosensor using alkaline phosphatase for determination of heavy metals. Ecotoxicol Environ Saf 51:206–209
36. Durrieu C, Chouteau C, Barthet L, Chovelon JM, Tran-Minh C (2004) A bi-enzymatic whole-cell algal biosensor for monitoring waste water pollutants. Anal Lett 37(8):1589–1599
37. Eguilaz M, Moreno-Guzman M, Campuzano S, Gonzalez-Cortes A, Yanez-Sedeno P, Pingarron JM (2010) An electrochemical immunosensor for testosterone using functionalized magnetic beads and screen-printed carbon electrodes. Biosens Bioelectron 26(2):517–522
38. Eltzov E, Marks RS, Voost S, Wullings BA, Heringa MB (2009) Flow-through real time bacterial biosensor for toxic compounds in water. Sens Actuators B 142(1):11–18
39. Evans SD, Johnson SR, Cheng YL, Shen T (2000) Vapour sensing using hybrid organic-inorganic nanostructured materials. J Mater Chem 10:83–188
40. Evtugyn GA, Eremin SA, Shaljamova RP, Ismagilova AR, Bidnikov HC (2006) Amperometric immunosensor for nonylphenol determination based on peroxidase indicating reaction. Biosens Bioelectron 22(1):56–62
41. Fan X, White IM, Shopova SI, Zhu H, Suter JD, Sun Y (2008) Sensitive optical biosensors for unlabeled targets: a review. Anal Chim Acta 620(1–2):8–26
42. Farré M, Brix R, Barcelo D (2005) Screening water for pollutants using biological techniques under European Union funding during the last 10 years. Trends Anal Chem 24(6):532–545
43. Farré M, Martínez E, Ramon J, Navarro A, Radjenovic J, Mauriz E, Lechuga L, Marco MP, Barcelo D (2007) Part per trillion determination of atrazine in natural water samples by a surface plasmon resonance immunosensor. Anal Bioanal Chem 388(1):207–214
44. Farré M, Barcelo D (2009) Biosensors for aquatic toxicology evaluation. Hdb Environ Chem 5J:115–160
45. Gan N, Yang X, Xie D, Wu Y, Wen W (2010) A disposable organophosphorus pesticides enzyme biosensor based on magnetic composite nano-particles modified screen printed carbon electrode. Sensors 10(1):625–638
46. Gilbert L, Browning S, Jenkins ATA, Hart JP (2010) Studies towards an amperometric phosphate ion biosensor for urine and water analysis. Microchim Acta 170(3–4):331–336
47. Girotti S, Ferri EN, Fumo MG, Maiolini E (2008) Monitoring of environmental pollutants by bioluminescent bacteria. Anal Chim Acta 608(1):2–29

48. Goure JP, Blum L (2009) Biosensors and chemical sensors based upon guided optics. In: Fouletier J, Fabry P (eds) Chemical and biological microsensors: applications in fluid media. Wiley, New York
49. Gong J, Wang L, Zhang L (2009) Electrochemical biosensing of methyl parathion pesticide based on acetylcholinesterase immobilized onto Au–polypyrrole interlaced network-like nanocomposite. Biosens Bioelectron 24(7):2285–2288
50. Grieshaber D, MacKenzie R, Vörös J, Reimhult E (2008) Electrochemical biosensors—sensor principles and architectures. Sensors 8(3):1400–1458
51. Gu MB, Gil GC, Kim JH (1999) A two-stage minibioreactor system for continuous toxicity monitoring. Biosens Bioelectron 14(4):355–361
52. Guan G, Liu B, Wang Z, Zhang Z (2008) Imprinting of molecular recognition sites on nanostructures and its applications in chemosensors. Sensors 8(12):8291–8320
53. Guedri H, Durrieu C (2008) A self-assembled monolayers based conductometric algal whole cell biosensor for water monitoring. Micro Chim Acta 163(3–4):179–184
54. Gupta AK, Nair PR, Akin D, Ladisch MR, Broyles S, Alam MA, Bashir RR (2006) Anomalous resonance in a nanomechanical biosensor. Proc Natl Acad Sci USA 103: 13362–13367
55. Habauzit D, Chopineau J, Roig B (2007) SPR-based biosensors: a tool for biodetection of hormonal compounds. Anal Bioanal Chem 387(4):1215–1223
56. Haes AJ, Van Duyne RP (2002) A unified view of propagating and localized surface plasmon resonance biosensors. Anal Bioanal Chem 379(7–8):920–930
57. Han TS, Sasaki S, Yano K, Ikebukuro K, Atsushi K, Nagamune T, Karube I (2002) Flow injection microbial trichloroethylene sensor. Talanta 57(2):271–276
58. Hansen KM, Thundat TT (2005) Microcantilever biosensors. Methods 37:57–64
59. Hansen LH, Sorensen SJ (2001) The use of whole-cell biosensors to detect and quantify compounds or conditions affecting biological systems. Microb Ecol 42(4):483–494
60. Hervas Perez JP, Sanchez-Paniagua Lopez M, Lopez-Cabarcos E, Lopez-Ruiz B (2006) Amperometric tyrosinase biosensor based on polyacrylamide microgels. Biosens Bioelectron 22(3):429–439
61. Hildebrandt A, Jordi R, Bragos R, Marty JL, Tresanchez M, Lacorte S (2008) Development of a portable biosensor for screening neurotoxic agents in water samples. Talanta 75(5): 1208–1213
62. Hleli S, Martelet C, Abdelghani A, Bessueille F, Errachid A, Samitier J, Burais N, Jaffrezic-Renault N (2006) Atrazine analysis using an impedimetric immunosensor based on mixed biotinylated self-assembled monolayer. Sens Actuators B 113(2):711–717
63. Hnaien M, Lagarde F, Bausells J, Errachid A, Jaffrezic-Renault N (2011) A new bacterial biosensor for trichloroethylene detection based on a three dimensional carbon nanotubes bioarchitecture. Anal Bioanal Chem 400:1083–1092
64. Hoa XD, Kirk AG, Tabrizian M (2007) Towards integrated and sensitive surface plasmon resonance biosensors: a review of recent progress. Biosens Bioelectron 23(2):151–160
65. Hwang ET, Ahn JM, Kim BC, Gu MB (2008) Construction of a nrdA: luxCDABE fusion and its use in escherichia coli as a DNA damage biosensor. Sensors 8(2):1297–1307
66. Imm JE, Han JA, Kim BK, Han JH, Park TS, Hwang S, Cho SI, Lee WY, Kim YR (2010) Electrochemical detectionof estrogen hormone by immobilized estrogen receptor on Au electrode. Surface Coatings Technol 205(Suppl 1):S275–S278
67. Inama L, Dire S, Carturan G (1993) Entrapment of viable microorganisms by SIO_2 sol-gel layers on glass Surfacestrapping catalytic performance and immobilization durability of S*accharomyces cerevisiae*. J Biotechnol 30:197–210
68. Ino K, Kitagawa Y, Watanabe T, Shiku H, Koide M, Itayama T, Yasukawa T, Matsue T (2009) Detection of hormone active chemicals using genetically engineered yeast cells and microfluidic devices with interdigitated array electrodes. Electrophoresis 30(19):3406–3412
69. Jaffrezic-Renault N, Dzyadevych SV (2008) Conductometric microbiosensors for environmental monitoring. Sensors 8(4):2569–2588

70. Jang E, Son KJ, Kimb B, Koh WG (2010) Phenol biosensor based on hydrogel microarrays entrapping tyrosinase and quantum dots. Analyst 135(11):2871–2878
71. Joseph Y, Guse B, Yasuda A, Vossmeyer T (2004) Chemiresistor coatings from Pt- and Au-nanoparticule/nonanedithiol films: sensitivity to gases and solvent vapors. Sens Actuators B Chem 98:188–195
72. Joshi KA, Prouza, Kum MM, Wang J, Tang J, Haddon R, Chen W, Mulchandani A (2006) V-type nerve agent detection using a carbon nanotube-based amperometric enzyme electrode. Anal Chem 78(1):331–336
73. Karnati C, Du H, Ji H, Xu X, Lvov Y, Mulchandani A, Mulchandani P, Chen W (2007) Organophosphorus hydrolase multilayer modified microcantilevers for organophosphorus detection. Biosens Bioelectron 22(11):2636–2642
74. Katz E, Willner I (2003) Probing biomolecular interactions at conductive and semiconductive surfaces by impedance spectroscopy: routes to impedimetric immunosensors, DNA-sensors, and enzyme biosensors. Electroanalysis 15(11):913–947
75. Khadro B, Namour P, Bessueille F, Léonard D, Jaffrezic-Renault N (2009) Validation of a conductometric bienzyme biosensor for the detection of proteins as marker of organic matter in river samples. J Environ Sci 21(4):545–551
76. Khanna VK (2008) Nanoparticules-based sensors. Def Sci J 58(5):608–616
77. Kim SJ, Gobi KV, Iwasaka H, Tanaka H, Miura N (2007) Novel miniature SPR immunosensor equipped with all-in-one multi-microchannel sensor chip for detecting low-molecular-weight analytes. Biosens Bioelectron 23(5):701–707
78. Kochana J, Gala A, Parczewski A, Adamski J (2008) Titania sol-gel-derived tyrosinase-based amperometric biosensor for determination of phenolic compounds in water samples. Examination of interference effects. Anal Bioanal Chem 391(4):1275–1281
79. Komaitis E, Vasiliou E, Kremmydas G, Georgakopoulos DG, Georgiou C (2010) Development of a fully automated flow injection analyzer implementing bioluminescent biosensors for water toxicity assessment. Sensors 10(8):7089–7098
80. Kong L, Huang S, Yue Z, Peng B, Li M, Zhang J (2009) Sensitive mediator-free tyrosinase biosensor for the determination of 2,4-dichlorophenol. Microchim Acta 165(1–2):203–209
81. Kotova VY, Manukhov IV, Zavilgelskii GB (2010) Lux biosensors for detection of SOS response, heat shock, and oxidative stress. Appl Biochem Microb 46(8):781–788
82. Kumar S, Kundu S, Pakshirajan K, Dasu VV (2008) Cephalosporins determination with a novel microbial biosensor based on permeabilized pseudomonas aeruginosa whole cells. Appl Biochem Biotech 151(2–3):653–664
83. Kuswandi B, Fikriyah CI, Gani AA (2008) An optical fiber biosensor for chlorpyrifos using a single sol-gel film containing acetylcholinesteraseand bromothymol blue. Talanta 74:613–618
84. Kwan RCH, Leung HF, Hon PYT, Barford JP, Renneberg R (2005) A screen-printed biosensor using pyruvate oxidase for rapid determination of phosphate in synthetic wastewater. Appl Microb Biotech 66(4):377–383
85. Lagarde F, Jaffresic-Renault N (2011) Cell-based electrochemical biosensors for water quality assessment. Anal Bioanal Chem 400:947–964
86. Lanyon YH, Tothill IE, Mascini M (2006) An amperometric bacterial biosensor based on gold screen-printed electrodes for the detection of benzene. Anal Lett 39t(7–8):1669–1681
87. Lee CS, Kim SK, Kim M (2009) Ion-sensitive field-effect transistor for biological sensing. Sensors 9(9):7111–7131
88. Lee JH, Youn CH, Kim BC, Gu MB (2007) An oxidative stress-specific bacterial cell array chip for toxicity analysis. Biosens Bioelectron 22(9–10):2223–2229
89. Lee JH, Park JY, Min K, Cha HJ, Choi SS, Yoo YJ (2010) A novel organophosphorus hydrolase-based biosensor using mesoporous carbons and carbon black for the detection of organophosphate nerve agents. Biosens Bioelectron 25(7):1566–1570
90. Lei Y, Mulchandani P, Wang J, Chen W, Mulchandani A (2005) A highly sensitive and selective amperometric microbial biosensor for direct determination of p-nitrophenyl-substituted organophosphate nerve agents. Environ Sci Technol 39(22):8853–8857
91. Lei Y, Chen W, Mulchandani A (2006) Microbial biosensors. Anal Chim Acta 568:200–210

92. Lei Y, Mulchandani P, Chen W, Mulchandani A (2007) Biosensors for direct determination of fenitrothion using recombinant Pseudomonas putida IS444 with surface expressed organophosphorus hydrolase. 2. Modified carbon paste electrode. Appl Biochem Biotechnol 136:243–250
93. Li J, Gao H (2008) A renewable potentiometric immunosensor based on Fe_3O_4 nanoparticles immobilized anti-IgG. Electroanalysis 20(8):881–887
94. Li M, Tang HX, Roukes ML (2007) Ultra-sensitive NEMS-based cantilevers for sensing, scanned probe and very high-frequency applications. Nat Nanotechnol 2:114–120
95. Li X, Zhang G, Bai X, Sun X, Wang X, Wang E, Dai H (2008) Highly conducting graphene sheets and Langmuir-Blodgett films. Nat Nanotechnol 3:538
96. Ligler FS (2009) Perspective on optical biosensors and integrated sensor systems. Anal Chem 81(2):519–526
97. Lin L, Xiao LL, Huang S, Zhao L, Cui JS, Wang XH, Chen X (2006) Novel BOD optical fiber biosensor based on co-immobilized microorganisms in ormosils matrix. Biosens Bioelectron 21(9):1703–1709
98. Liu L, Shang L, Liu C, Liu C, Zhang B, Dong S (2010) A new mediator method for BOD measurement under non-deaerated condition. Talanta 81(4–5):1170–1175
99. Liu S, Leech D, Ju HX (2003) Application of colloidal gold in protein immobilization, electron transfer, and biosensing. Anal Lett 36(1):1–19
100. Liu S, Yuan L, Yue X, Zheng Z, Tang Z (2008) Recent advances in nanosensors for organophosphate pesticide detection. Adv Powder Technol 19:419–441
101. Long F, He M, Shi HC, Zhu AN (2008) Development of evanescent wave all-fiber immunosensor for environmental water analysis. Biosens Bioelectron 23(7):952–958
102. Luckarift HR, Balasubramanian S, Paliwal S, Johnson GR, Simonian AL (2007) Enzyme-encapsulated silica monolayers for rapid functionalization of a gold surface. Colloids Surf B 58(1):28–33
103. Luong JHT, Maleb KB, Glennon JD (2009) Boron-doped diamond electrode: synthesis, characterization, functionalization and analytical applications. Analyst 134(10):1965–1979
104. Luppa PB, Sokoll LJ, Chan DW (2001) Immunosensors—principles and applications to clinical chemistry. Clin Chim Acta 314(1–2):1–26
105. Mann TS, Mikkelsen SR (2008) Antibiotic susceptibility testing at a screen-printed carbon electrode array. Anal Chem 80(3):843–848
106. Marchesini GR, Meulenberg E, Haasnoot W, Irth H (2005) Biosensor immunoassays for the detection of bisphenol A. Anal Chim Acta 528(1):37–45
107. Martin CR, Siwy ZS (2007) Learning nature's way: biosensing with synthetic nanopores. Science 317:331–332
108. Mauriz E, Calle A, Lechuga LM, Quintana J, Montaya A, Manclus JJ (2006) Real-time detection of chlorpyrifos at part per trillion levels ground, surface and drinking water samples by a portable surface plasmon resonance immunosensor. Anal Chim Acta 561(1–2):40–47
109. Mauriz E, Calle A, Manclus JJ, Montoya A, Hildebrandt A, Barcelo D, Lechuga LM (2007) Optical immunosensor for fast and sensitive detection of DDT and related compounds in river water samples. Biosens Bioelectron 22(7):1410–1418
110. Mita DG, Attanasio A, Arduini F, Diano N, Grano V, Bencivenga U, Rossi S, Amine A, Moscone D (2007) Enzymatic determination of BPA by means of tyrosinase immobilized on different carbon carriers. Biosens Bioelectron 23(1):60–65
111. Mitchell J (2010) Small molecule immunosensing using surface plasmon resonance. Sensors 10(8):7323–7346
112. Monk DJ, Walt DR (2004) Opticalfiber-based biosensors. Anal Bioanal Chem 379:931–945
113. Nakamura H, Suzuki K, Ishikuro H, Kinoshita S, Koizumi R, Okuma S, Gotoh M, Karube I (2007) A new BOD estimation method employing a double-mediator system by ferricyanide and menadione using the eukaryote *Saccharomyces cerevisiae*. Talanta 72(1):210–216
114. Ndobo-Epoy JP, Lesniewska E, Guicquero JP (2007) Nano-pH sensor for the study of reactive materials. Anal Chem 79:7560–7564

115. Notsu H, Tatsuma T, Fujishima A (2002) Tyrosinase-modified boron doped diamond electrodes for the determination of phenol derivatives. J Electroanal Chem 523(1):86–92
116. Nowicka AM, Kowalczyk A, Stojek Z, Hepel M (2010) Nanogravimetric and voltammetric DNA-hybridization biosensors for studies of DNA damage by common toxicants and pollutants. Biophys Chem 146(1):42–53
117. Palchetti I, Mascini M (2008) Nucleic acid biosensors for environmental pollution monitoring. Analyst 133(7):846–854
118. Patolsky F, Zheng G, Hayden O, Lakadamyali M, Zhuang X, Lieber CM (2004) Electrical detection of single viruses. Proc Natl Acad Sci USA 101:14017–14022
119. Perez Lopez B, Merkoci A (2009) Improvement of the electrochemical detection of catechol by the use of a carbon nanotube based biosensor. Analyst 134(1):60–64
120. Pingarron JM, Yanez-Sedeno P, Gonzalez-Cortes A (2008) Gold naoparticucle-based electrochemical biosensors. Electrochim Acta 53:5848–5866
121. Poghossian A, Ingebrandt S, Offenhäusser A, Schöning MJ (2009) Field-effect devices for detecting cellular signals. Semin Cell Dev Biol 20(1):41–48
122. Ponomareva ON, Arlyapov VA, Alferov VA, Reshetilov AN (2011) Microbial biosensors for detection of biological oxygen demand (a review). Appl Biochem Microb 47(1):1–11
123. Popovtzer R, Neufeld T, Ron EZ J, Rishpon, Shacham-Diamand Y (2006) Electrochemical detection of biological reactions using a novel nano-bio-chip array. Sens Acta B 19:664–672
124. Prodromidis MI (2010) Impedimetric immunosensors—a review. Electrochim Acta 55:4227–4233
125. Quan D, Nagarale RK, Shin W (2010) A nitrite biosensor based on coimmobilization of nitrite reductase and viologen-modified polysiloxane on glassy carbon electrode. Electroanalysis 22(20):2389–2398
126. Rahman MA, Shiddiky MJA, Park JS, Shim YB (2007) An impedimetric immunosensor for the label-free detection of bisphenol A. Biosens Bioelectron 22(11):2464–2470
127. Ramanathan M, Simonian AL (2007) Array biosensor based on enzyme kinetics monitoring by fluorescence spectroscopy: Application for neurotoxins detection. Biosens Bioelectron 22(12):3001–3007
128. Riu J, Maroto A, Rius FX (2006) Nanosens Environ Anal 69(2):288–301
129. Rodriguez-Mozaz S, Marco MP, Lopez de Alda M, Barcelo D (2004) Biosensors for environmental monitoring of endocrine disruptors: a review article. Anal Bioanal Chem 378(3):588–598
130. Rodriguez-Mozaz S, Lopez de Alda M, Marco MP, Barcelo D (2005) Biosensors for environmental monitoring. A global perspective. Talanta 65(2):291–297
131. Rodriguez-Mozaz S, Lopez de Alda M, Marco MP, Barcelo D (2007) Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography-mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. J Chromatogr A 1152(1–2):97–115
132. Rogers KR (2006) Recent advances in biosensor techniques for environmental monitoring. Anal Chim Acta 568(1–2):222–231
133. Sakaguchi T, Morioka Y, Yamasaki M, Iwanaga J, Beppu K, Maedac H, Morita Y, Tamiya E (2007) Rapid and onsite BOD sensing system using luminous bacterial cells-immobilized chip. Biosens Bioelectron 22(7):1345–1350
134. Sanchez-Acevedo ZC, Riu J, Rius FX (2009) Fast picomolar selective detection of bisphenol A in water using a carbon nanotube field effect transistor functionalized with estrogen receptor-α. Biosens Bioelectron 24(9):842–2846
135. Scarano S, Mascini M, Turner APF, Minunni M (2010) Surface plasmon resonance imaging for affinity-based biosensors. Biosens Bioelectron 25(5):957–966
136. Schöning MJ, Poghossian A (2006) Bio FEDs (Field-Effect Devices): state-of-the art and new directions. Electroanalysis 18(19–20):1893–1900
137. Sepulveda B, Sanchez del Rio J, Moreno M, Blanco FJ, Mayora K, Dominguez C, Lechuga LM (2006) Optical biosensor microsystems based on the integration of highly sensitive Mach-Zehnder interferometer devices. J Opt A 8(7):S561–S566

138. Shan C, Yang H, Han D, Zhang Q, Ivaska A, Niu L (2010) Electrochemical determination of NADH and ethanol based on ionic liquid-functionalized grapheme. Biosens Bioelectron 25:1504
139. Shitanda I, Takamatsu S, Watanabe K, Itagaki M (2009) Amperometric screen-printed algal biosensor with flow injection analysis system for detection of environmental toxic compounds. Electrochim Acta 54(21):4933–4936
140. Shulga O, Kirchhoff JR (2007) An acetylcholinesterase enzyme electrode stabilized by an electrodeposited gold nanoparticle layer. Electrochem Commun 9(5):935–940
141. Sohail M, Adeloju SB (2009) Fabrication of redox-mediator supported potentiometric nitrate biosensor with nitrate reductase. Electroanalysis 21(12):1411–1418
142. Soldatkin OO, Pavluchenko OS, Kukla OL, Kucherenko IS, Peshkova VM, Rkhypova VM, Dzyadevych SV, Soldatkin AP, El'skaya AV (2009) Application of enzyme multibiosensor for toxicity analysis of real water samples of different origin. Biopolym cell 25(3):204–209
143. Song Y, Li G, Thorton SF, Thompson IP, Banwart SA, Lerner DN, Huang W (2009) Optimization of bacterial whole cell bioreporters for toxicity assay of environmental samples. Environ Sci Technol 43(20):7931–7938
144. Stankovich S, Dikin DA, Domett GHB, Kohlass KM, Zimney EJ, Stach EA, Piner RD, Nguyen ST, Ruoff RS (2006) Graphene-based composite materials. Nature 442:282
145. Su S, He Y, Zhang M, Yang K, Song S, Zhang X, Fan C, Lee ST (2008) High-sensitivity pesticide detection via silicon nanowires-supported acetylcholinesterase-based electrochemical sensors. Appl Phys Lett 93:023113
146. Tag K, Riedel K, Bauer HJ, Hanke G, Baronian KHR, Kunze G (2007) Amperometric detection of Cu_2+ by yeast biosensors using flow injection analysis (FIA). Sens Actuators B 122(2):403–40905
147. Tatsuma T, Yoshida Y, Shitanda I, Notsu H (2009) Algal biosensor array on a single electrode. Analyst 134(2):223–225
148. Teles FRR, Fonseca LP (2008) Applications of polymers for biomolecule immobilization in electrochemical biosensors. Mater Sci Eng 28(8):1530–1543
149. Timur S, Anik U, Odaci D, Gorton L (2007) Development of a microbial biosensor based on carbon nanotube (CNT) modified electrodes. Electrochem Commun 9(7):1810–1815
150. Tschmelak J, Proll G, Riedt J, Kaiser J, Kraemmer P, Barzaga L, Wilkinson JS, Hua P, Hole JP, Nudd R, Jackson M, Abuknesha R, Barcelo D, Rodriguez-Mozaz S, Lopez de Alda MJ, Sacher F, Stien J, Slobodnik J, Oswald P, Kozmenko H, Korenkova E, Tothova L, Krascsenits Z, Gauglitz G (2005) Automated water analyser computer supported system (AWACSS).PartII: Intelligent, remote-controlled, costeffective, on-line, water-monitoring measurement system. Biosens Bioelectron 20(8):1509–1519
151. Tschmelak J, Kumpf M, Kappel N, Proll G, Gauglitz G (2006) Total internal reflectance fluorescence (TIRF) biosensor for environmental monitoring of testosterone with commercially available immunochemistry: antibody characterization, assay development and real sample measurements. Talanta 69:343–350
152. Turner APF, Karube I, Wilson Biosensors SW (1986) Fundamentals and applications. Oxford Science Publications, Oxford
153. Tymecki L, Glab S, Koncki R (2006) Miniaturized planar ion-selective electrodes fabricated by means of thick-film technology. Sensors 6(6):390–396
154. Van der Meer JR, Belkin S (2010) Where microbiology meets microengineering: design and applications of reporter bacteria. Nat Rev Microbiol 8(7):511–522
155. Védrine C, Leclerc C, Durrieu C, Tran-Minh C (2003) Optical whole-cell biosensor using Chlorella vulgaris designed for monitoring herbicides. Biosens Bioelectron 18(4):457–463
156. Vidal JC, Bonel L, Castillo JR (2008) A modulated tyrosinase enzyme-based biosensor for application to the detection of dichlorvos and atrazine pesticides. Electroanalysis 20(8):865–873
157. Viswanathan S, Radecka H, Radecki J (2009) Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA. Biosens Bioelectron 24(9):2772–2777
158. Vo-Dinh T (2008) Nanosensing at the single cell level. Spectrochim Acta Part B 63:95–103

159. Wang J (2004) Carbon-nanotube based electrochemical biosensors: a review. Electroanalysis 17(1):7–14
160. Wang L, Ran Q, Tian Y, Xu J, Xian Y, Peng R, Jin L (2010) Covalent grafting tyrosinase and its application in phenolic compounds detection. Microchim Acta 171(3–4):217–223
161. Wang S, Tan Y, Zhao D, Liu G. G (2008) Amperometric tyrosinase biosensor based on Fe_3O_4 nanoparticles–chitosan nanocomposite. Biosens Bioelectron 23(12):1781–1787
162. Wang WU, Chen C, Lin KH, Fang Y, LieberM CM (2005) Label-free detection of small-molecule-protein interactions by using nanowire nanosensors. Proc Natl Acad Sci USA 102:3208–3212
163. Wang X, Zhi L, Mullen K (2007) Transparent conductive graphene electrode for dye-sensitized solar cells. Nano Lett 8:323
164. Wei D, Bailey MJA, Andrew P, Ryhanen T (2009) Electrochemical biosensors at the nanoscale. Lab Chip 9(15):2123–2131
165. Wijaya C, Lenaerts S, Maricot J, Hastanin S, Habraken JP, Vilcot R, Boukherroub S, Szunerits S (2011) Surface plasmon resonance-based biosensors: from the development of different SPR structures to novel surface functionalization strategies. Curr Opin Solid State Mater Sci 15:208–224
166. Woutersen M, Belkin S, Brouwer B, van Wezel AP, Heringa MB (2010) Are luminescent bacteria suitable for online detection and monitoring of toxic compounds in drinking water and its sources? Anal Bioanal Chem 400(4):915–929
167. Xia W, Li Y, Wan Y, Chen T, Wei J, Lin Y, Xu S (2010) Electrochemical biosensor for estrogenic substance using lipid bilayers modified by Au nanoparticles. Biosens Bioelectron 25(10):2253–2258
168. Xu Z, Chen X, Dong S (2006) Electrochemical biosensors based on advanced bioimmobilization matrices. Trends Anal Chem 25(9):899–908
169. Xuejiang W, Dzyadevych SV, Chovelon JM, Jaffrezic-Renault N, Ling C, Siqing X, Jianfu Z (2006) Conductometric nitrate biosensor based on methyl viologen/Nafion®/nitrate reductase interdigitated electrodes. Talanta 69(2):450–455
170. Yanez-Sedeno P, Pingarron JM (2005) Gold nanoparticle-based electrochemical biosensors. Anal Bioanal Chem 382(4):884–886
171. Yildiz HB, Castillo J, Guschin DA, Toppare L, Schuhmann W (2007) Phenol biosensor based on electrochemically controlled integration of tyrosinase in a redox polymer. Microchim Acta 159(1–2):27–34
172. Yuan CJ, Wang CL, Wu TY, Hwang KC, Chao WC (2011) Fabrication of a carbon fiber paper as the electrode and its application toward developing a sensitive unmediated amperometric biosensor. Biosens Bioelectron 26(6):2858–2863
173. Zeijli H, Hidalgo-Hidalgode Cisneros JL, Naranjo-Rodriguez I, Liu B, Tansamani KR, Marty JL (2008) Phenol biosensorbased on sonogel-carbon transducer with tyrosinase alumina sol-gel immobilization. Anal Chim Acta 612:198–203
174. Zhang J, Lang HP, Huber F, Bietsch A, Grange W, Certa U, McKendry R, Guntgerodt HJ, Hegner M, Gerber C (2006) Rapid and label-free nanomechanical detection of biomarker transcripts in human RNA. Nat Nanotechnol 1:214–220
175. Zhang Z, Jaffrezic-Renault N, Bessueille F, Léonard D, Xia S, Wang X, Chen L, Zhao J (2008) Development of a conductometric phosphate biosensor based on tri-layer maltose phosphorylase composite films. Anal Chim Acta 615(1):73–79
176. Zhang Z, Xia S, Léonard D, Jaffrezic-Renault N, Zhang J, Bessueille F, Goepfert Y, Wang X, Chen L, Zhu Z, Zhao J, Almeida MG, Silveira CM (2009) A novel nitrite biosensor based on conductometric electrode modified with cytochrome c nitrite reductase composite membrane. Biosens Bioelectron 24(6):1574–1579
177. Zhao J, Zhi Y, Zhou W (2009) A tyrosinase biosensor based on ZnO nanorod clusters/nanocrystalline diamond electrodes for biosensing of phenolic compounds. Anal Sci 25(9):1083–1088
178. Zheng G, Patolsky F, Cui Y, Wang WU, Lieber CM (2005) Multiplexed electrical detection of cancer markers with nanowiresensor arrays. Nat Biotechnol 23:1294–1301
179. Ziegler C (2004) Cantilever-based biosensors. Anal Bioanal Chem 379:946–959

180. Zoski CG, Yang N, He P, Berdondini L, Koudelka-Hep M (2007) Addressable nanoelectrode membrane arrays: Fabrication and steady-state behavior. Anal Chem 79:1474–1484
181. Zhang Y, Zhang K, Ma H (2009) Electrochemical DNA biosensors based on gold nanoparticles/cysteamine/poly(glutamic acid) modified electrode. Am J Biomed Sci 1(2):115–125
182. Zhou Y, Zhi J (2006) Development of an amperometric biosensor based on covalent immobilization of tyrosinase on a boron-doped diamond electrode. Electrochem Commun 8(12):1811–1816
183. Zourob M, Simonian A, Wild J, Mohr S, Fan X, Abdulhalime I, Goddard NJ (2007) Optical leaky waveguide biosensors for the detection of organophosphorus pesticides. Analyst 132(2):114–120

Chapter 8
Respiratory Toxicity of Carbon Nanotubes

Sophie Lanone

Abstract Carbon nanotubes (CNT) are emblematic nanomaterials, and have generated a highly competitive international scientific research activity. Since their initial description in 1991, the understanding of their unique physicochemical properties led to a large number of actual applications and uses, as well as future developments. Because of these promising applications, there is an increasing concern regarding the consequences that could result from human exposure to CNT. Analysis of the existing literature shows that respiratory exposure to CNT can lead to the occurrence of pulmonary inflammation, the formation of granuloma, and the development of pulmonary fibrosis. The exact determinants of these effects still remain to be clearly identified, although intrinsic physicochemical characteristics of CNT (i.e. length, dispersion status, and residual catalyst content) seem to be of importance. Several critical issues still remain to be solved, such as the translocation of CNT outside the lungs and the occurrence of their biotransformation, which should open a new understanding to the respiratory effects of CNT.

S. Lanone (✉)
Inserm, U955, Équipe 4, 94000 Créteil, France
e-mail: sophie.lanone@inserm.fr

Faculté de Médecine, Université Paris Est, 94000 Créteil, France

Centre Hospitalier Intercommunal de Créteil,
Service de pneumologie et pathologie professionnelle, 94000 Créteil, France

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_8,

8.1 Introduction

Carbon nanotubes (CNT) are emblematic nanomaterials, and have generated a highly competitive international scientific research activity. CNT are cylinders of one or several (up to 20) concentric graphite layer(s) (single- or multi-walled CNTs respectively—SWCNT and MWCNT). Their diameter is in the order of the nanometer, and they can measure up to several micrometers in length. Since their initial description in 1991, the understanding of their unique physicochemical properties, such as mechanical, thermal, or electrical conductivity, led to a large number of actual applications and uses, as well as future developments in aerospace, automobiles, nanoelectronic, or nanomedicine. CNT are currently used in computers, aircraft airframe, and many sporting goods such as tennis rackets, bicycles, golf irons, sport shoes spikes, hockey sticks, or baseball bats (see *Woodrow Wilson International Center for Scholars* web site for inventory). Because of these actual and future applications, there is an increasing concern regarding the consequences that could result from human exposure to CNT.

In this chapter, the context of pulmonary exposure to nanomaterials, and CNT in particular, will be first exposed. Then, what is currently known about respiratory effects of CNT will be presented, along with the proposed underlying mechanisms. Finally, the remaining issues regarding pulmonary toxicity of CNT will be discussed.

8.2 Context of Pulmonary Exposure to CNT

The determinants of an exposure to CNT are multifactorial. They can be characterized by several factors, including: (1) in which environmental compartment are the nanomaterials?, (2) what is the context of exposure (occupational or not)?, and (3) which compartment of the human body is exposed?

8.2.1 Which Compartment of the Environment?

Because of the always increasing production and use of manufactured nanomaterials, and CNT in particular, their release in the environment is a more and more probable phenomenon. Several environmental compartments can be targeted: air, water, soil. Indeed, unintentional release of CNT in the air, during their production process, can happen, as well as the contamination of wastes, water, and/or soil in the vicinity of production sites. Exposure can also result from the particular uses of nanomaterials. CNT are for example proposed to be promising tools in the context of nanomedicine, and therefore, be associated to a direct exposure of the human body to them.

8.2.2 What Context of Exposure?

Exposure to CNT can occur in an occupational or a private context. Workers from nanotechnologies can be directly exposed at the time of production of CNT, as well as during their transport, storage, or during their incorporation in final products. Moreover, because of the life cycle of nanomaterials, and particularly their degradation, the general population can be exposed. Finally, because of the use of CNT in sporting goods for example, the general population can also be exposed when using them, such as tennis rackets or CNT-containing bicycles.

8.2.3 Which Compartment of the Human Body?

Four main portals of entry of nanomaterials in the human body can be considered; respiratory, digestive, cutaneous, and systemic compartments (in the context of nanomedicine particularly). Only the respiratory system will be considered in this chapter, as it represents a unique portal of entry for inhaled nanomaterials, but also because it receives the entire cardiac output. The respiratory system can therefore be exposed secondary to a systemic passage of CNT. Respiratory apparatus can be considered as an assembly of tubing, the airways, which allow the air to flow from the nose and mouth to the pulmonary alveoli. Extrathoracic (or upper) airways are composed of nose, mouth, pharynx, and larynx, while intrathoracic (or lower) airways are represented by conducting airways (trachea, bronchi), and respiratory airways, with pulmonary alveoli. These latter are the site of gaseous exchanges between air and blood. There are approximately 300 million alveoli in the adult lung, representing a very large exchange surface (circa 140 m^2, about the surface of a tennis court).

8.2.4 Deposition of CNT in the Pulmonary System

As for other nanomaterials, CNT can be deposited in the respiratory tract. However, the identification of the determinants ruling the site of deposition is considered as a remaining issue in the actual literature. When in suspension in the air, particles in general, and CNT in particular, form an aerosol. The behavior of this aerosol is largely conditioned by the size of the particles, which further condition the deposition mode of the particles. Moreover, as for other nanomaterials, CNTs have a tendency to form agglomerates and aggregates, which will also influence their site of deposition in the respiratory tract. Mathematical predictive models have been proposed to help understand this phenomenon. In these models, the respiratory tract is arbitrarily divided into three regions: nasopharyngeal (upper airways), tracheobronchial, and alveolar. From these models,

established for particles between 1 and 100 μm aerodynamic diameter, one can conclude that the smaller the particles are, the best they are able to deposit in the respiratory tract. Each region of the respiratory tract is the target for different classes of particles, the alveoli for example being the preferred deposition site of nanoparticles around 20 nm [1]. These differences of deposition site could result in differential effects of nanoparticles. It must be noted that such mathematical models have been proposed for ideal spherical nanoparticles, and may not be well suited for CNT in particular. Moreover, the calculations have been performed considering mouth breathing, at rest. It is easily imaginable that these parameters can be modified while breathing during an effort (higher volume of air breathed, greatest air flux perturbations), or in case of respiratory pathology (asthma, bronchitis). For example, it is predicted that deposition will be higher for nanoparticles in constricted airways. In accordance, a higher retention of nanoparticles has been demonstrated in obstructed or asthmatic airways [2–5].

8.3 Respiratory Effects of CNT

The existing literature regarding respiratory effects of CNT is relatively new, as the first study came out in 2004 [6]. Since then, there have been an always increasing number of studies dealing with respiratory toxicity of CNT, mainly after exposure of mainly mice or rats to either SWCNT or MWCNT. Several administration routes have been used so far; intratracheal or intrapharyngeal administration, and more recently, inhalation (by means of whole-body or nose-only exposure systems). The different studies used a large range of doses; 10–500 μg/mouse for intratracheal or intrapharyngeal administration [7, 8], and 0.3–30 mg/m^3 for inhalation exposure for example [9–11, 12]. The duration of exposure has also been wide; from 24 h up to 6 months (only a very limited number of studies evaluated later time points). Three major endpoints have been studied so far and will be described here. They are focused on specific immediate and/or long-term responses of lung tissue: occurrence of pulmonary inflammation, the formation of granulomas, and the development of lung fibrosis. A few more endpoints have been promptly studied and will be discussed at the end of this chapter.

8.3.1 Pulmonary Inflammation

The induction of an inflammatory response in the lung is the most reported phenomenon after pulmonary exposure to CNT. This inflammation is an early event, occurring as early as 6–24 h after the initial exposure, with the recruitment of neutrophils in the bronchoalveolar lavage (BAL) fluid [8, 13]. This neutrophil-driven infiltration is often reported as being transient, and usually resolved within 15 days after the initial exposure. The release of proinflammatory cytokines,

including tumor necrosis factor (TNF) alpha, interleukin (IL)-1ß, -6, monocyte chemoattractant protein (MCP)-1, or macrophage inflammatory protein (MIP)-2 (or CXCL-2) [8, 14, 15] occurs during this acute-phase inflammatory response. This is observable at the level of the BAL fluid, as well as in whole lung tissue homogenates (RNA and protein levels). This response is consistent with a foreign body response, and is often followed by the formation of multifocal granulomas.

8.3.2 Formation of Granulomas

The granulomas contain macrophages, and are usually surrounding CNT clusters, but can also appear distal, where dispersed CNT are believed to be present (although, because of the difficulty to observe dispersed CNT in biological media, it is hard to be confident on that matter). The presence of granulomas is documented to be a persistent event following CNT administration, as they can be still observable 6 months after the initial exposure [8, 13].

8.3.3 Pulmonary Fibrosis

The development of pulmonary fibrosis is the third biological consequence of CNT exposure that is well documented in the current literature [14, 16]. This pathological event can occur both within granulomas or distal to them, as diffuse interstitial and septal fibrosis. Fibrosis development has been described as soon as 15 days after the initial exposure to CNT. This is also a long-term event, since the fibrotic lesions can still be persistent after 6 months [15, 17]. The histological alteration is accompanied by the expression of markers of extracellular matrix deposition (collagen I and III), increase in hydroxyproline lung content, as well as the production of profibrotic mediators (i.e. transforming growth factor (TGF) beta).

8.3.4 Carcinogenic Effects

Only a limited number of studies have considered the carcinogenic potential of CNT so far. A study performed in mice exposed to SWCNT by inhalation showed the occurrence of mutation in the *k-ras* oncogene locus within the first week of exposure, and until the end of the experiment 4 weeks later [10, 11]. These findings were, however, not confirmed in a study using the pharyngeal aspiration route of exposure, implying that exposure route is a critical parameter to consider in terms of influencing the biological effects of CNT [14]. Because of the physical and chemical durability and fibrous shape of CNT, concerns have early been raised

that they may exhibit potentially significant health hazards similar to asbestos, i.e., development of mesothelioma. To date, no such effects of CNT have been described in response to pulmonary exposure of laboratory animals. However, evidences have been given that intraperitoneal as well as intrascrotal administration of MWCNT in mice or rats can lead to the formation of abdominal and/or thoracic mesothelioma [18, 19]. Moreover, it has been recently described that CNT can rapidly reach the pleura after pulmonary exposure to CNT by either inhalation or pharyngeal aspiration [20]. From these studies, it is clear that more toxicological research is necessary to determine the effective carcinogenic potential of CNT, but that some of the studies suggest proceeding with caution.

8.4 Proposed Underlying Mechanisms

8.4.1 Reactive Oxygen Species

Besides the development of pulmonary inflammation already described in this chapter, the presence of an oxidative stress is considered to be a major mechanism underlying pulmonary response to CNT exposure. Oxidative stress is defined by the existence of an imbalance between oxidants production and antioxidants defense, in favor of oxidants. Several approaches have been used to demonstrate the presence and role of an oxidative stress. First, the detection of biomarkers of oxidative stress such as lung protein thiols or malone dialdehyde (MDA) content is increased as soon as 24 h after the initial exposure to CNT [10, 11, 21]. Moreover, the presence, in the total lung, of a product of lipid peroxidation 4 hydroxynonenal (4-HNE), is rapidly increased after pharyngeal aspiration of SWCNT in mice [15], as well as protein carbonyls [10, 11]. Second, the role of oxidants in pulmonary response to CNT has been assessed using mice presenting low antioxidant defenses, thanks to a vitamin E-deficient diet. Exposure of such mice to CNT induced a higher cellular inflammatory response (total cell count in BAL, as well as total neutrophil content), accompanied by an increased secretion of two major inflammatory cytokines; TNF and IL-6 [10, 11, 21, 22]. Similar mice also developed an exaggerated fibrosis response, as demonstrated by increased TGF beta levels in the lungs, and increased thickness of alveolar walls [21]. Finally, a genetic approach using $gp91^{phox-/-}$ mice confirmed the implication of oxidants in CNT biological effects. Indeed, the absence of a functional NAD(P)H oxidase in these mice was associated to a diminished induction of collagen deposition, footprint of fibrosis development, in response to SWCNT administrated by pharyngeal aspiration 28 days earlier [10, 11].

8.4.2 Systemic Effects of Respiratory Exposure to CNT

As stated before, the occurrence of an inflammatory response, the formation of granuloma, and/or the development of pulmonary fibrosis have been the three major endpoints studied so far to describe pulmonary effects of CNT administration. More recent studies describe the systemic effects of CNT administered via pulmonary route. After inhalation of in whole-body chamber exposure, 0.3, 1 or 5 mg/m^3, 6 h a day, for 7 or 14 days, Mitchell and collaborators [23] have demonstrated that systemic immunity was affected. Interestingly, they observe an absence of pulmonary effects in terms of inflammation, granuloma formation, or fibrosis as well as tissue injury. In this study, the authors demonstrate that spleen-derived T cells show a suppressed T cell-dependent antibody response, a decreased proliferation of T cells following stimulation, as well as an altered natural killer cell's killing activity. This was the first evidence of systemic effects induced by CNT secondary to pulmonary exposure. Some methodological issues can however be raised; because of the experimental setting utilizing a whole-body chamber exposure, an ingestion of CNT because of the cleaning of the animal's fur and skin and the subsequent access of CNT to the systemic circulation cannot be ruled out. Since then, other studies, utilizing less physiologically relevant exposure routes (pharyngeal aspiration, intratracheal administration) allow concluding with better certainty on extrapulmonary effects of CNT. In such studies, as soon as 4 h after the initial exposure, increased systemic levels of inflammatory proteins (IL-1ß, CXCL1, CXCL2, IL-8rß, S100a8, Mac-1) as well as stress response markers [hypoxia inducible factor 3 (HIF-3a), matrix metalloproteinase (MMP)-9, arginase II, osteopontin, colony stimulating factor-1 (CSF-1), and insulin growth factor receptor 1 (IGF-1R)] have been described [24]. These markers were detected in lung as well as total blood and/or in serum, but, interestingly, some of them were detected exclusively in the blood of the exposed animals. In addition, total and active levels of plasminogen activator inhibitor 1 (PAI-1), a procoagulant acute-phase protein which is involved in inhibition of the fibrinolytic cascade, were significantly increased in the lungs as well as in the plasma of CNT-exposed animals. This study gives the evidence that 4 h after CNT deposition in the lungs, local, and systemic inflammatory as well as prothrombotic responses are activated [23–25]. This kind of systemic response, particularly if chronic and persistent, may trigger or exacerbate cardiovascular dysfunction and disease, such as atherosclerosis. Unfortunately, as no assessment of the presence of CNT outside the lungs of the animals was performed, one cannot conclude on the necessity or not for CNT to translocate outside the lung to have systemic effects.

8.4.3 Influence of Existing Pathologies

The literature on respiratory effects of CNT initially focused on healthy animals. However, more recent studies investigated the effect of CNT exposure in the

context of existing pathologies such as asthma or bacterial infections, or along with exposure to environmental contaminants such as ozone. Indeed, exposure to CNT occurring concomitantly with an installed pulmonary disease or pathogenic infections may modify (and potentially enhance) the natural pathological response. Murine models of asthma have been used to evaluate the potential effects of CNT in such pathological condition. SWCNT as well as MWCNT have been described to increase the early inflammation and the susceptibility to develop airway fibrosis compared to CNT alone or ovalbumin sensitization only (ovalbumin sensitization being a common method used to develop asthma in rodents). Moreover, CNT induced an increased expression of immunoglobulins (Ig) related to allergic response in blood [9, 26]. Bacterial infection is a common pathological condition. Effects of an exposure to CNT concomitantly with bacterial infection have been explored using Gram-positive (*Listeria monocytogenes*) and Gram-negative (lipopolysaccharide) bacteria. In both cases, CNT exposure in combination with bacterial infection was able to induce an increased airway fibrosis as compared to bacterial infection alone [27, 28]. A less reproducible event is the occurrence of an increased inflammatory acute and/or chronic response. Interestingly, pharyngeal administration of SWCNT following an exposure to *Listeria monocytogenes* induced a decreased bacterial clearance. Altogether, these studies demonstrate that CNT may interfere with existing allergies and with natural responses against pathogenic infections. However, studies are still needed to deeply understand the underlying mechanisms of these effects.

8.5 Determinants of CNT Biological Effects

When trying to figure out an Ariadne's thread to CNT effects after pulmonary exposure, scientists are quickly confronted with what could be considered as discordant results from the literature. It actually comes from the fact that CNT cannot be considered as a single material; the so-called physicochemical characteristics of CNT are to be taken into account to accurately evaluate their effects. These characteristics are the result of CNT synthesis and post-synthesis processes and will be described hereafter.

8.5.1 Synthesis Processes

Several processes are commonly used for CNT synthesis: arc discharge, chemical vapor deposition (CVD), and laser ablation. These methods share in common that energy (which can be either electrical, thermal, or high intensity light) is supplied to a carbon source (carbon monoxide, alcohols, or hydrocarbons for example), enabling the further production of carbon atoms that recombine together to result in the generation of CNT. The synthesis of CNT is therefore a complex process

resulting in a heterogeneous population of CNT presenting a whole set of various physical and chemical characteristics. Post-manufacturing processes such as (partial) removal of residual catalyst content may also lead to physicochemical characteristics different from that of the initially produced CNT. From the existing literature concerning biological effects of CNT, it clearly appears that several factors mainly related to the physicochemical characteristics of CNT are major determinants of their subsequent biological effects. The exact physicochemical determinant(s) conditioning CNT biological effects is/are unknown for now, but these factors include for example the length, catalytic residues nature and content, surface properties, etc. of CNT.

8.5.2 Length

Length has been accepted to influence fiber clearance, because it dictates the ability of phagocytic cells in general, and macrophages in particular, to completely internalize and further process the fibers. The influence of CNT length on their biological effects has been therefore assessed in a few studies, and from these data, it appears that shorter CNT have less biological effects than long CNT, in terms of reactive oxygen species production, inflammatory mediators release, and inflammatory cell recruitment [29, 30]. Longer CNT (with a cut-off at 5–10 μm) promote frustrated phagocytosis, with a failure of macrophages to entirely engulf CNT [31]. However, several issues must be raised concerning the effect of length in CNT biological effects, apart from those mentioned above. First of all, because of still remaining technical issues, it is difficult to measure accurately CNT length. Indeed, they have a tendency to entangle and aggregate, which complicates a linear measurement. Microscopy techniques (usually transmission electron microscopy, TEM) are often used to demonstrate (but not quantify) the differences of CNT length, but it necessitates an amazingly high amount of measurements from the investigator. Moreover, shortening of CNT is often obtained by grinding raw CNT materials, by the use of an agate ball mill, a process which can introduce structural defects in CNT [32]. This would lead to the modification of not only the length of CNT, but also other physicochemical characteristics of the CNT. In this regard, Wako and collaborators recently demonstrated that grinding CNT could modify inflammatory response in terms of localization of macrophages infiltrates [33]. These authors showed that intratracheal administration of ground MWCNT in rats lead to the infiltration of MWCNT-laden macrophages in the alveoli, whereas unground MWCNT-laden macrophages accumulated in the pulmonary interstitium. This was associated with the formation of smaller aggregates in the case of ground MWCNT, as compared to unground MWCNT.

8.5.3 Dispersion Status

Dispersion is often proposed as an important determinant of biological effects of CNT. Indeed, dispersion can condition the potency for CNT to form aggregates in solution. What is currently believed is that well dispersed CNT seem to preferentially induce the development of fibrosis, whereas less dispersed CNT lead to the formation of granuloma. A better dispersion of CNT can be achieved by the use of dispersing agents (such as surfactants), attachment of functional groups to CNT's surface (functionalization process), use of solvents, or mechanical treatment, such as grinding or sonication. Another way to achieve different dispersion status is the administration mode, as seen in a study by Li and coworkers. These authors demonstrated the formation of small aggregates after inhalation of CNT, as compared to the formation of larger aggregates consequent to intratracheal instillation of similar amounts of CNT [34].

8.5.4 Residual Catalyst Content

Finally, and directly resulting from their production process, the amount of residual catalyst particles present in CNT is believed to play an important role in determining CNT biological effects. Iron, nickel, and cobalt are the most common catalyst particles primarily used during CNT manufacture. Their amount in the final product varies with the manufacturing process, as well as the post-treatments undertaken to purify as produced raw CNT. Several studies suggest that the metal content (and particularly that of iron) of CNT can contribute to their effects probably via the induction of an oxidative stress [14, 35, 36]. However, these studies are not consistent in their conclusions; some show that a high content in catalyst residues is associated to increased biological effects, and some others report opposite findings. The discrepancies between the different studies may relate to the bioavailability of the catalyst residues in the various CNT samples; some catalyst residues are engulfed inside the carbon structure of CNT, although in some other cases, catalyst residues are present at the surface of the carbon structure, being therefore more accessible and prone to play a role in CNT's biological effects. Finally, the purification process (high temperature, strong acidification process) used to get rid of (at least in part) catalyst residues may introduce other physicochemical modifications in the so-called purified CNT, and therefore complicate the interpretation of the results.

As a general comment, care must be taken regarding the experimental design of the studies aimed to determine the importance of specific physicochemical determinant(s), in particular concerning the origin of the CNT utilized to perform the study. Indeed, as exposed earlier, fabrication processes for CNT synthesis are highly complex, and can lead to the production of a large variety of CNT. Trying to compare results obtained with CNT issued from different origins (producers, fabrication process) can be hazardous when it comes to giving straightforward conclusions.

8.6 Remaining Issues Regarding CNT Toxicity

Several specific endpoints have been either not or only poorly considered until now regarding CNT respiratory effects. This essentially concerns two major endpoints: the translocation of CNT as well as their further processing (*devenir*) after pulmonary exposure.

8.6.1 Translocation of CNT After Pulmonary Exposure

It is quite clear, given the actual literature on the subject, that CNT can have extrapulmonary effects after pulmonary administration. However, the underlying mechanisms are quite unclear, particularly regarding the need or absence of need for CNT to be translocated to the systemic circulation or other organs to have an extrapulmonary effect. There are some evidences of biopersistence of CNT after pulmonary exposure; Muller and collaborators early demonstrated that 60 days after the initial intratracheal administration, roughly 80 % of MWCNT was found inside the lungs of the exposed rats [17]. Interestingly, only 36 % of the initial dose of MWCNT was found inside the lungs when the authors considered ground MWCNT. Unfortunately, no indication on the localization (organ, body fluids) of the CNT has been achieved in this study. It is important to have in mind that observation of CNT in a biological context is a complex matter when considering the biodistribution of CNT. Optical and conventional electronic microscopy observations are of limited interest, especially in the case of small diameter SWCNT, because of limitations related to the spatial resolution of these techniques and to the low contrast of these materials [37]. A possibility might reside in the grafting of a functionalized group to the raw CNT, but this could affect the intrinsic physicochemical characteristics of the CNT, therefore modifying their biological effects. Another possibility is to take advantage of the residual catalyst present in the CNT, such as iron. However, it requires highly demanding techniques, such as synchrotron-based X ray fluorescence microscopy (μXRF) [38], which is clearly not convenient to use on a routine basis.

8.6.2 Devenir of CNT

Another important remaining issue to get a comprehensive understanding of CNT's biological effects is the question of their *devenir* and potential biotransformation in a biological context. Indeed, despite the earlier mentioned indications of biopersistance, along with evidences of CNT penetration inside cells, no actual data are available on that matter, although such data are very important to obtain since CNT biotransformation products could have their own biological effects and

therefore participate in CNT's reactivity and further toxicity. A few groups have demonstrated, yet essentially in test tubes, the possible modifications of SWCNT [39, 40]. However, more extensive research is clearly needed regarding this matter.

8.7 Conclusions

Given the existing literature on the subject, it can be concluded that respiratory exposure to CNT lead to the occurrence of pulmonary inflammation, the formation of granuloma, and the development of pulmonary fibrosis. The exact determinants of these effects still remain to be clearly identified, although intrinsic physico-chemical characteristics of CNT such as length, dispersion status, and residual catalyst content seem to be of importance. Several critical issues still remain to be solved, such as the translocation of CNT outside the lungs and the occurrence of their biotransformation. This should open a new understanding to the respiratory effects of CNT.

