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Shivendu Ranjan
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Nanoscience in Food and Agriculture 4

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Sustainable agriculture is a rapidly growing field aiming at producing food and energy in a sustainable way for humans and their children. Sustainable agriculture is a discipline that addresses current issues such as climate change, increasing food and fuel prices, poor-nation starvation, rich-nation obesity, water pollution, soil erosion, fertility loss, pest control, and biodiversity depletion.

Novel, environmentally-friendly solutions are proposed based on integrated knowledge from sciences as diverse as agronomy, soil science, molecular biology, chemistry, toxicology, ecology, economy, and social sciences. Indeed, sustainable agriculture decipher mechanisms of processes that occur from the molecular level to the farming system to the global level at time scales ranging from seconds to centuries. For that, scientists use the system approach that involves studying components and interactions of a whole system to address scientific, economic and social issues. In that respect, sustainable agriculture is not a classical, narrow science. Instead of solving problems using the classical painkiller approach that treats only negative impacts, sustainable agriculture treats problem sources.

Because most actual society issues are now intertwined, global, and fast-developing, sustainable agriculture will bring solutions to build a safer world. This book series gathers review articles that analyze current agricultural issues and knowledge, then propose alternative solutions. It will therefore help all scientists, decision-makers, professors, farmers and politicians who wish to build a safe agriculture, energy and food system for future generations.

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Nanoscience in Food and Agriculture 4

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We dedicate this book to our parents

Nano-materials: Anciently Present
But Recently Discovered

Nandita Dasgupta
Shivendu Ranjan

Preface

This book is the fourth of several volumes on *Nanoscience in Food and Agriculture*, published in the series *Sustainable Agriculture Reviews* (http://www.springer.com/series/8380). Nanotechnology, which is the use of techniques to build nanomaterials, is a fast emerging scientific topic. However, nanomaterials are not new; they have always occurred in nature. What is new is the methods that allow to synthesize unprecedented nanomaterials of tailored, fine-tuned properties, thus opening many applications in diverse fields. In particular, the high surface to volume ratio of engineered nanomaterials makes them often more efficient than in nature. Surprisingly, some nanomaterials even exhibit contrasting properties compared to their macro-counterpart. While nanomaterials are actually commercialized in various sectors, their use in food industries is still slowly emerging and debated. Results show that nanomaterials improve bioavailability, shelf life and nutrient delivery; they reduce nutrient loss and are essential in active packaging. Active packaging, also named intelligent or smart packaging, refers to packaging systems that help to extend shelf life, monitor freshness, display information on quality, improve safety, and improve convenience. Nevertheless, the potential toxicity of new nanomaterials should be studied before their use in consumer products (Fig. 1). This book presents comprehensive reviews on the principles, design and applications of nanomaterials in food, water and pharmaceutical sectors.

A nanocomposite is a multicomponent solid where one of the components has dimensions of less than 100 nm. It is a solid combination of a bulk matrix and nano-dimensional components differing in properties due to dissimilarities in structure and chemistry. In the broadest sense, nanocomposites include porous media, colloids, gels and copolymers. The principles and application of nanomaterials in food packaging, with focus on nanocomposites, are presented in the first two chapters by Ramos et al. and Ahmad et al. The synthesis and applications of nanoemulsions, which are stable systems containing two immiscible liquids, are then reviewed by Bhushani and Anandharamakrishnan in Chap. 3. Aiming at the safe design nanomaterials for food, Manickam et al. discuss recent advances on the genotoxicity of nanomaterials in Chap. 4. Chaurasiya and Hebbar then describe in Chap. 5 the use of reverse micelles as nanoreactors for the synthesis of nanomaterials and for the

Fig. 1 Nano-food products are reaching stores. This image has been modified and designed from a copyright-free image source by Nandita Dasgupta – VIT University, India

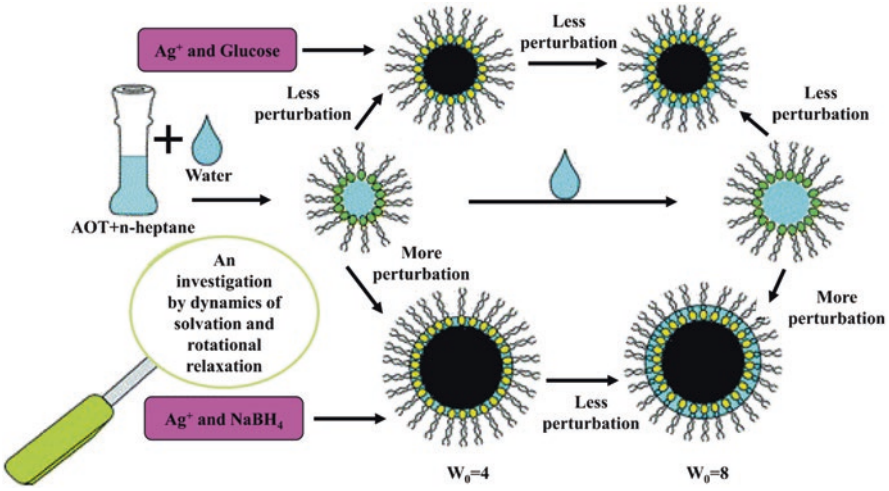

Fig. 2 Effect of the different reducing agents (glucose and sodium tetrahydroboride) on the size of silver nanoparticle synthesized in bis(2-ethylhexyl) sulfosuccinate sodium salt/n-heptane system. Sodium tetrahydroboride thus produces bigger size of silver nanoparticles, which causes more perturbation to reverse micelles. Chaurasia and Hebbar, Chap. 5

separation of biomolecules (Fig. 2). Chapter 6 by Paroha et al. reviews nanotechnology applications for the efficient delivery of coenzyme Q10, a health supplement.

The use of enzymatic nanosensor for detection of contaminants in food, water and agriculture is presented in Chap. 7 by Verma. Health supplements and nutraceuticals can be developed by nano-co-polymerization of natural products with

polyethylene glycol and polydimethylsiloxane, as reviewed by Pandey et al. in Chap. 8. Biofuel production from waste using nanotechnology is then discussed by Srivastava et al. in Chap. 9. Arsenic remediation by nanotechnologies, with emphasis on iron oxide nanomaterials, is presented by Paroda et al. in the last chapter.

Thanks for reading

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Contents

About the Editors

Shivendu Ranjan is currently working as DBT-research fellow, Department of Biotechnology, Ministry of Science and Technology, Govt. of India at VIT University, Vellore, Tamil Nadu, India. He is also serving for a nongovernment organization as an honorary director, Research Wing, Veer Kunwar Singh Memorial Trust, Chapra, Bihar, India. He is the founder-director at Xpert Arena Technological Services Pvt. Ltd., India (www.xpertarena.com); this company is dedicated to serve in online and offline sectors with a vision to simplify the education. He has also founded and drafted the concept for first edition of *VIT Bio Summit* in 2012 and the same has been continued till date by the university.

His area of research is multidisciplinary which includes, but not limited to, nano-food technology, nano-agri technology, nanobiotechnology, nano-toxicology, natural products technology, natural products chemistry, bio-business, food chemistry and food engineering. He has published many scientific articles in international peer-reviewed journals and also served as editorial board member and referee for reputed international peer-reviewed journals. He has bagged several awards from different organizations, e.g. best poster award, achiever award, research award, young researcher award, etc.

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She has published many scientific articles in international peer-reviewed journals and also served as editorial board member and referee for reputed international peer-reviewed journals. She has received Elsevier Certificate for "Outstanding Contribution" in reviewing from Elsevier, the Netherlands. She has also been nominated for Elsevier advisory panel for Elsevier, the Netherlands. She was the guest editor in *Journal of Chemistry* for the special issue entitled "Food Nanotechnology Opportunities and Challenges". She has received several awards from different organizations, e.g. best poster award, young researcher award, special achiever award, research award, etc.

Eric Lichtfouse, 56, PhD in organic chemistry at Strasbourg University, is a geochemist designing new materials and techniques to increase soil carbon sequestration at the French National Institute for Agricultural Research (INRA). He has invented the ^{13}C-dating method[1] allowing to measure the dynamics of soil organic molecules[2], thus opening the field of molecular-level investigations of soil carbon sequestration. Chief editor of the awarded journal *Agronomy for Sustainable Development*[3], he has raised the journal rank from 29/53 in 2003, with an impact factor of 0.56, to 2/81 in 2014, with an impact factor of 3.99, in the agronomy category. He is also chief editor and founder of the journal *Environmental Chemistry Letters*[4] and the book series *Sustainable Agriculture Reviews*[5]. He is lecturing scientific writing and communication in universities worldwide[6]. His publication assistance service at the INRA has founded the French-English newsletter *Publier La*

[1] http://dx.doi.org/10.1007/s10311-011-0334-2

[2] http://archive.sciencewatch.com/inter/jou/2010/10novAgrSusDev

[3] http://www.springer.com/journal/13593

[4] http://www.springer.com/journal/10311

[5] http://www.springer.com/series/8380

[6] http://fr.slideshare.net/lichtfouse/scientific-writing-and-communication, https://www.youtube.com/playlist?list=PLKEz5Pbi4p3By53Q0gclKPeSBTK2HJGK_

Science[7]. He has published the book *Scientific Writing for Impact Factor Journal*[8]. This textbook describes in particular the micro-article[9], a new tool to identify the novelty of experimental results. Further details are available on Slideshare[10], LinkedIn[11], ResearchGate[12], ResearcherID[13] and Orcid[14].

[7] http://www6.inra.fr/caps-publierlascience

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Chapter 1
Active Nanocomposites in Food Contact Materials

Marina Ramos, Alfonso Jiménez, and María Carmen Garrigós

Abstract The traditional application of food packaging materials as mere containers without food interaction has changed to the new concept of active packaging, where interactions between food, packaging materials and the environment increase food quality and shelf-life. This concept takes also into consideration the consumer safety and the nutritional characteristics of food. This has led to the design of active nanocomposites that improve the structural integrity and barrier properties of the packaging materials by the addition of nanomaterials such as nanoclays or metal nanoparticles, and that also increase their antimicrobial and antioxidant performance sing active additives and nanofillers. Here we review active nanocomposites in food packaging. We discuss the relevance, advantages, and limitations of active nanocomposites with respect to their safety and migration regulations.

Keywords Nanocomposites • Active packaging • Biodegradable and bio-based polymers • Nanofillers • Metallic nanoparticles • Nanoclays • Active nanocomposites • Antimicrobial activity • Antioxidant activity

1.1 Introduction

Packaging sector represents one of the main production sectors worldwide of plastic industries and have experienced a continuous growth by widening their portfolio and applications window. Plastics in the area of food packaging are adequate to maintain the quality and safety of food products from their processing to consumption, including storage and transportation. Packaging materials should provide mechanical, optical, and thermal protection while preventing unfavorable degradation factors or conditions, such as spoilage microorganisms, oxygen, moisture, chemical contaminants, light, external forces, high temperatures, etc. (Rhim et al. 2013a, b).

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The conventional polymers such as petroleum-based thermoplastics which have been extensively used since their introduction in the 1950–1960 decades due to exhibit a range of properties that makes them suitable for specific food applications, such as their large availability at relatively low cost and good physico-chemical performance in tensile and tear strength, good barrier to oxygen, water vapor, CO_2 and aromas, good processing capabilities, such as heat sealability, good aesthetic quality and so on (Siracusa et al. 2008). However novel bio-based and biodegradable plastics for food packaging applications have appeared to be consider a key enabler of innovation and alternative to permit a significant reduction in the environmental impact of packaging materials by reducing the waste disposal problems and alleviate the overdependence from petroleum and other non-renewable sources (Shameli et al. 2010; Rhim et al. 2013a, b). The raising trends for the industrial use of environmentally-friendly materials, such as poly(lactic acid) or thermoplastic starch, represent an interesting alternative to polymers derived from petroleum due to its renewable origin, biodegradability and biocompatibility (Raquez et al. 2013). Moreover, the idea of improving the quality and extending food shelf-life while maintaining their natural properties and conditions is gaining the interest of researchers and food industries.

The traditional concept for food packaging materials as mere containers with no interaction with food to avoid contamination has evolved by the use of other additives, permitting the interactions between food, packaging and the environment. This new concept is called active packaging, and it is associated to take into account the process of mass transfer and interaction between food and packaging materials without forget the consumer safety (Singh et al. 2011; Sanches-Silva et al. 2014). What the active packaging pretends is the addition of antimicrobial or antioxidant additives to extend the shelf-life of food products by limiting their spoilage effects caused by microorganisms or their lipid oxidation respectively (Valdes et al. 2015). Ramos et al. reported a study based on poly(lactic acid) with 8 wt% of thymol and organo-modified montmorillonite as active additive with antioxidant action for food packaging (Ramos et al. 2014b). The increasing demand for natural additives has resulted in studies based on natural active compounds, such as plant extracts or essential oils, which are categorized as Generally Recognized as Safe by the US Food and Drug Administration as well as the current European Legislation for materials intended to be in contact with food (Ramos et al. 2012).

Nanotechnology has been used in the last decade into a multidisciplinary field of applied science and technology, representing a revolution in many concepts in materials science. The novel properties and functions provided by nanomaterials has been considered to be used in food packaging by either improving key properties such as flexibility, gas barrier properties, temperature, moisture or thermal stability (Priolo et al. 2011; Ramos et al. 2014b) or by addition of nanoparticles with antimicrobial/antioxidant properties (Jin and He 2011; Fortunati et al. 2014; Ramos et al. 2014a, b). Copper, zinc, titanium, gold and silver nanoparticles and some of their metallic oxides have been proposed as active additives to extend food shelf-life and to provide affordable and safe innovative strategies to develop novel active nanocomposites (Llorens et al. 2012). Fukushima et al. reported that the highest thermo-mechanical

and mechanical improvements in PLA matrices were obtained upon the addition of 10 wt% of nanoclay, and they are associated with the good dispersion level observed by using wide angle X-ray scattering (WAXS) and to the high clay content (Fukushima et al. 2013).

The nanotechnology in combination with active packaging in food packaging has emerged a technologies that link the potential advantages of both technologies due to what these are achieved is to improve the structural integrity and barrier properties imparted by the addition of nanomaterials (either nanoclays or metal nanoparticles), and the increase in antimicrobial and/or antioxidant properties in most cases by the action of active additive and/or the own nanofiller.

This review contains an overview of the new advances and challenges in the food packaging sector focused on the use of active additives and nanofillers in novel active nanocomposites that are related to the extension of shelf life of packaged food with maintaining food safety and quality.

1.2 Food Packaging

Plastic industries are one of the main production sectors worldwide and have experienced a continuous growth by widening their portfolio and applications window. This increase in production has reflected in numerous important sectors of the World economy. In general terms, the plastics sector can be consider a key enabler of innovation of many products and technologies in important sectors of the global economy, such as healthcare, energy generation, aerospace, automotive, maritime, construction, electronics, packaging or textile.

With a continuous growth for the last decades, global plastic production reached 299 million tonnes in 2013, with a 3.9% increase compared to 2012 (Plastics-Europe 2015). This increase has been mostly observed in emerging countries, but it was not the case in the most developed economies. In particular, packaging sector represents 39.6% of the total plastics demand and almost half of them are used for food packaging in form of films, sheets, bottles, cups, tubs, or trays; followed by building and construction with 20.3% of the total European demand. Automotive is the third sector with a share of 8.5% and the rest of applications comprises a total of 31.6% of the European plastics demand (Plastics-Europe 2015).

Plastics are currently considered one of the four basic materials for food packaging, together with glass, metal or paper. Plastics are adequate to satisfy the main function for food packaging, i.e. to maintain the quality and safety of food products from their processing to consumption, including storage and transportation. Packaging materials should provide mechanical, optical, and thermal protection while preventing unfavorable degradation factors or conditions, such as spoilage microorganisms, oxygen, moisture, chemical contaminants, light, external forces, high temperatures, etc (Rhim et al. 2013a, b).

Table 1.1 General properties such as density, melting point, glass transition temperature, elastic modulus and elongation at break (general properties) of some common thermoplastics used in food packaging, where the general trends in thermoplastics physico-chemical properties can be noticed

	Low density polyethylene	High density polyethylene	Poly-propylene	Poly-styrene	Polyethylene terephthalate	Poly-amide
ρ (g cm^{-3})	0.92–0.94	0.94–0.95	0.90–0.91	1.04–1.12	1.37	1.05–1.14
T_m (°C)	120	137	168	250	256–260	185–260
T_g (°C)	−45 to −15	−45 to −15	−32 to −2	80–100	67–81	37–70
E (GPa)	0.15–0.34	0.98	1.1–1.6	2.7–3.4	3.5	0.7–0.98
ε_B (%)	300–900	20–50	200–1000	2–3	70	200–300

Density (ρ)
Melting temperature (T_m)
Glass transition temperature (T_g)
Elastic Modulus (E) at 25 °C, 65% relative humidity (RH)
Elongation at break (ε_B) at 20–25 °C and 65% RH
Adapted from Bastarrachea et al. (2011)

1.2.1 From Conventional Polymers to Bioplastics

Among the basic food packaging materials, petroleum-based thermoplastics have been extensively used since their introduction in the 1950–1960 decades. They exhibit a range of properties that makes them suitable for specific food applications, such as their large availability at relatively low cost and good physico-chemical performance in tensile and tear strength, good barrier to oxygen, water vapor, CO_2 and aromas, good processing capabilities, such as heat sealability, good aesthetic quality and so on (Siracusa et al. 2008).

The most important thermoplastics used in food packaging include low density polyethylene (LDPE), high density polyethylene (HDPE), polypropylene (PP), polystyrene (PS), polyamides (PA) and poly(ethylene terephthalate) (PET), which exhibit many common properties ideal for their use in packaging, such as light weight, low processing temperature (compared to metal and glass), variable barrier properties, good printability, heat sealability and ease of conversion into different forms (Lim et al. 2008). Table 1.1 shows some properties: (density, ρ; melting and glass transition temperature, T_m and T_g respectively; elastic modulus, E; and elongation at break, ε_B) of the main thermoplastic polymers with interest in food packaging.

There are some key properties that make thermoplastics one of the most favorable material families in food packaging applications. For example, thermal properties provide important information about criteria to be considered during processing and their possible resistance to high temperatures. Mechanical properties provide data on the ability of packaging materials to sustain their integrity under the influence of various stresses occurring during processing, handling, and storage of packaged food. Other important parameters, such as permeability to oxygen and water vapor, should be also considered and controlled to select the most adequate

polymer or composite materials for foodstuff with particular requirements (Bastarrachea et al. 2011).

But, despite the multiple advantages provided by conventional thermoplastics in their application to food packaging, the raising societal concerns about environmental issues and the most recent polices implemented by authorities at the international level have introduced some caution on their massive use and uncontrolled disposal. For instance, in 2012, according to the Association of Plastics Manufacturers in Europe, 25.2 million tonnes of post-consumer plastics ended up in the waste upstream, of which 62% was recovered through recycling and energy recovery processes while 38% still went to landfills (Plastics-Europe 2015). Non-biodegradable conventional thermoplastics cause a serious environmental problem since they are not easily degraded in nature taking centuries to be decomposed into their simple constituents and absorbed by the environment. This problem has become a general global concern, in particular from the beginning of the XXI century, since environmental problems, such as the climate change, CO_2 footprint and the noticeable shortage in fossil resources have accelerated the search for better concepts and sustainable alternatives to conventional plastics for packaging. The reuse and plastics recycling is one of the most advanced alternatives to disposal, but other possibilities are being studied for immediate implementation in massive production.

Novel bio-based and biodegradable plastics for food packaging applications should be developed by strictly following the guidelines for the efficient use of natural and renewable resources while keeping the properties of conventional thermoplastics to preserve food quality and safety for consumer and reducing waste disposal and CO_2 footprint by offering new recovery options. In general terms, novel bio-based and biodegradable plastics should be obtained and modified from raw materials with biological origin, or more precisely from renewable resources to permit a significant reduction in the environmental impact of packaging materials by reducing the waste disposal problems and alleviate the overdependence from petroleum and other non-renewable sources (Rhim et al. 2013a, b). In addition, they should be able to be treated by various recycling and recovery techniques to get waste streams easy for treating and versatile enough to get efficient processes such as composting, bio-refineries and so on.

According to European Bioplastic Association, bioplastics are plastics that are bio-based, biodegradable or both. There are three main groups of bioplastics based on their renewable/non-renewable origin and biodegradable/non-biodegradable character and it is shown in Fig. 1.1; (i) bio-based or partly bio-based, non-biodegradable plastics such as Bio-PE, Bio-PET and Bio-PA. These materials are characterized by their bio-based origin (most of them are obtained from sugar-rich fractions in the bio-ethanol production) and their properties are similar to those of the main commodities used in food packaging, such as polyolefins. However, they are non-biodegradable and their waste disposal is similar to the conventional thermoplastics; (ii) biodegradable plastics based on fossil resources, such as poly(butyrate adipate terephthalate) copolymer (PBAT) or poly(ε-caprolactone) (PCL). These biopolymers degrade fast under environmental conditions, but are obtained from petroleum. They show in general good properties for food packaging

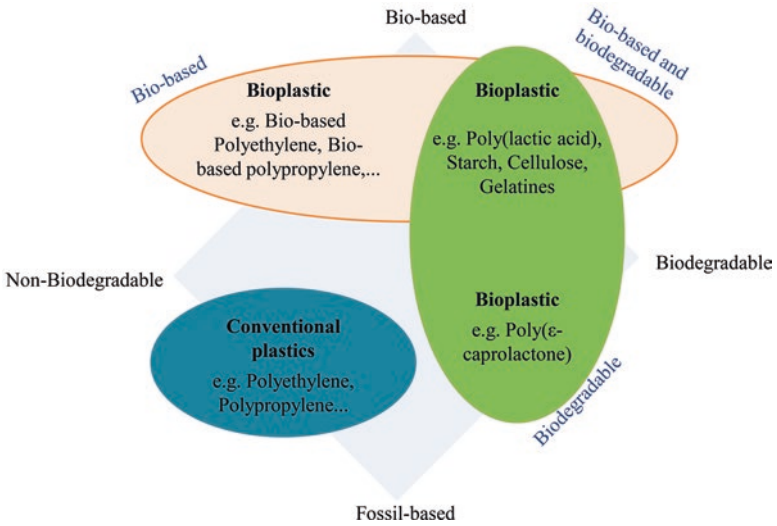

Fig. 1.1 Classification of plastics based on their renewable/non-renewable origin and biodegradable/non-biodegradable character

but their production costs are high and they are not yet competitive with conventional thermoplastics; and (iii) bio-based and biodegradable plastics, including poly(lactic acid) (PLA), cellulose, starch-based plastics, animal proteins and poly(hydroxyalkanoates) (PHA). These biopolymers are the ideal solution from the environmental point of view, but most of them fail in some of the main properties in food packaging, such as thermal resistance and barrier effect, making necessary their modification with additives to improve these characteristics.

Table 1.2 summarizes the most important commercial bio-based and biodegradable polymers and their main properties. We have focused our interest in this group by their high potential in becoming a sustainable alternative to conventional thermoplastics. Some of them are already produced at the industrial scale, such as poly(lactic acid), poly(hydroxyalkanoates) or starch-based polymers as shown in Fig. 1.2.

The current global production capacities of bioplastics have increased continuously and amounted to about 1.6 million tonnes in 2013 with almost 40% of the production destined for the packaging market which it is the current largest market segment within the bioplastics industry (Reddy et al. 2013; European-Bioplastics-Association 2015). This continuous growth in the bioplastics production for food packaging is also possible by the similar machinery and processing conditions to those traditionally used with conventional thermoplastics. Indeed, no special machinery is required for the processing of bioplastics, with just some changes in the processing parameters depending on the type of bioplastic.

In conclusion, all types of bioplastics used in the formulation of packaging materials offer one outstanding advantage over fossil-based products, as it is the use of renewable resources and the consequent intrinsic value increase in reducing the

Table 1.2 Physical data of some commercial bioplastics, where the main mechanical, thermal and structural properties can be observed

	Poly(lactic acid)	Polyhydroxybutyrate	Poly(ε-caprolactone)	Thermoplastic starch	Cellulose
T_m (°C)	130–180	140–180	59–64	110–115	–
T_g (°C)	40–70	0–5	−60	−20–43	–
E (MPa)	2050–3500	3500	390–470	400–1000	3000–5000
ε_B (%)	30–240	5–8	700–1000	580–820	18–55
TS (MPa)	48–53	25–40	4–28	100	100

Melting temperature (T_m)
Glass transition temperature (T_g)
Elastic Modulus (E) at 25 °C, 65% relative humidity (RH)
Elongation at break (ε_B) at 20–25 °C and 65% RH
Tensile strength (TS)
Adapted from Jamshidian et al. (2010)

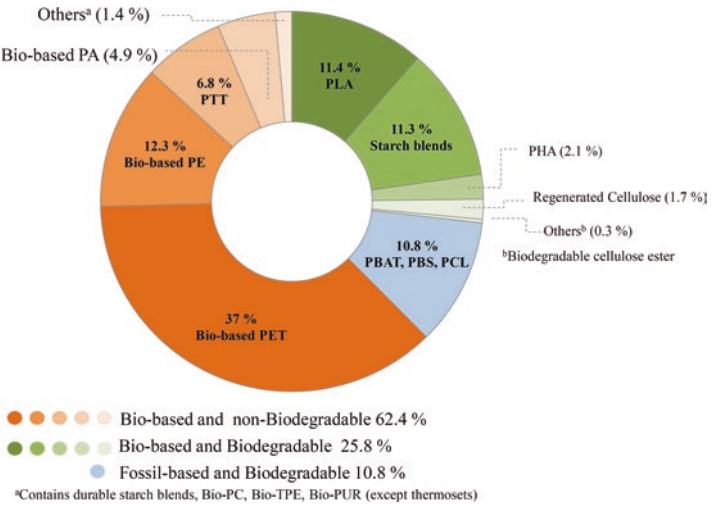

Fig. 1.2 Global production of bioplastics in 2013 (Adapted from European-Bioplastics-Association)**.** *PHA* poly(hydroxyalkanoates), *PET* polyethylene terephthalate, *PBAT* poly(butylene adipate-co-terephthalate), *PBS* polybutylene succinate, *PCL* poly(ε-caprolactone), *PLA* poly(lactic acid), *PTT* polytrimethylene terephthalate, *PE* polyethylene

environmental impact of packaging materials. Nevertheless, the use of bioplastics is still hampered by both, some poor properties and high production costs in comparison to conventional fossil-based plastics. The main challenge in the bioplastics research would be the improvement in properties without any further increases in the market price.

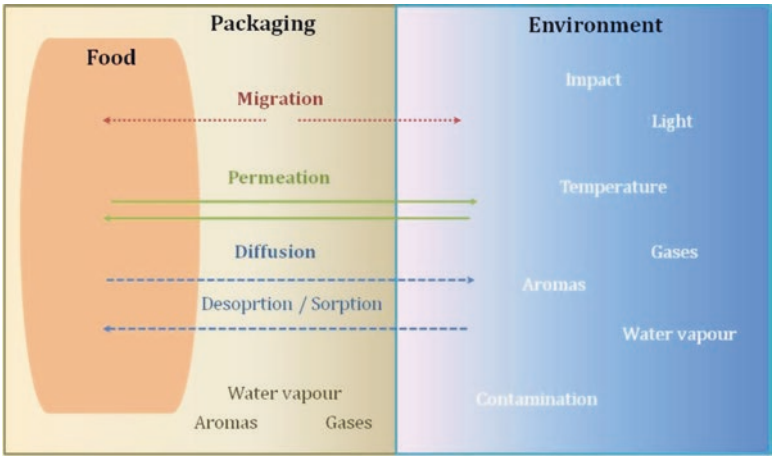

Fig. 1.3 Interaction processes between food packaging materials and the environment where the main transport phenomena in these three parts can be observed

1.2.2 Active Packaging

The traditional concept for food packaging materials as mere containers with no interaction with food to avoid contamination (i.e. passive packaging) has been changed in most cases by the use of other formulations where interactions have been permitted, promoted by the new concepts: *active and intelligent packaging*. In fact, research in new developments in food packaging materials have been aimed by the increase in consumer's preferences for fresh, natural, healthy and easy-to-prepare food, minimally processed and convenient from the nutritional point of view. Consequently, the food sector has experienced important changes aimed to meet the increasing consumer's demands while adapting to the global market, which implies an increase in time of food transport and distribution (Singh et al. 2011; Sanches-Silva et al. 2014).

The concept of active packaging and the developments associated to this change in paradigms for food packaging producers and consumers have to take into account the process of mass transfer and interaction between food and packaging materials (Fig. 1.3). This interaction could be divided into different individual processes, which could appear all together or be partially restricted in each particular case. These processes include migration of the material components (monomers or additives) to foodstuff, sorption and desorption of volatile compounds (flavours and aromas), changes in the food moisture content, permeability to gases and the possible degradation of food by external conditions (Silva-Weiss et al. 2013). All these physico-chemical processes should be carefully studied to design and develop new packaging systems that leave behind the simple and conventional passive container.

Active packaging includes the use of additives, which could be defined as 'freshness enhancers', able to diffuse and interact with food by increasing the packaging's functionalities, such as resistance to oxidation and microbiological spoilage or retention of natural aromas and flavors as well as the food quality and safety (López-Gómez et al. 2009).

The chemical nature of additives used is diverse, but all of them should play their role efficiently by scavenging or absorbing oxygen, CO_2, ethylene, moisture and/or odour and flavour taints; releasing oxygen, CO_2, water vapor, ethanol, sorbates, antioxidants and/or other preservatives and antimicrobials (antimicrobials); and/or maintaining temperature control to avoid the food overheating during transport and distribution. New developments in active packaging materials, methods and effects on food are under study in the last few years. These studies are based on chemical, physical or biological actions to modify and control the interactions between materials, food and the packaging headspace to achieve certain desired outcome (Gómez-Estaca et al. 2014; Mellinas et al. 2015; Valdes et al. 2015).

1.2.2.1 Antimicrobial and Antioxidant Active Packaging

Antimicrobial active packaging is focused on the reduction of the risk from pathogens to food and extension of shelf-life by limiting their spoilage effects caused by microorganisms. A wide range of agents with antimicrobial characteristics has been proposed, e.g. organic acids, bacteriocins, spice extracts, thiosulphates, enzymes, proteins, isothiocyanates, antibiotics, fungicides, chelating agents, parabens and metals (Sung et al. 2013). All of them could be incorporated into or coated onto food packaging materials to get the desired effect (Singh et al. 2011). However, some important features should be considered to design an efficient antimicrobial packaging: (i) development of system able to get the controlled release of the antimicrobial agent from the polymer film in the adequate time to maximize efficiency. The fast release of the antimicrobial agent from the packaging material to food is quite common and this is an important drawback of these systems, since the effect of the active compound is limited in time; and (ii) the use of harmless substances to food and not all the proposed antimicrobial agents are currently included in the current legislation, as chemicals intended to be in direct contact with food.

Antioxidant active packaging focuses on the improvement of the resistance to oxidation of lipids retarding the natural processes that can lead to organoleptic deterioration and reduction of shelf-life of food products. The use of active packaging materials with antioxidant properties is relevant in many types of food, but particularly for dried products and oxygen sensitive food (Gómez-Estaca et al. 2014). Moreover, some authors related that the addition of natural antioxidants to polymer matrices can protect the polymer from degradation during the processing, representing the double effect of protection for the material and food through controlled release mechanisms (Peltzer et al. 2010; Wu et al. 2014).

Both types of active food packaging systems can be divided into two main groups. This classification is based on the incorporation of the active additive to the

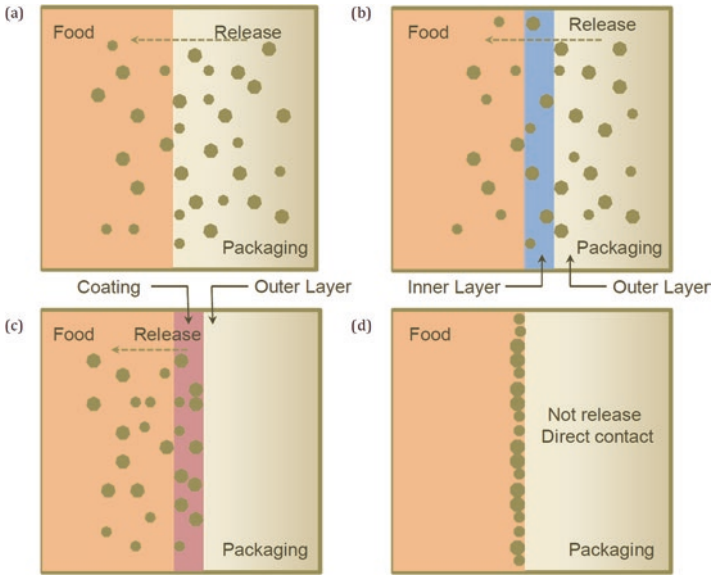

Fig. 1.4 Release of active substance in different applications of active packaging systems: films that allow the release of the active additive (**a**), (**b**) and (**c**); films that do not release the active additive (**d**) (Adapted from Bastarrachea et al. 2011)

packaging material and the interaction between the agent and foodstuff (Bastarrachea et al. 2011). Most of them are based on the use of thin films of the polymer where the active agent is embedded:

(i) Films that allow the release of the active additive by following a particular kinetic scheme. The antimicrobial or antioxidant agent can be incorporated to the material either within the matrix or onto the surface of the food packaging material. The incorporation strategy is relevant to control the release of active compounds from the material to the food surface to perform their specific action. Different approaches have been proposed:

   (a) Packaging systems which incorporate the active additive in a single layer, permitting a gradual release into food.
   (b) Packaging systems with an inner layer which can be useful in controlling the release rate of the antimicrobial and/or antioxidant compounds from the outer layer.
   (c) Packaging systems with a coating layer containing the active additive.

(ii) Films that do not release the active additive but there are a direct contact with food inhibiting the microbial growth or lipid oxidation on the food surface.

Figure 1.4 shows the scheme of these different interaction mechanisms between the active agent, packaging material and food.

All these systems for the incorporation of the active agents to the packaging material and further interaction with food have shown their potential in active systems, but some drawbacks related to the complete control of the release kinetics of the antimicrobial and/or antioxidant compounds to food have been described (Anbinder et al. 2015; Fuciños et al. 2015). Recent studies have proposed the use of different strategies for the incorporation of active compounds to the packaging material permitting a more efficient release to food. These new techniques are encapsulation which it is a process by which small particles of core materials are packed within a wall material to form capsules to protect bioactive compounds from adverse environment and also for the controlled release at targeted sites (Marques 2010; Ezhilarasi et al. 2013; Dias et al. 2014; Noronha et al. 2014; Wen et al. 2016), grafting which is one of the most promising method to functionalize the polymeric materials (Schreiber et al. 2013) and reinforcement with nanofillers some of them with intrinsic antimicrobial properties, in particular metallic nanoparticles (Llorens et al. 2012; Fortunati et al. 2013, 2014; Dias et al. 2014; Shankar et al. 2015).

The introduction of bioplastics to the packaging markets has permitted the design of systems where the active agents are incorporated into biopolymer matrices, giving a surplus to these formulations combining activity and sustainability. The proposal for new bioplastics, such as poly(lactic acid) or edible films and coatings, to substitute conventional plastics in active formulations has been recently reviewed (Rhim et al. 2013a, b; Mellinas et al. 2015) and many formulations have been proposed. Some of the most important of them are discussed below.

Manzanarez-López et al. reported a study based on poly(lactic acid) with 2.58 wt% of α-tocopherol as active additive with antioxidant action for food packaging (Manzanarez-López et al. 2011). The main optical and thermal properties were evaluated as well as the kinetics of diffusion of the active agent from the poly(lactic acid) matrix to ethanol and vegetable oil as food simulants. Their results showed a slower diffusion of α-tocopherol to soybean oil than to ethanol with 5.1 and 12.9% of release respectively after 60 days. Authors also studied the influence of temperature in the release kinetics by testing their systems at temperatures between 20 and 40 °C. The release of α-tocopherol from poly(lactic acid) films to soybean oil was enough to delay the oxidation of this oil stored at 20 and 30 °C, compared with the oil put in contact with pure poly(lactic acid) films with no active agent in their composition. Jamshidian et al. used solvent casting processing to obtain films based on poly(lactic acid) with natural antioxidants, including α-tocopherol, and synthetic phenolic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate or tert-butylhydroquinone (TBHQ) ((Jamshidian et al. 2013). They studied the release of all these antioxidants to different food simulants and calculated the kinetic coefficients. It was concluded that antioxidant active packaging was adequate for foodstuff protection and α-tocopherol can be used as a natural additive.

Wu et al. evaluated the antimicrobial activity of films based on poly(lactic acid) combined with poly(ε-caprolactone) and thymol as active agent (Wu et al. 2014). Results showed that the addition of thymol to the biopolymer matrix had a plasticizing effect by the decrease in the T_g values and poly(lactic acid) crystallinity, but it did not affect the thermal stability of films. The antimicrobial activity was also

evaluated, and they reported that films with thymol showed inhibition against two foodborne bacteria: *Escherichia coli* and *Listeria monocytogenes*.

Polysaccharides, lipids, proteins or their blends can be used as edible biopolymer matrices in active food packaging applications (Rhim et al. 2013a, b). Indeed, chitosan was proposed as edible matrix on bioactive coatings containing organic acids and nanoemulsions with carvacrol, mandarin, bergamot and lemon essential oils (Cruz-Romero et al. 2013). Gamma irradiation and modified atmosphere packaging were proposed as adequate techniques to increase efficiency of the active films in selected food (Severino et al. 2015). These authors confirmed the strong antimicrobial activity of carvacrol nanoemulsions against two Gram-negative pathogenic bacteria (*Escherichia coli* and *Salmonella typhimurium*). Authors stated that the bioactive coating with carvacrol onto the chitosan matrix resulted satisfactory in the total inhibition of these bacteria after 11 days of storage, highlighting the strong bactericidal effect of this coating.

The effects of the addition of butylated hydroxytoluene and green tea extracts on the physical, barrier, mechanical, thermal and antioxidant properties of potato starch films were reported by u Nisa et al. (2015). The antioxidant properties of these bioactive composites were evaluated by using the spectrophotometric method with formation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) complex. The DPPH method permitted the determination of the radical scavenging ability of both butylated hydroxytoluene and green tea extracts starch films after their contact with fatty food simulant (95% ethanol). The formation of metmyoglobin was monitored while the lipid oxidation was evaluated by using the thiobarbituric acid reactive substances (TBARS) method. Green tea extracts and butylated hydroxytoluene films were individually applied to fresh beef samples stored at 4 °C and room temperature for 10 days. It was concluded that the addition of butylated hydroxytoluene and green tea extracts resulted in the decrease in the concentration of metmyoglobin and thiobarbituric acid reactive substances values.

In conclusion, several types of active packaging with antimicrobial and/or antioxidant performance can be developed to decrease microbial proliferation and oxidation reactions, particularly in lipidic food. These effects would improve the final properties of food, particularly shelf-life and quality. The addition of antioxidant or antimicrobials active additives and the use of biopolymers have increased their use and their potential applications, representing a new kind of materials with clear possibilities of application in active food packaging.

1.2.3 Natural Additives

The selection of the most adequate natural compounds to be used as antioxidant or antimicrobial additives in active packaging formulations depends primarily on their activity against the targeted microorganisms and compatibility with the packaged food, while their continued release during storage and distribution is necessary to extend food shelf-life and quality (Manzanarez-López et al. 2011). But many other

Table 1.3 Potential additives from natural sources used in food packaging where the main current chemicals can be observed

Additives of plant origin
Plant-derived compounds:Phenolic compounds, quinines, saponins, flavonoids, tannins, coumarins, terpenoids and alkaloids
Essential oils
Plant extracts
Plant-by products
Fruit pomace, seeds, peels, pulps, unused flesh and husks

factors need to be considered in the design of active packaging systems, such as their specific activity, resistance of microorganisms to the additives action, release kinetics and mechanisms, storage and distribution conditions, physical and mechanical properties of the packaging materials and, organoleptic characteristics of food (Gómez-Estaca et al. 2014). All these factors should be carefully considered in agreement with requirements stated in legal regulations (Dainelli et al. 2008).

All these considerations have led to a search for natural additives to be used in active packaging formulations in substitution of synthetic additives. Many studies have been performed to propose the use of compounds obtained from natural sources with antimicrobial and/or antioxidant character (Srinivasan 2012; Silva-Weiss et al. 2013; Sung et al. 2013; Gómez-Estaca et al. 2014; Gyawali and Ibrahim 2014; Valdés et al. 2014, 2015).

Natural additives can be obtained from different sources including plants, animals, bacteria, algae and fungi or by-products generated from fruits and vegetables processing. Table 1.3 summarizes some of the potential additives obtained from plant origin proposed for their use in food packaging applications.

Essential oils are some of the mostly studied active agents obtained from plants, since they are fully renewable additives, easy to extract and highly efficient in their antimicrobial and/or antioxidant character. For example, the essential oil of *Mosla chinensis Maxim* and its methanol extract were studied to evaluate their antioxidant activity and their antimicrobial effect against eight bacterial and nine fungal strains (Cao et al. 2009). Results showed that this essential oil, whose main components are carvacrol (57%), p-cymene (14%), thymol acetate (13%), thymol (7%) and c-terpinene (2%) exhibited great potential against microorganisms, in particular against two Gram-positive bacteria ever-present in many food products, *Staphylococcus aureus* and *Listeria monocytogenes*. Moreover, high antioxidant activity was also reported for this essential oil.

In general terms, essential oils are rich in monoterpenes, sesquiterpenes, esters, aldehydes, ketones, acids, flavonoids and polyphenols (Ćavar Zeljković and Maksimović 2015). All these chemicals have demonstrated their antioxidant/antimicrobial character. Figure 1.5 summarizes names and molecular composition of some of the main bioactive compounds that can be obtained from plants, in particular essential oils and extracts. These compounds have been recently incorporated into or coated onto packaging films and their performance as active additives has

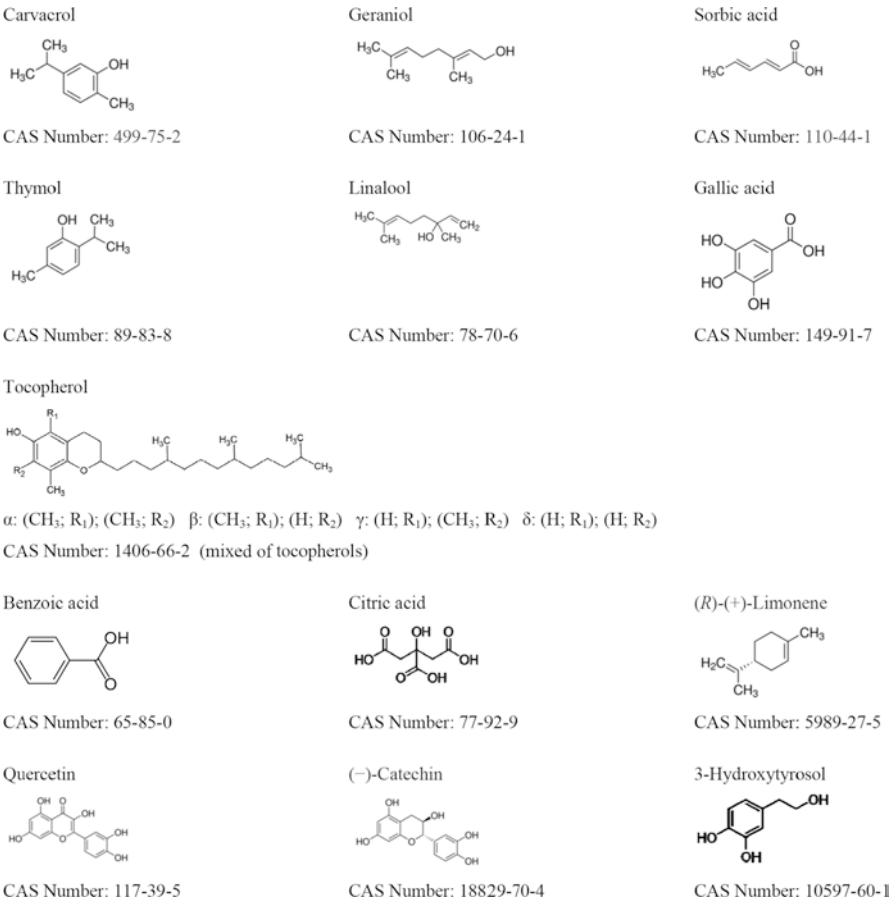

Fig. 1.5 Chemical structures of some natural additives incorporated in active food packaging with antioxidant/antimicrobial character

been assessed. Li et al. added different natural additives with antioxidants properties, obtained from green tea, grape seeds, ginger and gingko leaf, into gelatin-based films with good results in inhibition of the oxidation of selected food (Li et al. 2014). Sánchez-Aldana et al. used lime essential oil as antimicrobial agent on edible films with high activity against foodborne pathogenic bacteria (*Escherichia coli* O157:H7, *Salmonella typhimurium*, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*) determined by the agar disc diffusion method (Sánchez Aldana et al. 2015).