References

1. Witschger O, Fabries JF (2005) Particules ultra-fines et santé au travail, 2—Soures et caractérisation de l'exposition. Hygiène et Sécurité au travail 199(ND 2227):37–54
2. Anderson PJ, Wilson JD et al (1990) Respiratory tract deposition of ultrafine particles in subjects with obstructive or restrictive lung disease. Chest 97(5):1115–1120
3. Card JW, Zeldin DC et al (2008) Pulmonary applications and toxicity of engineered nanoparticles. Am J Physiol Lung Cell Mol Physiol 295(3):L400–L411
4. Chalupa DC, Morrow PE et al (2004) Ultrafine particle deposition in subjects with asthma. Environ Health Perspect 112(8):879–882
5. Farkas A, Balashazy I et al (2006) Characterization of regional and local deposition of inhaled aerosol drugs in the respiratory system by computational fluid and particle dynamics methods. J Aerosol Med 19(3):329–343
6. Lam C, James JH et al (2004) Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Toxicol Sci 77:126–134
7. Chou CC, Hsiao HY et al (2008) Single-walled carbon nanotubes can induce pulmonary injury in mouse model. Nano Lett 8(2):437–445
8. Tabet L, Bussy C et al (2011) Coating carbon nanotubes with a polystyrene-based polymer protects against pulmonary toxicity. Part Fibre Toxicol 8(1):3
9. Ryman-Rasmussen JP, Tewksbury EW et al (2008) Inhaled multi-walled carbon nanotubes potentiate airway fibrosis in murine allergic asthma. Am J Respir Cell Mol Biol 40:349–358
10. Shvedova AA, Kisin ER et al (2008) Inhalation versus aspiration of single walled carbon nanotubes in C57bl/6 mice: inflammation, fibrosis, oxidative stress and mutagenesis. Am J Physiol Lung Cell Mol Physiol 95:L552–L565
11. Shvedova AA, Kisin ER et al (2008) Increased accumulation of neutrophils and decreased fibrosis in the lung of NADPH oxidase-deficient C57BL/6 mice exposed to carbon nanotubes. Toxicol Appl Pharmacol 231(2):235–240
12. Ryman-Rasmussen JP, Cesta MF et al (2009) Inhaled carbon nanotubes reach the subpleural tissue in mice. Nat Nano 4:747–751

13. Tabet L, Bussy C et al (2009) Adverse effects of industrial multiwalled carbon nanotubes on human pulmonary cells. J Toxicol Environ Health A 72(2):60–73
14. Johnston HJ, Hutchison GR et al (2010) A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes: the contribution of physico-chemical characteristics. Nanotoxicology 4:207–246
15. Shvedova AA, Kisin ER et al (2005) Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. Am J Physiol Lung Cell Mol Physiol 289(5):L698–L708
16. Kayat J, Gajbhiye V et al (2011) Pulmonary toxicity of carbon nanotubes: a systematic report. Nanomedicine 7:40-49
17. Muller J, Huaux F et al (2005) Respiratory toxicity of multi-wall carbon nanotubes. Toxicol Appl Pharmacol 207(3):221–231
18. Sakamoto Y, Nakae D et al (2009) Induction of mesothelioma by a single intrascrotal administration of multi-wall carbon nanotube in intact male Fischer 344 rats. J Toxicol Sci 34(1):65–76
19. Takagi A, Hirose A et al (2008) Induction of mesothelioma in p53 +/− mouse by intraperitoneal application of multi-wall carbon nanotube. J Toxicol Sci 33(1):105–116
20. Mercer RR, Hubbs AF et al (2011) Distribution and persistence of pleural penetrations by multi-walled carbon nanotubes. Part Fibre Toxicol 7:28
21. Shvedova AA, Kisin ER et al (2007) Vitamin E deficiency enhances pulmonary inflammatory response and oxidative stress induced by single-walled carbon nanotubes in C57BL/6 mice. Toxicol Appl Pharmacol 221(3):339–348
22. Shvedova AA, Kisin ER et al (2004) Pro/antioxidant status in murine skin following topical exposure to cumene hydroperoxide throughout the ontogeny of skin cancer. Biochemistry (Mosc) 69(1):23–31
23. Mitchell LA, Gao J et al (2007) Pulmonary and systemic immune response to inhaled multiwalled carbon nanotubes. Toxicol Sci 100(1):203–214
24. Erdely A, Hulderman T et al (2008) Cross-talk between lung and systemic circulation during carbon nanotube respiratory exposure. Potential biomarkers. Nano Lett 9(1):36–43
25. Park E-J, Cho W-S et al (2009) Pro-inflammatory and potential allergic responses resulting from B cell activation in mice treated with multi-walled carbon nanotubes by intratracheal instillation. Toxicology 259:113–121
26. Inoue K, Koike E et al (2009) Effects of multi-walled carbon nanotubes on a murine allergic airway inflammation model. Toxicol Appl Pharmacol 237(3):306–316
27. Cesta MF, Ryman-Rasmussen JP et al (2010) Bacterial lipopolysaccharide enhances PDGF signaling and pulmonary fibrosis in rats exposed to carbon nanotubes. Am J Respir Cell Mol Biol 43(2):142–151
28. Shvedova AA, Fabisiak JP et al (2008) Sequential exposure to carbon nanotubes and bacteria enhances pulmonary inflammation and infectivity. Am J Respir Cell Mol Biol 38(5):579–590
29. Kolosnjaj-Tabi J, Hartman KB et al (2010) In vivo behavior of large doses of ultrashort and full-length single-walled carbon nanotubes after oral and intraperitoneal administration to Swiss mice. ACS Nano 4(3):1481–1492
30. Muller J, Huaux F et al (2008) Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: toxicological aspects. Chem Res Toxicol 21(9):1698–1705
31. Poland CA, Duffin R et al (2008) Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol 3(7):423–428
32. Fenoglio I, Greco G et al (2008) Structural defects play a major role in the acute lung toxicity of multiwall carbon nanotubes: physicochemical aspects. Chem Res Toxicol 21(9):1690–1697
33. Wako K, Kotani Y et al (2010) Effects of preparation methods for multi-wall carbon nanotube (MWCNT) suspensions on MWCNT induced rat pulmonary toxicity. J Toxicol Sci 35(4):437–446

34. Li JG, Li WX et al (2007) Comparative study of pathological lesions induced by multiwalled carbon nanotubes in lungs of mice by intratracheal instillation and inhalation. Environ Toxicol 22(4):415–421
35. Guo L, Morris D et al (2007) Iron bioavailability and redox activity in diverse carbon nanotube samples. Chem Mater 19:3472–3478
36. Liu X, Guo L et al (2008) Targeted removal of bioavailable metal as a detoxification strategy for carbon nanotubes. Carbon N Y 46(3):489–500
37. Porter AE, Gass M et al (2007) Direct imaging of single-walled carbon nanotubes in cells. Nat Nanotechnol 2(11):713–717
38. Bussy C, Cambedouzou J et al (2008) Carbon nanotubes in macrophages: imaging and chemical analysis by X-ray fluorescence microscopy. Nano Lett 8:2659–2663
39. Allen BL, Kichambare PD et al (2008) Biodegradation of single-walled carbon nanotubes through enzymatic catalysis. Nano Lett 8(11):3899–3903
40. Kagan VE, Konduru NV et al (2010) Carbon nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary inflammation. Nat Nano 5(5):354–359

Chapter 9
Fate and Health Impact of Inorganic Manufactured Nanoparticles

Armelle Baeza-Squiban, Sandra Vranic and Sonja Boland

Abstract Inorganic nanoparticles (NPs) either based on metal oxides (iron oxide, cerium oxide, titanium dioxide, silicon dioxide, etc.) or metals (gold and silver) have now wide applications. Consequently it increases the probability of unintended exposure that could affect workers as well as the general population including susceptible people. Inhalation, ingestion, and dermal contact are the main routes of exposure. Before reaching the epithelial barrier lining the respiratory tract, the digestive tract, or the skin, NPs get in contact with biological fluids and become covered by molecules present in these fluids forming the so-called "corona". The fate and the effects of NPs may be different according to the corona composition as the cell membrane does not interact directly with the NPs surface but with the NP surrounded by its corona. Endocytosis has been shown to be an important route of NPs uptake. However, the rate and mechanism of uptake seem to be cell-type dependent, cell density-dependent and vary for NPs of different size, charge, and other surface properties. Uptake is mostly an energy-dependent process, dependent on NPs size, shape, and charge. There is also some evidence of NPs exocytosis allowing NPs to cross epithelial barrier and enter systemic circulation. Different in vitro models have been proposed showing potential of different NPs to translocate. NPs biodistribution have been studied in different in vivo models after intravenous injection, oral ingestion, intratracheal instillation, or inhalation showing that smaller NPs can be better eliminated, but are also more widespread in secondary organs. Inhalation studies underline that NPs mainly remain at the site of exposure and only a low amount translocates. NPs health effects are widely studied. Toxicological studies performed on animals by intratracheal instillation have underlined that the most predominant effect of NPs is the

A. Baeza-Squiban (✉) · S. Vranic · S. Boland
Sorbonne Paris Cité, Laboratory of Molecular and Cellular Responses to Xenobiotics, Unit of Functional and Adaptive Biology (BFA), Université Paris Diderot, EAC CNRS 4413, 5 rue Thomas Mann, 75 205, Paris cedex 13, France
e-mail: baeza@univ-paris-diderot.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_9,

induction of lung inflammation characterized by the increase of immune cells, frequently macrophages and neutrophils, in the bronchoalveolar lavage and the increased release of pro-inflammatory mediators (cytokines and chemokines), and all this effects are dependent on dose, size, surface reactivity, and NPs composition. There is also evidence of some cardiovascular and neurologic effects of NPs. NPs toxicity mainly results from their ability to induce an oxidative stress resulting from the ability of NPs to directly or indirectly generate reactive oxygen species (ROS). Some studies have shown the role of specific interactions between NPs and proteins in cell activation or cell metabolism suggesting potential additional pathways of toxicity independent of oxidative stress. A better knowledge about the NPs properties involved in their toxicity is expected in order to propose NPs safe by design.

9.1 Introduction

Nanotechnologies have known a huge rise in the last 10 years and they are promised to continue their development. The unique properties arising at the nanoscale allow many applications in microelectronics, catalysis, physics, optics, cosmetics, drug delivery, imaging. Consequently exposure to nanomaterials will concern not only occupational settings where NPs are produced and used for inclusion in different items but also the general population and environment taking into account the life cycle of NPs from manufacturing to recycling and to final disposal.

Concern about NPs raises from the knowledge of the toxicologists and pathologists about the toxicity of airborne particles. Epidemiological studies have clearly underlined the link between cardiorespiratory health outcomes and exposure to particulate pollution in urban areas [21]. Especially the finest fraction of the aerosol constituted of nanoscale particles is considered to be responsible for such outcomes [44]. Their small size allowing them to reach the deep lung and to cross their air-blood barrier, their biopersistence, their surface reactivity, and their chemical composition altogether contribute to their toxicity. Engineered nanomaterials being elaborated to exhibit specific properties and reactivity, it is likely that these peculiar properties could induce unwanted effects toward biological systems.

In contrast to the fast development of nanotechnologies providing a large variety of nanomaterials for a great diversity of applications, nanotoxicology is far less advanced. Policy makers face social demand of a better knowledge of risk assessment of nanomaterials. For a sustainable development of nanotechnologies, there is an urgent need to ascertain whether exposure to NPs can lead to possible health risks for workers and consumers. Many toxicological investigations are currently performed in order to identify NP characteristics that are the most important to explain their fate and biological reactivity among size, surface area

(taking into account the porosity and roughness of the particle), shape, bulk chemical composition (including the crystal structure), surface chemistry (including lipophilicity as well as surface charge or coatings), and surface reactivity which is linked to the two latest (surface area and surface chemistry). For a defined chemical composition, reaching small size (<30 nm) produces dramatic changes in the crystalline structure that enhances their interfacial reactivity [3].

Our present review will focus on inorganic NPs including those based on metal oxides (iron oxide, cerium oxide, titanium dioxide, silicon dioxide, etc.) and metals (gold and silver). They represent a great amount of NPs currently used, such as TiO_2 and ZnO in sunscreens, SiO_2 in microelectronics, drug delivery and food additives, Ag in clothes and in food packaging. In addition this review will be restricted to the consideration of unintended exposure that could affect workers as well as the general population including susceptible people.

A key question in nanotoxicology concerns the ability of NPs to interact and cross biological barriers at the route of exposure. In case of efficient translocation, NPs have access to blood circulation allowing a systemic toxicity. Cellular mechanisms behind NP-induced effects are generally attributed to their potential to induce an oxidative stress. However, identification of the interaction of NPs with specific proteins could provide clues of new mechanisms of toxicity.

9.2 Fate of Nanoparticles

9.2.1 Main Routes of Exposure to Nanoparticles

The main routes of entry of NPs are the respiratory tract, the digestive tract, and the skin due to the presence of NPs in the atmosphere, in water or food and in cosmetics or wears.

The respiratory tract with its large surface area (around 120 m^2) represents an important surface of exchange with the air that in addition of the 15 m^3 of air that one adult breathes each day favors the penetration of NPs. The respiratory tract is subdivided in the airways that are involved in the air conduction and the alveoli where gas exchanges take place. The airways from the nose to bronchioles are covered by a mucociliary epithelium that is responsible for air clearance. The mucus produced by epithelial cells, covers the epithelium and traps microorganisms, dusts, and NPs present in the air. Due to the ciliary beat provided by ciliated cells, this mucus sheet is removed either by expectoration or ingestion. For an efficient mucociliary clearance, the viscoelastic properties of the mucus must be preserved as well as the efficiency of cilia activity. Recent studies underlined that the efficiency of the protection afforded by human mucus is different according to the types of metal oxides, ZnO being crossing better the mucus [38] and that positively charged functionalized polystyrene NPs impede mucin gel swelling by crosslinking mucins [11]. Alveoli are delimited by a very thin epithelium

composed of large and flattened type I alveolar cells making the distance between the air and the blood very short (2 μm). They are associated to type II alveolar cells involved in the production of surfactant, a tensioactive substance avoiding alveoli collapse at each breath. Moreover in alveoli, macrophages are present to phagocyte microorganisms or dusts that succeed to reach this distal part of the lung. There are some evidences that the phagocytic properties of macrophages toward NPs are less efficient than toward larger particles [63]. The deposition of NPs in the respiratory tract is described from predictive models [63] established from data obtained by the International Radiological Protection Commission. According to their size they will deposit in different regions of the lung: 5 nm diameter NPs can deposit all along the respiratory tract whereas those over 5 nm mainly deposit in the alveoli and those below will deposit in the upper airways. Such models having been established for normal respiration at rest, it will likely be different for an exercising person as well as for people suffering from respiratory diseases in which air flux are reduced and clearance mechanisms less efficient.

The gastrointestinal (GI) tract is another organ exhibiting also a large surface (240 m^2 only for the gut) to allow an efficient absorption of nutrients. The intestinal epithelium is composed of enterocytes that present microvillosities at their apical side to increase their exchange surface as well as goblet cells producing mucus to protect the intestinal mucosa. In addition, some Peyer plaques are dispersed in the epithelium and are involved in local immunity. They contain specialized cells, M cells, used by microorganisms to get access to the underlying lymphoid follicles. They are considered as a potential route of entry of NPs due to their transcytosis properties.

By comparison with the respiratory and digestive tracts, the skin has a limited surface (1.5–2 m^2). It is composed of the epidermis and the dermis. The epidermis has a protective function. It is a stratified epithelium and it is not vascularized. The outer layer is made of dead cells having high keratin content and forming the stratum corneum that is a waterproof and soft layer. The inner layer contains living cells responsible for the epidermis renewal. The dermis is a conjunctive tissue in which are the skin annexes such as sweat and sebum glands, follicle glands and blood vessels.

9.2.2 From the Bare NPs to "Hybrid Bionanoparticles"

Whatever the route of exposure, before reaching the epithelial barrier, NPs will get in contact with biological fluids such as the lung lining fluid in the respiratory tract, the sweat on the skin, the feed bolus and mucus in the GI tract, and the plasma in case of a systemic translocation. NPs being characterized by a high surface energy, they will immediately interact with the whole set of molecules present in their surrounding forming the so-called "corona". It results from the molecular interactions between chemical groups on the NP surfaces and the amino acid residues of the proteins or the lipids [31]. Consequently these interactions modulate the properties of the bare NP and what the cells interact with is not directly the NP

surface but the NP surrounded by its corona [89] corresponding to a hybrid bionanoparticle.

The size and surface properties (charge, curvature, and hydrophobicity) are NP determinants strongly involved in the formation of the so-called "hard" corona comprising proteins exhibiting high affinity with NPs [52]. The composition of the corona can be modified when the NP moves from one environment to another one as recently demonstrated by Lundqvist comparing the protein corona composition of either silica NPs or polystyrene NPs incubated with cytosolic fluid before or after an incubation in human blood serum [51]. For a given NP, its protein signature is environment dependent [83] but still keeps a fingerprint of its history.

In addition to the NP characteristics, the physicochemical properties of the proteins such as the surface charge and resistance to structural alteration will determine their affinity for the NP surface. Comparing the interactions of four model proteins on silica NPs, Turci et al. have underlined that both the distribution of charges at the surface of the protein and the structural protein stability control the extent of coverage of the NP surface [87]. However, in a more complex biological media, such as blood plasma, neither protein size nor charge significantly determine the protein composition of the corona on silica NPs that is also not simply correlated with the relative abundance of protein in the plasma [85].

According to the corona composition, NPs may exhibit different behaviors and toxicity. For example, magnetite NPs are more taken up by alveolar macrophages when the exposure is made in presence of the surfactant protein A, one of the protein of the alveolar surfactant, than in presence of albumin, the prevailing protein in plasma [69]. Gold NPs exhibit different toxicity toward epithelial cells according to the culture medium in which they have been suspended [54].

9.2.3 Cellular Internalization of Nanoparticles

The small size of NPs allows their interactions with biological entities, such as cells, cellular components, bacteria, and viruses which are not possible at the bulk scale. When a NP interacts with the plasma membrane of a cell, its entry inside the cell is generally achieved through a process termed "endocytosis" but uptake by non-endocytic processes could also occur. An understanding of the mechanisms of NPs internalization is essential as NPs uptake can greatly contribute to NPs toxicity especially for insoluble NPs. A better knowledge of the determinants involved in NP uptake could provide clues to create safe NPs.

9.2.3.1 Cellular Internalization by Endocytosis

Endocytosis is a multiple stage process. The cargo, here the NP, is engulfed in membrane invaginations that will pinch off to form membrane-bound vesicles, known as endosomes (or phagosomes in case of phagocytosis). These endosomes

deliver the cargo to various specialized vesicular structures, which enables sorting of cargo toward different destinations. Finally, the cargo is delivered to various intracellular compartments, recycled to the extracellular milieu or crosses cells (a process known as "transcytosis" in polarized cells).

In general, endocytosis can be divided into two categories: phagocytosis (the uptake of large particles) and pinocytosis (the uptake of fluids and solutes). Phagocytosis is a process by which macrophages engulf particles as large as 20 μm [26]. This process is characteristic of specialized professional phagocytes, such as macrophages, neutrophils, monocytes, and dendritic cells but could occur in non-professional phagocytes, such as fibroblasts, epithelial, and endothelial cells, but to a lower extent [65]. Pinocytosis is present in all cell types and has different mechanisms that depend on the cell origin, function, cargo, and receptors involved. Different classifications of pinocytosis are proposed. The most recent approach is based on the proteins involved in the different endocytic pathways: clathrin-dependent endocytosis (also known as clathrin-mediated endocytosis) and clathrin-independent endocytosis [17]. The clathrin-independent pathways are further classified as (1) caveolae-mediated endocytosis, (2) clathrin- and caveolae-independent endocytosis, and (3) macropinocytosis. Clathrin- and caveolae-independent pathways are sub-classified as Arf6-dependent, flotillin-dependent, Cdc42-dependent, and RhoA dependent endocytosis [55].

Endocytosis has been shown as the main mode of NP uptake in some studies [69, 76, 79]. However, it is difficult to draw general conclusions about cellular uptake of NPs, as the rate and mechanism of uptake seem to be cell-type dependent, cell-density dependent, and vary between NPs of different size, charge, and other surface properties.

In many studies uptake of NPs has been shown to be an energy-dependent process. The treatment of cells with NPs at 4 °C abolishes NP uptake and no NPs are found inside the cells [43]. However, the low temperatures not only slow metabolism but also change membrane rigidity. To be sure that the absence of NPs inside the cells is due to energy requirements cells can be exposed to metabolic inhibitors, particularly to NaN_3, known to block production of ATP. For some manufactured NPs this has completely blocked uptake confirming the importance of ATP for NP endocytosis process [79]. However, if NPs do enter the ATP-depleted cells, it indicates that some passive diffusion through cell membrane can take place.

Considering identification of particular endocytic pathways implied in NP uptake it is important to take into consideration the size of vesicles that can be formed. Indeed larger NPs or aggregates of NPs can be taken up by endocytic processes that lead to the formation of large vesicles (phagocytosis or macropinocytosis). Smaller NPs may also enter the cells by clathrin-mediated endocytosis (which lead to vesicle sizes between 150–200 nm), or caveolae-mediated endocytosis (forming vesicles of less than 100 nm). Indeed, no studies have unambiguously demonstrated the existence of larger caveolae able to accommodate uptake of larger NP aggregates. In addition the use of different endocytic inhibitors allowed identifying the endocytic pathways involved in NP uptake. It has been

shown that different NP types are taken up by different endocytic pathways, and even the same NPs exhibiting different surface properties are taken up by different mechanisms. Citrate stabilized gold NPs were predominantly taken up by macropinocytosis leading to the formation of large vesicles, while PEG-coated gold NPs were taken up by clathrin- and caveolae-dependent endocytotic pathway [6].

9.2.3.2 Cellular Internalization by Non-Endocytic Mechanisms

Internalization of NPs by non-endocytotic mechanisms has been shown for gold and TiO_2 NPs in red blood cells, used as a model of non-phagocytic cells as they do not have phagocytic receptors and no actin myosin system [68]. By sophisticated microscopic methods it has been shown here that NPs are internalized but never membrane bound. The mechanisms allowing the NPs to enter into these cells are not yet elucidated. This translocation of NPs may occur by unspecific means, including diffusion, transmembrane channels, and electrostatic, Van der Waals, hydration forces, or steric interactions, also called "adhesive interactions" in adhesion science and technology [66].

9.2.3.3 Role of Nanoparticle Characteristics in Their Internalization

Cells were generally found to internalize NMs in a *size*-dependent manner, with smaller NPs being better taken up. However, there are several reports showing that manufactured NPs in the size-range of 20–50 nm size are taken up more rapidly than smaller or larger NPs [12, 39, 40]. Interestingly, NP's uptake by macrophages seems to be less efficient as the size of NPs decreases. One of the main mechanisms to remove insoluble micrometer-sized particles from the lung surfaces is by phagocytotic uptake by lung macrophages. However, this is not the case for inhaled nanometer-sized particles that deposit along the entire respiratory tract. It seems that surface macrophages are not able to efficiently phagocytose NPs [24].

Particle shape has been considered to play an important role in both cellular interactions with NPs and the systemic distribution of NPs. However, general conclusions cannot be drawn as different NPs have different behavior in living systems. For silica NPs it has been shown that particles with larger aspect ratio (AR, the ratio of length to width) were taken up in larger amounts and had faster internalization rates [34]. By contrast cellular uptake studies of gold nanorods (GNRs) show that there is a decrease in uptake as the aspect ratio of GNRs increases [13]. Uptake of magnetic nanowires resulting from the controlled assembly of iron oxide ($-Fe_2O_3$) NPs, with diameters of 200 nm and lengths between 1 and 40 μm has also been reported in mouse fibroblasts [70].

Because particles with a positive *charge* will bind to the negatively charged cell surface, one would expect positively charged particles to be endocytosed more efficiently than negatively charged particles [88]. Amino-functionalized NPs of a size of 100 nm show a more than 40-fold increase in uptake compared to

unfunctionalized polystyrene [53]. However, carboxy functionalized NPs show also an enhanced uptake which might be due to interactions with molecules, for example, proteins that will bind to the NP surface. Taking into account all molecules that NPs will meet on their way from external milieu to the cell membrane, the bare NP as it has been produced and characterized is not what the cell will see. Presence of hard corona is important because of the interaction of adsorbed proteins with different membrane receptors susceptible to affect the internalization process itself. Indeed it has been demonstrated that the characteristics of the serum (heat inactivated or not) modulate the uptake of polystyrene NPs by alveolar epithelial cells in vitro [47].

9.2.3.4 Localization and Fate of Internalized NPs: Biopersistence, Dissolution, Exocytosis

NP localization and intracellular fate is dependent on NP type and properties, but mostly they move through the endosomal system from early to late endosomes to end up in lysosomes, organelles where they will be digested or stored [1, 75]. Soluble NPs, such as ZnO or CuO, can be dissolved in the cell culture medium but also in the lysosomes where acidic conditions favor their dissolution, thus exerting immediate cytotoxic effects mostly by ions that are released from these NPs [9, 82]. On the other hand, non-soluble NPs, such as TiO_2 or CeO_2, have a better biocompatibility, and they can even have protective effect [92]. Higher cytotoxicity of metal NPs compared to their micrometer size counterparts can thus be explained by a higher uptake rate of NPs that, once in the endosomal environment, can be better dissolved.

Manufactured NPs are sometimes found in the cell nucleus, but this finding is rather controversial. For example, this was the case for silica NPs found in the nucleus of the human epithelial HEp-2 cells [11]. In the work of Al-Rawi et al. the same silica NPs could not be detected neither in the nucleus nor in the mitochondria or in any cellular organelle of the HeLa cells [1]. This can be explained by difference in the cell type studied and by the fact that the experiment was performed on proliferative cells. To the best of our knowledge, no studies have identified the mechanisms involved in the presence of NPs in the nucleus: diffusion through the nucleus membranes, passage through nuclear pores, or incorporation into the nucleus at the end of mitosis. This last mechanism could explain differences among experiments with the same type of NPs according to the proliferative status of the cell type used. Singh et al. have recently reported that fluorescent cerium oxide NPs co-localized with mitochondria, lysosomes, and endoplasmic reticulum as well as being abundant in the cytoplasm and the nucleus of the normal human keratinocyte cell line (HaCat) [78]. On the other hand, Asati et al. have shown that ceria NPs are localized in the cytoplasm or lysosomes of different normal and cancer cell lines according to their charges and that this cellular localization will determine their cytotoxicity [2].

There are few reports about possible exocytosis of NPs from the cells and controversies according to the studies. Shapero et al. considered that there is no evidences of the export of silica NPs from the lung cell line A549 [75]. They consider that the apparent decrease of uptake is due to cell division: NPs are divided between daughter cells after the cell division. However, recently the exocytosis of mesoporous silica NPs from mammalian cells is demonstrated for the first time. The differences in the degree of exocytosis of mesoporous silica NPs between healthy and cancer cells is also reported in this study [80]. Exocytosis of gold NPs has also been reported recently [13]. Exocytosis could be one of the mechanisms explaining the translocation of NPs across epithelial barriers for further access to blood and biodistribution that has been already demonstrated (see Sect. 9.2.5).

9.2.4 Translocation of Nanoparticles Through Epithelial Barriers

The respiratory tract as well as the digestive tract and skin are lined by epitheliums that act as physical barriers. Functions of epithelial cells include secretion, selective absorption, protection, transcellular transport, and detection of sensation. However, their efficiency to protect body organs especially the lungs from the inhaled NPs is questionable since there is consistent evidence for cardiovascular effects associated with increased concentrations of ambient fine and ultrafine particles due to their ability to reach the blood stream [25]. Recent experimental studies with different NPs confirm their presence in the blood and secondary organs after inhalation or ingestion (see Sect. 9.2.5).

Conducting quantitative translocation studies in humans and animals has its limitations so most data are obtained by experiments done in vitro on different cell lines. Cultures on permeable membranes have been developed using different respiratory epithelium such as the Calu-3 (human lung epithelial) cell line or primary rat type II pneumocytes [28, 93] to study the translocation of NPs through the air-blood barrier. With such model, no translocation of quantum dots occurred through the intact epithelia regardless of the NP surface charge. A translocation was only observed in case of disruption of the cell–cell barrier by an oxidant insult [27]. By contrast it was shown a trafficking of fluorescently labeled polystyrene NPs across a monolayer of rat alveolar cells that was more important for positively charged NPs than negatively charged ones [93]. In addition, this translocation was shown to be transcellular but not via the known major endocytic pathways.

A more sophisticated model has been developed for in vitro study of the translocation of NPs through the air-blood barrier including the most relevant cell types of this barrier. It consists of a triple cell co-culture model established in two compartment cell chambers (Transwell system) using the widely used A549 human alveolar epithelial cell line or bronchial epithelial cells (16HBE) co-cultured with human

blood monocyte-derived macrophages on the apical side of the porous membrane and directly exposed to NPs and dendritic cells cultivated on the basal side of the membrane [67]. They demonstrated the interplay between these different cell types to process NPs. Especially they have shown that dendritic cells extend processes between epithelial cells through the tight junctions to collect NPs in the "luminal space" and to transport them through cytoplasmic processes between epithelial cells across the epithelium or to transmigrate through the epithelium to take up particles on the epithelial surface. They concluded that dendritic cells and macrophages appear to handle in a very effective manner a transcellular transepithelial transport of NPs from the "luminal side" to the base of the membrane. By this approach translocation of NPs in vitro was documented, however, no quantification of translocation could be made as observations were obtained by electron microscopy [5].

Recently Huh et al. developed a "lung-on-a-chip" device to study NP translocation [35]. In a device made of compartmentalized microchannels, alveolar epithelial cells are grown on the upper surface of a flexible microporous membrane whereas endothelial cells are grown on the other side of this membrane. Alveolar cells are exposed to air containing or not NPs whereas endothelial cells are fed with a flux of culture medium containing neutrophils. By applying vacuum to the side chambers of the culture chamber, there is a mechanical stretching of the membrane recreating the physiological breathing movements. The translocation of fluorescent NPs is 4-fold more important in case of the mechanical stretching and NPs can be observed in the underlying endothelial cells: more than 70 % of cells within mechanically active epithelium and endothelium internalized NPs, whereas this fraction was lower by a factor of 10 in the absence of strain [35].

Considering skin exposure to NPs, TiO_2 and ZnO have been extensively studied as they are constituents of sunscreens in which they act as effective barriers against ultraviolet damage to the skin. This raises the question about the possibility of penetration of NPs to deeper regions of the skin. In experiments where normal human skin was repeatedly exposed to TiO_2, these NPs either stay on the epidermis or are present in the strateum corneum but never penetrate or cross the viable layer of the epidermis. In addition, it was shown that NPs can accumulate and persist in hair follicles but until now there is no evidence of a passage toward the dermis by this route [9].

Whereas the healthy skin seems to be an efficient barrier toward NPs, other conditions such as damaged skin (wounds, erythema, eczema.) and flexures sites are suspected to be more susceptible to NP translocation. In vitro experiments with human skin submitted to mechanical flexions (20 flexions of 45 ° per min) have shown that in 50 % of skin samples flexions favor a low epidermal and dermal absorption of fluorescent NPs (0.5 and 1 μm) after a 60 min exposure whereas particles of larger size (2 and 4 μm) stayed localized on the strateum corneum [86]. A recent study investigated the penetration of TiO_2 and ZnO in sunscreen formulations by UVB sunburned skin in pigs [59]. In vitro studies performed to evaluate the presence of NPs in the perfusate concluded to minimal transdermal absorption. In vivo experiments revealed that TiO_2 NPs penetrated the upper layers (7 layers) of the stratum corneum of the normal skin whereas they penetrate deeper

(13 layers) into UVB-damaged epiderma. Completing microscopic investigations with TOF–SIMS analysis concluded to the presence of TiO_2 within epidermis and superficial dermis. Coated and uncoated Zn NP was localized to the upper layers of the stratum corneum whatever the type of skin. This study concluding to the slight enhancement of the penetration of TiO_2 and ZnO in sunscreen formulations by UVB-damaged skin but no transdermal absorption is not completely confirmed by another one focusing on ZnO. Comparing on human volunteers, two sunscreens ("nanosunscreen" containing 19 nm ZnO NPs and "bulk sunscreen" containing >100 nm ZnO particles) enriched with the stable isotope ^{68}Zn [29] in order to detect low quantities of Zn and distinguish naturally occurring Zn from dermally absorbed Zn, it was shown that the radioactive tracer was detected in blood after the 5-day application period and continued to increase beyond the 5-day application phase in contrast to those in urine. Levels of ^{68}Zn in blood and urine were higher for subjects receiving the nano sunscreen than for those receiving the bulk sunscreen. However, due to the solubility propensity of ZnO NPs, it is not known whether ^{68}Zn has been absorbed as ZnO NPs or soluble Zn or both.

9.2.5 Biodistribution of Nanoparticles

In order to investigate the body distribution of NPs, in vivo models are used where animals are exposed intravenously or by more relevant routes of exposure considering occupational or general exposition such as the respiratory and digestive tract. Body distribution should provide the answer about possible accumulation and harmful effects of NPs in the secondary organs after potential translocation from the lungs or the intestine to the systemic circulation.

Different experiments performed by intravenous injection of different types of NPs have underlined the role of their physico-chemical characteristics. For example, the biodistribution of gold NPs of different sizes (10, 50, 100, and 250 nm) was quantified in different organs by inductively coupled plasma-mass spectrometry after injection of the NPs in the rat tail vein. It reveals that it is size dependent with a widespread distribution for the smallest one. The 10 nm-gold NPs were present in various organ systems including blood, liver, spleen, kidney, testis, thymus, heart, lung, and brain, whereas the larger particles were only detected in blood, liver, and spleen [19]. Comparing the biodistribution of 1.4 nm with 18 nm radiolabeled-gold NPs after intravenous injection in rats, it was demonstrated that the 18 nm gold NPs were almost completely removed from the blood within 24 h by trapping predominantly in liver and spleen. In contrast, the 1.4 nm gold NPs were excreted through the kidneys as well as the hepatobiliary system, with significantly lower accumulation in liver and spleen [74]. In another study using radiolabeled gold NPs of 5 nm coated or not with poly(ethylene glycol), it was shown that NPs accumulated mostly in liver and spleen, with different kinetics dependent on PEG stabilization of NPs [49]. Gold NPs functionalized with long PEG chains exhibit a prolonged blood circulation and are cleared by the hepatobiliary pathway. A study in mice after intravenous injection

of fluorescent-dye labeled silica NPs of different sizes (50, 100, and 200 nm) confirmed the role of particle size on tissue distribution and excretion. As particle sizes increased, more particles were trapped by macrophages in the spleen and liver and remained there until 4 weeks after the single injection. Smaller particles were found in the urine and feces at higher concentrations and were cleared rapidly [14].

Concerning more realistic routes of exposure, a recent study investigated the biodistribution of radiolabeled gold NPs after oral ingestion [72]. 1.4, 5, 18, 80, and 200 nm gold NPs bearing negative charges were compared with 2.8 nm NPs bearing positive charges after intra-oesophageal instillation in rats. The highest accumulation in secondary organs was observed for the smallest NPs and those bearing negative charges. Notably, a higher accumulation in brain and heart was observed with 18 nm only.

The inhalation route has been studied in different studies using the intratracheal instillation or inhalation of radiolabeled NPs. Whatever the type or the dose tested (nanoceria 24 nm at 1 mg/kg [30], Fe_2o_3 144 nm at 16 mg/kg [95], gold NPs coated or not with PEG with 11–31 nm at 140 μg/kg [49], radioactively labeled iridium aerosol 17–20 nm at [73], it was observed a low but fast (10 min) translocation toward blood circulation and secondary organs such as liver and spleen that exhibit the highest level of accumulation. In all these studies, it was shown a high pulmonary retention of NPs that is sustained since 63 % of nanoceria are still present in the lungs 28 days after the exposure [30]. It has been shown that the major fraction of inhaled radiolabeled iridium NPs disappeared from the epithelial surface and were relocated within the epithelium and in the interstitium over 6 months. Interestingly it was also shown that from this interstitial NP fraction, Ir NPs continued to re-entrain back onto the lung epithelium adding to the macrophage-mediated clearance transport to the larynx and fecal excretions. Ir NP translocation into circulation was measurable, but the fraction was much less than that re-entraining back onto the epithelium [73].

The role of the physico-chemical characteristics of NPs in their ability to cross the air-blood barrier was investigated after bronchial instillation of a series of 16 near-infrared (NIR) fluorescent NPs exhibiting differences in size, charge, shape, and chemical composition [16]. NP trafficking toward blood and secondary organs was studied owing to real-time NIR fluorescence imaging. NPs translocation is primarily governed by the size of NPs, irrespective of chemical composition, shape, and conformational flexibility. A threshold size of 34 nm has been determined. Below this size, NP charges controlled the amount of translocation: NPs with cationic charges exhibit the lowest translocation. NPs <6 nm with zwitterionic charges reach the blood stream in 3 min and are rapidly cleared by the renal route in 30 min.

Altogether these studies underline that NPs mainly remain at the site of exposure and only a low amount translocates. Smaller NPs can be better eliminated, but are also more widespread in secondary organs. Evidences of the presence of NPs in secondary organs after single exposure underline the urgent need to investigate the health impact resulting from repeated exposures to NPs.

9.3 Major Health Effects of Nanoparticles

Due to the recent use of engineered NPs, epidemiological studies are not yet available. However, huge epidemiological data have been published on the health effects of airborne particulate matter (PM) stressing their short as well as long-term effects on chronic respiratory diseases and cancer [64]. In urban and industrial locations, these PM are mainly resulting from anthropogenic activities and are characterized by a heterogeneous size distribution and composition. In urban areas, combustion processes mainly contribute to the production of PM that exhibit a carbonaceous core with adsorbed organic compounds and metals and range in the fine and ultrafine modes. Ultrafine particles are NPs and a recent expert elicitation underlines their role in the cardiorespiratory effects [44], which have been related to particles translocation and/or systemic inflammation.

Several animal studies have been performed to identify the health effects of NPs. A frequent weakness in these studies is the relevance of the doses used that are generally very high to observe an effect and must only be considered as hypothesis-forming. In addition there are numerous discussions among toxicologists about the most appropriate metric for assessing the toxic dose of NPs. Classically in toxicology dose is related to the mass. However, in the field of NPs other metrics would be more relevant such as the number of NPs or the surface area [25, 62]. For low solubility particles, the inflammatory effects were related to surface area [58].

9.3.1 Respiratory Effects

Inhalation exposure to NPs being a technological challenge, many animal studies have used intranasal or intratracheal instillation. The relevance of such studies is limited as in such exposure, NPs are suspended in a fluid and animals are not exposed to an aerosol as in the real life. Some inhalation studies using industry-relevant and property-controlled NPs have shown the ability of Fe_2O_3 NPs to cause both pulmonary and cardiovascular effects whereas SiO_2 and Ag have no effect [81]. A 90 days inhalation (6 h/day, 5 days/week) of gold NPs (4–5 nm) by rats allows determining a lowest observed adverse effect level (LOAEL) of 20 $\mu g/m^3$ for which lung histopathological and functional changes are observed and a no observed adverse effect level (NOAEL) of 0,38 $\mu g/m^3$ [84].

The numerous studies performed by intratracheal instillation have underlined that the most predominant effect of NPs is the induction of a lung inflammation characterized by the increase of immune cells, frequently macrophages and neutrophils, in the bronchoalveolar lavage and the increased release of pro-inflammatory mediators (cytokines and chemokines). According to studies, the dose, size, surface reactivity, and composition are underlined as the determinant parameters. Furthermore comparison of the inflammatory potential of a series of

metal oxide NPs in rats exposed at equal surface area doses has shown that those that are inflammogenic (CeO_2, NiO, ZnO, CuO) exhibit unique inflammatory footprints in rats in terms of time course, types of infiltrating cells and inflammatory mediators [15]. For example, a 24 h exposure to NiO NPs was associated to a neutrophilic/mild cytotoxic pattern that was greatly amplified after 4 weeks whereas CuO NPs induce rapidly a neutrophilic/eosinophilic/severe cytotoxic pattern that was resolved after 4 weeks. These results suggest the possibility of inducing different pathologies according to the type of NPs.

Chronic inflammation resulting from biopersistent NPs and/or repeated exposures could lead to pulmonary diseases or exacerbation of existing lung disorders making susceptible people more reactive to NPs. Some studies have investigated the response to NPs of compromised animals. In a mouse model of asthma induced by exposure to diisocyanate, TiO_2 and gold NPs have been shown to aggravate lung inflammation and airway hyperreactivity [37].

9.3.2 Cardiovascular Effects

The possibility that NPs could have cardiovascular effects results from the numerous epidemiological and toxicological studies performed on human volunteers exposed to combustion derived-airborne ultrafine particles [56]. These cardiovascular effects could result from (i) particles translocation reaching the blood stream where they can directly interact with cardiovascular tissues to induce injury or inflammation, (ii) the release of inflammatory mediators at the site of deposition such as the lung that further produce a systemic inflammation (iii) NPs deposited in the lung that could act through a neural mechanism to alter cardiac autonomic function.

Inhalation of TiO_2 by rats impairs endothelium-dependent vasodilation in coronary arterioles due to microvascular oxidative stress [45, 46]. The inhalation of nickel NPs by mice (100, 150, or 900 $\mu g/m^3$) for 1, 3, or 5 consecutive days (5 h/day) [18] induce acute endothelial disruption and alter vasoconstriction and vasorelaxation. The long-term effects (79 $\mu g/m^3$, 5 h/day, 5 days/week for 5 months) of this nickel NPs in hyperlipidemic mice (apoprotein E-deficient) exacerbate the progression of atherosclerosis associated to an inflammation [41]. At that time no sufficient studies on a larger panel of NPs have been used in order to attribute such effects on a specific NPs characteristic.

9.3.3 Neurologic Effects

The demonstration of a translocation of NPs to the brain either from the olfactory epithelium in the nose or from the blood has stimulated studies to investigate the consequences of such exposure on the central nervous system [33]. Due to the low translocation rate, acute effects are not expected but long-term effects are likely

especially for biopersistent NPs [77]. For example, the intranasal instillation of four different types of TiO_2 particles every day for 30 days in mice induce morphological changes of neurons in the cerebral cortex and a disturbance of the monoamine neurotransmitter related to the physico-chemical characteristics of the NPs [94]. In another study, again concerning TiO_2 NPs, an intragastric administration to mice for 60 days induces impairment in spatial recognition memory associated to an accumulation of NPs and the induction of an apoptosis in the hippocampus [32].

9.4 Cellular Mechanisms of Toxicity

The oxidative stress is considered to be one of the underlying mechanisms of toxicity induced by NPs [60]. However, not all studies fit with this paradigm. Recent studies underline the role played by NPs-protein interactions in triggering other mechanisms of toxicity.

9.4.1 Role of the Oxidative Stress

In aerobic organisms, ROS are either generated within cells as by-products of necessary reactions such as in the mitochondria during oxidative phosphorylation processes or are intentionally generated, either in molecular synthesis or breakdown, as part of a signal transduction pathway or as part of a cell defense mechanism such as NADPH oxidase. To avoid the potential of ROS to cause oxidative deterioration of protein, lipid, and DNA, cells are equipped with a set of antioxidant enzymes and antioxidants molecules. When disequilibrium occurs between the amount of ROS and antioxidants, an oxidative stress arises in cells due to the loss of redox homeostasis.

NPs as direct or indirect generators of ROS

NPs are prone to disturb redox homeostasis in different ways: (i) their intrinsic ability to produce ROS, (ii) their uptake by cells leading to ROS production, and (iii) their ability to induce an inflammatory response.

The intrinsic ability of NPs to produce ROS in abiotic conditions can be due to their chemical composition such as the presence of redox active metals as well as to their surface properties due to the presence of oxidative groups functionalized on NPs or surface defects. Some particles, such as TiO_2 can be photoactivated and generate electron hole pairs [60]. Different assays are available to compare different NPs for their free radical generation in acellular conditions [4] such as electron paramagnetic resonance assay [50] and the dithiothreitol (DTT) assay. In this assay DTT oxidation is monitored resulting from the ability of redox active compounds associated with NPs to transfer electrons from DTT to oxygen generating superoxide.

NPs lacking intrinsic ability to produce ROS, can however, induce the intracellular production of ROS when they interact with target cells by mechanisms not clearly understood. Some NPs have been shown to interfere with the permeability transition pores in mitochondria increasing the production of superoxide anion. Others produce a depletion of the cellular glutathione, the most important antioxidant molecule [48, 60, 91]. A nice study from Hussain et al. [36] have clearly shown that two different NPs, carbon black, and TiO_2 both induces an oxidative stress dependent apoptosis of bronchial epithelial cells linked for carbon black NPs to their intrinsic ability to produce ROS and for TiO_2 to induce a lipid peroxidation.

Due to the role of oxidative stress in NPs cellular effects, models are currently developed to predict the oxidative stress potential of NPs especially oxide NPs [8] using reactivity descriptors to build the energy band structure of oxide NPs. Such approach could be the first step of a tier strategy in NPs testing but needing complementary approaches to predict heath effect. Indeed Lu et al. nicely demonstrated that the potency of oxide NPs in generating free radicals in vitro did not predict their inflammogenic potency [50].

NPs can indirectly produce oxidative damages by inducing an inflammatory response. The exposure to NPs can produce a pro-inflammatory response of target cells characterized by the release of pro-inflammatory mediators that will recruit inflammatory cells at the site of exposure. Such immune cells (macrophages and neutrophils) produce significant amount of ROS.

Oxidative stress-related effects

The consequences of the oxidative stress are dependent on the level of this stress according to a hierarchical response to oxidative stress developed by Nel [91].

At low level (Tier 1), ROS activate signaling pathways leading to the expression of oxidative stress responsive genes involved in the cellular defence. Detoxifying enzymes and antioxidant enzymes such as heme oxygenase-1 (HO-1), NADPH quinone oxidoreductase, glutathione-S-transferase, and superoxide dismutase, are induced as a result of the activation of the nuclear factor-erythroid 2-related factor 2 (Nrf2) transcription factor. Nrf2 binds to the antioxidant responsive elements (ARE) within the promoter of these enzymes and activates their transcription. Inactive Nrf2 is retained in the cytoplasm by association with Keap1, a protein that functions as a molecular sensor detecting changes in cellular redox state. Upon exposure of cells to oxidative stress, Keap1 is oxidized, allowing Nrf2 dissociation and translocation toward the nucleus to bind AREs and transactivates detoxifying enzymes and antioxidant enzymes. The activation of the Nrf2 pathway resulting to the induction of HO-1 expression has been demonstrated in bronchial epithelial cells exposed to CeO_2 NPs [22] but not in a model of mouse macrophages (RAW 264.7) with CeO_2 or TiO_2 NPs where only the soluble ZnO NPs induce HO-1 expression [92]. In another cell type, blocking Nrf2 expression increased apoptosis in Ag NPs-treated cells by reducing the level of HO-1 [42]. Such study illustrates that Nrf2-dependent HO-1 up-regulation plays a protective role in Ag NPs-induced cell death.

Whether the adaptive response induced by oxidative insult (Tier 1) is not sufficient to counteract the production of ROS, the level of oxidative stress

increases but is still moderated (Tier 2). ROS activate other signaling pathways leading to the expression of genes involved in the inflammation. The expression of various inflammatory cytokines, chemokines, and adhesion molecules results from the activation of the NF-κB transcription factor and its binding in the promoter of these inflammatory mediators. Inactive NF-κB is retained in the cytoplasm by association with IkBα in which phosphorylation favored by activation of upstream signaling kinases sensitive to oxidative stress will liberate NF-κB allowing its translocation to the nucleus. The exposure of macrophages (RAW 264.7) to different NPs (Ag, Al, Au) induced a significant generation of intracellular ROS associated to a nuclear translocation of NF-κB, an increased release of interleukin-6 (IL-6), and an induction of cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α) expression with a maximum effect for Ag NPs followed by Al whereas Au NPs has low inflammatory effect demonstrating the role of NPs composition in such responses [61]. Again Xia et al. comparing CeO_2, TiO_2, and ZnO NPs effects in mouse macrophages, has shown that only ZnO triggers Tier 2 by activating the JNK MAP kinases and inducing the release of TNFα likely in relation with its solubility [92]. The ability of ZnO to induce Tier 2 was also shown in another study using bronchial epithelial cells where it was observed that ZnO exposure induces rapid phosphorylation and degradation of IκBα, associated to an increase in NFκB transcriptional activity and increased IL-8 expression [90]. However ZnO-induced IL-8 expression being blocked in presence of inhibitor of endocytosis, authors claimed that ZnO effects were ZnO-uptake dependent. The need of the NPs internalization in the activation of stress-related signaling pathways was also shown both in macrophages and alveolar cells exposed to poor soluble SiO_2 NPs. It was observed an activation of the JNK and p38 MAP kinases leading to the activation of the ATF-2 transcription factor that was dependent to NPs size, coating, and uptake [57]. In the "lung-on-a-chip" device previously described (Sect. 9.2.4), the exposure of alveolar epithelial cells to 12 nm SiO_2 NPs for 5 h induced an increase in ROS production not only in alveolar cells but also in the underlying endothelial cells [35]. It was associated to the increased expression of ICAM-1 by endothelial cells allowing the capture of circulating neutrophils and their transmigration across the tissue–tissue interface and their accumulation on the epithelial surface. Such mechanical strain-induced oxidative responses were not observed when cells were exposed to other NPs such as gold NPs or polystyrene NPs.

When the level of oxidative stress becomes too important macromolecules, such as lipids, proteins, and nucleic acids are damaged and it can lead to cell death. Numerous studies using different cell types and NPs have shown their ability to induce cytotoxicity in a dose- and time-dependent manner. However, the mechanism involved was rarely studied. Comparing carbon black NPs and TiO_2 NPs, Hussain et al. have shown that both NPs induced a ROS-dependent apoptosis but through different molecular pathways. CB NPs induce apoptosis by a ROS-dependent mitochondrial pathway whereas TiO_2 NPs induce cell death through lysosomal membrane destabilization and lipid peroxidation [36].

9.4.2 Other Mechanisms Involved in NPs Toxicity

Recent experiments provide evidence of alternative explanations to oxidative stress to explain NPs toxicity.

As previously mentioned, NPs are decorated with a protein corona. The interaction of adsorbed proteins with different membrane receptors can trigger cascade of different cell signaling pathways without necessarily needing NPs uptake. The interaction protein/NPs can produce secondary and tertiary protein structure changes that can modify the protein function as shown for fibrinogen, a serum protein, when interacting with poly(acrylic-acid)-coated gold NPs [20]. This binding to NPs induces the unfolding of the fibrinogen domain containing the $\gamma^{377–395}$ sequence that is involved in the interaction with Mac-1 receptor expressed on the membrane of macrophages. It triggers a pro-inflammatory response in macrophages characterized by the increased release of IL-8 and TNF-α, two inflammatory cytokines.

The binding of cellular proteins to NPs following their uptake could give some clues to consider new mechanisms of toxicity. A study identifying the proteins adsorbed on NPs when they are incubated with a cellular extract revealed a different profile of adsorption according to the type of NPs considered [83]. Adsorbed protein classification according to their function revealed striking differences among NPs. For example, carbon nanotubes adsorb few proteins but exclusively cytoskeletal proteins whereas all TiO_2 NPs bind peroxiredoxin 1, annexin A2, and several ribosomal and cytoskeletal proteins. The relevance of such findings is highlighted by the recent study of Sanfins et al. [71] showing that carbon black NPs modifies the conformation of the arylamine N-acetyltransferase, a cytosolic enzyme involved in the xenobiotic metabolism. The enzymatic activity is inhibited and NPs-exposed bronchial epithelial cells exhibit a reduced ability to metabolize arylamines. This study provides evidence that NPs could interfere with metabolic pathways such as those involved in the xenobiotic metabolism. An interaction NPs-cytochrome P450, a phase I xenobiotic metabolism enzyme, has also been described [23]. Consequences of these interferences could be important in case of co-exposure NPs-toxics such as in occupational exposures.

9.5 Conclusion

In the last years a considerable progress has been made in the understanding toxicity of inorganic NPs although yet a conclusive overall picture of the hazard identification cannot be drawn. It appears that all inorganic NPs cannot be considered as a single hazard entity and each of them should be evaluated separately.

There are growing experimental data showing that whatever the route of exposure, NPs can enter the blood circulation and reach secondary organs allowing both local and systemic effects. Chronic exposure studies are now required to

examine the long-term fate and effects of NPs according to their biopersistence. Recent findings have also highlighted the formation of a protein corona on NPs producing a hybrid bionanoparticle which is the real object that cells interact with. A detailed knowledge of the dynamic formation of this protein coating around NPs is necessary in association with a better knowledge of its involvement in NP uptake to better predict the entry and behavior of NPs in the organism. Some studies have shown the role of specific interactions between NPs and proteins in cell activation or cell metabolism suggesting potential additional pathways of toxicity than the only role of oxidative stress.

This progress makes nanotoxicology still a very challenging field that should shed light in the next years on the NP determinants involved in their toxicity in order to propose NPs safe by design.

References

1. Al-Rawi M, Diabaté S et al (2011) Uptake and intracellular localization of submicron and nano-sized SiO_2 particles in HeLa cells. Arch Toxicol 85(7):813–826
2. Asati A, Santra S et al (2010) Surface-charge-dependent cell localization and cytotoxicity of cerium oxide nanoparticles. ACS Nano 4(9):5321–5331
3. Auffan M, Rose J et al (2009) Toward a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol 4(10):634–641
4. Ayres JG, Borm P et al (2008) Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential–a workshop report and consensus statement. Inhal Toxicol 20(1):75–99
5. Blank F, Rothen-Rutishauser B et al (2007) Dendritic cells and macrophages form a transepithelial network against foreign particulate antigens. Am J Respir Cell Mol Biol 36(6):669–677
6. Brandenberger C, Mühlfeld C et al (2010) Quantitative evaluation of cellular uptake and trafficking of plain and polyethylene glycol-coated gold nanoparticles. Small 6(15):1669–1678
7. Brunner TJ, Wick P et al (2006) In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol 40(14):4374–4381
8. Burello E, Worth AP (2011) A theoretical framework for predicting the oxidative stress potential of oxide nanoparticles. Nanotoxicology 5(2):228–235
9. Burnett ME, Wang SQ (2011) Current sunscreen controversies: a critical review. Photodermatol Photoimmunol Photomed 27(2):58–67
10. Chen EY, Wang YC et al (2010) Functionalized positive nanoparticles reduce mucin swelling and dispersion. PLoS ONE 5(11):e15434
11. Chen M, von Mikecz A (2005) Formation of nucleoplasmic protein aggregates impairs nuclear function in response to SiO2 nanoparticles. Exp Cell Res 305:51–62
12. Chithrani BD, Chan WC (2007) Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano Lett 7(6):1542–1550
13. Chithrani DB (2010) Intracellular uptake, transport, and processing of gold nanostructures. Mol Membr Biol 27(7):299–311
14. Cho M, Cho WS et al (2009) The impact of size on tissue distribution and elimination by single intravenous injection of silica nanoparticles. Toxicol Lett 189(3):177–183
15. Cho WS, Duffin R et al (2010) Metal oxide nanoparticles induce unique inflammatory footprints in the lung: important implications for nanoparticle testing. Environ Health Perspect 118(12):1699–1706

16. Choi HS, Ashitate Y et al (2010) Rapid translocation of nanoparticles from the lung airspaces to the body. Nat Biotechnol 28(12):1300–1303
17. Conner S, Schmid S (2003) Regulated portals of entry into the cell. Nature 422(6927):37–44
18. Cuevas AK, Liberda EN et al (2010) Inhaled nickel nanoparticles alter vascular reactivity in C57BL/6 mice. Inhal Toxicol 22(Suppl 2):100–106
19. De Jong WH, Hagens WI et al (2008) Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials 29(12):1912–1919
20. Deng ZJ, Liang M et al (2011) Nanoparticle-induced unfolding of fibrinogen promotes Mac-1 receptor activation and inflammation. Nat Nanotechnol 6(1):39–44
21. Dominici F, Peng RD et al (2006) Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295(10):1127–1134
22. Eom HJ, Choi J (2009) Oxidative stress of CeO2 nanoparticles via p38-Nrf-2 signaling pathway in human bronchial epithelial cell, Beas-2B. Toxicol Lett 187(2):77–83
23. Fröhlich E, Kueznik T et al (2010) Size-dependent effects of nanoparticles on the activity of cytochrome P450 isoenzymes. Toxicol Appl Pharmacol 242(3):326–332
24. Geiser M (2010) Update on macrophage clearance of inhaled micro- and nanoparticles. J Aerosol Med Pulm Drug Deliv 23(4):207–217
25. Geiser M, Kreyling WG (2010) Deposition and biokinetics of inhaled nanoparticles. Part Fibre Toxicol 7:2
26. Germain RN (2004) An innately interesting decade of research in immunology. Nat Med 10(12):1307–1320
27. Geys J, De Vos R et al (2009) In vitro translocation of quantum dots and influence of oxidative stress. Am J Physiol Lung Cell Mol Physiol 297(5):L903–L911
28. Geys J, Nemery B et al (2007) Optimisation of culture conditions to develop an in vitro pulmonary permeability model. Toxicol In Vitro 21(7):1215–1219
29. Gulson B, McCall M et al (2010) Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci 118(1):140–149
30. He X, Zhang H et al (2010) Lung deposition and extrapulmonary translocation of nano-ceria after intratracheal instillation. Nanotechnology 21(28):285103
31. Hellstrand E, Lynch I et al (2009) Complete high-density lipoproteins in nanoparticle corona. FEBS J 276(12):3372–3381
32. Hu R, Zheng L et al (2011) Molecular mechanism of hippocampal apoptosis of mice following exposure to titanium dioxide nanoparticles. J Hazard Mater 191(1–3):32–40
33. Hu YL, Gao JQ (2010) Potential neurotoxicity of nanoparticles. Int J Pharm 394(1–2): 115–121
34. Huang X, Teng X et al (2010) The effect of the shape of mesoporous silica nanoparticles on cellular uptake and cell function. Biomaterials 31(3):438–448
35. Huh D, Matthews BD et al (2010) Reconstituting organ-level lung functions on a chip. Science 328(5986):1662–1668
36. Hussain S, Thomassen LC et al (2010) Carbon black and titanium dioxide nanoparticles elicit distinct apoptotic pathways in bronchial epithelial cells. Part Fibre Toxicol 7:10
37. Hussain S, Vanoirbeek JA et al (2011) Lung exposure to nanoparticles modulates an asthmatic response in a mouse model. Eur Respir J 37(2):299–309
38. Jachak A, Lai SK et al. (2011) Transport of metal oxide nanoparticles and single-walled carbon nanotubes in human mucus. Nanotoxicology 1–9. Posted online on 29 Jul 2011
39. Jiang W, Kim BY et al (2008) Nanoparticle-mediated cellular response is size-dependent. Nat Nanotechnol 3(3):145–150
40. Jin H, Heller DA et al (2009) Size-dependent cellular uptake and expulsion of single-walled carbon nanotubes: single particle tracking and a generic uptake model for nanoparticles. ACS Nano 3(1):149–158
41. Kang GS, Gillespie PA et al (2011a) Long-term inhalation exposure to nickel nanoparticles exacerbated atherosclerosis in a susceptible mouse model. Environ Health Perspect 119(2): 176–181

42. Kang SJ, Ryoo IG et al. (2011b) Role of the Nrf2-heme oxygenase-1 pathway in silver nanoparticle-mediated cytotoxicity. Toxicol Appl Pharmacol 258:(1) 89–98
43. Kim JS, Yoon TJ et al (2006) Cellular uptake of magnetic nanoparticle is mediated through energy-dependent endocytosis in A549 cells. J Vet Sci 7(4):321–326
44. Knol AB, de Hartog JJ et al (2009) Expert elicitation on ultrafine particles: likelihood of health effects and causal pathways. Part Fibre Toxicol 6:19
45. LeBlanc AJ, Cumpston JL et al (2009) Nanoparticle inhalation impairs endothelium-dependent vasodilation in subepicardial arterioles. J Toxicol Environ Health A 72(24):1576–1584
46. LeBlanc AJ, Moseley AM et al (2010) Nanoparticle inhalation impairs coronary microvascular reactivity via a local reactive oxygen species-dependent mechanism. Cardiovasc Toxicol 10(1):27–36
47. Lesniak A, Campbell A et al (2010) Serum heat inactivation affects protein corona composition and nanoparticle uptake. Biomaterials 31(36):9511–9518
48. Limbach LK, Wick P et al (2007) Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. Environ Sci Technol 41(11):4158–4163
49. Lipka J, Semmler-Behnke M et al (2010) Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection. Biomaterials 31(25):6574–6581
50. Lu S, Duffin R et al (2009) Efficacy of simple short-term in vitro assays for predicting the potential of metal oxide nanoparticles to cause pulmonary inflammation. Environ Health Perspect 117(2):241–247
51. Lundqvist M, Stigler J et al (2011) The Evolution of the Protein Corona around Nanoparticles: A Test Study. ACS Nano 5(9):7503–7509
52. Lundqvist M, Stigler J et al (2008) Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc Natl Acad Sci U S A 105(38):14265–14270
53. Mailänder V, Landfester K (2009) Interaction of nanoparticles with cells. Biomacromolecules 10(9):2379–2400
54. Maiorano G, Sabella S et al (2010) Effects of cell culture media on the dynamic formation of protein-nanoparticle complexes and influence on the cellular response. ACS Nano 4(12):7481–7491
55. Mayor S, Pagano RE (2007) Pathways of clathrin-independent endocytosis. Nat Rev Mol Cell Biol 8(8):603–612
56. Mills NL, Törnqvist H et al (2007) Air pollution and atherothrombosis. Inhal Toxicol 19(Suppl 1):81–89
57. Mohamed BM, Verma NK et al (2011) Activation of stress-related signalling pathway in human cells upon SiO2 nanoparticles exposure as an early indicator of cytotoxicity. J Nanobiotechnol 9:29
58. Monteiller C, Tran L et al (2007) The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area. Occup Environ Med 64(9):609–615
59. Monteiro-Riviere NA, Wiench K et al (2011) Safety evaluation of sunscreen formulations containing titanium dioxide and zinc oxide nanoparticles in UVB sunburned skin: an in vitro and in vivo study. Toxicol Sci 123(1):264–280
60. Nel A, Xia T et al (2006) Toxic potential of materials at the nanolevel. Science 311(5761): 622–627
61. Nishanth RP, Jyotsna RG et al (2011) Inflammatory responses of RAW 264.7 macrophages upon exposure to nanoparticles: Role of ROS-NFκB signaling pathway. Nanotoxicology 5:502–516
62. Oberdörster G (2010) Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. J Intern Med 267(1):89–105
63. Oberdörster G, Oberdörster E et al (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823–839

64. Pope CA, Dockery DW (2006) Health effects of fine particulate air pollution: lines that connect. J Air Waste Manag Assoc 56(6):709–742
65. Rabinovitch M (1995) Professional and non-professional phagocytes: an introduction. Trends Cell Biol 5(3):85–87
66. Rimai DS, Quesnel J, Busnaina AA (2000) The adhesion of dry particles in the nanometer to micrometer-size range. Colloids and Surfaces A: Physicochemical and Engineering Aspects 165:3–10
67. Rothen-Rutishauser BM, Kiama SG et al (2005) A three-dimensional cellular model of the human respiratory tract to study the interaction with particles. Am J Respir Cell Mol Biol 32(4):281–289
68. Rothen-Rutishauser BM, Schürch S et al (2006) Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environ Sci Technol 40(14):4353–4359
69. Ruge CA, Kirch J et al (2011) Uptake of nanoparticles by alveolar macrophages is triggered by surfactant protein A. Nanomedicine 7(6):690–693
70. Safi M, Yan M et al (2011) Interactions between magnetic nanowires and living cells: uptake, toxicity, and degradation. ACS Nano 5(7):5354–5364
71. Sanfins E, Dairou J et al (2011) Carbon black nanoparticles impair acetylation of aromatic amine carcinogens through inactivation of arylamine N-acetyltransferase enzymes. ACS Nano 5(6):4504–4511
72. Schleh C, Semmler-Behnke M et al (2011) Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration. Nanotoxicology 6(1):36–46
73. Semmler-Behnke M, Takenaka S et al (2007) Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent reentrainment onto airways epithelium. Environ Health Perspect 115(5):728–733
74. Semmler-Behnke M, Kreyling WG et al (2008) Biodistribution of 1.4- and 18-nm gold particles in rats. Small 4(12):2108–2111
75. Shapero K, Fenaroli F et al (2011) Time and space resolved uptake study of silica nanoparticles by human cells. Mol BioSyst 7(2):371–378
76. Shukla R, Bansal V et al (2005) Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: a microscopic overview. Langmuir 21(23):10644–10654
77. Simkó M, Mattsson MO (2010) Risks from accidental exposures to engineered nanoparticles and neurological health effects: a critical review. Part Fibre Toxicol 7:42
78. Singh S, Kumar A, Karakoti A, Seal S, Self WT (2010) Unveiling the mechanism of uptake and sub-cellular distribution of cerium oxide nanoparticles. Mol Biosyst 6:1813–1820
79. Singh S, Shi T et al (2007) Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and ultrafine TiO2: role of the specific surface area and of surface methylation of the particles. Toxicol Appl Pharmacol 222(2):141–151
80. Slowing II, Vivero-Escoto JL et al (2011) Exocytosis of mesoporous silica nanoparticles from mammalian cells: from asymmetric cell-to-cell transfer to protein harvesting. Small 7(11): 1526–1532
81. Sotiriou GA, Diaz E et al (2011) A novel platform for pulmonary and cardiovascular toxicological characterization of inhaled engineered nanomaterials. Nanotoxicology 1–11. Posted online on 2 Aug 2011
82. Studer AM, Limbach LK et al (2010) Nanoparticle cytotoxicity depends on intracellular solubility: comparison of stabilized copper metal and degradable copper oxide nanoparticles. Toxicol Lett 197(3):169–174
83. Sund J, Alenius H et al (2011) Proteomic characterization of engineered nanomaterial-protein interactions in relation to surface reactivity. ACS Nano 5(6):4300–4309
84. Sung JH, Ji JH et al (2011) Subchronic inhalation toxicity of gold nanoparticles. Part Fibre Toxicol 8:16

85. Tenzer S, Docter D et al (2011) Nanoparticle Size Is a Critical Physicochemical Determinant of the Human Blood Plasma Corona: A Comprehensive Quantitative Proteomic Analysis. ACS Nano 5(9):7155–7167
86. Tinkle S, Antonini J et al (2003) Skin as a route of exposure and sensitization in chronic beryllium disease. Environ Health Perspect 111(9):1202–1208
87. Turci F, Ghibaudi E et al (2010) An integrated approach to the study of the interaction between proteins and nanoparticles. Langmuir 26(11):8336–8346
88. Verma A, Stellacci F (2010) Effect of surface properties on nanoparticle-cell interactions. Small 6(1):12–21
89. Walczyk D, Bombelli FB et al (2010) What the cell "sees" in bionanoscience. J Am Chem Soc 132(16):5761–5768
90. Wu W, Samet JM et al (2010) Phosphorylation of p65 is required for zinc oxide nanoparticle-induced interleukin 8 expression in human bronchial epithelial cells. Environ Health Perspect 118(7):982–987
91. Xia T, Kovochich M et al (2006) Comparison of the abilities of ambient and manufactured nanoparticles to induce cellular toxicity according to an oxidative stress paradigm. Nano Lett 6(8):1794–1807
92. Xia T, Kovochich M et al (2008) Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS Nano 2(10):2121–2134
93. Yacobi NR, Malmstadt N et al (2010) Mechanisms of alveolar epithelial translocation of a defined population of nanoparticles. Am J Respir Cell Mol Biol 42(5):604–614
94. Zhang L, Bai R et al (2011) Rutile TiO(2) particles exert size and surface coating dependent retention and lesions on the murine brain. Toxicol Lett 207(1):73–81
95. Zhu MT, Feng WY et al (2009) Particokinetics and extrapulmonary translocation of intratracheally instilled ferric oxide nanoparticles in rats and the potential health risk assessment. Toxicol Sci 107(2):342–351

Chapter 10
Impacts and Physico-Chemical Behavior of Inorganic Nanoparticles in the Environment

Melanie Auffan, Jerome Rose, Armand Masion, Jerome Labille, Corinne Chaneac, Mark R. Wiesner and Jean-Yves Bottero

Abstract The specific properties of engineered nanoparticles have been used in many fields (e.g., medicine, cosmetic, electronics, catalysis, and environment). Their increased production and use come along with questions about their environmental and human health impacts. Rather than doing case-by-case studies, our vision is to extract general principles from environmental pertinent examples that determine nanoparticles behavior and biological effects. In this chapter, we will discuss the case of TiO_2 (used as additive in sunscreen) in terms of environmental degradation of nanoTiO_2-based formulations, reactive oxygen species generation, colloidal stability in the water column, transport in porous media, and also ecotoxicological impacts.

M. Auffan (✉) · J. Rose · A. Masion · J. Labille · J.-Y. Bottero
Aix-Marseille University, CEREGE UMR 6635, 13545, Aix en Provence, France
e-mail: auffan@cerege.fr

CNRS, CEREGE UMR 6635, 13545 Aix en Provence, France

M. Auffan · J. Rose · A. Masion · J. Labille · C. Chaneac · Mark R.Wiesner · J.-Y. Bottero
International Consortium for the Environmental Implications of Nanotechnology,
Duke University, Durham, USA

C. Chaneac
Laboratoire de chimie de la matière condensée, UMR 7574 UPMC CNRS, Paris, France

Mark R.Wiesner
Center for the Environmental Implications of Nanotechnology,
Duke University, Durham, USA

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_10,

10.1 Introduction

Due to their novel properties, nanoparticles cannot be considered as other organic and inorganic xenobiotics in the environment, e.g., pesticides, dissolved metals, or also medicines. They are subjected to phenomena of classical and quantum physics. Their reactivity means that their surface atoms are labile; easily change their redox state, and highly reactive with respect to compounds in the environment. Considering the huge range of applications using nanoparticles, it seems reasonable to expect their dissemination in the environment at each step in their life cycle, from design through production to use and disposal of finished products. To date, the data available show that nanoparticles can cross biological membranes and distribute themselves within different compartments of living organisms, or can also induce a remote toxicity. Consequently, there is a need to elucidate how the nanoparticles can lead to adverse effects on organisms in their natural environment, considering not only their effects on target organs and life traits, but also their fate and transfers within the food webs.

In this chapter, we will take the example of TiO_2 nanoparticles to underline the complexity of the research issues related to the environmental fate of nanoparticles and the need for an interdisciplinary methodology. The titanium dioxide nanoparticles were chosen because the commercial production has been estimated at more than 10,000 tons/year for the years 2011–2014. Its worldwide production level has been estimated at approximately 2.5 million metric tons by 2025 [51]. TiO_2 nanoparticles are indeed widely used in industrial applications for their UV-absorbing (e.g., sunscreen formulation) or photocatalytic properties (e.g., self-cleaning surfaces). Currently, about 65 % of the production is used in personal care products (sunscreens, cosmetics), while the rest is used in industrial applications, such as plastics, catalysts, and ceramics. Recent studies have shown that nano-TiO_2 can be disseminated in the environment. For instance, the leaching out of TiO_2 nanoparticles from outdoor facades releases a few $\mu g.L^{-1}$ of TiO_2 into stream water [30]. The predicted environmental concentrations (PEC) range from 0.7–16 $\mu g.L^{-1}$ in water, and 0.4–4.8 $\mu g.kg^{-1}$in soils [41], and 40 $mg.L^{-1}$ in wastewater [35]. In sunscreens, TiO_2 is the main inorganic UV filter [44, 47]. For transparency and efficacy reasons, surface modified TiO_2 nanoparticles [21, 36] are often preferred to larger sized particles [48, 53].

10.2 Physico-Chemical Behavior of Inorganic Nanoparticles in Aqueous Media

The rapid development of nanotechnologies is related to the specific properties exhibited by particles at the nanoscale. Indeed, owing to their small size, nanoparticles possess properties that are often widely different from their conventional counterparts, such as mechanical resistance, electronic properties, thermal conductivity, and

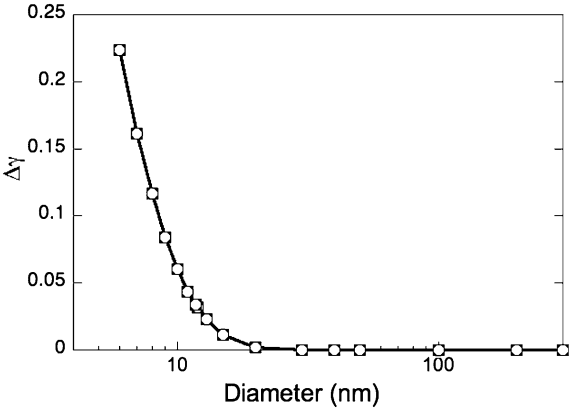


Fig. 10.1 Calculated variation of the interfacial tension $\Delta\gamma$ as a function of the particle diameters. Adapted from [29]

chemical reactivity [9]. To date, it seems that the physicochemical behaviors, the thermodynamics, the toxicology, and the ecotoxicology of nanoparticles are all properties that cannot be extrapolated directly from those of larger solids or xenobiotics (e.g., arsenic and trichloroethene). For instance, nanoparticles smaller than 10 nm have more than 20 % of their atoms at the surface and 1 nm nanoparticles have a surface energy estimated at 560 J/g [2], whereas the percentage of atoms at the surface and the surface energy (0.56 J/g) of micron-sized particles are negligible [2]. This size-dependent increase of the surface energy is illustrated in Fig. 10.1. Owing to their surface energy, very small nanoparticles are considered as thermodynamically metastable in suspension and the decrease of their surface energy is the driving force for their behavior and fate in the environment (e.g., adsorption, dissolution, oxidoreduction, aggregation). Therefore, it is impossible to study and understand the biological impacts of nanoparticles without a solid understanding of their extrinsic and intrinsic physicochemical properties in aqueous media [9].

10.2.1 Chemical Reactivity of Inorganic Nanoparticles in Solution

Nanoparticles are recognized for their ability to hold ions at their surface. This strong affinity for ions is in part due to their high specific surface area. However, the adsorption capacity cannot be explained simply by an increase in specific surface area, i.e., the adsorption capacity per gram. Another important parameter is the surface reactivity of nanoparticles, i.e. in that case, the adsorption capacity per nm^2 of surface. Recent studies have shown that iron oxide nanoparticles of diameter 10 nm can hold up to 10 As^{III}/nm^2 [66], 13 As^{V}/nm^2 [66], or 22–34 Co^{II}/nm^2 [61], whereas microscopic iron oxides can hold on average 1–4 atoms/nm^2 [3, 56]. The only way to explain such a high adsorption capacity per nm^2 is that adsorption mechanisms at the surface of nanoparticles differ from conventional mechanisms occurring at the surface of microparticles. One way to probe

adsorption sites on particles is to use specific chemical probes [1, 37, 62]. For example, As(III) has been used to probe the surface of nanomaghemites of diameter 6 nm. Brice-Profeta et al. [18] have shown that the occupation rate of the maghemite tetrahedral site by Fe (Fe[Td]) decreases as particle size decreases [18]. They also demonstrated the existence of a preferential iron octahedral layer at the nano-maghemite surface, i.e. a deficit of Fe[Td] at the surface of very small maghemite nanoparticles. X-ray diffraction revealed that 10 % of Fe[Td] sites are vacant before As(III) adsorption. The adsorption of As(III) led to an increase in the occupancy of the surface Fe[Td] sites, as revealed by X-ray diffraction, suggesting possible As adsorption at this very specific crystallographic surface sites. EXAFS at the As K-edge further indicated that As was chemically sorbed but with a surprising high Fe coordination number (3.1 $\pm$ 0.6). A careful examination of the coordination of the Fe[Td] site on the (111), (011), and (100) surface planes strongly suggested that As filled the Fe[Td] surface sites through the formation of tridentate, hexanuclear, corner-sharing (3C) surface complexes. Morin et al. [40] found the same As(III) complex at the surface of magnetite in sorption as well as co-precipitation experiments [40, 64]. At higher surface coverage, arsenite adsorbs on Fe octahedral Fe[Oh] surface sites through monodentate trinuclear complexes supposedly in a lattice position.

Even if adsorption of As(III) at the highly reactive vacant surface Fe[Td] sites on magnetite and maghemite can explain the uptake of 2 As/nm^2, it cannot explain the maximum amount observed for As adsorption (8 As/nm^2). Other factors need to be taken into account help to understand this unusually high level of As(III) uptake. Indeed nanoparticles are thermodynamically unstable compared with their microscopic counterparts. Adsorption of ions at the surface of particles decreases the energy (ΔG) of a system by $\mathbf{\Delta G = 3Vm\Delta\gamma/r}$, where Vm is the molar volume, γ is the difference in interfacial energy before and after adsorption, and r is the radius of the particles [29]. Therefore, the adsorption of a dense arsenite layer decreases—via radius increase—the energy of the system more than when adsorption occurs on larger particles of 20 or 300 nm. Whereas in macroscopic systems adsorption is mainly governed by chemical affinity and electrostatic bond strengths, for nanoparticles the decrease of free energy must be taken into account [14, 20].

10.2.1.1 The Example of TiO_2 Nanoparticles and Their Photocatalytic Activity

Nanoparticle surface reactivities can assume different forms (e.g., adsorption, aggregation), but the most interesting regarding TiO_2 nanoparticles is their photocatalytic activity. This can be considered as reactivity induced by an external influence, whereas chemical reactivity with respect to adsorbing molecules occurs spontaneously. The photocatalytic effect is not unique to nanoparticles, but there is a clear modification of these effects depending on the size of the particles.

Photocatalysis is usually described as a photochemical and catalytic reaction occurring at the surface of a solid, generally a semiconductor [23, 46, 54]. This reaction is initiated by the transition of an electron from the valence band to the conduction band under the effect of a light ray carrying energy hν greater than or equal to the energy difference between the two bands. It goes beyond the scope of this chapter to detail the complex mechanisms underlying photocatalysis, but we shall discuss some aspects of the problem that are related to particle size.

Titanium dioxide exists in three main (polymorphic) forms: rutile, anatase, and brookite. It has recently been shown that rutile is the most thermodynamically stable phase for particle sizes larger than 35 nm, and anatase for sizes smaller than 11 nm, while brookite turns out to be the most stable in the intermediate size range. The exact reason for these differences has not yet been fully elucidated, but it is certainly related to the nature of the exposed crystallographic faces. Apart from this indirect size effect, several authors have shown that there is an optimal size for photo-oxidation of organic substrates for a given polymorph. This optimum size is $\sim$7 nm for trichloroethylene [38], $\sim$11 nm for chloroform [63], and $\sim$25 nm for phenol [4]. These optimum sizes are thought to result from competing effects of the particle size on light absorption and scattering efficiency, charge-carrier dynamics, and specific surface area.

10.2.2 Dispersion States in the Water Column

Inorganic nanoparticles form colloidal suspensions, which can remain stable over very long periods. The dispersion/aggregation state of nanoparticles in the environment is a major concern for both exposure and toxicity from the cellular to the ecosystem scale. At this stage it is worth detailing that in our chapter aggregation is referred to an assembly of particles that are loosely attached to each other. It means that aggregation is a reversible phenomenon and that nanoparticles can aggregate in aquatic media and can disaggregate in the presence of stabilizing agents like proteins. Two types of stabilization depending on whether they involve electrostatic or steric interactions can occur to maintain the nanoparticles dispersed or aggregated [2, 19, 28, 49, 60].

The electrostatic stabilization of nanoparticles, described by the DLVO theory (due to Derjaguin and Landau, Verwey, and Overbeek), is specific to charged species in aqueous solution. This is a kinetic stabilization method which results from the balancing of attractive forces intrinsic to the chemical nature of the solid (Van der Waals forces) and repulsive forces of electrostatic origin between the charged surfaces. Under such equilibrium conditions, the particles disperse spontaneously under the action of Brownian motion. Electrostatic stabilization requires strict control of the pH and the nature and concentration of the ions in solution, and it has the great advantage that the particle surface is bare, in the sense that the surface atoms are in direct contact with the solution [11].

Steric stabilization involves a modification of the chemical nature of the surface by adsorption or grafting of a coupling agent, usually a polymer, which can form a corona or shell around the particles. This shell guarantees solvation of the particles and prevents them from aggregating by steric repulsion. By virtue of the modified surface state, collisions between particles (induced by Van der Waals forces) are quasi-elastic and redisperse the particles. The quality of the dispersion will depend on the compatibility between the polymer chains at the surface in relation to the nature of the solvent, since this guarantees good shell swelling and surface coverage [11].

Consequently, as a function of the nanoparticle surface properties and the physico-chemical properties of the aqueous media (e.g., pH, ionic strength) the suspensions of nanoparticles will be stabilized or destabilized in the water column. For instance, neutral pH and high ionic strength will decrease the repulsive forces between nanoparticles inducing their aggregation, while surface complexes modifying the hydrophobic–hydrophilic will favor their dispersion in the water column.

10.2.2.1 The Example of the Dispersion of Surface Modified TiO_2 Nanoparticles Used in Sunscreen

Dispersion experiments in water [8, 33] have been performed with the commercial T-Lite SF material (BASF, Germany), formulation used in sunscreen. It consists of a nano-TiO_2 core, coated with aluminum hydroxide [$Al(OH)_3$] as photocatalysis shield, and a polydimethylsiloxane (PDMS; [-$Si(CH_3)_2$-O-] repeat units) outer layer for easy incorporation in a cream. Due to the hydrophobic PDMS coating, the TiO_2 nanocomposite is initially hydrophobic. However, after only 30 min of stirring, the solid starts to disperse in the aqueous phase. Large agglomerates of ca. 2 μm are measured, along with smaller volume fraction of solids around 300 nm, corresponding to well-dispersed particulate byproducts (Fig. 10.2). Its relative contribution tends to increase rapidly with time, and reaches values up to 35 vol % after 48 h stirring. This submicron size allows the corresponding colloidal residues to remain stable in suspension when stirring is stopped. This easy dispersion of a hydrophobic material in water was explained by the gradual removal of the PDMS coating from the surface of the nanomaterial [8, 33]. The release of Si from the nanomaterial into solution during stirring, measured by induced-coupled plasma atomic emission spectroscopy (ICP-AES), revealed that most of the PDMS, which is the only Si source in the system, was no longer attached to the particle surface. The plateau obtained after 4 days of suspension corresponds to ca. 90 % of the Si initially presents in the system [33].

The aqueous degradation of an actual commercial sunscreen shows some similarities with the isolated TiO_2 nanocomposite [16, 39]. Like in the case of the T-Lite SF particles, no size distribution could be measured at $t = 0$ due to the hydrophobic character of the material, which prevented immediate dispersion in water. However, soon after mixing (30 min), some of the material was dispersed,

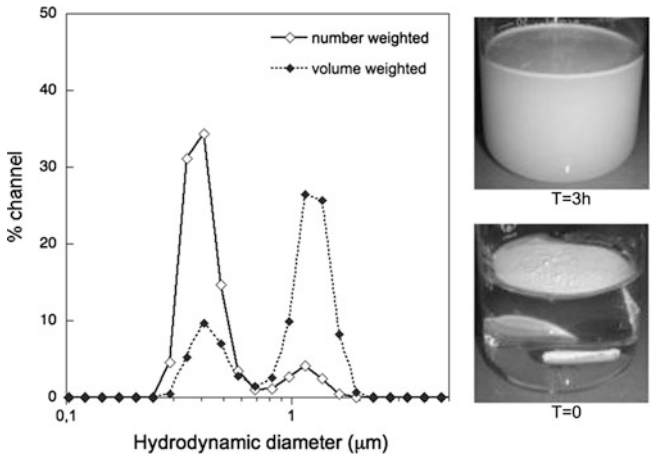


Fig. 10.2 Hydrodynamic diameters of the T-Lite SF suspended in water under stirring and daylight for 3 h. pH 5; [NaCl]) 0.01 mol/L; 25 °C; $[TiO2]_{initial} = 1$ g/L. Adapted from [7]

as indicated by a submicron size class on the particle size distribution. The relative weight of this submicron fraction keeps increasing during the 48 h during which the size distribution was monitored. This trend demonstrates a gradual transformation of the initially hydrophobic material into phases with a marked hydrophilic character. The particle size distribution of the stable suspension at 48 h settling reveals that the submicron size class has become predominant. The mass of this stable colloidal fraction represents between 20 and 40 % of the initial sunscreen, and these colloids contain less than 30 w/w % of the initial TiO_2. However, large agglomerates of several tens of micrometers are also detected. This larger size class is tentatively attributed to the agglomeration of organics into open and low density structures [16].

10.2.3 Transport Within Porous Media

The mobility or deposition of manufactured nanoparticles in porous media is a real concern for bioaccessibility. The diffusion column experiment is conventionally used to study the parameters determining the mobility or attachment of nanoparticles in a saturated porous medium [34, 57]. A mobile electrolyte solution circulates a column filled with a stationary porous medium. A volume of nanoparticles in suspension is then injected into the column. The breakthrough curve is typically box-shaped. Many parameters affecting the attachment of nanoparticles to the porous medium can vary in both the mobile—(pH, ionic strength, hydrodynamics) and in the stationary phase (porosity, mineralogy, grain size).

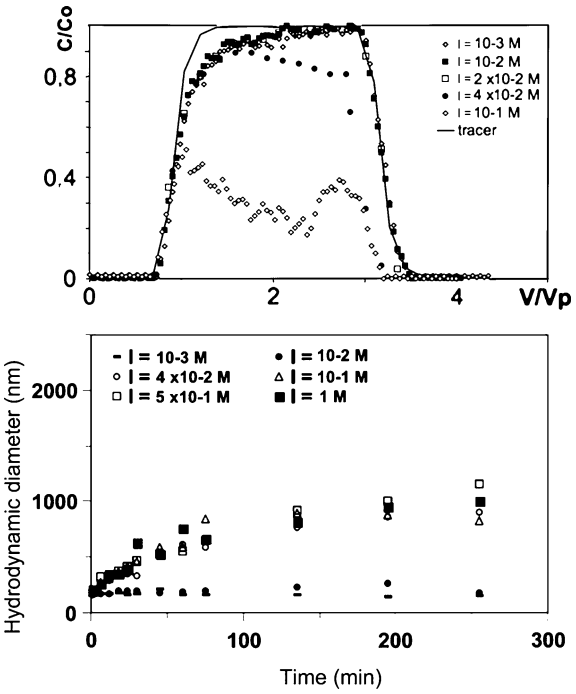


Fig. 10.3 *Top* Aggregation kinetics of TiO_2 nanoparticles (50 mg.L^{-1}) for different NaCl concentrations at pH 8. *Bottom* Breakthrough curves of TiO_2 nanoparticles for different ionic concentrations. C/C_0 is a ratio of the TiO_2 concentrations in the effluent to the influent of the column, V/Vp is the ratio of the eluted volume to total pore volume of the column. 50 mg.L^{-1}, pH 8, Darcy velocity = 0.002 cm.s^{-1}. Adapted from [57]

10.2.3.1 The Example of the Transport of TiO_2 Nanoparticles in Porous Media: Aggregation Versus Deposition

The pH has a strong influence on the mobility of TiO_2 nanoparticles within a sand column [57]. At pH = 3.6, sand grains and TiO_2 nanoparticles have opposite surface charges and retention of the nano-TiO_2 is quasi-total. As expected, when both the mobile- and the stationary solids are negatively charged (pH = 8), the deposition of nano-TiO_2 does not exceed 5 %. For pH values close to the IEP of TiO_2 (IEP = 5.5), a strong retention is observed, which is most likely due to self-aggregation of nano-TiO_2 inside the column.

The repulsive electrostatic forces between sand and TiO_2 surfaces which are predominant a low salt concentration, can be screened at higher ionic strength. The critical salt concentration, above which particle deposition increases, is around 4×10^{-2} mol L^{-1}. It is interesting to notice that this value coincides well with the critical coagulation concentration measured in liquid phase. This strongly suggests a link between the aggregation of nanoparticles and their apparent deposition. Two reasonable mechanisms can be proposed [57]: (i) steric effects make the deposition of larger aggregates much more likely than the deposition of small individual nanoparticles; and (ii) drag forces through porous aggregates favor their deposition onto a collector, while individual nanoparticles remain in the flow toward the column outlet Fig. 10.3.

It is also interesting to notice that, under conditions where nanoparticles are aggregated (pH = 6 or [NaCl] > 4×10^{-2} M), TiO_2 is eluted from the column at smaller V/Vp (ratio of the eluted volume to total pore volume of the column) values than the well-dispersed nanoparticles, which follow mainly the conservative tracer profile. This faster motion through the column is due to size exclusion effect, which causes aggregates to visit a smaller portion of the available pore network within the column. Therefore, the aggregates that do not attach to the stationary phase will have a shorter residence time in the column than the individual nanoparticles.

In this simple model system, where only one parameter was allowed to vary at a time, already several mechanisms controlling the mobility of nanoparticles have been identified. It is obvious that the determination of the mechanisms in a natural or at least a more realistic system will require investigating the influence of other crucial parameters (e.g. presence of organic matter, redox conditions, flow velocity, saturated/unsaturated cycling…), which may vary simultaneously and have opposite effects on the particle mobility.

10.3 Biological Impact of Inorganic Nanoparticles Toward Aquatic Organisms

The number of available publications dealing with the environmental impacts of nanoparticles (called nano-ecotoxicology [31]) is in constant increase. Studies regarding algae, protozoa, and invertebrates are more recent (since 2006) than the ones regarding bacteria (which started in the 1980s) (*ISI web of knowledge source*). Recent reviews examined a number of nanoparticles that are toxic to living organisms e.g., [25, 32, 42]. There are now obvious examples of the toxic effects of C_{60} fullerenes to bacteria, to daphnids; Nano-TiO_2, nano-CeO_2, nano-Ag, etc. can also be toxic to various organisms e.g. [25, 32]. It is noteworthy that missing or inconsistent physical–chemical characterizations of the nanoparticles prevent a useful analysis of contradictory toxicity reports. Indeed, nanoparticles can differ in many aspects due to environment driven variations in size, shape, redox properties, surface charge, crystal structure, chemical composition, adhesion to surfaces… and it is worth examining the influence of various physical–chemical properties of the nanoparticles on their toxic effects.

10.3.1 Effects of the Aggregation State on Ecotoxicity

Most of pioneer published results dealing with nanoparticles ecotoxicity mainly focussed on size effects. But the size was mainly measured by TEM (dry state) and was not characterized in nutritive or aquatic media. Indeed the dispersion state of nanoparticles (aggregation *versus* dispersion) in contact with living organisms can

change from one study to another. There is a generally accepted idea assuming that aggregation of nanoparticles impedes the determination of specific properties of nanoparticles. It is obvious that the transport and mobility are strongly affected (see previous sections). Nevertheless, intrinsic or specific 'nano' physical–chemical properties of nanoparticles are not necessarily affected by aggregation. In the particular case of surface chemical reactivity, Auffan et al. [10, 13] have shown that surface normalized quantity of probe molecules sorbed to iron oxide particles is strongly enhanced for particles smaller than 12 nm. This surface reactivity was not affected by aggregation [13]. The example of Nano-γFe_2O_3 in contact with *E. coli* can illustrate that the aggregation state as well as the size may not be the most important parameters in terms of toxicity [5, 6]. Indeed, the authors shown that 8 nm γFe_2O_3 had no toxic effect toward *E. coli* regardless of the aggregation state of the Nanoparticles.

However, the aggregation state is important to consider when dealing with exposure. On a large scale, aggregation/sedimentation of nanoparticles in aquatic environments will leave a small portion of the total mass of nanoparticles available for direct uptake by planktonic organisms, while the majority will be in contact with benthic organisms. In this case, sediments should be regarded as an important sink for nanoparticles discharged to the aquatic environment. Not only can the exposure pathway be different upon aggregation, but the mechanisms of internalization by cells can also vary. Internalization could involve a combination of large-scale phagocytosis of aggregates or a passive nonspecific mechanism if individual nanoparticles can diffuse through the cell membrane. Toxicity mechanisms can also change as a function of the aggregation state. Thill et al. and Zeyons et al. found that when the bactericidal effects of nanoparticles are attributed to direct redox effects, close contact is required between cerium dioxide nanoparticles and the bacterial wall [59, 67]. These authors also found that if the CeO_2 nanoparticles strongly interact with the exopolysaccharides excreted by bacteria, this close contact does not occur, the electrostatic interactions are reduced and the toxicity decreases [59, 67].

10.3.2 Effect of the Chemical Stability on Ecotoxicity

As function of their chemical composition, the redox states of nanoparticles can change in biological Eh/pH conditions, inducing bulk redox transformation, oxidative dissolution, and reductive dissolution. Indeed most elements exhibit various possible redox states that are or are not stable as function of Eh and pH values. It is obvious for all metals that they can exist under metallic or oxidized forms. But even when oxidized some elements can exist under various redox states. Metals like Fe, Mn, Cr, or rare earth like Ce illustrate such variation (Fe^{2+}, Fe^{3+}; Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{7+}; Cr^{3+}, Cr^{6+}; Ce^{3+}, Ce^{4+}…). Living organisms and especially eukaryotic cells metabolism cannot support high pH variation. For most eukaryotic cells pH is buffered in a 7–8 pH range. In the case of Eh, oxidative phosphorylation in mitochondria is based on a series of redox reactions at near circumneutral pH

for which potentials are in a -0, 32 (NAD^+/NADH) to 0, 29 V (cytochromes). Besides oxidative phosphorylation (respiration) extracellular Eh is generally controlled by thiol/disulfide redox systems (mainly GSH/GSSH and Cys/CySS) for which Eh vary in a −0.140/−0.08 V range. In such conditions many elements can be redox unstable that lead to electron exchange between NP surface and surrounding media. Then this could be the starting point of disequilibrium of the redox balance and then to oxidative stress. In the specific case of iron, Auffan et al. [5] have shown that nano zero valent iron as well as nano-magnetite (Fe_3O_4) can be toxic to *E. coli*. The study reported that the oxidation of iron in contact to bacteria can be at the origin of the toxicity. The potential of the redox couple characteristic of Fe^0 nanoparticles at pH = 7 is in a $Eh_{Fe2+/Fe0} = -0{,}40/-0{,}7$ V as function of the Fe concentration [50] which is lower than the typical Eh in biological media. This allowed the oxidation of Fe^0(s) in contact with *E. coli.* This oxidation is directly responsible of toxic effects through the generation of an oxidative stress, as demonstrated by an enhanced toxicity of these Fe Nanoparticles toward a mutant strain of *E. Coli* deprived of defense mechanism against oxidative stress. Other examples in the literature confirm the importance of the redox 'instability' of nanoparticles. Using XANES at Ce L_3-edge, Thill et al. [58, 67] and Auffan et al. [12] have shown that the cytotoxicity/genotoxicity of CeO_2 was related to the reduction of surface Ce(IV) atoms to Ce(III).

10.3.2.1 The Example of Phototoxicity of TiO_2 Nanoparticles

Several authors have shown that the presence of actives sites on nanoparticles that are able to generate ROS (reactive oxygen species) is involved in the toxicity of nanoparticles. Direct relationships between the specific surface area, ROS generation, and inflammatory effects induced by nanoparticles have been shown [43]. However, there are great debates as to whether or not size-dependent structural changes contribute to an increase of toxicity in a general sense e.g., [24, 65]). For instance, for a given mass, 20 nm-anatase TiO_2 nanoparticles are more toxic toward rats than 250 nm-anatase particles. But for a given specific surface area, the toxicity responses are similar whatever the size was [45]. However, other studies have reported anatase (present in greater proportions for TiO_2 crystallites <15 nm) to be more biologically active than rutile TiO_2 in terms of cytotoxicity or oxidative DNA damage [26, 52]. Jang et al. [27] have shown that the bactericidal effects increase as the size of nanoparticles decreases from 30 to 15 nm and the mass fraction of anatase increases. Braydish-Stolle et al. [17] demonstrated that 100 % anatase nanoparticles, regardless of size, induce cell necrosis and membrane leakage but do not generate ROS. In contrast, the rutile nanoparticles initiate apoptosis through formation of ROS. Therefore, it seems that links between size and crystal structure may play a role in mediating nanoparticle toxicity.

More than the size, the surface properties and its evolution over time also govern the ability of TiO_2 nanoparticles to generate ROS. Auffan et al. found that the altered T-Lite SF used in sunscreens do not generate superoxide anions in their experimental

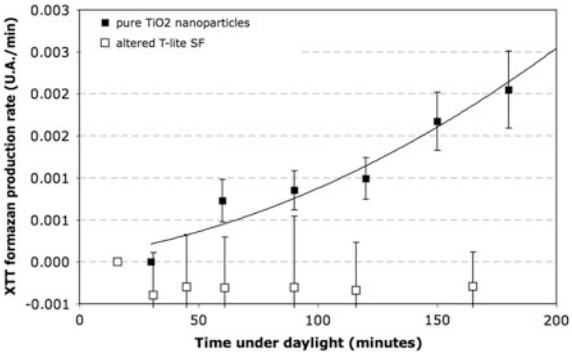


Fig. 10.4 Assessment of ROS generation by the altered T-Lite SF during 48 h under daylight at pH 5. XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was used to target and measure the O_2^- anions. Adapted from [7]

conditions [7], whereas bare nano-TiO_2 do even at lowest concentration (Fig. 10.4). The remaining Al-based layer at the surface after alteration (pH 5, UV light) prevents the chemical interactions between the Ti atoms of the surface of the nano-TiO_2 core and the O_2 and/or H_2O molecules from the solution. This inhibits the promotion of electron-/hole+ of the nano-TiO_2 core and the ROS generation.

10.3.3 Impacts of Inorganic Nanoparticles on Food Webs

Past experience with chemicals (e.g. methyl mercury, DDT, and PCBs) revealed dietary uptake at lower trophic levels and accumulation up the food chain to be an important route of contaminant exposure. In nano-ecotoxicology, it is now important to assess in complex systems, whether the organism responses correlate or not with results from studies performed on monoculture or breeding.

For instance, effects of nanoparticles were assessed toward biofilms and communities and the results are different compared to experiments performed on monocultures. For instance, TiO_2 nanoparticles from wastewater and seawater sludge have low toxicity on *V. fischeri* [55]. However, low concentration (5.3 mg L^{-1}) of TiO_2 nanoparticles added to a stream microcosm under ambient UV radiation [15] damaged the cell walls of planktonic microorganisms due to intercellular action of ROS and accumulated in the benthic biofilms. Cell membrane damages were more pronounced in free-living cells than in biofilm cells, indicating the protective role of cell encapsulation against TiO_2 nanoparticles.

Recent studies also focused on freshwater simplified food webs. One study regards the trophic transfer of TiO_2 nanoparticles in the food chain including *Daphnia magna* and its predator, the zebrafish *Danio rerio* [68]. 8-days-old *D. magna* were exposed to TiO_2 of 21 nm in diameter (0.1 and 1 mg/L) and transferred to the 3-month-old *D. rerio* tanks. No biomagnification of TiO_2 nanoparticles from daphnia to zebrafish was found, but the dietary intake was found to be one of the main nanoparticles exposure route for higher trophic level aquatic organisms.

However, these studies regarding short trophic links do not take into account important parameters, such as the colloidal destabilization of the nanoparticles, the interaction between the surface of the nanoparticles, and (in)organic molecules naturally occurring or bio-excreted, or the flux between compartments of the ecosystems (aqueous phase, sediments, biota). To work under more realistic scenario of exposure, few studies are performed on mesocosms. Ferry et al. [22] were the first to study the fate of positively charged Au nanoparticles (65 × 15 nm) in estuarine mesocosms containing sediments, biofilms, primary producers, filter feeders, grazers, and omnivores. While water and sediments represented 99 % of the total mass of the system, on a per mass basis the filter feeders and biofilms were the most effective sink of Au nanoparticles. The estimated mass of biofilms was less than 0.5 % but they recovered 60 % of nanoparticles. This high affinity is related to the negative surface charge of the biofilm interacting with the positively charged Au nanoparticles and to the large surface area developed by the biofilms. These results are interesting since biofilms offer (i) a route into the food web through grazing by detritovores and (ii) a route for mineralization through biofilm calcification.

10.4 Conclusion and Perspectives

This chapter discusses the ecotoxicity and the physico-chemical behavior of nanoparticles in the environment. It is obvious that assessing the biological impacts strongly depends on an accurate physical–chemical characterization of the properties and fate of nanoparticles. To date, many challenges need to be met to go further in the understanding of the risk related to nanotechnologies.

- Most of the studies focus on bare or free nanoparticles. To be more relevant with regard to the reality, it is crucial to assess the behavior of actual commercialized nano-products through their life cycle (as illustrated by the case of TiO_2 nanoparticles used in sunscreen).
- The current knowledge on the effects of nanoparticles toward organisms in ecosystems is limited to studies evaluating the interactions between one nanoparticle and one kind of organism. It is now necessary to develop systemic approaches taking into account the transfer and distribution mechanisms in the ecosystems as well as the toxicity all along the trophic chain. Such studies need to be performed in mesoscosms.
- Nano-ecotoxicity studies carried out at concentrations much higher than would ever be expected in the environment; provide necessary data regarding the mechanisms of nanoparticles toxicity. However, complementary studies on the impact of nanoparticles at concentrations comparable to those encountered in an environmental situation are required. Chronic exposures to low doses have also to be considered for future research.

Acknowledgments The authors would like to thank the CNRS and CEA for funding the International Consortium for the Environmental Implications of NanoTechnology and also the NSF and the US-EPA for funding the Center for the Environmental Implications of Nano-Technology. They also acknowledge financial support from the French National Agency (ANR) in the frame of the P2N/MESONNET project.

References

1. Academies des Sciences et Technologies de Paris (2004) Nanoscience, Nanotechnologies. wwwacademie-sciencesfr 18
2. Adamson AW, Gast AP (1997) Physical chemistry of surfaces. Wiley, New York
3. Al-Abadleh HA, Grassian VH (2003) Oxide surfaces as environmental interfaces. Surf Sci Rep 52(3–4):63
4. Almquist CB, Biswas P (2002) Role of synthesis method and particle size of nanostructured TiO2 on its photoactivity. J Catal 212(2):145
5. Auffan M, Achouak W, Rose J, Roncato MA, Chaneac C, Waite DT, Masion A, Woicik JC, Wiesner MR, Bottero JY (2008) Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward *Escherichia coli*. Environ Sci Technol 42(17):6730–6735
6. Auffan M, Decome L, Rose J, Orsiere T, De Meo M, Briois V, Chaneac C, Olivi L, Berge-Lefranc JL, Botta A, Wiesner MR, Bottero JY (2006) In vitro interactions between DMSA-coated maghemite nanoparticles and human fibroblasts: A physicochemical and cyto-genotoxical study. Environ Sci Technol 40(14):4367–4373
7. Auffan M, Pedeutour M, Rose J, Masion A, Ziarelli F, Borschneck D, Chaneac C, Botta C, Chaurand P, Labille J, Bottero JY (2010) Surface structural degradation of a TiO2-based nanomaterial used in cosmetics. Environ Sci Technol 44(7):2689–2694
8. Auffan M, Pedeutour M, Rose J, Masion A, Ziarelli F, Borschneck D, Chaneac C, Botta C, Chaurand P, Labille J, Bottero JY (2010) Surface structural degradation of a TiO2-based nanomaterial used in cosmetics. Environ Sci Technol 44(7):2689–2694
9. Auffan M, Rose J, Bottero JY, Lowry G, Jolivet JP, Wiesner MR (2009) Towards a definition of inorganic nanoparticles from an environmental, health, and safety perspective. Nat Nanotechnol 4:634–641
10. Auffan M, Rose J, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol 4(10):634–641
11. Auffan M, Rose J, Chaneac C, Jolivet JP, Wiesner MR, Bottero JY (2011) Surface reactivity of manufactured nanoparticles. In: Marano F, Lahmani M, Houdy P (eds) Nanotoxicology and nanoethics, vol Tome 4. Springer, Berlin
12. Auffan M, Rose J, Orsiere T, De Meo M, Thill A, Zeyons O, Proux O, Masion A, Chaurand P, Spalla O, Botta A, Wiesner MR, Bottero J-Y (2009) CeO2 nanoparticles induce DNA damage towards human dermal fibroblasts in vitro. Nanotoxicology 3(22):161–171
13. Auffan M, Rose J, Proux O, Borschneck D, Masion A, Chaurand P, Hazemann JL, Chaneac C, Jolivet JP, Wiesner MR, Van Geen A, Bottero JY (2008) Enhanced adsorption of arsenic onto maghemites nanoparticles: As(III) as a probe of the surface structure and heterogeneity. Langmuir 24(7):3215–3222
14. Auffan M, Rose J, Proux O, Borschneck D, Masion A, Chaurand P, Hazemann JL, Chaneac C, Jolivet JP, Wiesner MR, VanGeen A, Bottero JY (2008) Enhanced adsorption of arsenic onto nano-maghemites: As(3+) as a probe of the surface structure and heterogeneity. Langmuir 24:3215–3222
15. Battin TJ, Kammer FVD, Weilhartner A, Ottofuelling S, Hofmann T (2009) Nanostructured TiO2: transport behavior and effects on aquatic microbial communities under environmental conditions. Environ Sci Technol 43(21):8098–8104

16. Botta C, Labille J, Auffan M, Borschneck D, Miche H, Cabie M, Masion A, Rose J, Bottero JY (2011) TiO2-based nanoparticles released in water from commercialized sunscreens in a life cycle perspective: structures and quantities. Environ Pollut 159:1543–1550
17. Braydich-Stolle L, Schaeublin N, Murdock R, Jiang J, Biswas P, Schlager J, Hussain S (2008) Crystal structure mediates mode of cell death in TiO2 nanotoxicity. J Nanopart Res 11(6):1361–1374
18. Brice-Profeta S, Arrio MA, Tronc E, Menguy N, Letard I, Cartier dit Moulin C, Nogues M, Chaneac C, Jolivet JP, Sainctavit P (2005) Magnetic order in [gamma]-Fe2O3 nanoparticles: a XMCD study. J Magn Magn Mater 288:354
19. Cao G (2004) Nanostructures & nanomaterials: synthesis, properties & applications. Imperial College Press
20. Charlet L, Morin G, Rose J, Wang Y, Auffan M, Burnol A, Fernandez-Martinez A (2011) Reactivity at mineral-water interfaces, redox processes, and arsenic transport in the environment. CR Geosciences 343:123–139
21. Dransfield G, Guest PJ, Lyth PL, McGarvey DJ, Truscott TG (2000) dispersions. DPtoT-bisPN-a, J Photochem Photobiol B 59 (120e3) e Photoactivity tests of TiO2-based inorganic sunscreens. Part 1: Non-aqueous dispersions. J Photochem Photobiol B 59 (1–3):147–151
22. Ferry JL, Craig P, Hexel C, Sisco P, Frey R, Pennington PL, Fulton MH, Scott IG, Decho AW, Kashiwada S, Murphy CJ, Shaw TJ (2009) Transfer of gold nanoparticles from the water column to the estuarine food web. Nat Nanotechnol 4(7):441–444
23. Fujishima A (1999) TiO2 photocatalysis: fundamentals and applications. BKC, Inc., Tokyo
24. Gurr J-R, Wang ASS, Chen C-H, Jan K-Y (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213(1–2):66–73
25. Handy RH, von der Kammer F, Lead JR, Hassellöv M, Owen R, Crane M (2008) The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology 17:287–314
26. Hirakawa K, Mori M, Yoshida M, Oikawa S, Kawanishi S (2004) Photo-irradiated titanium dioxide catalyzes site specific DNA damage via generation of hydrogen peroxide. Free Radic Res 38(5):439–447
27. Jang H, Kim S, Kim S (2007) Effect of particle size and phase composition of titanium dioxide nanoparticles on the photocatalytic properties. J Nanopart Res 3:141–147
28. Jolivet JP, Chaneac C, Tronc E (2004) Iron oxide chemistry. From molecular clusters to extended solid networks. Chem Commun 5:481–487
29. Jolivet JP, Froidefond C, Pottier A, Chanéac C, Cassaignon S, Tronc E, Euzen P (2004) Size tailoring of oxide nanoparticles by precipitation in aqueous medium. A semi-quantitative modelling. J Mater Chem 14:3281–3288
30. Kaegi R, Sinnet B, Zuleeg S, Hagendorfer H, Mueller E, Vonbank R, Boller M, Burkhardt M (2010) Release of silver nanoparticles from outdoor facades. Environ Pollut 158(9):2900–2905
31. Kahru A, Dubourguier H-C (2010) From ecotoxicology to nanoecotoxicology. Toxicology 269(2–3):105–119
32. Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RH, Lyon DY, Mahendra S, Mclaughlin MJ, Lead JR (2008) Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ Toxicol Chem 27(9):1825–1851
33. Labille J, Feng J, Botta C, Borschneck D, Sammut M, Cabie M, Auffan M, Rose J, Bottero JY (2010) Aging of TiO2 nanocomposites used in sunscreen. Dispersion and fate of the degradation residues in aqueous environment. Environ Pollut 158 (12):3482–3489
34. Lecoanet H, Bottero JY, Wiesner MR (2004) Laboratory assessment of the mobility of several commercial nanomaterials in porous media. Environ Sci Technol 38(19):5164–5169
35. Lee BC, Kim SH, Shon K, Vigneswaran S, Kim SD, Cho J, Kim IS, Choi KH, Kim JB, Park HJ, Kim J-H (2009) Aquatic toxicity evaluation of TiO2 nanoparticle produced from sludge of TiCl4 flocculation of wastewater and seawater. J Nanopart Res 11:2087–2096
36. Lee WA, Pernodet N, Li B, Lin CH, Hatchwell E, Rafailovich MH (2007) Multicomponent polymer coating to block photocatalytic activity of TiO2 nanoparticles. Chem Commun 7(45):4815–4817

37. Madden AS, Hochella MF, Luxton TP (2006) Insights for size-dependent reactivity of hematite nanomineral surfaces through Cu2+ sorption. Geochim Cosmochim Acta 70(16):4095–4104
38. Maira AJ, Yeung KL, Lee CY, Yue PL, Chan CK (2000) Size effects in gas-phase photo-oxidation of trichloroethylene using nanometer-sized TiO2 catalysts. J Catal 192(1):185
39. Masion A, Auffan M, Labille J, Botta A, Solovitch N, Rose J, Bottero J-Y (2011) Environmental fate of nanoparticles: physical chemical and biological aspects, a few snapshots. Int J Nanotechnol (in press)
40. Morin G, Wang Y, Ona-Nguema G, Juillot F, Calas G, Menguy N, Aubry E, Bargar JR, Brown GE (2009) EXAFS and HRTEM evidence for As(3+)-containing surface precipitates on nanocrystalline magnetite: implications for as sequestration. Langmuir 25(16):9119–9128
41. Mueller NC, Nowack B (2008) Exposure modeling of engineered nanoparticles in the environment. Environ Sci Technol 42(12):4447–4453
42. Napierska D, Thomassen LCJ, Lison D, Martens JA, Hoet PH (2010) The nanosilica hazard: another variable entity. Part Fibre Toxicol 7(1):39
43. Nel A, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311(5761):622–627
44. Newman MD, Stotland M, Ellis JI (2009) The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens. J Am Acad Dermatol 61(4):685–692
45. Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113(7):823–839
46. Pelizzetti E, Serpone N (1986) Homogeneous and heterogeneous photocatalysis. Reidel Publishing Company, Dordrecht
47. Petrazzuoli M (2000) Advances in sunscreens. Curr Probl Dermatol 12(6):287–290
48. Popov AP, Priezzhev AV, Lademann J, Myllyla R (2005) TiO2 nanoparticles as an effective UV-B radiation skin-protective compound in sunscreens. J Phys D Appl Phys 38(15):2564–2570
49. Pottier AS, Cassaignon S, Chaneac C, Villain F, Tronc E, Jolivet JP (2003) Size tailoring of TiO2 anatase nanoparticles in aqueous medium and synthesis of nanocomposites. Characterization by Raman spectroscopy. J Mater Chem 13(4):877–882
50. Pourbaix M (1974) Atlas of electrochemical equilibria in aqueous solutions. National Association of Corrosion Engineers, Houston
51. Robichaud CO, Uyar AE, Darby MR, Zucker LG, Wiesner MR (2009) Estimates of upper bounds and trends in nano-tio2 production as a basis for exposure assessment. Environ Sci Technol 43(12):4227–4233
52. Sato T, Taya M (2006) Enhancement of phage inactivation using photocatalytic titanium dioxide particles with different crystalline structures. Biochem Eng J 28(3):303–308
53. Serpone N, Dondi D, Albini A (2007) Inorganic and organic UV filters. Their role and efficacy in sunscreens and suncare products. Inorg Chim Acta 360(3):794–802
54. Serpone N, Pelizzetti E (1989) Photocatalysis: fundamentals and applications. Wiley, New York
55. Sharma VK (2009) Aggregation and toxicity of titanium dioxide nanoparticles in aquatic environment-A Review. J Environ Sci Health A Tox Hazard Subst Environ Eng 44(14):1485–1495
56. Sigg L, Behra P, Stumm GN (2000) Chimie des milieux aquatiques, chimie des eaux naturelles et des interfaces dans l'environnement
57. Solovitch N, Labille J, Rose J, Chaurand P, Borschneck D, Wiesner MR, Bottero JY (2010) Concurrent aggregation and deposition of Tio2 nanoparticles in a sandy porous media. Environ Sci Technol 44(13):4897–4902
58. Thill A, Zeyons O, Spalla O, Chauvat F, Rose J, Auffan M, Flank AM (2006) Cytotoxicity of CeO2 nanoparticles for Escherichia coli. Physico-chemical insight of the cytotoxicity mechanism. Environ Sci Technol 40(19):6151–6156
59. Thill A, Zeyons O, Spalla O, Chauvat F, Rose J, Auffan M, Flank AM (2006) Cytotoxicity of CeO2 nanoparticles for *Escherichia coli*. Physico-chemical insight of the cytotoxicity mechanism. Environ Sci Technol 40(19):6151–6156
60. Turkevitch J, Stevenson PC, Hillier J (1951) A study of the nucleation and growth processes in the synthesis of colloidal gold. J Discuss Faraday Soc 11:55–75

61. Uheida A, Salazar-Alvarez G, Bjorkman E, Yu Z, Muhammed M (2006) Fe3O4 and gamma-Fe2O3 nanoparticles for the adsorption of Co2+ from aqueous solution. J Colloid Interface Sci 298(2):501–507
62. Villieras F, Michot LJ, Bardot F, Chamerois M, Eypert-Blaison C, Francois M, Gerard G, Cases JM (2002) Surface heterogeneity of minerals. Comptes Rendus Geosciences 334(9):597–609
63. Wang CC, Zhang Z, Ying JY (1997) Photocatalytic decomposition of halogenated organics over nanocrystalline titania. Nanostruct Mater 9(1–8):583
64. Wang YH, Morin G, Ona-Nguema G, Menguy N, Juillot F, Aubry E, Guyot F, Calas G, Brown GE (2008) Arsenite sorption at the magnetite-water interface during aqueous precipitation of magnetite: EXAFS evidence for a new arsenite surface complex. Geochim Cosmochim Acta 72:2573–2586
65. Warheit DB, Webb TR, Sayes CM, Colvin VL, Reed KL (2006) Pulmonary instillation studies with nanoscale TiO2 rods and dots in rats: toxicity is not dependent upon particle size and surface area. Toxicol Sci 91(1):227–236
66. Yean S, Cong L, Yavuz CT, Mayo JT, Yu WW, Kan AT, Colvin VL, Tomson MB (2005) Effect of magnetic particle size on adsorption and desorption of arsenite and arsenate. J Mater Res 20:3255–3264
67. Zeyons O, Thill A, Chauvat F, Menguy N, Cassier-Chauvat C, Orear C, Daraspe J, Auffan M, Rose J, Spalla O (2009) Direct and indirect CeO2 nanoparticles toxicity for *E. coli* and Synechocystis. Nanotoxicology 3(4):284–295
68. Zhu X, Wang J, Zhang X, Chang Y, Chen Y (2010) Trophic transfer of TiO2 nanoparticles from daphnia to zebrafish in a simplified freshwater food chain. Chemosphere 79:928–933

Chapter 11
Ecotoxicological Impact of ZnO and CdE (E = S, Se, Te) Quantum Dots on Microorganisms

Alice da Rocha and Roberta Brayner

11.1 Introduction

To produce unique products with novel properties, we need to manipulate materials at the nanoscale level [1]. In the world, these nanomaterials are being rapidly produced in large scale, and it was shown, in the past 10 years, that the nanomaterials have different toxicity profiles compared with bulk particles because of their small size and consequently, their high reactivity. In this moment, the toxicological and environmental effects of direct or indirect exposure to these manufactured nanomaterials are not completely elucidated [2].

Physicochemistry is essential to understanding the fate and behavior of nanoparticles in the environment, as well as uptake and distribution within organisms, and the interactions of nanoparticles with other pollutants. It has been reported that when the particle size decreases, there is a tendency to increase the toxicity, even if the same material is relatively inert in bulk form (e.g., SiO_2, carbon black, TiO_2, ZnO) [3, 4].

The nanoparticles, due to their nanoscale, shape, and consequently huge surface area, may interact more efficiently with biological systems, producing important toxicity. In addition, the surface area is directly correlated to many other physicochemical properties such as chemical reactivity, surface adsorption ability, surface charge, and so on. All these factors strongly dominate nanotoxicological behavior in vivo [3, 4].

Another important point of this study is the choice of the biological target. For example, there are different possible routes of environmental exposure to nanoparticles after release to the aquatic environment (Fig. 11.1). After entry of the

A. da Rocha · R. Brayner (✉)
Interfaces, Traitements, Organisation et Dynamique des Systèmes (ITODYS),
UMR 7086 CNRS, Université Paris Diderot, Sorbonne Paris Cité,
15 rue Jean-Antoine de Ba, 75205 Paris Cedex 13, France
e-mail: roberta.brayner@univ-paris-diderot.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_11,

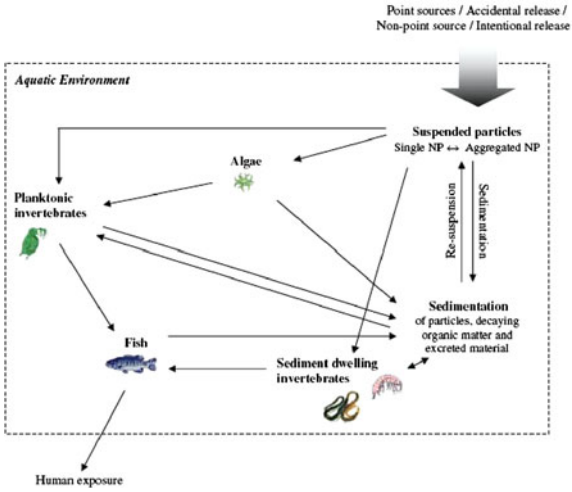


Fig. 11.1 Fate of the nanoparticles in aquatic environment [5]. Copyright from Springer

nanoparticles into the aquatic environment, the suspended particles will be taken up by planktonic or sediment dwelling invertebrates through different exposure routes (i.e., direct uptake from the water phase or through food uptake).

In this new multidisciplinary field, there are many challenges ahead and some controversies, but knowledge transfer from biology, toxicology, colloid chemistry, as well as material and geological sciences will enable us to improve (nano) ecotoxicology studies.

The aim of this review is to make an overview about the ecotoxicological impact of ZnO- and Cd-based nanoparticles on microorganisms. This work is focused on (i) the physicochemical properties of the nanoparticles in the culture media; (ii) the choice of the biological target; (iii) the interactions between the nanoparticles and the biological targets.

11.2 The Fate of the ZnO Nanoparticles in the Environment

11.2.1 Physicochemical Properties, Morphology, and Structure of ZnO Nanoparticles

Zinc oxide (ZnO) is used as pigment in paints, as fillers in rubber, and cover some papers, particularly because of its ability to absorb UV radiation. It also finds applications in creams, lotions, and other solar products. It has piezoelectric properties and is luminescent. Globally, thousands of tons of ZnO are produced annually.

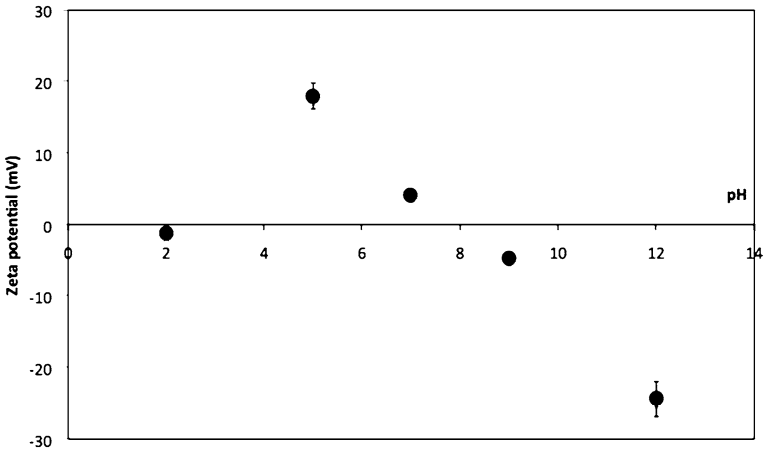


Fig. 11.2 Zeta potential of ZnO nanoparticles as a function of pH

The use of fine powders improves the technical properties of zinc oxide, including increasing its ability to absorb UV, and also its surface area available to catalyze some reactions (antibacterial, antifungal, adhesion, etc). In addition, ZnO nanoparticles are transparent, offering benefits including the preparation of cosmetic products such as sunscreens. In the laboratory, ZnO nanoparticles were more effective at absorbing UVA radiation than nanoparticles of TiO_2 [6].