Poly(ε-caprolactone) with α-tocopherol incorporated by nanoencapsulation technique (30, 50 and 70 wt%) was proposed in the production of biodegradable and antioxidant films based on methylcellulose (Noronha et al. 2014). Films were obtained by solvent casting and their mechanical and optical properties were determined together with their antioxidant performance and release kinetics. The incor-

poration of α-tocopherol to poly(ε-caprolactone) films produced a modification of their mechanical properties, decreasing their tensile strength around 60% when the nanocapsules percentage was high (70 wt%) and the elastic modulus decreasing around 70%. The films showed high antioxidant character by the incorporation of nanocapsules to permit the controlled release of α-tocopherol to food simulants.

Different natural additives have been selected to develop new active packaging systems in order to improve the final properties of food, since they have known antioxidant and antimicrobial properties. The main challenge to be overcome for a massive application of these chemicals obtained from natural sources is the need of improvement in extraction yields with higher efficiencies and lower costs than the current processes.

1.2.4 Antimicrobial Activity of Essential Oils

The bacterial susceptibility to essential oils and their extracts increases with the reduction in pH of food, since at low pHs the hydrophobic character of the oil increases, resulting in easier dissolution of the cell membranes of the target bacteria (Burt 2004). In addition, the most common bacteria, in particular pathogens, have lower reproduction rate at low pHs. The mechanism of action of essential oils against bacteria is not clear yet, since each compound present in the essential oil composition exhibits a unique mechanism of action that is specific to a particular range of food and microorganisms (Bastarrachea et al. 2011). Different mechanism have been identified: damage to the cell wall, interaction with and disruption of the cytoplasmic membrane, damage of membrane proteins, leakage of cellular components, coagulation of cytoplasm and depletion of the proton motive force. All these effects produce the microorganisms death by the modification of the structure and composition of the bacteria cells (Tajkarimi et al. 2010; Kuorwel et al. 2011; Calo et al. 2015).

Two main types of bacterial cell wall structures have been studied, permitting their classification in Gram-positive and Gram-negative organisms (Fig. 1.6). Both types of cells have external cytoplasmic membranes but some details make the difference between them, such as the presence of a thin peptidoglycan layer in Gram-negative bacteria (Aldred et al. 2009). In consequence, Gram-negative should be more resistant to essential oil. These outer layers contain lipids, proteins and lipopolysaccharides in their composition, preventing the penetration of hydrophobic compounds, such as essential oil compounds (Feng et al. 2000; Maneerung et al. 2008).

It was reported that phenols, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids and alkaloids present in essential oils or plant extracts are responsible of their antimicrobial activity (Kuorwel et al. 2011; Sung et al. 2013). However, the total antimicrobial activity of essential oil cannot be attributed entirely to the mixture of their main components, since these complex matrices produce synergies between major and minor compounds to increase the antimicro-

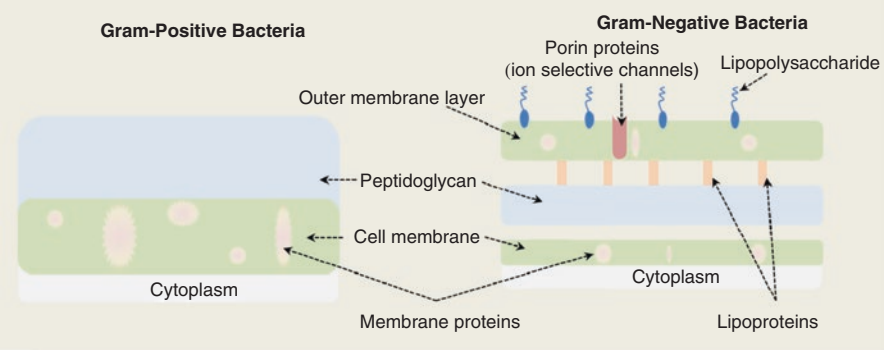

Fig. 1.6 The basic structure of bacterial cell wall structures of Gram-positive and Gram-negative bacteria which are two principal classes of pathogenic bacteria (Adapted from Aldred et al. 2009)

bial action (Sánchez-González et al. 2011). Different authors have reported the effectiveness of essential oils to inhibit different pathogenic and spoilage microorganisms, including Gram-positive such as *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* bacteria; Gram-negative such as *Escherichia coli, Salmonella enteritidis, Salmonella choleraesuis, Yersinia enterocolitica* and *Pseudomonas aeruginosa;* yeasts, such as *Saccharomyces cerevisiae, Candida albicans, Debaryomyces hansenii*; and molds such as *Alternaria alternate, Aspergillus niger, Botrytis cinerae, Aspergillus flavus, Penicllium roqueforti* (Kuorwel et al. 2011).

1.2.5 Antioxidant Activity of Essential Oils

On the other hand, essential oils and their main compounds are some of the most important additives to avoid food degradation by lipid oxidation due to their high reactivity with peroxyl radicals. The mechanism of action of these natural antioxidants in lipid oxidation reactions is focused on phenols and other compounds with hydroxyl groups presents in the essential oil. Hydrogen atoms from phenol hydroxyl groups could react with the peroxyl radicals produced in the early stages of the oxidation mechanisms to yield stable phenoxyl radicals and, consequently, result in the termination of the lipid peroxidation chain reactions (Mastelic et al. 2008; Amorati et al. 2013). However, the antioxidant activity of these phenolic compounds depends on the electronic and steric effects of their ring, substituents and the strength of hydrogen-bonding interactions between the phenol and the solvent in the essential oil (Mastelic et al. 2008).

A large variety of testing methods have been proposed to evaluate the antioxidant activity of natural additives, either as pure compounds or plant extracts. Some of them are methods based on inhibited autoxidation studies, which are better followed by monitoring the kinetics of oxygen consumption and the hydroperoxides forma-

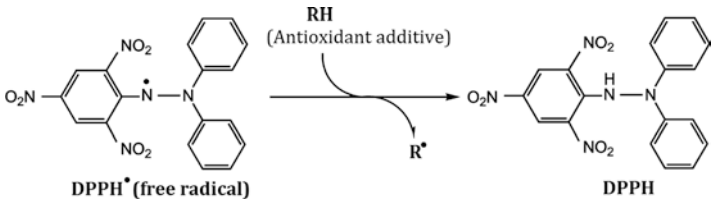

Fig. 1.7 Testing method proposed to evaluate the antioxidant activity of natural additives. Reaction is between the radical 2,2-diphenylpicrylhydrazyl (DPPH·) and antioxidant to form DPPH by which DPPH• free radical accepts hydrogen from an antioxidant

tion. The analytical determination of secondary oxidation products (e.g. carbonyl compounds) has been also used and it is the basic reaction of the majority of the current testing methods even though they do not involve substrate autoxidation. These methods can be direct, such as oxygen-radical antioxidant capacity (ORAC) or total oxidant scavenging capacity (TOSC). But the most common are the indirect methods, which are based on the reduction of persistent radicals, such as 2,2-diphenylpicrylhydrazyl (DPPH) and trolox-equivalent antioxidant capacity (TEAC) methods, or inorganic oxidizing species; e.g. such as ferric reducing antioxidant power (FRAP) and Folin-Ciocalteu methods (Sánchez-Moreno 2002; Amorati and Valgimigli 2015). 2,2-diphenylpicrylhydrazyl (DPPH) method is based on the theory that a hydrogen donor is an antioxidant and the antioxidant effect is proportional to the disappearance of DPPH• free radical in test samples. Figure 1.7 shows the mechanism by which DPPH• free radical accepts hydrogen from an antioxidant (Moon and Shibamoto 2009).

Barbosa-Pereira et al. based their studies in the evaluation of the antioxidant effectiveness of different commercial products containing natural antioxidants incorporated into LDPE matrices by their effect on the delay of lipid oxidation in salmon muscles (Barbosa-Pereira et al. 2013). The antioxidant activity of these films was tested using the DPPH method and the effect in salmon muscle was evaluated by using the TBARS method. Results demonstrated that the natural products used in this trial had important antioxidant effectiveness, being the mixture of natural tocopherols the natural product with the best results showing good possibilities to replace synthetic antioxidants in food packaging materials.

In conclusion, several types of antioxidant methods can be applied to determine the antioxidant activity of essential oils, demonstrating their potential to be used as active additives in bio-based and biodegradable formulations.

1.3 Nanotechnology in the Food Industry

Nanotechnology has developed in the last decade into a multidisciplinary field of applied science and technology, representing a revolution in many concepts in materials science. The novel properties and functions provided by nanomaterials and the

increasing possibilities to work at the nano-scale, between 1 and 100 nm, has resulted in advanced materials with a large number of potential applications, including food industry (Cushen et al. 2012). In this general context, nanofillers have gained some space as additives in food packaging materials by their action in improving some of their key properties, including mechanical and barrier performance.

There are many different terms to refer to nanofillers depending on their morphology, but the most accepted classification includes nanoparticles, nanofibrils, nanorods, nanocrystals and nanotubes (Zaman et al. 2014). In particular, nanoparticles are defined as discrete entities with their three dimensions in the nano-scale (lower than 100 nm). Nanoparticles show larger surface area, aspect ratio and higher number of surface atoms than their microscale counterparts (de Azeredo 2009). The research in the use of nanoparticles in food packaging materials has increased since their introduction has allowed creating, understanding, characterizing and using these compounds in material structures, devices and systems with novel and unseen properties and this is the main reason of their importance in food packaging applications (Cushen et al. 2012). In this area, nanotechnologies have offered innovation and technological advances all over the production chain; from primary production at the farming level, due to advances in pesticides efficiency and delivery, to processing and properties of the final food product to improve taste, colour, flavour, texture and consistency (Ezhilarasi et al. 2013; Mihindukulasuriya and Lim 2014). Other important features of the use of nanotechnologies in food industry include the increase in absorption and bioavailability of food and food ingredients (nutrients) by their nanoencapsulation. Different methods have been suggested to nanoencapsulate active principles into food packaging materials, such as spray drying and electrospinning showing promising results as novel delivery vehicles for supplementary food compounds working with an aqueous solutions at RT (Ghorani and Tucker 2015; Santos et al. 2015; Wen et al. 2016).

Table 1.4 shows some of the main nanofillers that have been studied for their incorporation into food contact materials to enhance their mechanical and barrier properties, to prevent their photodegradation and to preserve and extend the food shelf-life by their antimicrobial effect (Othman 2014).

1.3.1 Nanoclays

There are many commercial types of nanofillers that have been incorporated into polymer matrices to form nanocomposites. The first developments in this field focused in the use of layered silicates, also known as nanoclays. They typically have a stacked arrangement of silicate layers (nanoplatelets) with nanometric dimensions (de Azeredo 2013; Reddy et al. 2013).

The most common crystalline arrangement in layered silicates is called phyllosilicate structure, in particular the smectites which are based on 2:1 layers distribution made up of two tetrahedral coordinated silicon atoms forming an edge-shared

Table 1.4 Clasification of nanofillers used in food packaging applications according to their origin

Organic origin				Inorganic origin	
Natural biopolymers	Natural antimicrobials	Clays	Carbon nanofillers	Metals	ZnO
Chitosan	Nisin	Mica	Fullerenes	Silver	TiO
Chitin		Montmorillonite	Graphene	Copper	MgO
		Sepiolite	Carbon nanotubes	Gold	AgO
Starch		Laponite		Palladium	CuO
Cellulose				Iron	

octahedral sheet. These sheets show central holes where native metal atoms, such as aluminum or magnesium could be founded (Sinha Ray and Okamoto 2003; Reddy et al. 2013). The layers dimensions depend on the clay source and preparation technique, but most of them show thicknesses around 1 nm and their length vary from tens of nanometers to more than 1 micron. Hence, phyllosilicates show very high aspect ratio (surface-to-volume) and surface area (Alexandre and Dubois 2000).

Smectites are the most common phyllosilicate layered silicates due to their availability, low cost, significant enhancement in key properties of polymers and relative simple processability (de Azeredo 2009). Table 1.5 shows the characteristics of the main smectites and their structural characteristics.

Montmorillonites are the most widely studied nanoclays and they show high swelling capacity in aqueous media favoring the dispersion of silicates into their individual layers, making them adequate for the formulation of nanocomposites with polymers. However, this high swelling capacity makes montmorillonites hydrophilic and low compatible with hydrophobic polymers (Raquez et al. 2013). Therefore, the organo-modification of layered silicates is a requirement to get stable nanocomposites and the use of organo-modified montmorillonites produce an increase of the interlayer spacing due to the large volume of the organo-modifiying cations, favoring the dispersion of organo-modified montmorillonites into their individual layers in the polymer matrix and thereby improving in a high degree the service properties of nanocomposites, in particular their mechanical and barrier performance.

Table 1.6 shows some of the most common commercial organo-modified montmorillonites besides their characteristic features. In addition, the organic substituent can provide specific functional groups able to react with the polymer matrix or in some cases initiate the polymerization process to improve the strength of the interface between the silicate and the polymer matrix (Reddy et al. 2013).

Araújo et al. studied the influence of the clay organic modifier on the thermal stability of poly(lactic acid) based nanocomposites with different organo-modified montmorillonites (C30B, C15A and D43B) at different concentrations (3 and 5 wt%) (Araújo et al. 2014). All nanocomposites were submitted to thermo-oxidative degradation at 140 °C for 120 h by using an oven under air atmosphere. Authors reported that the better dispersion achieved with C30B could be associated to the

Table 1.5 Structural characteristics of common smectites (2:1 layered silicates)

Smectite group/formula 2:1 layered silicates	Cation	Interlayer cations	CEC (meq/100g)	Aspect Ratio
Saponites				
$Ca_{0.25}(Mg,Fe)_3((Si,Al)_4O_{10})(OH)_2nH_2O$	Mg^{2+}	Na^+	86.6	50–60
		Ca^{2+}		
		Mg^{2+}		
Montmorillonites				
$(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2nH_2O$	Al^{3+}	Na^+	110	100–150
		Ca^{2+}		
		Mg^{2+}		
Hectorites				
$Na_{0.3}(Mg,Li)_3(Si_4O_{10})(F,OH)_2$	Mg^{2+}	Na^+	120	200–300
		Ca^{2+}		
		Mg^{2+}		

Adapted from Bordes et al. (2009)

strong interactions between the carbonyl functions of poly(lactic acid) chains and the hydroxyl functions of the modifier, which improve the dispersion of this nanoclay through the poly(lactic acid) matrix. They calculated the interlayer spacing values (d-spacing values) by using Bragg's law and results showed high increases in d-spacing of nanocomposites with respect to the original matrix (1.60 nm for C30B and 1.65 nm for D43B).

In conclusion, among the large number of commercial nanofillers that have been incorporated into polymer matrices to form nanocomposites, montmorillonites are the most widely used by their abilities to be incorporated into different biopolymer matrices with clear improvement in the main physico-chemical and functional properties of these nanocomposites.

1.3.2 Metallic Nanofillers

Metallic nanofillers have found some space in the packaging technologies specially in active systems since they are able not only to enhance the barrier and mechanical properties when they are incorporated into materials in direct contact with food, but also to improve the food preservation and shelf-life by their antimicrobial performance (Jokar et al. 2010; Erem et al. 2013; Kanmani and Rhim 2014; Pagno et al. 2015). Copper, zinc, titanium, gold and silver nanoparticles and some of their metallic oxides have been proposed as active additives to extend food shelf-life and to provide affordable and safe innovative strategies (Llorens et al. 2012).

Table 1.6 Commercial organo-modified montmorillonites (OMMTs)

OMMTs/ designation	Organo-modifiying type[a]	CEC (meq/100 g)	Interlayer spacing (Å)	Weight loose on ignition (%)	Used by…
Supplier: Southern Clay Products (USA)					
Cloisite® Na/ CNA	none	–	11.7	7	Dias et al. (2014) and Shemesh et al. (2015)
Cloisite®10A/ C10A	$N^+(Me)_2$(benzyl)$(T)_2$	125	19.3	39	Shemesh et al. (2015)
Cloisite®15A/ C15A	$N^+(Me)_2(T)_2$	125	31.5	43	(Araújo et al. (2014) and Shemesh et al. (2015)
Cloisite®20A/ C20A	$N^+(Me)_2(T)_2$	95	24.2	38	Olivares-Maldonado et al. (2014) and Shemesh et al. (2015)
Cloisite®25A/ C25A	$N^+(Me)_2(C_8H_{17})$ (T)	95	18.6	34	Olivares-Maldonado et al. (2014)
Cloisite®93A/ C93A	$NH^+(Me)(T)_2$	90	23.6	40	Olivares-Maldonado et al. (2014), and Xia et al. (2015)
Cloisite®30B/ C30B	$N^+(Me)(EtOH)_2(T)$	90	18.5	30	Fukushima et al. (2011), Araújo et al. (2014), and Efrati et al. (2014)
Supplier: Laviosa Chimica Mineraria (Italy)					
Dellite® 43B/ D43B	$N^+(Me)_2(CH_2$-Ph) (T)	66	16.6		Scatto et al. (2013), Araújo et al. (2014), and Ramos et al. (2014a, b)

[a]Tallow (T): ~ 65% C18; ~ 30% C16; ~5% C14
Adapted from Bordes et al. (2009) and Reddy et al. (2013)

1.3.2.1 Silver Nanoparticles

Silver nanoparticles are those most widely used for the development of active packaging materials by their high surface-to-volume ratio which provides better contact with microorganisms, showing their efficiency in antimicrobial behavior compared to ionic silver. In addition, silver nanoparticles show unique properties in their electric, optical, catalytic, thermal stability performance (Dallas et al. 2011).

In the last decade, many researchers have reported the strong antimicrobial activity of silver, in particular when it is used as nanoparticles, against a wide variety of Gram-positive and Gram-negative bacteria, viruses and fungi (Kim et al. 2007; Rai et al. 2009; Sharma et al. 2009). However, the antimicrobial mechanism of the silver nanoparticles is a highly controversial subject, in particular when referred to materials in direct contact with food. These controversies are mainly due to the small dimensions required to achieve a significant antimicrobial effect, the requirement of an oxidized surface and the subsequently feasible exchange of silver ions. For these reasons, the mechanism of action is not been well known yet. The proposals for mechanisms suggested by several authors are supported by the morphological and structural changes found in the bacterial cells and the possibilities to silver nanoparticles penetrate inside the bacterial cell due to their attachment to the cell membrane (Reidy et al. 2013).

Rhim et al. proposed other mechanisms for the activity of silver ions and silver nanoparticles against bacteria (Rhim et al. 2013a, b). Silver ions interact with negatively charged groups in the enzymes and nucleic acids, causing direct damage to cell walls and membranes by structural changes and deformation that lead to disruption of metabolic processes followed by cells death. It has been reported that the increase in surface area in silver nanoparticles is associated with the high release rate of silver ions and consequently the electrostatic attraction between the negative-charged cell membranes and the positive-charged nanoparticles is improved causing direct damage to cell membranes (Kim et al. 2007). The accumulation of silver nanoparticles in the bacterial cytoplasmic membrane can also produce a significant increase in permeability with the result of silver nanoparticles entering into the bacterial cells and altering the respiratory chain, cell division and finally leading to cell death (Kim et al. 2007; Rhim et al. 2013a, b).

Sondi et al. studied the surface morphology of *Escherichia coli* inoculated in agar plates supplemented with silver nanoparticles from 10 to 100 μg cm^{-3} (Sondi and Salopek-Sondi 2004). Scanning electron microscopy (SEM) images of these bacteria cell walls showed changes in the treated bacterial cells resulting in major damage due to the formation of "pits" in their cell walls. The energy dispersive X ray analysis (EDAX) of these samples showed that silver nanoparticles were incorporated into the membrane of the treated bacterial cells since the characteristic optical absorption peak of silver at around 3 keV is observed due to surface plasmon resonance.

Silver nanoparticles used in research works can be synthesized by using some methods, either by ex situ synthesis by chemical reduction, as used in this work, or in situ in direct contact with bacteria cells (de Azeredo 2013). These methods

include the use of polymer matrices as carriers, biological macromolecules, mesoporous inorganic materials and hydrogels. Other environmentally-friendly approaches to obtain silver nanoparticles have been proposed by several authors (Sharma, Yngard and Lin 2009; Rajan et al. 2015). Extracts of *Skimmia laureola* have been used by Ahmed et al. to synthesize silver nanoparticles (Ahmed et al. 2015). Authors reported that the spherical nanoparticles obtained had a diameter around 40 nm and they showed antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*.

When silver nanoparticles are immobilized in food packaging polymers, they can display their antimicrobial activity by the release of metal ions due to the high water sorption created by the hydrophilic character of some biopolymer. The moisture sensitivity and the associated plasticizing effect due to the water sorption induce the uncontrolled release of immobilized nanoparticles besides the oxidation of silver, releasing silver ions gradually (Llorens et al. 2012). Echegoyen et al. studied the release of silver nanoparticles incorporated into polyolefins in two food simulants: ethanol 50% (v/v) and acetic acid 3% (v/v) at two testing conditions: 40 °C for 10 days and 70 °C for 2 h in three cycles (Echegoyen and Nerín 2013). Results showed that the overall migration of silver was far below the general maximum migration limits stated by the European legislation in all cases ensuring the possibilities of these formulations with silver nanoparticles in food packaging applications.

In conclusion, metallic nanoparticles, in particular silver nanoparticles, have demonstrated their ability to modify the final properties of nanocomposites when they are incorporated as nanofiller, from several points of view. They join the typical structural improvement of many nanoparticles with their intrinsic antimicrobial capacity, making them really attractive for active nanocomposites for packaging.

1.3.3 Nanocomposites in Food Packaging

The use of nanocomposites is gaining some space in the food and beverage packaging market, although it is not yet widely introduced by the increase in costs of the final material and the strict legislations regarding the use of materials in the nanoscale in food applications. Research is raising fast in this area and Table 1.7 summarizes some examples of nanocomposites in food packaging applications.

The use of nanocomposites in food packaging materials has resulted in improvement in some of their key properties, such as strength and flexibility, barrier properties, moisture stability and higher resistance to heat and cold (Restuccia et al. 2010; Cushen et al. 2012). For example, the addition of low amounts of several nanomaterials, either metal nanoparticles or nanofillers, to poly(lactic acid) matrices has resulted in improvement of poor thermal, mechanical resistance and gas barrier properties, which are essential characteristics to the packaging industry and have joined to key properties of poly(lactic acid), including thermoplasticity, high transparency and biocompatibility (Araújo et al. 2014).

Table 1.7 Representative examples of nanocomposite application in food packaging

Polymer matrix	Nanofiller	Amount	Processing	Effect of nanofiller	References
Quinoa starch	Au nanoparticles	2.5 and 5% (v/v)	Solvent casting (82 °C)	Inhibition of 99% against *Escherichia coli* and 98% against *Staphylococcus aureus*.	Pagno et al. (2015)
				Improvement in mechanical and optical performance, maintaining the thermal and barrier properties.	
Food grade agar	Ag nanoparticles	0, 0.2, 0.5, 1.0 and 2.0 wt%	Solvent casting (95 °C)	Increase in WVP and surface hydrophobic character.	Rhim et al. (2013b)
				Strong antimicrobial activity against *Listeria monocytogenes* and *Escherichia coli.*	
Gelatin-based	ZnO nanoparticles	N.R.	Solvent casting (80 °C)	Antibacterial activity against both Gram-positive and Gram-negative bacteria. Stronger activity against *Listeria monocytogenes.*	Shankar et al. (2015)
				Enhanced thermal stability.	
LDPE	TiO_2 nanoparticles	0.05, 0.08 and 0.11 g TiO_2 in 100 mL ethyl methyl ketone	Manual Coating	Antimicrobial activity of the films exposed to fluorescent and UV radiation increased with the TiO_2 nanoparticles concentration.	Othman et al. (2014)
PE	TiO_2 nanoparticles	3 wt%	Melt extrusion (130 °C)	Improved barrier properties.	Bodaghi et al. (2015)
				Excellent antimicrobial activity against *Pseudomonas spp.* and ethylene photodegradation.	

(continued)

Table 1.7 (continued)

Polymer matrix	Nanofiller	Amount	Processing	Effect of nanofiller	References
PLA	TiO_2 nanoparticles	1, 3.5 and 8 wt%	Melt blending (180 °C)	Improvement of E and crystallization temperature.	Fonseca et al. (2015)
				Antimicrobial activity increased under UV irradiation.	
LDPE	Ag nanoparticles	0.1, 0.3, 0.5, 3 and 5 wt%	Melt blending (140 °C)	Antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli.*	Jokar et al. (2010)
PLA	Silver/ montmorillonite	1, 5 and 10 wt%	Solvent casting (RT)	Migration levels of silver, within the legislation and high antimicrobial activity against *Salmonella spp*.	Busolo et al. (2010)
Corn starch	Chitosan-montmorillonite Laponite RD	5 wt%	Blending (RT)	Reinforcing effect: Improvement of E and tensile strength.	Chung et al. (2010)
Chitosan	C30B	5 wt%	Solvent casting (60 °C)	Reinforcing effect: Improvement of E and strength.	Rodríguez et al. (2012)
				Reduction of oxygen transmission rate (OTR).	
PLA	C30B Fluoro-hectorite/SOMASIF MEE	5 and 10 wt%	Melt blending (165 °C)	Acceleration in the degradation of PLA in compost at 40 °C.	Fukushima et al. (2013)
PLA	Cellulose nanocrystals from *Posidonia oceanica*	1 and 3 wt%	Solvent casting (RT)	Migration levels into two food simulants were well below the European legislative limits.	Fortunati et al. (2015)

N.R. not reported
RT room temperature
PLA poly (lactic acid), *LDPE* low density polyethylene, *PE* polyethylene

In general terms, the studies based on the use of poly(lactic acid) with nanofillers showed the clear increase in toughness and tensile strength of the biopolymer matrices after the addition of nanoclays and/or metal nanoparticles. For example, tensile properties of poly(lactic acid) based nanocomposites can be improved with the addition of C30B at different concentrations. The increase in elastic modulus and tensile strength compared with the unfilled poly(lactic acid) was around 40% and 50% respectively. Jollands and Gupta reported that the elastic modulus was around

4200 MPa for unfilled poly(lactic acid) and 5900 MPa for poly (lactic acid) with 4 wt% of C30B, while tensile strength was 32 MPa and 59 MPa, respectively (Jollands and Gupta 2010). Fukushima et al. also reported that the highest thermo-mechanical and mechanical improvements in poly(lactic acid) matrices were obtained upon the addition of 10 wt% of nanoclay, and they are associated with the good dispersion level observed by using wide angle X-ray scattering (WAXS) and to the high clay content (Fukushima et al. 2013).

Regarding thermal stability of poly(lactic acid), nanocomposites based on poly(lactic acid) with D43B showed higher thermal stability than C15A and C30B after thermo-oxidative degradation experiments due to the hydrophobic character caused by the benzene ring in the D43B structure (Araújo et al. 2014). As poly(lactic acid) is a hydrophobic polymer due to the presence of methyl groups, the D43B can be easily dispersed into the polymer structure. Moreover, the ability of D43B to absorb moisture is lower, than in the cases of C15A and C30B, retarding the poly(lactic acid) hydrolysis.

In general terms, gas barrier properties can be greatly improved with the inclusion of particulate nanomaterials into polymer matrices. The mechanism for this increase in barrier to gases is based on the higher tortuosity of the path to be followed by gas molecules in the presence of nanoparticles.

Carboxy(methylcellulose) films reinforced with montmorillonite improved significantly their barrier properties (around 50% in oxygen permeability). This effect was due to the high degree of exfoliation/intercalation reached in nanocomposites and the possible interactions between the polymer and the nanoclay (Quilaqueo Gutiérrez et al. 2012).

The use of nanocomposites in food packaging materials has resulted in clear improvement in some of their key properties and it has gaining some space in the food and beverage packaging market, in spite of the increase in costs of the final material and the strict legislations regarding the use of materials in the nanoscale in food applications.

1.3.3.1 Preparation and Processing

Processing techniques for nanocomposites should be optimized to obtain well-dispersed nanoparticles with high structural integrity and to minimize their adverse effects to the polymer matrix, such as their possible degradation at high temperatures.

Nanocomposites are usually obtained by using three main techniques: (i) in-situ intercalation where the layered silicates are swollen in a monomer solution before polymerization; (ii) solvent intercalation consisting of swelling the layered silicates in a suitable solvent to promote the diffusion of the macromolecular chains in the clay galleries; and (iii) melt-intercalation process where usual polymer processing in the molten state, such as extrusion, are used. Other techniques, such as electrospinning (Ghorani and Tucker 2015) and electrospraying (Tapia-Hernández et al. 2015) have been recently proposed for the preparation of homogeneous nano-

biocomposites. The use of supercritical conditions, such as supercritical CO_2, as blowing agent to obtain poly(lactic acid) based foams with C30B has been also reported (Keshtkar et al. 2014). Yang et al. used supercritical CO_2 to pre-disperse commercial organic montmorillonites with further solvent mixing with PS to form nanocomposites with significant dispersion and interfacial enhancement (Yang et al. 2014). X-Ray Diffraction (XRD) results showed that nanocomposites with C10A and C20A increased the d-spacing values and consequently the interlayer spacing of these nanoclays. Nanocomposites with C10A showed diffraction values $2\theta = 2.4°$ corresponding to an increase of the interlayer spacing from 1.05 to 2.68 nm and nanocomposites with C20A experienced an increase from 1.77 to 2.68 nm, suggesting that the polymer chains had been intercalated into the clay galleries.

Obviously, the melt intercalation process is highly preferred for food packaging producers since there is no need of organic solvents and the production can be easily scaled-up to industry.

1.4 Active Nanocomposites

Active nanocomposites are particularly useful in emerging technologies in food packaging due to their improved structural integrity and barrier properties imparted by the addition of nanomaterials (either nanoclays or metal nanoparticles), and the increase in antimicrobial and/or antioxidant properties in most cases by the action of active additive and/or the own nanofiller. Nevertheless, the selection of the most adequate antimicrobial and/or antioxidant agent to be combined with nanofillers is often a complex task by the lack of compatibility of many active compounds with the polymer matrix or by the poor heat resistance of active agents, polymers or nanofillers hampering their stability during processing.

The effect of active nanocomposites to food depends on the specific activity of each active agent against spoilage microorganisms and/or oxidation processes; besides the nanofiller and the polymer as well as other additives, such as plasticizers (Rhim and Ng 2007). In this sense, the polymer plays the most important role in the action of the additives and nanofillers by controlling the particle release or the homogeneous distribution of nanofillers through the matrix.

Much research is currently ongoing in this area by evaluating the possibility to mix different types of nanofillers (nanoclays, nanocelluloses, silver nanoparticles, etc.) with antimicrobial and/or antioxidant additives in conventional plastics or bioplastic. EOs obtained from rosemary (Abdollahi et al. 2012; Gorrasi 2015), clove ,cumin, caraway, marjoram, cinnamon and coriander (Alboofetileh et al. 2014) or Zataria multiflora Boiss (Shojaee-Aliabadi et al. 2014) have found their promising possibilities as active additives in active nanocomposites. Main compounds of essential oils, such as α-tocopherol (Dias et al. 2014), hydroxytyrosol (Beltrán et al. 2014), thymol (Ramos et al. 2014b) as well as plant extracts, such as pomegranate rind powder extract (Qin et al. 2015) or alcoholic extracts of red propolis (Costa et al. 2014), play an important role in novel active nanocomposites.

Nanocomposites with metal nanoparticles are gaining some space in research in active packaging, since the nanofillers could play a double role as nanofillers (increasing mechanical and barrier properties) and active agents with antimicrobial performance. Silver, gold and TiO_2 nanoparticles (Busolo et al. 2010; Bodaghi et al. 2015; Mihaly Cozmuta et al. 2015; Pagno et al. 2015) have been proposed in active formulations and will be briefly discussed below.

For example, gelatin-based antimicrobial films with silver nanoparticles and C30B were produced and characterized (Kanmani and Rhim 2014a). The antimicrobial activity was measured by the agar well diffusion and the colony count methods. Results showed that nanocomposites with silver nanoparticles and C30B exhibited different antimicrobial activity against Gram-positive bacteria. While the strong action of silver nanoparticles is well known, in the case of C30B the antimicrobial effect is mainly due to the strong activity of the organic modifier used in the modification of the native montmorillonite. The combination of C30B and silver nanoparticles into gelatin films showed their synergic effect against *Listeria monocytogenes*. The tensile strength of the antimicrobial nanocomposite with C30B and silver nanoparticles (20.8 ± 3.1 MPa) and the nanocomposite with C30B and no silver nanoparticles (19.5 ± 4.3 MPa) showed some increase when compared to the unfilled film (15.5 ± 3.9 MPa). However, no increase was observed in the film with silver nanoparticles and no C30B (15.3 ± 3.5 MPa). Similar increases in tensile strength of the active nanocomposite films by the addition of nanoclays, such as montmorillonite, have been frequently observed with other biopolymer matrices such as κ-carrageenan (Rhim and Wang 2014).

Lavorgna et al. synthesized antimicrobial nanocomposites by loading chitosan matrices with silver-montmorillonite nanoparticles by replacing Na^+ ions in native montmorillonite with silver ions by exchange reactions between both ions (Lavorgna et al. 2014). The silver-montmorillonite nanocomposites were submitted to XRD and X-Ray Photoelectorn Spectroscopy (XPS) analysis in order to have a deep understanding of their chemical structure. The main diffraction peaks assigned to the montmorillonite were modified in terms of shape and intensity and an additional peak characteristic of silver nanoparticles corresponding to the (1 1 1) plane reflection of silver appeared. In addition, the successful intercalation and the interaction between chitosan and silver nanoparticles led to the enhancement of the thermal stability of active nanocomposites with clear improvement of their tensile strength, mainly due to the better load transfer between matrix and fillers. Antimicrobial tests were performed and results showed that a significant delay in microbial growth was obtained after 24 h with the active nanocomposites.

Munteanu et al. studied the antibacterial property of silver nanoparticles and thymol the antioxidant activity of Vitamin E when they were combined by incorporation of these two active components within poly(lactic acid) nanofibers via electrospinning (Munteanu et al. 2014). Results showed the strong antimicrobial effect against *Escherichia coli, Listeria monocytogenes* and *Salmonella typhymurium*, and the antioxidant activity was determined as 94% by using 2,2-diphenylpicrylhydrazyl (DPPH) method.

Other studies showed that carvacrol and thymol can be also incorporated as active additives into polymer nanocomposites to enhance their antimicrobial and/or antioxidant properties (Tunç and Duman 2011; Efrati et al. 2014; Shemesh et al. 2015). Perez et al. reported that low density polyethylene based films with Nanomer® I44P and carvacrol at 5 and 10 wt% produced an increase of the interlayer spacing favoured for the addition of carvacrol. And the addition of nanoclay improved the cristallinity and reduces the permeability to oxygen. Similar results were obtained for Shemesh et al. (2015). In this study the authors presented a new approach to use montmorillonites and organo-modified montmorillonites, as active carriers for carvacrol, aiming to minimize its loss throughout the polymer compounding. Different nanoclays were pretreated with carvacrol, resulting in the oil molecules intercalation in between the clay galleries and enhanced carvacrol thermal stability. The active nanocomposites exhibited excellent and prolonged antimicrobial activity against *Escherichia coli* compared with binary system low density polyethylene with carvacrol films.

As emerging technology, active nanocomposites combine the potential of the use of nanofillers, and the antioxidant and antimicrobial abilities of several active additives. This combination improves the final properties of novel active nanocomposites focusing on food packaging.

1.4.1 End-of-Life for Active Nanocomposites

Polymer degradation are the processes that are cable to induce changes in polymer properties due to chemical, physical or biological reactions resulting in bond scission and subsequent chemical transformations. The changes produced are reflected in changes of material properties such as mechanical, optical or electrical characteristics, erosion, discoloration, phase separation or delamination, bond scission, chemical transformation and formation of new functional groups (Shah et al. 2008).

However, a biodegradable polymers should be biodegraded by the action of naturally occurring microorganisms in aerobic or anaerobic conditions, such as bacteria, fungi, and algae, but the processes vary considerably depending on the environment where they take place, since the microorganisms responsible for the degradation differ from each other and they have their own optimal growth conditions;, such as industrial composting plants, soil, fresh water, marine water. These microorganisms are able to produce changes in the chemical structure of biodegradable polymers reducing the long chains into simple chemical substances like water and CO_2 during aerobic biodegradation besides minerals with formation of other intermediate products like biomass and humic materials or water, methane and CO_2 during anaerobic biodegradation (UNE-EN_13432 2000; Shah et al. 2008; Vaverková et al. 2012; Araújo et al. 2014).

Biodegradation process through the action of microorganisms is also associated with chemical deterioration and depends of living organisms and it occurs in two steps (Shah et al. 2008): (i) fragmentation of the long polymer chains into lower

molar mass species by means of either abiotic reactions, such as oxidation, photodegradation or hydrolysis, or by microorganisms; and (ii) bio-assimilation of the polymer fragments by microorganisms and their mineralization.

This process can be evaluated under aerobic or anaerobic conditions by International standard method (UNE-EN_13432 2000).

The polymers characteristics, mainly mobility, tacticity, crystallinity, molar mass, type of functional groups and substituents present in their structure as well as plasticizers or additives in the polymer formulation also play an important role in the degradation rate and mechanism (Shah et al. 2008). In the case of nanocomposites, they are also submitted to biodegradation processes after their useful life. Relevant results have been obtained in the last decade showing a remarkable improvement in biodegradability for nanocomposites prepared with organically modified layered silicate.

Fukusima et al. studied the biodegradability of amorphous poly(lactic acid) and the corresponding nanocomposites prepared with organo-modified montmorillonite and modified kaolinite by using compost conditions at the laboratory scale at 32 °C (Fukushima et al. 2012). Results showed that the poly(lactic acid) biodegradation rate was significantly enhanced in nanocomposites due to the presence of terminal hydroxylated edge groups in modified kaolinite which started heterogeneous hydrolysis of the poly(lactic acid) matrix after absorbing water from the compost medium. However, in the early stages (initial 6 weeks in compost), organo-modified montmorillonite tended to delay the degradation of poly(lactic acid), likely due to its higher dispersion level into the polymer matrix as compared to modified kaolinite, causing a high barrier effect of organo-modified montmorillonites layers towards microbial attack on poly(lactic acid) ester groups, as well as reducing loss of oligomers which could catalyse poly(lactic acid) hydrolysis through chain end hydroxyl groups.

Disintegration under composting conditions in laboratory-scale tests of active nanocomposite films based on poly(lactic acid) with thymol and silver nanoparticles was also reported (Ramos et al. 2014a, b). The addition of both additives to poly(lactic acid) increased the disintegration rate in composting conditions highlighting the high influence of thymol in the diffusion process of water molecules through the polymer structure, promoting hydrolysis, due to the increase in chain mobility induced by the combined presence of the additives, as previously discussed.

In conclusion, the end-of-life of active nanocomposites or, in general terms, for all food packaging materials should be taken into account during the development of these systems. For this reason, the above-outlined tests are strictly necessary to assess the environmental sustainability of these biocomposites with active functionality. The main current challenge in this area would be to increase disintegrability and biodegradation of all the components of active nanocomposites.

1.4.2 Risk Assessment and Migration in Active Nanocomposites

The high surface-to-volume ratio and surface reactivity of nanofillers provide nanocomposites with enhanced properties and different migration levels. These effects and the presence of materials in the nanoscale in formulations intended to be in direct contact with food raise some potential health and environmental risks to be studied before using these active nanocomposites at the industrial scale (Sanchez-Garcia et al. 2010).

The potential toxicity, mutagenicity and carcinogenicity of some nanofillers have been put under discussion. The main concerns over the risks associated with the use of nanocomposites in food packaging are based on the lack of sufficient knowledge about nanofillers and the significance of their interaction at the cellular and molecular level in the human body (Huang et al. 2015). The evaluation of potential risks should be based on considering the properties of the nanomaterials and their transfer rate through cell walls. It should be noted that no migration of nanofillers should be considered in normal cases, but poor characteristics of packaging materials and the subsequent ingestion of food previously in contact with nanocomposites can be consider as a potential exposure route (Huang et al. 2015). Consequently, the investigation of the possibility to apply nanomaterials in food packaging and the clear assessment of the safety of these materials in contact with foodstuff is necessary to permit the commercial distribution of active nanocomposites. The first studies should be focused on migration analysis under controlled conditions to determine the real possibilities of nanomaterials to be considered a real hazard in food packaging.

Migration is the result of the diffusion, dissolution and equilibrium processes involving the mass transfer of low molecular weight compounds initially present in the packaging material into a food sample or food simulant (Manzanarez-López et al. 2011). Several factors, such as original concentration, particle size, molecular weight, solubility and diffusivity of the specific substance in the polymer, as well as the pH value, temperature, polymer structure and viscosity, mechanical stress, contact time, and food composition, are the main controlling parameters in migration studies (Song et al. 2011).

It has been stated that migration rates depend on mass transport parameters and the thermodynamic equilibrium between the materials in contact with food (Torres et al. 2014). Many factors are essential to estimate the magnitude of the migration process from packaging films into food or food simulants and to know the concentration change of migrating species with time. The key point in designing a specific migration model in food contact materials is the determination of two fundamental parameters, the diffusion and partition coefficients which are specific for each system. In most cases, the migration of a particular substance from a polymer packaging film is controlled by the molecular diffusion of the migrant in the film, which can be described by Fick's second law (Poças et al. 2012; Huang et al. 2015):

Table 1.8 Food simulants established by EU Regulation No 10/2011

Food simulant	Abbreviation
Ethanol 10% (v/v)	Food Simulant A
Acetic acid 3% (w/v)	Food Simulant B
Ethanol 20% (v/v)	Food Simulant C
Ethanol 50% (v/v)	Food Simulant D1
Vegetable oil	Food Simulant D2
Poly(2.6-diphenyl-p-phenylene oxide), also known as MPPO and TENAX®	Food Simulant E

$$\frac{\partial C_p}{\partial t} = D\frac{\partial^2 C_p}{\partial x^2} \tag{1.1}$$

where C_p refers to the concentration of the migrant in the packaging material at time t and position x, and D is the diffusion coefficient which measures the rate at which the diffusion process occurs, and it could be either a constant or a concentration-dependent value. Migration kinetics can be defined by the rate at which the transferred substances move through the system, which is characterized by the diffusion coefficient.

Active nanocomposites can be used in packaging applications as two-dimensional delivery systems based on the release of active additives to extend the shelf-life of food products by their action against microorganisms and oxidative degradation processes. Indeed, the main role of active packaging materials consists of the release of functional additives onto the food surfaces in a controlled and systematic process, depending on the consumer's nutritional needs and tastes, including mineral, probiotics, vitamins, phytochemicals, marine oils and other active agents.

Migration tests in food contact materials should cover all requirements established by the European Union Regulation No 10/2011 on plastic materials in contact with foodstuff. Although the best approach to test migration is to work with real food matrices, it is not often possible by the complex compositions of most foodstuff and analysis difficulties resulting in no reliable, tedious and time-consuming procedures. The current legislation marks the valid route to assess the mass transport processes by evaluating the specific and overall migration of targeted substances using food simulants (Commission_Regulation/(EU)/No-10/2011; Huang et al. 2015). Table 1.8 shows the food simulants which are selected as model systems according to the current legislation.

At the end of the contact period and depending on the selected food simulant, accurate analytical methods should be applied to determine the precise amount of migrant in contact with food under the test conditions. It is necessary to identify and determine the target substance(s) in the food or food simulants to estimate the specific migration level. However, no standardized analytical methods have been proposed up to now to identify and determine nanoparticles and/or active additives in food simulants.

Chromatography has been classically used for identification and quantification of migrated compounds in passive packaging materials, particularly for common additives, such as plasticizers or active packaging. Recent studies have proposed the use of different chromatographic techniques to evaluate the migration of active agents as gas chromatography-flame ionization detector (GC-FID)(Kuorwel et al. 2013; Muriel-Galet et al. 2015), gas chromatography–mass spectrometry (GC-MS) (Efrati et al. 2014) or high performance liquid chromatography-UV detector (HPLC-UV) (Muriel-Galet et al. 2015).

In most cases, a previous step to chromatographic analysis involves the preparation of an appropriate sample based on an analytical procedure to achieve a concentration and/or isolation of analytes by using sample preparation techniques such as solid phase extraction (SPE) (Ridgway et al. 2007; Viñas and Campillo 2014).

Inductively coupled plasma coupled to different detectors, such as mass spectrometer (ICP-MS), atomic emission spectrometer (ICP-AES) and optical emission spectrometer (ICP-OES) can be also used in quantitative and elemental analysis of potentially migrating nanofillers. These techniques are highly selective, sensitive and accurate, making them the most efficient in determining trace metal ions, such as those present in nanofillers and nanocomposites intended for the use in food packaging applications. For example, Lavorgna et al. quantified the concentration of silver released in aqueous solutions at room temperature from multifunctional active nanocomposites based on chitosan with silver-montmorillonite antimicrobial nanoparticles by using ICP-MS (Lavorgna et al. 2014). Artiega et al. also used this technique to evaluate the silver nanoparticles migration from commercial food containers. Results demonstrated that the amount of silver migrated increased with storage time and temperature although, in general, silver showed a low tendency to migrate into food simulants (Artiaga et al. 2015).