ZnO has generally been considered insoluble at a neutral pH. Although a trace of Zn^{2+} ions is an essential nutrient, it is toxic to microorganisms above a threshold concentration. Recent studies were focused on the stability of ZnO nanoparticles (aggregation) in aqueous environments as it directly affects their distribution and uptake [7–9]. It was shown that if ZnO nanoparticles are released into water systems, these particles could damage aquatic organisms, especially if free-Zn^{2+} ions are released [10–12]. However, their environmental impact and the mechanism of toxicity still have not been fully elucidated.

The zero point charge for ZnO is about pH 9 [13]. Zeta potential of ZnO nanoparticles with varying pH was measured after 30 min of contact between the nanoparticles and the pH solutions (Fig. 11.2). In this case, ZnO nanoparticles were suspended in MiliQ water ([Zn] = 10^{-3} M) adjusted to pH 2, 5, 7, 9, and 12 by addition of HCl or NaOH solutions.

ZnO nanoparticles are positively charged between pH 4 and 8 and negatively charged between pH 9 and 12. Moreover, at acidic pH, partial dissolution of the nanoparticles was observed. In this case, the zeta potential was negative.

The surface charge of nanoparticles is closely related to their state of aggregation. It was observed in all cases that the nanoparticle aggregation increases around the zero charge point. For ZnO nanoparticles it is around pH 9 (Fig. 11.3).

Another important parameter for the study of the toxicological impact of ZnO nanoparticles on microorganisms is the nanoparticle shape. It was shown that the

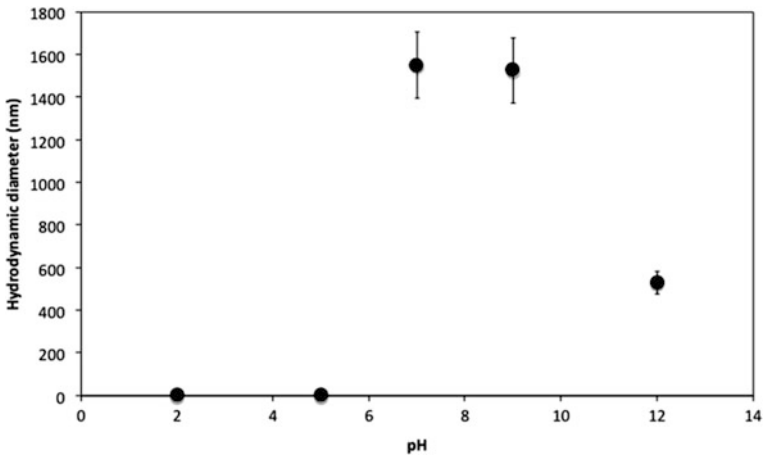


Fig. 11.3 Hydrodynamic diameter of ZnO nanoparticles as a function of pH

toxicity of rod-shaped particles to *Phaeodactylum triocornutum* was greater than that of the spheres [14].

The control of the size and shape of ZnO nanoparticles synthesized by the Polyol process was made using trioctylphosphine oxide (TOPO) by varying the hydrolysis ratio ($H = n_{H2O}/n_{Zn2+}$) [15–17]. By varying the hydrolysis ratio from 2 to 10 it is possible to obtain rod-shaped particles (Fig. 11.4).

Figure 11.5 presents XRD patterns of ZnO nanoparticles after Polyol forced hydrolysis reaction. In all cases, the lines can be indexed as pure ZnO wurtzite structure (*hcp* structure; Fig. 11.5a). Crystallite sizes inferred from X-ray line broadening compared to particle sizes obtained from TEM are quite similar. In addition, the increase of the (002) line of ZnO samples presenting anisotropic forms (rod-shaped particles; Fig. 11.5b) shows that the preferred growth orientation of these samples is along the *c*-axis. An isolated nanorod detected by high-resolution TEM (HRTEM) analysis is single crystalline (Fig. 11.5c). The analysis of HRTEM images revealed *hcp* structure with the same orientation confirming that the *c*-axis of this structure is parallel to nanorod revolution axis.

11.2.2 Ecotoxicological Studies Using ZnO Nanoparticles

One of the first studies about the toxicological impact of ZnO nanoparticles was made using *E. coli* bacteria as biological target [15, 16]. In this study, biocidal effects and cellular internalization was observed using ZnO nanoparticles functionalized with several protective agents such as TOPO, sodium dodecyl sulfate (SDS), polyoxyethylene stearyl ether (Brij-76), and bovine serum albumin.

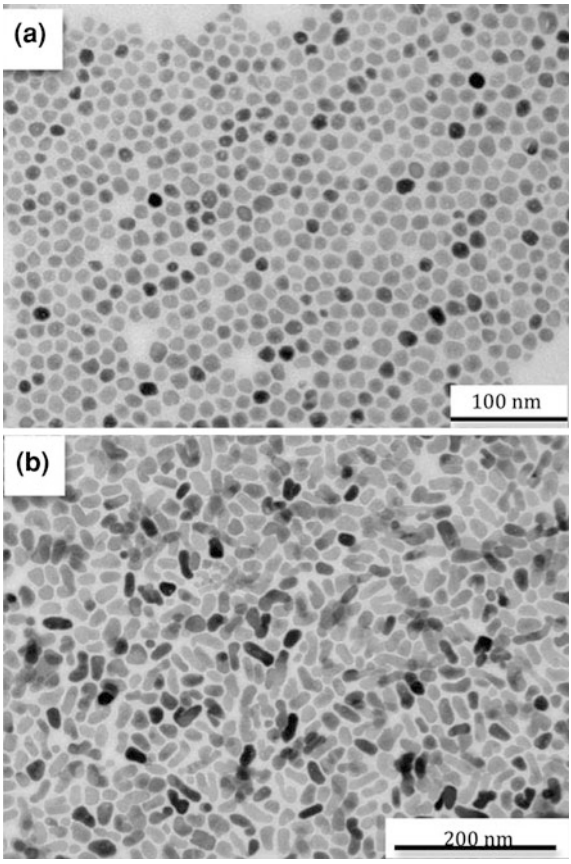


Fig. 11.4 TEM micrographs of ZnO nanoparticles with **a** $H = 2$ and **b** $H = 10$

Bacteriological tests were performed on solid agar plates with different concentrations of protective agents, as well as different concentrations of ZnO nanoparticles. The inoculated cells were estimated to be 200 colony-forming units per plate and all results were compared with a control without nanoparticles. ZnO-free lysogeny broth (LB) agar plates and polyol-LB (DEG) agar plates were used as control. It was observed that the number of bacterial colonies grown on the control, DEG and albumin plate is quite similar (~ 100 %) and growth is inhibited in the presence of SDS because of denaturalization of bacterial proteins (Fig. 11.6a).

TOPO and Brij-76 molecules promote bacterial growth after they are metabolized (Fig. 11.6a).

The same test was carried out with free-ZnO, varying the nanoparticles concentration from 10^{-2} to 10^{-3} M. In this case, the lethal dose was observed between 10^{-2} and 3.0 10^{-3} M (Fig. 11.6b).

In addition, *E. coli* cells were damaged, showing a Gram-negative triple membrane disorganization and, consequently, ZnO internalization after contact with

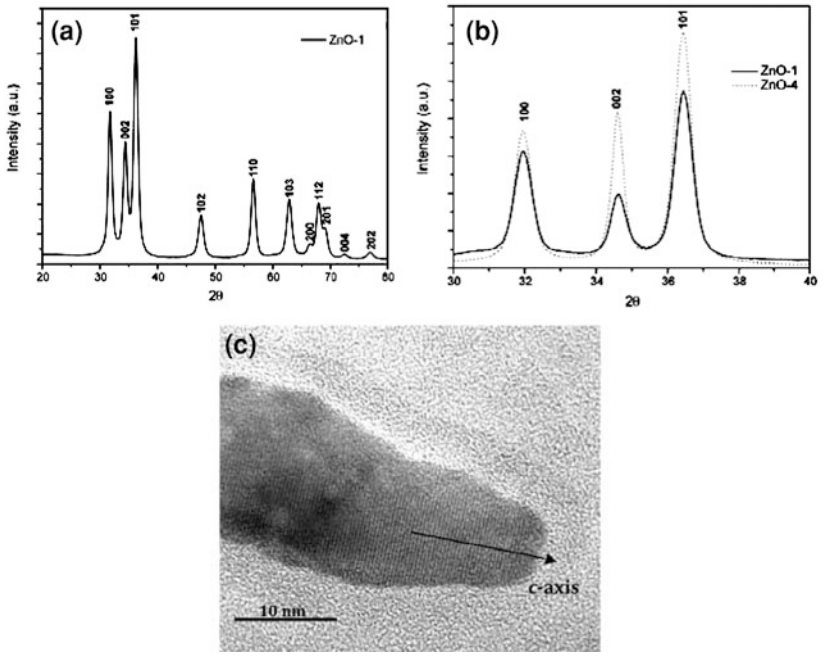


Fig. 11.5 XRD patterns of (**a**) ZnO spherical nanoparticles; **b** comparison between spherical and rod-shaped ZnO nanoparticles, **c** HRTEM of ZnO nanorod (growth orientation along *c*-axis)

nanoparticles concentration higher than 1.3 10^{-3} M and in the presence of some adsorbed molecules. It is important to know that at 1.3 10^{-3} M ZnO concentration the internalization was observed without bactericidal effects (Fig. 11.6b).

The same nanoparticles were also in contact with *Anabaena flos-aquae* cyanobacteria and *Euglena gracilis* euglenoid microalgae, two different biological targets [17]. These microorganisms are photosynthetic. For *Anabaena flos-aquae*, the photosynthetic activity after contact with ZnO nanoparticles decreased progressively due to the stress induced by the presence of the nanoparticles in the culture medium. After contact with ZnO-TOPO, this decrease was followed by cell death. On the other hand, after 10 days, a progressive increase was observed after contact with free-ZnO and ZnO-Brij-76 nanoparticles. In the case of *E. gracilis*, cell death was observed after contact with all nanoparticles. These behaviors were confirmed by Live/Dead tests using Trypan blue dye (Fig. 11.7). The cultures with free-*Anabaena flos-aquae* and free-*E. gracilis* were used as controls. For *Anabaena flos-aquae*, 75 % cell survival was observed after contact with free-ZnO and ZnO-Brij-76 nanoparticles and only 25 % was observed for ZnO-TOPO. In the case of *E. gracilis*, the percent of cell survival was near 10 % in all cases. These results are in agreement with the photosynthetic activities.

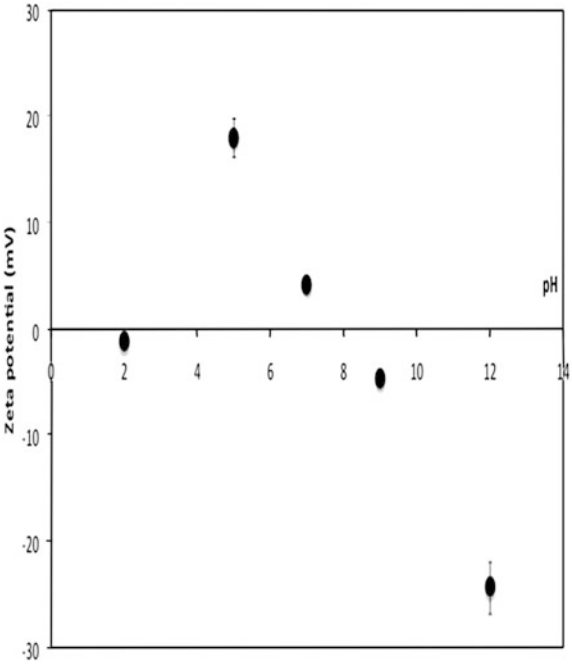


Fig. 11.6 Bactericidal tests as a function of **a** different protective agents and **b** ZnO concentration after incubation at 37 °C overnight

Comparing the behavior of *E. coli*, *Anabaena flos-aquae*, and *E. gracilis* microorganisms after contact with the same ZnO nanoparticles, it is possible to conclude that the toxicological impact depends on (i) the physicochemical properties of the nanoparticles (size, surface functionalization (TOPO, Brij-76, SDS, Albumin, etc.), (ii) the biological target (bacteria, cyanobacteria, microalgae. etc).

It was shown that it is possible to synthesize ZnO nanoparticles with different shapes. The next step is to show the effect of morphology of ZnO nanostructures on their toxicity to microorganisms [14]. In this work, four ZnO nanoparticles motifs, possessing distinctive sizes and shapes, were synthesized without adding protective agents (Fig. 11.8).

The influence of these nanoparticles morphologies on the toxicological impact using marine diatoms (*Thalassiosira pseudonana*, *Chaetoceros gracilis*, and *Phaeodactylum tricornutum*) was studied. It was observed that between 4.1 and 4.9 % of the Zn^{2+} from all types of nanoparticles dissolved within 72 h and was neither concentration nor morphology dependent. Addition of all nanoparticles at all concentrations tested (10, 20, 40, and 80 mgL^{-1}) stopped growth of *T. pseudonana* and *C. gracilis*, whereas *P. tricornutum* was the least sensitive, with its growth rate inversely proportional to nanoparticles concentration. Bioaccumulation of dissolved free Zn^{2+} in *T. pseudonana* was sufficient to promote cell death. Rod-shaped particles toxicity to *P. triocornutum* was noted to be greater than that of the spheres. The overall results suggest that toxicity studies assessing

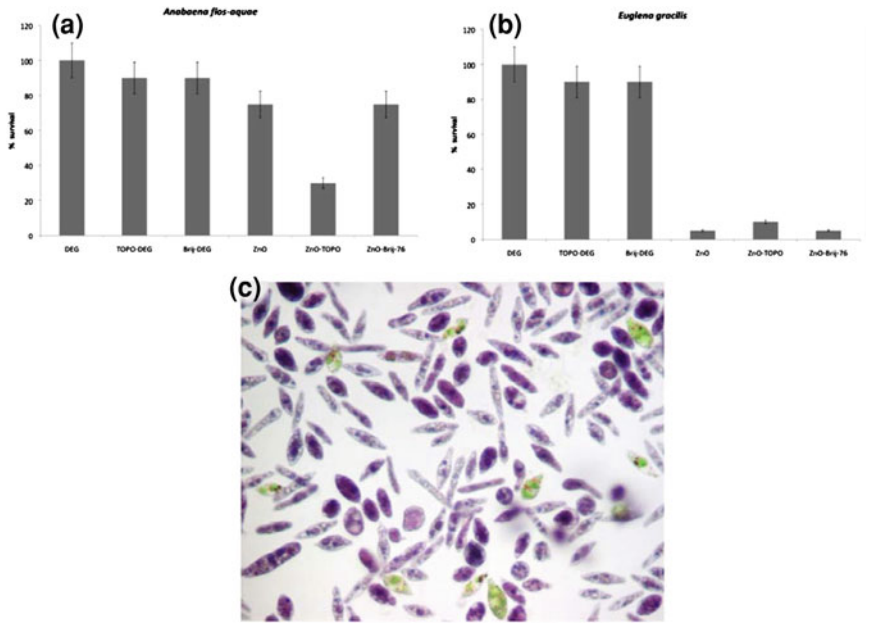


Fig. 11.7 Live/Dead tests using Trypan blue dye. Cellular viability (% survival) after contact with **a** *Anabaena flos-aquae* and **b** *E. gracilis*. **c** representative photonic micrograph of *E. gracilis* Live/Dead test after contact with free-ZnO nanoparticles. [Zn] = 10^{-3} M

the effects of nanoparticles on aquatic microorganisms need to consider both dissolution and cellular interactions of nanoparticles aggregates.

Algae play an important role in the aquatic ecosystem. Therefore, alga is one of the normally used model organism for the toxicity examination of toxicants and nanoparticles as well. Nanoparticles such as CuO [18], ZnO [17], TiO_2 [19], Ag [20], CeO_2 [21], and SiO_2 [22, 23] were all observed able to inhibit the growth of varieties of algae. In all cases, the toxicological mechanisms vary with the nanoparticles used and also with the biological target. In some cases, the toxicity is attributed to the dissolved ions from the nanoparticles, other studies show that the toxicity is due to the interaction between nanoparticles and algae. However, more studies are clearly needed to clarify both toxicological effects and their underlying mechanism of nanoparticles.

Comparative experiments were made using dissolved Zn^{2+} ions, nano-ZnO, and bulk-ZnO in contact with *Chlorella* sp. microalgae [24]. Dose–response curves of these Zn-based materials on the viability of algal cells are shown in Fig. 11.9. In all cases the algal viability decreased with increasing concentrations of the three zinc materials during the 6 days. The toxicity of nano-ZnO and free Zn^{2+} ions to the algae increased with time, whereas the toxicity of bulk-ZnO remained largely unchanged from the second day to the sixth day.

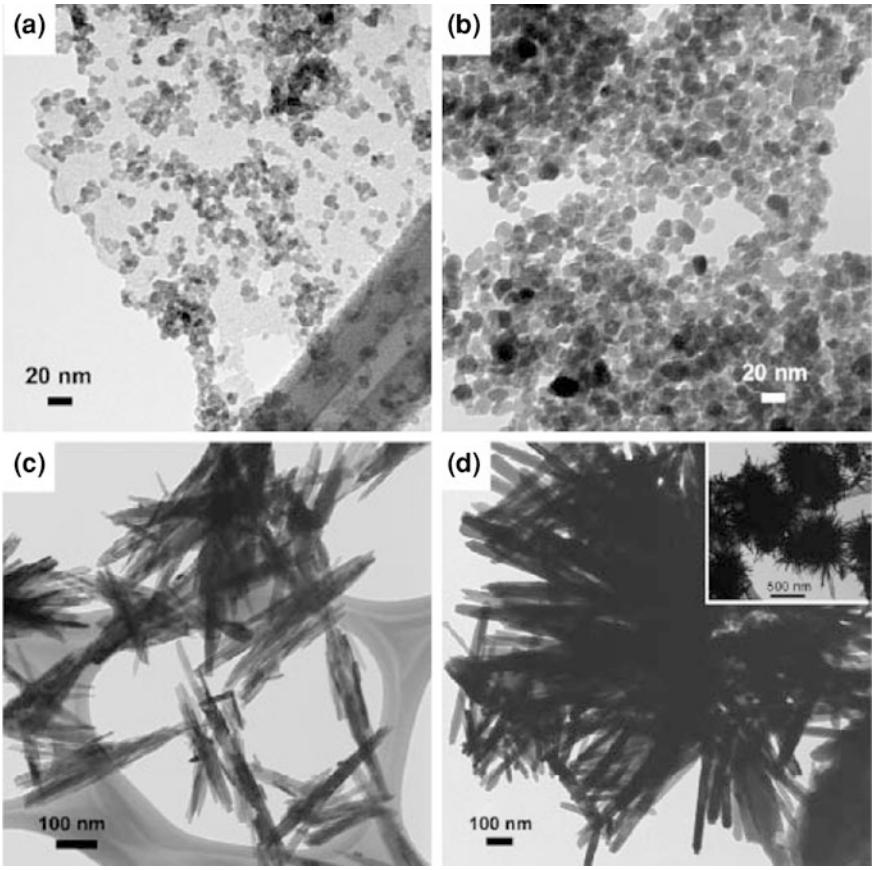


Fig. 11.8 TEM images of ZnO nanoparticles possessing different morphologies **a** small spheres; **b** large spheres; **c** nanorods; and **d** nanoneedles. *Inset* represents low magnified TEM images of nanoneedles [14]. Copyright from Elsevier

In this study, the toxicity ranking of the Zn-based materials at low concentrations ($<$50 mgL^{-1}) to the algae followed an order of bulk-ZnO $<$ nano-ZnO $<$ Zn^{2+}.

These three Zn-based materials were also tested in the presence of zebrafish (*Danio rerio*) [25]. It was found that nano-ZnO aggregated into irregular shapes in suspensions, and showed a relationship between its size distribution and concentration. It was also shown that dissolved Zn^{2+}, from nano-ZnO and bulk-ZnO in suspensions, were toxic to zebrafish, while the aggregation and sedimentation of nano-ZnO suspensions reduced the toxicity of nano-ZnO. However, the authors emphasized that Zn^{2+} ions may not be the main source of acute toxicity of nano-ZnO and bulk-ZnO to zebrafish.

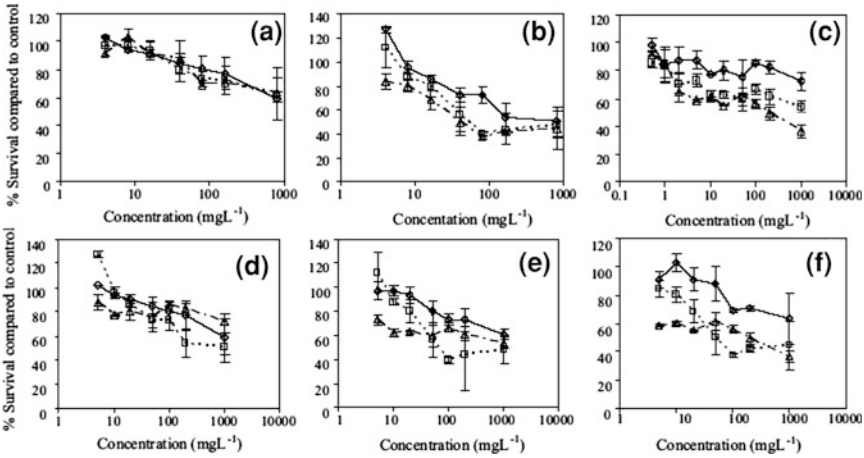


Fig. 11.9 Dose–response relationship *curves* of **a** bulk-ZnO; **b** nano-ZnO; **c** free Zn^{2+} ions solutions on the algal growth at the (*diamond*) second day; (*square*) fourth day; (*triangle*) sixth day. The other three *panels* show the dose–response relationship curves of (*diamond*) bulk-ZnO, (*square*) nano-ZnO, (*triangle*) free Zn^{2+} ions [24]. Copyright from Elsevier

These experimental results highlight the importance of a systematic assessment of toxicity mechanisms of metal oxide nanoparticles to determine definitively whether their toxicity is caused by nanoeffects.

11.3 Quantum Dots: CdE Nanoparticles

11.3.1 Physicochemical Properties, Morphology, and Structure of CdS Nanoparticles

In the last few years, considerable efforts have been devoted to synthesize II–VI semiconductor nanoparticles CdE (E = S, Se, Te) with narrow size distribution and anisotropic morphologies since their electrical and optical properties are sensitive to size and shape [26–29].

There are few ecotoxicological studies using these quantum dots in the environment. For example, it was shown that quantum dots coated with polymaleic anhydride-1-octadecene, and polyethylenimine decreased growth rates of Gram-positive *Bacillus subtilis* and Gram-negative *E. coli* but were not bactericidal [30]. On the other hand, U.S. Environmental Protection Agency test protocol ad fluorescence microscopy were used to determine the fate and effect of quantum dots having a protective organic coating (Qdot® 545 ITK™ Carboxyl Quantum Dots) using standard aquatic test organisms [31]. In this case, no lethality was measured following 48 h exposure of *Ceriodaphnia dubia* to quantum dots suspensions as high as

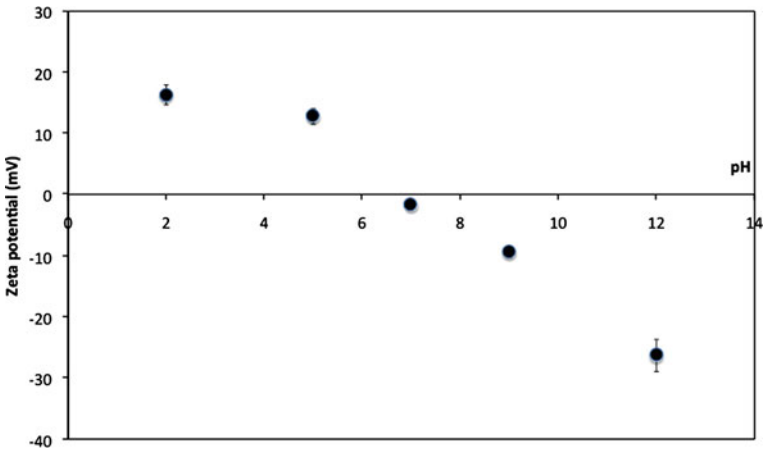


Fig. 11.10 Zeta potential of CdS nanoparticles as a function of pH

110 ppb, but 96 h median lethal concentration to *Pseudokirchneriella subcapitata* was measured at 37.1 ppb.

The zero point charge for CdS nanoparticles is about pH 7 [13]. Zeta potential of CdS nanoparticles with varying pH was measured after 30 min of contact between the nanoparticles and the pH solutions (Fig. 10). In this case, CdS nanoparticles were suspended in MiliQ water ([Cd] = 10^{-3}M) adjusted to pH 2, 5, 9, and 12 by addition of HCl or NaOH solutions [32]. CdS nanoparticles are positively charged between pH 2 and 6 and negatively charged between pH 8 and 12.

It was observed for ZnO nanoparticles that surface charge is closely related to their state of aggregation and consequently the nanoparticles aggregation increases around the zero charge point. For CdS nanoparticles, the same behavior was observed (Fig. 11.11).

As we showed, dissolution may be a critical step for some nanoparticles in determining fate in the environment and within the organisms. The drive force of dissolution depends on the nanoparticle solubility within a given environment as well as the concentration gradient between the particle surface and the bulk solution phase. In the case of CdS nanoparticles prepared by the Polyol process, the dissolution rate was about 40 % after 48 h in water.

The control of the size and shape of CdS nanoparticles prepared by the Polyol route was made using different sulfur-based molecules such as thiourea, sodium sulfide, and mercaptoic acid. Quasi-spheres, nanorods, nanotubes, and pyramids were obtained (Fig. 11.12).

Figure 11.13 presents XRD patterns of CdS nanoparticles prepared by the Polyol process. By varying the sulfur-based molecules, it is possible to control nanoparticles shape and structure. Pure CdS wurtzite structure (*hcp*) was obtained using thiourea molecule. These nanoparticles present quasi-spherical shape. On the other hand, by replacing thiourea with mercaptoic acid, pure blende structure (*cfc*) was observed with a pyramid shape.

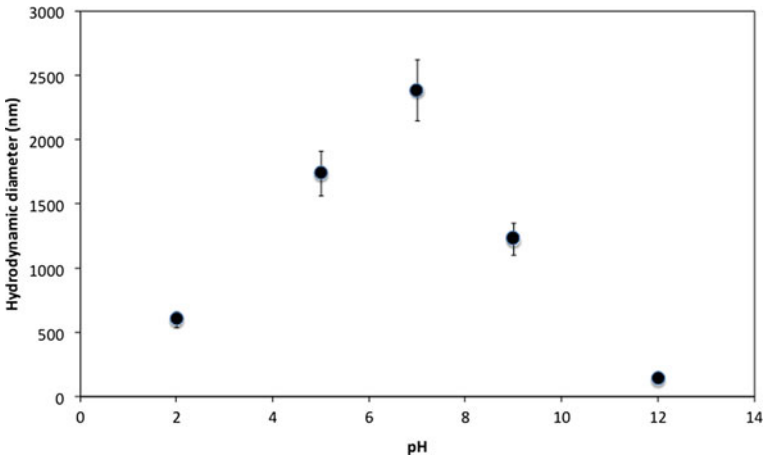


Fig. 11.11 Hydrodynamic diameter of CdS nanoparticles as a function of pH

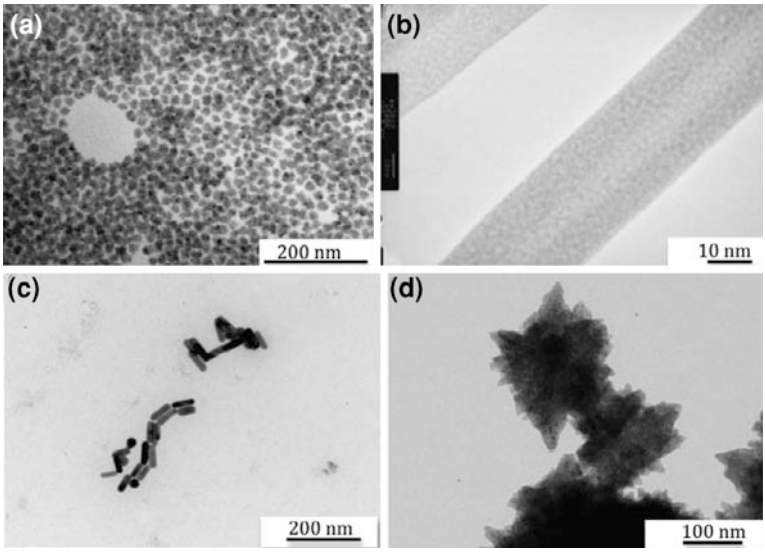


Fig. 11.12 TEM micrographs of CdS nanoparticles prepared by the Polyol route

*11.3.2 The Fate of CdE (*E = *S, Se, Te) Quantum Dots on Microorganisms*

The potential ecotoxicity of CdS quantum dots, synthesized by the Polyol process, was investigated using common *Anabaena flos-aquae* cyanobacteria and *E. gracilis* euglenoid microalgae [32]. In this study, it was observed that cadmium

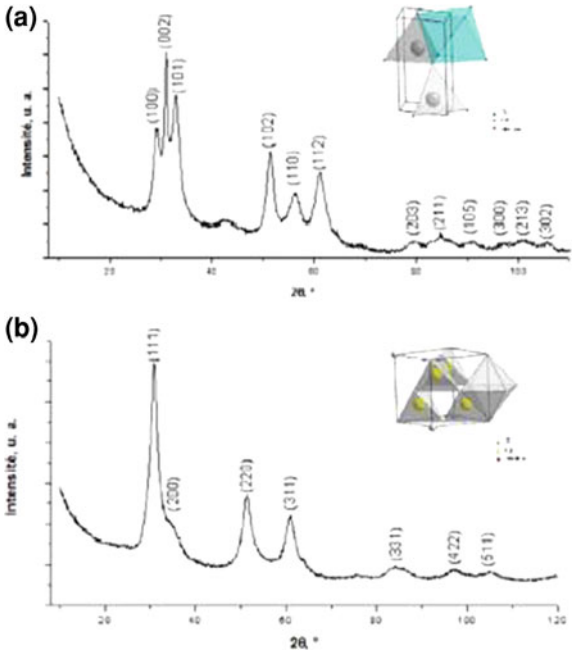


Fig. 11.13 XRD patterns of CdS nanoparticles **a** pure CdS wurtzite structure (*hcp*) prepared with thiourea; **b** pure CdS blende structure (*cfc*) prepared with mercaptoic acid

concentration, the addition of TOPO protective agent and the particle dissolution process in the culture medium, plays an important role during the ecotoxicological tests. It was shown that free Cd^{2+} ions, CdS, and CdS-TOPO were very toxic for *Anabaena flos-aquae*. However, for *E. gracilis* the photosynthetic activity was stable for more than 1 month in the presence of free Cd^{2+} ions. Moreover, the toxicity varies with CdS and CdS-TOPO nanoparticles concentrations.

TEM analyses of microorganisms ultrathin sections showed that the polysaccharides produced by *Anabaena flos-aquae*, after contact with CdS and CdS-TOPO nanoparticles, protect the cyanobacteria against particle internalization (Fig. 11.14). Moreover, the nanoparticles internalization was observed after contact with all nanoparticles in the presence of *E. gracilis* by endocytosis. All nanoparticles are inside vesicles formed by the cells.

CdSe quantum dots are also fluorescent semiconductor and widely used in photovoltaics and in diagnostics for stably labeling mammalian cells and bacteria. They are often capped with ZnS to enhance fluorescence [33]; core–shell configurations also stabilize Cd^{2+} surface atoms against dissolution, which increases biocompatibility [34, 35].

Cd^{2+} ions cause cellular toxicity by several mechanisms including interfering DNA repair [36] and metabolic proteins [37], membrane lipid peroxidation [38], substitution for physiological Zn^{2+}, and reactive oxygen species (ROS) formation [39].

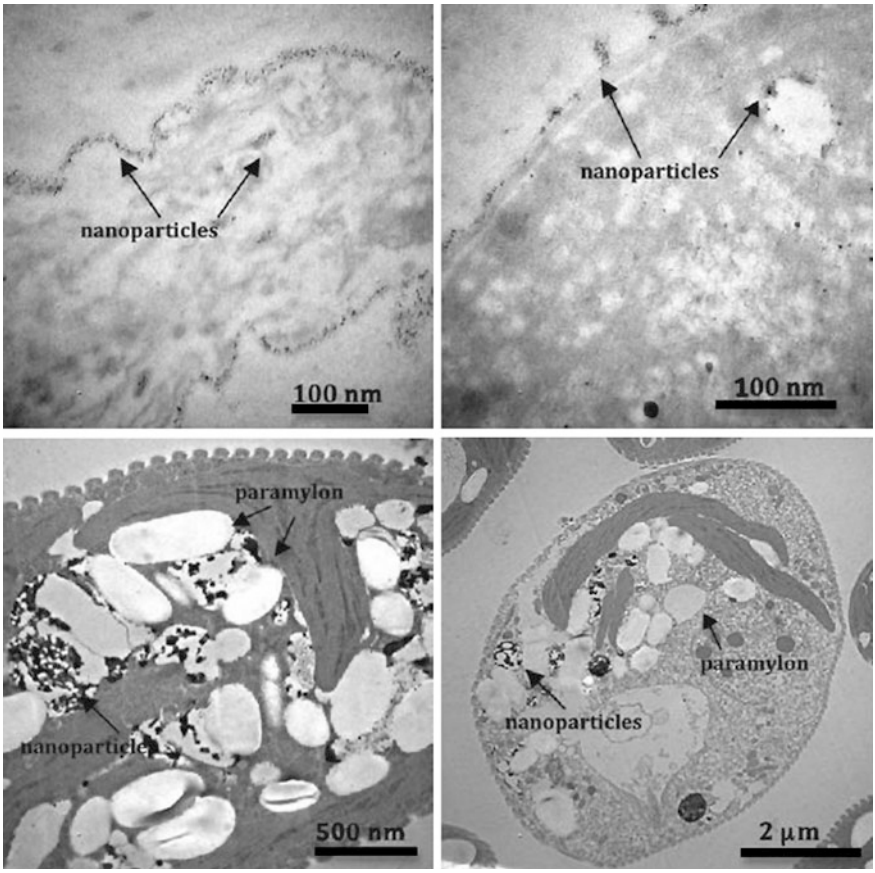


Fig. 11.14 TEM micrographs of (**a**) *Anabaena flos-aquae* after contact with CdS (10^{-3} M); **b** *Anabaena flos-aquae after* contact with CdS-TOPO (10^{-3} M); **c** *E. gracilis* after contact with CdS (10^{-3} M); **d** *E. gracilis* after contact with CdS-TOPO (10^{-3} M)

The effects of soluble cadmium salts versus CdSe quantum dots on the growth of planktonic *Pseudomonas aeruginosa* were studied [40]. In this study *P. aeruginosa* PG201 bacteria were cultured with similar total cadmium concentrations as either fully dissolved cadmium acetate or ligand-capped CdSe quantum dots, and cellular morphology, growth parameters, intracellular ROS, along with the metal and metalloid fates were measured. In this case, CdSe quantum dots dissolved partially in growth media, but dissolution was less in biotic cultures compared to sterile controls. Dose-dependent growth effects were similar for low concentrations for all samples, but effects differed above a concentration threshold of 50 mgL^{-1} where (i) the growth of CdSe-treated cells was more impaired, (ii) the membranes of CdSe-growth cells were damaged, and (iii) CdSe-growth cells contained CdSe cytoplasmic inclusions in addition to Se^0 and dissolved cadmium. In addition, for most concentrations, intracellular ROS were higher for CdSe versus free

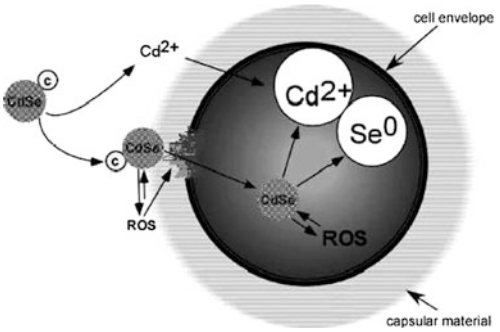


Fig. 11.15 Model of citrate-stabilized quantum dot interactions with *Pseudomonas aeruginosa* [40]. Copyright from American Chemical Society

Cd^{2+}-grown bacteria. *In fine*, quantum CdSe nanoparticles were more toxic to *P. aeruginosa* than Cd^{2+} ions, and were affected by cells through quantum dots stabilization, intracellular enrichment, and cell-associated decay (Fig. 11.15).

CdTe quantum dots are preferred for potential medical, diagnostic, and other basic research applications because of their unique intrinsic photophysical properties. Compared with traditional organic dyes, they are superior in many respects, such as their strong photostability and also fluorescence wavelength tunable by size [41, 42]. In the case of CdTe nanoparticles, the release of free Cd^{2+} ions is considered to be a major factor causing cytotoxicity. In order to reduce toxicity, but enhance the stability and biocompatibility of CdTe nanoparticles, different coating methods for bare CdTe have been developed.

Based on the same CdTe core, CdTe/CdS, CdTe/ZnS, and CdTe/SiO_2 nanoparticles were synthesized and their Cd^{2+} release rates were carefully studied based on dialysis using inductively coupled plasma mass spectroscopy [43]. In this study, the mechanism of Cd^{2+} release from Cd-based nanoparticles was proposed to be the result of surface oxidation, and Cd release can be considered as a first-order reaction. The results shown in Fig. 11.16a indicated the release rate of Cd from the bare CdTe nanoparticles is much greater than that for Te, and good linear relationships between Cd and Te concentration and the dialysis time were obtained for both Cd and Te.

The significant difference between the release rates of Cd and Te might be attributed to the different oxidation mechanisms they undergo. In fact, O_2 molecules oxidize chalcogenide atoms (S, Se, Te) located on the surface of the nanoparticles to form oxides. In the case of CdTe nanoparticles, Cd forms Cd^{2+} ions while Te forms TeO_2 on the surface [44]. The Cd^{2+} ions formed may diffuse into the dialysis solution, but TeO_2 formed can hardly dissolve in the dialysis solution, resulting in a much lower Te concentration and release rate compared with Cd. Furthermore, Fig. 11.16b demonstrates the linear relationship between the Cd concentration in the dialysis buffer solution and the dialysis time, suggesting that the corresponding Cd^{2+} released rate decreased in the order of CdTe > CdTe/CdS > CdTe/SiO_2 > CdTe/ZnS. In this work, the cytotoxicity of the CdTe nanoparticles was studied using *P. tricornutum* diatom microalgae. Figure 11.17 presents the growth curves of *P. tricornutum* cells exposed to Cd-based nanoparticles.

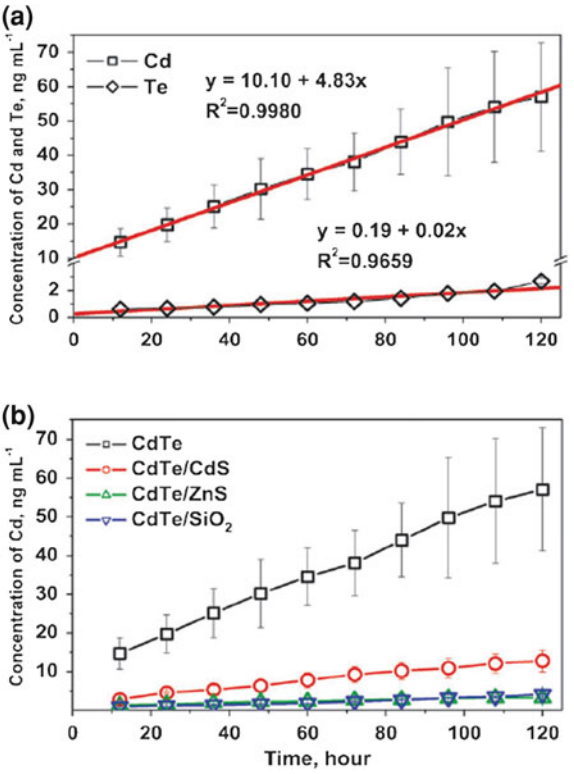


Fig. 11.16 **a** The concentration changes of Cd and Te released from bare CdTe nanoparticles in dialysis solution over time; **b** Cd released from CdTe, CdTe/CdS, CdTe/ZnS, and $CdTe/SiO_2$ in dialysis solution over time [43]. Copyright from The Royal Society of Chemistry

Based on these experimental results, it is possible to conclude that the cytotoxicity decreases in the order $CdTe > CdTe/CdS > CdTe/SiO_2 > CdCl_2 > CdTe/ZnS$. This result was in accordance with the Cd^{2+} ions release rates of the CdTe nanoparticles. On the other hand, the Cd content inside the cell of the CdTe exposed group was much higher than that in the other groups, suggesting that the uncoated and smallest CdTe nanoparticles might more easily have been taken up by the cells (Fig. 11.18).

It was found that water-soluble CdSe/ZnS nanoparticles have a high affinity for the *Chlamydomonas* sp. microalgae [45]. The adsorption of these nanoparticles to the algal cell surfaces is from a combined result of non-specific interactions, as well as possible reactions between the amine groups of the polysaccharides or glycoproteins in the algal cell wall and the carboxyl groups of the mercaptoundecanoic acid ligands coated on the nanoparticles surface. The porous structure of the algal cell wall also afforded ample binding sites for the nanoparticles. In this study, no clear evidence of nanoparticles internalization was found. This lack of internalization could be explained as a result of the thick cell wall, the size and aggregation state of the nanoparticles, and a lack of endocytosis capacity of the microalgae. The nanoparticles adsorption has been found to hinder the algal photosynthetic activity, as indicated by both reduced CO_2 depletion for

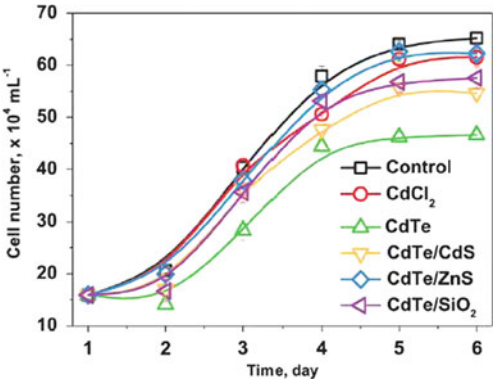


Fig. 11.17 Growth *curves* of *P. tricornutum* in the control group and exposed Cd-based groups at initial concentration of 300 ng mL^{-1} [43]. Copyright from The Royal Society of Chemistry

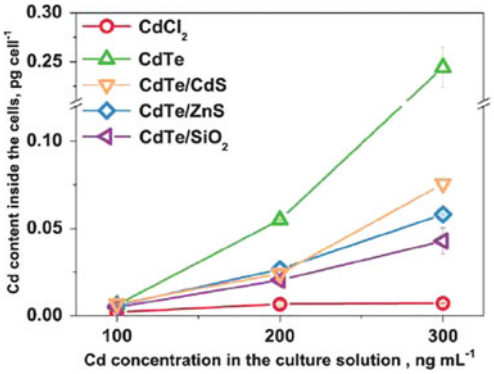


Fig. 11.18 The Cd contents inside *P. tricornutum* cells with respect to the Cd concentration in the culture solutions on the sixth day [43]. Copyright from The Royal Society of Chemistry

nanoparticles over 100 ppm and declined O_2 production for low nanoparticles concentration but no apparent algal cell death was observed in this study.

The bioaccumulation and effects of CdTe/CdS nanoparticles were also studied using *Chlamydomonas reinhardtii* microalgae [46]. In this case, the nanoparticles dissolution increased with decreasing pH and with increasing nanoparticles concentration. When exposed to CdTe/CdS nanoparticles, bioaccumulation was largely accounted for by dissolved Cd. Nonetheless, these nanoparticles were shown to be taken up by the cells and to provoke unique biological effects. Whole transcriptome screening using RNA-Seq analysis showed that the free Cd^{2+} ions and the CdTe/CdS nanoparticles had distinctly different biological effects.

11.4 Conclusions

Because of the environmental use of nanoparticles such as ZnO and quantum dots and their unintentional release, the exposure of algae, cyanobacteria, plants, and fungi to these new materials is a reality. However, at this moment there is some

lack of information on some key aspects, which prevents a better understanding and assessment of the toxicity and ecotoxicity of nanoparticles to these key ecosystem organisms. So, we can draw the following conclusions regarding the examples cited. The toxicological impact of nanoparticles on microorganisms depend on (i) the physicochemical properties of these nanoparticles (surface area, charge and functionalization, aggregation rate, dissolution, etc.), (ii) the structural and morphological properties of the nanoparticles, and also (iii) the biological target used for the tests.

The multidisciplinary approaches needed to address these questions stress the paramount importance of collaborative efforts between ecotoxicologists, toxicologists, biologists, and chemists.

References

1. Gerber C, Lang HP (2006) Nat Nantechnol 1:3
2. Guzman KAD, Taylor MR, Banfield JF (2006) Environ Sci Technol 40:1401
3. Hervé-Bazin B (ed) (2007) Les nanoparticles: un enjeu majeur pour la santé au travail? EDP Sciences, Paris
4. Kumar CSSR (ed) (2006) Nanomaterials—toxicity, health and environmental issues. Wiley-VCH, Weinheim
5. Baun A, Hartmann NB, Grieger K, Kusk KO (2008) Ecotoxicology 17:387
6. Pinnel SR, Fairhust D, Gillies R, Michnick MA, Kollias N (2000) Dermatol Surg 26:309
7. Zhang Y, Chen YS, Westerhoff P, Crittenden JC (2009) Water Res 43:4249
8. Zhou D, Keller AA (2010) Water Res 44:2948
9. Zhang Y, Chen YS, Westerhoff P, Hristovski K, Crittenden JC (2008) Water Res 42:2204
10. Xia T, Kovochich M, Liong M, Mdler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nel AE (2008) ACS Nano 2:2121
11. Zhu XS, Wang JX, Zhang XZ, Chang Y, Chen YS (2009) Nanotechnology 20:195103
12. Miller RJ, Lenihan HS, Muller EB, Tseng N, Hanna SK, Keller AA (2010) Environ Sci Technol 44:7329
13. Jolivet JP, Livage J, Henry M (2000) Metal oxide chemistry and synthesis: from solution to solid state. Wiley, Chichester
14. Peng X, Palma S, Fisher NS, Wong SS (2011) Aquatic Toxicol 102:186
15. Brayner R, Ferrari-Iliou R, Brivois N, Djediat C, Benedetti MF, Fiévet F (2006) Nano Lett 6:866
16. Brayner R (2008) Nano Today 3:48
17. Brayner R, Dahoumane SA, Yéprémian C, Djediat C, Meyer M, Couté A, Fiévet F (2010) Langmuir 26:6522
18. Aruoja V, Dubourguier HC, Kasemets K (2008) Sci Total Environ 407:1461
19. Hall S, Bradley T, Moore JT, Kuykindall T, Mirella L (2009) Nano Toxicol 3:91
20. Miao AJ, Schwehr KA, Xu C, Zhang SJ, Luo ZP, Quigg A, Santschi PH (2009) Environ Pollut 157:3034
21. Van Hoecke K, Quik JTK, Mankiewicz-Boczek J, De Schamphelaere KAC, Elsaesser A, Van der Meeren P, Barnes C, McKerr G, Howard CV, Van de Meent D, Rydzynski K, Dawson KA, Salvati A, Lesniak A, Lynch I, Silversmit G, De Samber B, Vincze L, Janssen CR (2009) Environ Sci Technol 43:4537
22. Van Hoecke K, De Schamphelaere KAC, Van der Meeren P, Lucas S, Janssen CR (2008) Environ Toxicol Chem 27:1948

23. Fujiwara K, Suematsu H, Kiyomiya E, Aoki M, Sato M, Moritoki N (2008) J Environ Sci Health Part A 43:1167
24. Ji J, Long Z, Lin D (2011) Chem Eng J 170:525
25. Yu LP, Xiong DW, Zhu WT, Sima XF (2011) J Environ Monit 13:1975
26. Peng X, Manna L, Yang W, Wickham J, Scher EC, Kadavanich A, Alivisatos AP (2000) Nature 404:59
27. Brus L (1986) J Phys Chem 90:2555
28. Manna L, Scher EC, Alivisatos AP (2000) J Am Chem Soc 122:12700
29. Peng ZA, Peng X (2001) J Am Chem Soc 123:1389
30. Mahendra S, Zhu H, Colvin VL, Alvarez P (2008) Environ Sci Technol 42:9424
31. Bouldin JL, Ingle TM, Sengupta A, Alexander R, Hannigan RE, Buchanan RA (2008) Environ Toxicol Chem 27:1958
32. Brayner R, Dahoumane SA, Nguyen JN-L, Yéprémian C, Djadiat C, Couté A, Fiévet F (2010) J Nanosci Nanotechnol 10:1
33. Hines MA, Guyot-Sionnest P (1996) J Phys Chem 100:468
34. Derfus AM, Chan WCW, Bhatia SN (2004) Nano Lett 4:11
35. Ryman-Rasmussen JP, Rivière JE, Monteiro-Rivière NA (2007) J Invest Dermatol 127:143
36. Hartwig A, Asmuss M, Ehleben I, Herzer U, Kostelac D, Pelzer A, Schwerdtle T, Burkle A (2002) Environ Health Perspec 110:797
37. Zimenkov YV, Salminen A, Efimova IS, Lahti R, Baykov AA (2004) Eur J Biochem 271:3064
38. Smeets K, Cuypers A, Lambrechts A, Semane B, Hoet P, Van Laere A, Vangronsveld J (2005) Plant Physiol Biochem 43:437
39. Nies DH (1999) Appl Microbiol Biotechnol 51:730
40. Priester JH, Stoimenov PK, Mielke RE, Webb SM, Ehrhardt Ch, Zhang JP, Stucky GD, Holden PA (2009) Environ Sci Technol 43:2589
41. Bruchez M Jr, Moronne M, Gin P, Weiss S, Alivisatos AP (2005) Science 307:538
42. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S (1998) Science 281:2013
43. Xu M, Deng G, Liu S, Chen S, Cui D, Yang L, Wang Q (2010) Metallomics 2:469
44. Yi X, Liou JJ (1995) Solid-State Electron 38:1151
45. Lin S, Bhattacharya P, Rajapakse NC, Brune DE, Ke PC (2009) J Phys Chem C 113:10962
46. Domingos RF, Simon DF, Hauser C, Wilkinson KJ (2011) Environ Sci Technol 45:7664

Chapter 12
Cerium Oxide Nanoparticles: Structure, Applications, Reactivity, and Eco-Toxicology

Mercedes Perullini, Sara A. Aldabe Bilmes and Matías Jobbágy

Abstract In this chapter, the physical, chemical, and ecotoxicological features of nanometric cerium oxide will be discussed on the basis of the recent research. In contrast with other oxides such as SiO_2, ZnO, ZrO_2, or TiO_2 with relevant industrial applications, ceria presents a unique redox chemistry that expanded its application to fields that take advantage of its chemical reactivity, as heterogeneous catalysis and detoxification of gaseous exhausts. In the past, several studies were strictly focused on the exploration of its eventual damage to environment and human health. CeO_2, as other rare earths oxides, is basically a low toxicity substance[1] and nowadays there is vast and increasing evidence pointing to its potential role as protective compound in terms of human health. The aim of this chapter is to offer a wide scope of description of the intrinsic physicochemical behavior of this unique compound, with deep emphasis in the inherent challenge that represents a definitive understanding of its surface chemistry. The apparent contradiction between toxicity and health benefits will be discussed according to the present evidence and the intrinsic limitations of these complex studies.

12.1 Introduction: Physicochemical Properties of CeO_2 and its Relevant Applications

Cerium that holds a $4f^2 5d^0 6s^2$ electronic configuration has a chemistry mostly governed by the trivalent and tetravalent oxidation states, in contrast with most of the lanthanides stabilized at the trivalent state exclusively. In natural environments,

M. Perullini · S. A. Aldabe Bilmes · M. Jobbágy (✉)
Laboratorio de Superficies y Materiales Funcionales INQUIMAE-DQIAQF,
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Ciudad Universitaria, Pab. II, C1428EHA, Buenos Aires, Argentina
e-mail: jobbag@qi.fcen.uba.ar

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_12,

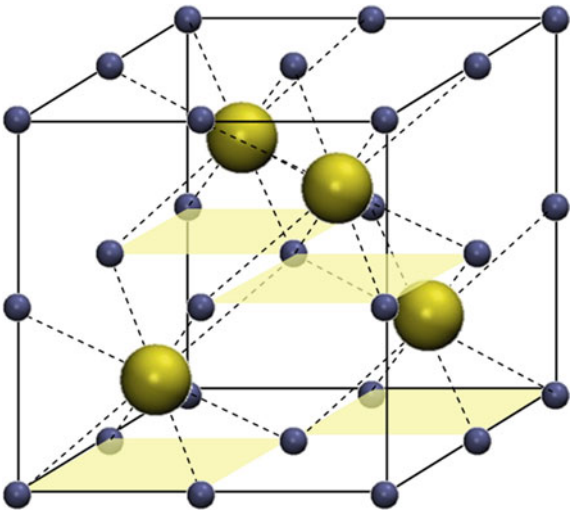


Fig. 12.1 Scheme of the cubic unit cell of CeO_2; gray spheres represent O^{2-} anions while yellow ones Ce^{4+} cations. Dotted lines indicate distance between the nearest 8 oxygen neighbors of each cerium atom. Colored planes indicate the base of the four subcells occupied by Ce^{4+} cations

this element typically lies in several minerals as alanite, bastanite, monazite, cerite, and samarskite, partially substituted by other trivalent rare earths. However, only bastnaesite, a hexagonal $Ce(OH)CO_3$ also containing fluoride anions and monazite, $CePO_4$, represents the most commercial sources. The relevant phase of this element from a technological point of view is its pure oxide, CeO_2, also known as Cerianite. This phase crystallizes in the Fluorite structure, which is named after the mineral form of CaF_2. It has a face-centered cubic unit cell (*f.c.c.*) with space group F*m3 m,* having a characteristic lattice parameter $a = 0.541134(12)$ nm [2]. In this structure, each cerium cation is coordinated by eight equivalent nearest neighbor oxygen anions at the corner of a cube, each anion being tetrahedrally coordinated by four cations (Fig. 12.1).

The pure oxides of cerium constitute a vast family of mixed valence compounds ranging from the fully oxidized CeO_2 form to the totally reduced Ce_2O_3, the *C*-type sesquioxide, regarded as a fluorite type in which 25% of the anion sites are vacant and ordered (a = 1.116 nm) [3], depending on the temperature and oxygen partial pressure [4]. Reduced ceria results from the removal of O^{2-} ions from the CeO_2 lattice, which generates an anion-vacant site according to the following scheme:

$$4\,Ce^{4+} + O^{2-} \rightarrow 4\,Ce^{4+} + 2e - /\square + 0.5\,O_2 \rightarrow 2\,Ce^{4+} + 2Ce^{3+} + \square + 0.5\,O_2 \tag{12.1}$$

where □ represents an empty position (anion-vacant site) originated from the removal of O^{2-} anions from the lattice, here represented as an oxygen tetrahedral site (Ce_4O). Electrostatic balance is persevered by the reduction of two cerium cations from the tetravalent to the trivalent state. Historically, the oxides of cerium

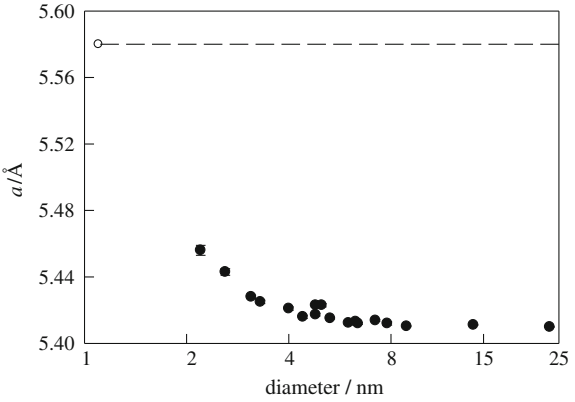


Fig. 12.2 Expansion of CNPs lattice parameter, *a*, as function of particle's diameter reported by Baranchikov et al.[24] Dotted line represents the lattice parameter of pure Ce_2O_3 sesquioxide and the empty dot the limit diameter predicted by several authors

in the range Ce_2O_3–CeO_2 were treated using the classical point-defect model of nonstoichiometry, in which oxygen-vacant sites were considered to be present in the lattice in a randomized fashion. However, bulk stoichiometric phases with ordered vacancies were described in terms of geometric models for defect ordering [5–10]. Beyond this, a lattice expansion results from the reduction of Ce^{4+} ions to Ce^{3+}, the radius of the Ce^{3+} ion being larger than that of Ce^{4+} (1.14 Å vs. 0.97 Å), according to the data of Shannon and Prewitt and in good agreement with the observations made over several doped forms of CeO_2 [11–13]. It was observed by several authors a lattice expansion of ceria when the crystal size drops to a few nanometers [14]; there is a general agreement in assigning that expansion to the stabilization of Ce^{3+} ion [14–19]. Early computer simulations [20] indicated that for substances with predominantly ionic type of bond (in particular, for oxides) the change in the unit cell parameter on passing to the nanostate is related to the change in the formal oxidation state of atoms. For the case of ceria nanoparticles (CNP), the extrapolation of lattice parameter values suggested that the *C*-type sesquioxide exists in the pure form once their diameter drops to less than 1.5–1.1 nm and most of Ce^{3+} ions are located near the surface. This observation is consistent with the enhanced stability of oxygen vacancies in the surface of ceria in comparison with oxygen vacancies in the bulk [21], and with the enhanced reducibility of small ceria clusters compared to bulk ceria [22]. Detailed inspections based on electron energy loss spectroscopy revealed that the oxygen nonstoichiometry of CNP can be envisaged as core–shell nanostructures; in relatively large particles, the core has a composition close to stoichiometric cerium dioxide and the surface is close to Ce_2O_3 [17, 23]. Very recently, a systematic structural exploration supported by Rietveld refinement of the nanostructures was reported, confirming that this common trend describes a vast population of particles obtained under diverse preparation methods [24] (Fig. 12.2).