Therefore, migration studies are necessary when novel food packaging systems should be developed, particularly when nanofillers are present since the total absence of risks to human health should be ensured. Because of the high surface-to-volume ratio and surface reactivity of nanoparticles it should be taken into account that a final compromise between the enhancement of final properties and different migration levels with the absence of toxicological effects to humans should be considered.

1.5 Legislation

The legislative framework associated to food contact materials includes many regulations, not always applicable in all countries, which have been discussed and turned on by considering legal and scientific assumptions applicable to the formulation, processing and use of materials intended to be in direct contact with food. The European Union (EU) has made great effort in unifying the legislation of different member countries, and this will the focus of this work, since it is the current applicable legislation in Spain.

Traditional packaging systems intended to come in contact with food must comply with the legislation set up by the European Union and extrapolated to the national level. But, the raising interest in production of active packaging systems has forced the EU and other administrations to set up the applicable legislation in food contact materials (Amenta et al. 2015). The legislative framework is represented in Fig. 1.8 specifically devoted to active packaging systems surrounded by green lines (Regulation_(EC)/No-1935/2004).

The introduction of active and intelligent food packaging systems, which are supposed to interact with food and/or the package headspace, represents the appearance of new challenges for the evaluation of their safety and their harmless character to human health. From the beginning of the research in active packaging for their possible commercial use it was clear that regulations limiting or even banning migration should be changed to avoid the incorrect use of packaging due to the wrong, the insufficient labeling or non-efficient operation of the packaging material (Dainelli et al. 2008). The Framework Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food contains some general provisions on the safety of active and intelligent packaging and sets the framework for the European Food Safety Agency (EFSA) evaluation process. Figure 1.8 shows the current legislation framework which is applicable to active packaging and all the aspects related with the Regulation (EC) No. 1935/2004.

However, since 2009 there was specific legislation devoted to active and intelligent materials. Regulation (EC) No, 450/2009 was published to cover this particular situation in the case of these materials designed to intentionally interact with food with specific rules for active and intelligent materials and also for their marketing. This regulation should be applied in harmonization with the general requirements established in the Framework Regulation (EC) No 1935/2004 (Commission_ Regulation/(EC)/No-450/2009). This regulation also mentions that the substances responsible for the active and intelligent functions can either be directly incorporated into the packaging material or contained in separate containers (e.g. sachets or labels). Moreover, it also describes the procedure for the authorization of active substances at the EU. The main requirement indicated in this regulation is based on the risk assessment that the EFSA should perform to all the active compounds to be proposed for their commercial use in the EU before their authorization. In addition, a list of substances or group/combination of them intended to be used in active and intelligent packaging materials should be drawn up following the risk assessment of these substances by the EFSA (Valdés et al. 2014). Therefore, only those substances included in the positive list of authorized substances drawn by the EFSA may be used as valid components of active and intelligent packaging materials and articles, with the exception of these substances already authorized in other EU legislation, such as food additives, flavorings, enzymes, etc (AINIA and EOI 2015).

The list of authorized substances is continuously growing after the positive evaluations by the EFSA, which allows the submission of new proposals for active substances, which must be accompanied by a risk assessment study (Restuccia et al. 2010).

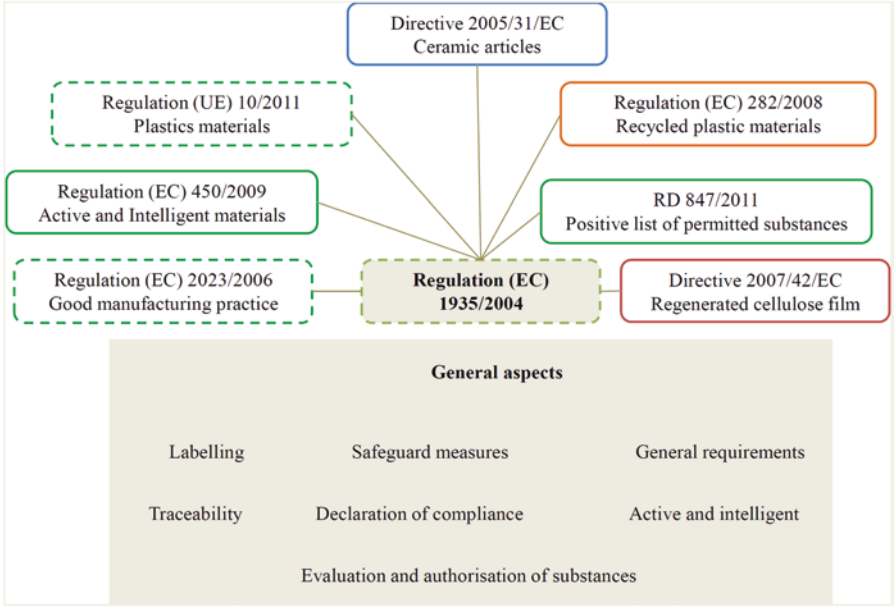

Fig. 1.8 Applicable legislation to food contact materials in the EU and general aspects of the Framework Regulation (European Commission) No 1935/2004 on materials and articles intended to come into contact with food and legislation applied to active packaging (surrounded by *green lines*)

All passive parts of active and intelligent food packaging systems are also subject to the EU legislation (Fig. 1.8). The Regulation (European Union) No 10/2011 and subsequent amendments and corrections (the last of them being introduced in February 2015), indicated the specific measures to be taken into account within the meaning of the Framework Regulation (EC) No 1935/2004 based on plastic materials and articles intended to come into contact with food. This regulation provides the overall migration limits admissible for materials in direct contact with food (10 mg per 1 dm^2 of food surface area or 60 mg per kg food) to ensure the safety of the final material or article. It also establishes the specific migration limits for substances incorporated inside the polymer matrix that can be released after the extended contact with food. These limits ensure that the material in contact with food does not pose a risk to human health. The basic rules on testing overall and specific migration levels for materials and articles are also described by using food simulants. In addition, it is indicated that the amount of active substances released from packaging materials could exceed the overall migration requirements indicated in the EU or national legislations if these substances have been approved as harmless by the EFSA. The transfer of these active substances to food should not be included in the calculation of the overall migration limit (Valdés et al. 2014).

On the other hand, as previously discussed, the growing concern associated with nanotechnologies and the human health has forced the legislative bodies to set up new regulations regarding the safe use of nanomaterials in food packaging applications (Bumbudsanpharoke and Ko 2015).

The EU proposes the use as a guideline and reference for nanotechnology applied in food contact materials of the European Food Information to Consumers Regulation (EU) No 1169/2011, which was published on the provision of prepacked food information to consumers on general food labeling and nutrition labeling (EFSA 2011). The main novelty of this regulation and the application to nanomaterials was that all food ingredients with a form of engineered nanomaterials must be indicated in the list of ingredients, warning consumers of their use (Bumbudsanpharoke and Ko 2015).

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Chapter 2
Nanopackaging in Food and Electronics

Nabeel Ahmad, Sharad Bhatnagar, Shyam Dhar Dubey, Ritika Saxena, Shweta Sharma, and Rajiv Dutta

Abstract Nanoscience has induced a profound revolution in all industrial domains, notably in the food and electronic industries. The food industry has constantly augment the quality, shelf life, safety and traceability of products. This has led to development of nanomaterials for food packaging and nanosensors to detect contaminations. Nanomaterials are to develop 'improved', 'active' and 'intelligent food packaging. Nanomaterials have also been conjugated with biobased polymers to develop environmentally friendly nanocomposites. This article review nanopackaging of food with emphasis on carbon nanotubes, nanosensors, nanowires, nanolaminates, nanocomposites, nanocrystals, biobased fillers for nanocomposite, and antimicrobial nanoparticles.

Keywords Nanotechnology • Food packaging • Electronic packaging • Nanocomposites • Carbon nanotubes

2.1 Introduction

As the population of the world keeps on increasing, the issues of food security, safety and preservation are steadily being thrust into the spotlight. Advances in food packaging technology have become one of the important tools to ensure the safety of the produced food worldwide. A large amount of food is being wasted annually on account of microbial contamination and exposure to deleterious components of

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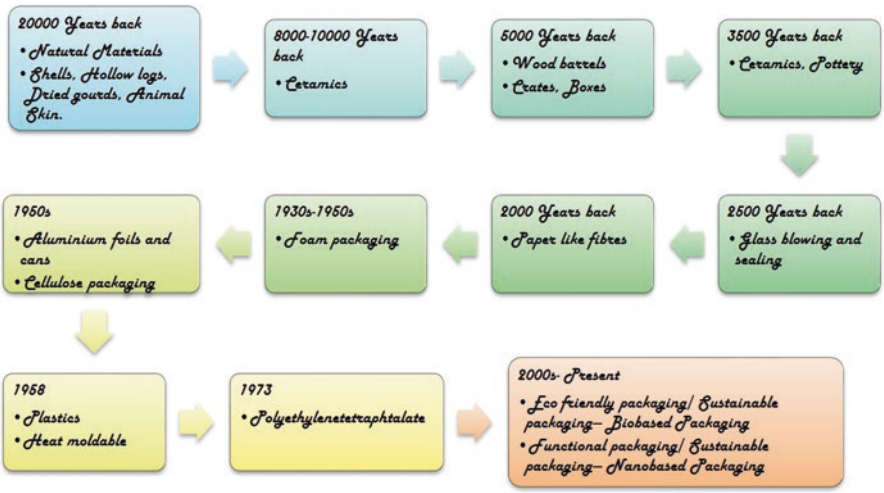

Fig. 2.1 Evolution of food packaging

environment due to deficiencies in existing packaging technologies. The evolution in packaging materials has usually kept pace with the requirements of the society as well as the new challenges thrown by technological advances in every era (Fig. 2.1). A shift towards inclusion of nanomaterials in existing packaging materials might help to combat this particular conundrum. These novel materials can not only serve the essential functions of food packaging viz., protection and preservation of food, maintenance of quality and safety and reduction in waste, but can also actively interact with the food to ensure all these functions in the real time. However, the food and beverage industry is always on prowl to search for efficient and novel technologies which could help them to reduce the deterioration of food, thereby maximizing the profitability.

The advancements in the field of nanotechnology have enabled the researchers to fabricate materials with entirely new properties in the quantum range, which can be harnessed to improve the properties of existing materials. Nanotechnology can be defined as the branch of science dealing with materials in the nanometer size range. At this range, the mechanical, electrical and chemical properties of the material become entirely different as compared to the bulk material and these properties form the basis of revolution promised by nanotechnology. Food and beverage industries are intent upon exploring the possibilities in this sector by conjoining it with food packaging. Numerous companies have been actively involved in this research to come up with packaging nanomaterials that can be employed on a mass scale (Chaudhry et al. 2008). These possible applications of nanotechnology in food packaging represent the latest step in the evolution continuum of the engineering marvels.

Food processing industry is a hyper competitive market and as such the industries desire an edge to overcome competition. The customers desire fresh and authentic food products complete with flavor. Therefore, packaging technology is gaining importance in today's scenario. The advances in the technology are therefore

focused on enhancement of shelf life and improvement in the safety and quality of product. As the research in field of nanotechnology has broken new grounds, the applications have expanded exponentially. Novel nanomaterials with distinct properties are being developed and researched rapidly. These fabricated materials are being researched for potential applications in therapeutics, diagnostics, drug delivery, food packaging among others.

As with any nascent developing cutting edge technology, majority of research is being conducted in the developed countries with higher allocation of resources devoted to research. USA, European Union, China, Australia and Japan have been among the major contributors in this field. With fast paced technology and need for the improvement in packaging technology, it is not unreasonable to think that nanomaterials can soon become a central player in this regard. This will result in new packaging materials flooding the market in upcoming years. The major bottleneck in this regard is the economic considerations and the technological knowledge required to create these novel packaging material including nanomaterials at a very large scale. If these materials can be fabricated at a large scale at low costs, it can indeed potentially conquer the packaging market.

2.2 Nanotechnology in Food Industry

Nanotechnology allows the researchers to imagine and create systems that can be used in wide spectrum of applications depending upon their properties on molecular scale. For example, alteration in molecular structure of plastic can help in fabrication of plastic with desired properties such as enhancement or reduction in gas or moisture permeability. Such plastics can then be used to tend to needs of specific products such as soft drinks and beer. Films incorporating nanomaterials can theoretically increase the tensile strength of the packaging material resulting in development of sturdier packaging material. It can also be used to actively prevent the contamination of foods. In future, it is expected that development of nanosenors can lead to development of visual indicators which can track the biochemical changes happening in the food, for ex., meat packaging that changes colour when putrefaction sets in. All these systems will be discussed comprehensively in the following sections.

Recently, developments of nanosensors to detect the variations in food and environment quality have become the latest focus in ever progressing nanotechnology. These sensors use electrically conducting polymers which can detect molecular signals to give information regarding the required parameters in real time. These nanosensors can monitor the nutrient content of the food continuously; thereby displaying the current statistics and helping the consumer to make an intelligent choice. On the other hand, this information can also be used to correctly assess the expiry of food too.

Although conjunction of nanotechnology with packaging technology can yield significant advantages, these achievements must be balanced by studying the impact of interaction of nanomaterials with food and environment. Right now, the studies

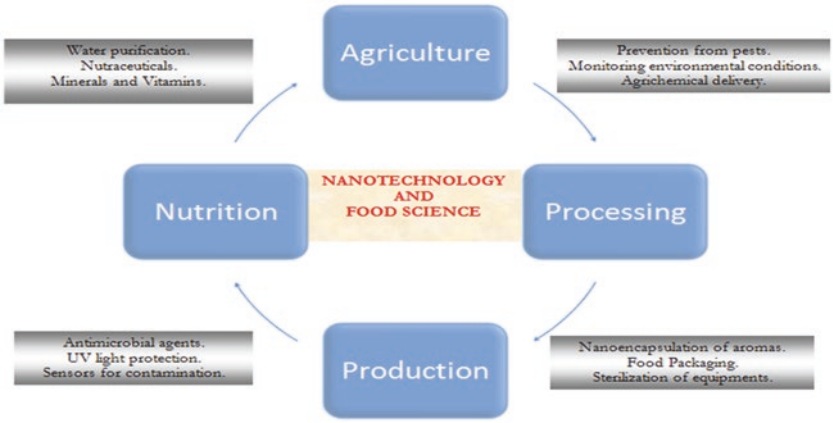

Fig. 2.2 Application of nanotechnology in food science

of impact on environment and biodegradation are very limited in comparison to studies conducted on food and nanomaterial interaction. A number of studies are required to ensure the food safety when nanoparticles are being used in the vicinity (Chen et al. 2006). Even though the use of nanotechnology is currently limited by technological barriers, researchers and industries have figured out the potential areas of interest in almost all the spheres of food industry. Nanotechnology can be used in pesticide, fertilizer formulation to modification of food flavor and properties to development of anti counterfeiting devices.

Undeniably, the most active area of food nanoscience research and development is packaging: According to a new market report published by Persistence Market Research "Global Market Study on Nano-Enabled Packaging For Food and Beverages: Intelligent Packaging to Witness Highest Growth by 2020", the global nano enabled packaging market for food and beverages industry was worth USD 6.5 billion in 2013 and is expected to grow at a CAGR of 12.7% during 2014–2020, to reach an estimated value of USD 15.0 billion in 2020. Even though these numbers are quite promising, complete potential of this technology is not yet being realized due to the fact that people are likely to be more receptive towards use of nanotechnology in external applications rather than where nanoparticles are directly in contact with food (Market attitude Research 2009; Siegrist et al. 2007, 2008).

This technology has the potential to revolutionize the entire food industry and applications in the commercial food systems are steadily gaining ground. Nanotechnology promises large repayments in terms of food safety, quality, and shelf life, provided the technological and economic challenges can be overcome (Stones 2009) (Fig. 2.2).

2.3 Food Packaging

Food packaging is not a current invention. In fact, the roots of food packaging can be traced to the time when humans started gathering food. It has always been an essential part of the food industry since the efforts to enhance the food life are being conducted since times immemorial. Nevertheless, packaging has evolved consistently with the evolution in the methods of food production. With time and requirements, old technology gets weeded and latest technology takes its place. Same is true for the food packaging industry. The latest step in this evolution is the involvement of nanotechnology in packaging and this was embraced by food industry in earnest. Currently, around 500 packaging products flaunting nanotechnology in one way or another are in commercial use. This market share of such products is expected to rise to 25% in the coming decade (Reynolds 2007). Nano packaging can also be designed to release antimicrobials, antioxidants, enzymes, flavors and nutraceuticals to extend shelf life. A number of nanotechnology products have already entered the market and several others are in fray. Most of these products either increase the packaging material strength or have antimicrobial or antioxidant properties. The success of these packaging materials will pave the way for use of more advanced technology such as nanosensors in future (Cha and Chinana 2004; El Amin 2005). These applications of nanotechnology have pushed this so far elusive technology in the centre of commercial scale production. This has become one of the areas where nanotechnology has successfully ventured out of lab and found its way into mass production. Already several companies are using these enhanced packaging materials to extend the shelf life and quality of food. The future researches are more focused on development of nanomaterials which can monitor the milieu of the food and properties of food itself in real time (Broody 2003). Researchers are focusing on development of packing materials which can provide visual identifiers for contamination and which could repair itself after wear and tear of packaging. Apart from technological drawbacks, the delay in incorporation of nanotechnology in food sector is subjected to legal guidelines and regulations relating to labeling of food and consumer health aspect.

Food packaging utilizing nanomaterials can be classified as:

- "Improved" polymer nanomaterials – The presence of the nanoparticles in the composite material improves the mechanical and chemical properties of the packaging but do not interact actively with the goods (Fig. 2.3).
- "Active" polymer nanomaterials – The packaging is compounded with the integration of nanomaterial in such a way that it allows the packaging to interact and control the goods and the environment, thereby actively dictating the terms of preservation (Fig. 2.3).
- "Intelligent" polymer nanomaterials – Incorporation of nanosenors and devices in the packaging to ensure the monitoring and rapid identification of state of the product. It may also serve to protect the genuine food items from cheaper imitations (Fig. 2.3).

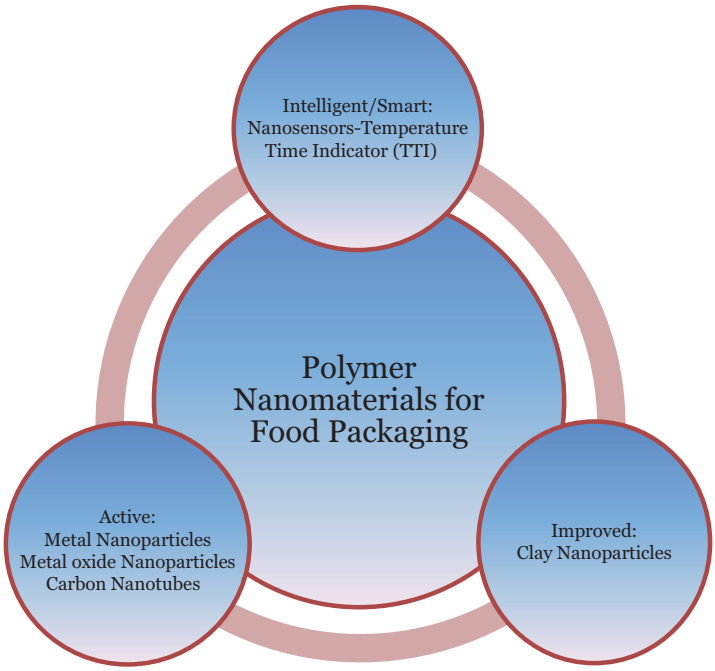

Fig. 2.3 Polymers used in food nanopackaging

2.3.1 "Improved" Polymers

The development of a large number of polymeric nanomaterials for food packaging has been influenced by the fact that due to change in properties of materials at nanoscale, inclusion of nanomaterials in the packaging matrix may be able to improve the properties of packaging on mechanical and chemical fronts (Wu et al. 2002; Pissis 2007). During the initial stages, the onus for improvement in packaging properties rested with clay nanoparticles. Integration of clay nanoparticles to the polymer matrix represents the class of forerunners in the improved food packaging materials. These nanoparticles tend to have a monolayer structure along with isolated layers via interlayer galleries (Paul and Roberson 2008; Ray and Okamoto 2003). To use these clay nanoparticles in the packaging, it is essential to obtain a homogeneous dissipation of these nanoparticles in the polymeric matrix. The structural characteristics of these nanocomposites are said to be dominated by entropic and enthalpic factors (Vaia and Giannelis 1997; Balazs et al. 1998; de Paiva et al. 2008). If the interaction between the matrix and the nanoparticles are deemed to be suitable in the terms of enthalpy, only then the homogeneous dissipation can be achieved. Generally, alkyl ammonium surfactants have been used to increase the strength of these interactions, thereby increasing enthalphy and improving the dissipation properties. The initial distribution of the nanoparticles in the matrix is

dependent upon surface energy of the clay particles which dictates the rate of diffusion and interaction with polar molecules (Kornmann et al. 2001; Mackay et al. 2006).

2.3.2 *"Active" Polymers*

The search for substances that actively participate in the control of the properties of the food and the surrounding environment has led to the development of active nanomaterials for food packaging. These materials include substances in the matrix which would interact with packaged food and environment by either releasing or absorbing some components. Although, the use of silver nanoparticles and its renowned applications as surface antimicrobial agent has dominated the idea of active packaging to a certain extent via development of antimicrobial containments, other ideas are gradually coming to light such as oxygen scavenging, removal or emission of carbon dioxide and ethylene removal etc. (Arora and Padua 2010). Apart from silver, other metallic nanoparticles such as gold or copper, metal oxide nanoparticles and carbon nanotubes have been associated with antimicrobial activity. The use of these materials to come up with active packaging polymers is steadily gaining traction. For now though, the research is more focused on silver, gold and zinc particles at nanoscale. These particles may interact straight from the surface of the packaging, but could also diffuse slowly through the food to interact with the organic component of their choice. Silver nanoparticles usage have been made commercially as early as 1984 when Sinanen Zeomic Co. Ltd., a Japanese company developed a composite of ionic silver and zeolites. Among the properties of silver, stability at high temperatures and its effectiveness as anti microbial agent against 150 different species at nanoscale are the more important ones (Kumar and Munstedt 2005; Liau et al. 1997). Nano silver is postulated to achieve the antimicrobial properties via several pathways such as: degradation of cell membrane, destroying the cell DNA and binding with electron donor groups in form of ions to disrupt the metabolism. (Sondi and Salopek-Sondi 2004; Li et al. 2008), (Morones et al. 2005) Silver nanocomposites have been reported to demonstrate efficient antimicrobial activity by many researchers.

2.3.3 *"Intelligent/Smart" Polymers*

Intelligent food contact materials are aimed at monitoring, identification and maintenance of the state of food product and its surrounding environment (Kerry and Butler 2008; Yam et al. 2005; Yam 2010). The major idea behind this technology is to give consumers a chance to make an intelligent choice regarding foodstuff they intend to purchase by giving them appropriate knowledge about some prominent factors such as freshness, nutritional content, storage conditions, contamination etc.

via visual indicators. Chief hurdles in the application of these materials in commercial market arise from the costs involved, real time performance and congruency with existing technologies. Initial advances in this field was development of device which could be integrated with packaging material to monitor the state of packaging itself and the time and temperature regime to which food has been subjected. This particular device could measure these parameters to estimate the expiry date of the product. The expiry date given on the product by the industry is determined on the basis of assumed storage conditions and distribution, but these predictions often fall short of the mark since perfect storage conditions are not always available in real life. Foods are constantly subjected to sub-optimal temperatures which could lead to faster deterioration. The initial applications were intended to overcome this scenario using devices called Time-temperature indicators. These devices enabled suppliers and consumers to find out if the food has been stored at requisite temperature and has not been subjected to temperature abuse (Taoukis and Labuza 1989). These devices may distinctly be classified in two ways: one which relied on temperature dependent migration of dye and other which generated a visual signal as a consequence of a certain chemical reaction. These indicators allowed the flexibility of choice for both consumers as well as suppliers.

A recent application of this system is to detect the presence of oxygen and other indicators which signal the initiation of spoilage of food. The excess amount of oxygen present over the food surface could lead to the growth of aerobic microorganisms, which could lead to deterioration. Therefore, presence of nanosensors in the packaging materials which could check the level of the oxygen present inside the packaging makes sense. Apart from this, nanosensors can be fabricated specifically to detect some chemical or biological changes associated with spoilage of food. (Ahvenainen 2003). Colorimetric oxygen sensors have been developed using Titanium oxide nanoparticles and crystalline Tin dioxide which can vary its color depending upon the amount of oxygen exposure (Mills and Hazafy 2009).

2.4 Nanomaterials in Food Packaging

A unanimous definition of nanotechnology is still not agreed upon although the general consensus is that it can be characterized by size in nanometer dimensions. Nanometer can be simplified to millimeter divided a million times. Even the flimsy sheet of a newspaper is 100,000 nanometers thick! Nanosized particles exist naturally and can be synthesized using various chemical, physical or biological techniques, but in accordance with the definition, they must be less than 100 nanometers at least in one direction. Special techniques such as Transmission electron microscopy, Scanning tunneling microscopy etc. are required to view these nanosized materials, since conventional techniques are not powerful enough to visualize these structures. Nano scale materials often have several advantages over their bulkier counterparts due to their enhanced properties such as unique mechanical, optical, chemical, electrical properties amongst others. Because of these properties,

nanomaterials are entering the realm of conventional technological fields and are ushering in a start of new class of technologies as well.

Nanotechnology is being steadily used in field of medicine to design novel pharmaceuticals, to develop targeted drug delivery, in mechanical engineering to enhance the strength of materials coupled with reduction in weight, in electronics to develop new electronic packaging technology and revolutionizing the era of miniature devices to mention a select few applications.

However, with this great opportunity knocking at our doorstep, we also need to know about the pitfalls of this technology. The potential impact of these nanomaterials on human health and environment is not clearly understood yet. A lot more studies are needed to elucidate these effects in quantifiable manner. Even familiar nanomaterials such as silver, gold can be hazardous to human at nanoscale. Penetration via skin, inhalation or ingestion of nanomaterials can lead to problems. For example, fibrous carbon nanomaterials can inflame the tissues in lungs similarly to asbestos. Similarly, the impact of nanomaterials in environment, biodegradability, interaction with various facets of environment is yet to be studied.

Nanomaterials in food packaging comprises of not only nanoparticles but also other nanostructured materials such as fibres, nanocomposites, nanostructured surfaces etc. (Ranjan et al. 2014). In accordance with ISO/TS 80004–1:2015 the main requirement in order that a material could be considered as an nanomaterials is either having whichever external dimensions in the nanoscale or having internal structure in the nanoscale (Fig. 2.4).

The basic function of the food packaging is concerned with increasing the shelf life of food products via use of different shielding methods and suitable coatings (Ahvenainen 2003; Coles et al. 2003; Hernandez et al. 2000). The cumulative effect of a suitable coating and correct packaging technology can uphold the product characteristics and can ensure freshness while the food is marketed and sold for consumption (Brown 1992; Stewart et al. 2002). Owing to the environmental concerns around disposal of food packaging, researchers are looking at prospects of development of biological based packaging materials that can serve the primary functions of the food packaging and are biodegradable as well (Tharanathan 2003). Recently, most of materials in vogue for food packaging come from fossil fuel sources, such as plastics and polythene and are virtually non degradable. These materials can easily address the basic functions of food packaging but in a long run they constitute an acute global environmental hazard (Kirwan and Strawbridge 2003) (Fig. 2.5). So far, the inclusion of biodegradable materials in food packaging has been a limited success because of inadequate mechanical and barrier properties. However, these biodegradable materials can be combined with conventional fossil fuel based materials or upcoming nanomaterials to come up with conjugates exhibiting better packaging and environmental properties compared to current packagings (Robertson 2012). This could lead to increase in development of edible and environmentally friendly films (Lagaron et al. 2005; Ray and Bousmina 2005). These films could achieve the essential goal of preservation and increase in the shelf life of the food along with reduction in packaging wastes (Labuza and Breene 1989). Different forms of nanomaterials being used in food packaging are being discussed below.

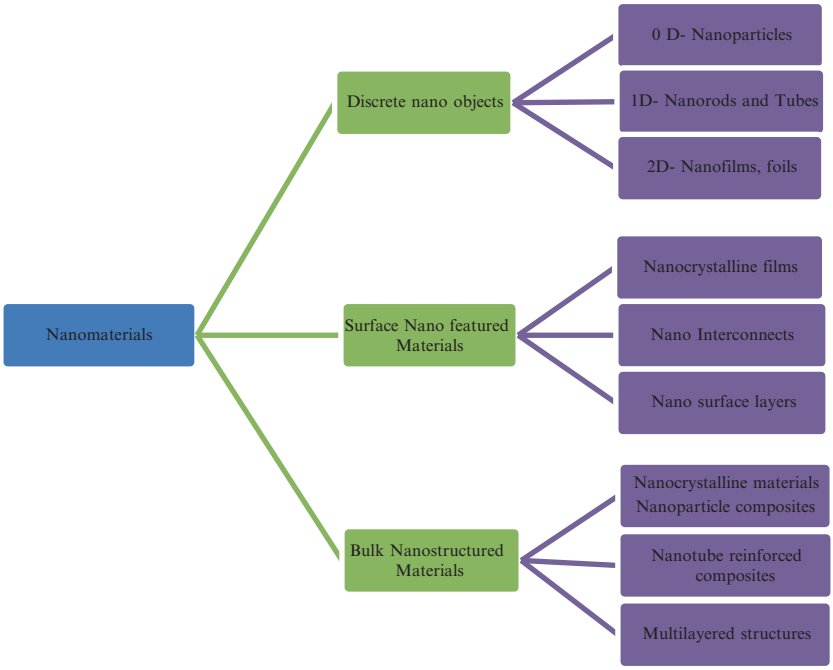

Fig. 2.4 Classification of nanomaterials based on their dimensions

2.4.1 Nano Emulsions

Nano emulsions have been deployed in food packaging applications and in sterilizing the packaging instruments and equipments. Nano emulsions have been reported to exhibit antimicrobial activity against *Staphylococcus aureus, Escherichia coli C 600* and *Listeria innocuous* (Ghosh et al. 2014; Terjung et al. 2012). Nanoemulsions of Carvacrol, limonene and cinnamaldehyde encapsulated in the sunflower oil droplets have been elucidated to have antimicrobial activity against *Escherichia coli*, *Lactobacillus delbrueckii* and *Saccharomyces cerevisiae* (Donsì et al. 2012). Vitamin E acetate nanoemulsion formulated utilizing the edible mustard oil and Tween-80 has been shown to have improved bioactivity, antioxidant, and antimicrobial activity. This material has potential applications in packaging of fruit juices (Dasgupta et al. 2016) Beverages containing encapsulated functional compounds such as coenzyme Q10, lycopene, lutein, β-carotene, omega-3, vitamins A, D3 and E, phytosterols and isoflavones have been developed by Nutralease (NutraLease 2011). Unilever has developed healthier ice cream with lesser fat content without compromising on taste through the application of nanoemulsions (Unilever 2011).

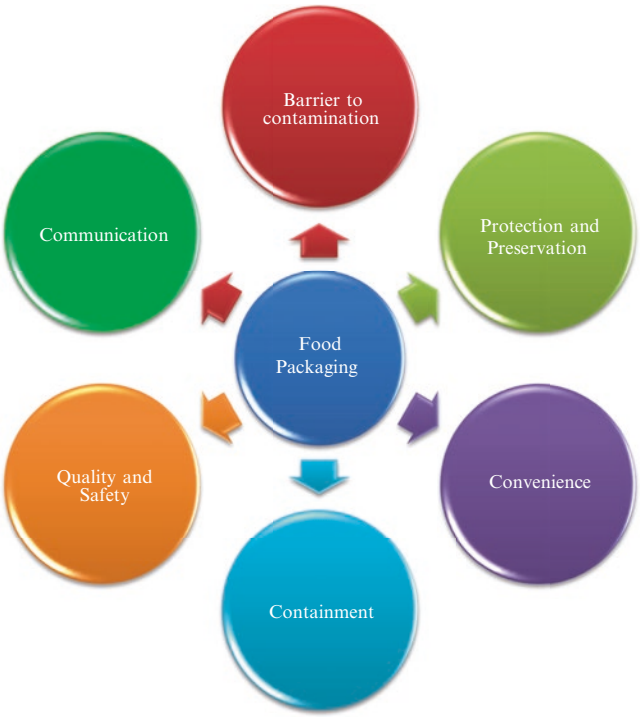

Fig. 2.5 Functions of food packaging

2.4.2 Antimicrobial Nanoparticles

Antimicrobial nanoparticles coating in the packaging material can prevent post contamination by inhibiting the microbial growth on food product. Metallic nanoparticles have been studied greatly in this regards. Silver, Gold and Zinc nanoparticles have been found to have an antimicrobial activity (Ahmad et al. 2014, 2015). Silver nanoparticles are being used for research and commercially to come up with functional food packaging extensively. It is evaluated for meat and meat products (Véronique 2008). Active paper packaging incorporating nanoparticles have been shown to be effective in protection of bakery products (Rodriguez et al. 2008; Gadang et al. 2008), silver oxide nanoparticles (Sondi and Salopek-Sondi 2004), Magnesium oxide and zinc oxide nanoparticles (Jones et al. 2008).

2.4.3 Polymeric Nanocomposites

Polymeric nanocomposites consist of polymer nanostructures combined with a matrix and can be used to generate monolayer films with improved mechanical and gas barrier properties. They contain 2–8% carbon nanoparticles, Nano clays, polymeric resins, nanoscale metals and oxides as nano inclusions by weight (Duncan 2011). Cellulose containing polymeric nanocomposites has been used to increase the tensile strength of the packing material (Othman 2014; Ghaderi et al. 2014).

2.4.4 Carbon Nanotubes

Carbon nanotubes are cylindrical nanostructures of carbon exhibiting a very high length to diameter ratio. They can be applied to enhance mechanical properties of packaging material. They have also been shown to possess an antimicrobial activity as evident by the reports stating the interaction between single walled carbon nanotubes and *Escherichia coli* leads to its death (Kang et al. 2007). Carbon nanotubes in conjunction with allyl isothiocyanate have been shown to reduce the microbial count of *Salmonella Choleraesuis* over a period of 40 days of packaging (Dias et al. 2013). Carbon nanotubes infused polyethylene films were tested over a period of 90 days for packaging of Mazafati dates and was found to inhibit fungal growth, thereby increasing the shelf life; although, a loss of sensory information was also reported (Asgari et al. 2014). Recently, Chemiresistive detectors using single-walled carbon nanotubes with cobalt meso-arylporphyrin complexes have been developed to detect amines produced during meat spoilage (Liu et al. 2015).

2.4.5 Nano Coatings

Conventionally, Waxy coatings have been used increasingly for some foods such as apples and cheeses. Recently, the use of nanotechnology has initiated development of edible nanocoatings which can be used with variety of foods as a packaging material. These nanocoatings have a thickness of 5 nm. These materials have potential to replace the current waxy coatings. Edible coatings are currently used on numerous food items such as fruits, vegetables, meats, chocolate, cheese, candies, bakery products etc. (Morillon et al. 2002; Cagri et al. 2004; Rhim 2004). These coatings could serve the essential functions of packaging by acting as moisture and gas barriers. PlasmaPlus® has developed a nanocoating for applications in soft drink industry. This material is transparent like glass but have better barrier properties such as lesser leakage of CO_2 or diffusion of oxygen as compared to conventional PET bottles (Plasmatreat). Even nanocoatings exhibiting antimicrobial activities are in the pipeline for development. The U.S. Company, Osmotic

Corporation announced in early 2007 that it has developed an edible antibacterial nanocoating, which can be applied directly to bakery goods (El Amin 2007).

2.4.6 Nano Laminates

Nanotechnology provides food scientists with a number of ways to create novel laminate films suitable for use in the food and dairy industry. A nano laminate consists of two or more layers of materials with nanometre dimensions that are physically or chemically bonded to each other. A variety of different adsorbing substances could be used to create the different layers, including natural polyelectrolytes (proteins, polysaccharides), charged lipids (Phospholipids, surfactants), and colloidal particles (micelles, vesicles, droplets). It would be possible to incorporate active functional agents such as antimicrobials, antibrowning agents, antioxidants, enzymes, flavours, and colors into the films. These functional agents would increase the shelf life and quality of coated foods. Researchers have developed a nanolaminate consisting of alternating alginate and lysozyme layers for the protection of Coalho cheese. The coating exhibited the Water vapour transmission rates and Oxygen transfer rate values of 1.03×10^{-3} and 1.28×10^{-4} g m^{-2} s^{-1} respectively. Lower values of mass loss, pH, lipidic peroxidation, microorganisms growth was noted after 20 days as compared to uncovered cheese (Medeiros et al. 2014).

2.4.7 Clay Nanoparticles and Nano Crystals

The barrier properties of dairy and food packaging materials are improved by incorporating as well as embedding Nano clays and nanocrystals. These materials are examples of improved polymers since they only impact barrier and mechanical properties rather than actively taking part in some reactions to increase the shelf life of the product. Clay-based nanocomposite in the packaging material offers improved shelf life, shutter proof, lighting weight and heat resistant (Ravichandran 2009; Ranjan et al. 2014). Nanobiocomposites films containing cellulose nanocrystals and poly (3-hydroxybutyrate) have been shown to exhibit better gas barrier and migration properties which can be harnessed for food packaging applications (Dhar et al. 2015) (Fig. 2.6).

2.4.8 Nano Sensors

Food preservation is one of the important aspects of food packaging industry. This aspect can be governed by continuous sensing of various parameters of food in real time. This is where nano sensors come into picture and can be of great importance.

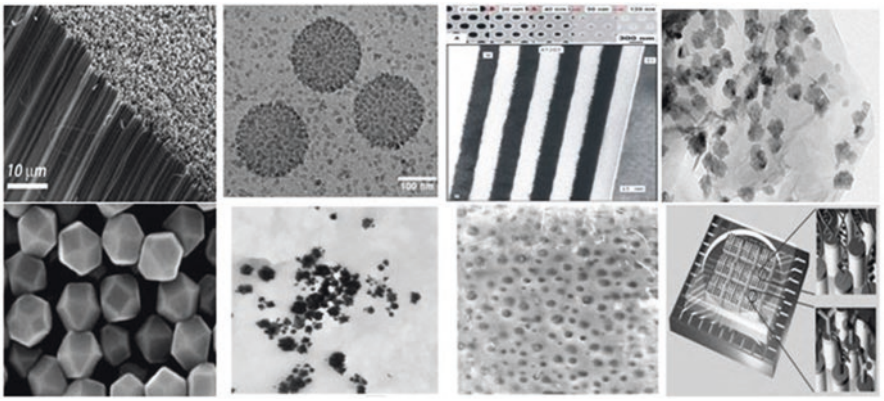

Fig. 2.6 Nanomaterials in food packaging. Clockwise from *top*: Carbon Nanotubes, Nanoemulsions, Nanolaminates, Nanocomposites, Nanosensors, Nanocoatings, Antimicrobial Silver Nanoparticles, and Nanocrystals

For examples, colorimetric changes can be induced into food packaging by incorporation of nanoparticles when food pathogens are encountered. Earlier, the determination of contamination in food was a tedious and a long exercise because the food had to be sent to the laboratory for testing and results could take days. Since, time of incubation is very crucial for growth of pathogens, nanosensors are deployed to reduce the detection time by as much margin as possible. This means that potentially the detection time can be reduced from days to hours or minutes (Bhattacharya et al. 2007). Commonly, the microorganisms responsible for spoilage of food are bacteria rather than fungi or molds. Major indication of spoilage of food is the pungent smell produced either due to decomposition of food or by the microbes themselves. The detection of these pungent odors can also serve as a useful tool in detection of spoilage, since it takes quite lesser amount of time as compared to tests for microbial growth or immunoassays (Warad and Dutta 2005). An electronic nose is one such equipment which can recognize and analyze several different specific components of odor to provide the user with the information regarding the chemical processes taking place in the food. This technology has been around for a long time but with its large size and high price, it usage has been limited. The focus has been on development of smaller, inexpensive and sensitive devices which can be incorporated in the packaging. Such systems consist of several components, namely: Sample handling system, Detection system and data processing system. These systems are modeled on human olfactory systems where sensors are being used instead of brain. These systems can be used for process monitoring, quality and sensory evaluation and assessment of other parameters (Peris and Escuder-Gilabert 2009). This e-nose can be used in food packaging material to detect odors released during rotting. This technology has been utilized for characterization of wines and tea (Di Natale et al. 1996; García et al. 2006; Yu and Wang 2007). The National Academy of Agricultural Sciences, New Delhi has proposed that Nano sensors can be used in

detection of nutrients, contaminants and soil fertility evaluation. Nanosensors can also be constructed using enzymes as major sensor because of their specificity towards ligands. Enzyme inhibition can be used to screen different contaminants although differentiation between different contaminants is rather difficult and non specific, for example it is difficult to distinguish between pesticide and heavy metals. Using this principle, a nanosensor to detect organophosphorus residue has been developed. This sensor utilizes Enzyme linked immunosorbent assay in which antibody is coupled to ferroferic oxide nanoparticles coated with 3-aminopropyl triethoxysilane using chemical means (Hu et al. 2010). Barcodes and other identification measures are an important requirement in food packaging encompassing all the products whether with short shelf life or a longer shelf life one. Development of miniaturized barcodes or tags can be useful for providing general information regarding the product and also with specific information on the basis of bioassays (Warad and Dutta 2005). Nano barcodes using nanostructures have been developed since they provide with lot more permutations, combinations than conventional ones and are durable, machine readable while still being very small in size (Nicewarner-Peña et al. 2001).

2.4.9 Biobased Fillers for Nanocomposites

Biodegradable substances are the materials that are capable of being broken down by microorganisms. Biodegradable polymers are the material in which degradation is assisted is at least in part by the action of microorganism. Under required environmental conditions, biodegradation leads to destruction of polymers without leaving any undue toxic or environmentally unfriendly residues. Depending upon the synthesis mechanism, these polymers can be divided into following classes: Polymers from biomass such as proteins, polysaccharides etc. Polymers synthesized using chemical methods such as polyethylene, PET, polyesters.

- Polymers synthesized from microbial sources such as bacterial cellulose, xanthan gum etc.

Currently, a lot of work is being going on application of various natural polymers conjoined with nanostructures to create biodegradable nanocomposites (Ray and Bousmina 2005). The most researched filler materials include starch, polyhydroxybutyrate (PHB), polylactic acid etc. These biopolymer nanocomposites have been found suitable for packaging applications.

2.4.9.1 Starch and Their Derivatives

Since, starch is available in humungous quantity in nature and can easily be obtained from plants or from current production technologies, is relatively inexpensive, starch and its derivatives have become one of the important materials with regards

to development of packaging technology (Gonera and Cornillon 2002; Vliegenthart et al. 1998). Starch does not have requisite material strength and mechanical properties to be used as a packaging material in an isolated manner, so it is used in conjuction with other material to form a composite having the required properties. It can also be chemically, thermally or mechanically modified to change its packaging properties. (Kim and Pometto 1994). Starch has been used in clay nanoparticles to come up with functional nanocomposites (Avella et al. 2005). Na-Montmorillonite-corn starch nanocomposites have been shown to increase the tensile strength of the material, although no antimicrobial activity was reported for the material (Heydari et al. 2013). Zinc oxide- soluble starch nanocomposites have been developed to provide the material with antimicrobial activity. This was accomplished by using starch as a stabilizer affixed onto cotton fibres and incorporating nano zinc oxide particles into this matrix (Vigneshwaran et al. 2006).

2.4.9.2 Polylactic Acid (PLA)

Polylactic acid is the polymer with major potential in field of bio based nanopackaging. This is because it can easily be produced in large amounts since its precursor, lactic acid, can be easily obtained via fermentation of carbohydrates. Polylactic acid- silver based nanoclay composites have been researched for potential applications as active food packaging. These films exhibited strong biocidal activites and increase in gas barrier. Although the silver from packaging migrated into the food, it was found to be within the limits specified by European Food Safety Agency (Busolo et al. 2010). Cellulose nanocrystals along with silver nanoparticles were infused in polylactic acid matrix to come up with a transparent material with high tensile strength and antibacterial activity (Fortunati et al. 2012). Storage capacity of polylactic acid has also been enchanced by usage of cellulose nanowhiskers. These composites were found to be thermally stable and increased the storage modulus of polylactic acid (Petersson et al. 2007).

2.4.9.3 Polyhydroxybutyrate (PHB)

Polyhydroxybutyrate is a polymer produced by numerous bacteria as a soure of energy reserves. This material is biodegradable and highly compatible with biological systems and hence is an important candidate for industrial applications. Polyhydroxybutyrate-co-valerate interlayers of zein nanofibers have been developed. The mechanical properties and barrier properties of the material was found to be dependent upon the production methodology (Fabra et al. 2013). These packaging are compatible with many foods such as dairy products, beverages, meat products etc.