Among reactive rare earth oxides, CeO_2 plays a key role in industrial catalysis due to the reversibility of its redox cycle that gas–solid equilibrium implies and

Table 12.1 Main gas–solid heterogeneous redox reactions driven by ceria at moderate temperatures

$CeO_{2-x} + x/2\ O_2$	→	CeO_2	(Eq. 12.1)
$CeO_2 + x\ CO$	→	$CeO_{2-x} + x\ CO_2$	(Eq. 12.2)
$CeO_2 + x/3\ C_nH_{2n}$	→	$CeO_{2-x} + nH_2O + n\ CO_2$	(Eq. 12.3)
$CeO_2 + x/2\ SH_2$	→	$CeO_{2-x} + x/2\ S + x/2\ H_2O$	(Eq. 12.4)
$CeO_{2-x} + x/2\ SO_2$	→	$CeO_2 + x/2\ S$	(Eq. 12.5)
$CeO_{2-x} + x/2\ NO_2$	→	$CeO_2 + x/2\ N_2$	(Eq. 12.6)

this property is regarded as oxygen storage capacity (OSC) [25]. CeO_2 has potential uses in two of the most important commercial catalytic processes as the three-way catalysis (TWC) [26] and the fluid catalytic cracking (FCC). It is also involved in solid-state reactions, as the removal of soot from diesel engine exhaust [27], for the catalytic wet oxidation of organics from wastewaters [28], as an additive for combustion catalysts and processes [29], and the emerging field of IT-SOFC fuel cell technology, either as a solid electrolyte [30] or anode [31]. Then, much effort is still focused in studying the role of ceria and its substituted forms in well-established industrial processes; [32] the most relevant reactions of ceria are summarized on Table 12.1.

Beyond the aforementioned reactions, more detailed exploration of CeO_2 reactivity at an atomic level revealed that the crystal plane of ceria dramatically affects its catalytic properties for CO oxidation [33]. Single-crystalline CeO_2 nanorods reveal that the predominantly exposed 001 and 110 planes are more reactive for CO oxidation in contrast with the 111 stable ones that prevail in irregular nanoparticles [33]. According to high-resolution transmission electron microscopy, different exposed crystal planes prevail on each kind of single crystal morphology: 111 and 100 for polyhedral, 110 and 100 for rods, and 100 for cubes. Reactivity is also affected by morphology; OSC measurements recorded at 400 °C revealed that reversible reduction takes place both at the surface and the bulk in the case of CeO_2 nanorods and nanocubes, but is restricted at the surface for the nanopolyhedra, just like the bulk one. This result suggests that high OSC materials might be designed and obtained by a shape-selective synthetic strategy [34]. A tuned morphology can improve the OSC achieving useful activities at a temperature 250 °C less than the recorded for irregular ones [35, 36]. Nowadays, there is a vast number of synthesis methods, including highly shape-selective ones, for the preparation of CNPs [37], ranging from mechanochemical procedures [38–44], high temperature combustion [45], and mild thermal decomposition [46, 47], to microwave- or sonochemical-based ones [48].

The basic aspect underlying this structure-dependent reactivity triggered in silico-based research; the formation of oxygen vacancies through depletion of oxygen from CNP (Ce_nO_{2n} with $n < 80$) was found to be greatly facilitated compared to extended surfaces, which explains the observed spectacular reactivity of nanostructured ceria [49–51].

The redox properties of elemental cerium also played a relevant role in the metallurgic industry, as an anticorrosion agent. The high affinity of cerium for oxygen and sulfur underlies the use of cerium-containing ferro-alloys to improve the physical properties of high strength low-alloy (HSLA) steels [52–66] or aluminum-based alloys [67]. In the iron casting process, cerium is considered to remove free oxygen and sulfur from the melt, improving significantly this oxidation resistance [68]. In electrolysis, self-forming anode technology is used whereby cerium oxide coatings are deposited onto conducting ceramic substrates. Cerium oxide provides an alternative to thorium oxide, a common additive in welding electrodes that is now being phased out for environmental reasons. The addition of cerium oxide to other oxides as zirconia produces a material with exceptional toughness and good strength [69, 70]. Cerium oxide-doped zirconia is also used in thermal barrier spray coatings on metal surfaces.

Beyond the chemical properties of cerium oxides, increasing attention has been paid to applications dealing with the interaction of this large-gap semiconductor phase with light. As might be expected, nanoconfinement affects the phase's intrinsic band diagram and CNPs exhibited noticeable changes in its absorptive properties as a function of size; Masui et al. [71] reported that a decrease in micelle stabilized particles sized from 4.1 to 2.6 nm is accompanied by an increase in the band gap energy (E_g) from 2.73 to 2.87 eV for indirect transition and from 3.38 to 3.44 eV for direct transition. Other researchers found a similar trend for colloidal CNP however the found E_g values markedly exceeded those reported earlier, probably due to the inherent high error involved in linearization of UV spectroscopy data [72]. This tendency observed for colloidal ceria was also reproduced in thin films of nanocrystalline cerium dioxide [73]. Zhang et al. [74] reported an expression linking the dependence of the CeO_{2-x} band gap for direct transition on the particle size of radius R and a relative dielectric constant of cerium dioxide equal to 24.5. Beyond this issue, it was stated that the observed change obeys to the increasing reduction of Ce^{4+}; increasing the energy difference between the O $2p$ and Ce $4f$ orbitals, resulting in a hypsochromic shift of the absorption band of this phase. In this scenario, it is not surprising to find differences among different colloids depending on the preparation procedure [75, 76]. The relative contribution of quantum confinement and partial reduction is still a matter of debate [77]. The ability of CNP to drive electron–hole splitting under light absorption was exploited for its potential application in solar cells, photocatalytic degradation of organic pollutants as well as photocatalysis sensitizing agents for TiO_2 [78–85]. UV-shielding property of certain nanocrystalline semiconductor materials is widely used in sunscreen cosmetics; most of inorganic UV-blocking filters are based on titanium dioxide (TiO_2) and zinc oxide (ZnO). However, it was reported that the aforementioned oxides can eventually exert certain degree of cell damage on brain cells [86], blood lymphocytes [87], and lymphoblast cells damage by titania nanoparticles [88]. Both zinc and titanium oxides nanoparticles exhibit remarkable photocatalytic activity under UV-irradiation, even immersed within sunscreen cosmetics, releasing reactive oxygen species (ROS) that can react with skin cells damaging their DNA [89–96]. Photocytotoxicity of titania against

fibroblasts has also been confirmed [97]. As an alternative to the aforementioned compounds and in accordance to its E_g, CeO_2 emerge as a friendly option, due to the intrinsic highly defective structure of ceria lattice, in which the recombination of free charge carriers (electrons and holes) forming upon UV-irradiation of ceria proceeds very rapidly compared to TiO_2. Additionally, UV-extinction coefficient of ceria is rather high, positioning this phase as a promising alternative UV-filter in sunscreen cosmetics [98–100]. CNPs exhibit protective properties against radiation-induced cellular damage, radiation-induced pneumonitis, and can prevent retinal degeneration by photons of harmful light [101, 102]. Very recently it was reported that the sun protection factor, the critical absorption wavelength, and the UVA/UVB-ratio of ceria nanoparticles are comparable to traditional oxide nanoparticles holding a dramatically lower photocatalytic activity. A recent report describes the protective character of CNPs for mouse fibroblasts (L929) and fibroblast-like cells of African Green monkey (VERO) exposed to UV-irradiation [103].

Concerning photoemissive properties of cerium ions, once isolated in a proper host are an essential luminescent component applied in several phosphors formulations. Upon excitation by energetic cathode-ray electrons, they produce useful light emission, finding application in numerous light sources such as compact fluorescent lighting and related devices [104–108]. However, there is increasing evidence pointing to CeO_2 even in the form of CNPs, as a suitable phase to host and activate several photoemitting centers as Eu, Tb, or Yb, with potential application in biolabeling [109–114].

Due to its intrinsic hardness, cerium oxide is the most efficient polishing agent for most glass compositions as well as a glass additive to diminish undesired Fe absorption preventing UV-driven damage or antireflective coatings [115–118]. Cerium oxide has a high refractive index, and is an opacifying agent in enamel compositions used as protective coatings on metals. Rare earth sulfides, among them also cerium, are used in glass and ceramics as colorants to replace toxic CdS. In certain glass compositions (at low weight percentages) along with comparable amounts of titanium oxide, cerium oxide produces a deep yellow coloration.

In the last decade, a vast amount of basic research was focused on exploring and elucidating the redox activity of CNPs in aqueous media, beyond all the well-established industrial applications of CNP in gas solid heterogeneous catalysis [119]. This particular issue was recently reviewed in great detail and the following section describes the main aspects of its fascinating biomimetic chemistry [120]. In a straight resemblance to enzymatic redox reactions, nanoceria with a high Ce^{3+}/Ce^{4+} ratio on its surface is able to reduce superoxide to peroxide, (see Scheme 12.1) playing the role or superoxide dismutase (SOD) [121]. However, the mechanism for the restoration of reduced nanoceria remains uncertain [122]. It was also shown that H_2O_2 is able to oxidize ceria from Ce^{3+} to Ce^{4+} in a reversible fashion, in the time scale of days [101, 123, 124]. Celardo et al. [125] proposed a comprehensive mechanism of regeneration of nanoceria, based on the combination of both the SOD mimetic [121] and the catalase-

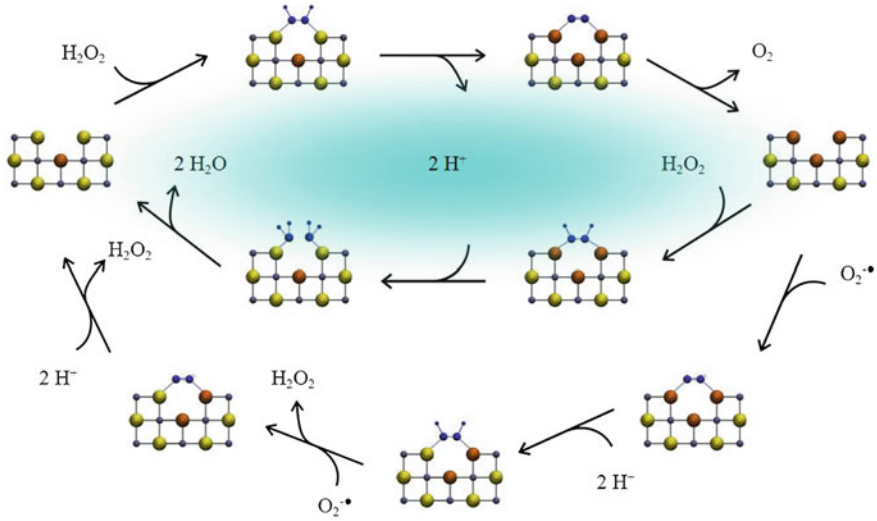


Fig. 12.3 Scheme of the main cycle for the oxidation of hydrogen peroxide by nanoceria via reduction by superoxide and the active site regeneration, according to the catalytic pathway proposed by Celardo et al.[125]. A surface oxygen-deficient site on the nanoceria (extreme left) offers a two Ce^{4+} binding site for H_2O_2, the release of protons is coupled with two-electron transfer to the two cerium ions and molecular oxygen is released from the now fully reduced oxygen vacancy site (*extreme right*). Superoxide binds to this site, and is reduced by one Ce^{3+}; the uptake of two protons releases H_2O_2. With the coordination of a second superoxide molecule, the oxygen vacancy site returns to the initial stable state, with two Ce^{4+}, releasing a second H_2O_2 molecule. A plausible reaction mechanism for hydrogen peroxide's disproportion can be envisaged as a shortcut of the former mechanism (*cycle around blue shadow*): The reductive site (*extreme right*) binds a second H_2O_2 molecule to the two neighbors Ce^{3+} centers, with a subsequent uptake of two protons and breakage of the O–O bond with transfer of electrons to the two Ce^{3+}, and release of the water molecules to regenerate the initial Ce^{4+} site (*extreme left*) in an analogous way to the previous scheme

mimetic activity [124] (see Fig. 12.1). Interestingly, by coupling both redox steps (superoxide to peroxide, peroxide to O_2) the paradoxical toxic effects of SOD enzymes observed for cell systems possessing low catalase levels could be skipped [126] (Fig. 12.3).

These simply coupling of redox cycles easily explain the ROS scavenging activity of ceria; however, it should be kept in mind that the degree of hydroxylation, the pH as well as the presence of competing anions as phosphates with high affinity for Ce^{3+} sites could severely affect the aforementioned pathway and the intrinsic activity of ceria [127]. CNP (unlike SOD) can inactivate also the hydroxyl radical OH [128]. This is in line with the recent discovery of the key role that Ce^{3+}, instead of oxygen vacancies, plays in the intracellular antioxidant effect on leukocyte cell lines [129]. In silico studies suggest that reduced ceria, CeO_{2-x}, in contact with molecular oxygen leads to the formation of peroxo, O_2^{2-}, and

superoxo, O_2^-, species. The formation of the former can be explained by the interaction of O_2 with two electron-donor oxygen vacancies at the ceria surface. In contrast, the latter form can be explained by direct interaction of O_2 with low-coordinated Ce^{3+} ions on reduced ceria nanoparticles [130]. Beyond the ability to catalyze these ROS scavenging cycles [131], CNP (3–5 nm in size) are able to hydrolyze phosphate ester bonds, cleaving phosphate groups from biologically relevant molecules [132]. Eventually, as can be envisaged from the inherent capacity of CNP to decompose NOx-carrying exhausts, Nitrogen reactive species (NOS) can also be effectively scavenged by CNP under physiological conditions [119].

Concerning the attempts to achieve a comprehensive exploration of the enzymatic mimetic reactivity and the eventual toxicity of CNP, several critical issues should be taken into account. Numerous factors limit the straight comparison of the results reported by different authors. As was stated by Celardo et al. [125], and in agreement with the dramatic shape-dependant reactivity found, CNPs obtained under different protocols could exhibit very different intrinsic surface reactivity. Instead of mass based concentrations, effective available surfaces seems to be a more robust parameter to define CNP activities, however, the coalescence of nanoentities in the form of larger aggregates, an expectable phenomenon that inevitably occurs in biological studies. In this sense, strategies to prevent agglomeration include capping nanoceria with organic compounds [133] provided that the external layer does not alter the ceria biological effects, and that it is biocompatible and biodegradable [134]. Citrate capping was recently shown to promote nanoceria cell uptake without cytotoxic effects [135].

Finally, another important source of uncertainty is the eventual release of Ce^{3+} ions to the liquid media, since the reduced form may be soluble in water for pH values lower than 7.5 [136], which are found in most of the reports. This could be critical for the biological effect of nanoceria, since soluble Ce^{4+} salts might be toxic in vivo [137]. Additionally, from a mechanistic point of view, Ce^{3+} ions demonstrated to rapidly react in aqueous solution with H_2O_2, producing hydroxyl radicals in a Fenton-type reaction [138]. These side effects have been the main hindrance for the development of catalase and SOD model compounds in the past [139] and the true limits between heterogeneous and homogeneous reactivity of cerium is still a matter of debate [140]. The high reactivity of ceria nanoparticles in aqueous suspensions and its role on assorted redox processes [134, 141] combined with their inherent low toxicity [142] open the gate for its application linked with the inactivation of some of the most toxic ROS, such as superoxide radical [121], hydrogen peroxide [124], and nitroxyl radical [143]. Nowadays, CNP is considered one of the most promising inorganic nanomaterials in the field of nanomedicine [120, 144–150].

12.2 Interaction of CNPs with the Environment and Representative Organisms

Before the industrial era the fate of cerium compounds was governed by the inherent geochemical cycle of rare earths, which is affected by several complex and interactive processes as any weathering process, including atmospheric phenomena, geologic activity, physical and chemical weathering, hydrologic cycles, etc. [151]. Once CNPs are exposed to reducing environments the eventual presence of free Ce^{3+} is unlikely since in the soil, like the other lanthanides, this cation is immobile under a wide range of pH conditions, due to the low solubility of its typical solid phases, such as carbonates, fluorohydroxides, and phosphates. Since lanthanides sorbs strongly to silicates and humic material, the bulk of the Ln content including cerium is associated with such colloidal particulates present in most natural waters [152]. The fate of CNPs in aquifers is controlled by the inherent complexity of colloidal physicochemistry, in general agreement with DLVO theory. Advanced modeling is subject of current research [153].

Concerning the anthropogenic sources of CNPs, the massive emission by refineries and automobiles [154, 155] into the atmosphere and hence, the whole environment has been a matter of concern since decades. Ten years ago, the National Institute of Health (USA) reported a comprehensive study summarizing the impact of these emissions on human health [156]. Another report from the Health Effects Institute also analyzes this topic in great detail [157]. More recently, the Environmental Protection Agency (USA) published a toxicological review of cerium oxide and cerium compounds, in the frame of the Integrated Risk Information System (IRIS) [158]. In the following section, the most recent research concerning the interaction of CNPs with the environment and the human health will be summarized and discussed. As a first reductionist approach to envisage the eventual environmental impact of a certain nonentity, toxicity assays performed over common wild microorganism can offer a first glance of the potential damage that those entities represent. Yet, this approach is matter of debate and has intrinsic limitations [159]. Most of the work on ecotoxicological effects has been done with algae and aquatic organisms. Some work has been done with bacteria as model organisms and few studies with seeds or plants in order to determine the effect of NPs in germination and the possible translocation in leaves. Data on ecotoxicological effects of CNPs are scarce and seems to be contradictory. However, most of published work does not compare similar shapes and surface derivatization conditions. The concentration doses are also very dissimilar. The effect of size on survival or growth has been established: the smaller the NPs, the more important the damage.

Table 12.2 Compilation of ecotoxicological studies performed with CNPs

Microorganism and reference	CNPs source, properties and concentration range	assays	observations
Ref. [160] *Pseudokirchneriella subcapitata*	Sigma Aldrich, 7–25 nm (TEM) aggregates 20–500 nm; $75m^2\ g^{-1}$, characterization: Ce(III):Ce(IV) by PEELS [a] (NPs 62 % surface Ce(III)), dissolution, redox potential, XRD, TEM, DLS, BET, zeta potential. 1–200 mg/L	IC50 [b] after 72 h compared to the controls Membrane damage by fluorescence of DNA-binding dye SYTOX® Green General oxidative activity with KI Production of OH radicals by coumarin assay ICP-AES phosphate Lipid peroxidation by TBARS [c] assay (linoleic acid as model fatty acid)	CNPs 6.5 times more toxic than micro (bulk) CeO_2. When normalised to surface area NPs are 4 times less toxic than bulk. 40–50 % more depletion of P for NPs Negligible dissolution in algal medium Increase of cell permeability with NPs. Oxidative stress is photocatalytic (not important in dark)
Ref. [161] *Pseudokirchneriella subcapitata* & Anabaena CPB4337 CPB4337	Sigma Aldrich 10–60 nm Charcaterization: BET, TEM, TXRF, DLS, zeta, ICP. Large aggregates (300-500 nm) [d], positive surface charge in culture media (negative in water). Low Ce dissolution, depend on particle size. 0–100 mg/L.	Growth inhibition by OD and direct cell counting ATP by production of oxyluciferin on *Pseudokirchneriella subcapitata* Luminescence inhibition of Anabaena CPB4337	EC50 after 24h has a minimum for NPs 13–22 nm no evidence of nanoparticle uptake by cells The presence of salts decrease toxicity of NPs, not that of dissolved cerium toxic mode of action by direct NP-cell contact cells with damaged membrane shortening and narrowing of filaments (Anabaena)
Ref. [162] *Pseudokirchneriella subcapitata Daphnia magna* and *Thamnocephalus platyurus* embryos of *Danio rerio*	14, 20 and 29 nm supplied by industry partners 61, 42 and $29m^2\ g^{-1}$ XANES Ce dissolution by ICP. 3.2–32 mg/L.	*P. subcapitata* l growth by cell counting and fluorescence of extracted chlorophyll. TEM for NP-cell contact Ammonia and phosphate (nutrient depletion)	EC10 for 2.6–5.4 mg/mL Decreased algal cell density not correlated to clusteringWeak NP or aggregates with algal cell interaction. No evidences of uptake or strong adsorption Negligible dissolution of CeO2 Toxicity is not due to depletion of nutrients. Reduction of growth when 60 % phosphate depleted (50 % reduction in growth rate in phosphate-free medium) No evidence of shading
		crustaceans: *Daphnia magna* and *Thamnocephalus platyurus* Living organisms after 24 h	No acute toxicity up to 1000 (D. Magna) and 5000 (T. platyurus) mg/mL
		living organisms after 72 h TEM for NP-embryos of *Danio rerio* contact	No acute toxicity up to 200 mg/mL Evidence of NP adherence

(continued)

Table 12.2 (continued)

Microorganism and reference	CNPs source, properties and concentration range	assays	observations
Ref. [163] *daphnia magna* neonates	NPs Sigma Aldrich < 25 nm or< 5 μm XRD, DLS In culture water NPs aggregates 3950 nm; micro particles, aggregates 2730 nm. 0–10 mg/L.	Survival and molting (count of carapaces) Acute (96 h) and chronica (21d) exposures. (0.01< C< 10 mg/L)	Only NPs inhibit moulting at 10 mg/L.. Inhibition of growth at 10 mg/ L and 0.01 mg/ L (not 1 or 0.1) Neonates 33 % smaller in size, absence of algae from the digestive tract and lack of lipid storage droplets due to reduced feeding or increased metabolism and/ or excretion rate.
Ref. [164] *daphnia magna Vibrio fischeri*	Synthesis by, oxidation with HMT. [74]64–640 mg/L	Median lethal concentration LC50 of *daphnia magna*	LC_{50}= 0.012 mg/mL ; HMT non toxic
		Decrease of *Vibrio fischeri* bioluminescence [e]	Inhibition ≥ 80 % at 0.064 mg/mL
Ref. [165] *daphnia magna* neonates & *Cyprius Carpio* trout hepatocytes	CNPs Sigma Aldrich < 25 nm or < 5 μm. XRD, DLS, TEM. 0–10 mg/L.	*daphnia magna* neonates lethality and shedding of the carapace	no mortality observed
		Cyprius Carpio tissue analysis by ICP-MS	no detection
		trout hepatocytes lactate dehydrogenase (LDH) release	no LDH release
Daphnia Magna neonates & *Chironomus riparius* larvae	synthesized by hydrothermal method in supercritical water; 15 and 30 nm (TEM); 56 and 9 m^2/g respectively. 1 mg/L	Mortality reproduction *in vivo* Comet assay for DNA damage	CNPs are genotoxic and induced DNA damage slight increase of mortality (higher with 15 nm CNPs) no significant changes in growth and reproduction
Ref. [166] zebrafish (*Danio rerio*)	Sigma-Aldrich, 10.2±1.5 nm by TEM. 0.5–5 mg/L.	Fish exposed to NPs in water column under semistatic 24 h and 7 days. ICP of fish tissues.	Significant uptake in livers of fish exposed to 0.5 mg/L but no in fish exposed to 5 mg/L. No uptake detectable in other tissues
Ref. [167] *Caenorhabditis elegans* in culture medium seeded with *Escherichia coli*	synthesized by hydrothermal method in supercritical water; 15 and 45 nm (TEM). 1 mg/L.	Growth, fertility and survival Semi-quantitative reverse transcription-polymerase chain reaction RNA interference feeding	CNPs exposure did not provoke significant effect on growth and survival. exposure to CNPs decrease of 28 % (15 nm) and 11% (45 nm) number of eggper worms increased expression of *cyp35a2* gene which has negative effect on fertility

(continued)

Table 12.2 (continued)

Microorganism and reference	CNPs source, properties and concentration range	assays	observations
Ref. [168] *Caenorhabditis elegans* feed with *Escherichia coli*	Synthesis by oxidation with HMT.[74] 8.5±1.5 nm by TEM; BET 107.8 m^2/g.1–500 nM	*E. coli* viability curves ROS with H2DCFDA Cellular uptake by TEM (up to 1 mM). Nematode *Caenorhabditis elegans* feed with *E.Coli* counting Accumulation of lipofucsin ROS with Juglone *In vitro* capture of $OH^{\bullet}$ by ABTS (in conditions where 0.001 % of total Ce is dissolved as Ce(III)). Uptake by TEM	CNPs act as an exogenous source of ROS CNPs found in intestinal lumen CNPs adhered to *E.Coli* cell membranes
Ref. [169] *Escherichia coli*	Rhodia from precipitation at low pH. Ellipsoidal 7 nm; 400m^2 g^{-1}, pzc 10.5 Ce(III): Ce(IV) by XANES. 0.46–730mg/L.	CFU on LB petri dishes	adsorption on cell membrane 50% survival 5 mg/L; no survival above 230 mg/L Reduction of NPs by bacteria as defense to oxidative stress Cytotoxicity requires direct spatial contact cell-NP
Ref. [170] *Nitrosomonas europaea*	Meliorum Tech needle-shaped 60nm length, 20 nm diameter (TEM); 93.8 m^2/g. pzc 7.5, 20 and 200 ppm	cell size by DLS after exposure to media with NPs morphological changes by TEM	no change in cell size NPs adhered to cell walls and distorsion of membrane no intrusion of particles in the celll
Ref. [171] 3-5 old maize plants	flame spray pyrolysis from cerium 2-ethylhexanoic in xylene (8 wt %), 37 nm, 110 m^2/g. 10μg (Ce) /L	exposure of plants to air with NPs irrigation of plants with NPs suspensions exposure of viable leaves to 10 ppm ceria suspensions Ce by ICP-MS TEM of leaves quenched in liquid N_2	agglomerated CNPs adsorbed on leaves exposed to ceria aerosol independent on illumination (open or closed stomata) incorporation of CNPs into leaves no translocation of ceria in new leaves no incorporation of CNPs by irrigation
Ref. [164] seeds	Synthesis by oxidation with HMT.[74] 64–640 mg/L	germination and roots length	0 % germination after 5 days exposed to 0.64 mg/L; 20 % exposed to 0.064 mg/L

(continued)

Table 12.2 (continued)

Microorganism and reference	CNPs source, properties and concentration range	assays	observations
Ref. [170]Soybean (*Glycine max*) seeds	cubic, NPs Meliorum Technologies. 7nm crystalline domain by XRDd. 10–4,000 mg/L.	DNA isolation and yield after treated with 2,000 and 4,000 mg L^{-1}. RAPD genotoxicology,Uptake by XANES.	All the CNPs concentrations significantly increased root elongation.. Ce in tissues increasing with concentration of NPs Roots uptake and store CNPs with same oxidation state as in NPs. At 2,000 and 4,000 mg mL^{-1} CNPs are genotoxic

[a] Parallel electron energy loss spectrometry
[b] Inhibitory concentration giving 50 % reduction in algal growth rate
[c] Thiobarbituric acid reactive substances
[d] In Allen and Arnon Modified Medium Diluted 1/10 and Adjusted to pH 6 in 2mM HEPES aggregates reach 2,000 nm
[e] Effective concentration, EC50, defined as the concentration that produces a 50 % light reduction measured after 5 and 15 min contact time

12.3 Interaction of CNPs with Cells and its Impact on Human Health

The mechanisms of cellular entry by NPs are a topic under intense debate [172–174]. On one side, given the small size CNPs (3–5 nm in many preparations), direct transport across the membrane has been claimed for uncovered NPs or NPs with hydrophobic coatings [175], whereas most of the work points to their incorporation by endocytosis, an active transport in which the cell encloses the objects in vesicles or vacuoles pinched off from its cytoplasmic membrane [176, 177]. The uptake of NPs involves their interaction with the non-rigid and non-uniform cell membrane [172]. The cell surface is heterogeneous both in phospholipid composition and presence of embedded proteins and other structures, and can be thought as patches with a length scale of 10–50 nm. If a NP were to interact with one patch at a time, the interaction energy would vary depending on NPs properties such as surface charge, surface roughness, and degree of curvature, as well as on its location on the cellular membrane. Moreover, the interaction of a particle with the phospholipid bilayer leads to a new particle surface different from the initial one. This introduces the concept of time-dependent dynamic interface that allows describing complex phenomena such as endocytosis [178, 179]. On the other hand, the mechanisms of direct entrance of NPs inside the cells (i.e., without endocytic compartments) are not less complex. It has been observed that subtle changes (for example, by tuning the non-specific binding forces of spiked uncoated particles or by modifying the arrangement of the ligands on coated NPs) allow or impede the direct penetration of the lipid bilayer [174]. The mechanisms of cellular uptake and further fate inside the cells may vary depending on NP properties (size, shape and surface chemistry) as well as on the cellular type. For instance, in a normal human keratinocyte cell line fluorescent-labeled nanoceria was found to be internalized via endocytic pathways, and further distributed throughout the cell [180].

It is worth mentioning that particles much larger than the membrane patch length, i.e., microparticles or big-size NPs aggregates, rarely enter non-phagocytotic cells. The surface properties of nanoparticles can drive their agglomeration into larger aggregates; in turn, these properties are also determined by the physicochemical scenario of the dispersion media, in particular the ionic strength, pH, and the eventual presence of complexing agents and capping macromolecules [181]. The properties of NPs assemblies interacting with a cell membrane are far to be a problem with a trivial solution. Moreover, multiple particles might form rafts with different properties than the sum over those of the single particles. Further complexity is added when one considers that the surface of NPs is usually covered with adsorbed molecules resulting from the synthesis process in order to get stable and monodispersed nanocrystals or specially designed for some purpose as for example for drug delivery or imaging [182].

Due to the high area to volume ratio, the shape (i.e. spherical, cubic, rod-like, triangular), the surface composition, the surface charge, and surface roughness

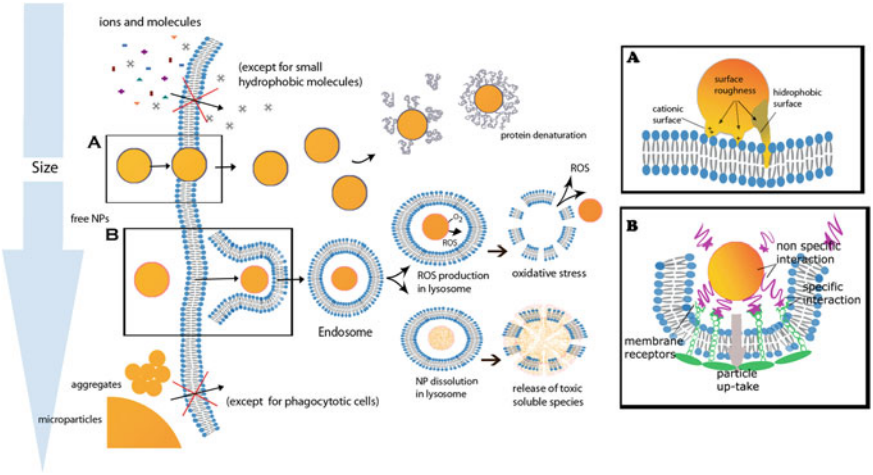


Fig. 12.4 Schematic representation of the mechanisms of NPs cellular uptake and toxicity. Insets depict the complex NP-cellular membrane interactions that allow the direct penetration of the lipid bilayer (A) or the specific ligand (adsorbed to the nanoparticle)-receptor (at the cell membrane) interactions that can drive the endocytosis pathway for cellular uptake (B)

play an important role in the properties of NPs that must be taken into account either when considering medical or technological applications, as well as their toxicity [173, 183]. The nanosize can also give them access to biological systems that are normally inaccessible to both single molecules [184] or to larger particles [185].

Figure 12.4 presents a simplified schematic representation of the mechanisms of NPs cellular uptake and toxicity. Three major mechanisms of NPs toxicity are shown. One common mechanism relays on the ability of NPs to organize around them a protein corona and generate adverse biological outcomes through protein unfolding, loss of enzymatic activity, and fibrillation [186]. For example, SiO_2-NPs have been shown to generate nucleoplasmic protein aggregates impairing normal nuclear function [187]. Another paradigm of NPs toxicity is the release of toxic ions when the thermodynamic properties favor particle dissolution in the biological environment [188]. It is worth mentioning that while most organisms live in rather neutral pH ranges, intracellular compartments (vesicles, lysosomes) and specialized organs (stomach) significantly extend the possibility for degradation or chemical modification. An example is ZnO that dissolves to form hydrated Zn^{2+} [189], inducing apoptosis in mammalian cells [190]. Limbach et al [191] demonstrated that metal-oxide NPs internalized in human lung epithelial cells by a so-called Trojan-horse mechanism provoked an up to eight times higher oxidative stress if compared to reference cultures exposed to aqueous solutions of the same metals. NPs can also interfere with the antioxidant defense mechanism [192], leading to reactive oxygen species generation, the initiation of an inflammatory response, and perturbation and destruction of the mitochondria causing

apoptosis or necrosis [193]. This last toxicity mechanism is generally attributed to nanoceria [169, 194, 195]. On the other hand, several studies have reported that CNPs are not nocive [196] or even that they mitigate oxidative stress at a biological level [197, 198] As will be shown, the discrepancies regarding the antioxidant/oxidant effects of cerium oxide NPs could be attributed to the fact that health effects vary significantly depending on the type of cells used for the study and the physicochemical characteristics of the used CNPs.

NPs have shown to produce cytotoxic, genotoxic, inflammatory, and oxidative stress responses in different mammalian cells [199, 200]. The nature of the interface between NPs and biological systems affects the in vivo biocompatibility and toxicity. Evaluation of NPs safety has to consider their interaction with proteins, DNA, lipids, membranes, organelles, cells, tissues, and biological fluids [172]. It is important to consider that extracellular nanoceria might also affect cell behavior, e.g., by ROS generation or scavenging, by adhering to and disturbing the plasma membrane, or by mimicking specific molecular interactions (i.e., ligand–receptor) and promoting intracellular signaling cascades.

The release of nanoparticles into the environment can occur through many processes, such as spilling and washing consumer products incorporating nanoparticles; during synthesis and production; as an accidental release during transport or use; from industries that exploit nanotechnology, for example wastewater treatment and drug delivery. The way of contact of NPs with the biological target is a key factor to take into account when assessing toxicity. As mentioned in the previous sections, CNPs has found increasing use in polishing and computer chip manufacturing [201, 202] but mainly as an additive to decrease diesel emissions [203]. Thus, the principal way of exposure to CNPs is the respiratory tract.

Figure 12.5 summarizes the biodistribution and the mechanisms of detoxification of CNPs administered by the gastrointestinal (GI) tract, intratracheal (IT) instillation, or by intravenous (IV) injection. In the center of the figure is depicted the lung deposition and extrapulmonary translocation of CNPs after intratracheal instillation, according to Xiao He et al. [204]. After administration, well-dispersed NPs of 6.6 $\pm$ 0.9 nm in diameter, aggregate in contact with the intratracheal fluid. The deposited nanoceria was slowly cleared from the host lung tissue (male Wistar rats), with an elimination half-life of 103 days. It was found that most of the particles on the surface of airways, i.e., before reaching the alveoli, (about 23 % in the cited study) were removed from the lungs within 1–2 days by mucociliary clearance, and swallowed by the animals into the GI tract and finally eliminated via feces. Following the same terminal, the main clearance route of aggregated-NPs deposited in alveoli, at 4–7 days post exposure, is phagocytosis by alveolar macrophages (AM) and AM-mediated re-entrainment into the airway lumen, with the consequent elimination via the GI tract. Moreover, it has been demonstrated that over 99 % of oral administered CNPs is eliminated through feces during the first 3 days after administration, rendering the absorption in the GI tract barely discernible. These results are illustrated in Fig. 12.5 (*left*).

At long term, the binding to proteins present in biological fluids would lead to the redispersion of the big size NPs aggregates and the protein-NPs binding affinity

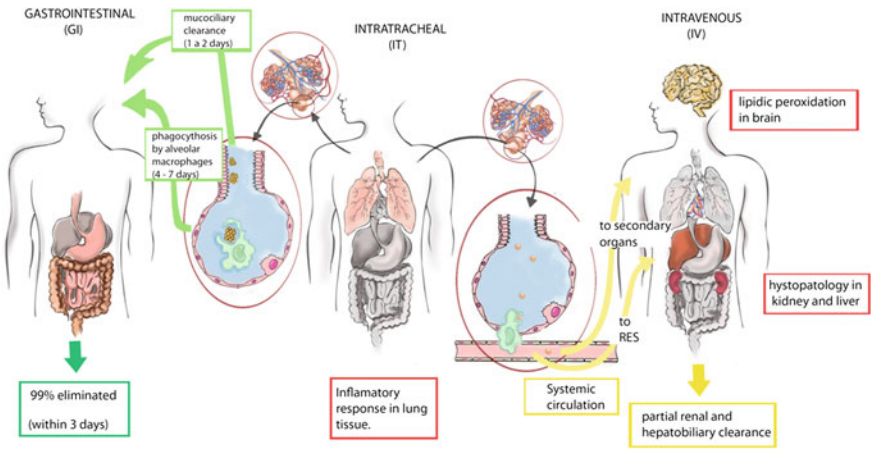


Fig. 12.5 Biodistribution and mechanisms of detoxification of CNPs

would also induce strong adhesion of NPs to the membrane or intracellular substances of cells in alveolar walls. This would aggravate the retention and interstitialization of NPs in the lung tissue, which has been shown to cause inflammatory responses such as granuloma and/or fibrosis through persistent damage to the lung [205].

Another important consequence of the disaggregation of CNPs is that small size particles are now able to penetrate through the alveolar-capillary barrier into the systemic circulation. It was proposed that NPs in blood are taken up by the phagocytic cells in tissues, so they would be accordingly accumulated in the phagocytic cell-rich tissues. Yokel et al. [206] studied the biodistribution and oxidative stress effects of a systemically introduced commercial nanoceria in mice. The used NPs were mostly platelets, highly crystalline, and had a bimodal size distribution (TEM average particle sizes: 8 nm and 24 nm). Zeta potential measurements showed that the system would be stable at physiological pH (-35 mV at pH 7.4). Different doses of CNPs up to 0.75 % animal weight were intravenously (IV) infused. The initial $t_{½}$ of ceria clearance from blood after termination of the infusion was 7.5 min. Tissue Ce concentration is dose dependent, being highest in the spleen while the factor *organ weight X Ce concentration* was found to follow the trend: liver > spleen > blood > brain. Ceria agglomerations were seen in the spleen (cytoplasmic localization), although no obvious histopathology was detected in this organ. By the contrary, some was observed in the liver and kidney. In contrast to the significant accumulation of ceria agglomerates in reticuloendothelial organs, much less ceria was seen in the brain (and almost no evidence of toxicity, except for some lipidic peroxidation in hippocampus), and no microscopic evidence of disruption of the blood–brain barrier (BBB) was observed. These results are illustrated in Fig. 12.5 (*right*).

The reticuloendothelial system (RES) is the first line of defense against xenobiotic intrusion. Thus, the spleen, liver, and kidney constitute a specific subpopulation of organs with phagocytic potential to take up ceria, but the aggregation state of the NPs is determinant, because only external bodies >100 nm in size are recognized by RES. Small size NPs exert a higher toxicity, as they can injure the target tissue by entering to non-phagocytotic cells in one hand, and in the other hand by penetrating into the systemic circulation reaching secondary organs. As a counterpoint, they may be (at least partially) eliminated by renal (NPs< 8 nm) or hepatobiliary clearance (NPs< 20 nm) [172].

While admittedly it is impossible to perform risk assessment and management without in vivo toxicological data, the strategy of using animal studies as the primary means of analysis method when confronted with the great diversity of commercial NPs and exposure conditions is unsustainable. Efforts have been done to develop predictive in vitro toxicological screening to rank NPs for priority in vivo testing: target-specific and predictive in vitro science that utilizes mechanisms of injury and toxicological pathways to guide the judicious use of in vivo studies [173]. Toward this end, quick screening approaches can be used to speed up the safety analysis on a scale that commensurate with the rate of expansion of nanotechnology development [207]. Moreover, in vitro analysis can be based on detection methods that are more difficult to utilize in vivo, such as tracking of radioactive marks, and may provide complementary insight at the molecular and subcellular level [208]. Due to the redox couple Ce^{3+}/Ce^{4+}, the main toxicity exerted by CNPs is via oxidative stress and their intracellular toxicity can be assessed according to the *hierarchical oxidative stress paradigm* (HOSP) [173], in which the different levels of oxidant stress have been classified as antioxidant defense (Tier 1), pro- inflammatory (Tier 2), and cytotoxic (Tier 3) cellular responses.

Since the respiratory tract is the first target attack by CNPs aerosols, many of the in vitro studies are being performed in lung epithelial cells. Studying cytotoxic effects in a human bronchoalveolar carcinoma-derived cell line, significant dose- and time-dependent ROS generation, lipid peroxidation, and cellular membrane breakage (revealed by LDH levels in culture medium) were reported [194], thus confirming that cellular damage caused by CNPs results from elevated oxidative stress. Park et al. [195, 209] showed that different sizes of CNPs (15, 25, 30, 45 nm) cause dose-dependent ROS increase, glutation (GSH) decrease, and induced antioxidant defense genes such as heme oxygenase-1, catalase, glutathione-S-transferase, and thioredoxin reductase (HOSP-Tier 1). It was also reported that the increased ROS induced by these NPs triggered the induction of pro-inflammatory pathways, as revealed by nuclear factor kappa-B (NFκB) augmented expression (HOSP-Tier 2). Moreover, morphological changes to these cells such as chromosome condensation and apoptosis were observed (HOSP-Tier 3). Surprisingly, the authors did not find significant differences in toxicity among NPs with different sizes (and hence, with different surface areas). They assumed that it may be due to the aggregation state of NPs inside the cells, determining similar surface areas regardless of the NP size.

By means of X-ray absorption spectroscopy, Auffan et al. [210] defined the causal mechanisms linking the physico-chemical properties of CNPs (ellipsoidal crystallites; hydrodynamic diameter in solution of 15 nm) with their biological effects. They examined the potential in vitro cytotoxicity and genotoxicity toward human dermal fibroblasts and the interactions occurring at the NP/biological media interface. They found that even though NPs stability at physiological pH (7.4) would be expected (isoelectric point pH 7.9–10.5), the suspension was destabilized after 24 h in the culture medium, as a consequence of interactions with proteins and salts that neutralized the NPs charge, decreasing electrostatic repulsion. Concerning the oxidation state of CNPs, 8 ± 2 % of cerium was reduced to trivalent state in the abiotic culture medium, but no increase in Ce^{3+}/Ce^{4+} ratio was detected after 24 h of incubation with fibroblasts. TEM images showed that during the incubation with fibroblasts, CNPs aggregates were adsorbed onto the cell membrane and further internalized into the cytoplasm inside vesicles. No mitochondria or nuclear presence of NPs was detected. The cytotoxicity (found to be similar to that of the positive control, TiO_2-NPs) was evaluated in terms of cell viability, which decreased 20–40 % for concentrations larger than 1.5 g/L. Genotoxicity was studied by monitoring DNA single strand breaks (SSB) formation and micronuclei (MN) induction, and was found to be even greater than that observed for TiO_2-NPs. They found a strong dose-dependent effect in chromosome damage caused by CNPs, generated by oxidative stress. Additionally, the genotoxicity was compared to that caused by micro-CeO_2 (particle size = 320 nm), finding that CNPs are much more genotoxic per unit of mass, but that the toxicity effects become similar when nano- and micro-CeO_2 doses are normalized by surface area. Another important conclusion is that genotoxic effects appeared at concentrations 2–3 orders of magnitude lower than the concentration at which cytotoxic effects occurred, highlighting the importance of taking into account DNA damage effects in risk assessment studies.

To study particle-cell interactions in cell culture systems representing the airway epithelial barrier, it is important to mimic the in vivo interactions of particles with cells as closely as possible. As stated before, the physical and chemical properties of NPs rapidly change when suspended in biological fluids or artificial culture mediums. To simulate accidental exposure to NPs in a relevant state of agglomeration and surface coating, Rothen et al. [211] directly combined the synthesis of NPs to the exposure of alveolar epithelial cell cultures at the air–liquid interface in a glove box. The deposition of the particles was monitored by TEM. In contrast to other studies performed with particles in suspension, in the present study a homogeneous distribution of CNPs with only a few aggregates was found on culture cells. No cytotoxic reaction and no remarkable change in the cytoskeleton or cellular ultrastructure of the epithelial cells due to particle exposure (highest dose 0.024 mg/cm^2) were observed. However, they report short-term (30 min) dose-dependent decrease in epithelial tightness and increase in permeability, possibly due to disorganization of the tight junctions and long-term decrease of lamellar body volumes. This last effect may be due to surfactant release triggered by NPs.

Paradoxically, other studies with ceria nanostructures showed high biocompatibility [212]. CNPs have been shown to serve as free radical scavengers [213, 214] providing protection against chemical, biological, and radiological insults that promote the production of free radicals. The intracellular CNPs promote cell longevity and decrease toxic insults by virtue of their antioxidant effects [215], preventing the accumulation of ROS, reducing the activation of the apoptotic response and death of the cells [216], and avoiding retinal degeneration induced by intracellular peroxidases [213]. Additionally, on previous studies, CNPs showed no toxic effect on normal breast epithelial cells (CRL 8798) and only a slight effect on breast cancer (MCF-7) cells at concentrations >50 nM [101]. Furthermore, CNPs selectively conferred radioprotection to the normal cells (CRL 8798) as compared with the tumor cells (MCF-7), representing a novel approach to the protection of normal cells from radiation-induced cell damage in vitro on normal lung fibroblast cells (CCL 135) and in vivo on athymic nude mice [102].

Thus, CNPs may offer a novel therapeutic alternative for scavenging environmentally elevated ROS. Recently, the use of nanoparticle-based antioxidants as a potential treatment for hepatotoxicity, which is a life-threatening problem, was explored. One obvious use of the nanoparticles would be for enhancing the performance of antioxidants, such as those normally present in the body or those administered as medicines for this kind of injury. It was shown that CNPs provided protection against Monocrotaline (MCT), a plant-derived pyrrolizidine alkaloid that causes oxidative veno-occlusive disease of the liver [217]. Electron microscopic examinations of liver samples from rats receiving CNPs alone demonstrated a homogeneous intrahepatocellular distribution of nanoparticles without phenotypic alteration of hepatocellular architecture. Liver samples obtained from the CeO_2 + MCT group also demonstrated regular intracellular distribution of nanoparticles and, importantly, did not exhibit alterations in cellular morphology, which is likely to be due to CeO_2 protection against MCT-elevated oxidative damage to the liver. This puts in evidence the protective effects of cerium oxide nanoparticles against hepatic oxidative damage.

This apparent discrepancy may be due to the surface oxidation state of nanoceria to scavenge superoxide or act in a catalytic manner, to the aggregation state of particles (that will depend not only on the their isoelectric point, but also on the interaction with particular biomolecules present at the biological fluid) and, even more important, to the pH micro conditions of the biological matrix (for instance subcellular organelle) that hosts the NPs. A. Asati et al. [212] synthesized polymer-coated nanoceria with enhanced aqueous stability and unique pH-dependent antioxidant activity, demonstrating optimal antioxidant properties at physiological pH, and behaving as an oxidase at acidic pH [131]. As shown in Figure X, the ability of NPs to permeate the cellular membrane depends on the hydrophobic/hydrophilic properties of their surface. Thus, the polymer coating and functionalization of the NP's surface play a major role in cellular uptake and subcellular localization. In turn, as mentioned before, the pH in the medium surrounding the NPs (highly acidic in lysosomes or neutral in cytoplasm) will play a critical role in NP's beneficial (antioxidant) *vs.* harmful (oxidant) properties. The same authors

showed that CNPs coated with either poly-acrylic acid (negatively charged), aminated poly-acrylic acid (positively charged), or dextran (with no charge), present a completely different uptake pattern in several cell lines, including cardiac myocytes (H9c2) and human embryonic kidney (HEK293) normal cells and lung (A549) and breast carcinoma cell lines (MCF-7) [218]. Positively charged NPs internalized in all cells except for breast carcinoma, and localized preferentially in the lysosomes, resulting toxic to these cells. Negatively charged NPs were internalized only by lung carcinoma, localizing in lysosomes and consequently exhibiting toxicity selectively to this cell line. NPs with neutral charge resulted nontoxic to normal or cancer cells, as these NPs primarily localized in the cytoplasm of these cells.

12.4 Concluding Remarks

In the context of this book it should be remarked that CNPs seem to be more a promise than a danger for life, both from a chemical and a biological perspective. From the evidence presented in previous reports as well as this chapter it can be concluded that the toxicity of CNPs to human health is mainly related with airborne particles that straightly affect the respiratory track, lungs, and subsequently other organs. However, at the same time, the same CNPs are offering increasing evidence pointing toward their unique ability to catalytically decompose ROS under physiological conditions. This apparent contradiction is an expectable result from an inherently complex system that requires deep and systematic investigation under very controlled experimental boundary conditions. Regarding this, some critical issues should be summarized in order to give guidance for future research on reactivity and/or toxicity of CNPs.

Since the cerium centers located on CNP's surface can be easily reduced to the trivalent state, much attention should be paid to the redox conditions. In the reduced state, the surface is more susceptible to irreversible chemical transformations as partial dissolution or other transformations as inner sphere coordination of phosphate or carboxylic groups. This will result in the modification of the inherent redox reactivity as well as the surface charge, affecting the intrinsic ability of CNP's to diffuse through tissues and cell membranes, due to electrostatic repulsion, steric hindrance due to the binding of bulky macromolecules, or, eventually, massive agglomeration of CNPs into large micrometric clusters.

A common observation in nanotextured systems is that not only particle size but also shape governs several relevant properties of nanoparticles. This is particularly valid for the CNPs, beyond the particle size, the morphology should be known and controlled since it dramatically affects the surface reactivity and eventually, the rate of dissolution.

Acknowledgments MP, SAB and MJ are members of CONICET. We are deeply indebted to Cecilia Speadlieri for her critical reading of this chapter.

References

1. Lambert CE, Barnum EC, Shapiro R (1993) J Am Coll Toxicol 12:617
2. J. 34-394
3. Adachi G, Imanaka N (1998) Chem Rev 98:1479–1514
4. Zinkevich M, Djurovic D, Aldinger F (2006) Solid State Ionics 177:989–1001
5. Ray SP, Cox DE (1975) J Solid State Chem 15:333–343
6. Ray SP, Nowick AS, Cox DE (1975) J Solid State Chem 15:344–351
7. Knappe P, Eyring L (1985) J Solid State Chem 58:312–324
8. Touzelin B (1981) J Nucl Mater 101:92–99
9. Kummerle EA, Guthoff F, Schweika W, Heger G (2000) J Solid State Chem 153:218–230
10. Hull S, Norberg ST, Ahmed I, Eriksson SG, Marrocchelli D, Madden PA (2009) J Solid State Chem 182:2815–2821
11. Shannon RD (1976) Acta Crystallographica Section A 32:751–767
12. Shannon RD (1976) Acta Crystallogr. 32:751–767
13. Kim D-J (1989) J Am Ceram Soc 72:1415–1421
14. Tsunekawa S, Sivamohan R, Ito S, Kasuya A, Fukuda T (1999) Nanostruct Mater 11:141–147
15. Zhang F, Chan SW, Spanier JE, Apak E, Jin Q, Robinson RD, Herman IP (2002) Appl Phys Lett 80:127–129
16. Deshpande S, Patil S, Kuchibhatla SV, Seal S (2005) Appl Phys Lett 87:1–3
17. Wu LJ, Wiesmann HJ, Moodenbaugh AR, Klie RF, Zhu YM, Welch DO, Suenaga M (2004) Phys Rev B 69
18. Tsunekawa S, Sivamohan R, Ohsuna T, Kasuya A, Takahashi H (1999) k. Tohji, Mater Sci Forum, pp 439–445
19. Tsunekawa S, Sahara R, Kawazoe Y, Ishikawa K (1999) Appl Surf Sci 152:53–56
20. Tsunekawa S, Ito S, Kawazoe Y (2004) Appl Phys Lett 85:3845–3847
21. Sayle TXT, Parker SC, Catlow CRA (1994) Surf Sci 316:329–336
22. Cordatos H, Ford D, Gorte RJ (1996) J Phys Chem 100:18128–18132
23. Mullins DR, Overbury SH, Huntley DR (1998) Surf Sci 409:307–319
24. Baranchikov AE, Polezhaeva OS, Ivanov VK, Tretyakov YD (2010) Cryst Eng Comm 12:3531–3533
25. Trovarelli A, De Leitenburg C, Boaro M, Dolcetti G (1999) Catal Today 50:353–367
26. Kaspar J, Fornasiero P, Graziani M (1999) Catal Today 50:285–298
27. Lahaye J, Boehm S, Chambrion P, Ehrburger P (1996) Combust Flame 104:199–207
28. Matatov-Meytal YI, Sheintuch M (1998) Ind Eng Chem Res 37:309–326
29. Liu W, Flytzani-Stephanopoulos M (1995) J Catal 153:317–332
30. Steele BCH (2000) Solid State Ionics 129:95–110
31. Putna ES, Stubenrauch J, Vohs JM, Gorte RJ (1995) Langmuir 11:4832–4837
32. Trovarelli A (1996) Catal Rev-Sci Eng 38:439–520
33. Zhou KB, Wang X, Sun XM, Peng Q, Li YD (2005) J Catal 229:206–212
34. Mai HX, Sun LD, Zhang YW, Si R, Feng W, Zhang HP, Liu HC, Yan CH (2005) J Phys Chem B 109:24380–24385
35. Zhang J, Kumagai H, Yamamura K, Ohara S, Takami S, Morikawa A, Shinjoh H, Kaneko K, Adschiri T, Suda A (2011) Nano Lett 11:361–364
36. Wang D, Kang Y, Doan-Nguyen V, Chen J, Kuengas R, Wieder NL, Bakhmutsky K, Gorte RJ, Murray CB (2011) Angewandte Chemie-Int Edition 50:4378–4381
37. Li M, Liu Z, Hu Y, Shi Z, Li H (2007) Coll Surf a-Physicochem Eng Aspects 301:153–157
38. Gopalan S, Singhal SC (2000) Scripta Mater 42:993–996
39. Tsuzuki T, McCormick PG (2001) J Am Ceram Soc 84:1453–1458
40. Lim G, Lee JH, Kim J, Lee HW, Hyun SH (2004) Designing, processing and properties of advanced engineering materials, Pts 1 and 2, pp. 1105–1108
41. Li YX, Zhou XZ, Wang Y, You XZ (2004) Mater Lett 58:245–249

42. Hadi A, Yaacob II, Gaik CS (2006) Functional Materials and Devices, pp 105–110
43. Hadi A, Yaacob II, Gaik CS (2006) Functional Materials and Devices, pp 252–256
44. Hadi A, Yaacob II (2007) Mater Lett 61:93–96
45. Mokkelbost T, Kaus I, Grande T, Einarsrud MA (2004) Chem Mater 16:5489–5494
46. Jobbagy M, Sorbello C, Sileo EE (2009) J Phys Chem C 113:10853–10857
47. Jobbagy M, Marino F, Schobrod B, Baronetti G, Laborde M (2006) Chem Mater 18:1945–1950
48. Wang H, Zhu JJ, Zhu JM, Liao XH, Xu S, Ding T, Chen HY (2002) Phys Chem Chem Phys 4:3794–3799
49. Esch F, Fabris S, Zhou L, Montini T, Africh C, Fornasiero P, Comelli G, Rosei R (2005) Science 309:752–755
50. Migani A, Vayssilov GN, Bromley ST, Illas F, Neyman KM (2010) Chem Commun 46:5936–5938
51. Migani A, Vayssilov GN, Bromley ST, Illas F, Neyman KM (2010) J Mater Chem 20:10535–10546
52. Arenas MA, Bethencourt M, Botana FJ, De Damborenea J, Marcos M (2001) Corros Sci 43:157–170
53. Arenas MA, Conde A, De Damborenea JJ (2002) Corros Sci 44:511–520
54. Creus J, Brezault F, Rebere C, Gadouleau M (2006) Surf Coat Technol 200:4636–4645
55. Ferreira MGS, Duarte RG, Montemor MF, SimÃµes AMP (2004) Electrochimica Acta 49:2927–2935
56. Hinton BRW, Wilson L (1989) Corros Sci 29(967–975):977–985
57. Lu YC, Ives MB (1993) Corros Sci 34(1773–1781):1783–1785
58. Lu YC, Ives MB (1995) Corros Sci 37:145–155
59. Mitra SK, Roy SK, Bose SK (1993) Oxid Metals 39:221–229
60. Montemor MF, SimÃµes AM, Ferreira MGS (2001) Prog Org Coat 43:274–281
61. Montemor MF, SimÃµes AM, Ferreira MGS (2002) Prog Org Coat 44:111–120
62. Montemor MF, Trabelsi W, Zheludevich M, Ferreira MGS (2006) Prog Org Coat 57:67–77
63. Nazeri A, Trzaskoma-Paulette PP, Bauer D (1997) J Sol–Gel Sci Technol 10:317–331
64. Seal S, Bose SK, Roy SK (1994) Oxid Metals 41:139–178
65. Wang C, Jiang F, Wang F (2004) Corros Sci 46:75–89
66. Wang H, Akid R (2008) Corros Sci 50:1142–1148
67. Bethencourt M, Botana FJ, Calvino JJ, Marcos M, Rodriguez-Chacon MA (1998) Corros Sci 40:1803–1819
68. Patil S, Kuiry SC, Seal S, Vanfleet R (2002) J Nanoparticle Res 4:433–438
69. Tsukuma K (1986) Am Ceram Soc Bull 65:1386–1389
70. Tsukuma K, Shimada M (1985) J Materials Science 20:1178–1184
71. Masui T, Fujiwara K, Machida K, Adachi G, Sakata T, Mori H (1997) Chem Mater 9:2197–2204
72. Tsunekawa S, Fukuda T, Kasuya A (2000) J Appl Phys 87:1318–1321
73. Nie JC, Hua ZY, Dou RF, Tu QY (2008) J Appl Physs 103
74. Zhang F, Jin Q, Chan SW (2004) J Appl Phys 95:4319–4326
75. Yin LX, Wang YQ, Pang GS, Koltypin Y, Gedanken A (2002) J Coll Interface Sci 246:78–84
76. Zhang YW, Si R, Liao CS, Yan CH, Xiao CX, Kou Y (2003) J Phys Chem B 107:10159–10167
77. Patsalas P, Logothetidis S, Sygellou L, Kennou S (2003) Phys Rev B Condens Matter Mater Phys 68:351041–3510413
78. Corma A, Atienzar P, GarcÃ-a H, Chane-Ching JY (2004) Nat Mater 3: 394–397
79. Xie YB, Yuan CW (2003) Appl Catal B-Environ 46:251–259
80. Li FB, Li XZ, Hou MF, Cheah KW, Choy WCH (2005) Appl Catal a-Gen 285:181–189
81. Coronado JM, Maira AJ, Martinez-Arias A, Conesa JC, Soria J (2002) J Photochem Photobiol A-Chem 150:213–221
82. Bamwenda GR, Arakawa H (2000) J Mol Catal A-Chem 161:105–113