2.4.9.4 Polycaprolactone (PLC)

The properties of this polymer such as high elongation capacity and easy availability are the major reasons for its commercial potential. It can be used in medical as well as agricultural industry (Nakayama et al. 1997). Polycaprolactone nanocomposites have been developed in conjunction with biocide thymol using casting methodology. These materials can be used as active packaging materials with antimicrobial activities (Sanchez-Garcia et al. 2008).

2.5 Structure and Morphology of Packaging Nanomaterials

The initial packaging material that was introduced into the market was manufactured using clay nanoparticles as a filler material to modify the material strength and barrier properties of the composite. Therefore, detailed analysis is available regarding the structure of these nanomaterials. These materials consist of nanolayers separated by interlayer galleries (Ray and Okamoto 2003; Pissis 2007; Paul and Robeson 2008). The morphological arrangement was found to be influenced by the values of entropy and enthalphy (Vaia and Giannelis 1997; Balazs et al. 1998; de Paiva et al. 2008). On the basis of these values, the different arrangement that were obtained is non-intercalated, intercalated, exfoliated and flocculated. These arrangements were in turn dependent upon the values of interfacial interaction of polymer matrix and filler. Intercalated structures are composed of alternating polymer and clay layers repeated at every few nanometers. When in such structures, the edges of silicate layers interact due to formation of hydroxyl bonds, flocculation of layers takes place and such an arrangement is called flocculated nanocomposites (Ray and Okamoto 2003). Exfoliated arrangement occurs when silicate layers are suspended in polymer matrix and layers are far apart leading to complimentary interactions with matrix (Luduena et al. 2007).

The functional properties of these silica nanocomposites are dependent upon the nature of adsorbing substances used in the nanocomposites (Fig. 2.7). Apart from that, the total number of layers, sequence of the layer and external and internal layer prevalent during formation determine the packaging properties of the material.

Fig. 2.7 Morphological arrangements of silica nanocomposites. From *left*: Intercalated, Intercalated with flocculated and exfoliated (Ray and Okamoto 2003)

Active materials can also be prepared by incorporating substances that provide antimicrobial activity, antioxidant activity, flavours etc. This could lead to a potential increase in shelf life and quality of food products (Weiss et al. 2006).

2.6 Effects of Nanopackaging on Food

Nanotechnology has revolutionized many facets of food industry such as improved texture and flavor encapsulation, nutrient and bioactive delivery system, microbiological control and food processing and packaging. Nanomaterials have the capacity to interact with food components, including biopolymers such as proteins, carbohydrates and fats. Nanoparticles in the packaging could undergo oxidation leading to generation of reactive oxygen species. The polysaccharides possess hydroxyl groups that could react with electron accepting nanoparticles. The proteins and nucleic acids consist of aromatic rings such as benzene aromatic rings that may interact with inorganic nanoparticles with adsorbed cations. The antioxidants, flavours and pigments also contain aromatic ring structures. Interaction with nanoparticles will lead to decrease in the activity of enzymes. The specific proteins that possess sulphur-containing groups like thiol are known to interact with silver and gold. The consistency and sensory properties of food may be altered by a variety of nanoparticles due to the formation of various polymorphic compounds and redistribution and crystallization of fats. Moreover, water and minerals of the food can also interact with nanoparticles. They can change its characteristics since some nanoparticles such as Silicon dioxide can absorb water. The bioavailability of vitamins may be modified on interaction with nanoparticles (Simon and Joner 2008). Thus nanomaterials are capable of reacting with almost all food components but their effects depend on their number and dimensions and it needs further investigation for quality assurance.

The effect of Nano packaging on food is dependent upon the makeup of the material. For example, the polymeric matrix will control the dispersion of nanoparticles, which could lead to change in the properties of nanomaterials. This has become an important aspect of research these days and could be translated into development of commercial products.

Current studies have revealed that nanomaterials can interact with eukaryotic and prokaryotic cells and can induce cell death via cytotoxicity (Nel et al. 2006; Brayner et al. 2006). This could be utilized in food industry to control the degradation and spoilage of food via antimicrobial activity and also it should be taken care that these nanoparticles don't react with the consumers in adverse way. Metal nanomaterials have been found to generate maximum antimicrobial activity and this fact is being utilized in generation of active packaging. The mechanism by which nanoparticles generate antimicrobial activity has not been clearly elucidated but a few mechanisms have been put forward. Nanoparticles may adsorb the nutrients via its large surface area and might starve the cells of essential nutrients (Geys et al. 2010). Food packagings involving silver nanoparticles have already been launched

on the market whereas gold, zinc nanoparticle, metal oxide packaging is in fray (Dekkers et al. 2007). Silver nanoparticles have the ability to decompose ethylene which could facilitate an increase in shelf life of fruits and vegetables (Hu and Fu 2003). Several studies of efficacy of silver nanoparticles as a packaging material in various forms have been studied. Various studies involving nanocomposite polyethylene film with Ag nanoparticles, coating of nanoparticles on surface, absorbent pads containing Ag nanoparticles etc. have been shown to increase the shelf life of different food systems (Li et al. 2009; An et al. 2008; Fernandez et al. 2009). Titanium oxide nanoparticles have been used to retard the growth of *Escherichia coli* in fresh produce (Chawengkijwanich and Hayata 2008). Calcium carbonate nanoparticles have been used to develop a xanthine amperometric sensor to gauge the quality of (Shan et al. 2009). Currently, not much research has been carried out on dairy products and cereal foods and as such it seems reasonable to develop packagings for these sectors next.

Among all the hoopla surrounding the use of nanotechnology in food, a general theme of concern is migration of nanostructures from packaging into the foodstuff and reaching our body. Silver is currently being used more prominently as compared to other materials and as such more data is available on interaction of silver with cellular components (Hansen et al. 2008). The silver nanoparticle affected human lung carcinoma and glioblastoma cells in a dose dependent manner. They cause reduction in adenosine triphosphate (ATP) content, deoxyribonucleic acid (DNA) damage and chromosomal aberrations, increased Reactive oxygen species (ROS) production, thus reporting the carcinogenic, genotoxic, cytotoxic and ant proliferative potential of silver nanoparticles (AshaRani et al. 2008). Clay nanocomposite films can be utilised in the food packaging sector as a passive material owing to their low overall migration limit (Avella et al. 2005). The migration potential of nanoparticles from polymer matrix is yet to be studied in detail for different nanocomposites (Chaudhry and Castle 2011). Still, more research is required for quality and safety assessment of nanomaterials coming in contact with foods.

2.7 Packaging Materials Being Used in Industries Currently

In recent years, nanotechnology has steadily been coming into focus of food packaging industry. Many reports have claimed a gradual increase in the market share of nano enabled packaging applications to the tune of almost US$ 8 billion by 2016. Food and beverage sections are major employers of these packaging systems with bakery, meat products, carbonated drinks and bottled water taking up the largest chunk. Few of these systems have already been deployed, though many are still under development. In Asia-Pacific region, Japan is the leading contender among all other countries in respect to current market share and is expected to grow by a margin of 13% annually. Since, this technology is being mostly deployed in developed countries, it is not a surprise that currently improved and active packaging is being used in United States, Australia and Japan. Meanwhile, the technology has not yet

caught the imagined speed in European countries because of legislative restrictions and also due to lack of public support. Other factors in this regard could be the questions over efficiency of some products and lack of research on environmental aspects apart from the costs involved. Apart from possible exception of use of clay nanoparticles as fillers in packaging applications, fabrication of other nanoparticles such as gold and silver is quite costly, especially when compared to the benefits achieved in the final packaged product. Therefore, the majority of the research is still languishing in the confines of laboratory rather than playing their role magnificently in the industrial revolution. These astounding prospects for advancement will continue to be ignored by the profit oriented commercial packaging industries until the manufacturing becomes more affordable in terms of cost. Even after a promising performance of few nanomaterials inside the laboratory settings, the barrier of industrial applications is being broken quite slowly but surely. A number of industrial giants such as Honeywell, Mitsubishi Gas and Chemical, Bayer, Triton Systems and Nanocor have accepted the changing technology and are progressively applying this technology at large scale. But other than that, generally, there is still reluctance for the global usage on account of variations in the packaging, unfavorable interaction of packaging material with foods and technological bottlenecks in the production of polymer nanocomposites. Major technological questions arise in the pre and post polymerization methods of fabrication of polymeric nanocomposites. Pre polymerization processes often determine the degree of polymerization, which is instrumental in achievement of better yields with good control. The degree of dispersion of the fillers in the nanocomposite is generally dependent upon the post polymerization processes. The optimization of the production conditions is a complicated process, especially when the change in scale of production is taken into account. Taking a technology from realms of a small scale lab preparation to a full blown packaging material is a comprehensive task, to say at least. The requirement of sophisticated instruments and equipments specifically for nanomaterials and limitations in the use of currently existing machinery with these materials constitute a major headache for the industry. Accepting this technology means recalibration of existing technologies and capital investments in the form new equipments, which is not an easy task for an average food packaging facility.

Presently, clay nanoparticles constitute the majority of share in the commercial applications of nanoparticles and account for almost 75% of the applications. Nano clay has been used as an embedded material in fabrication of plastics and nylon films to increase the tensile strength and reduce the gas permeability of the packaging material. This leads to an extension in shelf life of oxygen sensitive food products. Nano clays have also been incorporated in the packaging of beer, carbonated drinks in form of a multilayer film. A low cost nanocomposite using clay as filler as a coating for juice cartons have been developed by Bayer polymers. Nanocor® has come up with a nanocomposite material containing Polyethylenetetraphlate and clay nanoparticles to be used in beer bottles. With this packaging material, the shelf life of beer increased to about 30 weeks as compared to 11 weeks in the regular storage bottles. Other than this, silicon oxide coating has been used on PET bottles by plasma deposition method to come with a 100 nm layer of coating by SIG

Chromoplast P. This packaging material is claimed to increase the life of 350 ml bottle of carbonated soft drinks by three times to almost 25 weeks. These thin coatings can also be used on the surface to beer bottles for extension in shelf life. Clays and silicon oxides are the part of emerging improved packaging, but the sphere in which nanotechnology is expected to make a monumental impact is the active and intelligent packaging. Silver nanoparticles have already been used in packaging to impart antimicrobial properties, for example The Sharper Image FresherLonger(TM) Miracle Food Storage have been infused with 25 nm of silver nanoparticles which results in decrease of bacterial growth by 98% in 24 h. Other companies are using similar strategy to counter microbial growth in the stored foods. These include: Blue Moon Goods in the US, Quan ZhouHu Zeng Nano Technology in China, and A-DO Global in South Korea. All these companies claim results similar to that of Sharper Image. Sinanen Zenomics, a Japanese corporation have commercialized Zeomic, an antimicrobial agent combining zeolite with silver ions to manufacture food packaging films. The three dimensional structure of Zeolites enhances the uptake of ionic silver and forms the basis for antimicrobial activity. This product has been licensed by US FDA, US EPA and European BPR among others. Agion Technologies have come up with an antimicrobial agent consisting of zeolites and silver ions which is being used in a wide range of products by the company to reduce the microbial growth. Nanocor has developed a range of gas barrier resins using Nylon resins to come up with a material exhibiting significantly enhanced gas barrier properties. These can be used as a coating on PET bottles and have been approved for use as a non contacting layer. On the similar lines, a nanocomposite named AegisOXCE have been developed by Honeywell using an oxygen-scavenging nylon which can serve as a coating for the high-oxygen barrier demands of plastic bottles used for beer and other flavored alcoholic beverages. This leads to an increase in the shelf life of oxygen sensitive beverages. These examples incorporating antimicrobial agents in form of silver and nylon as oxygen scavenger are important as the pioneering applications of nanomaterials in food packaging.

The era of smart nanomaterials for food packaging has been initiated with the research on time/temperature indicators (TTI). Major applications are currently limited in absence of technological breakthroughs and convoluted interactions between multiple components which can reduce the accuracy of the measurement. Biosensors using bacterial antibodies conjugated to fluorescent dye have been developed for detection of microbial contamination in foods. Presence of bacteria in the food can be detected with the presence of visible dye instantaneously. Such sensors have been developed for a number of microbial contaminants such as *Staphylococcus enterotoxin B*, *E. coli*, Salmonella sp., and *Listeria monocytogenes* (Liu et al. 2007). One such sensor has been developed by Timestrip to detect a temperature breach as well as duration of the temperature breach. Several allergenic proteins such as nuts, gluten etc. can also be detected using these nanosensors. The freshness of the product can be surveyed on multiple parameters such as amount of pathogens, spoilage, chemical contaminants or tampering. These nanosensors possess numerous advantages over conventional detection techniques such as rapid detection, simplistic techniques, reduction in processing costs and equipments etc. These intelligent food

packaging materials are beneficial not only in terms of contamination detection but they can also provide us with reliable information about various parameters and spoilage of foods in a matter of seconds rather than subjecting the food sample to extravagant testing which require a lot of time and effort. As such, this intelligent packaging can have a dramatic impact in ensuring the food security for the entire world. If conventional technologies are replaced with these intelligent packaging materials, it would reduce time of testing for contamination from 5 to 7 days to few minutes. Moreover, these sensors have no issues with transportation as compared to other bulky equipments employed for detection of microbes currently.

2.8 Advantages of Nanomaterials in Food Packaging

2.8.1 Improvement of Mechanical Properties

Main aim of packaging must be to safeguard food from spoilage caused during shipment and also from contamination caused by external factors. Nanofibers with a high aspect ratio (the ratio of length to width, e.g. > 300) can be used for strengthening the physical properties of the packaging material Accordingly carbon nanotubes and cellulose nano fibers can provide more rigidity in comparison to the usual materials that are being used for packaging. Thus, even a short supply of nanomaterials is adequate to improve the tensile strength of the nanocomposites (Siro and Plackett 2010).

2.8.2 Improvement of Barrier Properties

Packaging of food provide security of food from damaged that can be caused by any external environmental factors and thus helps in maintain the freshness of the product. With the advancement in technology packaging materials with light-weight are being preferred hence, nanocomposites that can provide boosted barrier performance are being used. Vacuum-deposited aluminum coatings on plastic films are being used for packaging snack foods, confectionery and coffee. Even the planes of glass containers are being applied with oregano silane using plasma or other high temperature technology. Newly nanocomposites have been used in such a form that they hindered in the route of products out of the packaged material thus reducing the rate of diffusion.

2.8.3 Active Packaging

Active packaging interacts chemically or biologically with its contents to increase the shelf-life of the product. This packaging remains active even after when the product is packed and reduces food quality deterioration. It is an interactive packaging that is proposed to sense any changes occurring in the internal and external factors and hence changing its attributes accordingly that ultimately change the internal packaged environment. Desiccants are generally used in this technology as they absorb water vapor from the packaged food and even from the packaged headspace that could degrade the product. Even oxygen scavengers are used in this technology that remove or either reduce the level of oxygen form the interior packaged environment. This helps in extending the shelf-life of the product that is being packaged. Nano encapsulates can also be used to discharge additives such as preservatives or colors onto the food surface.

2.8.4 Surface Biocides

Biocidal agents are chemical substances that are used to suppress microorganisms that are harmful for any manufactured product. Thus the main intensions of using these agents are to maintain the hygienic condition without having any destructive effect on food. They can also play an important role in cleaning of equipments. They are also used in reusable containers and inside liners of refrigerators and freezers. Their one time use as a packaging material is unconfirmed. Because of their small-size nanoparticles have a large surface area to mass ratio and when applied with chemicals, they may have a real effect as a biocidal agent.

2.9 Toxicological Effects

Nanoparticles have gained a lot of attention in the recent time because of the ease in the way that they can be synthesized and manipulated. So, they can be used in a variety of fields. It has been predicted that the production of nanoparticles will increase many fold in the coming years and thus their massive production will definitely lead to their environmental exposure. The lack of knowledge regarding the interaction of nanomaterials with environment and at cellular level has become a major hurdle in defining safety protocols for the use of the nanotechnology in food industry. Due to this, different countries and associations in the world are following different safety procedures and standard. This variability means that not all the products in the market conform to same security norms. This has led to a situation where myriad of products utilizing nanotechnology in some form are available in market, with or without clearing stringent control measures and standard testing

procedures. The research over the toxicity potential of nanomaterials in biological systems and subsequent risk assessment has not been exhaustive. In food sector, there are very few studies available detailing these interactions and very few among these are useful in assessment of toxic potentials to these materials (Tiede et al. 2008; Card and Magnuson 2010). Since, most of the measurement of toxicity is done by using the procedures and instruments that have been used conventionally; obtaining information regarding nanomaterials is a tedious task since their bulk properties are different. Therefore, novel or modified strategies should be developed to target this particular problem. Some studies have pointed out the negative effects of nanoparticles on the tissues, such as inflammation, oxidative stress and initiation of tumor formation (Carlson et al. 2008; Ahmad and Kumar 2011). Different parameters of nanoparticles such as its morphology, reaction potential, physical and chemical properties determine the overall properties of this material. Therefore, it is possible that the toxicological properties of a certain nanoparticle have a variation because of the synthesis method used, molecules presents in vicinity and overall conditions. Hence, the risk assessment approach should incorporate all these factors and should be devised for individual cases (Munro et al. 2009).

Generally, during safety evaluation of a new material, its physical and chemical properties are studied to find out the procedures essential for safe storage, handling and disposal (Handy and Shaw 2007). Once these properties have been studied, advanced testing procedures are carried out to determine the toxic potential of the material. Several tests such as mutagenicity test, oral toxicity, dermal toxicity test etc. are carried out to determine the response on cellular structures. Since materials at macroscale have entirely different properties at nanoscale, even the nanomaterials fabricated from conventional materials have to be studied for toxicity. Cui et al. (2005) has studied the effect of single-walled carbon nanotubes on human embryo kidney cell proliferation and found out that nanotubes negatively impacted the cell growth and cell turnover. Similarly, size and morphology of silver nanoparticles have been shown to affect microbial and cellular toxicity (Dasgupta et al. 2015). Such materials exhibiting toxicity may never be used in food industry but it is equally important to appreciate the knowledge gained and as such these toxic effects should be kept in mind during development of these materials. Another important issue while drafting regulations is to consider the possible migration of nanomaterials from packaging to foods. These tests can be modeled upon the existing tests to check the migration of chemical species. This could form the basis of initial risk assessment.

Since these novel materials are expected to have novel properties and novel toxic reactions and mechanisms, some unique outlook on the traditional safety and control procedures is required to study their interactions. Even then, the existing knowledge on the behavior of ultrafine and small particles such as asbestos and aerosols might provide initial pointers for the research (Maynard 2010).

2.10 Environmental and Health Issues

The major issues regarding nanopackaging currently are the effect of these nanomaterials on human health and the environment. Firstly, it's needed to be assessed that whether the migration of nanomaterials in food can have an impact on health and secondly, what will be the implications of disposal of this material on the environment (Barlow et al. 2009). Whether nanomaterials can be recycled and if they can fit the existing recycling modules remains to be seen. Due to the lack of information on these fronts, the market regulations and consumption of this technology has been hindered to an extent (Dasgupta et al. 2015).

There are several aspects to these concerns, some unique to nanopackaging and other general concerns regarding nanomaterials. Lack of knowledge regarding understanding of uptake of nanomaterials orally and impact of any unique byproducts produced as a result of interaction of nanomaterials and food product. Next, there is currently very little knowledge available regarding the acceptable levels of exposure. This problem is actually twofold, in sense that we don't know the acceptable limits to migration of nanomaterials from surface of packaging to food and we don't know how to measure the amount of migration (Tiede et al. 2008). As of now, it is being assumed that because of inherently fixed or embedded nature of nanostructures in packaging material, migration is not an issue and as such doesn't pose any threat to the consumer (Bakoš et al. 2008). Although, the lack of analytical ability to measure this migration means that this supposition cannot be fairly tested. Lack of studies on influence of nanomaterials on environment is a serious future concern. The various factors important in this regards are reuse, recycle and disposal. Disposal is a general concern for all the packaging materials and is not limited to nanopackaging (Tiede et al. 2009). Reuse and recycle of this material is subjected to the properties of the material and their suitability to the currently existing recycling technologies. So, the question is whether these materials can be recycled or should they be disposed or can a middle ground be found. More importantly, it has to be studied how these recycling strategies are going to affect the properties of the materials. It remains to be seen how the mechanical, chemical, biological and toxicological properties will be altered. Size also raises question regarding the conventional technologies, that is, whether the current technologies will be able to adapt to the nano sizes.

Requisite research is needed to alleviate all these concerns. Some problems have to be researched specifically for nanopackagings and others are concerns that are generally related to nanotechnology or packaging technologies. The researches on levels of toxicity and degree of dangers should be addressed before ubiquitous use of this technology is made in the industry.

2.11 Regulations on Use of Nanotechnology in Foods

Existing regulations for use of nanotechnology in food are inadequate to safeguard the consumers but that is only because of lack of knowledge regarding the risks. Different regulatory bodies have sought to recommend stringent and specific standards and protocols but in absence of concrete knowledge, the groundwork laid is insufficient. Specific standards and testing protocols have been recommended by the European Union regulations for food and food packaging for usage of nanotechnology in food applications. Food safety regulations have also been introduced in India but are not sufficient to safeguard the interests of the consumers as well as producers. There are several factors that are responsible for lack of specific regulations, such as:

Because of unique structure and properties of nanomaterials at quantum scale, these materials do not act in the conventional manner and hence do not follow the current guidelines for assessment of chemical products which makes it difficult to standardize a protocol or a procedure. Moreover, toxicity from such materials is a potential problem, since neither the mechanism nor the exposure limits have been defined. (Chaudhry et al. 2008; Miller and van Schaik 2008).

The Food and Drug Administration (FDA) has provided its perspective on nanotechnology on its Web site (http://www.fda.gov/nanotechnology/). FDA has the power to regulate the products rather than technology and as such its website states that:

> This guidance is intended to describe the factors you should consider when determining whether a significant change in manufacturing process for a food substance already in the market:
>
> - Affects the identity of the food substance;
> - Affects the safety of the use of the food substance;
> - Affects the regulatory status of the use of the food substance; and
> - Warrants a regulatory submission to FDA.

FDA has adopted a very open minded and broad stance over the regulations on use of nanotechnology in food by offering to study the context of every particular case and then decide upon the regulations required. The FDA website states the following in this regards; “FDA will continue to regulate nanotechnology products under its existing statutory and regulatory authorities, in accordance with the specific legal standards applicable to each type of product under its jurisdiction. FDA intends to ensure transparent and predictable regulatory pathways grounded in the best available science” (FDA 2016).

2.12 Microelectromechanical Systems in Food Packaging

Microelectromechanical systems (MEMS) consists of mechanical systems and are manufactured using similar microfabrication techniques used to create integrated circuits such as deposition and etching of silicon or non-silicon base. The key to biological applications lies in the features such as analyte sensitivity, electrical responsiveness, temporal control, and feature sizes similar to cells and organelles (Grayson et al. 2004). MEMS can be used to provide quick diagnosis of contaminants and pathogens during food production, processing, storage and handling stages.

The materials for microfabrication of Microelectromechanical systems (MEMS) include silicon glass and polymers. Silicon is the most suited material and its micromachining start with high purity single crystalline silicon wafers. The techniques like wet/dry etching and physical and chemical deposition can be used to make microchannel and layers respectively (Gad-el-Hak 2001). The material glass has suitable features like high chemical resistance, high electrical insulation, high mechanical strength and a broad range of optical transmission. The polymers can also be used for architecture of Microelectromechanical systems (MEMS) as they have dominance over silicon and glass owing characteristics like chemical and biological compatibility, optical transparency, good thermal properties, high electrical insulation and a set of dynamic surface characteristics The most commonly used polymers include PMMA (Acrylic, Plexiglass, Perspex, Lucite), SU-8 and PDMS.

The principle of biosensors is based on the detection of presence of a particular compound in the external environment. It combines a biologically active element with a physical or chemical transducer. BioMEMS are driven towards micro or even nano arrays due to rise in number of analytes and decrease in size. The integration of biosensors and BioMEMS is a major breakthrough in detection technology in biological samples (Bhattacharya et al. 2007). The mechanical detection methods utilize micro fabricated cantilevers and are based on the physical, chemical or biological stimuli affecting the mechanical features of micromechanical transducers and the developing change can be quantified. It can be quantified by using piezo resistive or piezoelectric materials on the surface of the cantilevers (electronic), laser reflecting from the cantilever surface into a quadrature position detector (optical) and many more (Sarid 1991).The micromechanical oscillators coated in common nutritive layers is able to detect active growth of *E. coli* cells within 1 h. Micro-fabricated Uncoated as well as gold-coated silicon cantilevers were functionalized with concanavalin A, Fibronectin or immunoglobulin G and used for the fast quantitative detection of *Aspergillus Niger* and *Saccharomyces cerevisiae*. It can detect the target fungi in a range of 103–106 CFUml-1 and finds application in agricultural diagnostic and food and water quality monitoring (Nugaeva et al. 2005). The electrical or electrochemical detection system make use of measurement of conductance changes associated with variation in the medium iconicity (conductance), current variation at an electrode as a result of redox process (amperometric) and change in electric potential at the electrodes as a result of ions formed by a

redox process (potentiometric) (Spichiger-Keller 2008).The microbial assays via light addressable potentiometric sensors for monitoring hydrogen ion was realized using *E. coli*. This would be suited for routine analysis in the fermentation monitoring and food analysis (Seki et al. 2003).The semiconducting single-wall carbon nanotubes with controlled attachment of the redox enzyme glucose oxidase was found to induce variation in conductance in response to change in pH. Moreover, it is also capable of measuring enzyme activity and uses nanotechnology for its implementation (Besteman et al. 2003). The microfluidic technique for concentrating bacterial cells from a dilute sample, by factors on the order of 104–105 and detection of their metabolic activity by purely electrical means. The impedance-based detection at the microscale drastically reduced detection times for dilute bacterial samples, being able to efficiently concentrate and capture the cells in an extremely small volume (Gómez-Sjöber et al. 2005). The benefits of biochemical and chemical sensors include fast measurements and high sample throughput, versatility, ease of use, reagent free or reagent poor operation, front-line analysis, low waste, continuous measurements, no consumption of the analyte, enzymatic turnover of the analyte, disposable or exchangeable elements and consumer friendliness (Spichiger-Keller 2008). Microelectromechanical systems (MEMS)-based technologies on integrated circuit chips are extremely cost-effective and offer benefits in food and agriculture industry. The Dairy industry could profit from MEMS as it could help save money and improve productivity per cow and improve quality of products. Current detection methods for mastitis in dairy cow are slow and require manual sample preparation. Real-time early detection could save a large percentage of mastitis-related costs, as well as produce a higher-quality product that may even result in longer milk shelf life. The permittivity based sensor would also be used for real-time sensing of pathogens in milk. Furthermore, the hyperspectral imaging systems based on Microelectromechanical systems (MEMS) can detect certain bandwidths of light from the infrared to the near-infrared range. This system can be used to detect areas in a crop field that may be infected with various fungal diseases or that require irrigation. The food spoilage can also be prevented through early visualization of conditions not apparent to the naked eye. The counterfeit wine labels can also be detected using hyper spectral imaging system (Smith and Gottfried 2008).

2.13 Nanoelectromechanical System

Nanoelectromechanical System (NEMS) devices are a class of devices integrating electrical and mechanical functionality on the nanoscale. It is logically miniaturized microelectromechanical system. As their name suggests they are having dimensions in nanometer range. In the food-analysis market, Nanoelectromechanical System (NEMS) are already in use and might serve as developing tools in food preservation. They can act as active sell by devices and control the storage environment of the packaging system. NEMS systems technology was used to detect trans-fat content

in foods via digital transform spectrometer (DTS) produced by Polychromix. Nanoelectromechanical System (NEMS) because of advanced transducers could be used in food quality-control devices for specific detection of chemical and biochemical signals. NEMS are suited for the food safety and quality because of their ability to detect and monitor any adulteration in packaging and storage conditions (Canel 2006).They are advantageous to food technology as possessing features like portable instrumentation with quick response, low costs, and smart communication through various frequency levels. The principle of nanocantilevers is based on their ability to detect biological-binding interactions, such as between antigen and antibody, enzyme and substrate or cofactor, and receptor and ligand, through physical and/or electromechanical signalling (Hall 2002). Nanocantilevers proved successful in the detection of contaminant chemicals, toxins, and antibiotic residues in food products (Frómeta 2006).

2.14 Nanomaterials in Electronic Packaging

The era of nanotechnology is slowly but surely making its way across the threshold of conventional technologies. The field of electronics can already be considered being into the nano era since the size of integrated circuits reached below 100 nm. With the 22 nm complementary metal-oxide semiconductor (CMOS) in development, nanotechnology has well established itself into the field of electronics.

The consistently increasing demand for miniaturization, high performance at a low cost for microelectronics has considerably shifted the attention towards packaging technologies, system on chip (SoC) and system in package (SiP) being the premier among them. 3D system packaging is the only way to further reduce the volume and bulk of the microelectronics products available today and this is where nanotechnology comes into picture. The aim of nanopackaging is to develop nanoscale passives, interfaces and interconnections, which can be compressed into highly miniaturized systems. The standard definition of nanopackaging, according to IEEE can be considered as following: “Nanopackaging can be defined as the process of interconnecting, powering, cooling, and protecting the nanocomponents made of nanomaterials to form electronic and bioelectronic systems for greatly improved functionality and cost.”

Over last 60 years, there has been exceptionally improvement in the speed and reliability of the microprocessor. According to Moore’s law, the number of transistors in semiconductor devices or integrated circuits (ICs) would double approximately every 2 years. This has more or less held true for the active components of integrated circuits (ICs), but passive components has been unable to follow the miniaturization trend completely because of issues with material development and fabrication technologies. The different issues with passive components and interconnect technology has been main hurdles in achievement of miniaturization of devices. In the subsequent part, the various issues concerning both these technologies and the materials developed for resolution of these issues have been discussed.

2.15 Materials Used in Nanopackaging

2.15.1 Carbon Nanotubes (CNTs) in Nanopackaging

Carbon nanotubes are promising materials that can improve the overall properties and performance of electronic devices at nanoscale. Carbon Nanotubes possess certain excellent properties like extraordinary mechanical, electrical, thermal and electrochemical properties that have given rise to several possible applications: field emission devices (Fan et al. 1999), electronic circuits, devices, and interconnects (Naeemi et al. 2005), nanoscale sensors and batteries (Endo et al. 2000), separation membranes (Che et al. 1998), super capacitors, drug delivery systems (Pastorin et al. 2006), and composite materials—both polymeric and metal matrix filled. Recent research has been focused on fabricating carbon nanotube metal matrix and polymer-based composites. By introduction of carbon in carbon based composites the properties has been improved to great extent but large scale manufacturing is still an uphill task.

Carbon nanotubes (CNTs) have to overcome certain shortcomings like to prepare a homogenous composite, uniform dispersion of the tubes, agglomeration and better quality. Ratio and the composition of various compositions of carbon nanotubes (CNTs) during fabrication along with a sound technique have provided better results. When using these carbon nanotubes for electronics, proper bonding would result in better electron transfer and interaction between the two materials. Strong bonding can also achieved by good mechanical strength.

Carbon nanotubes (CNTs) as Filler Materials – In near future, carbon nanotubes can replace metals like copper in electronic packing (Wu 2004). Carbon nanotubes can be coupled with copper, epoxy composites, solders, and solder joints for various packaging applications. In copper electromagnetic resistance can increase without having a compromise with its conductivity. The various parameters which could decide fate of carbon nanotubes in interconnected applications possibly are: density of carbon nanotubes in the area, its interaction with copper and nanotubes orientation in the matrix. Solder joints – Homogenous inclusion of the carbon nanotubes in the solder joints hasn't only affected the electrical properties but also the thermal and mechanical properties, since shear strength increases as result of this inclusion (Bal and Samal 2007).

2.15.1.1 Carbon Nanotubes Fabrication

Synthesis of MWCNTs – In the reaction chamber, graphite is used as a substrate in the vapor mixture of benzene and hydrogen which is then made to react. After a series of high temperature treatments, finally MWCNTs are obtained in relatively smaller yields as compared with to that of arc discharge method.

In arc discharge method, two graphite electrodes are placed with an inert gas. Then an arc is struck between these two electrodes and finally MWCNTs of better yield and good quality are produced.

In electrochemical growth method, the MWCNTs produced suffer with defective walls and innermost cylinder consists of chemical remnants (Morris 2006).

In the catalytic method, due to nucleation, the fiber-like growths are produced over the surface of substrate. Here the catalytic particles contained the pretreated substrate and final reaction is initiated after exposing these to temperature treatment in chamber.

(i) **Arc Discharge and Laser Ablation-** Arc discharge and laser were the pioneer methods used for the bulk synthesis of SWNTs in relatively large (gram) amounts (Dai 2002a, b). Both of these methods share some similarities like condensation of hot gaseous carbon atoms produced from the evaporation of solid carbon. For the fabrication of single-wall tubes, a metal catalyst is also required in the arc-discharge system. In this process, two grapheme rods are placed at low pressure (between 50 and 700 mbar) with inert gas filled environment. Electrodes are kept at different potentials for spark generation and distance in between them 1 mm during the process which is about 1 min. After cooling and depressurization the CNTs are collected. For preparation of high quality of CNTs laser ablation method is used. Here intense laser is used for the ablation of carbon target placed in furnace at 1200 °C (Guo et al. 1995). During the process some inert gases carry the synthesized nanotube to the copper collector. Intense laser pulses ablate a carbon target which is placed in a tube-furnace heated to 1200 °C. During the process some inert gas like helium or argon flows through the chamber to carry the grown nanotubes to the copper collector. After the cooling of the chamber the nanotubes ablation and the by-products, like fullerenes and amorphous carbon over-coating on the sidewalls of nanotubes can be collected. After the cooling, the CNTs are collected along with by-products like fullerenes and amorphous carbon over-coating on the sidewalls of nanotubes. If the pure carbon is used as substrate in both of the methods (Arc Discharge and Laser Ablation) then SWNTs can be prepared. Since, both of the process takes place at a very high temperature so certain part of product is lost. Product is produced in the form of powder and amount of purification is quite high, although controlled synthesis of the CNTs is not possible by these methods (Fig. 2.8).

(ii) **Chemical Vapor Deposition-** In 1993, first time the carbon annotates was synthesized through CVD method. During the process the substrate are covered with metal catalysts, like nickel, cobalt, iron, or a combination at 700 °C. Finally growth starts with the passage gases like gas like nitrogen, hydrogen or argon, and some hydrocarbon gas like acetylene (C_2H_2) or methane (CH_4) (Isaacs et al. 2010). after the conversion of carbon reaches about 90%, carbon annotates are collected.

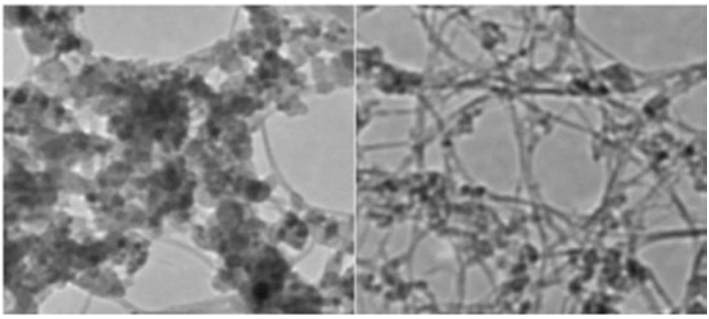

Fig. 2.8 Comparison of SWCNT produced by Arc discharge (*left*) and laser ablation (*right*) synthesis of singlewalled carbon nanotubes (Hornbostel et al. 2006)

(iii) **Pulsed Corona Discharge Method-** In corona pulsed discharge method, there is an electrode with high DC voltage which in turn is surrounded by the carbon containing gas (mostly methane) and finally an electric discharge is produced at atmospheric pressure is produced. In this method, pulse voltage should be ($\leq$7 kV) with pulse width 12 μs at a repetition rate of about 1 kHz. Here successful production of hydrogen and CNTs is due to high voltage electric field near the surface of the inner electrode (Morris 2006).

(iv) **Electrochemical Deposition of CNT-Cu Composites-** Electrochemical Deposition of CNTCu Composites is an ideal method to fill the TSVs with Cu-CNT composite for reducing cost and low technology handling issues. Cu-CNT composite can be used for filling the TSVs, which in turn reduce its cost of production. The technology serving this purpose is co-deposition of CNTs with any metal at room temperatures through electrochemical deposition (Aryasomayajula and Wolter 2013). CNTs are hydrophobic in nature which may be a hurdle for uniform distribution in water based solution. Various surfactants are used for solving above problem. Surfactants like Cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), Octadecyltrimethylammonium bromide (OTAB), and other cationic surfactants which introduce a positive charge on nanotubes and help in preventing flocculation. Functionalizing the nano surfaces with the group like COOH, OH by using different ratio of acids opens the cap of nano tubes. This is a way of electochemical deposition during, which breaking of van der Waals force takes place due to prevention from agglomeration and covalent bonding is strengthened between filler and matrix. There is a novel concept of electro co-deposition, in which copper was deposited in the presence of an external magnetic field to orient the nanotubes as they deposit. After evalution, it was found that nanotubes exhibit very good thermal and electrical properties in the radial direction of orientation along with good mechanical in axial direction.

2.15.1.2 Electrical Transport in Perfect Nanotubes

Electrical transport properties of carbon nanotubes have created many possible applications of the nanotubes in nanoscale electronic devices. The CNTs are nearly perfect 1D conductors and many mesoscopic phenomena such as single-electron charging, resonant tunneling through discrete energy level has been observed at low temperature (Popov 2004). Tunnelling conductance in CNTs shows temperature dependent power law suppression at high temperature. Due to optical or zone boundary phonons, there is scattering mechanism in metallic tubes. At low temperature, new phenomena may be generated due to the scattering and coherent backscattering processes. Transport experiments consisting of two- and four-probe measurements on individual MWNTs SWNT bundles, and isolated SWNTs. There was logarithmic decrease in the concductance with decreasing temperature and saturation below T 0:3 K was during the resistance measurement of an individual MWNT down to T ¼ 20 Mk (Langer et al. 1996). Magnetic field was kept zero during whole process. A pronounced and positive magneto resistance was when a magnetic field, perpendicular to the tube axis was applied. With weak 2D localization, temperature dependence of conductance was observed in the presence of magnetic field.

(i) Electrical transport through junctions- Since CNTs are sensitive on aspect of their electronic properties which is dependent on their structure, they are perfect candidate for preparation of for preparation of metal-semiconductor, semiconductor-semiconductor and metal-metal junctions. The chirality of the two CNTs should be seamlessly or same otherwise chirality of CNTs network can be defective leading to defect in its electronic properties (Dunlap 1992).
(ii) Another method to realize electrical transport through junctions consists of chemical doping of segments of the nanotube. Nanotube junctions are generally constructed from Y-branched nanotubes (Fuhrer et al. 2000) or from crossed nanotubes. It is a proven application of CNTs in fabrication of nanoscale devices.

2.15.1.3 Mechanical Properties

Due to carbon-carbon sp2 bonding carbon nanotubes (CNTs) are very stiff in nature and possess high axial strength. It requires a detailed study of elastic response, the inelastic behavior and buckling, yield strength and fracture of carbon nanotubes for their application. Theoretical studies and experimentation have been going on for exploring the full potentials of these properties (Popov 2004)

2.15.1.4 Thermal Properties

Phonon based determination of the specific heat and thermal conductivity of carbon nanotube systems is done. There is phonon domination at low

temperature in these properties and is due primarily to acoustic phonons. Below room temperature and above 1 K measurements yield linear specific heat and thermal conductivity (Lasjaunias et al. 2002).

2.15.1.5 CNTs as Field Emitters

Exploiting the nanometer scale radius of curvature, carbon nanotubes are used as field emitter arrays in displays. During their application as the enhancement emitter, emission can be obtained at very low threshold potential due to large enhancement factor (Bandaru 2007). So this high sensitivity towards electrical fields can be employed in the, in gas ionization sensors, vacuum microelectronics and X-ray generation (Yue et al. 2002).

Advantages of carbon nanotubes in cold cathode devices are:

(i) Instantaneous turn-on,
(ii) High power,
(iii) Low control voltage operation, along with long lifetimes and miniaturization.

Two of the important advantages are low power consumption and operation at room temperature as compared to other processes like emission, field emission induced.

2.15.1.6 Bio-chemical Sensors

With ample of desirable advantages in the properties, carbon nanotubes has allowed their uses in fabrication of Bio-Chemical Sensors. These have high carrier mobilities and chemical sensitivity, high sensitivity towards electrical fields which can be potentially used to detect changes in the surrounding electrochemical potential.

SWNTs are chemically inert which creates a hurdle for the covalent attachment and physisorbtion/noncovalent attraction (Dai 2002a, b). Surfaces of carbon nanotubes can easily be functionalized or manipulated for achieving proper chemical sensing and specificity by appropriate methods. For example, by using the size and hydrophilic effect, the helical protein known as streptavidin which is used in many biochemical assays can be crystallized over carbon nanotubes (CNTs) surface. Carboxylic acid can also be attached on hydrophilic nanotube surface by preventing the protein attachment which is prerequisite for analyte binding (Star et al. 2003). There have also been attempts to anchor a bio-receptor molecule to nanosurfaces. Analyte can cause depletion/accumulation of carriers in the channel, resulting in the electrical property change through chemical gating in the FET (field-effect transistor) without having gate. Sensor using such properties of carbon nanotubes has been

used in both gas and liquid environments. A gas sensor with semiconducting SWNTs and having a three orders of magnitude change in the electrical resistance was observed during its exposure to oxidizing and reducing gases such as NO_2 and NH_3 with a sensitivity of 2 ppm and 0.1% respectively.

That sensitivity was further explored during various sensor based analyzations (Bandaru 2007).

Future prospects -As the dimensions are reduced to great deal, it gives rise to new physical effects mainly based on the quantum mechanics considerations apart from classical electromagnetic phenomena. In near future heterogeneous metal-semiconductor junctions or homogeneous metal-metal/semiconductor-semiconductor junctions can be fabricated, since carbon nanotubes can be synthesized both as metallic and semiconducting forms and this can be greatly exploited for future device design. Potentials of SWNT field effect transistors in biosensing can engender new applications according to their chemical and mechanical stability. In Field emission displays through high density integration of carbon nanotubes, extensive application can be developed. Through optimal harnessing of various sensitive electrical phenomena exhibited by carbon nanotubes, multifunctional devices for autonomous sensing powered by thermoelectric effects can be developed. Silicon technology had proved to be very reliable and adaptable and carbon nanotubes can be grown in integration with silicon CMOS and as flexible polymer substrates, so carbon nanotubes easily be embraced into the Silicon fold as another nanostructured material. Novel techniques and methods along with different architectures should be developed for exploring maximum possible applications.

2.15.2 Nanowires

A nanowire (NWs) is a nanostructure, having the diameter below 100 nm. Another definition can also be defined as the ratio of the length to width being greater than 1000. Alternatively, it can be defined as structures with thickness or diameter constrained to tens of nanometers or less and an unconstrained length. At nano scale, quantum mechanical effects come into play, so they are also called wires, since the conduction phenomenon takes place. Common wires are constructed from metal rods while nanowire cannot be produced by wiredrawing and it does not necessarily comprise of a metal or one single material. Rod-like colloidal structures are normally mentioned as nanowire in certain literature. They should rather be regarded as rods or crystal needles if they consist of metals.

Over a decade or more, parallel developments and advances in the synthesis of 1-dimensional nanowires (1-D-NWs) by “bottom up” method with precise control on the chemical compositions, morphologies (Boston et al. 2014), and sizes which contributed their fabrication in to various nano devices NW Field Effect Transistors (NWFETs), LEDs, complimentary inverters, complex logic gates, lasers, and chemical sensors. Now the CMOS has been scale down to nanometers along with

maximum benefit at this size. These efforts have motivated the cost effective integration of NWs in devices and circuits for electronic as well as optoelectronic applications.

2.15.2.1 Fabrication of the Nanowires

Initially, Possin described the synthesis of the nanowires by the metal deposition inside etched tracks of high-energy charged particles in mica. This method was also used to form NWs in track-etched polymers too (Possin 1970).For the production of nano wires, exo-templates play an important role by serving as the scaffold which remains after metal filling by the formation of composites (Hulteen 1997), e.g., dipole storage devices. If the exo-templates are dissolved then suspended single wires or more complex metal nanostructures are formed. Anodic aluminum oxide (AAO) and tracketched polymer membranes (TEM) are most used exo-templates.

(i) **Anodic aluminum oxide (AAO) template method-** Most of the manufacturing techniques applied is expensive and time consuming processes. In this situation, anodic alumina templates based method of nano wire synthesis is a promising alternative (Javey et al. 2004). Here controlled geometrical features serve as 3D masks in various deposition procedures and are also easy to construct in house. AAO are used as the mould during the electrodeposition which is a common technique for nanostructures fabrication (Fig. 2.9). From this technique nano wires with high aspect ratio (Gelves et al. 2006) and nano materials with layers of anisotropically conductive or magnetically polarizability in the dielectric matrices has easily prepared. AAO has high stability so NWs of metals with the low redox potential can produced by high pressure filling with molten metal's (Morris 2006).

(ii) **The colloid-chemical approach**- Homogenous nano materials can be produced in the salt solution with low aspect ratio. The nano wires diameter up to the 15 nm and length to several micro meters can easily be produced from this approach (Vasilev et al. 2005). Nano rods produced through seed mediated method can be subjected to elongation by attaching the seeding nonmaterial with the solid support. Rod like micellar template like cationic surfactant CTAB can be used for the production of suspended cylindrical gold during this process (Jana et al. 2001).

(iii) **Photolithography-** Classical photolithography like deep UV lithography (Goolaup et al. 2005; Gubbiotti et al. 2005) colloid mask/nanosphere lithography and modern technologies, with nano imprint (cold) lithography (Cheung et al. 2006) which is the most developed one among them, all have generated metal NWs directly on planar substrates. During top-down approach, photolithographically generated spaces in the resist layer can be filled while producing the stretched wires (Fig. 2.10). Step-edge lithography (SEL) (Prober

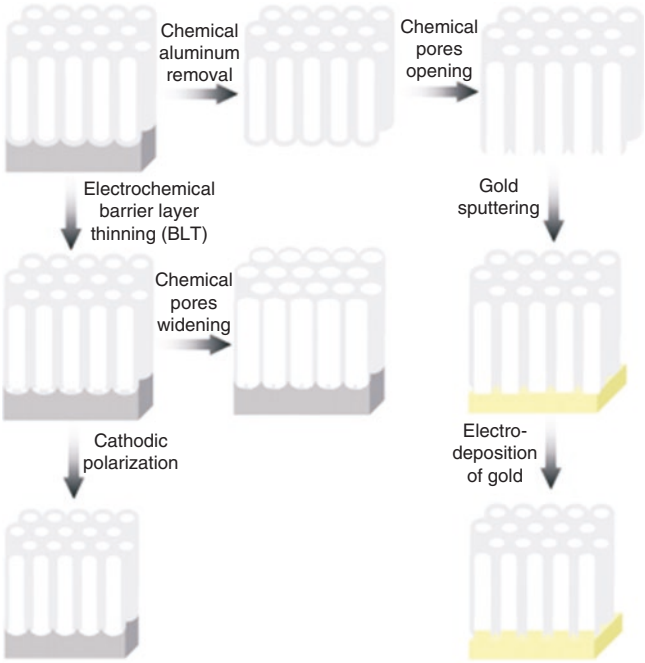

Fig. 2.9 Various approaches in preparation of AAO template on metallic electrode for fabrication of nanowires (Stepniowski and Salerno 2014)

et al. 1980), which is a Mask fewer alternatives based on substrate steps to fabricate wires by deposition. Principles of this can produce molybdenum NWs having 15 μm diameter and length up to 500 μm.

(iv) **Self Assembly**- There are certain chemicals which have property of supramolecular assemblies or self-assembling in a particular environment (Fig. 2.11) e.g. Hydroquinone in aqueous solution can act as exotemplate, self assembling itself to chessboard-like arrays of very narrow rectangular pores (Landman et al. 2000). These pores have been used as silver ion reducing templates to produce stable NW arrays of 0.4-nm in diameter with micrometer in length (Hong et al. 2001). Bioparticles e.g., tobacco mosaic (Nam et al. 2006) virus along with molecular endo-templates, characterized by inner (bio) molecular scaffold like proteins, lipids, and DNA are also alternatives. Fabrication through this method explores the advantages of inherent 3D self-assembly and directed labeling (Morris 2006). Under natural stimuli complex, Nanotubular networks can be generated on artificial substrates followed by gentle metal. Such an approach has been used in case of for DNA and lipid tubules.

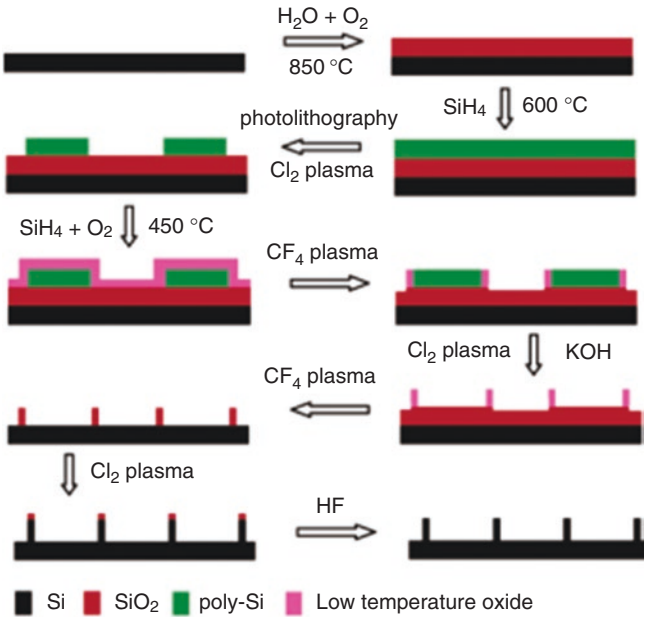

Fig. 2.10 Schematic of size reduction lithography (Choi et al. 2003)

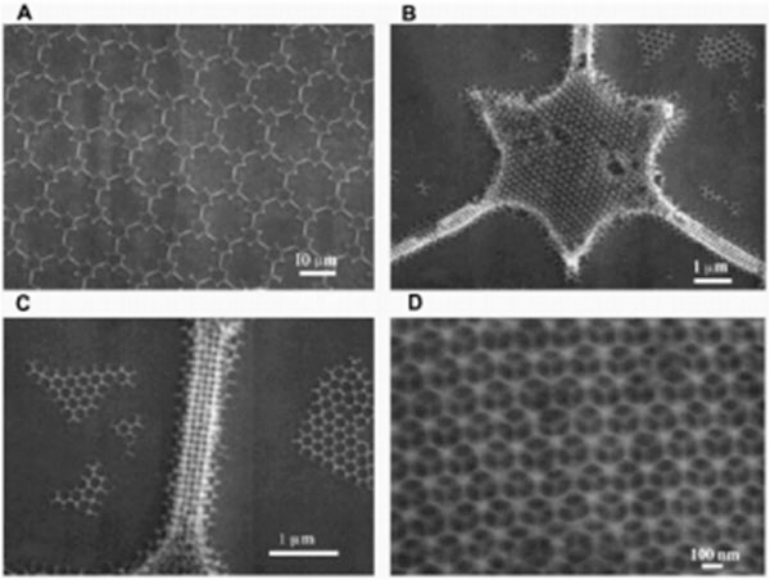

Fig. 2.11 Scanning electron microscopy images of colloidal self assembly (Parviz et al. 2003)

2.15.2.2 Properties of Nanowires

Optical Properties for measuring the electronic structures of nanowires, optical methods can be a fruitful aspect since the measurements are sensitive to quantum confinement and require minimal sample preparation in case of optical measurements. The wavelength of light used during sample characterization is usually smaller than the wire length and larger than that of diameter, so light used during the operation probes both sample and substance on which it rests (if the wire is embedded in the template). That is why even though optical properties have been proved to be an important tool characterizing nanowires; the interpretation of these measurements is not always straightforward The Dielectric Function Here effective medium theory can be used for optical properties of nanowires with diameter smaller than the wave length of light, noting that observable optical properties of materials can be related to the complex dielectric function (Aspens 1982). Effective medium theories have been successfully applied to model the nanowire and substrate as one continuous composite with a single complex dielectric function. This theory is based on shift in the plasma frequency in according to the percentage of nanowire material which is contained in the composite under observation.

Optical Properties characterization Variety of methods and techniques are available for the characterization of nanowires, to distinguish their properties from their parent bulk materials. One of the differences in properties is related to geometric differences like the small diameter size and the large length-to diameter ratio (also called the aspect ratio), while another focuses on quantum confinement issues.

The most basic optical technique for characterization of nanowires is the infrared spectra, in order to measure the reflection and/or transmission of a nanowire to determine the frequency dependent real and imaginary parts of the dielectric function. This technique has also been usefully deciphering the band gap dependence or relation in gallium nitride nanowires in 10–50 nm range in comparison to bulk values (Lee et al. 2001) since Hall Effect measurements cannot be made on nanowires.

Photoluminescence (PL) or fluorescence spectroscopies are widely used techniques for direct probing nanowires while neglecting the effect of host material. Properties like the optical gap behaviour, oxygen vacancies in ZnO nanowires (Zheng et al. 2002), confinement effect in INP nanowires (Gudiksen et al. 2002), and strain in Si nanowires (Lyons et al. 2002). One of the limiting factors in the resolution of photoluminescence (PL) optical imaging of a nanowire is wavelength of light, so the sample is placed very close to the detector and hence there is no chance for light to diffract of light can be resolved. Through above techniques, samples much smaller than the wavelength of light can be resolved. This technique is called as near-field scanning optical microscopy (NSOM) (Johnson et al. 2001) and has been used to successfully characterize the nanowires. For measurement of electronic band structure and determining the magnetic properties of nanowires in relation to bulk properties magneto optics has been successfully applied.

Electrical properties Electrical properties, including resistivity and temperature coefficient of resistance, were characterized for Cu nanowires with a width range of

90–330 nm (Huang et al. 2008). Results showed that the electrical properties were influenced by size and charge and size. Resistivity was size dependent having good agreement with the theoretical models. It was also demonstrated that as the diameter of NWs decreased values of the temperature coefficient of resistance also reduced. Current carrying capacity of nanowires are limited as compare to the CNTs. reliability has been demonstrated for board level interconnects by copper deposition in etched ion tracks (Lindeberg et al. 2003). More accurate current measurements of individual metal NWs has become accessible by the single pore etching and plating. Nanoindent contact has been applied on AAO templates (Fusil et al. 2005) which were electrochemically filled and whose diameter had been reduced up to 100 nm through polishing, use in systemic wire measurement. By above adjustment, charge up to 109 A/cm^2 could be injected into single Co/Cu NWs which in turn useful in studying magneto transport properties while other measurement techniques faces hurdles like contact resistance.

2.15.2.3 Mechanical Properties

Thermal Stability Due to the large surface area-to-volume ratio in nanowires and other nanoparticles, the thermal stability of nanowires is different from that of the bulk material. Latent heat of fusion and the melting point of the material are reduced in nanostructures. Above properties had been demonstrated in three types of nanowire systems: porous matrices impregnated with a plurality of nanowires, individual nanowires sheathed by a thin coating, and individual nanowires. Through differential scanning calorimetry (DSC) study of the melting freezing of matrix-supported nanowires had been performed.

One can perform the study of melting and recrystallization of individual nanowires by using sheathed nanowires because shell layer provides confinement for liquid phase within the inner cylindrical volume. During the melting of nanowires, there was the disappearance of the electronic diffraction pattern. The melting started from ends and advanced to the centre followed by increase in the temperature. During the cooling the recrystallization of nanowires takes place while following super cooling.

In case of free standing wire like copper nanowire, there is least interaction between surrounding and nanowire and their diameter isn't confined as in case of sheathed nanowires. During the thermal treatment, there is fragmentation of wires into linear array of metal spheres. This temperature response was result of Rayleigh instability which lead the nanowires to long cylindrical segments and finally to more spherical at higher temperatures. Here thinner nanowires are more vulnerable towards these transformations at higher temperature. According to the above observations annealing and melting are surface phenomenons which are dominated by the surface diffusion of atoms on the entire surface. Manipulation of Nanowires for indulging the nanowires in applicative process there should certain manipulations through contact free techniques by placing them one by one and not individually, since these are very sensitive. Examples of contact free delicate handling principles

are magnetophoresis, electrophoresis, dielectrophoresis, capillary forces, and interaction with focused laser light (laser tweezers), often in combination with hydrodynamic streaming. Gold nanowires with 70–350 nm in diameter has been assembled in the electrode gap through positive electrophoresis (Fusil et al. 2005). Magnetic field (Hangarter et al. (2007) have also been used to arrange and assemble the nickel nanowires in parallel. Surfactants can also participate in the assembly of nanowires.

Nanowire Interaction with Electromagnetic field because of high aspect ratio of nanowires, their resonance shift can be observed but the dimension of those should less than one fourth wave length of the external field. Non linear behavior of nanowires in light or high frequency magnetic field can be used for generating active and passive photonic structures (Schider et al. 2003). There is also possibility of NWs to produce stop-band filters or antenna structures. NWs can be applied in new integrated optical devices due to their negative magnetic permeability and dielectric permittivity in the visible and the near-infrared on the parallel pair's arrangement in a matrix (Morris 2006).

Magnetoresistance of single nickel nanowires studied at the 10–300 k showed its dependency on size (Rheem et al. 2007). Different values of transverse and longitudinal magnetoresistance were obtained as compare to thin layer when diameter was altered. Above results open new ways for NW arrays for new memory device architectures.

2.15.2.4 Future Aspects of Nano Wires

Recent success in microelectronics applications has opened the new gateway of packing. Modern packaging in the field of photonics, optical waveguide and fibre integration, (bio) microfluidics joining, thermal management, wire-, wafer-, and flipchip bonding, soldering and encapsulation has not only proved its utility on the basis of performance but sustainability has also has a great role too.

Due the unique properties and technology of nanowires photo detectors (NW PDs) physics and technology of nanowires photo detectors offer numerous insights and opportunities for nanoscale optoelectronics and photo voltaic. With the help of direct growth and transfer-printing techniques these nanowires photo detectors has been successfully integrated to CMOS-compatible substrates and various low-cost substrates. This enhances and facilitates the entry of this technology module in the semiconductor foundries (Logeeswaran et al. 2011).

Biophysics and cell biology Nano topographics are known to trigger biological process like phagocytosis and cell differentiation. Through nanowire arrays traction force generated by moving animal cells can be accurately measured (Dalby et al. 2004). The influence of substrate adhesion when cells were applied on gold nanaolawn was studied in cooperation with cell biologist (Katsen-Globa et al. 2006). The interaction of the cell surface with micro electronics important for neuroprosthetic device development which another area importance for microelectronic packaging. In Chemistry-NWs, depicted as nanoelectrode ensembles, have also

been evaluated for applications in the field of analytical chemistry along with applications in wet chemistry and gas analysis

2.15.3 Nanocomposites

A nanocomposite is a matrix to which nanoparticles have been added to improve a particular property of the material. Nanocomposites provide the potential benefit for high speed, high density miniaturized advanced packaging. These are very unique materials with a range of promising applications which is attributed to their small dimensions, excellent physical and chemical properties along with remarkable strength.

Many nanocomposites have been considered for electronic packing applications by using a printable polymer where different active filler can be used in the same functional polymer. Emphasizing on large scale production, there is scope of improvement in terms of flexible and cost effective printable processes, low processing temperature and materials. Printing processes have several advantages such as selective deposition, repair and re-print capability. However, printed features with desired properties, thickness and tolerance present significant challenges. For electronic packaging of nanocomposites, ink-jet printing, screen printing and contact printing are few of the common technologies. Concentration of the nanocomposite and its viscosity plays a vital role in printing process. Ink jet printing prefers low viscosity range from 7 to 10 cp while screen/contact printing prefer higher viscosities thixotropes (100,000–150,000 cp) with thickness of 10–25 micron. Conducting composites favor ink-jet printing for transistors but can use screen/contact printing for making lasers which is a surface phenomenon. Ink-jet or screen printing can induce different features in embedded resistors, capacitors, and conducting circuit lines.

2.15.3.1 Fabrication of Nanocomposite

There are two groups of polymers that have been used as polymer matrices in the synthesis of nanocomposites. These are non-conducting polymers and intrinsically conducting polymers. Examples of conducting polymers which have been used in nanocomposite fabrication are polyaniline (PANi), polypyrrole (PPy) and poly (3, 4-ethylene dioxythiophene) (PEDOT). Conducting polymer nanocomposites are usually prepared by in-situ polymerization where the monomer is chemically oxidized in the presence of the filler (Gangopadhyay and De 2000). Direct mixing of the conducting polymer and carbon nanotubes and electrochemical polymerization has also been used. The solution casting method is mainly used for preparing nanocomposites of non-conducting polymers. When fillers with diameters in order of nanometer range are used to reinforce a polymer, the resulting product which is a blend of the nanofiller along with polymer is called a nanocomposite.

The design and fabrication of nanocomposites for a particular type of application requires careful selection of the specific type of polymer matrix and the method of fabrication like as dissolving and casting, in-situ polymerization, melt blend and extrusion favoring the aim. The chemical or electrochemical oxidation of aniline results in the formation of polyaniline Pyrrole, a five member ring, can be polymerized chemically and electrochemically as well, results in the formation of polypyrrole. The PEDOT (Ha et al. 2004) is an intrinsic conducting polymer due to its remarkably high conductivity, environmental stability and, transparency and is used in flexible organic electronics. Since it is insoluble, it has limited applications in devices. This problem can be resolved by its combination with the water-soluble poly (styrenesulfonic acid) (PSS) but it is compromised with low conductivity. PEDOT fabrication follows the same mechanism as that of pyrrole.

Polythiophene is another important intrinsically conducting polymer whose matrix is preferred for nanocomposite fabrication. This conjugated polymer forms environmentally and thermally stable materials. Polythiophene materials has various application like electrical conductors, electrochromic or smart windows, photoresists, antistatic coatings, batteries, electromagnetic shielding materials, electrodes, new types of memory devices, nanoswitches, polymer electronic interconnects, nanoelectronic and transistors. Polythiophene can be fabricated by in situ polymerization at room temperature in chloroform ($CHCl_3$) using ferric chloride ($FeCl_3$) as an oxidant.

2.15.3.2 Electrical and Thermal Conductivity of Nanocomposites

Many reports had proved that after using nanocomposites as filler in carbon nanotubes, there has been an observed increase in thermal conductivity compared to the corresponding polymers. There is also a significant increase in thermal conductivity when graphite nanofibers are used as fillers. The rule of mixtures model is based on the on the average weightage of conductivity of matrix and filler which is suitable for unidirectional composites with continuous fibers, While the inverse rule of mixtures neglects the thermal conductivity of short fiber. Heat flux law models and Ohm's law models based on electrical series resistance analogy can also be used for same purpose (Jones et al. 2010).

Capacitors and resistors Polymer nanocomposites like $BaTiO_3$ epoxy with high dielectric constant nanocomposite are used for fabrication of thin film embedded capacitors. The capacitor fabrication is based on a sequential build-up technology employing a first etched Cu electrode, then applying nanocomposite in the desired pattern and laminated within a printed wiring board (PCB). All this process was performed at high temperature/pressure lamination used to fabricate capacitors in multilayer printed circuit boards. Sequential buildup technology for capacitor fabrication is followed here.

Inductors can be formed by the ink jet printing of spiral structures. The quality of the inductor depends on spacing in the spiral and resistance. High resistance is not suitable for good inductor due to high thermal loss. For increase current carrying

capacity or conductance there is deposition multi metal layer on ink jet printed lines. High conductance causes the generation of high magnetic field which leads to high inductance in small packages Electroless Cu, immersion gold, electroless Gold, electroless Palladium, electroless Nickel, etc. have been used for fabrication of multi metal layers.

Conducting adhesives for interconnects Nanocomposites can be used as used as conductive joints for high frequency and high density interconnect applications since they have low volume resistivity. Fillers in metal to metal can provide better electrical conductivity while polymer resins are for better process ability and mechanical robustness. Nan composites can be filled in the conductive joints formed during composite lamination using an electrically conductive adhesive. Composite structure is provided after lamination of the adhesive-filled joining cores with circuited sub composites.

Magnetically active Nanocomposites Magnetic nanocomposites have excellent microwave absorption properties, so they are excellent absorbers in radiations shielding. These nanocomposites are used by high density recording media (Yamamoto et al. 1997) and in Microwave based communication system due to less crystalline anisotropy (Gotic et al. 1998; Pannaparayil et al. 1988). Magnetically active nanocomposites are used to control inductance in the circuit and may also find its application as passive magnetic devices. For preparation of passive magnetic devices nanoparticles are embedded into an epoxy matrix. According to recent work on the fabrication and characterization of nanocomposites, the electrical and thermal conductivity properties depends on the type of polymer matrix, the post-production treatment, and the dispersion method.

Future prospects Due to various properties and advantages of the nanocomposites, its applications have been growing at rapid in other fields. These fields are:

- Drug delivery systems
- Anti-corrosion barrier coatings
- Lubricants and scratch free paints
- UV protection gel
- New fire retardant materials
- New scratch/abrasion resistant materials
- Superior strength fibers and films

Improvement in the existing mechanical properties of nanocomposites has resulted in numerous automotive and general/industrial fields. These include impellers and blades for vacuum cleaners, door handles, engine covers and intake manifolds and timing belt covers.

2.16 Conclusion

The technological advancements made in the food packaging sector have indeed aided the food and beverage industry to improve the quality and shelf life of the products. The development of novel packaging materials and sensors has led to

increased protection and recognition of contamination, without any degradation in the aroma, texture and flavor of foods. Advances in the arena of nanosensors and NEMS are increasingly impacting the detection, preservation and safety aspects of food industry. Some concerns still linger over the outcomes of the interaction between nanomaterials and human body, but most of these concerns have been dissipated in the wake of recent scientific evidences. The research on futuristic novel packaging materials, which can change color in presence of contamination, has entered well within the realms of possibility.

Miniaturization of digital devices using nanopackaging techniques had been hampered by the challenges of fabrication of nano sized passive components and interconnects technology in the past. Recent advances in nanotechnology has made it possible to reduce the size of passives to nano scale by utilizing unique methodologies and materials, thereby increasing the odds of development of embedded passives consequently leading to compact and high quality devices. In summary, nanotechnology has started to emerge over and above the limitation of books and labs and thus is influencing our lives.

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Chapter 3
Food-Grade Nanoemulsions for Protection and Delivery of Nutrients

Anu Bhushani and C. Anandharamakrishnan

Abstract Nanoemulsions are kinetically stable systems containing two immiscible liquids, water and oil, stabilized by a layer of surfactant material with droplet diameters in the nano range, of less than 200 nm. They are classified as oil-in-water, water-in-oil or multiple nanoemulsions, depending on the relative spatial organization of the oil and aqueous phases. Nanoemulsions find many applications in food systems such as protection and delivery of food bioactives, encapsulation and controlled release of flavor or antimicrobial compounds and preparation of low-fat food products. Further, the kinetic stability, optical transparency and unique rheological characteristics of nanoemulsions have a major role in formulation of beverage emulsions. Bioactive compounds such as curcumin, beta-carotene, resveratrol, catechins, omega-3-fatty acids and others, when encapsulated in nanoemulsions, possess increased stability, improved solubility, higher intestinal retention time due to mucoadhesive property and enhanced absorption via direct uptake from the gastrointestinal tract. Food grade, biocompatible nanoemulsions are economically and industrially viable for production by employing scalable top-down high energy approaches such as high pressure homogenization. Thus, the distinct advantages of nanoemulsions over conventional emulsions are the chief reasons behind the improved research and development efforts in the food industry. This chapter focuses on the high energy and low energy approaches for the fabrication of nanoemulsions, characteristics and advantages of nanoemulsions and their applications in the food industry.

Keywords Food Nanotechnology • Nanoemulsions • Efficient Delivery • Emerging Technology • Opportunities

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3.1 Introduction to Food Grade Nanoemulsions

Nanotechnology has gained increased attention from researchers belonging to diverse fields. Advantageously, the concept of nanoscale has prominent relevance in the food industry, such that, it can be applied in all phases of food cycle 'from farm to fork' (Anandharamakrishnan 2014). The main applications of nanotechnology in food industry include nanodelivery of nutrients, nanomaterials for packaging and nanosensors for pathogen or contaminant detection (Rashidi and Khosravi-Darani 2011). Among these distinct applications, enormous R&D efforts are undertaken to develop highly stable and bioavailable nutrients at nanoscale for oral delivery. The concept of '*nutrient nanodelivery*' using food grade ingredients has several approaches such as lipid based delivery, biopolymer based delivery or surfactant based delivery.

Lipid based delivery systems have marked advantages over biopolymer and surfactant based delivery systems. The foremost advantage is that industrial scale up is feasible with high encapsulation efficiency and low toxicity. In general, lipids are used as nanocarriers for food bioactives with low solubility and intestinal permeability. Nanoencapsulation using lipids not only enhance the solubility properties of active compounds, but also, improve the chemical and gastrointestinal stability, bioavailability and sustained release properties of the encapsulated bioactive ingredient. Common lipid based delivery systems are nanoemulsions, composed of liquid lipid; solid lipid nanoparticles, composed of solid lipids and nanostructured lipid carriers containing a mixture of liquid and solid lipids (Fathi et al. 2012).

Nanoemulsions are kinetically stable systems containing two immiscible liquids such as water and oil, stabilized by a layer of surfactant material with droplet diameters principally less than 200 nm, however, the acceptable limit is up to 1000 nm. Based on the relative spatial organization of the oil and aqueous phases, they are classified as oil-in-water (O/W), water-in-oil (W/O) or multiple (W/O/W or O/W/O) nanoemulsions. O/W nanoemulsions are primarily used for entrapment of lipophilic bioactives e.g. curcumin, beta-carotene and W/O systems for entrapment of hydrophilic bioactives e.g. polyphenols (Sanguansri and Augustin 2006). This chapter will briefly discuss the differences in the composition and properties of nano-, micro- and conventional emulsions. Further, the techniques for formation of nanoemulsions, food applications and instability mechanisms are highlighted in detail.

3.2 Fundamental Aspects of Conventional, Micro and Nanoemulsions

The morphological difference among conventional, nano and microemulsions is depicted in Fig. 3.1. Conventional emulsions or macroemulsions, are systems containing two immiscible liquids, generally oil and water, with one of the liquids dispersed as spherical droplets i.e. the dispersed phase in the other called the continuous

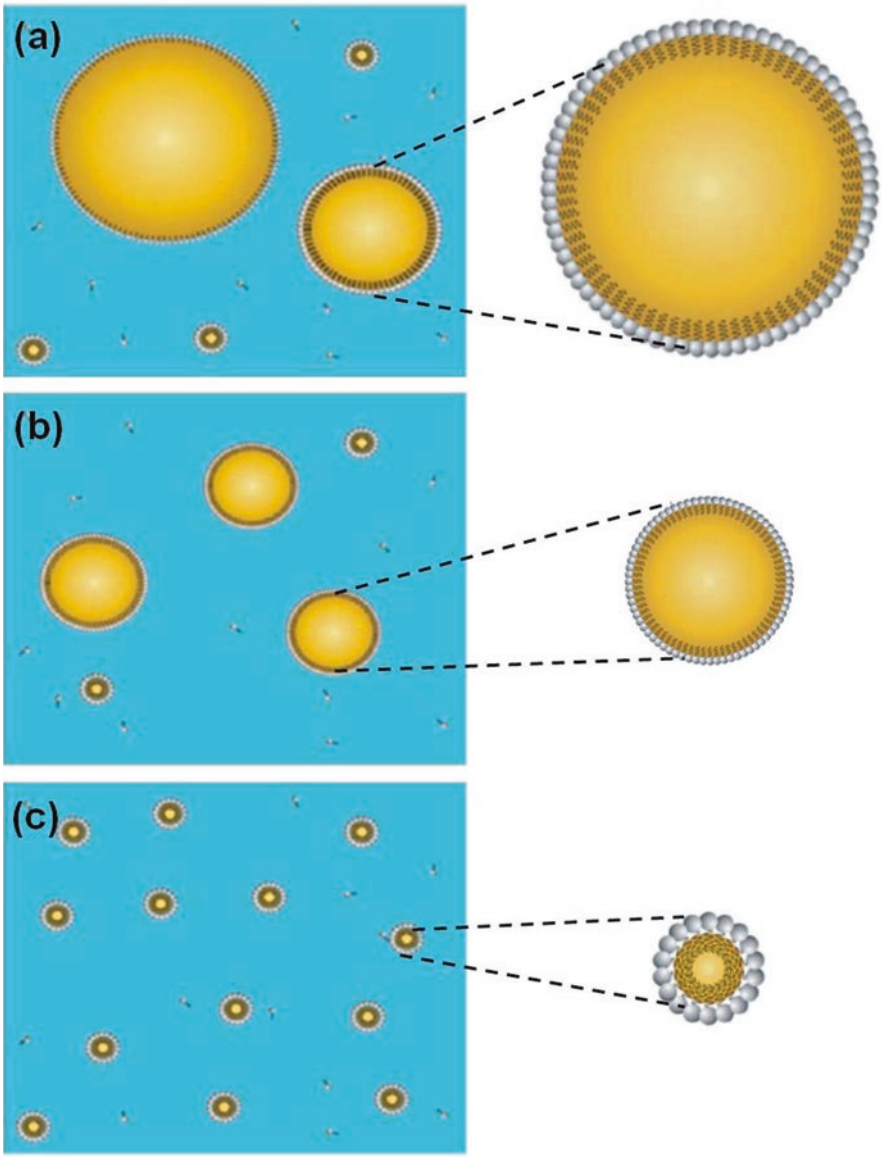

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Fig. 3.1 The image clearly depicts the structural differences between (**a**) conventional, (**b**) nano and (**c**) microemulsions, especially with respect to size and surfactant to oil ratio (Modified from Piorkowski and McClements 2014)

phase. The diameter of the conventional emulsion droplets lies between 0.1 μm and 100 μm. These emulsions are thermodynamically unstable and the physical stability of these emulsions is lower than micro or nanoemulsions. The droplet diameter of conventional emulsions are approximately equivalent to the wavelength of the light,

hence strong scattering of the incident light make these emulsions appear opaque of turbid (Dickinson 1992).

In contrast to conventional emulsions, nano- and microemulsions have droplet diameters less than 200 nm. Whilst, the difference in nano and micro emulsions can be explained based on energetics. Nanoemulsions or miniemulsions are kinetically stable but thermodynamically unstable systems. They are transparent or translucent systems as their droplet size is less than 25% of the wavelength of visible light. Due to the kinetic and nanoscale properties, nanoemulsions, have enhanced stability to gravitational separation and aggregation than conventional emulsions (Tadros et al. 2004; Wooster et al. 2008; Devarajan and Ravichandran 2011). On the other hand, microemulsions or swollen micelles are thermodynamically stable, transparent, isotropic systems containing oil and water stabilized by surfactant and co-surfactant molecules (Flanagan and Singh 2006). However, the thermodynamic stability of microemulsions is governed by factors such as composition and temperature. A change in these parameters will cause an alteration in the stability of system and result in formation of phase separated systems or nano/conventional emulsions (McClements 2012a). Dilution or temperature fluctuations are known to cause dramatic changes in microemulsions, whereas nanoemulsions are stable in conditions of stress. Further, micro and nanoemulsions are distinguished based on their composition, wherein, microemulsions have high surfactant to oil ratio (more than one) and nanoemulsions have low surfactant to oil ratio (Anton and Vandamme 2011). These properties have major influence on the end application. For instance, in case of beverage applications, the optical properties of microemulsions are preferred over conventional or nanoemulsions. However, thermodynamic instability associated with temperature or composition changes and usage of high concentration of synthetic surfactant, limit the use of microemulsions in soft drinks (Piorkowski and McClements 2014). Therefore, recent studies have attempted to facilitate the food applications of microemulsions by using food grade oils and surfactants such as soy bean oil (Lin et al. 2014; Ma et al. 2016), canola oil (Abbasi and Radi 2016), medium chain triglycerides (Roohinejad et al. 2015; Chatzidaki et al. 2015), essential oils (Ma and Zhong 2015; Ma et al. 2016), flavor oils (Rao and McClements 2012) and soy lecithin (Lin et al. 2014; Chatzidaki et al. 2015).

The formation of the different kinds of emulsions is dependent to an extent on the surfactant to oil ratio i.e. SOR and the emulsification technique employed. Based on a study by Rao and McClements (2011), the kinetic energy barrier in the oil-surfactant-buffer system and limitations in mass transport prevents them from reaching a stable form of microemulsion and the application of heat is an effective means to overcome the mentioned energy barrier. The study summarized the need for heat treatment (90 °C for 30 min) to produce stable microemulsion at high Tween 80 to lemon oil ratio (SOR more than 2) compared to conventional and nanoemulsions which can be formed by high energy methods such as microfluidization or ultrasonication.

Hence, with the above gist on the basic aspects of nanoemulsions and its difference from conventional and microemulsions, it would be easier to appreciate the formation methods, food applications and instability mechanisms associated with

nanoemulsions. The rest of the chapter deals with the various properties of food-grade nanoemulsions and its application in nutrient delivery.

3.3 Techniques for Formation of Nanoemulsions

In general, there are two approaches for the production of nanoemulsions, namely high energy and low energy methods. High energy approach utilizes the common types of forces such as compression, shear and impact for disruption of the oil phase and aqueous phase containing emulsifier to form stable nano droplets (Mason et al. 2006). Conversely, low energy approach utilizes the internal energy of the combined oil, aqueous and emulsifier system for formation of stable nanoemulsion by spontaneous emulsification/phase transition processes (Solans and Solè 2012) or use relatively low external energy for membrane emulsification process.

3.3.1 High Energy Nanoemulsification Methods

Nanoemulsification by high energy techniques involve utilization of mechanical devices or homogenizers to produce intense disruptive forces that are sufficiently high to break the emulsion droplets and subsequently maintain the stability and morphology of formed submicron or nano sized droplets (Schubert and Engel 2004). The emulsification process is a combination of two opposite 'elementary reactions' namely droplet disruption and droplet coalescence (Vankova et al. 2007). Thus achieving a balance between these two opposing processes is essential for the formation of stable nanoemulsions. In general the factors that influence the formation and stability of nanoemulsions can be classified as instrumental and solution parameters. The instrumental factors that determine the size of the emulsion droplets are the type of homogenizer used, interaction chamber, impingement and plunger design, feed rate, operating pressure, number of cycles and temperature of homogenization. The solution properties include the volume fraction of the various components of emulsion system namely oil, water and surfactant, viscosity ratio of the oil and aqueous phase, concentration and properties of active ingredient e.g. curcumin, beta-carotene, dispersed in oil phase, concentration of co-surfactant if used and concentration of solid nanoparticles used in case of pickering nanoemulsions. The widely used high energy techniques for nanoemulsification are high pressure valve homogenizer, microfluidizer and ultrasonicator (McClements and Rao 2011). Figure 3.2 illustrates the basic properties of high pressure and ultrasonic systems employed for production of nanoemulsions. In practice, a rotor-stator device is used to produce coarse emulsions from the oil-water-surfactant system, which is followed by the application of high energy system for formation of nanoemulsions.

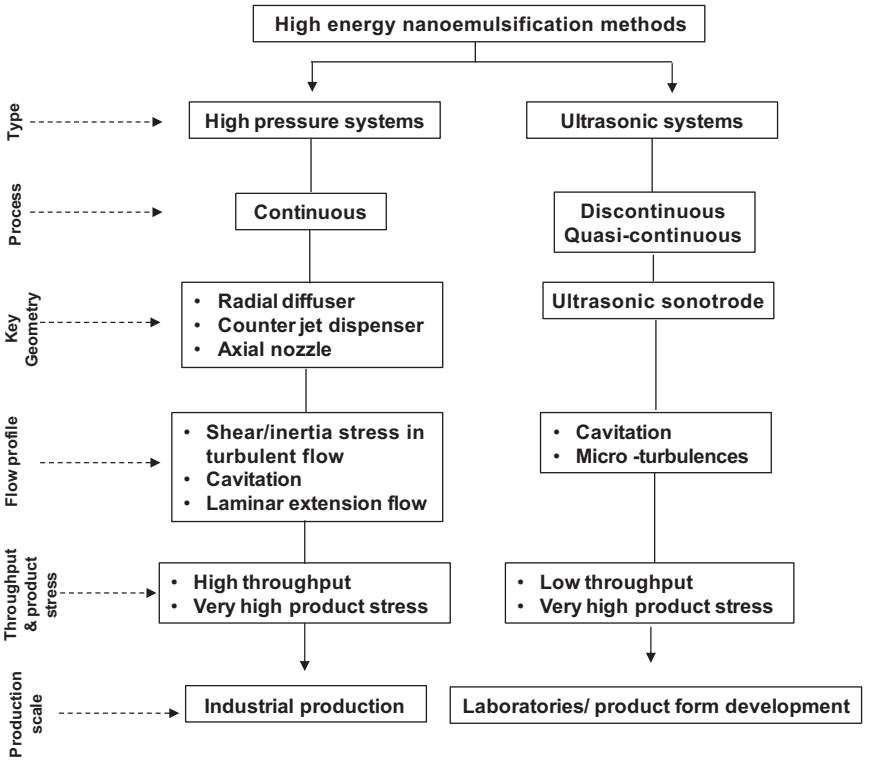

Fig. 3.2 The differences in the operating process, geometry, fluid flow profile, throughput, product stress and scalability for the two commonly used high energy nanoemulsification methods namely high pressure and ultrasonic systems are illustrated (Modified from Schultz et al. 2004)

3.3.1.1 High Pressure Homogenization

Homogenization is a term used to refer any process which reduces the relative heterogeneity of a system. Emulsification by high pressure homogenization is a continuous process with a high product throughput, making it an industrially feasible technique. High pressure homogenizers achieve homogeneity in coarse emulsion and form nanoemulsion by means of valves and plunger system which creates series of restrictions to the liquid flow and exert pressure ranging from 50 MPa to 700 MPa. Here, along with the high pressure, other forces such as shear, turbulence and cavitation act on the emulsion droplets. These forces define the hydrodynamics or flow profile of coarse emulsion inside the homogenizer that in turn influence the formation of nanoscale droplets. The major fluid flow profiles within homogenizer are *laminar*, *turbulent* and *cavitational* representing well-defined, irregular and complex flow respectively. Further, the flow regime of the droplets within the homogenizer is a combination of these flow profiles (Tadros 2009). During emulsification, the balance between the viscous and inertial forces acting on the droplet determines

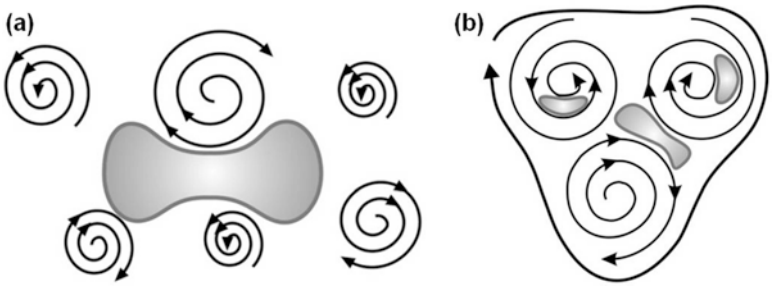

Fig. 3.3 Schematic representation of the (**a**) turbulent inertial and (**b**) turbulent viscous regime of emulsification in turbulent flow. It can be observed that in turbulent inertial regime, the droplet is larger than the smallest eddies formed, whereas, in turbulent viscous regime, the droplets are smaller than the smallest eddies formed (Reprinted with permission of Elsevier Ltd. from Vankova et al. 2007)

the fluid flow profile either laminar or turbulent. An estimation to which force dominates can be studied using the Reynolds number (R_e) which is the ratio of the inertia and viscous forces as given below:

$$R_e = \frac{u_a \times d_h \times \rho_c}{\eta_c} \tag{3.1}$$

where, u_a is the average fluid flow velocity; d_h is the hydrodynamic diameter of the orifice as characteristic length scale; ρ_c and η_c are the density and dynamic viscosity of the continuous phase respectively. It is stated that when the viscous forces generated within the fluid dominate the inertial forces, the flow is laminar (low R_e, i.e. R_e less than or equal to 1000) and when the inertial forces dominate; the flow changes from laminar to turbulent (high R_e, i.e. R_e greater than or equal to 2000). Based on this theory, the different flow regimes of emulsification are classified in to (i) laminar-viscous regime, (ii) turbulent-viscous regime and (iii) turbulent-inertial regime. For systems having water in the continuous phase, the regime is mostly turbulent-inertial. For systems possessing high viscosity of the continuous phase (i.e. η_c = 0.1 Pas), the regime is turbulent-viscous. For systems with very high viscosity (high η_c) or small homogenizing apparatus (low d_h), the regime is nearly laminar-viscous. Laminar regime is characterized by simple shear, rotational and elongational flow. In turbulent-inertial regime, as the droplets are larger than the smallest turbulent eddies formed, inertial forces cause disruption of the droplet. On the other hand, in turbulent-viscous regime, the droplets are smaller than the smallest eddies formed, leading to droplet disruption by shear stresses inside and between the eddies (see Fig. 3.3) (Walstra and Smulders 1998; Vankova et al. 2007).

Hence, based on the mechanism of droplet disruption within the homogenizer, the homogenizer nozzles can be broadly classified in to two types as shown in Fig. 3.4. The standard homogenizer nozzle and the relatively new microfluidizer nozzle are based on the disruption of droplets due to inertial forces in turbulent flow. In standard

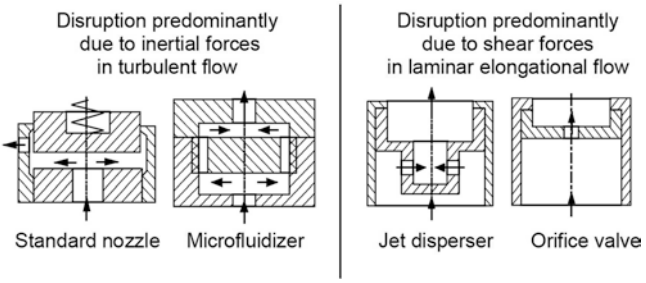

Fig. 3.4 Classification of nozzles used for homogenization based on the mechanism of droplet disruption (Reprinted with permission of John Wiley and Sons from Stang et al. 2001)

nozzle, the crude emulsion is pumped through a valve at high pressure and droplet disruption takes place at the radial junction of the valve seat and valve plug. Whereas, in microfluidizer, the pathlines of the coarse emulsion is divided in to two channels, which are redirected over the same plane at right angles and made to impinge against each other in the interaction chamber, thereby causing high shear, turbulence and cavitation in the exiting emulsion pathline. On the other hand, when the disruption of droplets is due to shear forces in laminar elongational flow, the nozzles are termed as jet disperser or orifice valve. In jet disperser, fine bores (diameter: 0.3–0.5 mm) are employed to create jets of crude emulsion and droplet disruption is brought about by the collision of the jets at high pressure. In orifice valve, droplet disruption occurs in three stages namely elongation of the droplet in front of the valve, deformation inside the valve and turbulent disruption of flow behind the valve (Stang et al. 2001; McClements 2004; Köhler et al. 2007).

In general, the influence of the homogenization parameters such as pressure and number of cycles and solution parameters such as surfactant concentration on the physicochemical stability of nanoemulsions have to be optimized and understood before developing a bioactive delivery system (Uluata et al. 2015; Mehmood 2015). For instance, a study by Qian and McClements (2011) reported that the mean droplet diameter decreased with increase in pressure and number of passes, exhibiting a linear log-log relationship between droplet diameter and homogenization pressure. Similarly, a study by Tan et al. (2015) revealed that the concentration of the bioactive compound encapsulated i.e. lutein and the surfactant i.e. Tween 80 used had significant influence on the particle size of the nanoemulsions prepared by high pressure homogenization method.

3.3.1.2 Ultrasonication

Sonication refers to the process of utilizing sound energy to agitate particles in a sample and when frequencies of greater than 20 kHz is used, the process is referred to as *ultrasonication*. In ultrasonication, sound waves propagate in to the liquid media briefly, the oil-water-emulsifier system and cause interfacial unstable waves

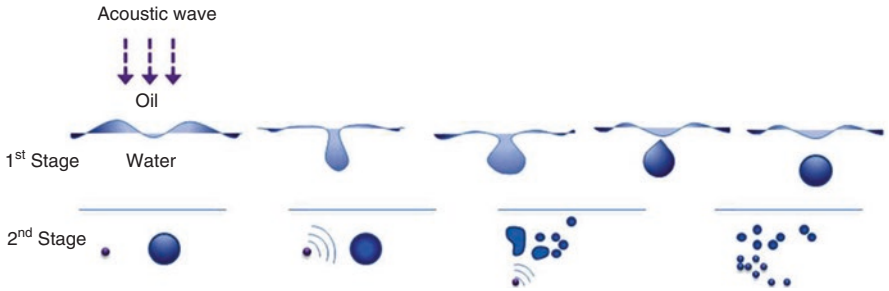

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Fig. 3.5 Schematic of the two stages namely droplet formation (1st stage) and break up (2nd stage) involved in formation of nanoemulsion by ultrasonication method (Reprinted with permission of Elsevier Ltd. from Sivakumar et al. 2014)

leading to the formation of droplets by eruption of oil phase in to the water-emulsifier system (Li and Fogler 1978a). Also, the acoustic waves cause sinusoidal pressure variations leading to alternating high pressure i.e. compression and low pressure i.e. rarefraction cycles. Herein, during rarefraction, the acoustic waves create cavitation i.e. the formation of voids or bubbles in the liquid, which grows rapidly and implode during compression cycle. The collapse of these microbubbles cause extreme turbulence and shear force in the liquid, leading to the generation of high velocity liquid jets which disrupt the droplets in the vicinity of the collapsing bubble. These series of reactions along with an increased local temperature; generate intense disruptive forces sufficient to form nanoemulsions with narrow particle size distribution (Li and Fogler 1978b; Kentish et al. 2008; Leong et al. 2009). This two stage process, involving droplet formation and break up, for formation of nanoemulsion by ultrasonication is represented schematically in Fig. 3.5 (Sivakumar et al. 2014). The parameters affecting the formation of nanoemulsions by ultrasonication are the sonication amplitude and duration. Amplitude has a direct effect on the intensity of sonication, which determines the droplet disruption rate of the emulsion system. Ultrasonication duration effect the rate of adsorption of surfactants to the droplet surface thereby influencing the formation of stable nanoemulsions. However, it has been reported that the chances of 'over-processing' are less in ultrasonication as against high pressure techniques used for nanoemulsion formation (Lin and Chen 2006; Leong et al. 2009).