83. Xiao JR, Peng TY, Li R, Peng ZH, Yan CH (2006) J Solid State Chem 179:1161–1170
84. Ji P, Zhang J, Chen F, Anpo M (2009) Appl Catal B-Environ 85:148–154
85. Hernandez-Alonso MD, Hungria AB, Martinez-Arias A, Fernandez-Garcia M, Coronado JM, Conesa JC, Soria J (2004) Appl Catal B-Environ 50:167–175
86. Long TC, Tajuba J, Sama P, Saleh N, Swartz C, Parker J, Hester S, Lowry GV, Veronesi B (2007) Environ Health Perspect 115:1631–1637
87. Kang SJ, Kim BM, Lee YJ, Chung HW (2008) Environ Mol Mutagen 49:399–405
88. Wang JJ, Sanderson BJS, Wang H (2007) Mutat Res-Genet Toxicol Environ Mutagen 628:99–106
89. Linsebigler AL, Lu G, Yates JT Jr (1995) Chem. Rev. 95:735–758
90. Herrmann JM (1999) Catalysis Today 53:115–129
91. Serpone N, Dondi D, Albini A (2007) Inorganica Chimica Acta 360:794–802
92. Brezova V, Gabcova S, Dvoranova D, Stako A (2005) J Photochem Photobiol B: Biol 79:121–134
93. Dunford R, Salinaro A, Cai L, Serpone N, Horikoshi S, Hidaka H, Knowland J (1997) FEBS Letters 418:87–90
94. Uchino T, Tokunaga H, Ando M, Utsumi H (2002) Toxicol in Vitro 16:629–635
95. Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, Ausman KD, Warheit DB, Colvin VL (2006) Toxicol Sci 92:174–185
96. Hidaka H, Kobayashi H, Koike T, Sato T, Serpone N (2006) J Oleo Science 55:249–261
97. Wamer WG, Yin JJ, Wei RR (1997) Free Radic Biol Med 23:851–858
98. Yabe S, Sato T (2003) J Solid State Chem 171:7–11
99. Li RX, Yabe S, Yamashita M, Momose S, Yoshida S, Yin S, Sato T (2002) Mater Chem Phys 75:39–44
100. Truffault L, Ta M-T, Devers T, Konstantinov K, Harel V, Simmonard C, Andreazza C, Nevirkovets IP, Pineau A, Verona O, Blondeau J-P (2010) Mater Res Bull 45:527–535
101. Tarnuzzer RW, Colon J, Patil S, Seal S (2005) Nano Lett 5:2573–2577
102. Colon J, Herrera L, Smith J, Patil S, Komanski C, Kupelian P, Seal S, Jenkins DW, Baker CH (2009) Nanomed-Nanotech Biol Med 5:225–231
103. Zholobak NM, Ivanov VK, Shcherbakov AB, Shaporev AS, Polezhaeva OS, Baranchikov AY, Spivak NY, Tretyakov YD (2011) J Photochem Photobiol B-Biol 102:32–38
104. Chien W-C, Yu Y–Y (2008) Mater Lett 62:4217–4219
105. Pan YX, Wu MM, Su Q (2004) J Phys Chem Solids 65:845–850
106. Pan YX, Wu MM, Su Q (2004) Mater Sci Eng B-Solid State Mater Adv Technol 106:251–256
107. Ebendorff-Heidepriem H, Ehrt D (2000) Optical Materials 15:7–25
108. Zych E, Brecher C, Glodo J (2000) J Phys-Condens Matter 12:1947–1958
109. Babu S, Cho J-H, Dowding JM, Heckert E, Komanski C, Das S, Colon J, Baker CH, Bass M, Self WT, Seal S (2010) Chem Commun 46:6915–6917
110. Kumar A, Babu S, Karakoti AS, Schulte A, Seal S (2009) Langmuir 25:10998–11007
111. Liu XH, Chen SJ, Wang XD (2007) J Luminescence 127:650–654
112. Wang Z, Quan Z, Lin J (2007) Inorg Chem 46:5237–5242
113. Ansari AA, Singh SP, Malhotra BD (2011) J Alloys Compd 509:262–265
114. Woan K, Tsai YY, Sigmund W (2010) Nanomedicine 5:233–242
115. Tsai MS (2004) Mater Sci Eng B-Solid State Mater Adv Technol 110:132–134
116. Lee SH, Lu ZY, Babu SV, Matijevic E (2002) J Mater Res 17:2744–2749
117. Kosynkin VD, Arzgatkina AA, Ivanov EN, Chtoutsa MG, Grabko AI, Kardapolov AV, Sysina NA (2000) J Alloys Compd 303:421–425
118. Krogman KC, Druffel T, Sunkara MK (2005) Nanotechnology 16:S338–S343
119. Karakoti A, Singh S, Dowding JM, Seal S, Self WT (2010) Chem Soc Rev 39:4422–4432
120. Ivanov VK, Shcherbakov AB, Usatenko AV (2009) Russian Chem Rev 78:855–871
121. Korsvik C, Patil S, Seal S, Self WT (2007) Chemical. Communications :1056–1058
122. Das M, Patil S, Bhargava N, Kang JF, Riedel LM, Seal S, Hickman JJ (2007) Biomaterials 28:1918–1925

123. Heckert EG, Karakoti AS, Seal S, Self WT (2008) Biomaterials 29:2705–2709
124. Pirmohamed T, Dowding JM, Singh S, Wasserman B, Heckert E, Karakoti AS, King JES, Seal S, Self WT (2010) Chem Commun 46:2736–2738
125. Celardo I, Pedersen JZ, Traversa E, Ghibelli L (2011) Nanoscale 3:1411–1420
126. Miao L, St DK (2009) Clair. Free Radic Biol Med 47:344–356
127. Singh S, Dosani T, Karakoti AS, Kumar A, Seal S, Self WT (2011) Biomaterials 32:6745–6753
128. Xue Y, Luan Q, Yang D, Yao X, Zhou K (2011) J Phys Chem C 115:4433–4438
129. Celardo I, De Nicola M, Mandoli C, Pedersen JZ, Traversa E, Ghibelli L (2011) ACS Nano 5:4537–4549
130. Preda G, Migani A, Neyman KM, Bromley ST, Illas F, Pacchioni G (2011) J Phys Chem C 115:5817–5822
131. Asati A, Santra S, Kaittanis C, Nath S, Perez JM (2009) Angewandte Chemie -Int Edition 48:2308–2312
132. Kuchma MH, Komanski CB, Colon J, Teblum A, Masunov AE, Alvarado B, Babu S, Seal S, Summy J, Baker CH (2010) Nanomedicine-Nanotech Biol Med 6(6) 738–744
133. Vincent A, Inerbaev TM, Babu S, Karakoti AS, Self WT, Masunov AE, Seal S (2010) Langmuir 26:7188–7198
134. Karakoti AS, Singh S, Kumar A, Malinska M, Kuchibhatla SVNT, Wozniak K, Self WT, Seal S (2009) J the Am Chem Soc 131: 14144–45
135. Safi M, Sarrouj H, Sandre O, Mignet N, Berret JF (2010) Nanotechnology 21:145103
136. Hayes SA, Yu P, O'Keefe TJ, O'Keefe MJ, Stoffer JO (2002) J Electrochem Soc 149:C623–C630
137. Huang SF, Li ZY, Wang XQ, Wang QX, Hu FF (2010) Ecotoxicol Environl Saf 73:89–93
138. Heckert EG, Seal S, Self WT (2008) Environ Sci Technol 42:5014–5019
139. Iuliano L, Pedersen JZ, Ghiselli A, Pratico D, Rotilio G, Violi F (1992) Arch Biochem Biophy 293:153–157
140. Peng Y, Chen X, Yi G, Gao Z (2011) Chem Commun 47:2916–2918
141. Babu S, Velez A, Wozniak K, Szydlowska J, Seal S (2007) Chem Phys Lett 442:405–408
142. Haley TJ (1965) J Pharm Sci 54:663–670
143. Ivanov VK, Shcherbakov AB, Ryabokon IG, Usatenko AV, Zholobak NM, Tretyakov YD (2010) Doklady Chem 430:43–46
144. Hirst SM, Karakoti AS, Tyler RD, Sriranganathan N, Seal S, Reilly CM (2009) Small 5:2848–2856
145. Rzigalinski BA, S. Seal, D. Bailey, S. Patil (2009) University of Central Florida Research Foundation Inc
146. Seal S, Patil SD, Haldar MK, Mallik S University of Central Florida Research Foundation Inc
147. Sugaya K, Merchant S, Seal S, Georgieva P, Vrotsos M University of Central Florida Research Foundation Inc
148. McGinnis JF, Chen J, Wong L, Sezate S, Seal S, Patil S (2008) University of Central Florida Research Foundation Inc; University of Oklahoma
149. Sicard C, Perullini M, Spedalieri C, Coradin T, Brayner R, Livage J, Jobbagy M, Bilmes SA (2011) Chem Mater 23:1374–1378
150. Karakoti AS, Tsigkou O, Yue S, Lee PD, Stevens MM, Jones JR, Seal S (2010) J Mater Chem 20:8912–8919
151. Hedrick JB (1995) J Alloys and Compounds 225:609–618
152. Choppin GR (1991) Eur J Solid State Inorg Chem 28:319–333
153. Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR (2008) Environ Toxicol Chem 27:1825–1851
154. Kitto ME, Anderson DL, Gordon GE, Olmez I (1992) Environ Sci Technol 26:1368–1375
155. Olmez I, Gordon GE (1985) Science 229:966–968
156. http://ntp.niehs.nih.gov/files/Ceric_oxide2.pdf
157. http://pubs.healtheffects.org/getfile.php?u=295

158. http://www.epa.gov/iris/toxreviews/1018tr.pdf
159. Neal AL (2008) Ecotoxicology 17:362–371
160. Rogers NJ, Franklin NM, Apte SC, Batley GE, Angel BM, Lead JR, Baalousha M (2010) Environ Chem 7:50–60
161. Rodea-Palomares I, Boltes K, Fernandez-Pinas F, Leganes F, Garcia-Calvo E, Santiago J, Rosal R (2011) Toxicol Sci 119:135–145
162. Van Hoecke K, Quik JTK, Mankiewicz-Boczek J, De Schamphelaere KAC, Elsaesser A, Van der Meeren P, Barnes C, McKerr G, Howard CV, Van De Meent D, Rydzynski K, Dawson KA, Salvati A, Lesniak A, Lynch I, Silversmit G, De Samber B, Vincze L, Janssen CR (2009) Environ Sci Technol 43:4537–4546
163. Gaiser BK, Biswas A, Rosenkranz P, Jepson MA, Lead JR, Stone V, Tyler CR, Fernandes TF (2011) J Environ Monit 13:1227–1235
164. Garcia A, Espinosa R, Delgado L, Casals E, Gonzalez E, Puntes V, Barata C, Font X, Sanchez A (2011) Desalination 269:136–141
165. Gaiser BK, Fernandes TF, Jepson M, Lead JR, Tyler CR, Stone V (2009) Environl Health 8
166. Johnston BD, Scown TM, Moger J, Cumberland SA, Baalousha M, Linge K, van Aerle R, Jarvis K, Lead JR, Tyler CR (2010) Environ Sci Technol 44:1144–1151
167. Roh JY, Park YK, Park K, Choi J (2010) Environ Toxicol Pharm 29:167–172
168. Zhang H, He X, Zhang Z, Zhang P, Li Y, Ma Y, Kuang Y, Zhao Y, Chai Z (2011) Environ Sci Technol 45:3725–3730
169. Thill A, Zeyons O, Spalla O, Chauvat F, Rose J, Auffan M, Flank AM (2006) Environ Sci Technol 40:6151–6156
170. Fang X, Yu R, Li B, Somasundaran P, Chandran K (2010) J Coll Interf Sci 348:329–334
171. Birbaum K, Brogioli R, Schellenberg M, Martinoia E, Stark WJ, Guenther D, Limbach LK (2010) Environ Sci Technol 44:8718–8723
172. Nel AE, Maedler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M (2009) Nat Mater 8:543–557
173. Nel A, Xia T, Madler L, Li N (2006) Science 311:622–627
174. Verma A, Uzun O, Hu Y, Hu Y, Han H-S, Watson N, Chen S, Irvine DJ, Stellacci F (2008) Nat Mater 7:588–595
175. Taylor U, Klein S, Petersen S, Kues W, Barcikowski S, Rath D (2010) Cytometry Part A 77A:439–446
176. Zhao F, Zhao Y, Liu Y, Chang X, Chen C, Zhao Y (2011) Small 7:1322–1337
177. Stark WJ (2011) Angewandte Chemie-Int Edition 50:1242–1258
178. Baca HK, Carnes E, Singh S, Ashley C, Lopez D, Brinker CJ (2007) Acc Chem Res 40:836–845
179. Baca HK, Ashley C, Carnes E, Lopez D, Hemming J, Dunphy D, Singh S, Chen Z, Liu N, Fan H, LÃ³pez GP, Brozik SM, Werner-Washburne M, Brinker CJ (2006) Science 313: 337–341
180. Singh S, Kumar A, Karakoti A, Seal S, Self WT (2010) Mol Biosyst 6:1813–1820
181. Li Z, Sahle-Demessie E, Hassan AA, Sorial GA (2011) Water Res 45:4409–4418
182. Jares-Erijman EA, Jovin TM (2003) Nat Biotech 21:1387–1395
183. Kahru A, Savolainen K (2010) Toxicology 269:89–91
184. Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, Bruinink A, Stark WJ (2006) Environ Sci Technol 40:4374–4381
185. Oberdorster G, Oberdorster E, Oberdorster J (2005) Environ Health Perspect 113:823–839
186. Linse S, Cabaleiro-Lago C, Xue W-F, Lynch I, Lindman S, Thulin E, Radford SE, Dawson KA (2007) In: Proceedings of the National academy of Sciences of the United States of America 104:8691–8696
187. Chen M, von Mikecz A (2005) Exp Cell Res 305:51–62
188. Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Casey PS (2007) Environ Sci Technol 41:8484–8490
189. Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nel AE (2008) ACS Nano 2:2121–2134

190. Gojova A, Guo B, Kota RS, Rutledge JC, Kennedy IM, Barakat AI (2007) Environ Health Perspect 115:403–409
191. Limbach LK, Wick P, Manser P, Grass RN, Bruinink A, Stark WJ (2007) Environ Sci Technol 41:4158–4163
192. Li N, Hao MQ, Phalen RF, Hinds WC, Nel AE (2003) Clin Immunol 109:250–265
193. Xiao GG, Wang MY, Li N, Loo JA, Nel AE (2003) J Biol Chem 278:50781–50790
194. Lin W, Huang Y.-W, Zhou X.-D, Ma Y (2006) Int J Toxicol 25: 451–457
195. Park E-J, Choi J, Park Y-K, Park K (2008) Toxicology 245:90–100
196. Pierscionek BK, Li Y, Yasseen AA, Colhoun LM, Schachar RA, Chen W (2010) Nanotechnology 21(3): 035102
197. Schubert D, Dargusch R, Raitano J, Chan SW (2006) Biochem Biophys Res Commun 342:86–91
198. Niu J, Azfer A, Rogers LM, Wang X, Kolattukudy PE (2007) Cardiovasc Res 73:549–559
199. Lewinski N, Colvin V, Drezek R (2008) Small 4:26–49
200. Medina C, Santos-Martinez MJ, Radomski A, Corrigan OI, Radomski MW (2007) British J Pharm 150:552–558
201. Limbach LK, Li YC, Grass RN, Brunner TJ, Hintermann MA, Muller M, Gunther D, Stark WJ (2005) Environ Sci Technol 39:9370–9376
202. Madler L, Stark WJ, Pratsinis SE (2002) J Mater Res 17:1356–1362
203. Jung HJ, Kittelson DB, Zachariah MR (2005) Combust Flame 142:276–288
204. He X, Zhang H, Ma Y, Bai W, Zhang Z, Lu K, Ding Y, Zhao Y, Chai Z (2010) Nanotechnology 21
205. Park E-J, Cho W-S, Jeong J (2010) Yi J.-h, Choi K, Kim Y, Park K. J Health Sci 56:387–396
206. Yokel RA, Florence RL, Unrine JM, Tseng MT, Graham UM, Wu P, Grulke EA, Sultana R, Hardas SS, Butterfield DA (2009) Nanotoxicology 3:234–248
207. Damoiseaux R, George S, Li M, Pokhrel S, Ji Z, France B, Xia T, Suarez E, Rallo R, Maedler L, Cohen Y, Hoek EMV, Nel A (2011) Nanoscale 3:1345–1360
208. Simonelli F, Marmorato P, Abbas K, Ponti J, Kozempel J, Holzwarth U, Franchini F, Rossi F (2011) Ieee Transactions on Nanobioscience 10:44–50
209. Park B, Donaldson K, Duffin R, Tran L, Kelly F, Mudway I, Morin JP, Guest R, Jenkinson P, Samaras Z, Giannouli M, Kouridis H, Martin P (2008) Inhalation Toxicol 20:547–566
210. Auffan M, Rose J, Orsiere T, De Meo M, Thill A, Zeyons O, Proux O, Mason A, Chaurand P, Spalla O, Botta A, Wiesner MR, Bottero J-Y (2009) Nanotoxicology 3:161–U115
211. Rothen-Rutishauser B, Grass RN, Blank F, Limbach LK, Muehlfeld C, Brandenberger C, Raemy DO, Gehr P, Stark WJ (2009) Environ Sci Technol 43:2634–2640
212. Perez JM, Asati A, Nath S, Kaittanis C (2008) Small 4:552–556
213. Chen J, Patil S, Seal S, McGinnis JF (2006) Nat Nanotech 1:142–150
214. Rzigalinski BA, Bailey D, Chow L, Kuiry SC, Patil S, Merchant S, Seal S (2003) Faseb J 17:A606–A606
215. Patil S, Sandberg A, Heckert E, Self W, Seal S (2007) Biomaterials 28:4600–4607
216. Fu PP, Xia QS, Lin G, Chou MW (2004) Drug Metab Rev 36:1–55
217. Amin KA, Hassan MS, Awad E-ST, Hashem KS (2011) Int J Nanomed 6:143–149
218. Asati A, Santra S, Kaittanis C, Perez JM (2010) ACS Nano 4:5321–5331

Chapter 13
Nanomaterials from Renewable Resources

Niki Baccile

Abstract Among the 12 principles that define green chemistry, the use of renewable resources is of paramount importance in the perspective of building a sustainable society. The effort that has been put, in the past 25 years, into the research and development of new materials with controlled nanoscale structures and properties has not specifically taken into account the possibility of employing natural resources. This trend has, nevertheless, changed in the past 10 years. In this chapter, some examples of nanomaterials and nanoscale-related processes are overviewed through the prism of sustainability. In particular, it will be shown how natural resources, from proteins to carbohydrates and more complex organisms, can be employed as precursors in the synthesis of nanomaterials.

13.1 Introduction

In 1998, 12 principles were proposed by Warner and Anastas to identify a new field in chemical science: green chemistry [1]. Focusing on both short-and long-term sustainability, the main philosophy introduced by the 12 principles is to develop a new form of chemistry in which energy consumption, renewable sources, and final impact of a given product are taken into consideration during the development process of new compounds and/or materials. Such restrictions in the *chemist's playground* have immediate effects on the development process of scientific knowledge. At an industrial scale, the recently approved REACH (Registration,

N. Baccile (✉)
Laboratoire de Chimie de la Matière Condensée de Paris,
CNRS-UPMC-Collège de France, 11 place M. Berthelot, 75005 Paris, France
e-mail: Niki.baccile@upmc.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_13,

Evaluation, Authorization, and Restriction of Chemicals) regulation issued by the European Community [2] has set important limitations to the production and use of specific chemical compounds of particular toxicity. What has happened? The combination of increasing environmental concerns dictated by large-scale accidents occurred to chemical plants (Seveso 1976, Bhopal 1984) and long-term pollution due to unhealthy chemical practise, has motivated the scientific community to realize that approach to chemical science needs to be profoundly reconsidered. In addition, limited access to fossil sources in the long run, envisioned energy crisis and a raising interest towards renewable resources have contributed to adapt old tools to the never-ending quest for new chemical compounds, and materials. Green chemistry, whose boundaries are circumscribed within the 12 principles, has became today an entirely new field of both academic and industrial research: development of new catalytic supports, use of less hazardous solvents, stoichiometric efficiency, reduction of waste, recycling, use of renewable resources, design of safe, and nontoxic compounds are just some general examples of the new research perspectives.

This contribution will show how these ideas can be applied not only to general chemistry but also to the field of nanoscience, to which chemistry brings a heavy contribution. Some examples concerning the conception of "green" nanomaterials will be provided and, in particular, I will focus on the employment of renewable feedstock in the synthesis of materials with nanoscale-related properties. In the first part of the chapter, I will propose few examples in which renewable resources have been successfully used to develop large-scale chemicals and more "classical" materials. Then, a large number of classified examples concerning the employment of biomass, processed or not, in the development of nanomaterials will be largely discussed.

Biomass is acquiring more and more importance in the field of material science; it can be used as such, or as intermediate, in the synthesis of chemicals and materials. In the fabrication process of plastics, for instance, carbohydrates are largely employed: the Italian chemical group Novamont [3] commercializes bio-based plastic products (MaterBi©) since the 1990s. In the same spirit, chemical intermediates can be obtained on a large scale from bio-based resources, which generally have the advantage of providing biodegradable compounds with very low toxicity. This is the case of a certain number of monomers, like lactic acid, hydroxymethylfurfural, isosorbide, used in the production of bioplastics, and obtained from the dehydration of glucose. Interestingly,the assumption that bio-based products are not toxic is not generally true. Furfural, for instance, an intermediate used in the synthesis of polyfuran resins, is highly toxic even if it can be obtained in large amounts from the dehydration of xylose, a pentose sugar.

Nanoscience is a relatively new, highly interdisciplinary, field of research which focuses on the development and control of nanoscale objects and the understanding of their mechanisams of formation and interactions. The effect of the perspectives imposed by sustainability on the domain of nanoscience is very recent, as shown by the first review article marking the field of green nano-synthesis in 2007 and which mainly focuses on the synthesis of nanoparticles (NP) [4]. Nevertheless, nanoscience does not limit itself to the fabrication of NP but it describes a much wider class of organic, inorganic and hybrid materials whose shape, size, functionality, and properties is extremely difficult to summarize in a simple way [5]. In the next sections, a number of

examples chosen from a short list of selected classes of nanomaterials obtained from renewable resources will be described. This chapter is not meant to give the reader an extensive view of what "green nanoscience" is like but rather the author's personal interpretation of what it looks like today and what it can be.

In Sect. 13.2, the specific concept of green chemistry is contextualized in the more recent development of biorefinery. Biopolymers are a wide range of organic compounds that can derive, directly or indirectly, from carbohydrates. The use of biopolymers in polymer and nanoscience applications is discussed in Sect. 13.3. The third section (sect. 13.4) of this chapter deals with the synthesis of inorganic NP, where greener approaches are compared to classical synthesis methods. Finally, sol–gel science is shortly mentioned in Sect. 13.5 while self-assembly of a number of natural compounds are discussed in Sect. 13.6.

The reader should be aware that many of these topics have already been covered by extended specific reviews, to which one should refer for more detailed pieces of information.

13.2 From Biorefinery to "Nano"-Biorefinery

The design of ecofriendly products from plant-derived feedstock has became one of the targets in many industrial applications. Biopolymers, biofibers and biosurfactants probably represent the mainstream exploitation of renewable resources for large-scale production in the fields of plastics, composite materials, detergency, cosmetics, emulsifiers. First of all, one should make a difference between using a bio-based product "as such" with respect to processed compounds. For instance, poly(hydroxyalcanoates) (PHA) and poly(lactic acid) (PLA) are two families of largely studied polymers derived from carbohydrates. At an industrial scale, PLA is obtained by classical polymerization of lactic acid monomer, which is produced by fermentation of glucose. PHAs, on the contrary, are directly produced by specific microorganisms and plants, requiring no additional chemical processing after recovery. Natural polymers like cellulose and lignin are also widely studied and used as such but chemical modification can be employed to improve dispersion, adhesion, flexibility, etc. Cellulose esters, for instance, are largely used as biodegradable plastics in common products like toothbrush and screwdriver handles [6].

The conception of a closed cycle in which biomass is harvested and processed for energy and chemical synthesis purposes while byproducts are eventually recycled is long dated and can be probably traced back to the first oil crisis in the 1970s, when I investments in bio-based chemical plants would have been competitive with classical oil-based plants. The biorefinery concept, after being abandoned for several years, has been largely developing in the past 10 years. Several plants in Europe [7], as shown by the Pomacle-Bazancourt biorefinery in north France, efficiently run on a closed cycle basis and which includes crops production, harvesting, food supply for human nutrition, post-harvesting treatment of biomass derivatives (nonfood byproducts) for the production of fuel (ethanol) but also of fine chemicals (monomers, solvents,

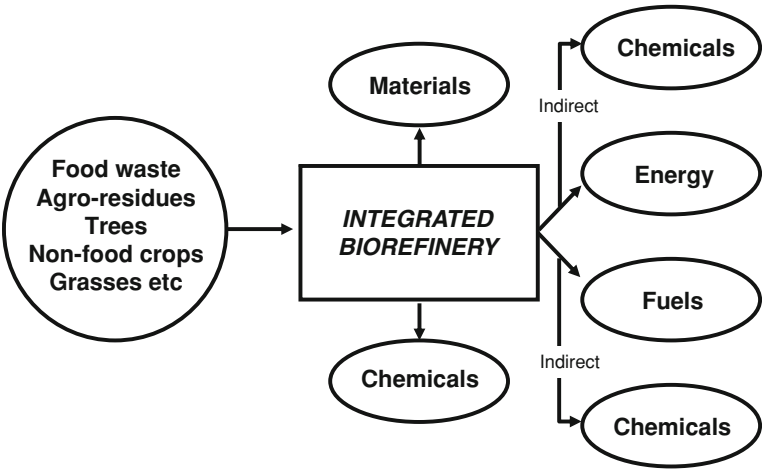


Fig. 13.1 The integrated biorefinery as a mixed feedstock source of chemicals, energy, fuels, and materials. Reprinted with permission from [9]. Copyright 2006 The Royal Chemical Society

surfactants), platform molecules, biopolymers, and biomaterials [8]. Strong collaborations with biology-related pilot plants allow the development of even more complex biorefineries where biocatalytical processing and white biotechnology (through enzymatic and microbial processing) are highly demanded, and, in some cases, they constitute a tangible reality (Fig. 13.1).

Future generations of biorefinery will undoubtedly be much more complex than what it looks today and it will definitely include nanoscience-related processes and materials at different extent. For instance, new efficient catalysts can be integrated in the process of biomass processing (cellulose treatment, for instance); these materials can be obtained via nonsustainable chemical synthesis but they can also result from the newly developed hybrid bio-inorganic nanomaterials, which would be the case of supported enzymes, microorganisms or a combination of both. Bio-sourced end by-products can be exploited for the synthesis of new materials, as shown by the recent development of carbohydrate-based carbonaceous materials described further down in the chapter. A fully developed, highly integrated and multidisciplinary, *nano-biorefinery* is still far from being a reality but the author hopes that this chapter will offer the reader a new perspective of what that can be like.

13.3 Biopolymers

In this section we will discuss few classical and more advanced applications of biopolymers. The following classes of biopolymers will be overviewed: PLA and PHA, polysaccharides, oil/fats-based compounds.

13.3.1 From Biopolymers for Plastics to Bio-Block Copolymers, the Case of PLA and PHA

Plastics application PLA is obtained from renewable resources like corn and sugar beet and it is meant to replace plastics on a large scale. It is obtained both by direct condensation of lactic acid or by ring-opening polymerization of the cyclic lactide dimer; recent advances in processing design and catalysis optimization increased the interest towards up-scaling perspectives of this compound, whose 100 % degradation by microorganisms constitutes its greatest advantage over petro-derived polymers. Through a fine control over its polymerization, co-polymerization and branching degree, a number of applications have been developed already such as grease and oil resistance, low-temperature heat sealability, good barrier to flavors, films and bags for packaging, fibers, foams, and extrusion coatings [10].

PHA refers to a large family of polyesters of hydroxyalkanoic acids, which are synthesized by microorganisms grown on a number of substrates like whey, wheat bran, rice bran, starch, molasses, waste vegetables and plant oil, etc. PHAs are biodegradable, like PLAs, and their chemical structure and physical properties can be tuned by choosing the proper organisms source (e.g., *Cupriavidus necatror* or *Escherichia coli*) or recombinant bacteria (*E. coli*). PHAs are used in everyday's plastic products like shampoo bottles and cosmetic materials but also in films, diaphragms, additives, foils, hot-melt adhesives, etc. More developed applications of PHA can be found in medicine like hosts materials for drug delivery and contrast agents [11]. Physical properties like thermo-resistance, plasticity, stiffness, etc., can be obtained by developing copolymers whose blocks are constituted of either PLA or PHA and a variety of compounds like fatty alcohols containing OH groups (ricinoleic acid), terpenes (menthide derived from menthol or classical isoprene). Copolymerization between PLA and PHA are also reported [12].

Application in nanoscience. Despite their importance in polymer chemistry, functional block copolymers have gained a large interest in the field of nanoscience. A rational design of the blocks may provide selective hydrophilic, thermoretractile, soluble, and pH-responsive properties. These compounds are currently used as structure directing agents, coatings for NP, and drug delivery capsules. To this regard, PLA and PHA-derived block copolymers have been synthesized and tested in the past few years. For instance, the lamellar self-assembly properties of crystallized Poly([*R*]-3-hydroxybutyric acid), PHB, a biodegradable and biocompatible polyester produced by many microorganisms, were shown by X-ray diffraction (XRD) when PHB is degraded and copolymerized through a transesterification reaction with poly (ethylene glycol), PEG [13]. More work on the synthesis, self-assembly, and bio-medical applications of PLA-based amphiphilic block copolymers was recently reviewed by Oh [14]. Specific properties like bio-recognition, hydrophilic, thermotropic, can be tuned by a wise selection of the complementary blocks: PEG, peptides, polyurethanes, polysaccharides, poly(meth)acrylates (Fig. 13.2).

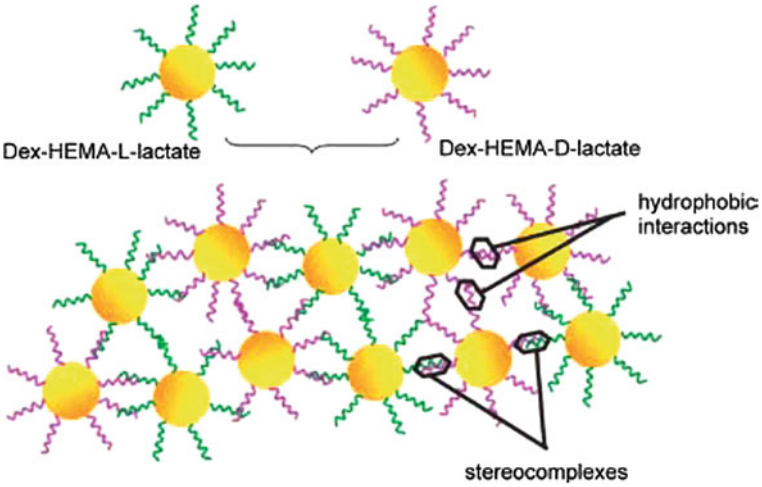


Fig. 13.2 Schematic representation of the concept of a self-assembling hydrogel composed of interconnected dextran-based (Hydroxyethyl methacrylate-derivatized dextran, Dex-HEMA) microspheres functionalized with L- or D-oligolactate chains. Inter-lactate hydrophobic interactions favor the self-assembly of the dextran beads and the formation of a hydrogel. Reprinted with permission from [15]. Copyright 2008 American Chemical Society

13.3.2 Vegetal Biopolymers

Polymers of vegetal origin probably constitute one of the largest source of renewable feedstock on earth with the advantage of being readily available and not geographically restricted. Wood-based products, tannins, hemicellulose, cellulose, lignins, starch, natural gums, are the most common examples but other compounds can be introduced in this category like alginates, carrageenans, pectin, chitosan. A nice collection of works describing the synthesis, recovery, and use of these compounds can be found in Ref. [16]. Starch or starch derivatives, for instance, are largely used in many domains: ethanol production for energetic purposes, adhesives, osmotic agents, water retention, thermostability, etc. Many important carbohydrates are also obtained from it: glucose, cyclodextrins, trehalose, maltose. Alginates are extracted from algae or produced from microbes and are mainly used, in presence of Ca^{2+} or other valence-2 ions, as gelling agents for food products, soft capsules, or dental applications.

A large number of biopolymer-derived materials is nowadays developed in nanoscience-related applications; in particular, pH or thermo-responsive properties are particularly targeted. For drug delivery applications, Bachelder et al. [17] have developed a biodegradable acid-sensitive acetal-modified dextran, a bacterial-derived glucose homopolysaccharide. Dextran was made water insoluble by grafting acetals on the OH group in position 2 of the sugar and this property was used to upload hydrophobic drugs within the biopolymer. At pH = 5, the acetal is hydrolyzed and dextran becomes water soluble again, thus releasing the cargo.

In the domain of hierarchical porous inorganic materials, cellulose was used as template. Several authors have reported on the use of cellulose filter papers as preferential scaffold to grow titanium dioxide from sol–gel chemistry in the perspective of developing porous photovoltaic devices. The soaking method was exploited [18] to make a network of TiO_2 particles whose diameter is between 45 and 150 nm. The layer-by-layer deposition and soaking approaches were combined together to obtain a hybrid titania/poly(vynilalcohol) fibrous nanostructured membrane where cellulose is eventually washed out in a NaOH/urea solution. The final material keeps the flexibility of the initial membrane and it displays swelling properties in various solvents [19].

Hybrid polysaccharide/inorganic interfaces are also largely studied in the scope of better understanding the biomineralization process in nature and put in evidence the role of functional sugar-based compounds in the precipitation of mineral salts, like carbonates, phosphates, etc. Biomineralization is an omnipresent phenomenon in many living organisms and it is partly responsible for the existence of fascinating hierarchical nano/micro structures, as observed in bone, diatoms, etc. A recent review describes the types of functional polysaccharides (hydroxylated, polycarboxylated, and sulfated) which were explored to understand their role in the mineralization process of calcium carbonate in both living organisms and lab-scale experiments [20].

In a different field and perspective, α-D-polysaccharides like starch can be transformed into mesoporous supports by controlling water adsorption, swelling degree, and temperature under microwave-assisted synthesis [21]. The group of Clark at the University of York (UK) has developed several methods to exploit starch-based materials for different purposes. In particular, they obtained micro and mesoporous polysaccharide-based matrices from amylopectin/amylose mixture by reaching depolymerization conditions at temperatures values above 150 °C. The specific pore volume and texture vary with temperature and amylopectin percentage in the mixture, the higher its content, the lower the exposed volume. Authors interpreted this result as depending on the crystalline influence of native amylopectin, which disfavors the formation of a porous gel.

Finally, increasing research activities are developed in the synthesis and applications of nanocellulose materials [22]. Nanocellulose is a broad term that refers to a set of three different nanosized cellulose-derived materials: microfibrillated cellulose (MFC), nanocrystalline cellulose (NCC) and bacterial nanocellulose (BNC), briefly described in Table 13.1. In particular, MFC is obtained by processing native cellulose via a mechanical treatment delaminating the fibers and liberating the microfibrils or by less energetic physical, chemical, or enzymatic processing. Eliminating the amorphous part in cellulose fibers by acid hydrolysis will result in the formation of NCC, whose flexibility is lower with respect to MFC. Alternatively, nanocellulose can be directly extracted from the biofilm obtained by specific aerobic bacteria of the genus *Gluconacetobacter*. Nanocellulose is highly demanded in food and emulsion/dispersion applications, medical, cosmetic, pharmaceutical and hygiene/adsorbent products, plasticizer for packaging, etc.

Table 13.1 The families of nanocellulose materials

Type of nanocellulose	Selected references and synonyms	Typical sources	Formation and average size
Microfibrillated cellulose (MFC)	Microfibrillated cellulose [1], nanofibrils and microfibrils, nanofibrillated cellulose	Wood, sugar beet, potato tuber, hemp, flax	Delamination of wood pulp by mechanical pressure before and/or after chemical or enzymatic treatment diameter: 5–60 nm length: several micrometers
Nanocrystalline cellulose (NCC)	Cellulose nanocrystals, crystallites [2], whiskers [3], rodlike cellulose microcrystals[4]	Wood, cotton, hemp, flax, wheat straw, mulberry bark, ramie, Avicel, tuncin, cellulose from algae and bacteria	Acid hydrolysis of cellulose from many sources diameter: 5–70 nm length: 100–250 nm (from plant celluloses); 100 nm to several micrometers (from celluloses of tunicates, algae, bacteria)
Bacterial nano cellulose (BNC)	Bacterial cellulose [5], microbial cellulose [6], biocellulose [7]	Low-molecular-weight sugars and alcohols	Bacterial synthesis diameter: 20–100 nm; different types of nanofiber networks

Reprinted with permission from [22]. Copyright 2011 John Wiley and Sons

13.3.3 Carbon Nanomaterials from Vegetal Biopolymers

Since the discovery of fullerenes [23] and the ongoing research in the field of carbon nanotubes and graphene, carbon-based nanostructures have attracted a large interest worldwide for their mechanical stability, optic and electronic properties, thermal conductivity, etc. Porous carbons are also widely studied for many applications going from environmental remediation to catalytic supports and materials for energy storage. Nevertheless, the common feature for this class of materials is constituted by the energy-demanding conditions for their synthesis like plasma discharge, laser ablation, chemical vapor deposition, or use of hazardous chemical compounds. In all cases, new research fields are growing to make carbon-based materials (nanotubes, NP, foams, aerogels, powders, etc.) from renewable resources. For instance, both indirect and direct methods were developed to make mesoporous carbons supports from different sources. Indirect methods involve the use of silica with long-range organized mesoporosity as hard templates during pyrolysis of glucose, a classical carbogenic precursor. Silica is eventually dissolved using HF or concentrated NaOH solutions [24]. Direct methods use the soft templating approach employing a block copolymer that behaves as template and a resorcinol–formaldehyde resin as carbogenic precursor [25]. These interesting methods have paved the way to new routes to conceive carbons with controlled organized porosity at the nanoscale, in contrast to

classical activated carbons, whose structure is still matter of debate today. Unfortunately, precursors used in the synthesis of mesoporous carbons are in either case toxic (HF, resorcinol, formaldehyde). For this reason, new routes are being explored where starting compounds are renewable and innocuous.

The recent re-discovery of the hydrothermal treatment of carbohydrates has motivated much research in the field of porous carbons from renewable resources. This very simple method consists of treating at about 160–180 °C for few hours any type of carbohydrate source. Dehydration of the sugar moieties into furna-based bricks drives the formation of a complex carbonaceous structure where furans and aromatic units are intimately connected; starch-derived carbons, Starbons© [26], a material in which expanded starch is treated at high temperature under hydrothermal conditions, are a nice example. It was shown that Starbons is actually a mesoporous carbonaceous material (no long-range order in the pore distribution is observed), whose aromatization extent is directly linked to either the process temperature or to a post-synthesis calcination step. In parallel, a similar technique, but with broader ambitions in terms of the nature of the feedstock employed, has been developed. A review on the ongoing research in the field of the so-called "hydrothermal carbonization" has been published recently [27]. In particular, many different precursors can be used as carbogenic compounds (as long as they contain carbohydrates), from simple, mono or disaccharides, to more complex polysaccharides [28]. Cellulose can also be used but higher temperatures are required to break the strong hydrogen bonds forming its crystalline structure [29]. A lot of work has been done so far to adapt the hydrothermal carbonization process to the synthesis of functionalized, hybrid and porous NP, nanofibers, aerogels, etc. Applications as energy-storage materials or CO_2 filters were largely explored (Fig. 13.3).

More classical carbon-based nanomaterials can also be obtained under environmental conditions where initial compounds are wax or natural gas. Endo et al. [32] have obtained multiwalled carbon nanotubes by reacting, at 1,000 °C for 6 h under air, city gas (mixture of methane, ethane, propane, etc.) on stone garnet sand (SiO_2, FeO, Fe_2O_3, Al_2O_3, etc.), which acted both as support, and catalyst. Interestingly, they also have shown the employment of an easy method to remove the support in water by a simple sonication step. A second example concerns the synthesis of carbon NP from candle soot [33]. Authors have shown interesting fluorescent properties of the NP, whose diameter is below 2 nm, under a single-wavelenght UV excitation at $v = 312$ nm and after a preliminary treatment in 5 M HNO_3 to make them dispersible in water. The fluorescent emission spectrum of the particles shows that, according to the purified fractions, a broad range of emission wavelengths in the visible are obtained, from 400 to 600 nm.

13.4 Inorganic Nanoparticles

The synthesis of metal, metal oxide, and semiconductor NP has been largely developed in the past 15–20 years. Optical, catalytic, sensing and magnetic properties have motivated the research activity in this field and a large number of

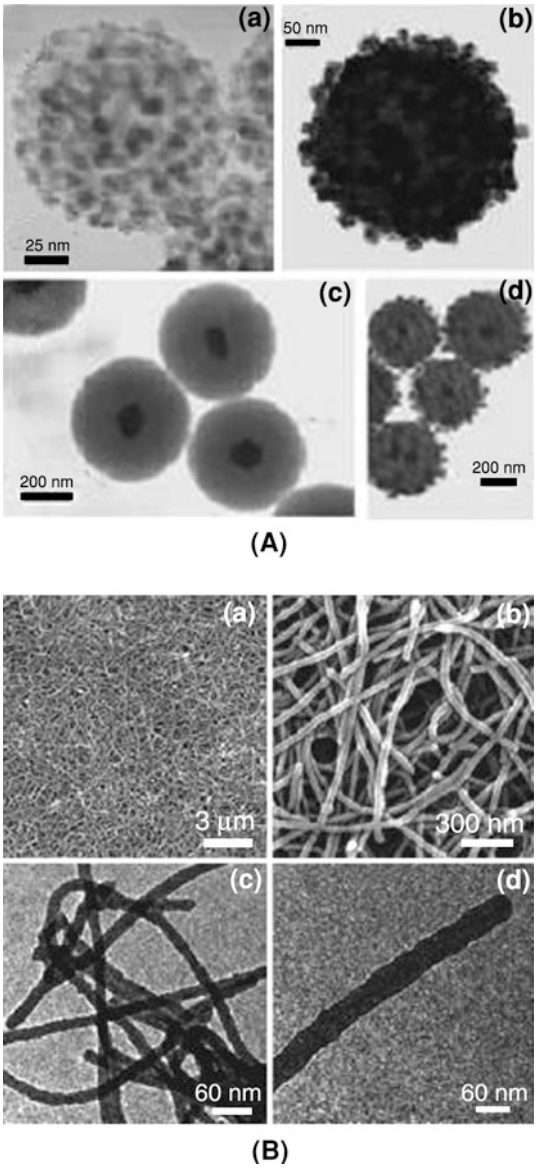


Fig. 13.3 Carbon-based nanomaterials derived from hydrothermal processing of biomass. **A** TEM pictures of (a) Au-cored carbon spheres from hydrothermal reduction and encapsulation method; (b), (c) silver-cored carbon spheres from encapsulation of silver nanoparticle seeds, and (d) layered structure with a silver core, a platinum shell, and a carbon interlayer. Reprinted with permission from [30]. Copyright 2004 John Wiley and Sons. **B** SEM (a–b) and TEM (c–d) images of carbon nanofibers obtained through the hydrothermal carbonization technique. Reprinted with permission from [31]. Copyright 2006 American Chemical Society

methods has been developed according to the nature, type, and final targeted application. Several common features can be highlighted.

(1) The synthesis of metal NP generally employs strong reducing agents; (2) precursors can be both water soluble and insoluble. In the latter case, they may express toxicity concerns; (3) surface ligands are a common feature to almost all types of NP. Ligands are employed to modify the particles' surface properties:

hydrophilicity, specific functionalities, coupling agents, etc. In many cases, the chemical nature of surface ligands is incompatible with the green chemistry principles; (4) remediation and recovery of NP is a problem which is generally not addressed in the initial phase of material conception.

Many of these points have been nicely discussed in a recent review paper from Dahl et al. [4]. In this section, some specific examples will be highlighted to let the reader understand how this field can has been developed in a "greener" way through the use of natural resources (plant-based molecules, proteins, microorganisms). A brief overview recycling methods will finally close this section.

13.4.1 Classical Synthesis

The synthesis of metal NP from their corresponding water soluble salts requires the use of strong reducing agents like sodium borohydride, even if less aggressive compounds, like citrate or carbohydrates, can be used instead with fairly good results. Unfortunately, the instability towards pH and ionic strength changes and towards additional organic molecules have demanded new synthesis pathways. For instance, one of the most reliable approaches in the field of gold NP is the so-called Brust method involving the use of hydrogen tetrachloroaurate, $HAuCl_4$, as gold precursor, dodecanethiol as capping agent, $NaBH_4$ as reducing agent, and toluene as solvent. Ecofriendly variations to this protocol were done by employing a monophasic medium, more polar solvents, different capping agents, etc. It is well-known that gold has a strong affinity towards mercaptans, hence thiol-containing compounds are classically used as surface capping agents, despite their general toxicity. Nevertheless, thio-modified common nontoxic compounds like glycine or succinic acids can be used, but also long-chain amphiphilic thiols, peptide moities, and SH-free aminoacids like lysine were also employed. Even more efforts were done in the field of semiconductor NP, like cadmium-based quantum dots. Typically, the synthesis of CdSe nanocrystals employs the use of highly toxic, pyrophoric, dimethyl cadmium in presence of trioctylphosphine oxide (TOPO), an expensive ligand. In order to overcome these problems, greener routes have been explored. For instance, the use of air-stable precursors of zinc and cadmium were tested to make CdSe or ZnSe NP. On the other side, paraffin and oleic acids replaced TOPO and the results in the synthesis of cubic CdSe seems to be quite promising.

13.4.2 Greener, Chemistry-Based, Approach to Nanoparticle Synthesis

Even if the review work by Dahl et al. [4] is quite extensive, many different strategies, only mentioned in their paper, are acquiring a lot of attention since. In particular, use of proteins, polysaccharides, fatty acids, lipids, amino acids and micro-organisms in

the synthesis of both metal oxides and metal NP of various shapes is becoming largely explored. Few examples, classified by metal type, are given below.

Gold. Nune et al. [34] have shown a very simple way to produce NP from $NaAuCl_4$ and an aqueous solution of Darjeeling tea leaves, very rich in flavins, rubigins, catechins, and other phytochemicals. The reaction was carried out at room temperature and particles did not show appreciable agglomeration. Authors have shown that catechins and theaflavins play an important role both as reducing agents of Au(III) to Au(0) and as stabilizers. Instead of tea leaves, Engelbrekt et al. [35] have used a starch/glucose mixture in water at room temperature in a buffered solution, adapting a previously published synthesis method [36]. In particular, starch and glucose have a double function: reducing agents and stabilizers while the control over the type of buffer was also extremely important to obtain a dispersed solution of gold NP.

Iron. Iron oxides NP are of paramount interest due to their superparamagnetic properties in their magnetite or maghemite structures. Their application in drug delivery, bio-separation, cancer detection direct most of the research in this field. The synthesis of these materials is quite straightforward and in particular, the co-precipitation method of a Fe(III)/Fe(II) mixture under pH-conditions provides good quality iron oxide nanocrystals. The synthesis is particularly inexpensive and it employs nontoxic compounds but, whenever good dispersion independent of pH and ionic strength is required, some additional efforts are required to keep the synthesis impact as low as possible. The literature on iron oxide nanoparticle synthesis is extremely wide and it is not meant here to make a summary. Two examples are discussed. In the first one, Liu et al. [37] used a combination of sodium citrate, Fe(III) and ethylene glycol, which served as reducing agent while citrate was used as stabilizer. They obtained superparamagnetic iron oxide particles of tunable size, between 80 and 400 nm and each composed of 5–10 nm NP. The final material shows high magnetization and good biocompatibility. In the second example, Park et al. [38] have used ionic liquids to manipulate size and shape of iron oxyhydroxide NP. The synthesis was carried out in water using 1-butyl-3-methylimidazolium chloride and Fe(III) salts under ionothermal conditions. According to the selected temperature, nanorods and isotropic β-FeOOH particles could be synthesized between 80 and 200 °C.

Quantum dots. Quantum dots semiconductor nanocrystals have attracted a large interest because of their broad range of light emission in the visible spectrum and only depending on the size, usually below 10 nm. Their importance as traceable markers in biology-related applications requires, though, a greater effort in reducing their toxicity, mainly due to the employment of cadmium, TOPO, and organic solvents. Several, more sustainable, aqueous based, methods to produce quantum dots have been developed recently. Ayele et al. [39] have developed an aqueous/ethanolic route under microwave radiation. They used less toxic $Cd(Ac)_2 2H_2O/Na_2SeSO_3$ precursors and oleic acid as capping agent, which was shown by means of FT-IR spectroscopy to be at the nanoparticle surface via the COOH function. Authors have shown that particle size (and bandgap) could be tuned from 2.3 to 2.7 nm as a function of temperature and residence time in microwave oven. A similar, Cd-free, synthesis of Mn-doped ZnSe nanocrystals was reported by Zhu

et al. [40], who also used a water/ethanol mixture under microwave conditions and oleic acid as capping agent and which was replaced by mercaptopropionic acid, more compatible with Zn^{2+} ions than COO^{-}.

13.4.3 Recycling

Recycling of NP represents a minor field of research but whose importance grows faster and faster. Due to the impossibility to filter NP with common methods (e.g., use of filter paper) or to the high energetic cost of classical separation methods like centrifugation and solvent evaporation, some research groups actually focus on the smart recover of NP from batch solutions. Magnetic separation proved to be an efficient approach whenever a magnetic phase is included in the particle structure. For instance, gold NP can be immobilized on the surface of silica-coated magnetic iron oxide NP. After using gold as catalysist in oxidation reactions, the employment of an external magnetic field allows the efficient recovery of the Au-SiO_2-Fe_3O_4 system and its re-use. A more general approach includes the use of antisolvents, such as liquid CO_2 or CO_2/water, to recover Au, Ag, ZnS, TiO_2 NP from the parent surfactant-rich solution. Control of the physico-chemical properties of the colloïdal NP/surfactant/solvent solutions can also be successfully used. In a typical water-in-oil microemulsion synthesis medium of (surfactant-stabilized) Au NP, it is possible to concentrate, at the end of the reaction, the NP in the upper oily phase by adding an excess of water to the system or by employing the cloud point phenomenon of the surfactant, whose solubility depends on the temperature. Temperature and pH-responding properties can also be interesting when using specific stimuli-responsive block copolymers like poly(N-iso-propylacrylamide), PNIPAM, and poly(2-vinyl-pyridine), P2VP as capping agents. More examples can be found in Ref. [41].

13.4.4 Microorganisms-Derived Nanoparticles

One of the key features of nanoscience is the ideal integration among many disciplines including, but not limited to, chemistry, physics, and biology. In particular, the bottom-up strategy integrates with increasing interest the concepts of biomimetism, bioinspiration, and even biosynthesis. Each of these topics could be easily related to green chemistry and they would definitely deserve to be treated independently and in more detail. Here, only few examples follow.

A relatively new approach consists of using living organisms (bacteria, yeasts, algae, fungi, human cells, viruses, etc.) to fabricate both metal and metal oxide NP. Magnetobacteria are well-known microorganisms to naturally produce magnetic Fe_3O_4 NP. For this reason, a broad number of organisms has been tested as bioreactors for a wide range of metal-based NP. Dahl et al. [4] provides a short paragraph on this topic but a more extensive review has been published only recently by

Narayanan and Sakthivel [42]. Microbe-derived NP is certainly one of the greenest methods to produce nanomaterials but one should not disregards some peculiar drawbacks of this time consuming, hard-to-handle, difficult to control, and low-yield method. In particular, monodispersity and release of the NP from the microorganisms core are still a clear challenge. Sonication and detergents are common methods to overcome this problem, impacting, of course, the re-use of the microbial population. Many studies to improve this approach and make it competitive concern an accurate strain selection and genetic manipulation of the micro-organism.

A large number of bacteria was used to accomplish this task (one can refer to Table 13.1 in Ref. [42] for a detailed list), so far. In particular, *Predomicrobium*-like, *Shewanella algae* and *Plectonema boryanum* bacteria have been reported to reduce Au^{3+} to Au^0 through an intracellular mechanism and to form octahedral or cubic-shaped NP according to the type of salt. *Clostridium thermoacetic* and *Klebsiella pneumoniae* were reported to make Cd-based quantum dots semiconductors from Cd^{2+} salts. Fungi and viruses have also been tested. Similarly to bacteria, fungi can produce NP via intra and extracellular bioreactions. On the contrary, the external capsid of viruses, in particular the tobacco mosaic virus (TMV) was used to template, for instance, iron oxides, CdS, PbS nanocrystals, as well as amorphous SiO_2. Using a selection-through-evolution approach, Belcher et al. [43] have reported the synthesis of, among others, CdS nanowires by selecting the peptides composition of the filamentous capsid of the M13 bacteriophage.

Viruses have also been used as exo and endo templates for material synthesis because their outer and inner part of the capsid can be used as structure-directing agent or hollow host for the synthesis of nanomaterials (not necessarily NP). Among the large number of examples reported in the literature and summarized in Ref. [44], one can retain, for instance, the use of viruses as template for the synthesis of porous silica materials, where the virus generates, after removal, pores of 50 nm in length. The use of biotin-streptavidin conjugation enables to build up 3-D arrays of viral NP: viruses are initially biotinilated and, by the intermediate of streptavidin, NP can be grown on their surface. The inner part of the capsid can be used to make both metal NP and wires by impregnation with metal salts and controlling precipitation through pH change or metal reduction. Such a broad variety of chemical routes is possible because of the possibility to tune the inner and outer surface chemistry of the virus via genetic manipulation. Many native viruses display amine groups from lysine and carboxylates from aspartic and glutamic acids. Nevertheless, genetic manipulation allows the introduction of thiol-containing cysteine groups, thus allowing stronger interactions with gold, palladium, or platinum (Fig. 13.4).

13.5 Sol–Gel Chemistry

Sol–gel science has imposed, since the 1980s, new perspectives in the synthesis of ceramic materials under soft conditions. The sol–gel process represents today an extremely rich way of making functional nanomaterials at different scales. To this

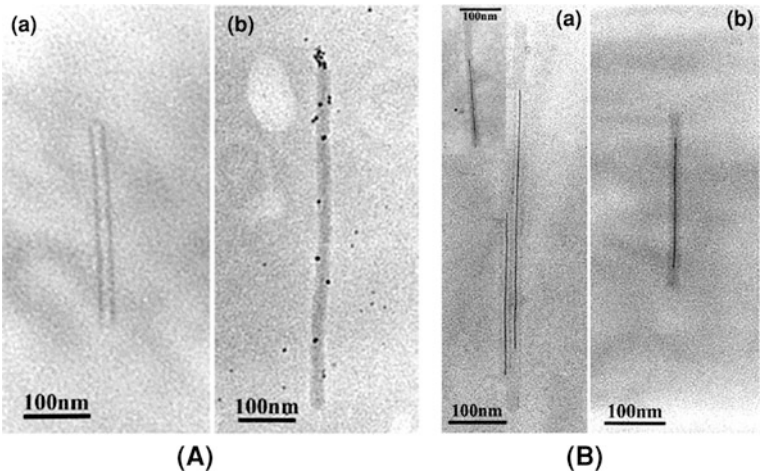


Fig. 13.4 Tobacco Mosaic Virus (TMV)-derived nanomaterials. **A** (a) TEM image of TMV. Only the contours appear dark; the coat proteins and the central channel are transparent. (b) TEM image of TMV after Pd(II) adsorption and reduction. **B** (a) TEM image of TMV after Pd(II) activation, followed by electroless deposition of Ni. Two adjacent virion aggregates are filled with wires. Energy resolved scanning TEM images proved that the dark wire is indeed composed of Ni. Inset: A single virion is filled with a 200 nm long wire with a ca. 3 nm diameter. (b) TEM image of TMV after Pd(II) activation, followed by electroless deposition of Co. The virion is filled by a 200 nm long wire with a ca. 3 nm diameter. Reprinted with permission from [45]. Copyright 2003 American Chemical Society

regard, it is important to briefly mention sol–gel in this chapter. In a recent review, [46] our group has given a broad perspective of possible sustainable approaches involved in the field of sol–gel chemistry, which refers to the aqueous and/or hydroalcoholic synthesis of metal oxides. Typical precursors are metal alcoxydes, $M(OR)_x$ (M = Si, Ti, V, Al, Zr, etc.…; R = CH_3, CH_2CH_3, etc., x = valence of the metal center), but aqueous silicates and even metal salts can be easily used, contributing to reduce the impact of this approach. The intrinsic added value of sol–gel science depends on the low-energetic chemical process involved (room temperature conditions are quite common), the variety of the possible output in terms of material processing (thin films, powders, monoliths, fibers), the "simple" chemical reactions involved (hydrolysis-condensation) and biocompatibility. Developed since the 1970s, the fundamental science of the sol–gel technique was developed mainly in the 1980s while more advanced nanoscience-related applications, including hybrid organic–inorganic, mesostructured and bioinspired nanomaterials, were largely looked at since the mid1990s. More added values in terms of sustainability of the sol–gel process were provided by the development of pure aqueous conditions with respect to hydro-alcoholic ones, use of biomass-derived precursors, enzymes as catalysts instead of mineral acids and bases, natural porogens in sol–gel derived micro-meso-macro porous materials, biomacromolecules such as proteins or carbohydrates to develop new kinds of hybrid organic–inorganic materials, etc. For more information, the reader can refer to Ref. [46].