The design of ultrasonicator can be altered based on the end applications. Generally, ultrasonic probe with linear, exponential or stepped design is employed for production of nanoemulsions. Studies have shown the effectiveness of ultrasonication as a technique in par with other high energy techniques such as microfluidization. Results obtained from a study by Tang et al. (2013) revealed that both microfluidization and ultrasonication produced nanoemulsions with droplet diameters in the range of 150–170 nm. Also, Jafari et al. (2006) stated ultrasonication to be a more convenient technique in terms of operation and cleaning than microfluidization. Recently, nanoemulsions of food bioactives such as curcumin (mean droplet diameter: 141.6 ± 15.4 nm) and quercetin (mean droplet diameter: 52 ± 10 nm) were

prepared by ultrasonication method using whey protein concentrate and saponin as emulsifiers respectively (Sari et al. 2015; Kaur et al. 2016). Further, flavor compound, D-limonene, has been nanoemulsified using ultrasonication with droplet diameter as less as 27.4 ± 1.4 nm (Li and Lu 2016). Similarly, essential oils such as cumin, pepper and fennel oil nanoemulsions were prepared with mean droplet diameters of 52.89 nm, 82.08 nm and 59.52 nm respectively, for anti-quorum sensing and anti-biofilm properties (Venkadesaperumal et al. 2016). These studies collectively affirm the efficacy of ultrasonication as a method to produce nanoemulsions with lower polydispersity and higher physical stability.

3.3.2 Low Energy Nanoemulsification Methods

Low energy nanoemulsification methods, as the name suggests, use either low external energy or the intrinsic physicochemical properties of the system containing surfactant, co-surfactant, oil and water to form nanoemulsions. Low energy techniques are facile, low cost, energy efficient, non-aggressive to encapsulated compound and capable of producing nanoemulsions with small particle sizes compared to high energy techniques (Anton and Vandamme 2009). Solans and Solè (2012) classifies the low energy emulsification methods based on the occurrence of changes in the surfactant spontaneous curvature during the process. If the nanoemulsion is formed without a change in the spontaneous curvature of surfactant, the process is referred to as self emulsification. However, if changes occur, the emulsification process is termed as phase inversion method. Apart from these, membrane emulsification is also classified as a low energy emulsification method (Sanguansri and Augustin 2006; Silva et al. 2012).

3.3.2.1 Spontaneous Emulsification

Spontaneous or self emulsification is an exciting phenomenon which has attracted great scientific interest over the years. The scientific appeal is partly due to the uncertainties in the various mechanisms that are involved in the process. By large, spontaneous emulsification is a process in which an emulsion is produced as a result of mixture of two liquids (i.e. aqueous and organic) at ambient conditions. The aqueous phase consists of water with a hydrophilic surfactant and the organic phase consists of oil, lipophilic surfactant and water-miscible solvent (Bouchemal et al. 2004). These two phases are thermodynamically stable, however, when mixed, they reach a non-equilibrium state. At the non-equilibrium stage, there is rapid diffusion of the water-miscible solvent from the organic to the aqueous phase, causing an increase in interfacial area which in turn leads to a metastable emulsion state (see Fig. 3.6). Thus, the underlying mechanisms that lead to formation of nanoemulsions

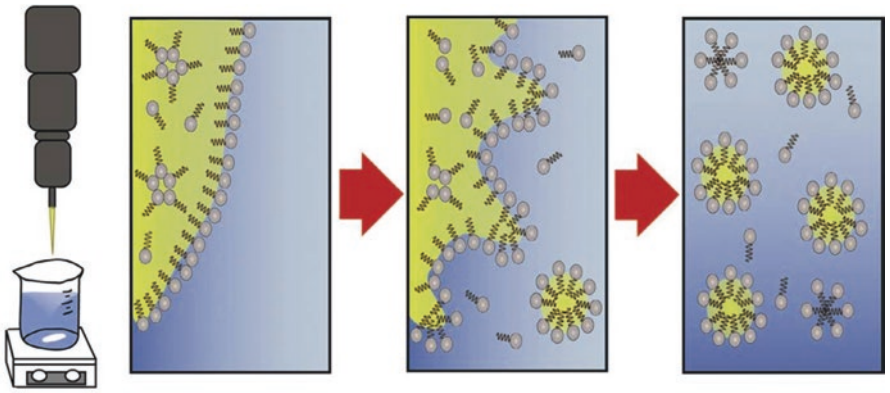

Fig. 3.6 Schematic representation of the formation of nanoemulsions by spontaneous emulsification process that occurs when an organic phase is added in to an aqueous phase at constant stirring (Reprinted with permission of Elsevier Ltd. from Komaiko and McClements 2015)

in spontaneous emulsification process are the interfacial turbulence, diffusion and stranding, negative interfacial tension and dispersion (Miller 1988; Anton et al. 2008; Anton and Vandamme 2009; Santana-Solano et al. 2012).

Spontaneous emulsification process can also take place by making use of the internal energy release while diluting the continuous phase of microemulsions at constant temperature. Herein, there is no associated change in the spontaneous curvature of the surfactant. In the pharmaceutical industry, nanoemulsions produced by the above described method are referred to as self-nano-emulsifying drug delivery systems (Solans and Solè 2012). However, these self-nano-emulsifying drug delivery systems are also used for delivery of food based bioactives such as curcumin (Joshi et al. 2013), lutein (Shanmugam et al. 2011) and turmeric essential oil (Zhao et al. 2010). For food applications, generally, Food and Drug Administration approved class 3 solvents such as ethanol, acetone or ethyl acetate are used in formulations. The commonly used oils include medium chain mono-, di- or triglycerides of capric and caprylic acids, long chain monoglycerides and fatty acid esters (Makadia et al. 2013). In recent years, works have focused on employing self emulsification as a low energy nanoemulsification technique for development of food bioactive nanodelivery system. For instance, vitamin E nanoemulsions with mean droplet diameter less than 50 nm have been obtained by self emulsification method (Saberi et al. 2013a). Similarly, resveratrol (Davidov-Pardo and McClements 2015), fish oil (Walker et al. 2015) and vitamin D (Guttoff et al. 2015) have been nanoemulsified by self emulsification technique. These nanoemulsions are highly relevant as delivery vehicle of flavors, antimicrobials, vitamins or antioxidants for incorporation in to beverages and clear liquid products (Saberi et al. 2013b).

3.3.2.2 Phase Inversion

Nanoemulsification by phase inversion method makes use of the internal chemical energy released in the system during phase transitions. The phase transition process involves change in the spontaneous surfactant curvature from positive to negative or vice versa. In addition, phase transitions occurring in surfactant film with average zero curvature such as lamellar liquid crystalline phase or bicontinuous phase also lead to formation of nanoemulsions by this method (Bilbao-Sáinz et al. 2010; Solans and Solè 2012). These phase transitions are triggered by either change in temperature referred as phase inversion temperature or composition referred as phase inversion composition.

Phase Inversion Temperature

The temperature at which the solubility of the surfactant is almost equal in both the oil and aqueous phases is referred to as the phase inversion temperature or the hydrophile-lipophile balance temperature. The nanoemulsification process by phase inversion temperature method takes advantage of the changes caused in the physicochemical properties (i.e. optimum curvature and solubility) of non-ionic surfactants at this critical temperature. Precisely, the process for production of O/W nanoemulsion by phase inversion temperature method involves heating of a surfactant-oil-water mixture near or slightly above its phase inversion temperature and then cooling immediately with constant stirring. At the phase inversion temperature, due to the balance in the hydrophilic and lipophilic properties of the surfactant, extremely low interfacial tension can be attained which promotes emulsification process. In addition to it, when the emulsion passes through the phase inversion temperature, the surfactant curvature tends towards unity, leading to formation of a highly dynamic interface, promoting emulsification. However, at this point, the barriers that resist coalescence process are low. Hence, once the nanoemulsion droplets are formed, the temperature is shifted far from the HLB temperature, thereby enabling the formation of stable nanoemulsions (Gutiérrez et al. 2008). Schematic representation of the formation of nanoemulsion by phase inversion temperature method is given in Fig. 3.7. In Figure, block (a) represents a macroemulsion with hydrophilic surfactants. When the temperature is increased (block b), the surfactants gradually become hydrophobic and get solubilized in to the oil droplets. When the system reaches the phase inversion temperature, bicontinuous microemulsions are formed (block c) and when the temperature is brought above the phase inversion temperature, the surfactants become lipophilic and the emulsion is inverted. Finally, when the system is cooled by water dilution, the surfactants become hydrophilic and this change induces rapid migration of the surfactants to the aqueous phase. According to Anton and Vandamme (2009), the turbulence generated during this final stage induces the formation of nanoemulsions with mechanism similar to spontaneous emulsification.

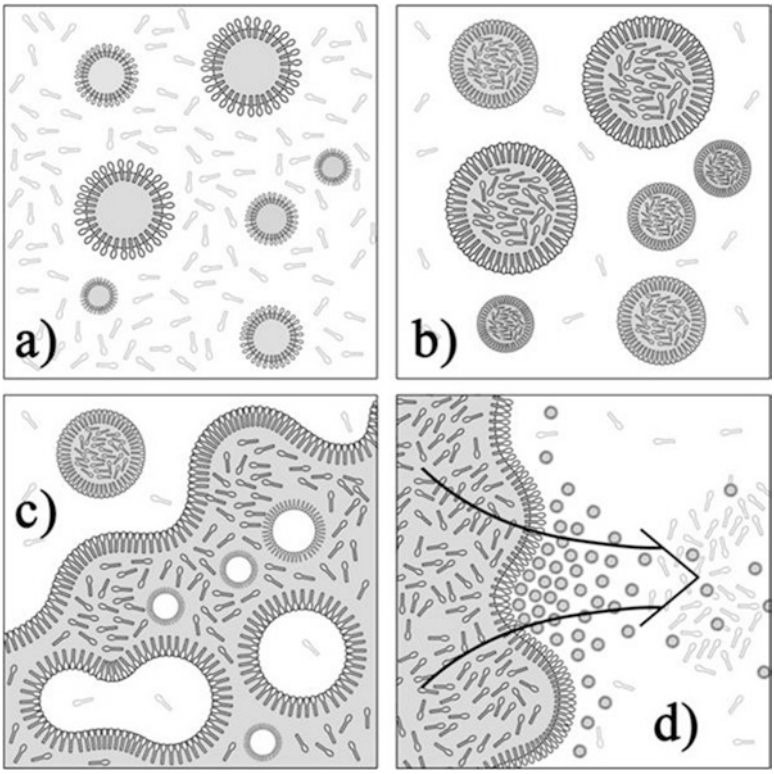

Fig. 3.7 Pictorial representation of the formation of nanoemulsions by the phase inversion temperature method. Briefly, the hydrophilic surfactants in the macroemulsion system (**a**) gradually becomes hydrophobic on increase in temperature (**b**) leading to the formation of bicontinuous microemulsions at the phase inversion temperature (**c**), beyond which the surfactants become lipophilic and the emulsion is inverted (**d**) (Reprinted with permission of Elsevier Ltd. from Anton and Vandamme 2009)

Phase Inversion Composition

In phase inversion composition method, water or oil is progressively added to either a mixture of oil-surfactant or water-surfactant respectively. For the formation of O/W nanoemulsion, water is added progressively to the initial W/O microemulsion system. As the volume fraction of water increases in the system, the surfactant chains get hydrated and the surfactant spontaneous curvature changes from negative to zero, thereby achieving a transition composition. Beyond the transition composition, the structures with zero curvature separate in to metastable droplets. These very small droplets contain oil (O/W nanoemulsion), indicating a very high positive curvature of the surfactant layer (Solans and Solè 2012; Anton et al. 2008) (see Fig. 3.8). On comparison with phase inversion temperature method, phase inversion composition is advantageous for large scale production due to the simplicity involved in the process.

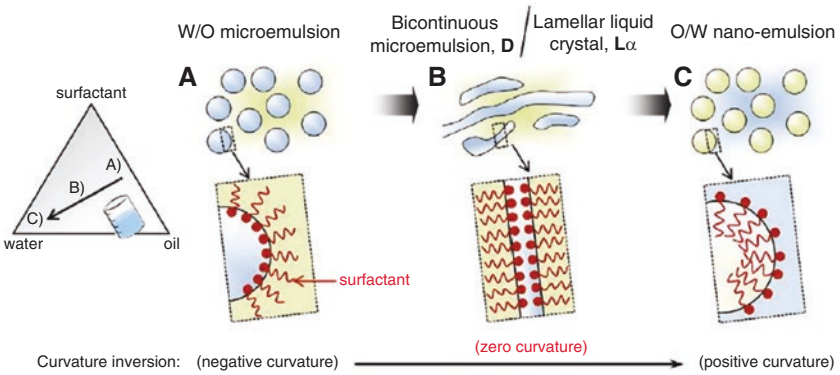

Fig. 3.8 Pictorial representation of the formation of oil-in-water nanoemulsions by the phase inversion composition method. Briefly, on addition of water to the water-in-oil microemulsion system (**a**), a transition composition is achieved with zero curvature (**b**), beyond which oil-in-water nanoemulsions containing surfactant with positive curvature is formed (**c**) (Reprinted with permission of Elsevier Ltd. from Solans and Solè 2012)

Emulsion Phase Inversion

As discussed in the previous sections, phase inversion temperature and phase inversion composition techniques involve a change in the temperature or composition of the oil-water-surfactant system leading to a transitional phase inversion from one system to another. On the contrary, emulsion inversion point or the emulsion phase inversion method involves a change in the emulsion system (O/W or W/O) through a catastrophic phase inversion when an aqueous phase is titrated in to a mixture of oil and hydrophilic surfactant (Fernandez et al. 2004; Ostertag et al. 2012). During titration, an unstable W/O emulsion will be formed due to the higher volume ratio of oil phase. When water is gradually added, the system (especially surfactant curvature) passes through different structures such as bicontinuous phases, lamellar phases and multiple emulsions. This is typically referred to as the catastrophic phase inversion. Due to these sudden changes, the instability in the system has to be overcome by constant stirring, until complete inversion of the emulsion takes place. This process is depicted in Fig. 3.9 (Ostertag et al. 2012). The critical factors determining the process are the flow rate of the aqueous phase during titration with oil phase, type and concentration of surfactant used and surfactant to oil ratio (Sajjadi et al. 2004; Thakur et al. 2008; Jahanzad et al. 2009).

Phase inversion methods for production of food grade nanoemulsions have been used mainly for flavors, essential oils and hydrophobic bioactives. A notable instance would be the preparation of lemon oil nanoemulsion with mean diameter of 100 nm by phase inversion temperature method. The study revealed the efficacy of sodium caseinate for use as emulsifier in preparation of nanoemulsion by phase inversion temperature method (Su and Zhong 2016). Also, D-limonene nanoemul-

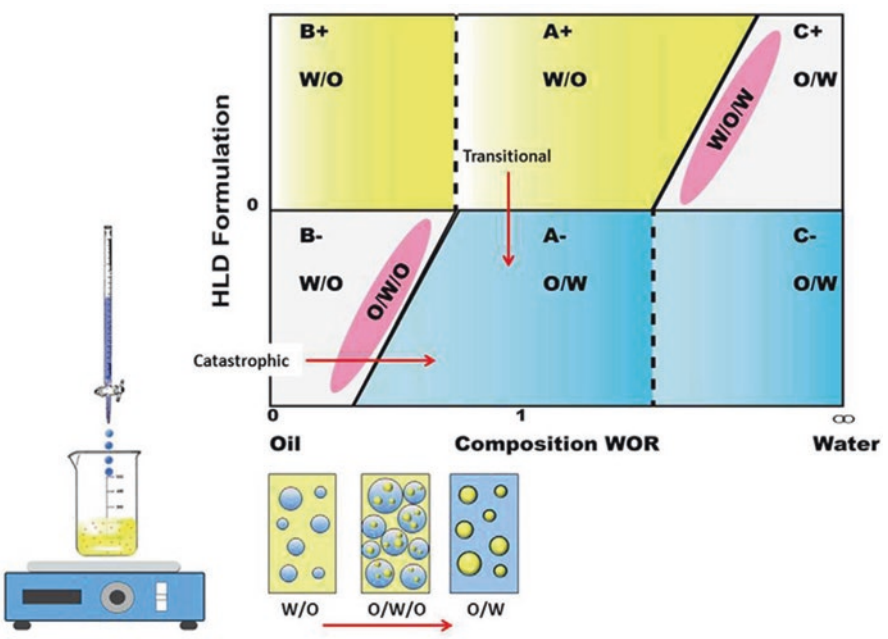

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Fig. 3.9 Pictorial representation of the emulsion phase inversion method, wherein, when an aqueous phase (from burette) is titrated against an organic phase (in beaker) at constant stirring, catastrophic phase inversion as illustrated in the formulation-composition map occurs, leading to change in the system from W/O to O/W nanoemulsion (Reprinted with permission of Elsevier Ltd. from Ostertag et al. 2012)

sions (mean diameter less than 100 nm) have been prepared by emulsion phase inversion method using olive oil and Tween 80 as oil and surfactants respectively (Li et al. 2013). Curcumin nanoemulsions with droplet diameters less than 250 nm have been prepared using soybean oil, Tween 80 and glycerol as oil, surfactant and cosolvents respectively. However, curcumin loading was reported to be low (0.07%) compared to high energy techniques (Borrin et al. 2016). Similarly, the effect of oil types (short, medium & long chain triglycerides) on the formation of stable vitamin E nanoemulsions by emulsion phase inversion method was investigated by Hategekimana et al. (2015). It was observed that the long chain triglyceride based vitamin E nanoemulsion had better stability against environmental stresses such as wide changes in temperature, pH and ionic strength and possessed better long term stability. More studies are necessitated to develop food grade nanoemulsions using phase inversion methods that utilize lower amount of surfactants, cosolvents for possible incorporation in food systems.

3.3.2.3 Membrane Emulsification

Membrane emulsification is a process in which emulsions are formed by forcing a dispersed phase to permeate through a membrane in to the continuous phase under the influence of low external pressure. The mechanism behind the formation of nanoemulsions involves two stages, the first being the growth of droplets at the tip of the membrane pore and the second being the detachment of the formed droplets from the pore tip (Peng and Williams 1998; Charcosset 2009). Conventional emulsification methods disrupt the droplets by laminar or turbulent flow. However, in membrane emulsification, small droplets are formed at the surface of a microporous membrane. Thus, due to the lower energy requirement (10^4–10^6 Jm^{-3}) as against the conventional mechanical processes (10^6–10^8 Jm^{-3}); membrane emulsification is classified as a low energy technique. This is one important phenomenon which renders membrane emulsification process suitable for processing shear-sensitive substances. Also, a distinct advantage of this technique is that the droplet size of the nanoemulsion formed is chiefly controlled by the choice of the membrane and not by the droplet disruption forces generated in the system (Schröder et al. 1998; Charcosset et al. 2004). A typical experimental setup for membrane emulsification is presented in Fig. 3.10. Here, three manometers namely M1, M2 and M3 are used to monitor the pressure at the inlet and outlet of membrane device and the pressure vessel, respectively. Initially, the aqueous phase will be pumped through the membrane unit and when it reaches the outlet, the oil is pumped at constant pressure through the membrane. Here, oil phase permeates through the pores of the membrane in to the aqueous phase and emulsification initiates immediately. The nanoemulsions thus formed will be stabilized under constant stirring for 15 min before further use (Laouini et al. 2012). The types of membranes used for formation of nanoemulsions are Shirasu Porous Glass, ceramic, polymer and stainless steel membranes.

The predominant forces that act on the droplets that are formed during membrane emulsification are the interfacial tension, static pressure difference, drag, dynamic lift, buoyancy and inertial forces. The magnitude of these forces changes as the size of the droplet increases (Schröder et al. 1998; Schröder and Schubert 1999; Charcosset et al. 2004). The critical parameters that influences the process are the size, wettability and permeability of the membrane pores; surfactant concentration and type; viscosity and density of continuous and dispersed phases; interfacial tension; temperature and transmembrane pressure (Charcosset 2009). Food bioactives namely vitamin-E (Laouini et al. 2012) and beta-carotene (Trentin et al. 2011) have been nanoemulsified using membrane emulsification technique. Eventhough there is moderate growth in the use of membrane emulsification for preparation of emulsions in the food processing industry, its nanoscale applications are still in its infancy. This may be due to the demerit of the technique i.e. low dispersed phase flux through membrane. Hence, scope exists in overcoming the limitation and improving the overall efficiency of the system.

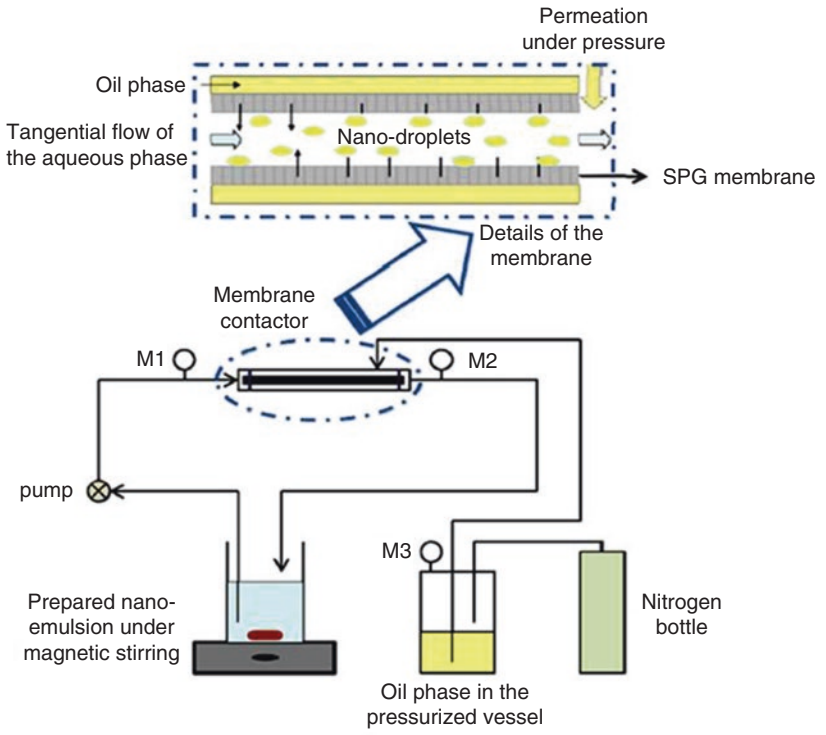

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Fig. 3.10 Illustrative experimental set up for the formation of nanoemulsions by membrane emulsification. M1, M2 and M3 represent the manometers used to monitor the pressure at the inlet of membrane, outlet of membrane device and the pressure vessel, respectively (Reprinted with permission of Elsevier Ltd. from Laouini et al. 2012)

3.4 Role of Nanoemulsions in Protection and Delivery of Nutrients

Nanoemulsions have diverse applications in the food industry for protection and delivery of nutrients, flavors and antimicrobial agents. The following sections will discuss the specific food based applications of nanoemulsions. Table 3.1 gives a detailed review of the latest research works on the use of nanoemulsion as a delivery vehicle for food bioactives and antimicrobial compounds.

Table 3.1 Recent works on nanoemulsion based protection and delivery of nutrients

Core material	Emulsification technique used	Major structural components	Inferences	Reference(s)
Food bioactives				
Curcumin	High pressure homogenization	Oil phase: Medium chain triglyceride Emulsifier: Whey protein isolate	Improved permeation of curcumin in nanoemulsion form as assessed by Caco-2 model.	Li et al. (2015)
Curcumin	High pressure homogenization	Oil phase: Corn oil Emulsifier: Lactoferrin For multilayer nanoemulsion: Alginate	Multilayer nanoemulsion had distinct physicochemical stability wherein, the alginate coating helped control the rate of lipid digestion and release of curcumin in simulated gastrointestinal tract.	Pinheiro et al. (2016)
Curcumin	Ultrasonication	Oil phase: MCT Emulsifiers: Whey protein concentrate and Tween 80	Improved chemical stability to high temperature, varying ionic strengths (0.1–1 M) and pH (3.0–7.0). Sustained release of curcumin in simulated gastrointestinal model.	Sari et al. (2015)
Docosahexaenoic acid (DHA)	High speed homogenization (HSH); High pressure homogenization (HPH); Combination of HSH + HPH	Oil phase: DHA Emulsifiers: Tween-40	An enhanced physical and chemical stability for a period of 100 days was observed for nanoemulsions prepared using combination of high speed and high pressure techniques.	Karthik and Anandharamakrishnan (2016)
Resveratrol	High pressure homogenization	Oil phase: Peanut oil Emulsifier: Soy lecithin, sugar ester	Sustained release and enhanced transport of resveratrol through cell monolayers.	Sessa et al. (2014)

Resveratrol	Spontaneous emulsification	Oil phase: grape seed oil and orange oil Emulsifier: Tween 80	Improved chemical stability of resveratrol with 88% retention in nanoemulsions after UV exposure.	Davidov-Pardo and McClements (2015)
Quercetin	High pressure homogenization	Oil phase: Medium chain triglyceride Emulsifiers: Tween 80, lecithin and Span 20	High bioaccessibility of quercetin in nanoemulsion form (~60%) compared to free form (~7%)	Aditya et al. (2014)
Green tea Catechins	High pressure homogenization	Oil phase: Sunflower oil Emulsifiers: Acidified soy protein dispersion	Improved in-vitro bioaccessibility and Caco-2 cell monolayer permeability of catechins in nanoemulsion form compared to unencapsulated form.	Bhushani et al. (2016)
Conenzyme Q10	Microfluidization	Oil phase: Long chain fatty acid (Heptadecanoic acid), carrier oil (corn oil) Emulsifier: Tween 80	Enhanced digestion in simulated small intestinal model and increased oral bioavailability in rat model.	Cho et al. (2014)
Vitamin E (acetate form)	Spontaneous emulsification	Oil phase: Vitamin E oil Emulsifier: Tween 80 Cosolvents – Propylene glycol and ethanol	Cosolvents had a significant effect on the stability of Vitamin E nanoemulsions and diluted nanoemulsions showed better storage and thermal stability than undiluted nanoemulsions	Saberi et al. (2013a)

(continued)

Table 3.1 (continued)

Core material	Emulsification technique used	Major structural components	Inferences	Reference(s)
Vitamin E (acetate form)	Microfluidization	Oil phase: Orange oil and vitamin E, Emulsifier: Saponin/ lecithin/ whey protein isolate/gum arabic	Concentration and type of surfactants had a major influence on the stability of nanoemulsions. Saponin stabilized emulsions were stable over a range of pH values and salt concentrations compared to lecithin. Whey protein isolate was capable of producing nanoemulsions with smaller droplet diameters than gum Arabic at relatively low concentration. However, gum Arabic stabilized emulsions were stable to flocculation at pH 5 and at high ionic strength	Ozturk et al. (2014) Ozturk et al. (2015a)
α-tocopherol	High speed dispersion homogenizer	Oil phase: α-tocopherol oil Emulsifiers: Span 80, Tween 80 Additional ingredients: Glycerol, nopal mucilage	Significant reduction in the pectin methylesterase and polyphenol oxidase activity, along with reduced browning was observed when tocopherol nanoemulsion was used as coating on fresh cut apples. This effect was enhanced by the addition of nopal mucilage which improved the tocopherol activity.	Zambrano-Zaragoza et al. (2014)
Vitamin D	Spontaneous emulsification	Oil phase: Vitamin D and MCT Surfactants: Tween 20/40/60/80/85 Cosurfactant: Sodium dodecyl sulphate (SDS)	Oil phase composition, surfactant to oil ratio (SOR) and surfactant type and stirring conditions had a significant effect on the initial particle size of emulsions formed. Tween 80 and SDS stabilized nanoemulsions at SOR ≥ 1 and high stirring speed (800 rpm) had better storage stability.	Guttoff et al. (2015)
Vitamin D3	Microfluidization	Oil phase: MCT/corn oil/ fish oil/mineral oil/ orange oil Emulsifier: Saponin	Carrier oil type had high influence on the lipid digestion and bioaccessibility of vitamin D3. Nanoemulsion prepared using corn or fish oil showed high bioaccessibility of vitamin D3.	Ozturk et al. (2015b)

Carotenoid (astaxanthin or lycopene)	High pressure homogenization	Oil phase: Linseed oil Emulsifier: Tween 20	Improved digestibility, release properties and bioaccessibility (> 70%) of carotenoid in nanoemulsion form.	Sotomayor-Gerding et al. (2016)
Beta carotene	Microfluidization	Oil phase: Corn oil Emulsifier: Tween 20	Improved lipid digestion and carotene bioaccessibility in nanoemulsion (mean dia 0.2 μm) compared to conventional emulsion (mean dia: 23 μm)	Salvia-Trujillo et al. (2013)
Fucoxanthin	Microfluidization	Oil phase: MCT/LCT/ non-digestible oil Emulsifier: Tween 80	Fucoxanthin had highest solubility in mixed micelles formed for nanoemulsions containing LCT. However, the bioavailability of fucoxanthin was similar for all three oil types.	(Salvia-Trujillo et al. (2015a, b)
5-Demethyltangeretin (citrus flavonoid)	Microfluidization	Oil phase: MCT Emulsifier: β-Lactoglobulin	Enhanced cellular uptake and bioactivity in nanoemulsion form compared to free form.	Zheng et al. (2014)
Pterostilbene	Microfluidization	Oil phase: Flax seed/ olive oil Emulsifier: Tween 20	Improved bioaccessibility of pterostilbene in flax/ olive oil based nanoemulsions. However, trans-enterocyte transport (Caco2 model) of pterostilbene was higher in olive oil than flax oil based nanoemulsion.	Sun et al. (2015)
Silybin (flavolignans)	Two stage high pressure homogenization	Oil phase: sunflower/ olive/castor oil Emulsifier: Tween 80	Carrier oil type significantly influenced silybin solubility and bioaccessibility. Castor oil provided highest storage stability compared to sunflower and olive oil.	Calligaris et al. (2015)

(continued)

Table 3.1 (continued)

Core material	Emulsification technique used	Major structural components	Inferences	Reference(s)
Flavors and essential oils				
Sweet fennel oil	High pressure homogenization	Oil phase: Sweet fennel oil Emulsifiers: Tween 20 and Span 80 (1:3)	High kinetic stability of nanoemulsions formulated based on the pseudo-ternary phase diagram. Sweet fennel oil retained the antioxidant activity in nanoemulsion form and can be explored as an antioxidant topical formulation.	Barradas et al. (2015)
D-limonene	Ultrasonication	Oil phase: Sweet fennel oil Emulsifiers: Span 85 and Brij 98 Cosolvent: Propylene glycol	Physical stability of D-limonene nanoemulsions were affected by storage temperature and duration. Highest stability was observed at 25 °C as against 4 °C and 50 °C.	Li and Lu (2016)
Eugenol and Beta carotene	High speed homogenization	Oil phase: Eugenol, soybean oil Emulsifiers: Lecithin, whey protein isolate	Eugenol improved the physicochemical stability of beta carotene loaded nanoemulsion when exposed to heat (ambient, 60 °C, 80 °C) and UV radiation (254, 302, 365 nm).	Guan et al. (2016)
Eugenol	Microfluidization	Oil phase: LCT (Canola oil)/MCT Emulsifiers: Tween 80, Span 80	Nanoemulsions showed better stability and release properties in simulated GI medium than conventional emulsions. LCT based nanoemulsions had high release percentage of eugenol (84%)	Majeed et al. (2016)
Clove oil	Ultrasonication	Oil phase: Clove oil Emulsifiers: Tween 80, Span 80	Clove oil nanoemulsions had no significant change in droplet diameter during storage up to 6 months. The prepared nanoemulsion has potential as nanopesticide.	Shahavi et al. (2015)

Black seed oil	Ultrasonication	Oil phase: *Calendula* infused black seed oil Emulsifiers: Tween 80, lecithin	Calendula infused black seed oil nanoemulsion demonstrated wound healing properties and protection against radiation in *in-vitro* cell culture models.	Gumus et al. (2015)
Oregano/ Thyme/ Lemon grass/ Mandarin essential oils.	Microfluidization	Oil phase: Essential oil Emulsifiers: High methoxyl pectin, Tween 80	Lemongrass or mandarin based nanoemulsions had better storage stability than Oregano or thyme nanoemulsions due to the higher adsorption of pectin in the oil surface of the former. The study opens up the application of essential oils in stable form in beverages.	Guerra-Rosas et al. (2016)
Cumin essential oil	Ultrasonication	Oil phase: Oleic acid Emulsifier: Tween 20 Co-surfactant: Ethanol	Transdermal application of the essential oil nanoemulsion showed improved antioxidant and hepatoprotective properties in rat model.	Mostafa et al. (2015)
Antimicrobial compounds				
Mandarin essential oil	High pressure homogenization	Oil phase: Sunflower oil, Mandarin essential oil Emulsifiers: Glycerol monooleate, Tween 20	Green beans on treatment with Mandarin nanoemulsion based chitosan coating in combination with gamma irradiation or Modified atmospheric packaging or high hydrostatic pressure, demonstrated antimicrobial activity *Escherichia coli*, *Salmonella Typhimurium* or *Listeria innocua* during storage for a period of 14 days.	Severino et al. (2014) Severino et al. (2015) Donsi et al. (2015)
Volatile oil of *Stenachaenium megapotamicum* (Spreng.) Baker	Spontaneous emulsification	Oil phase: Volatile oil Emulsifier: Polysorbate Co-surfactant: Ethanol	The minimal inhibitory concentration and minimal fungicidal concentration against dermatophytic fungi were reduced when the volatile oil was in nanoemulsion form than as bulk oil.	Danielli et al. (2013)

(continued)

Table 3.1 (continued)

Core material	Emulsification technique used	Major structural components	Inferences	Reference(s)
Carvacrol, Limonene and Cinnamaldehyde essential oils	High pressure homogenization	Oil phase: Sunflower oil, essential oil Emulsifier(s): Lecithin/Pea protein/Sucrose palmitate/ Combination of glycerol monooleate and Tween 20 (1:1)	The antimicrobial activity of the essential oil nanoemulsions was highly dependent on the formulation which determines the concentration of the active compound in the aqueous phase. Sucrose palmitate and combination of glycerol monooleate and Tween 20 had quick bactericidal activity (2 hr) whereas; lecithin and pea protein had evident activity after prolonged time (24 hr).	Donsi et al. (2012)
Eugenol	Ultrasonication	Oil phase: Sesame oil, Eugenol Emulsifier: Tween 20/ Tween 80	Eugenol nanoemulsion exhibited antibacterial activity against *Staphylococcus aureus* and when incorporated in orange juice, there was a significant reduction in the native heterotrophic bacteria population. These transparent essential oil based nanoemulsions can be used as preservatives in selected foods.	Ghosh et al. (2014)
D-limonene organogel	High pressure homogenization	Oil phase: D-limonene organogel (Monostearin, MCT, D-limonene) Emulsifier: Tween 80	There was an increased antimicrobial activity when D-limonene was encapsulated in to the organogel-based nanoemulsion when compared to free D-limonene.	Zahi et al. (2015)
Sunflower oil	Low energy method	Oil phase: Sunflower oil Emulsifier: Tween 80 Co-solvent: Ethanol	Sunflower oil nanoemulsion reduced the microbial count and improved the organoleptic properties of Indo-Pacific king mackerel (*Scomberomorus guttatus*) steaks stored at 20 °C for 72 h.	Joe et al. (2012)
Trans-cinnamaldehyde	Microfluidization	Oil phase: Trans-cinnamaldehyde oil Emulsifier: Tween 20	Trans-cinnamaldehyde nanoemulsion exhibited antimicrobial activity against *Salmonella* *Typhimurium* and *Staphylococcus aureus* in water as well as real food system such as water melon juice.	Jo et al. (2015)

Thymus daenensis essential oil	Ultrasonication	Oil phase: Essential oil Emulsifiers: Tween 80, lecithin	The antibacterial activity of the essential oil nanoemulsion against *Escherichia coli* was significantly higher in nanoemulsion form than pure oil form.	Moghimi et al. (2016)
Eucalyptus oil	Ultrasonication	Oil phase: Eucalyptus oil Emulsifier: Tween 80	Eucalyptus oil nanoemulsion had enhanced antibacterial activity against *Staphylococcus aureus* and possessed wound healing properties as demonstrated in rat model.	Sugumar et al. (2014)
Basil oil	Ultrasonication	Oil phase: Basil oil Emulsifier: Tween 80	Basil oil nanoemulsions possessed bactericidal activity against *Escherichia coli* even in diluted form. This food grade nanoemulsion can be explored as a preservative in food products.	Ghosh et al. (2013)
Lemongrass essential oil	Microfluidization	Oil phase: Lemongrass essential oil Emulsifier: Tween 80 and coating solution (sodium alginate)	Lemongrass nanoemulsions exhibited inactivation of *E.coli* when used as edible coatings on fresh-cut *Fuji* apples. Further, colour and firmness of the apples were maintained during the storage period of 2 weeks.	Salvia-Trujillo et al. (2015a, b)
Thyme oil	High shear homogenization	Oil phase: Thyme oil Emulsifiers: Sodium caseinate, soy lecithin	Thyme oil nanoemulsions resulted in quicker reduction of bacteria in milk at 21 °C compared to free thyme oil.	Xue et al. (2015)

3.4.1 Encapsulation of Food Bioactive Compounds

Nanoencapsulation is the process of entrapping a bioactive material such as nutraceuticals, vitamins, antioxidants, essential fatty acids or oils within a protective wall material at the nano scale (Sanguansri and Augustin 2006). According to the concept proposed by the House of Lords Committee, the upper limit set for nanoscale materials intended for food applications in less than 1 μm (Klaessig et al. 2011). However, for nanoemulsions, droplets with radius less than 100 nm have been regarded as appropriate (McClements and Rao 2011). Nanoemulsions are chiefly employed for encapsulating lipophilic compounds in the oil phase of oil in water system and hydrophilic compounds in the water phase of water in oil system.

In general, food grade nanoemulsions should possess few desirable characteristics. Briefly, it should be able to encapsulate a substantial amount of the bioactive ingredient within the system (loading capacity) in its active form until delivered at the site of action (encapsulation efficiency and active compound retention); it should enhance the chemical stability of the encapsulated compound during processing, storage or digestion in the gastrointestinal tract; it preferably should possess specific or controlled release properties that in turn aid in enhancement of the bioavailability of the compound; it must ideally mask off-flavor, off-odour or after taste of encapsulated bioactives, thereby widening its scope of food applications; it should be compatible with the food matrix or beverage intended for fortification and should not affect the overall acceptance and sensory characteristics of food; it should be composed of generally regarded as safe (GRAS) ingredients that are cost effective and should be fabricated using economically feasible techniques that are implementable at large scale (McClements 2015).

Recent years have witnessed a tremendous growth in research on nanoemulsion based delivery systems for nutraceuticals such as curcumin, resveratrol, catechins, quercetin; vitamins such as D3, carotenoids and oils such as α-tocopherol and docosahexaenoic acid. According to the biopharmaceutical classification system, many nutraceuticals can be categorized under class II, III or IV which represent compounds with high permeability and low solubility; low permeability and high solubility; or low permeability and low solubility respectively (Oehlke et al. 2014). For instance, curcumin is a lipophilic molecule (log P_{ow} = 3.07) with low water solubility (0.052 g/L) and low permeability (apparent permeability coefficient in the range of 1 x 10^{-7} cm/sec) (McClements 2012b). Hence, it is can be grouped under class IV type molecule. Herein, O/W nanoemulsions aid in enhancing the solubility and permeability of curcumin and enable incorporation in aqueous food systems such as beverages. A study by Li et al. (Li et al. 2015) showed the improved permeation of curcumin in Caco-2 cell model when encapsulated in whey protein stabilized, medium chain triglyceride based nanoemulsion system. Similarly, sustained release of curcumin in the gastrointestinal tract can also be achieved by means of nanoemulsions and multilayer nanoemulsions (Sari et al. 2015; Pinheiro et al. 2016). Further, the surface charge on the emulsion droplets plays a critical role in its digestive behavior and hence the targeted release properties of encapsulated curcumin. Pinheiro et al. (2013)

studied this concept in detail by using three different emulsifiers namely Tween 20, sodium dodecyl sulphate and dodecyltrimethylammonium bromide with non-ionic, anionic and cationic properties respectively. The morphological changes in the different emulsifiers stabilized nanoemulsions during simulated *in-vitro* digestion can be seen in Fig. 3.11. It was observed that DTAB stabilized curcumin nanoemulsions that possessed positive charge were least stable during *in-vitro* digestion, leading to an increase in droplet diameter and reduced curcumin bioavailability. On the other hand, Tween 20 stabilized nanoemulsions retained the emulsion structure during digestion thereby increasing its free fatty acid release and bioavailability.

Another nutraceutical compound of interest is resveratrol, which is poorly water soluble (0.021 g/L) and crystalline in nature. Hence, while encapsulating resveratrol in to nanoemulsions, it should be taken in to consideration that the loading concentration is below the saturation level of the compound. Sessa et al. (2014) loaded 0.01 wt% of resveratrol in to 9% peanut oil based nanoemulsions, stabilized by 1% soy lecithin and 0.3% sugar ester and reported an enhanced permeability through Caco-2 cell monolayers. Davidov-Pardo and McClements (2015) also enhanced the chemical stability of resveratrol against ultra violet radiation using grape seed oil and orange oil based nanoemulsion system. The authors also stated 100 ug/ml of resveratrol as the optimum loading concentration to prevent crystallization during processing or storage.

Compounds of carotenoids family are shown to have very low bioavailability due to high molecular mass (more than 500 g/mol), very low water solubility (10^{-5} to 10^{-9} g/L) and poor thermal and photostability (Chen et al. 2014). Nanoemulsion based delivery of carotenoids such as beta carotene, astaxanthi, lycopene, fucoxanthin has improved its solubility, chemical stability, bioaccessibility and release properties to a larger extent than conventional emulsion or unencapsulated forms (Salvia-Trujillo et al. 2013, 2015a; Sotomayor-Gerding et al. 2016). Studies have also shown the effect of carrier oil on the solubilisation of beta-carotene in to mixed micelles for efficient transport in to the intestinal epithelial cells. Beta-carotene nanoemulsion formed using long chain triglycerides as carrier oil was reported to enhance carotene bioaccessibility than medium chain triglyceride or non-digestible oil such as orange oil (Qian et al. 2012a). Similarly, the emulsifier type also has an important role in the chemical stability of carotene nanoemulsions, wherein globular protein (beta lactoglobulin) stabilized nanoemulsion systems are reported to possess higher chemical stability than non-ionic surfactants (Tween-20) stabilized systems (Qian et al. 2012b).

On the other hand, alpha-tocopherol, coenzyme Q10 and docosahexaenoic acid from algal or fish oil have very low water solubility (almost insoluble) and moderate intestinal permeability. Hence, delivery systems for such compounds should be capable of enhancing loading efficiency, water solubility and bioavailability of the bioactive compound (Cho et al. 2014; Ozturk et al. 2014, 2015a). Recently, Karthik and Anandharamakrishnan (2016) demonstrated the improved physical and chemical stability of docosahexaenoic acid nanoemulsions during storage of up to 100 days when processed through a combination of high energy techniques such as high speed and high pressure homogenization. The homogenous DHA nanoemulsion droplets with globular structure and low droplet diameter (less than 88 nm) can be seen in Fig. 3.12a.

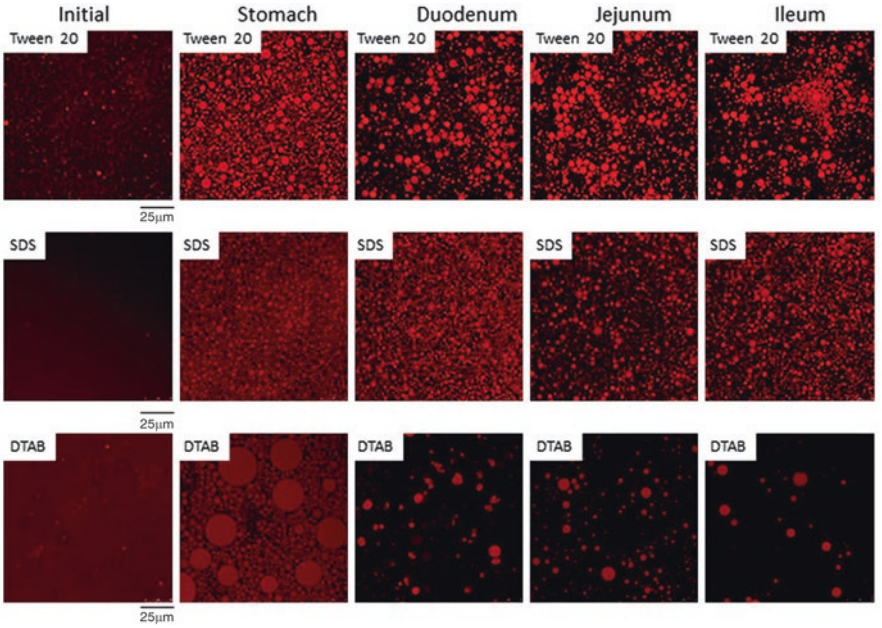

Fig. 3.11 Confocal images of Tween 20, sodium dodecyl sulphate (SDS) and dodecyltrimethylammonium bromide (DTAB) stabilized curcumin nanoemulsions during simulated *in-vitro* digestion is presented. Note the increase in droplet diameter of DTAB stabilized nanoemulsion compared to Tween 20 and SDS in stomach and small intestinal conditions (Reprinted with permission of The Royal Society of Chemistry from Pinheiro et al. 2013)

The above discussed nutraceuticals which can be distinguished based on their solubility and permeability profiles; however, few nutraceuticals have complexities associated with it. For example, catechins, a group of flavanols present in green tea and cocoa, have varying solubility profiles of their individual compounds namely catechin, epicatechin and their gallic acid conjugates. Hence, delivery of such compounds through nanoemulsion system present challenges in improving its encapsulation efficiency as well as storage stability. Recently, Bhushani et al. (2016) developed a soy protein based nanoemulsion system (Fig. 3.12b) with improved chemical stability, *in-vitro* bioaccessibility and Caco-2 cell monolayer permeability of green tea catechins.

Hence, a holistic approach should be adopted for protection and delivery of sensitive nutrients in its bioactive form. For instance, the effect of harsh food processing operations involving heat or radiation e.g. pasteurization, on the chemical stability of encapsulated nutrient has to be assessed. Secondly, it has to be noted that, results of many *in-vitro* studies fail to produce the same effect in *in-vivo* conditions. Therefore, it would be more effective to conduct *in-vivo* experiments in rodent models to evaluate the oral bioavailability of the encapsulated nutrients. Further, the effect of food matrix or co-ingested compounds on the gastrointestinal digestion and release profile of bioactive containing nanoemulsions has to be studied systematically.

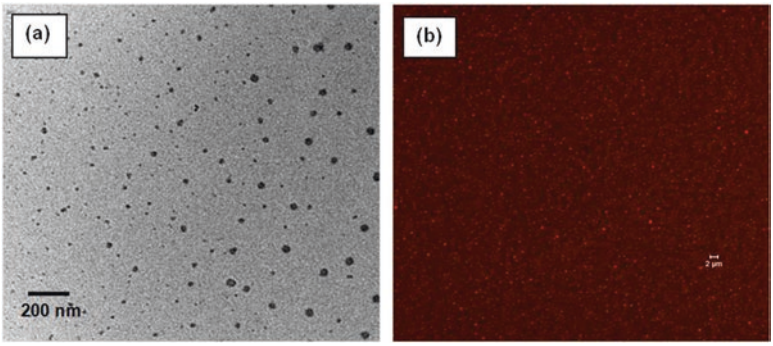

Fig. 3.12 (**a**) Transmission electron micrograph of Docosahexaenoic acid nanoemulsion and (**b**) confocal image of green tea catechins nanoemulsion stabilized by soy protein isolate depicting the oil droplets in nano range (Reprinted with permission of The Royal Society of Chemistry from Karthik and Anandharamakrishnan 2016 and of Elsevier Ltd. from Bhushani et al. 2016).

3.4.2 Controlled Release of Flavor Compounds

Flavors have a unique niche and value in the food industry as it has an influence on the overall sensory appeal of the food product. Encapsulation for flavor compounds aid in improving the chemical stability of the volatile aroma compounds during processing, storage and utilization. Essential or volatile or ethereal oils obtained from aromatic plants or herbs also have flavor characteristics in association with their antioxidant and antimicrobial properties. The limitations associated with the use of flavor compounds in foods are low solubility, poor chemical stability to light, temperature or presence of oxidants and intense flavor at high concentrations of the compound (Burt 2004; Madene et al. 2006). Nanoemulsions are suitable delivery vehicle for flavors and aroma/essential oils as they improve the solubility and chemical stability of the compound for food applications. Further, nanoencapsulation prevents undesirable changes in the flavor compound due to oxidation or related chemical reactions occurring in the food system.

Sweet fennel oil nanoemulsions with droplet diameters as low as 52.91 nm were prepared by Barradas et al. (2015) using high pressure homogenization technique. The antioxidant activity of the compound was retained in the nanoemulsion form and the authors envisage its possible application as antioxidant vehicle in topical formulations. Similarly, D-limonene, a terpene that is a major flavour element of citrus oils (lemon, orange, mandarine, lime), is a GRAS compound and used as a flavoring agent in beverages and baked foods. As with other essential oils, D-limonene also possesses antioxidant, bactericidal and therapeutic properties. However, the compound is heat labile, wherein at high temperatures it forms isoprene molecules, and is prone to oxidation (Sun 2007). D-limonene encapsulation in nanoemulsion system (mean droplet size: 27.4 ± 1.4 nm) has shown to increase its physical stability especially when stored at ambient temperatures (25 °C) (Li and Lu 2016). Clove essential oil and its flavor component namely

eugenol has also been nanoemulsified using non-ionic surfactants (Tween 80, Span 80) to enhance their storage stability and release properties in the gastrointestinal environment (Shahavi et al. 2015; Majeed et al. 2016). Also, eugenol when incorporated in to beta-carotene nanoemulsion, exhibited a synergistic effect in enhancing the physicochemical stability of the latter to high temperature and radiation (Guan et al. 2016).

Important aspects that have to be considered before formulating a flavour oil nanoemulsion are the chemical properties of flavour molecules, concentration to be loaded in the carrier oil phase, type of surfactant used to stabilize the nanoemulsion and the use of any co-solvents or co-surfactants. Hence, it is evident that flavour or essential oil nanoemulsions with suitable optical properties (transparent or translucent emulsions), kinetic and physicochemical stability have promising applications in the beverage industry.

3.4.3 Preparation of Low-Fat Foods

Apart from the oil in water and water in oil nanoemulsions, the concept of double or multiple emulsions are gaining interest in recent years. This is due to the enhanced functionality conferred by the emulsion systems by virtue of their structural configuration with regard to oil and aqueous phases. Multiple emulsions are complex systems which can be broadly classified as water-in-oil-in-water (W/O/W) or oil-in-water-in-oil (O/W/O). In W/O/W system (precisely, $W_1/O/W_2$), primary water droplets (W_1) are held within oil droplets that are dispersed with in the secondary continuous water phase (W_2). To develop this intricate system, two different types of emulsifiers are used – lipophilic emulsifier to stabilize water (W_1) droplets in the oil and hydrophilic emulsifiers to stabilize the oil in continuous water phase (W_2) (McClements 2012c; Dragosavac et al. 2012). Fabrication of multiple emulsions require controlled emulsification processes for efficiently handling the two distinct interfacial layers, one at the primary aqueous phase and one at the secondary continuous phase. To this end, membrane emulsifiers or micro-channel devices aid to process the emulsions with low shear stress and with higher control on the droplet diameters (Nisisako 2008). Research is still in its nascent stage with regard to development of bioactive encapsulated double emulsions in nanoscale. However, the inner water droplets (W_1) are in most cases with dimensions in the nano range.

An interesting application in the use of these multiple nanoemulsions is for the development of low calorie foods. Precisely, the structural design of the W/O/W emulsion makes it more viscous than the O/W emulsion for the same fat content. For instance, mayonnaise which is an emulsion of oil, emulsifier (lecithin of egg yolk) and vinegar or lemon juice can be prepared with lower fat content by introducing the concept of multiple emulsions. The water droplets (W_1) inside the oil droplets indirectly reduce the total fat content without compromising the sensory appeal of the product. Hence, the reduced fat content directly reflects on the total calorie of the product. Studies by researchers at Leatherhead Food International (United

Kingdom) have demonstrated a possible reduction of up to 40% of fat content from the traditional mayonnaise recipe (Dieroff 2011). Enormous scope exists in application of structural design principles in colloidal systems for development of food products with tailored properties.

3.4.4 Encapsulation and Release of Antimicrobial Compounds

As described earlier, an application of encapsulating flavour or essential oils is for augmenting their application as antimicrobial agents. Numerous studies have been carried out to use nanoemulsions as encapsulation structures for delivery of food grade, GRAS antimicrobial agents such as mandarin essential oil, eugenol, limonene, carvacrol, trans-cinnamaldehyde, eucalyptus oil, basil oil, thyme oil, lemongrass essential oil and so on. The antimicrobial activity of mandarin essential oil nanoemulsion in combination with techniques such as gamma irradiation, modified atmospheric packaging or high hydrostatic pressure have been employed for improving the shelf life of green beans. It was shown that the combination treatment was effective against *Escherichia coli*, *Salmonella Typhimurium* or *Listeria innocua* during storage for a period of 14 days (Severino et al. 2014, 2015; Donsì et al. 2015). Studies have also revealed that the antimicrobial activity of volatile oils is enhanced in nanoemulsion form than in free form (Danielli et al. 2013). However, the antimicrobial activity of the compound is highly dependent on its rate of release from the encapsulation structure to the site of action. For instance, if the antimicrobial agent is highly localized in the aqueous phase of the nanoemulsion system, its activity is quick compared to it being directly associated with the lipophilic phase (Donsì et al. 2012). A classic food application of antimicrobial nanoemulsions was explored by Ghosh et al. (2014). The authors encapsulated eugenol in to sesame oil based nanoemulsions (mean diameter: <100 nm) and demonstrated its antibacterial activity against *Staphylococcus aureus* in model food system i.e. orange juice. The study opens avenues for use of essential oil transparent nanoemulsions as natural preservatives in beverage industry. Also, lemongrass essential oil nanoemulsion had antibacterial activity against *Escherichia coli* when used as edible coatings on fresh-cut Fuji apples, without any notable change in the firmness and colour of apples during storage for two weeks (Salvia-Trujillo et al. 2015a, b). Similarly, thyme oil nanoemulsions had enhanced antibacterial activity in milk at 21 °C compared to free thyme oil (Xue et al. 2015). Apart from food applications, antimicrobial nanoemulsions also have relevance as topical formulations for wound healing applications (Sugumar et al. 2014).

In all these studies, essential oil nanoemulsions are shown to have improved antimicrobial activity than the compound per se in its free form. This is due to the ability of the nanoemulsions to fuse with the cellular components of bacteria or envelope of virus and destabilize the structure of the microorganism, thereby causing leakage of intracellular components and cell death. Due to this unique, non-specific mode of action, there are no chances for development of resistant microbial

strains (Karthikeyan et al. 2011; Moghimi et al. 2016). A practical implication of this theory can be explained by the work by Joe et al. (2012), wherein, sunflower oil nanoemulsions were used as an antimicrobial agent for preservation of Indo-Pacific king mackerel (*Scomberomorus guttatus*) steaks stored at 20 °C for 72 h.

3.5 Instability Mechanisms Associated with Food Grade Nanoemulsions

Emulsion stability is a term used to refer to the ability of an emulsion to resist changes in its physical and chemical properties during storage (McClements 2004). In some cases, the term stability also includes the ability of nanoemulsions to protect against microbial contamination or degradation (Qadir et al. 2016). Nanoemulsions, due to their high kinetic stability and nano scale droplets, are known to have higher stability than conventional emulsions. However, as they are thermodynamically unstable systems i.e. free energy of formation is greater than zero, they have the tendency to destabilize over storage. Thus, the overall stability of nanoemulsions is highly dependent on the emulsification type, composition, environmental conditions and duration of storage. A detailed review of the various challenges associated with stability of food grade nanoemulsions can be found at Karthik et al. (2015). Nanoemulsions breakdown due to one or more of the following mechanisms: (i) gravitational separation; (ii) aggregation and (iii) ageing or ripening (Fig. 3.13). When gravitational forces act on the nanoemulsion system, the instability is characterized by either creaming or sedimentation. Creaming involves an upward movement of dispersed phase droplets that typically have low density than the surrounding medium and sedimentation involves downward movement of droplets that have higher density than the continuous phase. When droplets aggregate, either flocculation or coalescence occurs. Flocculation is the aggregation of two or more droplets without losing their individual integrity, whereas, coalescence is the merging of two or more droplets and formation of one large droplet. On the other hand, ageing in nanoemulsion system is typically expressed as Ostwald or compositional ripening. In Ostwald ripening, large oil droplets are gradually formed at the expense of small droplets primarily due to diffusion of oil through the continuous water phase. This process is driven by an imbalance in the Laplace pressure in the curved surfaces of the large and small oil droplets. In contrast, compositional ripening is driven by the heterogeneity of the droplet composition, wherein, the concentration gradients drive the mass exchange, causing changes in droplet diameters. Apart from all these mechanisms, a prolonged coalescence at high intensity, will eventually lead to separation of oil phase on top of the nanoemulsion system, a process referred to as oiling off (Taylor 1998; Peña and Miller 2001; McClements 2004; Solans et al. 2005; Gutiérrez et al. 2008). In case of multiple emulsions (W/O/W or O/W/O), besides the above discussed instability mechanisms, they are also prone to stability issues with the inner phase and between the double emulsion

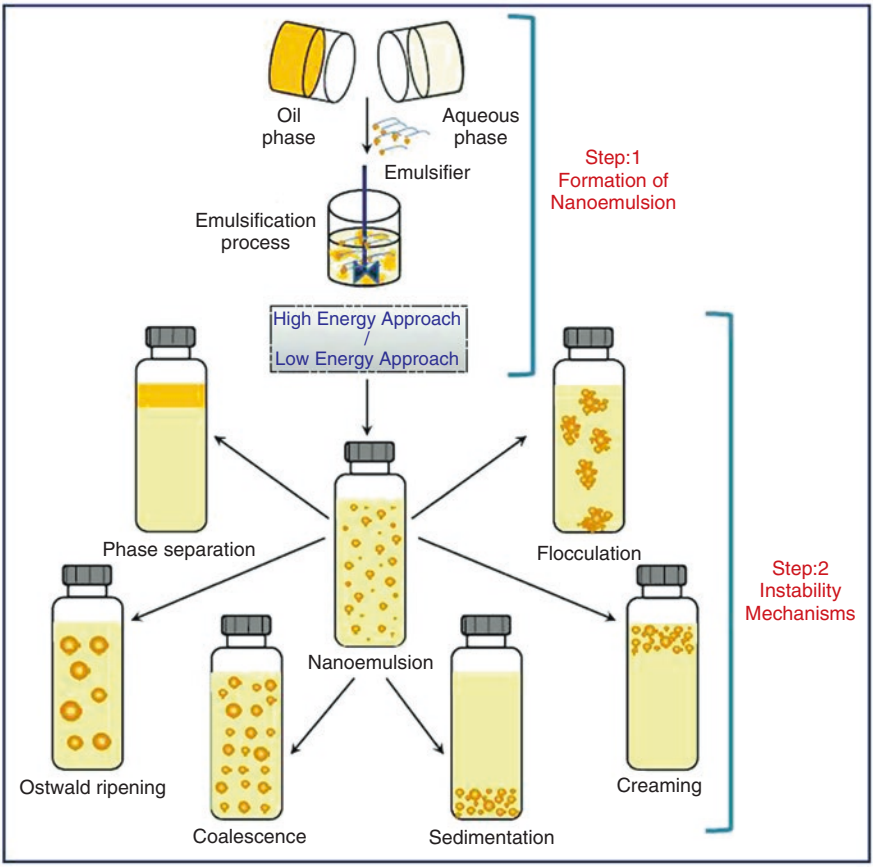

Fig. 3.13 The major instability mechanisms associated with oil-in-water nanoemulsions such as phase separation, Ostwald ripening, coalescence, sedimentation, creaming and flocculation are illustrated (Reprinted with permission of Taylor & Francis from Karthik et al. 2015)

droplets. The inner water or oil droplets can undergo coalescence, shrinkage, growth or even displacement/expulsion from the immediate continuous phase (Benichou et al. 2004). The above discussed mechanisms reduce the storage stability of nanoemulsion and also impair the chemical stability of the encapsulated nutrients.

Several approaches have been reported in literature to improve the storage stability of nanoemulsions. One common approach is to form nanoemulsions with droplet diameters in narrow range, such that the system is homogenous, typically characterized by low span value and low polydispersity index. Homogenous nanoemulsions can be achieved by using appropriate emulsification technique with suitable surfactants and optimized surfactant to oil ratio. However, care has to be taken to avoid over processing of emulsions during emulsification that increases the chances of droplet coalescence. Another method is to reduce the diffusion of oil droplets that lead to Ostwald ripening, by incorporating a significant volume of

insoluble oils such as long chain triacylglycerols, in the oil phase. Hence, it is essential to produce stable nanoemulsions as their food applications are directly dependent on its physicochemical and storage stability.

3.6 Conclusion

Nanoemulsions, being kinetically stable systems, present various advantages over conventional emulsions for protection and delivery of nutrients. They can be fabricated by using either high energy or low energy techniques. Nanoemulsions have promising applications in the food industry for development of fortified foods; oral delivery of nutrients; sustained release of flavors and incorporation of antimicrobials for preservation of foods. An emerging perspective to the application of nanoemulsions is by employing them as excipient foods. Nanoemulsions as excipient foods have functional properties when co-ingested with nutrient rich foods. Briefly, excipient foods do not have functionality on its own; however, they have the ability to improve the functionality of nutrients available in natural foods by altering their bioaccessibility and absorption. Future scope exists in this field of research and development for betterment of nutrient bioavailability by employing tailor made nanoemulsions for specific nutrients.

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Chapter 4
Genotoxicity of Nanomaterials in Food

Venkatraman Manickam, Ranjith Kumar Velusamy, Rajeeva Lochana, Amiti, Bhavapriya Rajendran, and Tamizhselvi Ramasamy

Abstract Nanomaterials are finding applications in the fields of food, agriculture, pharmaceuticals, catalytic industry, electronics and cosmetics. The use of nanomaterials in industries are widely appreciated due to their unique properties. Nonetheless, beside benefits, some nanomaterials are toxic. Nanomaterial Toxicity is often due to particular toxicokinetic and toxicodynamic properties, catalytic functions and inflammatory potential. Diverse genotoxic effects have been identified using different types of genotoxicity tests.

Here we review nanomaterials in food and pharmaceutical industries. We then discuss the genotoxicity of nanomaterials in food and health products. Toxic effects can be modified by functionalization, net particle reactivity, agglomeration and the functional environment.

Keywords Nanomaterials • Genotoxicity • Nanofood • Food printing • Supplements • Inflammation • Reactive oxygen species • DNA-damage • Oncogene activation • Cell cycle arrest • Cancer • Clay minerals • Food packaging

4.1 Introduction

Of the top ten nanotechnological applications proposed for the upliftment of developing countries while reaching the United Nation Millennium Development Goals, around half of them directly or indirectly associated with food and human intake based applications. It is clearly evident that several countries have initiated the focus on nanotechnology in order to strengthen their capacity and sustainable growth of their economies (Salamanca-Buentello et al. 2005). As a result, recently the nanomaterial applications are the emerging technological innovation in the fields of

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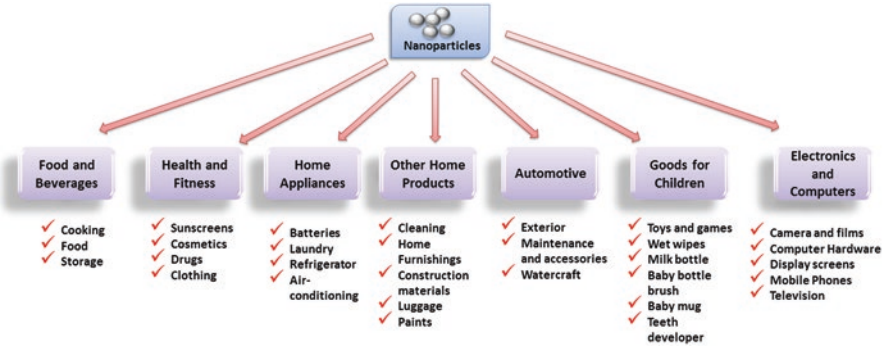

Fig. 4.1 Application of Nanomaterials in Consumer Goods: Nano materials find applications in various consumer items. Some of the industries where the nanomaterials are being applied include Food and Beverages, Health and Fitness, Home appliances, Other Home Products, Automotive, Goods for Children, Electronics and Computers (Reported from Sharma et al. 2012)

food industry, diagnostics, drug delivery systems, nanoproducts, nanodevices, and electronics. Consequently consumers worldwide are exposed to poorly studied or uncharacterized nanomaterials through foods, drugs, cosmetics, food color additives, food containers, paints and surface coatings (Fig. 4.1). These nanomaterials which increase health risks in addition to ever-increasing damage caused to our environment (Sahu and Casciano 2009). In line with the fast progress in the development and application of nanomaterials in different industries, to regulate them, many countries and ministries has established agencies to monitor and define the regulations, generation, management, and transfer of nano engineered knowledge. Their primary goal is to ensure safety through comparative analysis of the results derived through partner institutions and agencies.

European Commission Delegated Regulation (ECDR) has defined nanomaterial as 'any intentionally manufactured material, containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm to 100 nm' (Guo and Chen 2015). Currently around 1600 or more nanotechnology-based consumer products are being developed. Among them the majority are focusing primarily over the human-directed applications like health, nutrition and fitness. The regulatory mechanism regarding production, handling or labeling of nanotechnology based products are insufficient and thus it is necessary to scrutinize toxic effects of nanomaterials with regard to regulatory and risk avoidance purposes.

Many structural elements of regular food components, for example proteins and polysaccharides are nanodimensional in nature. These nanostructures (less than 300 nm) in the form of macromolecules, molecular assemblies, nanostructures, tiny particles, and interfaces are ubiquitously present in our daily foods of all forms (Fig. 4.2). Upon processing or cooking, these natural nanoforms provide the shaping to the microstructures in many foods. Thus the natural way of food nano-fabrication

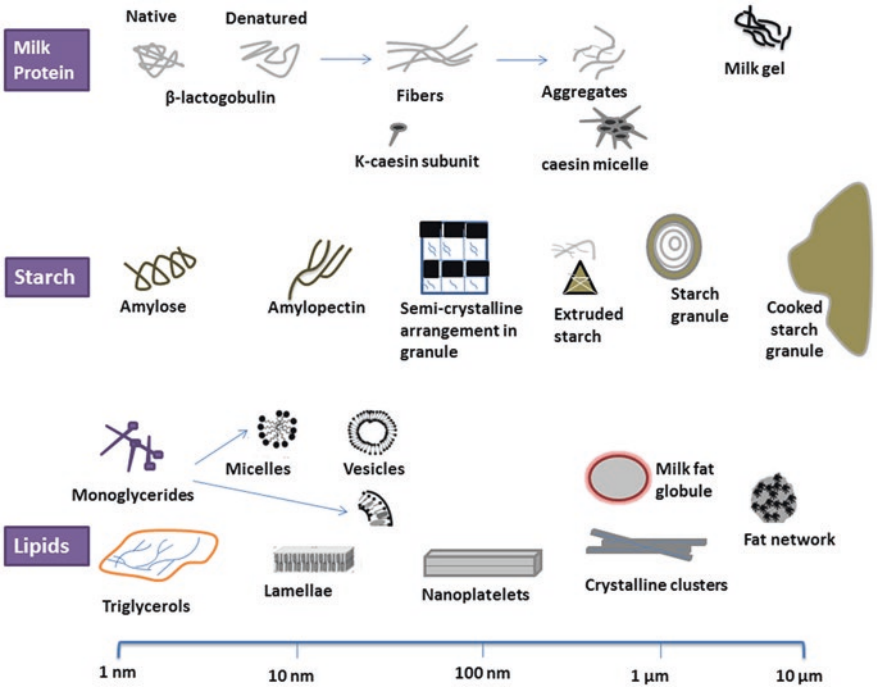

Fig. 4.2 Natural Nanofood forms: Proteins, carbohydrares, and lipids are present as macromolecular form in natural agricultural or animal products, which inturn transform into nanostructural forms during processing. These provide the properties like texture, flavor and shelf-life (Reviewed in Aguilera 2014)

utilizing cells, fibers, gels, emulsions, foams, and liquids, which provide the foods with various properties like texture, flavor, shelf-life, and nutritional value. Within our body upon digestion and after being liberated from the food matrix, these nanostructures again form new nanosized particles, complex aggregates, and self-assembled colloidal nanostructures (Aguilera 2014).

Inspired from these natural knowledge, food scientists came up with nanotechnological innovative ideas for (a) improvement in food processing, packaging and safety, (b) enhancing flavor and nutrition, (c) functionalizing foods for delivery of medicines and supplements, (d) enhancing large scale, cost-effective mass production. Some examples of food and associated products where great progress been made include processed, nanostructured food, nanocarrier systems for delivery of nutrients and supplements, organic and inorganic nanosized additives for food, supplements and animal feed, food packaging applications including surface-functionalized nanomaterials, nanocoatings on food contact surfaces, nanosized agrochemicals, water decontamination, and nanosensors for food labeling.

These novel food products are mainly to address and enhance the availability of often limited supply of food, and in improving the quality of available food and drinking water to the poor.

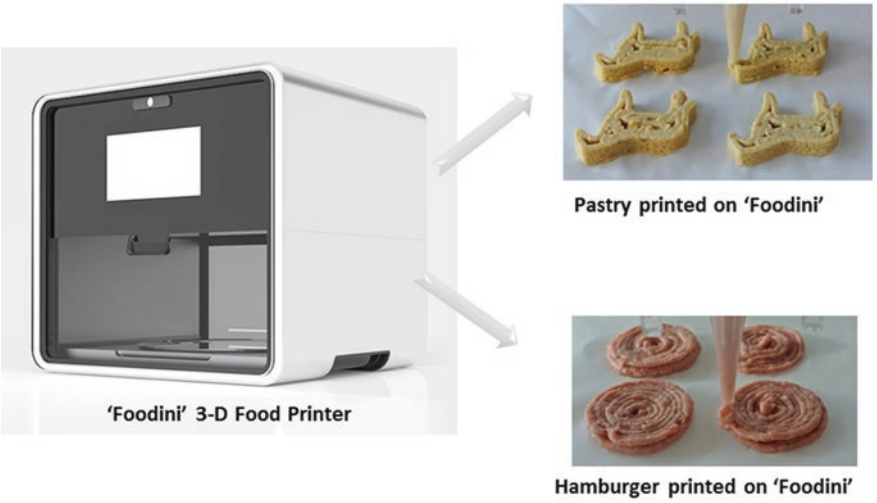

Fig. 4.3 3D Food printing: Currently it is possible to print food at industry level or for an individual's taste using three-dimensional (3D) printers. These designer foods could be engineered for desirable nutrients, texture, aroma, color, flavor, satiety and mouthfeel. Picture shows the 'Foodini' printer model, and the printed pastries and hamburger for army soldiers (As reported from Krassenstein 2014)

Accordingly, a recent 3D-Printing Industry reports states that food 3D-printing is now possible with the help of nanotechnology, by applying 3D food printers. Some of the reported list of food printers include 'ChefJet' from 3D Systems, 'Foodini' from Natural Machines, 'f3d 3D printer', Systems and Materials Research Corporation's special food printer for NASA, 'Choc Creator' from Choc Edge, ZMorph's 'Chocolate Extruder', **'**Discov3ry extruder' from Structur3d, **'**3D Fruit Printer' from Dovetailed**,** food printing by the Dutch research group TNO, and Germany-based Biozoon's food printer. Using these printers, specifically tailored, cartridge based, individualized 3D printed meals are now possible (Fig. 4.3) (Molitch-Hou 2014).

Nevertheless at the other side, there are reports about potential risks to human health arising from these ingested nanomaterials. For instance, the nanomaterial based DNA damage which could transform into cancer, heart or brain disorders. Additionally there is also risk of unintentional entry of nanoparticles into our food chain, for example, after being released from food packaging, or products of plant or animal origin and leaching out from coatings of processing equipments. Recently, increasing safety data have demonstrated that these nanoparticles could induce toxicity *in vivo* under a variety of exposure conditions, irrespective of route of administration like oral, inhalation, or via hypodermic injection. Potential toxic responses are expected at different molecular and functional levels, and some of which include cytotoxicity (cell death and proliferation modulations), genotoxicity, organ level toxicity, hemo-compatability problems, and problem with immunological responses.

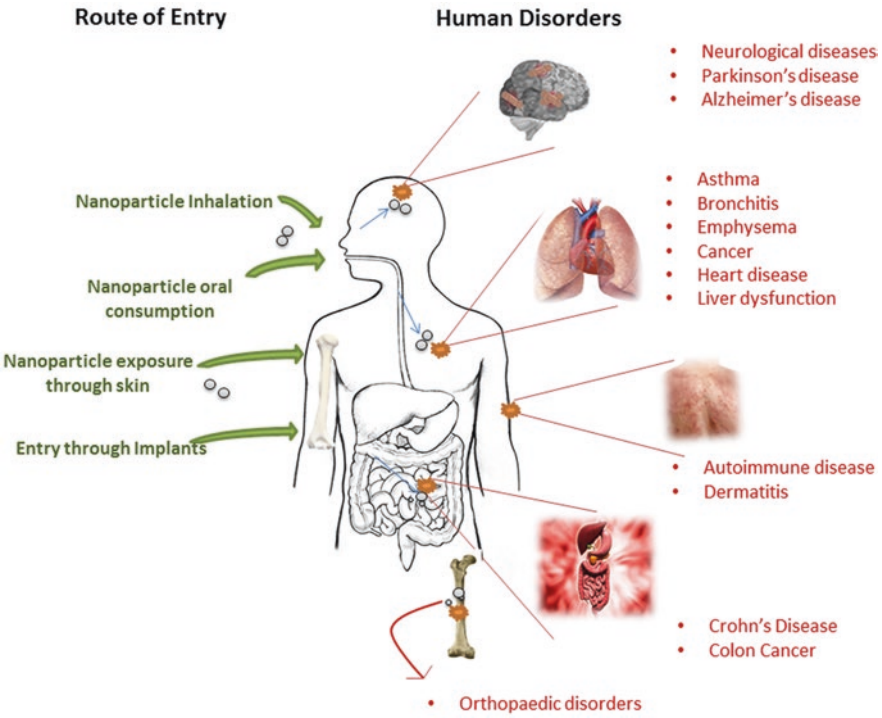

Fig. 4.4 Systemic entry and human disorders from nanomaterials: Nanomaterials from our daily life can enter through different routes like entry through oral, skin, respiratory and from implanted engineered products (if any). The suspected disorders expected from the toxicity of Nanomaterials are listed in the picture (Kumar and Dhawan 2013)

Genotoxicity could accumulate progressively over the period of time resulting in abnormal situations like cancer. Some of the reported disorders from the usage of nanomaterial include bronchitis, asthma, cancer originating at lung, colon and liver, Parkinson's disease, Alzheimer's disease, Crohn's disease, and heart disease (Kumar 2006). In addition, free radical induction from nanomaterials could lead to faster aging linked wrinkles, immune disorders like inflammation, autoimmune disease and metabolic disorders like weight gain and digestive problems (Fig. 4.4).

Currently there exist wide gap in knowledge especially about toxicology behind food based engineered nanomaterials, and if unanswered may lead to delayed adoption or even rejection of these innovative technologies, and also to inability in dealing with risks and uncertainties (Pray and Yaktine 2009). Clear understanding of the mechanisms and causes of nanomaterial toxicity will allow us to find effective solutions while at the designing level stage itself. Along with other toxicological profiling, genotoxic evaluation of nanomaterials has attracted much more attention among material safety regulation and risk assessment procedures (Chen et al. 2014a).

To make aware of the oncoming trend of food borne nanotoxicity and health issues in line with lifestyle disorders, here we provide with the overview of

nanotechnological applications in food industry and the available genotoxic information with respect to mechanism of toxicity, methods of study and reported genotoxic impacts and the conclusive future prospects. This could help creating awareness while implementing nanotechnological advances for the benefit of society, the environment, and the global economy.

4.2 Nanomaterials in the Food Industry

The definition on 'Nanotechnology' while framing United Nations Millennium Development Goals utilizing nanomaterial is 'the study, design, creation, synthesis, manipulation, and application of functional materials, devices, and systems through control of matter at the nanometer scale (1–100 nanometers), that is, at the atomic and molecular levels, and the exploitation of novel phenomena and properties of matter at that scale'. In food industry, any food is 'nanofood' when nanoparticles, nanoengineered techniques or tools are used during cultivation, production, processing, or packaging of the food (Garber 2006). Today knowingly or unknowingly many food industries are using nanomaterials. Food processing techniques followed for centuries utilize nanomaterials and nanotechnological measures using many different types of molecules and processes in an undefined manner. Only recently the comprehensive scientific perspective on developing, utilizing and visualizing micro and nano-structural technology are gaining momentum. However, predominantly today's consumers are generally ill informed about the nature and applications of nanotechnology in consumer-related products (Reisch et al. 2011).

Normally nano-molecules exist even in the natural unprocessed and processed foods. For instance the structural components and the macromolecules or polymeric components on digestion, breaks down into smaller molecules of ordered structures like cells, fibers, gels, emulsions, foams, and liquids. These smaller nano-units, in turn give foods the texture, flavor, shelf-life, and other nutritional significance. Traditionally nanomaterials in the forms of colloids like emulsions, micelles, mono- and bi- layers are applied in the food industry. The top-down approach like dry-milling technology is used commonly to obtain wheat flour of fine nano sized particle which improves the water-binding capacity of wheat flour. The same process improves the antioxidant activity of green tea powder after making them into 1000 nm sized particles (Shibata 2002; Sozer and Kokini 2009).

Today use of nanomaterials in food industry gained a great deal of interest due to various applications. Mainly nanotechnological measures are applied for the creation of functionalized foods like bringing in new textures and tastes, to design lesser calorie foods for specific applications, value added nutritional foods and for targeted strategy against clinical conditions or metabolic modulation in case of inborn errors, immune disorders, diabetic, weight and obesity controlling (Pray and Yaktine 2009). Thus nanoparticles can be the food ingredients in the form of flavors, antioxidants, antimicrobials, and bioactive compounds. In the food packaging industry, it provides low permeability and provides high strength packaging products.

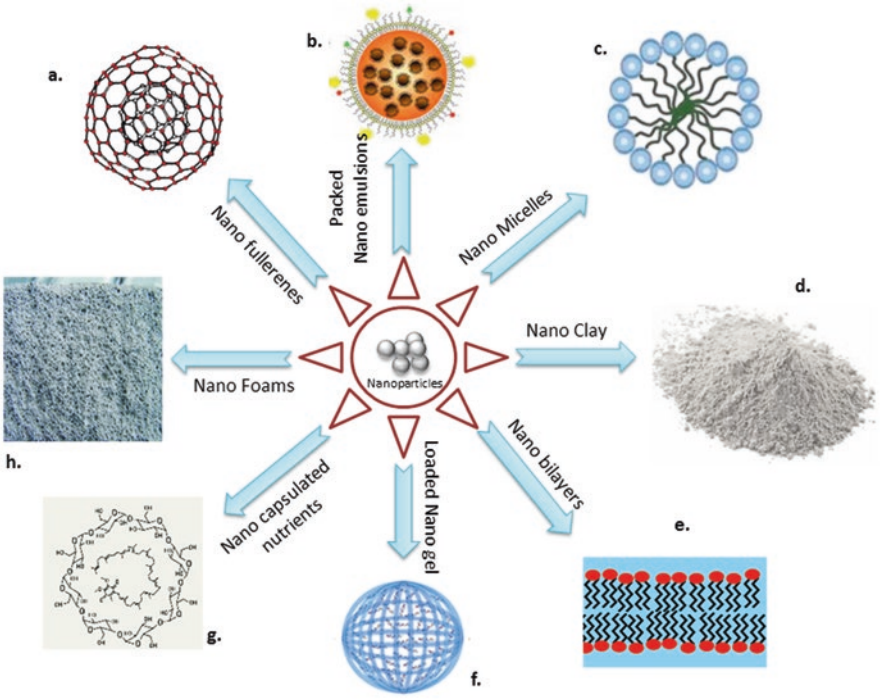

Fig. 4.5 Nanostructured Products in food industry: Multiple nanoforms like (*a*) fullerenes, (*b*) emulsions, (*c*) micelles, (*d*) Clays, (*e*) lipid bilayered structures, (*f*) gels, (*g*) encapsulated carriers, (*h*) foams are widely used for food and pharmaceutical industries

For detection of microorganisms and toxins in foods, magnetic engineered nanoparticles are applied in biosensors (Ranjan et al. 2014).

Currently various nanomaterial forms like nanotubes, fullerenes, nanofibres, nanowhiskers, nanosheets are used in the food industry (Cushen et al. 2012). They are applied in the form of nanoscale delivery systems like microemulsions, liposomes, biopolymeric nanoparticles, cubosome, solid lipid nanoparticles (SLNs), fibers, monolayers and nanosensors, microgels (Fig. 4.5) for encapsulation, protection, and delivery of lipophilic nutrients, vitamins, and nutraceuticals into the food (Sozer and Kokini 2009; McClements 2015).

Function wise these varying nanostructural types and engineered variants are studied in food industry to create novel structures, in introducing new functionalities, or aimed at enhancing and ensuring food safety. Specifically they increase food material bioavailability, enhance the taste, texture and consistency of foodstuffs, or to mask an undesirable taste or odor, and to alter the particle size, size distribution, potential agglomeration and surface charge (Powers et al. 2006). In addition to maintaining and enhancing the quality of food, for consumer care industry nanotechnology helps in water purification, fortification with vitamins and minerals, and also as antimicrobials (Fig. 4.6) (Duncan 2011).

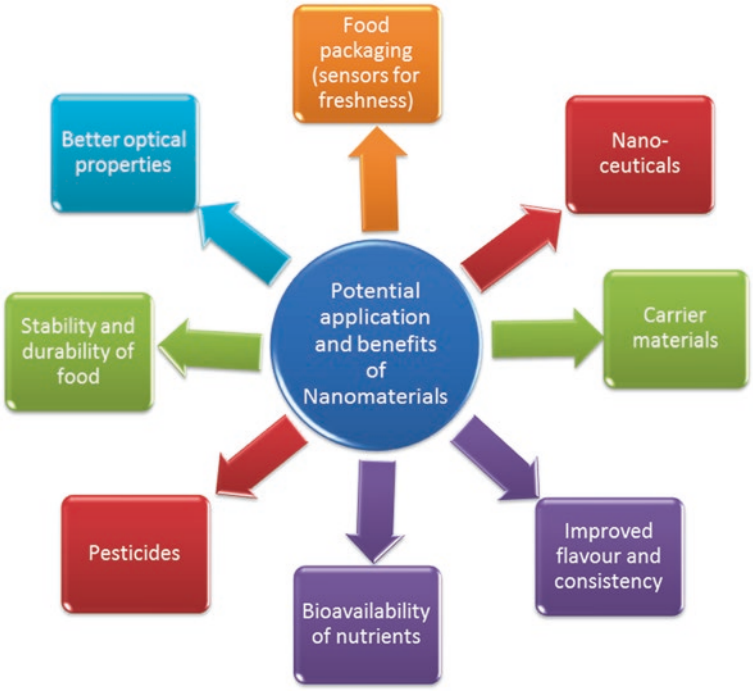

Fig. 4.6 Potential applications and benefits from nanomaterials (UK Parliamentory Report 2008)

According to a recent report, with regard to food and dietary supplementation industry some of the already commercialized products include (a) 'Nano green tea', where nanotechnology helps improving the bioavailability of selenium, thus enhancing the antioxidant effect, (b) 'Canola Active Oil' having nano-phytosterole capsules to reduce cholesterol absorption and thus in reducing the risk of cardiac diseases, (c) nanostructured carotenoid in enhancing the water solubility and thus in systemic availability, (d) Neosino capsules as dietary supplements in the form of nano-vitamins, nano-calcium, nano-magnesium and nano-silicon, (e) Aquanova, where micellas are used to improve the solubility of insoluble nutrients like vitamins A, C, D, E, K, beta-carotene, omega fatty acids (Fig. 4.7), (f) Nutralease, a fortifying nano-vehicles to carry nutraceuticals and drugs (UK parliamentory report on nanotechnology 2008).

Likewise, nanomaterial based engineered food technology are used routinely for sensing volatile compounds, detecting microorganisms, to improve the packaging and obtain the product information. For instance, nanotechnology has already led to the availability of devices that detect a combination of sub-optimal temperature and expiring shelf life using color-changing labels. Specifically this application includes the technology like detecting ripeness in the form of color-changing 'RipeSense' labels, or to detect pathogens and micro-organisms (Fig. 4.8).

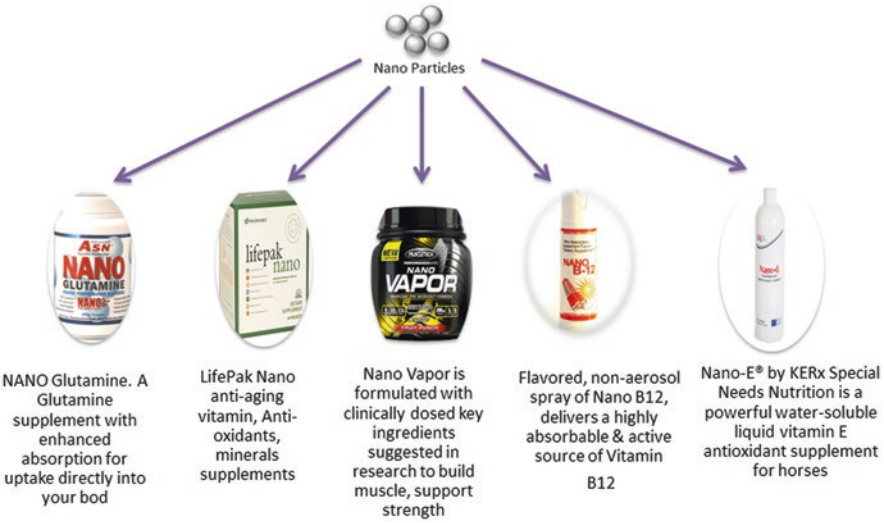

Fig. 4.7 Some of the nano-formulated products applied in food industry for supplementation and enrichment purpose, which include vitamins, aminoacids, minerals, antioxidants, and other biomolecules

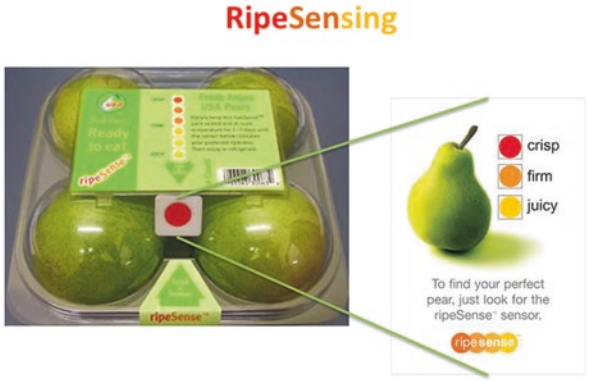

Fig. 4.8 'RipeSense': Smart sensing technology in fruit packaging, where changes in color indicate the ripeness of packed fruits. The initial *red* color indicates raw, crisp product, which gradually changes to *orange* and finally to *yellow* upon ripening. The RipeSense sensor works by reacting to the vapor phased aromas released by the fruit as it ripens. Using these sensors, it is possible by the consumer to choose the ripeness according to preference among pears, kiwifruit, melon, mango, avocado and stone fruit

Nanoemulsions are catching-up the conventional emulsions owing due to their varying size availability with the range of 20–200 nm, and thus for the delivery and bioavailabilty in health and nutrition industry. These lipid based delivery systems include liposomes, solid lipid nanoparticles, nanostructured lipid carriers, emulsions, micelles. These carrier materials provides the advantage of enriching bioactive

products, thus the health benefits to consumers without losing their nutritional properties, and as well as in increasing the profitability of food industries. Importantly they give desired food qualities like texture, aroma, color, flavor, satiety, mouthfeel and rheological properties to the products. Thus these carrier forms are preferred choice in delivering various functional lipophilic compounds of food industry including nutraceuticals, cosmeceutical, drugs, antioxidants, flavors and antimicrobial agents (Aditya and Ko 2015; Nandita et al. 2015).

Nanomaterials like carbon nanotubes (CNTs) are unique with their intrinsic mechanical, physical, and chemical properties. The carbon nanotubes are mainly applied in biomedical applications in the fields of drug delivery, targeted cancer therapies, biosensing, bio-imaging, and tissue engineering. Except for the biosensing, their application in food industry is generally limited.