Fig. 13.5 DNA nanotechnology: examples of 2D and 3-D assemblies of DNA strands. **A** folding ▶ paths. (a) square; (b) rectangle; (c) star; (d) disk with three holes; (e) triangle with rectangular domains; (f) sharp triangle with trapezoidal domains and bridges between them (*red lines* in inset). Dangling curves and loops represent unfolded sequence. Second row from *top*, diagrams showing the bend of helices at crossovers (where helices touch) and away from crossovers (where helices bend apart). Color indicates the base-pair index along the folding path; *red* is the 1st base, *purple* the 7,000th. *Bottom* two rows, AFM images. All images and panels without scale bars are the same size, 165 nm times . Scale bars for lower AFM images: (b) 1 microm; (c–f) 100 nm. Reprinted by permission from Macmillan Publishers Ltd: [Nature] [48], copyright 2006. **B** Hierarchical assembly of DNA-based 3-D objects. (a) A sequence-symmetric three-point-star unit was assembled from three copies of strand M, three copies of strand S, and one central strand L. The central strand contains a single-stranded loop (*red*), which provides flexibility. Depending on loop length and total DNA concentration, the formation of tetrahedra (90 % yield), dodecahedra (76 % yield), or bucky balls (69 % yield) is favored. *b* Cryo-electron microscopic images of DNA dodecahedra and corresponding projections expected from this structure. Reprinted by permission from Macmillan Publishers Ltd: [Nature] [49], copyright 2008

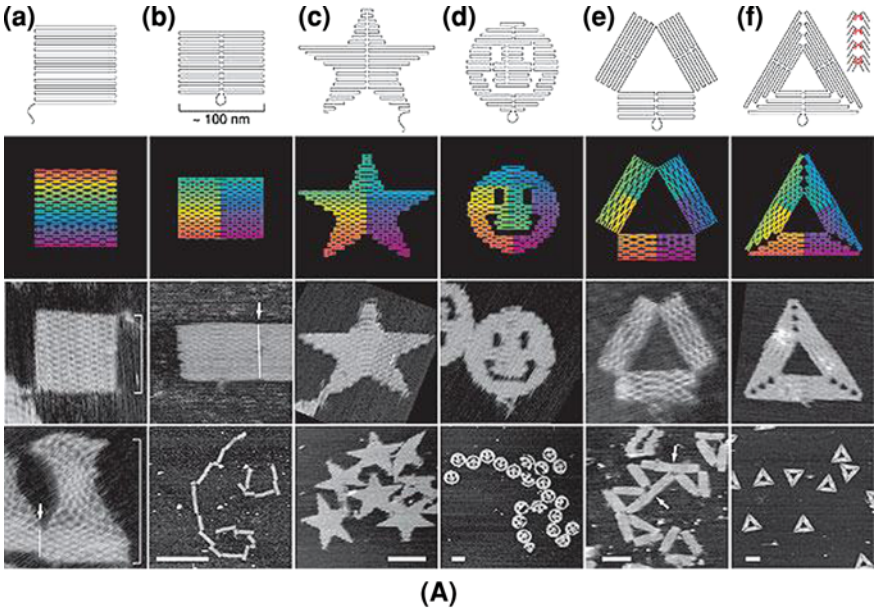
13.6 Self-Assembly

Self-assembly is a well-known and largely developed process that is generally driven by weak intra- and inter-molecular interactions in many organic and organo-metallic compounds. Self-assembly plays a major role in the formation of surfactant-derived micellar aggregates, DNA double helix and collagen triple helix formation, etc., and many of these compounds play a key role in the development of nanomaterials with complex 1D, 2D and 3-D organizations. Classical organic chemistry is of course the best way to produce molecules with interesting and tunable self-assembling properties; a precise control over the molecular shape, size and nature of functional group leads to an infinite variety of compounds with different properties: stimuli-responsive micelles, self-healing materials, tunable size, shape and surface charge, etc.

What ideas can green chemistry concepts bring to this widely developed domain? If many compounds are obtained through organic synthesis, an even larger number can actually be found in, or derived from, nature. Typically, direct extraction and purification from plants and animal (e.g., proteins, DNA), chemical processing of natural building blocks (e.g., polypeptides) and microbial synthesis (e.g., proteins, biosurfactants) are the main approaches to the recover and, in a sense, discover old new compounds with interesting unknown properties. The molecular library is in principle infinite and the studies of the properties of this type of compounds is still in its infancy. Of course, in many cases, purification, isolation, and available amount are important drawbacks.

13.6.1 DNA

One the of the most futuristic domain of nano-engineering is best represented by the field of DNA nanotechnology, which consists of building mechanically stable 2D and 3-D nanostructures of arbitrary shape by only employing DNA strands having complementary base sequences. The very interesting aspect of this



technique is that, by choosing the right pair of bases and by performing an intelligent design of the strands, one can achieve the synthesis of particularly complex shapes. Nevertheless, in most cases, the approach to simple 3-D structures like cylinders, tetrahedral, crosses, polyhedra, etc., hides complex molecular modeling studies involving synthetic DNA building blocks composed of well-designed strands. More pieces of information can be found in Ref. [47] (Fig. 13.5).

13.6.2 Proteins

In the field of biomaterials, no other organic compound has such an importance as proteins have. Widely present in simple and complex organisms, proteins have been largely employed as natural organic scaffold for material synthesis; the large amount of functional groups (COOH, NH_2, OH, etc.) is responsible for important intramolecular interactions highly influencing the protein shape, self-assembling, stimuli-responsive properties and, very importantly, its surface charge. These features altogether provide an extreme importance to the functional use of proteins in material science. For instance, proteins can be used as individual templates for nanomaterials synthesis; globular ferritin-like proteins are used to generate spherical NP, while tubular TMV allows the synthesis of nanorods instead, as described in Sect. 3.5. Nevertheless, multidimensional assemblies of proteins are also possible. Many examples of 1D, 2D and 3D protein assemblies are reported: 2D assemblies of ferritin cages or liquid crystal properties of M13 bacteriophage and collagen in the synthesis of bone-like structures [4, 50] are just some examples. Proteins can also be interpreted as natural polyelectrolytes and, to this regard, they can be used in association with charged systems, like surfactants, to create new complex systems with tunable flexibility, solubility, and unexpected lyotropic behavior [51].

13.6.3 Biosurfactants

Natural surfactants are a fascinating field of research due to the richness of their molecular structures and the broad range of synthesis pathways. Several works largely cover this field and one of the most comprehensive one was published recently [52]. The cleansing and cosmetic industries, motivated by the need of reducing the impact of petro-derived detergents, had a large influence on the development of this domain since the 1970s. Nevertheless, in most cases, only few physico-chemical properties like CMC, cleansing power, viscosity, foaming ability were studied in great detail. The development of extraction techniques, genetic engineering and importance of surfactants in the synthesis protocol of many nanomaterials allowed a recent rediscovery of this field by materials scientists, chemists, and physico chemists.

Biosurfactants can be extracted from plants and microorganisms. In this case, they are generally used as such but further modification is also possible. When microorganisms are employed, genetic manipulation is also an interesting way to tune the final molecular structure. Yields are generally low but some specific compounds are obtained in large amounts, as is the case of saponin, a glycoside-containing terpene that can be found in many plants. Classical organic chemistry approach, on the other hand, can be employed to make surface active compounds from natural molecules like carbohydrates, fatty acids, etc. For instance, cardanol, a mixture of alkylphenols obtained from cashew nut shell, can be used in combination with glucose to make cardanol-based glycolipids. These compounds were reported to form helical nanofibers of several micrometers in length [53] (Fig. 13.6).

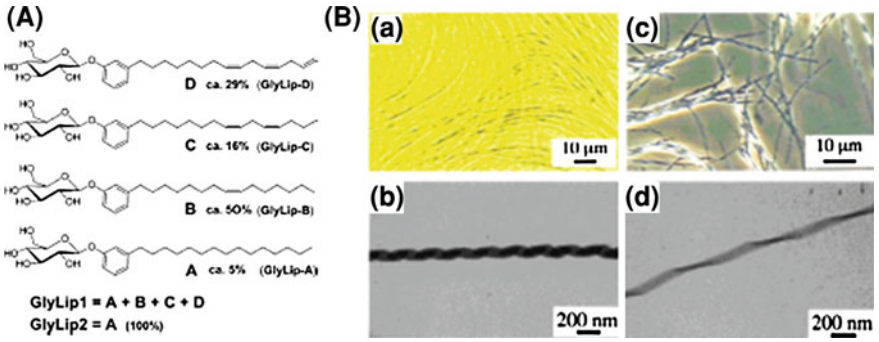


Fig. 13.6 Cardanol-based compounds and supramolecular structures. **A** Chemical structures of cardanol-based glycolipids with different degrees of unsaturation; **B** Self-assembled fibers from GlyLip1 *a*, of an individual coiled nanofiber of GlyLip1 *b*, self-assembled fibers of GlyLip2 *c* and an individual coiled twisted nanofiber of GlyLip2 *d*. Reprinted with permission from [54]. Copyright 2001 John Wiley and Sons

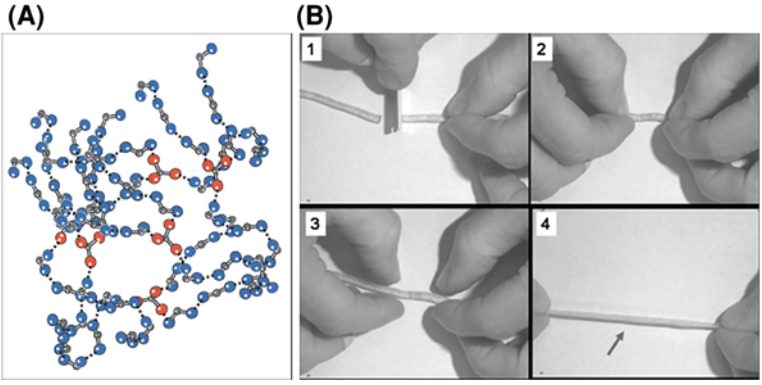


Fig. 13.7 Supramolecular assembling of bio-based resources (short-chain symmetric fatty acids, urea, diethylene triamine) into self-healing hydrogen-bonded rubber. **A** Schematic view of a reversible supramolecular network formed by mixtures of ditopic (*blue*) and tritopic (*red*) molecules associating by directional interactions (represented by *dotted lines*). Reprinted by permission from Macmillan Publishers Ltd: [Nature] [55], copyright 2008. **B** Demonstration of the thermotropic self-healing properties of the supramolecular rubber developed by Lebler et al. [46]. © Laboratoire Matière molle et chimie (CNRS-ESPCI)

13.6.4 Supramolecular Assemblies

Cooperative supramolecular assembling is an important way of building up materials and was largely developed in the 1980s. The use of natural compounds is certainly a strong restriction in the development of functional supramolecular materials but some important examples exist and are worth mentioning. The group of Leibler [55] has recently described the synthesis of a thermoreversible rubber

with self-healing properties. By employing widely abundant compounds obtained (short-chain symmetric fatty acids, urea, diethylene triamine) from renewable resources, it was possible to synthesize a glassy plastic material behaving, at temperatures higher than its glass-transition temperature (Tg = 28 °C), like a soft rubber. Interestingly, the material, soluble in benzyl alcohol, is exclusively composed of hydrogen-bonded monomers. The presence of 10 w/w % dodecane confers rubber-like and, most importantly, self-healing properties to the final material (Fig. 13.7).

13.7 Conclusion

The world of nanomaterials has been developing for the past 25 years at a fast pace but only recently the idea of sustainability, which has been affecting research practises in chemistry since late 1990s, is being considered in this field, as well. To this regard, the goal of this chapter was to show how renewable organic feedstock, whose wise exploitation is undoubtedly one of major concerns in the optics of building a true sustainable society, can be nicely transformed, thanks to the prism of the chemist, into valuable nanomaterials.

Specific examples extracted from the fields of nanoparticle synthesis, self-assembly, sol–gel chemistry, carbon nanomaterials, supramolecular chemistry, functional polymers have been analyzed. In particular, it was shown how carbohydrates, proteins, DNA, lignin, biologically derived amphiphiles, microorganisms can be nicely employed in a direct way or after chemical or biochemical transformation as building blocks for the synthesis of nanomaterials or studied as such. In a long-term perspective, green nanomaterials in their broadest sense, which includes interaction with the fields of biology and biotechnology, will probably integrate the so-called biorefineries, making a smart, closed-up, circle of both energy, and material production at reduced environmental cost. In addition, from the point of view of toxicological impact, the slogan *going green* can undoubtedly introduce interesting new perspectives concerning safety, both as a side effect of material production (e.g., exposed workers) and as direct result of the material's employment. Of course, the fact of being "natural," of "natural origin," "bio-derived," etc. does not necessarily mean lack of caution. The production of microbial-derived materials obtained from pathogenic organisms should be wisely controlled; health issues concerning NP, disregarding their synthetic pathway, is still matter of debate; working with DNA or antigens may still represent a factor of risk.

Finally, the goal of this chapter was to show that even in the very recent field of nanomaterials synthesis, another way, which is growing and growing, is actually possible and, very interestingly, its exploration has already started.

References

1. Anastas PT, Warner JC (1998) Green chemistry: theory and practice. Oxford University Press, New York
2. EC regulation N 1907/2006
3. http://www.materbi.com/NorthAmerica/default.asp?id=414
4. Dahl JA, Maddux BLS, Hutchison JE (2007) Chem Rev 107:2228–2269
5. Ozin GA, Arsenault AC (2005) Nanochemistry, a chimica approach to nanomaterials. The Royal Society of Chemistry, Cambridge, UK
6. Mohanty AK, Misra M, Drzal LT (2002) J Polym Environ 10:19–26
7. http://biorefinery-euroview.eu/biorefinery/public/index.html
8. Clark JH (2007) J Chem Technol Biotechnol 82:603–609
9. Clark JH, Budarin V, Deswarte FEI, Hardy JJE, Kerton FM, Hunt AJ, Luque R, Macquarrie DJ, Milkowski K, Rodriguez A, Samuel O, Tavener SJ, White RJ, Wilson AJ (2006) Green Chem 8:853–860
10. Drumright RE, Gruber PR, Henton DE (2000) Adv Mater 12:1841–1846
11. Akaraonye E, Keshavarz T, Roy I (2010) J Chem Technol Biotechnol 85:732–743
12. Robertson ML, Hillmyer MA, Mortamet A-C, Ryan AJ (2010) MRS Bull 35:194–200
13. Ravenelle F, Marchessault RH (2002) Biomacromolecules 3:1057–1064
14. Oh JK (2011) Soft Matter 7:5096
15. Van Tomme SR, Mens A, van Nostrum CF, Hennink WE (2008) Biomacromolecules 9:158
16. L'Actualité Chimique, Nov–Dec 2002, 6–87; in French
17. Bachelder EM, Beaudette TT, Broaders KE, Dashe J, Fréchet JMJ J Am Chem Soc doi:10.1021/ja803947s
18. Caruso RA, Schattka JH (2000) Adv Mater 12:1921–1923
19. Gu Y, Huang J (2009) J Mater Chem 19:3764–3770
20. Arias JL, Fernández MS (2008) Chem Rev 108:4475–4482
21. White RJ, Budarin VL, Clark JH (2008) ChemSusChem 1:408–411
22. Klemm D, Kramer F, Morits S, Lindstroem T, Ankerfors M, Gray D, Dorris A (2011) Angew Chem Int Ed 50:5438–5466
23. Kroto HW, Heath JR, O'Brien SC, Curl et RF, Smalley RE (1985) Nature 318:162–163
24. Ryoo R, Joo SH, Jun S (1999) J Phys Chem B 103:7743
25. Tanaka S, Nishiyama N, Egashira Y, Ueyama K (2005) Chem Commun 16:2125
26. Budarin V, Clark JH, Hardy JJ, Luque R, Milkowski K, Taverner SJ, Wilson AJ (2006) Angew Chem Int Ed 45:3782–3786
27. Titirici M-M, Antonietti M (2010) Chem Soc Rev 39:103–116
28. Titirici M-M, Antonietti M, Baccile N (2008) Green Chem 10:1204
29. Falco C, Baccile N, Titirici M-M (2011) Green chem 13:3273−3281
30. Sun XM, Li YD (2004) Angew Chem Int Ed 43:597–601
31. Qian H-S, Yu S-H, Luo L-B, Gong J-Y, Fei L-F, Liu X-M (2006) Chem Mater 18:2102–2108
32. Endo M, Takeuchi K, Ahm Kim Y, Park KC, Ichiki T, Hayashi T, Fukuyo T, Iinou S, Su DS, Terrones M, Dresselhaus MS (2008) ChemSusChem 1:820–822
33. Liu HP, Ye T, Mao CD (2007) Angew Chem Int Ed 46:6473–6475
34. Nune SK, Chanda N, Shukla R, Katti K, Kulkarni RR, Thilakavathy S, Mekapothula S, Kannan R, Katti KV (2009) J Mater Chem 19:2912–2920
35. Engelbrekt C, Sørensen KH, Zhang J, Welinder AC, Jensen PS, Ulstrup J (2009) J Mater Chem 19:7839–7847
36. Raveendran P, Fu J, Wallen SL (2003) J Am Chem Soc 125:13940–13941
37. Liu J, Sun Z, Deng Y, Zou Y, Li C, Guo X, Xiong L, Gao Y, Li F, Zhao D (2009) Angew Chem Int Ed 48:5875–5879
38. Park HS, Lee Y-C, Choi BG, Choi YS, Yanga J-W, Hong WH (2009) Chem Commun 27:4058–4060
39. Ayele DW, Chen H-M, Su W-N, Pan C-J, Chen L-Y, Chou H-L, Cheng J-H, Hwang B-J, Lee J-F (2011) Chem Eur J 17:5737–5744

40. Zhu D, Jiang X, Zhao C, Sun X, Zhang J, Zhu J–J (2010) Chem Commun 46:5226–5228
41. Myakonkaya O, Hu Z, Nazar MF, Eastoe J (2010) Chem Europ J 16:11784–11790
42. Narayanan KB, Sakthivel N (2010) Adv Coll Interf ci 156:1–13
43. Mao C, Solis DJ, Reiss BD, Kottmann S, Sweeney R, Georgiou G, Iverson B, Belcher AM (2004) Science 303:213–215
44. Evans DJ (2008) J Mater Chem 18:3746–3754
45. Knez M, Bittner AM, Boes F, Wege C, Jeske H, Maiss E, Kern K (2003) Nano Lett 3:1079–1082
46. Baccile N, Babonneau F, Thomas B, Coradin T (2009) J Mater Chem 19:8537–8559
47. Simmel FC (2008) Angew Chem Int Ed 47:5884–5887
48. Rothemund PWK (2006) Nature 440:297–302
49. He Y, Ye T, Su M, Zhang C, Ribbe AE, Jiang W, Mao CD (2008) Nature 452:198–202
50. Whyburn GP, Li Y, Huang Y (2008) J Mater Chem 18:3755–3762
51. Perico A, Ciferri A (2009) Chem Europ J 15:6312–6320
52. Kjellin M, Johansson I (2010) Surfactants from renewable resources, Wiley Series in Renewable Resources. Wiley, Chichester
53. Vemula PK, John G (2008) Acc Chem Res 41:769–782
54. John G, Masuda M, Okada Y, Yase K, Shimizu T (2001) Adv Mater 13:715–718
55. Cordier P, Tournilhac F, Soulié-Ziakovic C, Leibler L (2008) Nature 451:977

Chapter 14
Emerging Questions for Emerging Technologies: Is There a Law for the Nano?

Stéphanie Lacour

Nanotechnologies are a rapidly growing field of researches and applications. Their interdisciplinary scope, as well as the wide range of products they permit, qualified them, early, as enabling or general purpose technologies [1]. Almost all the fields of social life, from research, innovation, work safety, health, consumption, etc., to waste treatment, are, thus, affected by their development. These fields are already framed by legal norms. Thus, a rapid answer to the question we have raised might be, yes, there is plenty of law for the Nano [2].

However, as everyone can observe, this answer needs to be tempered. Indeed, if scientific and technological activities and attainments must logically be integrated into the existing legal and, broadly, normalized frameworks, it must also be raised that these should also be re-evaluated in view of the specificities that the emerging technologies are bringing about. This is not too easy, because of the intrinsic complexity of nanotechnologies as well as of the normative framework.

On the one hand, grouping activities and objects arbitrarily merely, according to physical dimension in the order of nanometers, nanotechnologies are still emerging in many ways, whereas some of their concrete application are already flooding the market and are having a concrete impact on health and environment as well as also on worker's and consumer's life.

In order to consider the social impacts of this complex reality, looking at laws is not sufficient. Confronted with a very broad and heterogeneous emerging field on the other hand, the normative landscape appears to be destabilized and fragmented and numerous other norms are challenging the legal system so as to give an appropriate framework to the "nanoworld".

S. Lacour (✉)
French National Scientific Researches
Center (CNRS) Centre d'études sur la COopération juridique Internationale (CECOJI),
Paris, France
e-mail: lacour@ivry.cnrs.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_14,

In the nano-arena, knowledge is still under construction and the uncertainties that society has to deal with are outstripping health and environmental issues to reach the social behavior of various scientific and technological stakeholders. Working on the existing normative frameworks and looking at those which are especially built in order to foster the responsible development of nanotechnologies can help the development of knowledge on uncertainty and of complexity framing, both applied to the nano-arena and extended to future emerging technologies issues.

Indeed, nanotechnology is unique and complex enough to challenge the usual normative frameworks for emerging technologies (I); their study could make sense in future scientific and technological contexts (II).

14.1 The Complexity of Nanotechnology as a Normative Challenge

Nanotechnology is obviously a complex field of study, assuming that complexity is a "web of heterogeneous components inseparably associated" [3]. Spearhead of European scientific and technological policies, the research it generates is multifaceted. Often described as inherently interdisciplinary and highly applied, definitions similar to those given to technosciences [4], the areas of investigation it includes are, in fact, far more diverse, ranging from the most basic research in small disciplinary fields to large interdisciplinary cooperation and applications. Applications, existing or planned, are also extremely varied.

Faced with such profusion, one of the first issues the regulation has to deal with is, of course, that of the concepts, which should allow the development of a relevant normative framework for each stage of the life cycle of nanotechnology (Sect. 14.1.1). In addition, the whish not to make in nanotechnology the same mistakes as those that have delayed the development of biotechnology, is pushing public authorities to promote a responsible development of nanotechnologies (Sect. 14.1.2).

14.1.1 The Central Challenge of Concepts

What is nanotechnology? Is this world designed well enough to understand and forge a critical view of the present regulating trends or is it just a portmanteau word [5], fashioned in the 1990s in order to foster broadly the development of researches and which should now be replaced by more accurate and defined concepts? A careful examination of scientific and technological policies on nanotechnology that have been published, worldwide, can help to measure the evolution undergone by the concepts used, despite their relative youth (Sect. 14.1.1.1). The continued use, by all stakeholders, of a common lexical field, built

on the prefix "*nano*", does not mean that the significance of these concepts is now stabilized. Furthermore, it raises questions about the ability of the public authorities to impose more stringent standards for the management of these technologies (Sect. 14.1.1.2).

14.1.1.1 The evolution of Concepts: From Nanotechnology to the French "Substances in a Nanoparticulate State"

The same trend can be noted in the various documents which have been published by public authorities, both French and European. According to a meta-analysis type of approach, nanotechnologies have gradually come to encompass problems involving health and the environment (nanoparticles and nanomaterials), problems inherent to funding research (nanosciences, nanotechnologies, nanosystems), and sometimes more specific queries about the industrial strategies to be adopted (nanoproducts). It is therefore noteworthy that, starting with a very wide-reaching definition "nanosciences and nanotechnologies" in 2004, the European Commission progressively reduced its field of intervention to nanoparticles and nanomaterials in the years following the enactment of its plan of action.

Such a trend can be seen in the first 2007 report on the plan of action because it includes a clause intending to specify the precise scope of the immediate concerns of the Union: "While N&N offer a number of beneficial applications, the potential impact on the environment and human health of certain "nanomaterials" and "nanoproducts" is not yet fully understood" [6].

The terminological turning point was confirmed a year later. Directed to list the regulatory texts applicable to nanotechnological development, the European Commission chose to restrict its inventory to texts that were capable of regulating nanomaterials [7]. In 2009, the distribution of areas of competency was clearly and definitively established on a European level. Indeed, the second report on the implementation of European strategy for nanosciences and nanotechnologies does states that it requires "[…] deepening the research efforts and roadmaps for key nanotechnology sectors, to enhance innovation and competitiveness. This is considered inseparable from advancing fundamental understanding of how nanomaterials throughout their life cycle interact with living organisms, to ensure a high safety level and protection of human health and the environment." [8].

However, the European Parliament, known for its very critical view of the Commission's position on nanotechnology development, did not pick up on this subtle change in the definition of the interventive scope [9]. By recentering the definition efforts, *per se,* on the term nanomaterials, the European Commission seems to be enacting this exclusion or, at the very least, to endorse a new distribution of roles among public authorities—those responsible for protecting health and the environment in terms of nanomaterials—and the scientific authorities—in charge of supervising more freely nanotechnological development. The French position, denoted by the seemingly original word choice of 'substances in a nanoparticulate state', seems to be following the same direction, even if the shift is less marked.

Such a change in vocabulary is nothing new. Similar adjustments in the socially competent arenas of public and scientific powers have already been investigated in the past, through the concepts of "science" and "trans-science", or through "risk assessment" and "risk management", as detailed in Sheila Jasanoff's work [10]. This type of dissociation does not clarify the contents of the various fields targeted, but its immediate effect is to remove certain stakeholders from sectors where specific regulations are being considered.

14.1.1.2 Definitions as an Entire Issue

Although we perhaps tend to perceive them as self-evident, or as having an obvious technological or scientific basis, definitions are truly the products of human activity. They are social constructions, meaning that they arise from concrete actions, from operations on language and from transactions, before possible institutional acceptance. In the nano-arena, as we saw, the activity of giving names to the concepts was crucial, but even when the issue is limited to nanomaterials, it still seems difficult to construct an appropriate regulation, as it is illustrated by the current debates surrounding their definition.

The first and main question to be solved is certainly the need of definition. A positive answer was expectable in the context of the European Union, both because of its regulative traditions—usually based on opening lists of definitions, created to insure a proper interpretation of the main concepts in various existing cultural and legal national frames—and because of the increasing role of this question in the current balance of power between the European Commission and the European Parliament [9]. It also occurred, more surprisingly, in the particular French context, where, the "substances in a nanoparticulate state", being neither a scientific concept nor a legal concept, the Parliament was free to define them as it chose or even to refuse to enter in the vicious circle of their definition.

An international echo of this controversy can be found in the exchanges between Andrew D. Maynard [11] and Hermann Stamm published in "Nature" last summer [12]. Even if the controversy between them can be seen as the expression of their position facing the challenge of choosing a precise type of regulation rather than concerning the need of definition, the manner they are taking issue is indicative of the trouble that it raises.

Several elements contribute to create a particularly difficult and perhaps insoluble problem of definition, whether it applies, as in the European Union, to nanomaterials, or, as in France, to the "substances in a nanoparticulate state".

First, because the policy makers lack a reliable census of nanomaterials, to begin their work, they are forced to make this census. This constraint leads to a vicious circle in which they get lost. Searching for nanomaterials requires distinguishing them from all other types of materials or chemicals, otherwise their proposed legal status and, hence, the rules of law attached to it, would lose their effectiveness. To distinguish them from other types of materials, it seems necessary to determine what makes them unique, while having a relatively accurate idea

of what is research and technical means that could easily circumvent the rules. Furthermore, at the request of public authorities, which are overwhelmed by the highly technical nature of the case in the particular field of nanomaterials [13], scientific considerations are perhaps considered more than elsewhere. Anticipating the fact that regulation is intended to protect health and environment, experts are naturally seeking the limits of the proposed definition, by inference, in the characteristics of nanomaterials which are known as likely to cause health or environmental dangers. But the existing knowledge on the characterization's needs to understand better the nanomaterials and their dangers and, thus, the existing knowledge on the risks associated with nanomaterials are still very fragmentary [14]. The proposed definition, despite its apparent scientific nature, will thus necessarily be arbitrary and will not satisfy all those concerned. The purpose of the regulation is not here, at least initially, to supervise the development of known objects, but at the very least to understand them, it might be prudent to avoid excessive zeal in the definitions.

Whether in Europe, where the question of the definition of nanomaterials has been raised as a preliminary to a major regulatory intervention by the European Commission [15], or in France, which only arrived at a later stage—after "Grenelle's Acts" that laid down the outline of the legal regime that will now apply to substances in the nanoparticle state—the results are disappointing. The failure is clear. Yet, Parliament was free to define them as it pleased, if not to define them at all, leaving to legal scholars and, ultimately, judges, the burden of doing so. Such a practice is, by no means, foreign to our regulatory system, at least in France. The terminological or partially normative definition [16], which are understood as accessory statements, do not meet to the usual frame of French law, which usually conforms to the injunctions of Portalis, that "the office of the law is […] to establish principles […], and not go into detail on issues that may arise on every subject" [17].

The need to define, nevertheless, is exposed to criticism and the result reflects the ambiguity of the interface, between law, science, and technology, which seems to ignore the fact that the rule will be subject to interpretation. The apparent technical nature of the chosen definition does not resolve, far from it, all the questions that may arise in the application of the text.

The very real difficulties encountered in attempts to define the subject of a regulation of nanomaterials testify the difficulties the governments faces in implementing their commitment to the promotion of the responsible development of nanotechnology.

14.1.2 The Responsible Development of Complex Technologies

In the regulation of nanotechnology, the legal fiction of the gradual adaptation of an existing framework is certainly insufficient. The difficulties met in the adoption

of an appropriate definition are an illustration of the gaps still existing in the texts of law. Rapid changes in the technologies surrounding nanoscale undoubtedly calls for the implementation of a framework reflecting them [18], able of rapid adaptation. This framework must also take careful not to undermine the foundations of non-stabilized economic developments, which appear to be promising. The renewed mistrust of our society in front of the scientific and technological progress is, finally, a factor, not to be disregarded.

All these specificities remove the desired framework for the development of nanotechnology from the legal positivist utopia set out by Hans Kelsen. They seem to be more flexible and renewed forms of regulation, than what is sometimes called more modern governance [19]. The same is true of the evolution of scientific research. Its current models are far from the ivory tower of Merton [20]. In the words of Sheila Jasanoff [21], they must avoid the drift toward a form of sequestration of knowledge that would be detrimental to all society. All these complex issues are essential premises for the responsible development of nanotechnology, a goal that is highlighted by public authorities (1). Its implementation, especially through public debate (2) is probably insufficient.

14.1.2.1 Responsible Development as a Leitmotiv

The concept of responsible development is central to the terminology that accompanies the plans and strategies which have been published in the field of nanotechnology. It is true in France, where it can be found in the Committee of precaution and prevention report, in 2006 [22], but also in the opinion of the Economic and Social Council in 2008 [23] and in the report of the National Consumption Council in 2010 [24]. This concept is also applied to the particular case of scientific research, according the opinion that the Ethics Committee of Sciences of the CNRS, published in 2006 [25]. The whish to promote responsible development of nanotechnology is thus emerging as one of the reasons that have led authorities to organize a national public debate on the issue, in France, in 2009 and 2010 [26].

The concept also appears in the literature of other European countries such as United Kingdom, where the Royal Academy of Sciences was the first, in 2004, to publish a lengthy report on the issue of opportunities and uncertainties of nanotechnologies in which several paragraphs are devoted to it [27].

However, the issue of the responsible development is not strictly European. It also appears in Canadian [28] and U.S [29] reports and in the documents accompanying the work of international organizations, such as the OECD [30]. Nevertheless, it is in the texts of the European Commission that this terminology is most considered. The Communication of the European Commission “Towards a European strategy for nanotechnology” [31] mentions it repeatedly. The “safe, integrated and responsible strategy”, advocated in its action plan [32] is a continuation.

Generally advocated in response to concerns about the development of nanotechnologies and, more broadly, technologies in general, the need for responsible development of nanotechnologies is based on findings from the previous experiences of appearance and development of poorly controlled new technologies. Examples of asbestos and biotechnology, particularly GMOs, are often cited as typical of those should be avoided.

Later, I will give examples of some concrete applications of this concept in terms of applied norms. In terms of governance, however, a line was crossed in 2008 with the adoption of a Recommendation from the European Commission on a Code of Conduct for responsible nanosciences and nanotechnological research [33]. This was originally intended to be reviewed every 2 years and to promote integrated, safe, and responsible nanosciences and nanotechnologies for the benefit of the whole society in Europe. This code of conduct, for member states of the European Union, as well as more broadly, for those to whom the Commission refers as those concerned, namely "Member States, employers, research funders, researchers and more generally all individuals and civil society organizations engaged, involved or interested in N&N research", states that "research activities in N & N are conducted in accordance with the precautionary principle, anticipating potential impacts their opportunities in the environment, health and safety and taking due precautions, the level of protection, while encouraging progress for the benefit of society and the environment". The concrete measures that it suggests are grouped into sub-themes and aim not only to implement the precautionary principle by a set of rather general incentives, but also to "implement good governance research" and "promote an inclusive approach".

They add few little practical recommendations to those that were issued until 2008 by various French and European scientific and ethics committees, in which the issue of risks associated with the development of nanotechnology was set out. However, this Code has the merit of placing the issue of governance and specifically the discussion of scientific and technological choices at the heart of the debate related to the responsible development of nanotechnology.

It is regrettable that this recommendation has received little support from the member countries of the European Union. If are to believe the intermediate results of opinion polls and surveys of those concerned already carried out under the NanoCode project [34], 80 % of the concerned interviewed expressed support for the Code of EU, but only 20 % of organizations had formally adopted it, and only the Netherlands did so as a member state. Neither did, the European Commission keep its promises [35] regarding the review of that Recommendation as well as of all the policy texts it has published to support responsible development of nanotechnology, such as the Action Plan of 2005. No revised text was published in 2010, any more than in 2009, leaving the issue of responsible development of nanotechnology at present in abeyance in Europe.

Finally, it is regrettable that no major public debate has been organized on the development of nanotechnology at European level, as seemed to be heralded by the plan of action of 2005 [6] and as the second report of implementation of this plan also seemed to encourage the Commission to do so in 2009 [36].

This absence of the organization of direct public debates was partially offset, at least in France, by the organization of a national public debate on policy development for nanotechnology. The means given to this debate, however, were clearly insufficient for a real illumination of the issues that were discussed there.

14.1.2.2 Responsible Development Through Public Debates

A lot of informal public debates have been carried out, in France, since 2006. In 2007, a very large colloquium was even organized, in Paris, to check the issues that had been discussed during a large set of previous initiatives. On this occasion, and despite this wealth of public meetings, the government decided to organize a national debate about nanotechnological development. This engagement lasted until 2009, when the French Environment Round Table, also known as the "Grenelle de l'Environnement" [37] concerning nanotechnologies began to be applied.

The debate was launched on the 15th of October 2009 by the French national committee on public debate (CNDP). A Special Committee, on development and regulation of nanotechnology, is historic, because of its theme and of the procedure that was followed. No less than seven Ministers charged the CNDP with the organization of a national debate on the subject [38]. The theme set by the government and agreed in its decision of the 4th of March 2009, by the CNDP was to debate: "general options for development and regulation of nanotechnology". However, in deciding to charge the CNDP to organize this debate, the Government thereby subjected it to the constraints of the legal framework concerning it [39]. Several elements of the regime of public debate, as it exists in current law, illustrate the difficulty of the latter to meet the criteria of "dialogic democracy" [40] desirable in the field of emerging technologies. This institution is made for debates rooted in the linking of projects and territories. When its fields of competence were further opened, in 2002, to broader topics of discussion, the following processes, delays, especially, were not adapted, and the form of concrete public debates, which is the in-house CNDP expertise field, was not adapted either.

This situation led to a rather disappointing result, for two main reasons. The first of these reasons is that the French authorities did not bother to answer in any manner whatsoever, the report and appraisal published by the CNDP and prepared by the special committee. In spite of the concern of members to hold a timely discussions on time, despite the difficulties to be overcome because of "noise" made by opponents of nanotechnology [41], despite their determination to pursue the debate while extensive publicity was made by the French government concerning the guidelines of its policy on nanotechnology [42], it appears that silence should be understood as a definitive answer. It is obvious that such a response does not satisfy anybody. The other reason, it is that few people who were ultimately willing or able to participate in these discussions. Public debates were largely disregarded, either because of the public themselves or opponents,

this preventing any exchange of opinion to be heard. The online debate has likewise been little pursued.

We do not intend, here, to give a sociological analysis of this debate, but to try to see how the changes made later in the Code of Environment might help improve the quality of such debates. By its expansion, in Article L 121-10 of the Code, the assumptions of referral to the CNDP, in particular by the incorporation of the issue of general options for sustainable development [43], the "Grenelle n°2 Act" [44] opens the possibility of a profound renewal of the role given to participatory democracy in science and technology policies. In doing so, it is in line with previous reforms that are marked by a steady source of law governing the development of debate in local or national projects since the early 1980s [45]. If passed after the organization of a national public debate on nanotechnology, a law could have taken greater advantage of its teachings [46].

A discussion of options of general national interest in sustainable development obviously implies public information. The time to debate differs from other debates on infrastructure projects or local equipments can be laid. The topics, often technical, as well as the scope of the issues raised, often international, as well as the relevant public…, everything differs between these two types of public debates. It is not surprising that these national debates have been included only in 2002 within the scope of competence of the CNDP. The scope of this extension of the legal framework for public discussion, however, has not really been considered. It is probable that the CNDP, whose competencies are large in terms of practical organization of debates, will eventually develop a doctrine and appropriate methodologies for them, just as it did, in the past, for the territorial debates. It is unfortunate that it remains constrained by a legal framework that seems ossified, in many of its aspects. Therefore, the time limits provided in the article L. 121-11 of the Code of the environment, often insufficient for complex and territorially broken debates, as evidenced by the debate on nanotechnology.

Furthermore, despite the welcome adoption, which is to be welcomed, of the 7th article of the Charter of the environment, which states that "everyone has the right, under the conditions and limits defined by law, to access to information about the environment held by public authorities and participate in the development of public decisions affecting the environment", French law remains behind the building of Europe's right of citizens' information, because it has no general principle of justification for administrative decisions. Thus, rules put in by article L. 121-10 al.3, and explicitly bearing on by the owner of the debate on the disclosure requirements, greatly risk to disappoint the public. This obligation is not subject to any time limit; neither is the form of information specified. Such inaccuracy is regrettable. The doctrine in its overwhelming majority does not argue for a replacement but only a complement of dialogic democracy next to the procedures of representative democracy. Many are concerned about the loss of credit that these devices suffer when, from reading the decisions that followed, the public feels that it is being ignored or, worse, that it has been used to ensure the social legitimacy of actions it disapproves.

Finally, the French Code of the environment is now extending the sphere of the legal concept of public debate to public information and public participation following the debate. This precision is supplemented by a reading of Article L. 121-13-1 of the Code, which sets out the conditions for monitoring the phase which follows the public debate on the assumption that it was intended for a project. However, it exceeds this framework, as is now mentioned in the section related to the missions of the National Committee on Public Debate and to the scope and purpose of public debate. It should, therefore, apply to proceedings provided for broader debates. This is a considerable extension of the powers of the CNDP. This opening of public debate would then imply that the total independence of the CNDP would be followed by a more relative autonomy in the realm of issues for which it would seek to ensure effective monitoring. It also implies that sufficient resources would be allocated for the administrative authority, means that far exceed, the personnel, time, and investment, those that required by the previous definition of public debate. Those, citizens involvement in the debates could be more continuous, and they could participate in the concrete development of policy guidelines. The regulatory part of the Environmental Code has not been amended to reflect these changes, but it's never too late to do.

Nanotechnologies, due to their complexity and generality, posed, in all these respects, problems which are difficult to resolve in the space provided by public debates as built nowadays, they can still serve as an example for the future. In this, as on many other aspects, they are an exemplary field of study for legal scholars.

14.2 The Nanotechnology's Normative Challenge as an Example

The general purpose characteristic of nanotechnology is, in addition to its complexity, of particular interest for the exploration the resources offered by various norms in framing emerging technologies. They arouse hopes as much as they inspire fears. As we have seen, they create a breeding ground for the concept of responsible development. Behind this, and beyond the wish of the public to be more involved in the discussion of strategic options for their development, hide strong principles, which find here an opportunity to register their declarations of intent to seek an effective implementation. The precautionary principle is obviously one of these, and its interrelation with other principles in force in specific areas of law sought during the life cycle of nanotechnology raises new problems. Despite the difficulties faced, by public debates on scientific and technological choices, in our legal system, the various discussions held on nanotechnology since 2007 have all focused on the characteristics: complexity, general purpose, public funding, and significant promise for the future together with increasingly unequal distribution of wealth between the North and South that question the evolution of our regulatory system. The uncertainty is part of the latter, as is the issue allowing technical, social, and economical innovations to take place. However, where the

first calls for a reactivation of pre-existing principles and may ultimately allow for a better control of the tools which could be used for a concrete implementation and for the articulation of these principles (Sect. 14.2.1), others are sources for a questioning in depth of the faculty of our legal systems to meet the challenges for which they were built (Sect. 14.2.2).

14.2.1 Reactivating Fundamental Principles to Face Uncertainty

If we take the time to carefully realize the uncertainty associated with nanotechnology, is different from what usually pertains to Law in the field of science and technology. Where our legal system mainly sees uncertainty in terms of uncertain risks, it is striking that this feature likewise pertains to the scientific principles used and studied on this scale, to the benefits expected. The statement could be seen as a truism. It is, also, true not merely in the field of nanotechnology [47], even if the promises of benefits that have been used to promote the latter are, particularly spectacular [48].

To consider the problem of uncertainty in terms of the relationship between uncertain promises of benefits and uncertain risks, however, allows for some of the criticisms that have been made in respect to the instrument that the law favors for the management of uncertainty: the precautionary principle (Sect. 14.2.1.1). The proposal also seems to be a renewed focus on the interactions of this principle with other principles in the various branches of special rights that may be expected to concern the life cycle of nanotechnology (Sect. 14.2.1.2).

14.2.1.1 The Precautionary Precaution at the Heart of Responsible Development

In France, the development of nanotechnology is, marked by the reference to the precautionary principle. The first committee of experts which ruled on the issue was the Committee of prevention and precaution [22], in 2006. Such a referral is not surprising. As they are emerging technologies, nanotechnologies are logically placed in the wake of the development of previous science and technologies, and, have thus, inherited the symbolic weight of a principle which had also just been adopted as a constitutional one [49]. At the European level, the approach was similar even if the reference to this principle has been less formal, at least initially. The European Action Plan for nanosciences and nanotechnologies [6] as well as the communication that preceded it in 2004 [31], placing the consequences of nanotechnology's development at the heart of the approach of the authorities, reminded that "an essential element of this responsible strategy for N & N is the integration of health, safety and environmental issues in the technological

development of N & N, […] to steer developments in a way that preserves the negative societal impact". By 2005, however, the matter of nanoparticles and nanomaterials was submitted to the scientific committee on emerging and newly identified health risks (SCENIHR) [50], and the 2008 Communication on regulatory aspects of nanomaterials [7] completed the merger, by stating that "when the scale of a risk is unknown, but concerns are so strong that management measures are deemed necessary, as is currently the case for nanomaterials, measures must be based on the precautionary principle".

Faced with risks such as those feared in the field of nanotechnology, the precautionary principle as defined in our Charter of the environment and even in previous laws [51], might however seem completely swamped. The criticism of the narrowness of its definition in French law has been mitigated. Yet, because of the scope the European Union Tribunal of First Instance gave to it by interpreting it as a general autonomous principle of Union law and allowing the grasp of the risks potentially caused not only for the environment but also for public health and safety [52]. This interpretation may be reinforced, here, by the complexity of its subject, the environment [53], and the undeniable progress of its logic in the field of risk assessment.

To think that a scientific background, as perfect as it may be, is not enough to understand the world in all its complexity and its richness is not to question the integrity or the scientific expertise usually called by the government to decide on the risks of technology. The segmentation of knowledge has certainly done nothing to improve the situation, but the scientists of the past, steeped in the humanities, never claimed omniscience. It is, in fact, impossible for experts to carry, as part of a scientific approach, all the legitimate conflicting views that the complexity of the debate displays. Modern sociology, analyzing the controversies leading to the decision-making in science does not lead to other conclusions and it also focuses on the scientific divisions themselves, particularly through the quarrels of experts [54]. The violence expressed at present by concerns often referred as societal, in the controversies related to new technologies and their products, is certainly no stranger to this recognition. As demonstrated by recent public debates organized by the CNDP, the risk of nanotechnology is not an area easily contained in a narrow discussion. On the contrary, whatever the subject set for discussion, the social concerns expressed often exceed the expectations and claims for an open framework in which the common future should be discussed. To be sure, these expectations include the implementation of the precautionary principle, but they cannot be reduced to it. Together with the precautionary principle and sometimes faced with its practical implementation, other principles and methods must be sought in the development of nanotechnology.

14.2.1.2 Around the Precautionary Principle: Other Principles and Methods

Designed as it is to support the uncertain risks of damage, the precautionary principle is often presented, in the area of legal tort, as following, the prevention principle both logically and chronologically. Thus, the logic of repairing damages caused by the technical work would be moved to their prevention, which would then progress to an earlier consideration of still emerging risks, and to the promotion of precautionary positions. Therefore, always in a chronological context, the implementation of this principle would logically be limited on the one hand by the scientific demonstration of uncertain risks, without which nothing would be began, and on the other hand by the ability to measure the probability of these risks, by shifting certainties and implementing the principle of prevention [55].

If such a description of the relationship between these two principles is rightly advocated, however, it is on two conditions. On the one hand, it deals only with the description of the principles of precaution and prevention in the area of the liability of public authorities concerned with technological developments. On the other hand, it is realized only in cases where the interaction between these principles is seen through a simple and well-defined object. These two conditions are not met where we observe the relationship between these two principles in several distinct branches of law (e.g., tort law and employment law) or if the review bears on a complex object (e.g., nanotechnology). And yet this issue has found echoes in both these registers.

First, because through their employers, workers are now in contact with nanomaterials, it is necessary to think of protective measures to be adopted toward them. The question deserves to be raised all the more that the risks associated with these nanomaterials are currently still very uncertain, even though if the available knowledge on the subject is increasing [50]. The implementation of the precautionary principle might be considered as following general risks prevention principles to workers, as part of employment law. In the workplace as in the field of public health or the environment, two hypotheses should therefore be distinguished in terms of nanomaterials. In one case, the risks associated with these products are known or at least probable and preventive measures would have to apply. In the second case, these risks remain uncertain according to present scientific knowledge, and it is appropriate to refer to the precautionary principle in this case. This interpretation should however be compared to the way in which our current labor law has interpreted the general risks prevention principles to workers.

Under French law, the Labor Code provides that “the employer shall take the necessary measures to ensure safety and protect the physical and mental health workers. These include: (1) Actions of prevention of occupational hazards; (2) to information and training; (3) The establishment of an organization and appropriate resources. The employer shall ensure the adaptation of these measures to take account of changing circumstances and aim to improve existing situations”. Since 2002 and the litigation relating to asbestos, this obligation of the employer has been interpreted as an obligation of result [56]. No mention is made here of the

precautionary principle, and protection is exclusively considered, at work, through the risks prevention principles. What, then is to be done in the presence of a risk which still remains uncertain, in the present state scientific knowledge? The existence of such a risk is well known in the field of nanomaterials. Would it be sufficient to engage the employer's liability? A negative answer to this question emerges in the field of civil or administrative liability (depending on the status of the employer). Should it have any effect on the extent of the preventive measures he is required to implement in the organization of work?

The interpretation given to the precautionary principle in its field of election might indeed result, in this case, in a decline of measures intended to protect the health and safety of workers or, alternately, in such a strengthening that any commercialization of N & N would become illusory.

Second, in a certain number of fields including for example the regulation concerning chemical products, the legal standard may be evaluated both from the point of view of the expressed and embodied principles, and of the standards and technical documents necessary for putting them into practice. Such standards and technical documents are expressly included in a "new" approach put forth since the mid 1980s by the European Commission for the construction of the European Common Market [57]. In the matter of nanoparticles and nanomaterials, "the public authorities prefer to make use of a more instrumental approach which is both integrated and open to evolution, conjugating the use of voluntary approaches, adoption of recommendations, and guides of best practices and adaptation" [58] rather than the elaboration of a specific legislation. This approach has manifested itself especially since 2008 [7]. Thus, in the light of the efforts of the European Commission, it seems possible to assert that the accurate texts for risk management of nanomaterials, to be adapted from a legal point of view, are simply the technical documents which offer an explanation of the application of existing legislation, apparently without any need to further investigate the content of the existing legislation. This interpretation of the texts of European Union has met with a strong opposition of the part of the European Parliament [9]. The explanation of this contradiction in the interpretation of the text can be found in the very heart of the pertinent regulatory disposition.

This in particular is the case for the REACH regulation [59], which seeks to establish a high level of protection for human health and environment while ensuring the free circulation of chemical substances within the Common Market, by regulating their manufacture, distribution, and marketing, and their use. It is fair to point out that this regulation innovates through abolishing the long-established distinction between existing substances and new ones, so that all chemical substances are treated equally. Another innovation is "the principle that it is for manufacturers, importers and downstream users to ensure that they manufacture, place on the market or use such substances that do not adversely affect human health or the environment." (Article 1 § 3). The limits of the regulation concerning nanomaterials have been widely commented upon. The most obvious of them is that, in the text, registry is mandatory only for chemicals which are manufactured or imported in quantities greater than one ton per year and by manufacturer or

importer. It is easy to take this first argument to illustrate the difficulties which the legal system must confront in its understanding of the nano-specificities [60]. It is sufficient to realize that the reglementary threshold of one ton per year and per operator could be difficult to reach on the European market for nanomaterials, with maybe rare exceptions, and may be easily circumvented by accounting tactics between actors in the sector. In theory, greater risk in quantity requires better knowledge of those risks. The Commission states that below the obligation of registry a certain threshold would render the system impractical.

Such a statement, from our point of view, only hides the magnitude of the modifications which are necessary in the heart of REACH's legislation itself, at least if one wishes to take correct account of the availability of nanomaterials in the marketplace. In this case, it is not because of the small quantities of manufacture or importation that the law has a problem, it is because the units of measure used to determine the threshold of applicability are inappropriate. The effects of such products are related to the distribution of particle size rather than to their total mass, as pointed out by the experts of SCENIHR [14]. Simply lowering the threshold is not enough. A complete review of the legislation is clearly necessary to take account of relevant characteristics, so that REACH has some meaning with respect to nanomaterials. Its various requirements seem irrelevant in relation to nanomaterials in view of the lack of standardization of technical means adapted to a risk evaluation for these products.

This situation requires a profound and fundamental questioning of the established legal system, which has been built on categories adapted to large scale chemicals, but have been brought into play in the new fields of nanomaterials. Behind the scenes, there are major interests among the concerned parties on the method employed to accompany the evolution of the standards framework, as well as on the room allowed for the expression of diverging opinions. In this case, not only a concept imported from a different legislative branch is in question, but the way in which the legal principles have been thought out within a coherent legal framework.

14.2.2 Renewing Some Questions About our Legal Systems Ability to Keep Their Goals

Nanosciences and nanotechnologies are both really interdisciplinary and innovative, complex and enabling researches and technologies. In terms of research, an isolated scientist can be baffled by the extraordinary variety of effects that has on the scale of a billionth of a meter. The use of complex instrumentation, which has, since 1980, been necessary to the field to expand, further accentuates the need for complementary skills. Teams exploring this area are therefore frequently made up of scientists and engineers coming from many different disciplines, such as chemistry, physics, biology. In addition, the same nanotechnology innovation, coming from a research laboratory or from the center of research and development of a company's chemical

sector, may receive applications in a variety of products, from cements to electronics, pharmacy or even sports items, after a phase of research and development which tends to accelerate. Such features raise obvious curiosity at first, hence the success, perhaps, of the research in the scientific world. They also stir up lust, as innovation is seen as the guarantee of bundle of power on very large markets and a breeding ground for revenue. Optimization of the results of nanotechnology research is, therefore, one of the major issues to which their development is exposed, and the legal tools of that valuation, patents at first, are logically sought.

Indeed, the rush toward patents in the nanotechnology arena has already begun. Like the gradual extensions of the realm of patentability initiated by the United States in the 1980s, nanopatents are about to alter the legal landscape of the innovation economy, of research and development, and of industry—no doubt to an unprecedented extent because of the scope covered by these technologies [61, 62]. Any such race for patents will inevitably prompt departures from the norm, which will occur both at the core of the system of industrial property law, which has been in place since the French revolutionary times, at the end of the 18th century, and in its philosophy.

In addition to the boundaries of matter that are crossed by nanotechnologies on the scientific and technological levels, other lines have surely been crossed as well—lines that are initially less obvious, but whose consequences may prove important over time. At a moment when the marketing of nanotechnology applications is only in its infancy, there is perhaps still time to consider the upheavals these technologies could cause in the patent system and, more broadly, the innovation economy.

From a global point of view, the very delineation of the scope of nanotechnologies confronts patent law with complex problems of definition. The emergence and characteristics of this technology are also giving rise to a reassessment of the criteria for patentability that could be prejudicial to innovation.

Patent applications for inventions in the realm of nanotechnologies are generally being filed at a very early stage [62], and across a very broad range of subject matter, and it would seem that in many instances their characterization as inventions should be questioned. Logically nanopatents feature the same characteristics as the subject matter they are meant to protect: blending fundamental and applied science, they upset the distinction that had been laid down between discoveries and inventions.

Despite the vigor with which it is being called into question, the subject matter of patent law continues, in many legal systems, to be inventions [63]. Because of this restriction on subject matter, standard-setting legislation generally excludes discoveries from the realm of patentability, although in most cases neither of these concepts is defined. The diacritical function of the notion of invention is justified, essentially, by other distinctions which make a patent a very particular tool. By setting invention, which is patentable, apart from discovery, which is not, patent law involves the difference between what exists and creation, between science and technology, or more precisely between fundamental science and the applications thereof. Pouillet put it eloquently: “patent law is written in the interest of industry

and not in the interest of science" [64]. Consequently even if the contours of the notions of invention and discovery can at times seem quite blurred, it is to this dichotomy that we believe one should refer to when seeking to reach the basics of the purpose of patent law.

It so happens that this is precisely one of the points that seem most difficult to put into practice with regard to nanopatents. As stated earlier, innovations in the realm of nanotechnologies exhibit quite distinctive characteristics, one of which is that they emanate fairly frequently from public research institutions [65], and, more generally, that they represent very fertile ground for collaboration not limited to scientific disciplines among themselves, but involving ever closer association with competencies of a more technological nature.

Moreover, "bottom-up" nanotechnologies, which are considered the most promising, involve the manipulation of atomic-level building blocks of elements that in some cases exist in their natural state, but which nonetheless give rise to massive patenting as soon as the basic principles of the technology have been laid. This in itself is not a problem if the patents in fact cover only one or more specific technical applications of the elements in question. It would seem, however, that even under these circumstances the fuzzy boundary between the products discovered and their applications has at times been crossed.

Other characteristics of nanotechnologies, including the fact that they are profoundly interdisciplinary and empowering, raise a number of problems with regard to conditions for patentability. The first of these difficulties, which results from the industrial application criterion, is the slight separation of science and its applications within nanotechnology [65]. Caught by the speed at which this area of science and technology is developing, and under pressure from government agencies impatient for results on the international economic scene to protect the fruits of their efforts, people applying for patents of invention are in many cases still at a stage in the development of the subject of their application at which it is extremely difficult to project any actual technical applications or to get past the stage of what some authors do not hesitate to categorize as abstract ideas [66].

The two other conditions for patentability—novelty and an inventive step—entail a comparison of the invention with what is known as the state of the art. This state of the art may be defined, broadly, as the sum total of knowledge in the public domain before the patent application was filed, i.e., the invention's prior art. But such a comparison obviously entails knowing the state of the art, which can prove difficult in fields in which the technology in question is recent and complex. Such is the case for nanotechnologies.

Moreover, the difficulty in ascertaining the relevant state of the art is compounded by another complexity. Many of the inventions emerging from nanotechnologies are interdisciplinary, but to determine whether the inventive step and non-obvious conditions are met entails assessing the presumed knowledge of the person skilled in the art. But which person, skilled in which art? [67] How could any single individual possess the basic knowledge needed to create interdisciplinary teams as extensive as those that were necessary, for example, to develop a DNA biochip: biologists, medical doctors, physicists, electronics engineers—none

of whom is superfluous and each of whom is fully part of the necessary synergy of talent?

Lastly, these findings are heightened by two practical considerations, the importance of which is undeniable. The first is the time allotted for examining patent applications by the examiner(s) of the office in question. Here, the EPO can be cited as a model, and the quality of its examination process is frequently praised. Some other offices, however—and by no means the least among such offices, since they include the USPTO—exhibit greater difficulties. At the USPTO, during the two, if not two and a half, years that it takes for the average application to be examined, examiners will in fact actually work on it for about 18 h [67]. If one factors in the increasingly condemned flight of patent examiners to more highly paying businesses once they are properly trained in the examination of applications as complex as those involving nanotechnologies, the first practical barrier turns out to be a substantial one. The second practical consideration is even more sensitive, and more specific to the field of nanotechnologies. Comparing an invention to the state of the art requires that the description of that state in the patent application and supporting arguments meet certain criteria. The first of these involves the vocabulary that is used. Examiners are not equally familiar with all languages. This is undoubtedly even more the case if a field of knowledge is very recent, and if its own vocabulary has not yet taken shape [68]. These semantic variations have repercussions in patent law, one of the major strengths of which they paralyze by mitigating the effects of the review of past art. There can be no doubt that they also result in partial blockage of the patent's unveiling effect. What will be the informational value of the contents of a patent that cannot be found because it is classified incorrectly due to dubious vocabulary?

Such effects are unfortunate, especially for stakeholders in the system themselves, be they in science or industry. A patent issued wrongly, or too broadly, offers none of the promised advantages to the community in terms of increasing scientific knowledge, and it may also, for others, block the marketing of certain products or promising research. One can, at least, regret the absence of serious consideration to specify what a patent can and should cover [69], as an instrument of social well-being, if one believes that patents should continue to play their proper role in the innovation economy and, maybe, play a better role in the future humanity's health.

References

1. Larédo P, Delemarle A, Kahane B (2010) Dynamics of nano sciences and technologies: policy implication. STI Policy Review, INAUGURAL ISSUE, N 1, vol 1, pp 43–62, ISSN :2093-3053, STEPI: Science and Technology Policy Institute, 2010. http://www.stipolicyreview.net/index.asp
2. See Communication from the European Commission to the European Parliament, Counsel and the European Economic and Social Committee. COM (2008) 366 final, Regulatory Aspects of nanomaterials, p 11: "Current legislation covers in principle the potential health, safety and environmental risks in relation to nanomaterials."

3. Morin E (2005) Introduction à la pensée complexe. Éditions du Seuil, Paris, p 158
4. Hottois G (2004) Philosophie des sciences et philosophie des techniques, sous l'égide du Collège de France. Odile Jacob, Paris
5. This expression has been used many times during the national debate on nanotechnology's governance which has been organized by the French National Committee on Public Debate (CNDP) in 2009 and 2010. See http://debatpublic-nano.org/informer/bilan_debat.html, especially p 24. An interesting sociological and political analysis of this concept can be found in Brice Laurent, "Les politiques des nanotechnologies. Pour un traitement démocratique d'une science émergente, Charles Léopold Mayer Editions, Sept 2010, p 26 et s
6. Communication from the Commission (2007) Nanosciences and nanotechnologies: an action plan for Europe 2005–2009. First implementation report 2005–2007 COM(2007) 505
7. Communication from the Commission (2008) Regulatory aspects of nanomaterials COM(2008) 366
8. Communication from the Commission (2009) "Nanosciences and nanotechnologies: An action plan for Europe 2005–2009. Second implementation report 2007–2009" COM(2009) 607
9. See the European Parliament resolution of 24 April 2009 on the regulatory aspects of nanomaterials, P6_TA(2009)0328, in which the Parliament "calls on the Commission to review all relevant legislation within two years to ensure safety for all applications of nanomaterials in products with potential health, environmental or safety impacts over their life cycle, and to ensure that legislative provisions and instruments of implementation reflect the particular features of nanomaterials to which workers, consumers and/or the environment may be exposed."
10. Jasanoff S (1987) Contested boundaries in policy-relevant science, social studies of science, vol 17(2), pp 195–230 (May 1987)
11. Maynard AD (2011) Don't define nanomaterials. Nature 475:31
12. Stamm H (2011) (JRC) Nanomaterials should be defined, Nature 476:399
13. The demand is expressed very clearly in Europe, where the Commission grasps the SCENHIR of scientific definition for nanomaterials regulation, (http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_q_024.pdf.) It is identical to the United States, where the White House released in June 2011 a memo arguing that science-based approach should be used by federal agencies to set rules to protect human health and the environment from nanotechnology, without creating unnecessary trade barriers further or stifle innovation. See http://www.whitehouse.gov/sites/default/files/omb/inforeg/for-agencies/nanotechnology-regulation-and-oversight-principles.pdf
14. Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR)—08 December 2010—Opinion on the Scientific Basis for the Definition of the Term "nanomaterial". The work of the SCENIHR, seized by the European Commission for a review of the scientific definition of nanomaterials, perfectly illustrates the uncertainties that still prevail in this area
15. See Background note and Draft Commission Recommendation on the definition of the term "nanomaterial" "The Commission intends to use the definition for the term "nanomaterial" contained in this Recommendation as an overarching, broadly applicable reference term for any Union communication or legislation addressing nanomaterials." http://ec.europa.eu/environment/consultations/nanomaterials.htm
16. Truchet D (2003) Les définitions législatives, in La confection de la loi, dir. Académie des sciences morales et politiques, Roland Drago, p 80
17. Portalis (2004) Discours préliminaire au code civil, in Motifs et discours prononcés lors de la publication du code civil, Bordeaux: Éditions Confluences, 2004
18. Chevallier J (2001) La régulation juridique en question. Droit et société 3(49):834
19. See EU DEEPEN project reports : Davies Sarah, Macnaghten Phil, and Kearnes Matthew (ed), "Reconfiguring Responsibility: Lessons for Public Policy", Durham: Durham University. Et Ferrari Arianna and Nordmann Alfred (ed), "Reconfiguring Responsibility: Lessons for Nanoethics", Durham University, Durham

20. Merton RK (1942) The normative structure of science. In Storer N.W. (ed)
21. Jasanoff S "Transparency in public science: purposes, reasons, limits", in Law and Contemporary problems, "Sequestered Science: The Consequences of Undisclosed Knowledge", Summer 2006, Volume 69, Number 3, p 21 et s. : "Yet modern societies' increasing dependence on science has proceeded hand in hand with developments that disable most citizens, even the most technically expert, from effectively addressing the larger set of questions: Is it good science; what is it good for; and is it good enough? Science has not only become infused with multiple social and political interests; it is also in danger of escaping effective critical control"
22. Avis du CPP Nanotechnologies, Nanoparticules—Quels dangers, Quels risques?, mai 2006
23. Avis du Conseil économique et social, Les nanotechnologies, Juin 2008
24. Rapport sur les nanotechnologies du groupe de travail du Conseil national de la consommation, Juin 2010
25. COMETS Enjeux éthiques des nanosciences et nanotechnologies, 12 octobre 2006
26. See the report published by the CNDP in April 2010. http://www.debatpublic-nano.org/informer/bilan_debat.html
27. The Royal Society and the Royal Academy of Engineering, Nanoscience, Nanotechnology: Opportunities and Uncertainties (2004) http://www.nanotec.org.uk/
28. See "Regulatory Governance Initiative" of Carleton University in Ottawa, Ontario and its report entitled "International Regulatory Approaches to the Governance of nanotechnology." http://www.nanonorma.org/ressources/sources-internationales/Nanotechnology-Regulation-Paper-April2009.pdf/view
29. See the report of the U.S. National Nanotechnology Initiative's strategy for research on aspects of nanotechnology-related environmental, health and safety research. http://www.nanonorma.org/ressources/sources-internationales
30. The OECD Working Party on Nanotechnology (WPN) was established in March 2007 to advise upon emerging policy issues of science, technology and innovation related to the responsible development of nanotechnology. http://www.oecd.org/document/8/0,3746,en_21571361_41212117_41226376_1_1_1_1,00.html
31. See the European Commission's Communication. Towards a European Strategy for Nanotechnology COM(2004) 338
32. See "Nanosciences and nanotechnologies: An action plan for Europe 2005–2009" (COM(2005) 243)
33. European Commission recommendation on a code of conduct for responsible nanosciences and nanotechnologies research (C(2008) 424 final)
34. http://www.nanocode.eu/files/reports/nanocode/nanocode-consultation-synthesis-report.pdf
35. This promise was made in the recommendation for a Code of Conduct following the Council's request, V. 12959/1/08 REV 1 (2891st Council meeting "Competitiveness")
36. Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee, "Nanosciences and Nanotechnologies: An action plan for Europe 2005–2009. Second Implementation Report 2007–2009", COM(2009)607 final
37. The "Grenelle" refers to a debate which gathers the opinion of government representatives, professional associations and Non Governmental Organisations (NGOs) on specific subjects. The debate is ultimately designed to help the government to adopt a position or legislate. Launched in July 2007 and involving various working groups and public consultations, the Environment's Grenelle was closed by a round table in October 2007 and its conclusions were published
38. Referral letter as referred to in Decision n° 2009/16/NANO/1 March 4, 2009 on general options for development and regulation of nanotechnology from the National Commission of public debate. The letter of referral itself is available at the following address. http://www.nanonorma.org/news/la-cndp-saisiede-lorganisation-dun-debat-public-national-sur-les-nanotechnologies
39. On this issue, see Lacour S Débat public et régulation dans les nanotechnologies. Évaluation d'un couple moteur, in La régulation des nanotechnologies. Clair-obscur normatif, Larcier,

2010. The regulatory frame of French public debates lies in the Code of Environment, article L. 110-1 and followings
40. For further developments on the notion of dialogic democracy, See Michel Callon, Yannick Barthe and Pierre Lascoumes, "Agir dans un monde incertain", published by Editions du Seuil in 2001
41. About this "noise", see the post Jean-Noël Jouzel published on the website of the program NanoNorma for the month of July 2010 available at http://www.nanonorma.org/lettres-dactualite/Historique%20des%20billets%20dhumeur%20NanoNorma.doc/view
42. For example, the statement of May 5, 2009, made by the research minister, Valerie Pécresse, entitled "Nano-Innov: a plan for nanotechnology", whose subtitle says, even though the CNDP has received the organization of the debate, "give industry the means to succeed French turn of nanotechnology. That's what the plan Nano-INNOV based, among other things, the creation of centers for the integration of nanotechnology in Grenoble, and Toulouse, Saclay. € 70 million will be spent on this plan in 2009. "
43. Yannick Rumpala, "Is " Sustainable development "calling for more democracy? When the "sustainable development" meets the "governance" " Vertigo, Volume 8, Number 2/October 2008
44. Loi n° 2010-788 du 12 juillet 2010 portant engagement national pour l'environnement, article 246
45. Pissaloux J-L (2011) La démocratie participative dans le domaine environnemental. Revue française d'administration publique 1(137–138):123–137
46. Lacour S Réguler l'incertain dans les rapports entre sciences, technologies et droit. Les débats publics sur les options générales d'intérêt national en matière d'environnement, de développement durable ou d'aménagement sont-ils une solution?, Lettre de la Mission Droit et Justice n°37
47. For a discussion of the use of economic promises in the modes of governance of innovation in general, see S. Jasanoff, "*Governing Innovation*", text for the symposium "Knowledge in Question", May 2009
48. Chateauraynaud F (2005) Nanosciences et technoprophéties. Le Nanomonde dans la matrice des futurs. Paris, Document du GSPR, mai 2005
49. See Constitutional Act n°2005-205 of 1 March 2005 which conferred constitutional value in the Charter of the environment and its Article 5 on the precautionary principle
50. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR). Opinion on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies, 10 mars 2006
51. Loi n° 95–101 du 2 février 1995, relative au renforcement de la protection de l'environnement
52. Tribunal of First Instance, November 26, 2002, Artegodan, Case. Ff T-74/00, Rec., 4945, pt. 183: "Although it is only mentioned in the Treaty in relation to environmental policy, the precautionary principle […] a broader scope. It is intended to apply, to ensure a high level of protection of health, consumer safety and the environment in all policy areas of the community. "
53. Ost F (1995) La responsabilité, fil d'Ariane du droit de l'environnement. Droit et Société n°30/31, p 305
54. Callon M, Law J, Rip (eds) (2005) Mapping the Dynamics of Science and Technology, The MacMillan Press Ltd, London, 1986. B. Latour, Reassembling the social: an introduction to actor-network-theory. Oxford University Press, Oxford
55. Trébulle F-G (2009) Les fonctions de la responsabilité environnementale : réparer, prévenir, punir, in La responsabilité environnementale. Prévention, Imputation, Réparation, Chantal Cans (dir.), Dalloz, p 31
56. Soc., 28 février 2002, pourvois n° 00-13.172, n° 99-18.389, n° 99-17.201: "Under the contract of employment binding him to his employee, the employer is obliged to him for a safety obligation of result, especially with regard to occupational diseases contracted by the employee due to products manufactured or used by the company, the failure to do so has the character of a gross negligence within the meaning of Article L. 452-1 of the Code of Social

Security, where the employer knew or should have been aware of the danger to which the employee was exposed, and has not taken the necessary steps to preserve"

57. Politique de nouvelle approche entreprise en 1984 et objet d'une résolution du Conseil des communautés européennes en 1985 : Cons. CE, résolution 7 mai 1985 : JOCE n° C 136, 4 juin 1985, p. 1 à 9
58. Hervé-Fournereau N La sécurité sanitaire et environnementale vis-à-vis des nanomatériaux, in Dossier thématique Droit et nanotechnologies, sous la direction de S. Lacour, Cahiers Droit, Sciences et Technologies n°1, CNRS éditions, p. 57 et s
59. Regulation (CE) n°1907/2006, concerning registration, evaluation and authorization of chemical substances, and restrictions applicable to these substances (REACH). Cf. For example : E. Brosset, Le règlement communautaire en matière de produits chimiques, petite explication de texte, RJE, n°1/2008, p.5
60. Thieffry P (2010) La prise en compte du risque des nanoparticules et nanomatériaux. Information et réglementation en amont. Mise en perspective juridique", in La régulation des nanotechnologies. Clair-obscur normatif, dir. S. Lacour. Editions Larcier, Paris, pp 181–208
61. See Lemley MA Patenting Nanotechnology vol 58. Stanford Law Review, pp 601, January 2005
62. See Coriat B (2002) Le nouveau régime américain de la propriété intellectuelle. Contours et caractéristiques clés. Revue d'économie Industrielle, No 99, 2nd quarter 2002, pp 17
63. This is, of course, the case in French law, where Article L. 611-10, par. 1 of the Intellectual Property Code (CPI) stipulates that "Patents may be granted to inventions…" See also the European Patent Convention, Article 52 of which states that "European patents shall be granted for any inventions …", or the TRIPS Agreement, Article 27 of which applies to "any inventions, whether products or processes, in all fields of technology …" For its part, American law does not accord the same value to this notion. There, patents are categorized as utility patents, and, according to Title 35 U.S.C. 100, "the term 'invention' means invention or discovery". However, regarding the basis for the utility requirement and the distinction between products of nature and products of man, the United States Supreme Court until excluded scientific discoveries from the scope of patenting (see Brenner v. Manson, 383 U.S. 519, 1966). But this precedent has been mitigated in recent years due to the effects of biotechnology patents
64. Pouillet (1909) Traité théorique et pratique des brevets d'invention, Paris, p 13
65. The proportion of patent applications to the EPO by public research institutions is very much higher in the field of nanotechnologies than in other areas. This holds true for the USPTO as well. Along these lines, see Masatura Igami and Teruo Okasaki, Capturing Nanotechnology's Current State of Development via Analysis of Patents, OECD, op.cit
66. Zekos GI (2006) Patenting abstract ideas in nanotechnology. J World Intellect Prop 9(1):113–136
67. See Lemley MA, Shapiro C (2005) Probabilistic Patents. J Econ Perspect 19(2):75698 Spring 2005
68. See Berger M Growing Nanotechnology Problems: Navigating the Patent Labyrinth, on www.nanowerk.com
69. On this question, and on the need to raise it whenever a new, still largely unknown, technology enters into the scope of patenting, see Eisenberg R (2000) Analyse this: a Law and economics agenda for the patent system, vol 53(6) Vanderbilt Law Review

Chapter 15
Nanomaterials in Political Life: In the Democracies of Nanotechnology

Brice Laurent

Abstract How to deal with nanomaterials in democratic societies? Answering this question requires an understanding of the political qualities of nanomaterials. Rather than discussing "political impacts" that could be assessed once they are properly identified, this paper argues that nanomaterials are inherently political in so far as they are uncertain objects, connected to the future developments of nanotechnology, and tied to public concerns and the mobilization of various publics. It starts by discussing the political dimensions of nanomaterials through the examples of a European "network of excellence" and a carbon nanotube development project in a private company. The paper then describes three democratic formations enacted by the management of nanomaterials. These formations rely on the arrangement between the definition of nanomaterials, expectations about the future, the identification of public concerns, and the mobilization of various publics. I contrast an international "science-based" expertise, a European moral space, and French attempts for the responsible development of nanomaterials.

15.1 Introduction

How to deal with nanomaterials in democratic societies? The answer to this could seem straightforward. As technical objects, nanomaterials would be best studied and controlled with appropriate expertise, which could then inform policy makers if specific measures are to be taken in order to mitigate the potential risks of these substances. For the rest, nanomaterials are produced and used by private

B. Laurent (✉)
(CSI—Mines ParisTech), Paris, France
e-mail: brice.laurent@mines-paristech.fr

R. Brayner et al. (eds.), *Nanomaterials: A Danger or a Promise?*,
DOI: 10.1007/978-1-4471-4213-3_15,

companies, integrated in market products, and live their lives as many other chemicals do, only with a different (and maybe wider) range of potential applications.

In this chapter, I argue that this reading oversimplifies the circulations of and discussions about nanomaterials, and that it is no less than the theory and practice of democracy that are at stake with these substances. I demonstrate this point by discussing the "political dimensions" of nanomaterials, and by reflecting on the ways in which these entities are dealt with in democracies.

The question of democracy has both an empirical and a normative dimension. I am more interested here in the empirical exploration of the various democratic constructions experimented with in order to deal with nanomaterials. Instead of an abstract reflection on the ways in which democracy should be organized about nanomaterials (asking questions such as "who should participate in the public management of nanotechnology?" or "is it necessary to ask people what they think about the development of nanomaterials?"), I propose empirical descriptions of the ways in which democracy functions with nanomaterials.

As previous works have shown, the public management of technical issues organizes democratic life.[1] It distributes roles and responsibilities across public and private bodies, lay and expert actors. It defines legitimate public issues and ways of dealing with them. It organizes decision-making processes. Analyzing these phenomena requires that one describes the instruments through which technical issues are dealt with. Controversies among the actors involved[2] and differences across countries[3] may then illustrate the range of democratic constructions enacted by these instruments.

In the vein of these works, I discuss in this chapter the democratic constructions that the definition and public management of nanomaterials enact. Thus, I describe different technico-political constructions that associate the definition of "nano ness" and its related concerns with the collective organization of democracy.[4] This exploration is situated within a stream of studies interested in the "coproduction" of knowledge and political order.[5] It touches on important political science questions (how does democracy function with technical issues?) using tools and methods developed in science studies. In the meantime, it also raises a very practical issue: that of the legitimate treatment of complex issues in democratic societies.

---

[1] See [9, 10] about the science/policy boundary; Jasanoff [11] about risk perceptions; Barthe [3] for an example about nuclear waste; [7] for a critique of risk/benefit evaluation.

[2] A canonical example of controversy about risk evaluation and management is Brian Wynne's study of Cambria sheep farmers, which describes the opposition between the scientific methodology of British administrative experts, and the local knowledge of farmers [29].

[3] International comparison can thus illustrate the variety of technico-political arrangements on which risk regulation is based [13].

[4] I use materials based on my work on "technologies of democracy" and the problematization of nanotechnology [19–21].

[5] The term was introduced and discussed by [12].

I start this chapter by discussing the ways in which nanomaterials have a political life. These objects are connected to global development programs on nanotechnology. They are produced in conjunction with public concerns. They are meant to be represented for and discussed with a variety of publics. Having then described the political life of nanomaterials, I turn to an analysis of the types of democratic constructions that are expected to deal with it. I contrast an international, a European, and a French democratic formation. The three of them define modes of collective and legitimate actions, as well as the "nano ness" of nanomaterials.

15.2 The Political Life of Nanomaterials

Nanomaterials are constructed in industrial and university laboratories, developed in view of applications, measured and studied by scientific instrumentation. Nanomaterials are not elements of a simple "natural category" that could be unearthed in order to build relevant innovation policy or risk regulation. The novelty of these materials and their "nano ness" itself needs to be defined. This section argues that nanomaterials have various "political" dimensions. In so far as they are connected to programs of development, public concerns, and publics, and are of an uncertain "nano" identity, they live a "political life".

I describe the components of the political life of nanomaterials through the use of two main examples: that of a European project called *Nano2Life*, and that of a French company producing carbon nanotubes. These examples will allow me to describe the interconnections between the development of nanomaterials and the construction of programs for the development of nanotechnology. Nanomaterials will appear as entities associating material elements with anticipations about the future, but also public concerns and publics.

15.2.1 Nanomaterials and Their Programs

The development of nanomaterials in laboratories, particularly for biomedical applications, is supported, for instance, by large-scale research projects funded by public bodies. Consider for instance the European project called *Nano2Life*, launched in 2004, which was the "single European network of excellence funded under the 6th Framework Programme of the European Commission". *Nano2Life* gathered 23 research institutions in ten different countries across Europe. As a "network of excellence", it did not add new research projects to those conducted by the partners. Rather, it hoped to "reduce fragmentation in European nanobiotech" by undertaking various common initiatives, such as training programs in nanotechnology, exchange programs among partners, sharing of scientific equipment, and coordinating long-term research objectives among the partners.

Nanomaterials, as they appear in the *Nano2Life* European networks, are not passive entities expected to be unproblematically discovered and harnessed by researchers. They are connected to the development of the European research policy regarding nanotechnology. Thus, part of *Nano2Life*'s activities was the organization of "foresight exercises", through which the project could "identify the future applications or techniques to focus the research efforts on".[6] The coordinator of *Nano2Life* and other members of the networks participated in the writing of a "vision paper", which was the first step in the establishment of a "European Technology Platform" for nanomedicine ("ETP nanomedicine").[7]

The road map that emerged from the ETP nanomedicine and *Nano2Life* was meant to coordinate European research and define objectives for the next nanotechnology policy initiatives. It identified problems to be solved and potential outcomes. For instance, it defined "devices for drug delivery" as "targeted applications", and then pointed to "key R&D priorities" (e.g., biocompatibility of materials and miniaturized systems), needed technologies (e.g., "nanocapsules"), the "challenges" to be met (e.g., the stability of the device), and the diseases supposed to be cured (cancer, diabetes, or cardiovascular disease).[8] The road map considered that nanotechnology required the early identification of promising domains, and the definition of research funding flows. Fundamental and applied research had to come together, and the road map heralded "public–private partnerships" as instruments through which nanotechnology could be developed according to the objectives defined, with limited public funding support. As scholars interested in technico-economic instruments have shown,[9] road maps such as the European nanomedicine one are not descriptions of a world already given, but contribute to shaping it by organizing collective action.

As this example illustrates, nanomaterials live their lives in the science policy world, in which the organization of laboratory research is determined by road maps and policy instruments expected to realize the "potential" of nanotechnology. This implies an organization targeted at the development of certain applications and meant to foster interdisciplinary research practices and industry/academic partnerships. The production of and research about nanomaterials is thus directly connected to the definition of short- and long-term objectives for the development of nanotechnology, and to operations meant to organize scientific and technological research. This is not the case only for academic laboratories. The development of nanomaterials in private companies is also directly linked with national and international science policy research. Ameka,[10] a leading French

---

[6] www.nano2life.org; accessed January 12, 2011.

[7] European Technology Platforms are coordination mechanisms organized at the initiative of the European Commission and scientific actors. They are meant to contribute to the making of the European research policy.

[8] Nanomedicine vision paper: 30.

[9] See [26] for an example about Moore's Law.

[10] The name is fictional.

producer of carbon nanotubes, decided to enter the nanotubes markets in anticipation of future growth of the domain, and in tight connections with the American and French developments programs for nanotechnology. As its production grew, the modalities of its industrial activities were discussed in conjunction with national and regional nanotechnology policies, and the company was part of numerous local and national programs of support for the development of nanomaterials.

15.2.2 Nanomaterials and Their Concerns

The political life of nanomaterials does not lie only in their connections with public science policy programs and private industrial strategies. As nanomaterials began to appear as both a promising economic market and an interesting research domain, they were also associated with public concerns that were to be taken into account.

Thus, the European nanotechnology policy is supposed to address the "Ethical Legal and Social Aspects" of the domain.[11] These "aspects" comprise the question of informed consent in clinical trials, the potential threats to privacy through the applications of nanoelectronics, and the issues of nanomaterials' potential risks. The "integration" of ELSA in nanotechnology programs means that research projects related to the "implications" of nanotechnology are funded as part of nanotechnology programs, some involving social scientists, others led by toxicologists or environmental scientists. In *Nano2Life*, ethicists and scientists were supposed to work closely together, so that an "ethics board" could publish about the potential concerns related to nanomedicine, while scientists could be trained about the "societal implications" of their research. In other cases, the importance of the integration of nanotechnology concerns is such that some speak about a "safety by design" approach, which would bring materials scientists, biologists, and toxicologists together in the making of new nanomaterials with a collection of precisely tailored properties—among which are toxicological properties.[12] In all cases, this contributes to making nanomaterials a part of public concerns related to potential health risks and ethical issues.

This has implications for private companies as well. The leader of the nanotube project at Ameka described humorously the transformation of the production of carbon nanotubes in his company into a source of public concerns:

> As soon as the production pilot (for nanotubes) was there, then the director of communication, the direction of the environment, the technical direction, the direction of the

[11] Hullmann [8].

[12] Kelty [15] describes the development of the "safety by design" approach by the American chemist Vicky Colvin, who was actively involved in the making of nanotechnology federal programs.

> plant, the préfet, the sous-préfet, the president of the region, the archbishop… Everyone was rushing there… there were so many people coming to see the reactor that we couldn't work anymore. It had become so present in the media… At the same time, nanomaterials were becoming a priority for the whole company. And there was pressure to control what we were doing. (Interview with the author)

That companies are concerned about the risks of nanomaterials depends on a variety of factors, among which the anticipation of potential controversies and the fact that the risks of nanomaterials might well be risks for the development of nanotechnology.[13] This results in different industrial strategies, among which the withdrawal of "nano claims" for industrial products is not to be targeted as "nano".[14] Ameka adopted a different strategy, based on the containment of nanotubes. This was a consequence of its decision to "apply the precautionary principle". But it also resulted from the technical difficulties Ameka met in attempting to mobilize other approaches, most notably predictive toxicology, that is, the demonstration of a causal link between this or that characteristic of the substance and measurable hazards.

Indeed, predictive toxicology soon proved too complex to undertake. What interests a company such as Ameka is its overall properties for products to sell to its customers. Production processes are thus tailored according to the observed properties of the end products, so that Ameka's nanotubes fit in the customers' products. This renders the detailed characterization of nanotubes unnecessary, which is not incidental. Indeed, measurement and identification techniques are not always available for nanomaterials, and not standardized. Ameka's nanotubes are woven together in ways that make detailed characterization impossible. The issue becomes even trickier when one considers the other carbon nanotubes produced by Ameka's competitors. As they are developed in view of specific properties, their physical characteristic (length, diameter, etc.) are potentially all different from each other. This points to a central issue for nanomaterials, that of their characterization and identification as "new" substances. The question is important, because it displaces the "political" dimension of nanomaterials from science policy programs and public concerns to the very definition of nanotechnology objects. This leads me to discuss the ways in which one can differentiate nanomaterials from "non nano" substances.

15.2.3 Defining Nano Ness

Distinguishing between substances relies on the classifications of chemicals (e.g., Mendeleev's table for chemical elements), and instrumented identification technologies (e.g., mass measurement and chemical composition evaluation

[13] MacCarthy and Kelty [23].

[14] The case of nano silver in the U.S. is paradigmatic for that matter.

devices). Once the industry is involved, it is also a matter of regulators and jurists, who need to identify substances in order to manage them (e.g., through the control of industrial products, the limitation of populations' exposure, or the labeling of consumer goods). Thus, regulatory texts define what an "existing substance" is, and thereby perform an ontological work. In Europe, the registration, evaluation, and authorization of chemicals regulation (REACH) defines a "substance" in this way:

> Substance: means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.[15]

REACH considers that two substances are distinct if they have different chemical composition (i.e., they are made of different elements), or different "physical parameters", defined as such, in a non-exhaustive manner:

> The other specific main identification parameters to be added depend on the substance. Examples of other main identifiers can be elemental composition with spectral data, the crystalline structure as revealed by X-ray diffraction (XRD), Infra Red absorption peaks, swelling index, cation exchange capacity, or other physical and chemical properties.[16]

Measuring these physical characteristics implies that instrumentation is available in order to identify them in an unambiguous manner.[17] In the US, the toxic substance control act (TSCA) considers as "existing" a substance listed in the TSCA inventory. A substance is "new" if it is not listed in the inventory, that is, if it can be distinguished from the existing ones. The criteria used to draw the distinction are relative to the chemical composition and physical parameters, most notably crystalline arrangements, isotopy, and allotropy. In the American as in the European case, measurable criteria are used to establish the existence of substances. In the latter, the existence of a substance implies constraints for industrialists, in particular that of information distribution through legally defined instruments (e.g., "safety data sheets" for the REACH regulation). This then allows the European administration to impose restrictions on the most dangerous substances.[18]

---

[15] REACH, title I, chap. 2, art. 3:1.

[16] European Chemicals Agency, [26] Guidance for identification and naming of substances under REACH, ECHA: 29.

[17] Instruments need to be standardized in order for their measures to be comparable. See [25] for the work needed to do so.

[18] The two texts follow different approaches: REACH forces industries to provide information (controlled by the European Chemicals Agency, ECHA), and to demonstrate the safety of their products [6]. TSCA asks EPA to demonstrate the existence of risks in order to impose restrictions, while the federal agency cannot force industries to provide data on their products. See (Sachs 2009) for a comparison between the two texts and a critique of TSCA. Sachs insists on the limited number of cases where EPA could impose restriction measures.

Thus, what makes a chemical exist is an infrastructure made of legal texts, standardized measurement instruments, technico-administrative instruments such as labeling and registration dossiers, and institutions able to stabilize the criteria being used. Through the instruments on which it is based, the data it circulates, the standardized measures it mobilizes,[19] and the management tools it constructs, this infrastructure defines existences for chemicals[20] at the same time as it defines the problem of the health and safety risks of substances and ways of dealing with it.

Nanomaterials raise additional issues. If two substances differ from each other because of the size of their components, then the criterion of the chemical composition cannot be used to distinguish "nano" from "non nano" (the atomic composition is the same). The distinction according to physical characteristics—for instance, crystalline arrangements—could be possible, but is not straightforward. Consider for instance the case of Ameka's carbon nanotubes. Regulators distinguish between graphite and diamond, two varieties of carbon.[21] According to the same logic, one could consider that nanotubes are an allotropic variety of carbon, and can thus be made "existing". But if the atomic structure is considered as a criterion, why not then differentiate between "multi walls" and "single walls", "rigid" and "flexible", diameters inferior or superior to a certain limit? The question is all the more acute as companies do use these criteria (and many others) to differentiate between their products when they patent them.[22] In order to know the parameter that matters for toxicological regulation, it would be necessary to establish a link between the physical or chemical characteristics chosen as a criterion and toxicological properties. This is predictive toxicology, the very approach that Ameka was unable to undertake because of characterization issues.

Consequently, the definition of nano ness is a crucial stake as soon as it is connected to the construction of regulation. This stimulates considerable debates, as to whether nanomaterials should be considered "nano" solely because of their size, or because of their (toxicological) properties. I will get back to this important point in the second section of this chapter. At this stage, one can notice a central aspect of the political life of nanomaterials: the very definition of "nano ness" can be discussed.

---

[19] About the importance of standardization activities in scientific practice see [16, 17].

[20] This example is another illustration of the importance of the study of classification operations in order to understand the constitution of the social [4].

[21] European Chemicals Agency, [26] Guidance for identification and naming of substances under REACH, Helsinki, ECHA. Another case is that of substances "of unknown or variable compositions, complex reaction products, or biological material" (UVCB), which encompasses compounds of several chemical elements produced by organic synthesis, or biological materials themselves. This latter case does not apply to nanotubes (made of a single element, carbon), nor to other nano substances (as their chemical composition is not questioned).

[22] A few court cases has been filed at the time of writing, but they all confirmed the patentability (and thus the novelty) of nano substances based on different processes, and having different size characteristics than already patented ones [2].

15.2.4 Nanomaterials and Their Public Lives

Nanomaterials are not just objects of interest for scientists and policy makers. They are expected to be developed for industries to use and consumers to buy. The growth of the market for nanomaterials was heralded soon in the development of nanotechnology programs as a reason for public and private investments in the field. "Publics" were not only considered as potential consumers, they could be potential threats, if too scared by possible health risks or long-term ethical concerns. Accordingly, the European nanotechnology policy, in heralding the "societal dimension" of nanotechnology, insisted on the need…

> to establish an effective dialog with all stakeholders, informing about progress and expected benefits, and taking into account expectations and concerns (both real and perceived) so as to steer developments on a path that avoids negative societal impact.[23]

It meant that nanotechnology's public was yet another component of projects such as *Nano2Life*, which included in its objectives the "education of society", and the "dialogue with civil society". The former related to training programs for students, and materials aimed to communicate the outcomes and objectives of *Nano2Life*. The latter pointed to the identification of public concerns, either related to "risks" or "ethics".

The call for "public dialogue" and the consideration of "citizens' expectations and concerns"[24] was not limited to Europe.[25] Following the reports released by the U.S. National Science Foundation about the "societal implications of nanotechnology", in which the need for "two-way communication with the public" had been expressed,[26] the US Nanotechnology Act required that US nanotechnology programs

> provide (…) for public input and outreach to be integrated into the Program by the convening of regular and ongoing public discussions, through mechanisms such as citizens' panels, consensus conferences, and educational events, as appropriate[27]

Integrating "the public" in nanotechnology policy implies the construction of specific devices: instruments expected to represent nanotechnology for "the public", to "inform about progress and expected benefits" (to reuse the language of the European Action Plan), and also devices aiming to "take into account expectations and concerns", which requires on the one hand to "make the public

---

[23] European Nanotechnology Action Plan: 8.

[24] Hulmann [8] p 12.

[25] Cf. [14, 24] for comments about the importance of the deliberation theme in nanotechnology policy, and its consequences for the involvement of social scientists. This latter question is important, and will be discussed at further length in other parts of the dissertation (particularly in chapters 3 and 6).

[26] Roco and Bainbridge [27].

[27] U.S. Congress, "21st Century Nanotechnology Research and Development Act", S.189 (P.L. No. 108–153).

speak", and, on the other, to mobilize what the public says in ways that can be said to have taken it into account. The development and public management of nanomaterials is tied to these devices, in ways that can vary. How are "publics" (and which ones) expected to transfer their expectations and concerns into the material constructions of nanomaterials and the definition of nanotechnology programs? The second section of this chapter will provide three different answers to this question.

As for Ameka, the construction of carbon nanotubes was undertaken in public watch (cf. 15.2.2). More than that, the company adopted a strategy of "transparency" about its activities. This implied an involvement in the public initiatives meant to monitor the industrial production of nanomaterials, and an active participation in the design and staging of public exhibitions in science museums about the companies' activities in the production of nanomaterials. For Ameka, the material control of carbon nanotubes through containment was also a discursive control about what was said about the company, who should be informed about its activities, and by whom.

15.2.5 Nanomaterials in Political Life

What should we learn from the previous descriptions? First, the development of nanomaterials occurs in conjunction with the formulation of nanotechnology policy. Second, the question of their potential impacts is raised as soon as they are developed. Third, the characterization of nanomaterials is not an easy task. In the case of Ameka, containment allowed the company not to raise the issue of characterization, but this has no reason to work for less stable objects. Fourth, nanomaterials are expected to meet their "publics" in one way or another. Nanomaterials are not neutral objects lying passively in laboratories for their "impacts" to be discovered. Their very existence is inherently connected to the definition of public problems and ways of dealing with them. They have political lives: they are objects to be defined, which are connected to futures to decide about, public concerns to manage, and publics to engage.

What are the consequences for the organization of democracy? The answer is not straightforward, as it requires a definition of what is meant by "democracy". As a working hypothesis, I consider that democracy is at stake in places where oppositions are voiced and organized for the treatment of public problems. This will allow me to describe, in the following section, three "democratic constructions" (that is, three modes of organizing public problems and defining ways of dealing with them) that shape the definition and management of nanomaterials as much as they are shaped by the components of the political lives of these substances. As it will appear below, these constructions connect the definition and management of nanomaterials with the planning and organization of nanotechnology programs.

15.3 Democratic Constructions

In this section, I follow up on the previous discussion in order to examine the ways in which the political life of nanomaterials results in different democratic constructions. I consider three examples: that of the international standard-making processes about nanotechnology, the European nanotechnology policy, and current French initiatives for the collective management of nanotechnology and nanomaterials.

15.3.1 International Expertise for Nanotechnology

The standardization of nanomaterials is discussed at the international standardization organization (ISO), where a "technical committee" on nanotechnology (TC229) was created in 2005. The work of TC229 is interesting, because it connects the various aspects of the political life of nanomaterials: the committee is expected to define "nano ness" in order to address public concerns related to the potential risks of nanomaterials, and ensure that stakeholders interested in their development (industries, environmental or consumer organizations, and states) are heard.

Standardization at ISO TC229 is done according to a "science-based" process. According to this process, definitions are to be crafted independently from considerations related to the potential risks of nanomaterials. It based the definitions on that of the nanoscale, as going approximately from 1 to 100 nm. The 1–100 nm size limit is a science policy concept stated in various policy reports.[28] It is both an umbrella term able to bring together the many research projects related to the exploration of properties emerging at the atomic scale, and a technological indication characterizing new properties and products. It is considered as a "typical but not exclusive" dimension.

Following the definition of the nanoscale, TC229 defined nano objects as substances with at least one dimension within the nanoscale.[29] Nanomaterials, then, were defined as either nano-objects, or "nanostructured materials", that is, materials displaying nanoscale regularities.[30] Defining nanomaterials as such means that some existing entities become "nano", and that differences are drawn among entities that were previously considered identical. This could be difficult to accept for companies if additional requirements are asked for nanomaterials. But

[28] The American National Nanotechnology Initiative, the 2004 British *Royal Society* report, and the O.E.C.D. used the 100 nm size limit, as an indication of a size range where new properties may emerge. The 1 nm inferior size limit was added by TC229 in order not to limit the scope of the substances qualified as "nano".

[29] Nanotechnologies—Terminology and definitions for nano-objects—Nanoparticle, nanofibre and nanoplate, ISO/TS 27687:2008.

[30] Nanotechnologies—Vocabulary—Part 1: Core terms, ISO/TS 80004-1: 2010.

the international agreement was eventually possible at TC229 because of the "science based" process. Basing the process "on science" implies that the identification criteria are used "only to describe" the nanoscale patterns. Consequently, the linear logic of the definition of the scale, objects, and nanomaterials avoids defining nanomaterials according to properties that are linked to a "political" objective, that is, in the language of ISO, linked to national regulatory choices. Thus, ISO constructs a boundary between (international) science and (national) politics through a nanoscale-based definition of nanomaterials.

This is important because it means that attempts to define nanomaterials based on their toxicological properties cannot succeed in the international arena. There would be indeed other possibilities for the definition of nanomaterials, in which the "nanoness" would be characterized by properties not necessarily related to size. Thus, researchers propose to define inorganic nanoparticles "from an environment, health and safety perspective."[31] This would lead to define nanomaterials according to "size related properties instead of size itself."[32] For instance, the specific surface area, the oxidation rate, or the ion release rate could be considered as criteria. Defining "nano ness" according to properties other than size was mentioned during the discussions at TC229. The idea was consistent with the TC229 mandate, which included the standardization of "the properties of nanoscale materials that differ from the properties of individual atoms, molecules, and bulk matter."[33] But the logic of the property-based definition could not be successful at ISO. Indeed, "nano properties" vary from one chemical to the other, and the product of company X to the company Y. Measuring instruments for particle size, surface reaction, or crystalline states are not uniform. And, as the example of Ameka's carbon nanotubes show, the characterization of potential "nano-properties" faces considerable technical difficulties.

This is not only a problem of time available to build technical infrastructures. Indeed, if a property and a corresponding instrument were selected to define nanomaterials, then the ISO members holding the technology were favored at the expense of those who would then be forced to buy it. This is problematic in the context of international negotiation. But a deeper problem lies in the fact that the property-based definitions threaten the logic of the "science based" process itself. They ground the definition of nanomaterials on risk management considerations: this is exactly the "political choices" that international standardization is expected to keep at bay. Contrary to the property-based definitions, the size criterion avoids addressing the cases of each material. It is both a technical requirement, and a criterion for science policy. It is not related to any binding regulation for nanomaterials. Thus, the 1–100 nm size limit can fit in the standardization body, contrary to definitions based on physical and chemical properties of substances that cannot rely on the infrastructure they need, and threaten to tie the definition of nanomaterials with a "political" regulatory objective.

---

[31] Auffan et al. [1].

[32] Auffan et al. [1] p 641.

[33] TC229 Business Plan.

ISO is not the only place where international discussions occur about the collective management of nanomaterials. At the organization for economic cooperation and development (OECD), nanomaterials are dealt with at the Working Party on Manufactured Nanomaterials (WPMN), where the problem of "nano ness" is dealt with in a different fashion than at ISO. At WPMN, reference materials are tested and characterized, the evaluation of risks is supposed to follow, and meant to be carefully separated from national political decisions. In this case, the "science-based" process is not grounded on the size criteria, but on material objects chosen after negotiations among the participants in WPMN. The expertise thereby produced is expected to be separated from another one about the public, treated in a separate body of the OECD. The working party on nanotechnology (WPN) is in charge of "policy expertise", and undertakes projects related to the policy framework for the development of nanotechnology and public engagement mechanisms. This division of labor between WPN and WPMN is not incidental, and is central in the everyday work of the OECD.[34] It is another manifestation of the importance of international "science-based" expertise, that is, independent from national regulatory choices. This means that neither the evaluation of nanomaterials' risks, nor the consideration of the modes of doing "public engagement" may displace the boundary between the work of the international organization and the national choices of the country members. This boundary is of course itself a political construct, that defines what can be discussed and what cannot, what is considered as "objective science", and what lies in the "political realm". Accordingly, it constructs a way of doing public engagement in which the preferred mode of action is the measure of public perception regarding an unquestioned technical reality, so that one can measure the perceptions of the public in order to tailor the communication programs adequately.[35]

15.3.2 A European Moral Space

In Europe, the development of nanomaterials is done within a strategy for the "responsible innovation" in nanotechnology. This means that the European programs of support for nanotechnology contend that the development of nanomaterials is supposed to be conducted in conjunction with risk and ethics studies, and in permanent "dialogue" with the European public.

This translates into the organization of the European research policy, as the example of *Nano2Life* illustrates. "ELSA" projects are funded, and are supposed to ensure that nanotechnology is developed according to "European values" such as sustainability, solidarity, transparency, and inclusion. The various science policy instruments mobilized by the European Commission all participate in the

[34] Laurent [22].

[35] For a discussion of "the political science of risk perception", see [11].

production of a European "moral space" characterized by an attention to principles and values. For example, ethics reviews ask all European projects to define their objectives in accordance with European values. They encourage the use of a "Code of Conduct" expected to define a "European approach" to the research and production of nanomaterials. They suggest specific adaptations of research practices to the case of nanomaterials, for instance by calling for the introduction of exposure limitation device. They encourage the creation of "ethics board" in European projects and the organization of training sessions for scientists to learn about the implications of their work.

The practice of "responsibility" is thus delegated to laboratory scientists, guided by general principles such as those presented in the "Code of Conduct". In the meantime, the European public is to be kept informed, needs to deliberate and dialog about potential applications. The Commission has recently introduced the idea of the "scientific understanding of the public" in order to point to the needed dialog with the public, and the "constant monitoring" of European public opinion supposedly necessary for the efficacy and legitimacy of European nanotechnology policy.[36] The idea of "scientific understanding of the public" would mean that European research programs adapt their objectives to the expectations and constraints of the European public. These initiatives are the components of an approach to the responsible innovation in nanotechnology in which no additional legal constraints are imposed on private industrial actors, and the development of nanotechnology is not hindered by negotiations among stakeholders about future regulations.

This approach is not uniformly accepted within the European Union. While the projects funded with the "ELSA" part of nanotechnology programs herald "lay ethics" and "deliberation" as central concerns, they are often critical of the ways in which the Commission is implementing these very objectives.[37] Within the European institutions, the European Parliament (EP) has voiced an opposition to the initiatives of the European Commission. The EP does not consider that the existing regulatory framework is sufficient to deal with nanotechnology issues. The EP's critical opinion regarding the activities of the European Commission toward nanotechnology was made explicit in an almost unanimous resolution that responded, in 2009, to the Commission's communication regarding the "regulatory aspects of nanomaterials."[38] This communication had explained how the

[36] European Commission [5].

[37] For example, the promoters of projects calling for the redefinition of the framing of public issues by deliberating publics consider that "scientific understanding of the public" reproduces a separation between unproblematic "publics" and unproblematic "applications of nanotechnology".

[38] European Parliament resolution of 24 April 2009 on regulatory aspects of nanomaterials (2008/2208(INI), hereafter "EP resolution"). 362 votes were casted in favor of the resolution, and four against (five MPs abstainted). The resolution responded to the "Communication from the Commission to the European Parliament, the Council and the European Economic and Social Committee regulatory aspects of nanomaterials" (SEC(2008) 2036). It also answered the conclusion of the Competitiveness council on 25 and 26 September 2008 (12853/1/08 REV 1 RECH 264 COMPET 311, Subject: "Council conclusions on responsible nanosciences and nanotechnologies research").

"principle of safety" was operationalized by the European institutions as regards nanomaterials. It had mentioned the code of conduct and the ethical review process. The Communication had concluded that the existing regulatory framework was efficient, and that no adaptation was necessary to deal with nano substances' potential risks. In its resolution, the European Parliament noted the "limited value" of the Communication "due to the absence of information about the specific properties of nanomaterials."[39] Consequently, the EP:

> did not agree (…) with the Commission's conclusions that (a) current legislation covers in principle the relevant risks relating to nanomaterials, and (b) that the protection of health, safety, and the environment needs mostly to be enhanced by improving implementation of current legislation.[40]

The position the EP defended was based on the "consideration of all nanomaterials as new substances."[41] Accordingly, the EP added amendments specifically targeted at nano substances in regulatory texts. For instance, the EP added an amendment to the November 2009 cosmetic regulation that asked companies to label products containing nanomaterials. In this amendment, nanomaterials were defined as follows:

> 'Nanomaterial' means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale 1–100 nm.[42]

This definition restated the 100 nm size limit that had been used at ISO. It also added two conditions, insolubility and biopersistence, which made it clear that the definition was to be used as an instrument for the regulation of risks for human health.[43] Through this amendment, the EP solidified a legal existence for nanomaterials for the first time. It later undertook similar regulatory actions for the novel food and the biocide directives, in which it added amendments requiring additional risk evaluation for nanomaterials.

Hence, the EP considered that new entities were to be created within the European regulation. Thereby, it challenged the Commission by pushing an ontological argument for the existence of nano substances. By representing the

---

[39] EP resolution: "Whereas" P.

[40] EP resolution.

[41] EP resolution: 9.

[42] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products: Art. 2.1, alinea k.

[43] In translating the 100 nm size limit into a regulatory text, the EP had also to eliminate the adverb "approximately" that was used in the ISO definition. The constraints of legal writing solidified this rigid limit. This caused NGOs to worry about the possibilities offered to companies wishing to escape the mandatory labeling to use slightly bigger than 100 nm substances (e.g., 110 nm) but nonetheless displaying enhanced properties because of their sizes. This was a reason for the EP to consider the nanomaterials amendment as a first step, which could be "adjusted and adapted" according to "technical and scientific progress and to definitions subsequently agreed on at international level" (article 2.3).

concerns of the European public through the electoral mechanism, it considers that the consultation of "lay publics" and the "scientific understanding of the public" brought forward by the Commission were not the only ways in which nanotechnology's publics were to be involved in European decision-making.

The story does not end there, as the European Commission itself released in October 2011 a recommendation "on the definition of nanomaterial".[44] The recommendation "invited Member States, the Union agencies and economic operators" to use a definition of nanomaterials based on the size distribution of the particles in a given material. It also suggested to use, when technically feasible, a specific surface area criterion. My objective is not to discuss the details of the definition eventually proposed by the Commission, but to highlight a few characteristics of the political life of nanomaterials in the European democracy. First, the initial reluctance of the Commission to regulate nanomaterials eventually resulted in a non-binding recommendation that constituted a new category of substances. As the Parliament had wished, new entities have indeed been created. But like the Commission's previous activities meant to ensure the responsible development of nanomaterials, this ontological work remains flexible and does not result in additional legal constraints. Second, the definition of nanomaterials within the European institution is tied to the connection between the public management of nanomaterials and the exercise of European values and principles. This results in attempts at crafting "policy-based approaches", translating, for instance, in definition criteria for nanomaterials that are linked to the potential toxicological properties of the substances (e.g., specific surface area, which implies a higher reactivity). The difference with ISO's "science-based" nanomaterials could not be clearer: as international nanomaterials are supposed to be independent of national policy choices, European ones are to be defined according to a "policy-based approach" consistent with European values and principles.

The European nanotechnology policy and the current debates about the definition and management of nanomaterials enact a democratic formation based on common values and principles. While the operationalization of these principles can be controversial (as the opposition between the Commsission and the Parliament shows), the public management of nanomaterials is directly connected to the moral principles that are supposed to ground the European identity. More than that, it is tied to the construction of the democratic legitimacy of the European institutions.

15.3.3 French Responsible Development

A last example I want to discuss relates to the French nanotechnology policy. The notion of the "responsible development" of nanotechnology is central in France as

[44] Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU).

in other places. But the operationalization of the "responsible development" took a different shape there than within the European institutions. While the development of nanotechnology became a priority of the French government and local administrative bodies, it also stimulated the experimentation of devices meant to ensure the collective management of nanotechnology.

The situation is contrasted though, as French actors oscillate between an ambition based on the development of nanotechnology for economic growth, and the desire to ensure the collective responsible management of nanotechnology. The best illustration of this ambivalence is certainly the case of the city of Grenoble, in the French Alps, where nanotechnology research has been a priority of the local administrative bodies.[45] This has resulted in the constant support to nanotechnology research projects, as illustrated by a research center called *Minatec*, part of the Commissariat à l'Energie Atomique (CEA, a national research institution), and proudly supported by the local public bodies. In Grenoble, the call for the responsible development of nanotechnology originated from a vocal anti-nanotechnology critique. It forced the local actors to adapt their programs to take the management public concerns into account, and stimulated the organization of public meetings devoted to the exploration of nanotechnology public concerns. This was not satisfactory for the anti-nanotechnology activists who blamed a "parody of democracy" for not questioning the objective of nanotechnology development for economic interests.

In this context, the French public bodies have appeared somewhat uncertain about how to deal with this critique and the public concerns of nanotechnology. The organization of a national debate on nanotechnology was an attempt on the part of the French government to ensure a collective discussion expected to "enlighten public choices" about nanotechnology. But many of the public meetings set up throughout the country were interrupted by anti-nanotechnology activists, and the organizers were eventually forced to cancel the final public meetings. This last example shows that the general objective of "responsibility", despite its importance for private and public actors, raises challenges for the conduct of public policy, as French actors do not attempt to ground the action on a "science-based process" independent of "political" choices, or on "principles" expected, as European principles are, to ensure the legitimacy of collective action.

Like the European Parliament, the French government introduced a definition of "nano ness" as it tried to introduce a mandatory declaration of "substances in nanoparticulate state" (*substances à l'état nanoparticulaire*),[46] defined as:

> Engineered substances characterized by one or more external dimensions, or internal structure, in the 1–100 nm size range, including under aggregates or agglomerate forms potentially bigger than 100 nm but conserving properties typical of the nanoscale.

[45] Laurent [18].

[46] This followed a proposition originated from the "Grenelle de l'Environnement", a national consultation process about environmental regulation, launched by Nicolas Sarkozy after his election in 2007.

The draft decree asked for the declaration of substances in nanoparticulate state, and of materials that used mixtures in which "substances at the nanoparticulate state were included but not linked" (that is, "potentially extracted or rejected in normal conditions of use"). This proposition has attracted many criticisms, especially from the industrialists, who contended that operationalizing the definition was not technically feasible, because of the lack of standardized measurement instruments. They also criticized the proposed threshold for the declaration,[47] which would include, for them, far too many objects in the scope of nano products.

Other instruments could appear much more acceptable for industries than those (like mandatory declaration) that required a solidification of the boundary between "nano" and "non nano". At ISO TC229, France leads a project on "control banding", which ambitions to develop instruments for industrial companies to manage uncertainty. In this case, nanomaterials are related to known substances in order to situate industrial processes in "bands" associated with safety features (e.g., confinement, or simple protection of workers with gloves and masks within a lower risk band). "Control banding" does not draw a boundary between "nano" and "non-nano". This is also the case of a project initiated in 2008 by an official in charge of nanotechnology at the Ministry of Health (and an active member of French delegations in international arenas), and then led by the French association of normalization (AFNOR). This project aims to develop a "nano-responsible tool". This tool intends to define principles for industry wishing to produce, use, or market "responsible" nanomaterials. It is addressed to any producer of substances considered as "nano" because of size-related properties. The format is that of a list of questions that an industrial using the tool would have to answer. Questions comprise, for instance, "What are the main physical and chemicals characteristics of the substance? Is the release of nanoparticles in the atmosphere possible during the production process? In what ways is the exposition to nanoparticles possible during the product lifecycle?" Accordingly, industrialists using the tool would be prompted to adapt their practices to account for the uncertainties of their product. They would be proposed to use methods such as containment, diffusion of information for customers, or substitution of the new products in favor of better-known substances. The tool is currently being developed throughout a collaborative process involving industrialists and civil society organizations. It is expected to account for technical uncertainties, as well as expectations and concerns of civil society.

Thus, the nano responsible tool aimed to make producers internalize the potential externalities of nanomaterials. It connects the development of nanomaterial products with the expectations and concerns of public administration, and of consumers and environmental groups. It is in this connection that uncertainties about the objectives of the project might appear. They relate, for example, to certification. Certification would publicly recognize producers, distributors, and

[47] In the draft decree: "500 g for declarations before May 1st, 2013", "100 g before May 1st 2014", "10 g for later declarations".

users of "responsible" nanomaterials. It would articulate the construction of standards with the implementation of regulations, by allowing regulators and the broad public to track industrial activities. That many participants are reluctant to certification highlights the ambivalence of the objectives of integrating externalities to ensure the "responsibility" of nanomaterials. On the one hand, "responsibility" is supposed to be a label for distributors and consumers to choose among many products. On the other hand, manufacturers want to avoid solidifying the difference between "responsible" and "not responsible" to render possible a strategic navigation in a situation where regulations are not determined and risks are difficult to prove. As for civil society organizations, some of them questioned the potential of the nano-responsible tool to redirect the development of nanotechnology. Indeed, the tool assumes that the development of nanomaterials, however "responsible" they are, is the objective of this collaborative project. Moreover, it is based on the internalization of the expectations and concerns of "civil society", which then loses the possibility for external critique.

The nano responsible tool is part of a particular democratic construction, in which the "responsible development" of nanomaterials is conducted throughout a series of experiments, some (like the national public debate) highly visible and contested, others more confined and less confrontational. While the overall objective of responsibility is widely accepted, its translation in the actual conduct of public action is uncertain. This results in the introduction of new categories (such as "substances in nanoparticulate state") and in the experimentation of devices that are expected to deal with the situation of uncertainty regarding both the very definition of nanomaterials and the expectations and concerns of nanotechnology's "publics".

15.4 Conclusion. In the Democracies of Nanotechnology.

Nanomaterials have political lives, and these lives are lived in democracies. Organizing democracy with nanomaterials means organizing oppositions, allocating roles, defining public issues, and constructing publics. This is not a sole "social" matter separated from the "technical" details of the production of nanomaterials. Rather, the modalities of the democratic game shape the collective management and, ultimately, the very definition of "nanomaterials", as much as the uncertainties about the characterization and application of nanomaterials shape the ways of organizing democracy.

In this chapter, I have argued that nanomaterials are part of global programs for the development of nanotechnology, contribute to shape public concerns, are addressed to various publics, and eventually are of an uncertain identity open for negotiation. This has led me to describe three contrasted democratic formations, characterized by their treatment of nanomaterials and the ways in which they ground the legitimacy of public and private action.

I have described an international formation, in which the separation between "international expertise" and "national policy choices" is crucial, a European one, in which the "responsibility" of European principles is ensured through their (contested) operationalization in science policy instruments, and a French experimental formation in which public actors attempt to open public policy choices and the very construction of nanomaterials to collective discussion. In these three cases, the definitions of public problems and the allocation of roles for nations, industries, and civil society organizations differ. These three formations articulate the definition of nanomaterials ("science-based", "policy-based", and experimented in various devices), expectations about future developments (left to national decision-making, tied to European values and principles, collectively experimented in public debates or the nano-responsible tool), the identification of public concerns (public perceptions to manage, safety and ethical risks to take care of, uncertainties to deal with), and the mobilization of various publics (lay public to know about, lay publics to engage, emerging concerned publics to involve). Hence, nanomaterials live different political lives in the international, European, and French spaces, and this enacts different democratic constructions.

What could then be the path for a "democratization" of nanomaterials and the associated nanotechnology programs? Rather than indicating what the "most democratic" approach could be, I have preferred to describe the democratic formations enacted by the collective management of nanomaterials. I have not attempted to evaluate initiatives according to an "ideal" of democracy. Accordingly, the analysis of the political life of nanomaterials that I have proposed here does not mobilize external criteria through which the collective management of technical issues might be evaluated. But it describes the investments needed for social orders to be stabilized, and of the possibilities for alternative constructions (e.g., the property-based definitions at ISO, or the opposition voiced by the European Parliament in Europe). Thus, the approach I have adopted proposes an empirical analysis of technical objects attached to the practicalities of the conduct of democratic life. It reintroduces oppositions and uncertainties in techno-political arrangements, and it is in this sense that it contributes to the democratization of nanotechnology.

References

1. Auffan M et al (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective Nat Nanotechnol 4:634–641 October 2009
2. Baluch A, Radomsky L, Maebius S (2005) In re Kumar: the first nanotech patent case in the federal circuit. Nanotechnol Law Bus 2(4):342–346
3. Barthe Y (2009) Les qualités politiques des technologies: Irréversibilité et réversibilité dans la gestion des déchets nucléaires. Tracés 16(1):119–137
4. Bowker G, Star L (1999) Sorting things out. Classification and its consequences. MIT Press, Cambridge
5. European Commission (2010) Communicating nanotechnology: why, to whom, saying what, and how. European Commission, Luxembourg

6. Fisher E (2008) The 'perfect storm' of REACH: charting regulatory controversy in the age of information, sustainable development, and globalization. J Risk Res 11(4):541–563
7. Fisher Franck (1990) Technocracy and the politics of expertise. Sage, London
8. Hullmannn A (2006) The economic development of nanotechnology—An indicator based analysis, European Commission, DG Research, Unit "Nano S&T—Convergent Science and Technologies". http://www.ist-mona.org/pdf/EU_Nanotechno_report_nov2006.pdf
9. Jasanoff Sheila (1986) Contested boundaries in policy-relevant science. Soc Stud Sci 17(2):195–230
10. Jasanoff Sheila (1992) Science, politics and the renegotiation of expertise at EPA. Osiris 7:192–217
11. Jasanoff Sheila (1998) The political science of risk perception. Reliab Eng Syst Saf 59:91–99
12. Jasanoff S (2004) States of knowledge. The coproduction of science and social order. Routledge, London
13. Jasanoff S (2005) Designs on nature. Science and democracy in Europe and the United States. Princeton University Press, Princeton
14. Kearnes M, Wynne B (2007) On nanotechnology and ambivalence: the politics of enthusiasm. Nanoethics 1(2):131–142
15. Kelty C (2009) Beyond implications and applications: the story of safety by design. Nanoethics 3(2):79–96
16. Latour B (1989) Science in action. Open University Press, Milton Keynes
17. Latour B (1990) Drawing things together. In: Lynch M, Woolgar S (eds) Representations in scientific practice. MIT Press, Cambridge, pp 19–68
18. Laurent B (2007) Diverging convergences. Innov Eur J Soc Sci Res 20(4):345–358
19. Laurent B (2010) Les Politiques des Nanotechnologies. Pour un traitement démocratique d'une science émergente. Charles Léopold Mayer, Paris
20. Laurent B (2011a) Democracies on trial. Assembling nanotechnology and its problems. PhD dissertation, CSI-Mines ParisTech
21. Laurent B (2011) Technologies of democracy: experiments and demonstrations. Sci Eng Ethics 17(4):649–666
22. Laurent B (2011c) Producing international expertise about technologies of democracy, paper presented at the Innovation in Governance Forum, Berlin, May 2011
23. MacCarthy E, Kelty C (2010) Responsibility and nanotechnology. Soc Stud Sci 40(3): 405–432
24. Macnaghten P, Kearnes M, Wynne B (2005) Nanotechnology, governance and public deliberation: what role for the social sciences? Sci Commun 27(2):268–287
25. Mallard A (1997) Compare, standardize and settle agreement. On some usual metrological problems. Soc Stud Sci 28(4):571–601
26. Miller P, O'Leary T (2007) Mediating instruments and making markets: capital budgeting, science and the economy. Account Organ Soc 32:701–734
27. Roco M, Bainbridge W (eds) (2001) Societal implications of nanoscience and nanotechnology. Springer, Dordrecht
28. Sachs N (2009) Jumping the pond: transnational law and the future of chemical regulation. Vand L Rev 62: 1817–1869
29. Wynne B (1992) Misunderstood misunderstanding: social identities and public uptake of science. Pub Underst Sci 1:281–304