The future applications of nanobiotechnology having close connection with food and health industry involves the development of *in vivo* biosensors (Fortina et al. 2005), utilization in biological circuit design, bioproduction systems, synthetic biology, drug delivery, medical diagnostics and disease therapy. Manipulation of materials at nanoscale has shown tremendous advantage of enhancing the sensitivity, selectivity and lowering the cost of diagnosis and other implied applications (Savaliya et al. 2015). In this regard, an oncoming nanotechnological modality tested under food industry includes radio frequency identification devices (RFID) in the chip forms, which can sense and reveal the freshness quotient and the suitability of the food product for consumption.

4.2.1 Nanomaterial in Food Packaging and Pharmaceutical Industry

In case of food industry-associated nanoproduct based packaging, the inorganic particles like clay along with surfactants when added to the biopolymeric matrix, helps ensure the sustainable bio-degradability of packaging material, or could help with other functionalities (Fig.4.9) (Dasgupta et al. 2015a). To provide maximum food security and simultaneously to ensure nutritional value at competitive prices, other than the traditional passive packaging, active and intelligent food packaging are attempted by food industries. In ‘active food packaging’, antimicrobials, antioxidants, and controllers of moisture, odor, and gases are usually added as active agents. Likewise in ‘intelligent or smart food packaging’, information about food or its surrounding environment, anti-theft indicators, locating devices, and time-temperature sensors are incorporated through nanoengineering. A typical example of this application is the addition of nanoclays in the plastic beer bottles (Ramos et al. 2015).

Among the lipid based delivery systems, along with liposomes and emulsions, solid lipid nanoparticles (SLNs) are widely accepted for the delivery systems in the food and pharmaceutical industries (Fig. 4.10). Special characteristics like stability

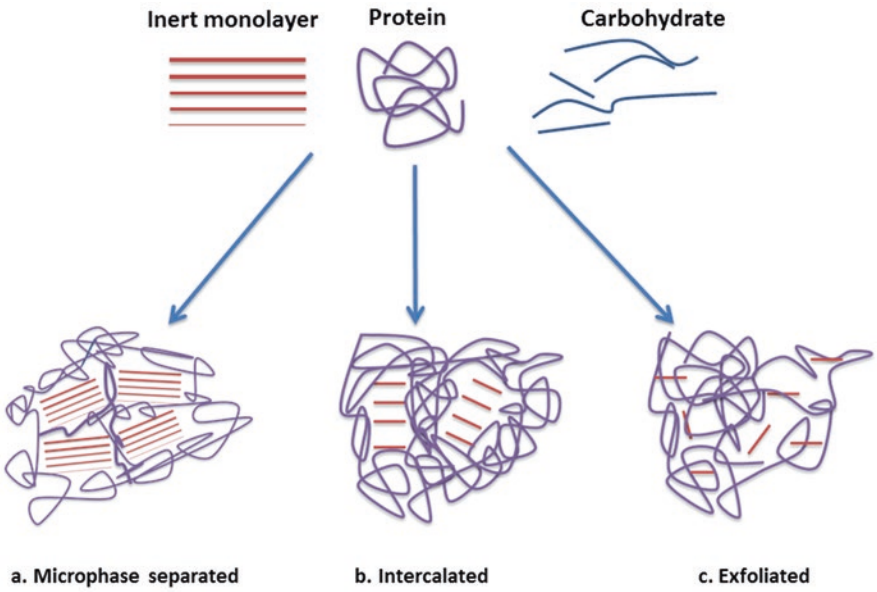

Fig. 4.9 Polymer-clay nanocomposites in food packaging: Structural and property alterations in a polymer matrix by including montmorillonite clay favor beneficial mechanical and thermal properties, moisture stability, and flame and weather resistance properties. Different hybrid types like (**a**) microphase-separated, (**b**) intercalated, and (**c**) exfoliated hybrids are explained diagrammatically

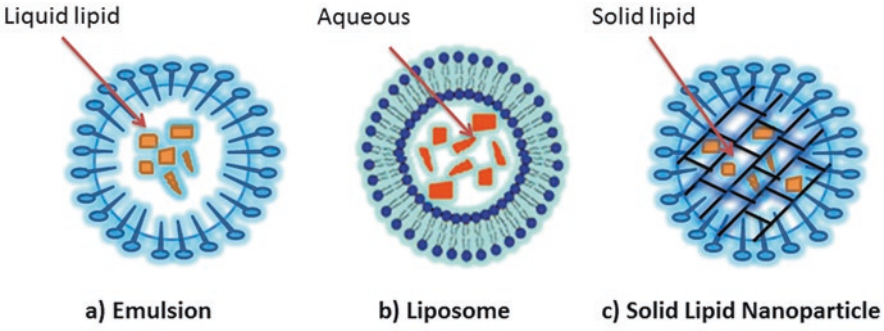

Fig. 4.10 Lipid based delivery systems: Different lipid based delivery vehicles like (**a**) emulsions, (**b**) liposomes, and (**c**) solid lipid nanoparticle are commonly applied for food bioactive molecules during supplementation and therapeutic drug delivery. These lipid system enhances taste and provide stability in gastro intestinal track (Adopted from Aditya and Ko 2015)

in the complex systems, non toxic composition, avoidance of organic compounds while in production, high entrapment efficiency, and the established industrially feasible method for production makes them the favorable choice as the carrier systems (Aditya and Ko 2015).

Some of the commercially available or near commercialization stage food nanotechnologies, and those related with respect to food matrix, food packaging, pharmaceutical, water purification systems as reviewed before (Cushen et al. 2012), and in the nanotechnology products and applications database of Nanowerk resource page (NanoWerk 2016) are

(a) Ag nanoparticles for the antimicrobial role in 'FresherLonger' plastic storage bags, Sunriver Industrial nanosilver fresh food bag (Huang et al. 2011; SCENIHR 2014).
(b) Oilfresh Nanoceramic inserts in deep fat fryers for inhibiting the polymerization of the frying oil
(c) Unilever's work on reduced fat ice cream
(d) Karate ZEON, an insecticide used on food crops for controlled release of active component lambda cyhalothrin through nano-encapsulation
(e) Bayer's 'Durethan KU 2601' packaging film, for improved O_2 barrier and improved gloss
(f) 'Ecosynthetix Adhesive' for burger containers having the advantage of lower heat activation temperatures, less water requirement for wetting, and reduced drying times compared to traditional adhesives
(g) Emulsiphan/EmPAC (Cornerstone), nanoemulsion-based drug delivery system for direct delivery into cancer cells of cancer-fighting drugs
(h) FluidCrystal NP (Camurus) for delivering amphiphilic and lipophilic drug compounds with solubility limitations, and for encapsulation of sensitive drug compounds
(i) Medusa (Flamel Technologies), nanogel for the extended release of a broad range of biological molecules and of small molecules
(j) TopScreen DS13 (TopChim), recyclable water based barrier coating formulation consisting of a biopolymer with monodispersed distribution of nanoparticles of regular shape. Used during the production of paper or cardboard for packaging
(k) NanoCeram-PAC (Argonide), powdered activated carbon for greater external surface area and more rapid adsorption of soluble contaminants like chlorine, iodine or toxic organic elements
(l) Nanofiltration Systems (Eurodia), nanofiltration membrane for pressure driven separations in food processing
(m) NANOWEB, nanofiber technology for advanced microfiltration required in industries lie food and beverage, liquid medium and process liquid filtration
(n) NanoClear, nanotechnology polymer based inexpensive purification of water at the molecular level.

Other than these industry linked products, food scientists are working on many more nanoproducts and the partial list of reported nanomaterials in the food industry are listed below in the Table 4.1.

With the rapid growth of the field, and because of the insufficient regulatory and risk measurement protocols, already consumer food products are sold with nano additives like iron in nutritional drink mixes, micelles that carry vitamins, minerals,

Table 4.1 Some nanomaterials developed for food or its allied industries

Nanomaterials	Application	References
Silver nanoparticles	Nanoparticle application for their effective antimicrobial role	Yu et al. (2007), An et al. (2008), Li et al. (2009), and Tankhiwale and Bajpai (2009)
Titanium dioxide (TiO_2)	TiO_2 powder-coated packaging film is widely used as a photocatalytic disinfecting material	Chawengkijwanich and Hayata (2008)
Food-grade TiO_2	Common additive (E171) in many types of foods, personal care, and other consumer products. Highest amount found in candies, sweets, and chewing gums, toothpastes and select sunscreens	Weir et al. (2012)
TiO_2 nanocomposite materials	Packaging films for a variety of oxygen-sensitive food products	Xiao-e et al. (2004)
TiO_2 doped with silver	Improved photocatalytic bacterial inactivation	Page et al. (2007) and Pratap Reddy et al. (2007)
Nanocrystalline SnO_2	As O_2-sensors in oxygen-free food packaging systems	Mills and Hazafy (2009)
Carbon nanotubes	Antibacterial properties of Carbon nanotubes puncturing on microbial cells causing irreversible damages and leakage of intracellular material	Kang et al. (2007)
Ultralong carbon nanotube-based membranes	Potable water purification appliances with superior desalination, disinfection and filtration properties based on ultrahigh specific adsorption properties	Yang et al. (2013)
Carbon black and polyaniline based nanosensors	Identification of food borne pathogens based on specific response pattern	Arshak et al. (2007)
Polymer-nanoclay composites	*Food packaging applications,* barrier properties	Adame and Beall (2009)
Biomolecule immobilizing matrices	Biosensing applications in food industry	Ahuja et al. (2007)
Cellulose nano-reinforcements	Improvements in barrier properties of polymers by addition of cellulose nano-reinforcements	Paralikar et al. (2008) and Svagan et al. (2009)
	Hydrophobization on cellulose surfaces by reactions involving hydroxyl groups, such as esterifications and acylation with fatty acids	Mohanty et al. (2001) and Freire et al. (2008)
Edible methylcellulose films with plant essential oils	Anti-microbial films	Otoni et al. (2014)

(continued)

Table 4.1 (continued)

Nanomaterials	Application	References
Intercalated nanocomposites	Ordered multilayer structure with alternating polymer/inorganic layers, i.e., two layers of tetrahedral silica sheets filled with a central octahedral alumina sheet	Weiss et al. (2006)
Food-grade lipid nanoparticles as nanoemulsion forms	To encapsulate, protect, and deliver hydrophobic functional components, such as oil-soluble flavors, colors, preservatives, vitamins, and neutraceuticals	McClements (2013)
Zein ultra-fine fibers loaded with gallic acid	Nutrient delivery utilizing Electro-spinned natural biopolymer	Neo et al. (2013)
Curcumin encapsulated seaweed iota-carrageenan	Neutraceutical delivery systems	Janaswamy and Youngren (2012)
Nanoscale enzyme immobilization systems	Lactase or cholesterol reductase to packaging materials in increasing the value of food products and for consumers with enzyme deficiencies	Fernández et al. (2008)
Nanosensors	*To* know the changes during storage (e.g., temperature, relative humidity, oxygen exposure), In measuring degradation products or microbial contamination	Bouwmeester et al. (2009)
Nanosensors integrated food packaging systems	Spoilage-related changes, pathogens and chemical contaminants, real-time status of food freshness	Liao et al. (2005)

and phytochemicals in oil, and zinc oxide in breakfast cereals. 'Friends of the Earth' reporting that among more than hundred food industry associated products identified to contain nanoparticles, dangerously none of them had any labeling information about the presence of nanomaterials, or safety testing status conducted by any government agencies (Sorrentino et al. 2007; Sozer and Kokini 2009; Read 2011). Thus it is right time to make aware of the health hazard problems associated with direct or indirect usage of nanomaterials in the food products.

Although for the past 20 years many nanotechnologies have been gaining access into commercial use, nevertheless it is very difficult to find out how many 'nano' consumer products are on the market and which product could be called 'nano'. To raise awareness and to update nanomaterial or nanotechnological information, the 'Project on Emerging Nanotechnologies' maintains an extensive list on inventory of nanotechnology-based consumer products already introduced in the market. This live, well maintained and updated inventory is a best resource for consumers, citizens, policymakers, and others who may be interested in knowing nanotechnological innovations in the marketplace. To date, this inventory maintains the information of more than 1600 manufacturer-identified nanotechnology-based consumer products

Table 4.2 Category-wise application of nanomaterials in consumer care products

Role in customer care application	Nano-technical principle
Dietary supplements/health applications	Silver, nano-organics, calcium, gold, silicon dioxide, magnesium, ceramics
Antimicrobial protection	Using silver, titanium dioxide and other nanoparticles
Protective coatings	By applying titanium dioxide and silicon dioxide
Environmental treatment	Nanotechnology to treat air and water in home
Cosmetic products	By applying silver nanoparticles, titanium dioxide, nano-organics, gold

introduced into various markets (Project on Emerging Nanotechnologies 2013). Among the 1244 benefit based nanomaterials repository, purpose-wise categories among the nanotechnological applications are sorted below in Table 4.2 (Vance et al. 2015). Though many of these innovations are still at the concept level (Tables 4.1 and 4.2), many have already entered into the market.

Although consumer products could directly or indirectly induce toxic measures, the potential for genotoxicity is very high among the food and beverage industry as the product of these industry are consumed directly. Within the food and related industries, the total numbers of reported nanomaterial applications by year 2015 are 117 products, and which includes 15 products in cooking, 20 products for storage, and 69 products for supplementation purposes. Likewise, though not utilized directly like food materials, still there exists the potential genotoxic hazard from the usage of nanomaterials in the health and fitness industry. There are about a total of 907 nanomaterial listings under health and fitness category, and the highly relevant numbers of nanomaterials among the sub-categories for genotoxicity potential are as below.

Filtration – 44
Cosmetics – 174
Personal Care – 344
Sunscreen – 40
Supplements – 17 products

Recent development in advancement of microscopic and other nano-analytical techniques have resulted in deeper understanding, and thus faster progression into novel nanotechnological applications. However at the product consumption point, still the acceptance of these high technological, innovative, to be ingested products by consumers are not up to the mark. Unfortunately, the existing database on safety evaluation for bulk materials is no longer valid when the same is extrapolated and applied for the safety assessment of nanomaterials. Thus along with the advancement of engineered nanomaterials and to clarify the hazardous assessment, there follows a new multidisciplinary, nanotoxicological evaluation science. This new nanotoxicology field aims to understand the toxicological and environmental impacts of engineered nanomaterials when they are exposed to humans and the environment (Zhao et al. 2013). Here in the coming sections the nanogenotoxic aspects associated with food bound engineered nanoparticles are discussed.

4.2.2 Consumer Acceptance of Nanomaterials in Food

Currently we are urgently in the need to explore the innovative strategies for promoting healthy eating. Thus today the concerns of food availability and nutritional intake are much greater in lesser-developed countries compared to the advanced economies like United States, Europe, and Japan. Nanotechnological modifications in food address the issues of food availability, nutritional enhancement, and affordability of quality food. With the advent of technological innovations day by day, uncharacterized side effects are also continuously evolving at a faster pace. With unique physicochemical properties and functions of engineered nanomaterials, there arise the undefined biological hazards to environment and humans from exposure like inhalation, ingestion, skin uptake (Zhao et al. 2013).

Today in developed economies, the public perception linked health awareness programs like opting for fresh and organic foods, avoiding genetically engineered foods or junk and packaged foods limits the public interest towards the nanotechnologically designed foods of finely controlled structure and functionality. Consumer skepticism is mainly associated with the health consequences and unknown environmental effects of engineered foods. With respect to the similarly innovative genetically modified ingredients in food products, it was concluded that, evaluation that considers the costs and benefits, with a special preference for public health protection is necessary prior to future development (Nelson 2001). Accordingly organization like 'Organic Consumers Association' are calling for stringent government regulation on nanofoods at least until more safety testing are completed (Garber 2006). In European Union, the Directorate General of Health and Consumer Protection has set up the 'Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)', which focuses on risk assessment of nanotechnological applications.

Additionally, the hidden risk of nanomaterials derived from workplace exposures and non-food industry based contamination into the environment is so prevalent. For instance, the nanomaterial derived from water treatment (O'Brien and Cummins 2010a), automobile, fuel exhaust, pharma/neutraceutical treatment (Kumar et al. 2014) of veterinary or poultry sources, which could enter silently into the environment. Again the risk of penetrating into the food-chain has to be seriously considered. Thus it is apparent that with technological advancement of food, we are inviting hidden risk factors and it is time to look into human and other living organism's well being and sustainability of the environment. Also by applying focused attempts over improving biocompatible surface engineering, it is possible to achieve minimalistic toxicity of biocompatible food materials. Through proper governmental guidelines and legislation, by applying thorough risk assessing toxicological screening procedures, it is possible to legalize the launch or withdrawal of commercial products to protect the unaware-consumers and the environment from potential hazards (O'Brien and Cummins 2010b).

4.3 Genotoxicity from Nanomaterials

Because of the size advantage associated with nanomaterials, they could enter into our body through almost all possible entry routes like inhalation, ingestion, skin penetration, intravenous injections or from the implanted medical devices. Once entered into the human or animal system, there are different ways through which cells uptake nanomaterials and mediate the potential interaction with intracellular macromolecules. Figure 4.11 provides the thematic representation of proposed internalization routes observed for nanomaterials.

In the human body, the major contributor of toxic effects arising from the food industry based nanomaterials is due to their increased surface area-in-contact (Dowling 2004), and thus highly favored intercellular and intracellular transport through adhesion and other uptake machineries. Another prevalent but an ignored area is the unforeseen risks from the use of nanomaterials in food-packaging materials and the resulting toxicity effects (Sozer and Kokini 2009). After accessing into

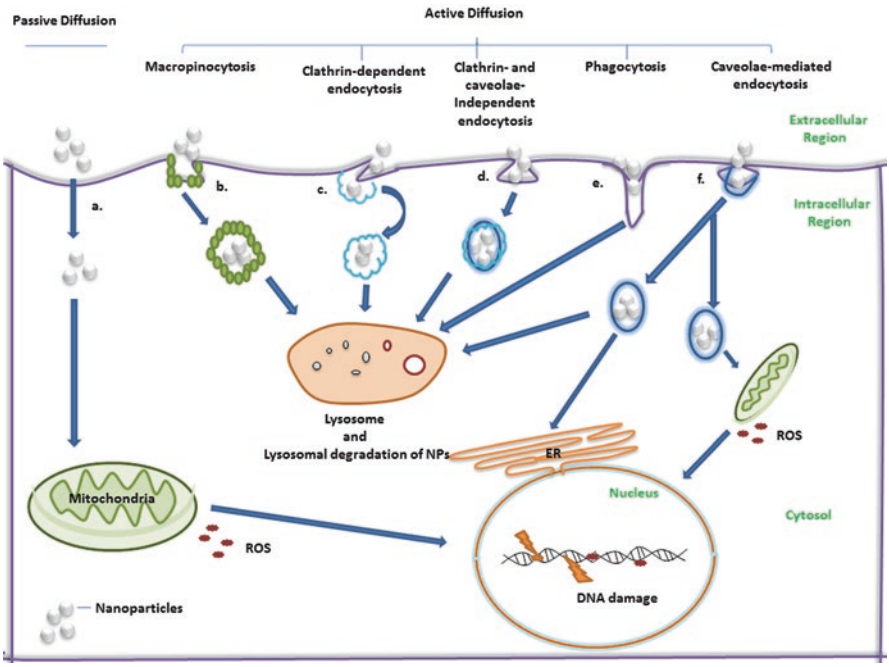

Fig. 4.11 Intake and trafficking of nanomaterials: At the cellular level nanomaterials may be internalized via (*a*) energy-independent passive diffusion, or through energy requiring (*b*) macropinocytosis, (*c*) clathrin dependent endocytosis, (*d*) clathrin and caveolae independent endocytosis, (*e*) phagocytosis, or (*f*) caveolae-mediated endocytosis. The transporting early endosomal vesicles then fuse to phagolysosomes or endosomes. They may also be carried to the cytosol, and from the cytosol they enter into subcellular compartments such as the nucleus, mitochondria or into the endoplasmic reticulum. Through transcytosis recycling endosomes could cross out of the cell. Also exocytosis happens through macropinocytosis

the cells because of the predominant risk of persistent, non-dissolvable, non-biodegradable nature of nanoparticles, they are not swiftly cleared out of the cellular system (Tiede et al. 2008) and thus causing the toxico-kinetic problems.

Currently in food industry, the toxicological screening, understanding, and risk assessments about nanotechnological applications are highly limited. There is serious lack of consumer understanding, government scrutiny, labeling procedures and testing methodologies with respect to risk assessment (Garber 2006). With the advancement of nano-engineered products into the food industry and with the shortage of proper toxicological characterization, there exists immense scope to improve our understanding of nanomaterial induced toxicity profiling and regulatory modalities.

4.3.1 Studying Nanomaterials Based Genotoxicity

Toxicology follows the general principle that any materials when going beyond the tolerable dose are poisonous. Many of the times nanomaterials behave toxically, because of their quantum physics derived material effects like size, large surface area, and accessibility when compared with their bulk counterparts. Also for the same reasons, they create uncertainty in measuring material toxicity profiling thus escaping stringent risk assessment scrutiny protocols (Guo and Chen 2015).

While studying the toxic profile, it is essential to study the behavior of nanomaterials both in physiological media and in the biological test systems. As the nanomaterial toxicity studies are prone to discrepancies, i.e. the same nanomaterial could elicit differing reactions based on the cellular origin or organ, it is crucial to use the wide array of cell types from differing origin thereby giving the systemic coverage (Wottrich et al. 2004). According to the NanoCare collaborative initiative, some of the commonly used model cell lines representing the organs of lung, skin and gastrointestinal-tract for evaluating nanomaterial toxicity are listed below in Table 4.3 (Nau and Krug 2009).

Also it is noteworthy that there arises the size and shape dependent fate of the nanomaterial toxicity within the body due to their dynamic and kinetic properties like deposition, clearance and translocation. For example the pharmaco-dynamic properties like cytotoxicity, necrosis and mutagenicity of nanomaterial can be influenced through particle size, and thus thorough evaluation is necessary (Sahu and Casciano 2009).

The aim for nanotoxicological profiling is to confirm the involvement of particle or particle-chemistry derived cellular responses in consistently detectable manner, so that the reliable particle testing methodologies utilizing different organs and cell systems can be developed in giving the advanced preventive warning. Already there exist some operational procedures in characterizing and comparing nanomaterials both in *in vitro* cellular system and at the *in vivo* animal models. By utilizing the existing and newly derived toxicological datasets and unrestricted sharing among

Table 4.3 Commonly used cell lines in nanomaterial genotoxicity studies

Cellular origin	Strain name	Cell type
Human (*H. sapiens*)	A549	Lung adenocarcinoma cell
	CaCo-2	Colon carcinoma cells
	CaLu3	Lung adenocarcinoma line
	HaCaT	Skin keratinocyte cell
	T84	Colon carcinoma epithelial cells
	MonoMac6	Monocyte-macrophage like leukaemia cells
Mouse (*M. musculus*)	NIH-3T3	Embryonal fibroblast cells
Rat (*R. norvegicus*)	NRK-52E	Kidney epithelium like cells
	Raw 264.7	Transformed macrophage cells
	RLE-6TN	Lung alveolar epithelial cells
Canine (*C. familiaris*)	MDCK	Kidney epithelial cells
	MDCK II	Kidney epithelial cells

different agencies, the knowledge created could be utilized for risk free application of the nanomaterials in the welfare of the common people (Nau and Krug 2009).

In connection to this regulatory decision-making process, consensus guidance documents released by the Organization for Economic Co-operation and Development (OECD) and the International Conference on Harmonization (ICH) recommend certain genotoxicity assessment tests. These accepted assay system include studying accumulated DNA breaks, gene mutations, and chromosomal alterations. Usually studying these genetic abnormalities apply bacterial *Salmonella* based mutagenicity test (the Ames test); *in vitro* mammalian cell assays such as the Comet assay, the mouse lymphoma gene mutation assay (MLA), and the micronucleus (MN) assay and *in vivo* animal experimentations (Kumar and Dhawan 2013; Magdolenova et al. 2014).

It is accepted that *in vivo* animal model derived experimental data has the advantage of revealing toxicological kinetics and systemic biodistribution of test compounds in test animals, thus providing the realistic genotoxicity information of different tissues and organs. The most common among the *in vivo* animal based assays includes *in vivo* micronucleus and Comet assays. It is worthwhile as reviewed by Guo and Chen that instead of high percentage of positive outcomes in *in vitro* studies, usually the *in vivo* based Comet and micronucleus assay yielded high negative results possibly from the inherent higher DNA repairing capability of animal models (Guo and Chen 2015). Thus when reviewing the information on hazardous nature using *in vivo* animal studies, caution has to be exercised while extrapolating results or mechanisms for the human hazard characterization and subsequent human risk assessment (Oberdörster et al. 2007).

Some preferred *in vitro* cellular system and the key assays used to measure genotoxicity include mutagenesis in epithelial cells from the lungs of the treated animals using bronchoalveolar lavage fluid (BAL), using hypoxanthin-phosphoribosyltransferase (HPRT) assay, neutrophil content in bronchoalveolar lavage fluid from

Table 4.4 Commonly used genotoxicity study tools

Genetic toxicity monitoring tools	Cell/tissue samples
Chromosomal aberrations (CA)	Lymphocytes
Sister chromatid exchanges (SCE)	Lymphocytes
Micronuclei (MN)	Lymphocytes
Point mutations (e.g., *hypoxanthine-guanine phosphoribosyl transferase* gene)	Lymphocytes and other tissues
DNA adducts	DNA isolated from cells/ tissues
Protein adducts	Haemoglobin, albumin
DNA strand breaks	DNA isolated from cells/ tissues
Oncogene activation	DNA or specific proteins isolated
Mutations/oncoproteins	Various cells and tissues
DNA repair	Isolated cells from blood samples

chronic inflammation (Driscoll et al. 1997), correlation between bronchoalveolar lavage fluid based neutrophil content and the level of DNA strand breakage measured by *in vivo* comet assay in the lung epithelial cells of the treated animals (Knaapen et al. 2002).

Evidently in case of TiO_2 nanoparticles, hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutation assay and comet assay based DNA strand breaks proved to be the more sensitive tests in evaluating the genotoxic effects. These assays were carried out in *in vivo* study on adult male Sprague-Dawley rats and in *in vitro* Chinese hamster lung fibroblasts (V79 cells) (Chen et al. 2014b). Likewise, both *in vivo* micronucleus and Comet assays are highly preferred specifically for the regulatory, decision-making purposes by the regulatory bodies (International Council for Harmonisation 2012; OECD 2013). Recently a modified comet assay using bacterial enzymes helps to detect oxidized DNA bases and thus in quantifying the induced oxidative DNA damage (Shukla et al. 2011).

Interestingly, Knaapen et al., has reviewed about the *in vitro* co-culture study models involving inflammatory cells and various other cell types (like lung epithelial cells), while establishing the inflammatory phagocyte (macrophages, neutrophils) associated secondary DNA damage and mutagenesis in neighboring non-inflammatory cells (Knaapen et al. 2006). It is also been believed that the Ames test and the chromosome aberration test are not reliable tests for nanomaterials (EFSA Scientific Committee 2011). Cumulatively the genotoxicity markers in genetic monitoring of nanomaterial exposure and the most commonly used cell/tissue samples listed by Encylopedia of Occupational Health & Safety are reproduced below (Table 4.4) (Stellman 1998).

This list is a generalized one for all types of toxic evaluations in pharmaceutical, clinical diagnostics and environmental studies, and thus the study techniques, target cells, molecules analyzed are the same for monitoring genetic toxicity in the food industry too.

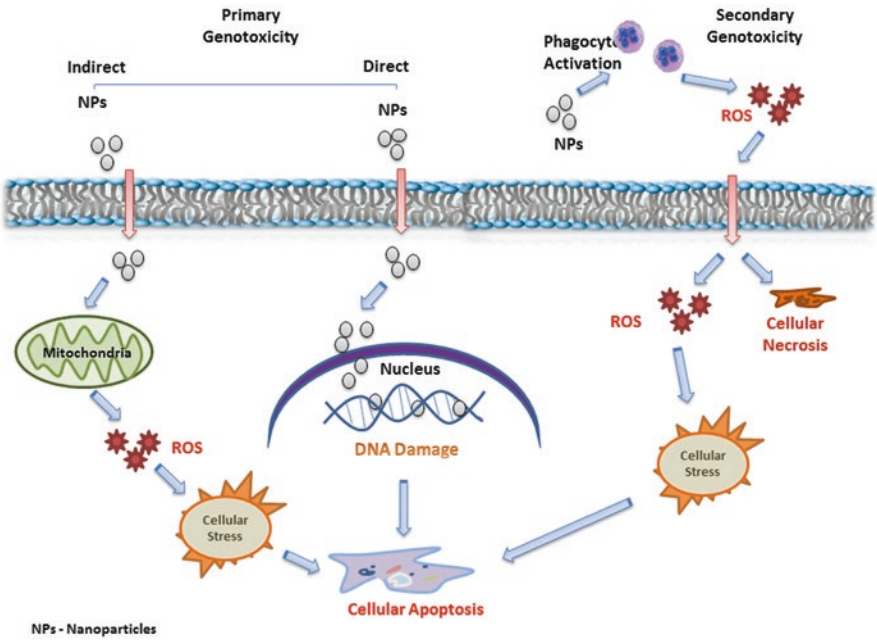

Fig. 4.12 Scheme showing DNA damage induction machineries: Generally nanomaterials are considered to induce genotoxicity through multiple routes, Direct action of nanomaterials on nuclic acids and induction of DNA damage (primary genotoxicity), Indirect DNA damage through formation of free radicals like ROS and RNS which enter inside and acts on the nucleic acid to cause damage (primary genotoxicity), or through secondary genotoxicity route, where phagocytes activation, i.e. macrophage and neutrophil cells will be recruited extracellularly and which secretes inflammation mediators like free radicals that causes genotoxic damages (Magdolenova et al. 2014)

4.3.2 Potential Mechanisms of Genotoxicity

Cellular genotoxicity involves association of genetic damages which could arise from intake of any extraneous sources including the nanomaterials. Genotoxicity could arise in the absence of any inflammatory reactions (primary genotoxicity). This primary genotoxicity could be due to direct physical interaction between the nanoparticles and the cellular genomic DNA contents.

This direct interaction of nanoparticles with the genomic DNA, or disruption in the elements in mitotic spindle apparatus have been reported for the particulate material of asbestos, crystalline silica, and for some specific nanosilver materials. Also it has been reported about the indirect existence of primary genotoxicity which happens through formation of reactive oxygen species (ROS) by nanoparticle-activated target cells, or from depletion of intracellular antioxidants (Fig. 4.12). Quartz particles may cause the primary genotoxicity, despite indirectly through oxidative attack of the genomic DNA by mitochondria-derived reactive oxygen

species (ROS) (Li et al. 2007). Likewise through the formation of peroxynitrite, TiO_2 and C_{60}-fullerenes nanoparticles caused the mutagenic effects (Xu et al. 2009). ZnO nanoparticles, for instance, by modifying the optimal levels of hydroperoxide ions, reactive oxygen species, malondialdehyde level, and lactate dehydrogenase activity leads to genotoxicity such as DNA fragmentation (Kumar et al. 2011). Other reported primary genotoxic machineries involves disturbance of membrane stability (Cveticanin et al. 2010), inhibition of various DNA-repairing machineries, reduced ATP levels which in turn may contribute to altered repairing processes in the nucleus (Wojewódzka et al. 2011).

Alternatively for the secondary genotoxicity, nanomaterial activated macrophages and neutrophils could elucidate genetic damages additionally during inflammatory processes. These inflammatory associated DNA-damages arise from reactive oxygen, nitrogen species (ROS/RNS) and other mediators however of phagocytes activation (macrophages and neutrophils activation) (Fig. 4.12). Evidently, oxidative and nitrosative stress were mainly associated with enhanced inflammatory response and genotoxicity of ZnO nanoparticle observed in human monocyte cells (Senapati et al. 2015). Likewise, chronic inflammation from prolonged exposure of nanoscale granular biopersistent particles could elucidate secondary genotoxicity without any primary chemical toxicity (Hartwig 2013).

During genotoxicity, there exist some physical and chemical parameters of nanomaterial characteristics that can affect critically the generation of reactive oxygen species. The elaborate list of nanomaterial characteristics reported todate include size, shape, particle surface, surface charges, surface-exposing groups, particle dissolution, metal ion release from nanometals and nanometal oxides, UV-light activation, aggregation, interaction mode with cells, inflammation, and pH of the medium (Fu et al. 2014). For instance, the surface/interface properties like elastic stiffness of nanostructured materials could dramatically change the surface physical properties of the engineered material, and thus the net apparent material properties to a novel one (Fig. 4.13). However to date, the effects of surface properties on toxic machineries are not studied in detail (Eremeyev 2015).

Owing to oxidative stress resulting from overproduction of reactive oxygen species and failure to carryout normal physiological redox-regulated functions, various deleterious toxic effects occurs which ultimately leads to DNA-damage, unregulated cell signaling, change in cell motility, cytotoxicity, apoptosis, and cancer initiation (Bouwmeester et al. 2009). Prolonged exposure to nanoparticles leads to systemic effects on different organs affecting immune, inflammatory, excretionary and cardiovascular system (Fig. 4.4).

Redox-regulation not only influence the genotoxicity of human and animal cells, even the marine organisms like bivalve mollusk *Scrobicularia plana* and the ragworm *Hediste diversicolor* when exposed with sub-lethal concentrations of metal-based nanoparticles, there were cellular nanoparticle accumulation and associated alteration in glutathione S-transferase and catalase markers. Eventually genotoxicity and apoptosis were frequently observed with these marine endobenthic species supporting the primary universal role of oxidative stress in mediating genotoxic measures (Mouneyrac et al. 2014). Overall when genotoxicity were reported, there

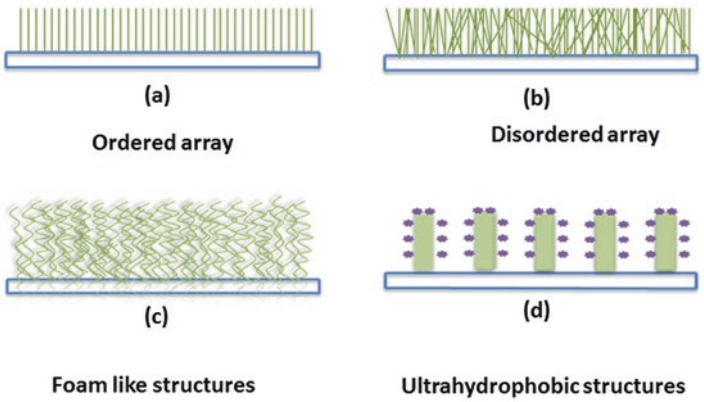

Fig. 4.13 Net surface physical properties of the engineered materials: Nanomaterial surfaces could be of various microstructures like (**a**) ordered array, (**b**) disordered array, (**c**) foam like structures, and (**d**) ultrahydrophobic structures. Such regular or irregular structures of nanocrystals, nanotubes and nanowires can be obtained by various methods, and that provides variations in surface properties (Eremeyev 2015)

were DNA single-strand breaks, double-strand breaks, oxidative DNA or chromosomal damage thus leading to net DNA fragmentation. In animal model studies, along with the tested pregnant animals, even their offspring were found to have DNA-deletions when TiO_2 nanoparticles were administered *in utero* during pregnancy, thus highlighting the teratotoxicity and the severity of damage inflicted (Trouiller et al. 2009).

Once genotoxic changes introduced, there will be activation of cellular repairing system predominately through DNA-damage sensing p53 tumor suppressor signaling pathway. This could leads to cell cycle arrest at different phases, DNA repair or senescence. If uncontrollable amount of damage inflicted by the nanoparticles, then there will be induction of apoptosis and phagocytosis of severely damaged cells takes place (Fig. 4.14). However prolonged exposure and persistence of highly inflamed situation could ultimately leads to transformation of cells and to cancer.

One of the major signaling response, DNA damage response is activated by nanomaterial exposure based DNA double-strand breaks and replication-blocking lesions. Microarray derived, global gene expression based signaling analysis suggested that silver-nanoparticle induced toxicity is from response against reactive oxygen species and DNA damage, chromosome instability, mitosis inhibition, and immune response induction, along with specific pathway like JAK-STAT signal transduction pathway (Xu et al. 2012). Similarly human alveolar A549 cells when exposed with TiO_2 nanoparticles, there were significant induction of double strand breaks and cell cycle arrest. Simultaneously these TiO_2 nanoparticles enhanced the expression of ATM, P53, CdC-2 and decreased the expression of ATR, H2AX, Cyclin B1, thus confirming the genotoxic potential of TiO_2 nanoparticles and the remedial repairing process following the exposure (Kansara et al. 2015). Accordingly,

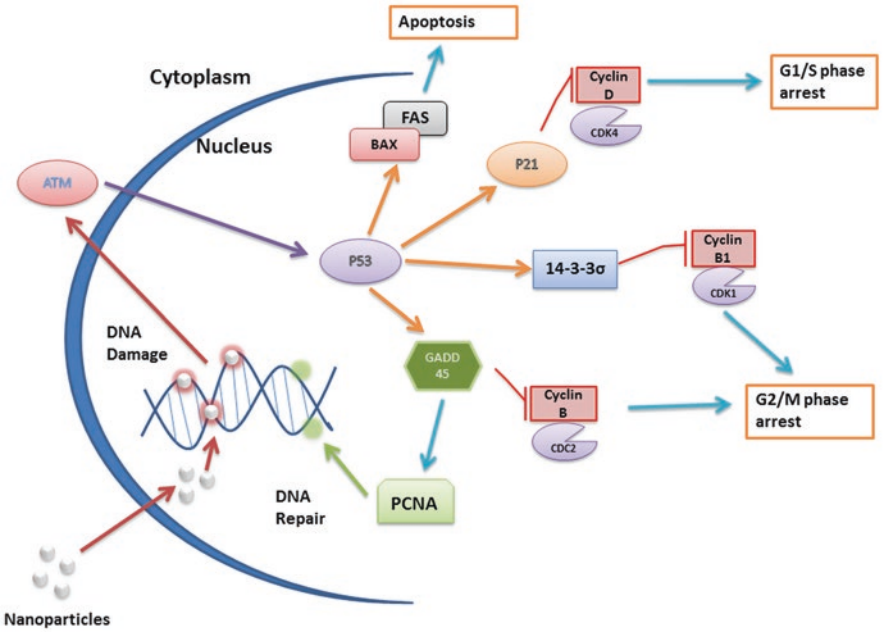

Fig. 4.14 p53 Activation: Chronic exposure and disposition with Nanoparticles could induce DNA damage, and which is sensed by serine-threonine kinases like ATM. These kinases activate cellular stabilization process through p53 phosphorylation and activation. Activated p53 transcription factors mediates cell cycle arrest (G1, G2 arrest), senescence, DNA repair, or when repairing is not sufficient, ultimately leading to apoptotic cell death (Brown et al. 2009)

kinases including ataxia telangiectasia-mutated (ATM) and ATM-and-Rad3-related (ATR) are activated which in turn activates multiple factors involved in cell cycle control and apoptosis. These factors ultimately regulate apoptosis, inflammation, and transformational process following genotoxic stress. Some of the prominent transcription factors include p53, breast cancer-associated protein 1 (BRCA1), Nuclear factor kappa B (NF-κB) and activated protein-1(AP-1) (Christmann and Kaina 2013).

As there is balancing role between apoptosis and inflammation, both apoptotic and anti-apoptotic genes primarily get modulated upon nanoparticle exposure. Some of the array of DNA damage and apoptosis-related genes upregulated by nanoparticle exposure include DNA-damage inducible transcript 3 (DDIT3), caspase 1, and cysteine peptidase (CASP1) genes and superoxide dismutase 2, glutathione reductase 1. Likewise the balancing apoptosis inhibitors like BCL2 interacting protein (HRK), BIK (BCL2-interacting killer, apoptosis inducing), Fas apoptotic inhibitory molecule 3 (FAIM3), apoptosis inhibitor (FKSG2), GADD45A are also upregulated with prolonged nanoparticle exposure (Xu et al. 2012).

4.4 Factors Influencing Genotoxicity

There are different factors which could affect the extent of genotoxicity. Based on the nanomaterial functionalization methods, structurally varying modifications have been incorporated during the synthetic process of nanoparticles. In a nanomaterial therefore, substituent inorganic minor elements, or elements from any masking, or stabilizing inorganic coating may change the toxicological effect. Other than the inorganic coating, organic coatings or any methods adopted during functionalisations of nanomaterials, or presence of any catalyst materials could also modulate the toxicity profile. Thus the net particle reactivity due to altered surface area, mass and/or particle number, surface properties play a major role while studying the toxico-kinetic and toxico-dynamic responses (Warheit et al. 2007).

Compared to their bulk counterparts, toxico-kinetic and dynamic properties could change due to nanomaterial shape, size and state of agglomeration. Simplest nanomaterial form like the orally taken quantum dots could breach the integrity of the human intestinal lumen cell lining, and thus nanoparticles ingested through food or in drinking water could possibly cross the human intestinal barrier (Koeneman et al. 2009). In contrast, it is reported that the phagocytizing ability of macrophages are altered by the particle agglomerate size. That is, the agglomerate size could also affect the particle penetration ability through cell membranes, and translocation through the tissues, lymph or circulatory system (Nemmar et al. 2001; Renwick et al. 2001).

Biological or chemical environment where nanoparticles are introduced also affect its toxic features, and as nanoparticles behave differently when placed in different environments there is possibility of inconsistencies in size information. In fact the characterization data to be correlated with toxicity profile are dependent upon characterization phase, sample preparation methods, and the measurement technique followed.

With respect to the environmental effect and toxicity variations on nanoparticles, European Food Safety Authority (EFSA) report on ‘Scientific Network for Risk Assessment of Nanotechnologies in Food and Feed’ confirms the existence of widespread differences in toxicological results. For their conclusion they accessed recent research results of orally exposed nanomaterials present in the food/feed chain (European Food Safety Authority report 2012). For consistent reliable results they suggest the genotoxicity tests in line with the European Union based initiative measure like ‘Nanogenotox’ project. In addition to genotoxicity studying, they recommend focusing further on study design, the test materials used, sample preparation methods, toxico-kinetics based tissue distribution of nanomaterials, and the final results interpretation.

During genotoxicity studies, in addition to the nanoparticle availability at organ and cellular level, levels of intra-nuclear bioavailability of elemental ions and thus their cellular homeostasis could seriously affect genomic stability. For instance, CuO nanoparticles when compared to their CuO microparticulate counterparts and water soluble copper chloride ($CuCl_2$), highest intra-nuclear bioavailability of

copper ions were observed only in case of CuO nanoparticles. This elevated nuclear availability of elemental copper ions correlated well with high DNA strand breaks and pronounced inhibition of poly ADP-ribosylation even without excess H_2O_2 (Semisch et al. 2014).

With respect to size dependent genotoxicity, SiO_2 nanoparticles of sizes 6, 20, 50 nm has particle size based enhancement in genotoxic and cytotoxic potential, when peripheral blood lymphocytes were examined using sister chromosome exchange study (Battal et al. 2014). In case of silver and titanium dioxide nanoparticles there were reports of specific high dose dependent genotoxicity, however in contrast, this effect was not observed or insignificant in case of low doses (Dobrzyńska et al. 2014). Thus great care has to be given while fixing the study dose alongside the application linked dose of nanomaterial, before interpreting about the genotoxic results.

In addition to the pharmaco-kinetics and dynamical property based variation, additional level of variation could arise from the evaluation procedure itself. The study on genotoxicity with SiO_2 nanoparticles reported about individual experiment dependent variations in genotoxicity while compared among MN assay, peripheral blood lymphocytes based sister chromosome exchange, and Comet assays (Battal et al. 2014). Interestingly, the results from the March 2013 report of 'Nanogenotox' which is based on different types of TiO_2, synthetic amorphous silica and multiple walled carbon nanotubes (MWCNT), it was reported that *in vitro* comet assay and the micronucleus assay gives varied cell specific results among different cellular systems. Surprisingly, *in vitro* mouse lymphoma assay were not raising any doubts. Thus when *in vitro* comet assay are used, the results has to be scrutinized seriously before proceeding to *in vivo* based genotoxic studies (NanoGenoTox partnership 2013).

According to Landsiedel et al., any optimization for genotoxic tests, and the testing conditions for extrapolating genotoxicity results on human risk might consider the following factors (Landsiedel et al. 2009)

1. Knowing what nanomaterial has been tested, and in what form
2. Recognizing that nanomaterials are not all the same
3. Considering about uptake and distribution of the nanomaterial
4. Taking into account the nanomaterials specific properties
5. Using standardized methods
6. Using *in vivo* studies to correlate *in vitro* results
7. Learning about the mechanism of nanomaterials genotoxic effects.

Finally many food and feed producers might not be aware themselves that they are using nanomaterials, and as a result the availability of genotoxic information on currently existing food based nanomaterials are highly limited. Today developing countries and their population are in urgent need for food availability, nutritional content, and affordability to pay for food. As a consequence we are not in the position to leave these engineered foods untouched. Also we are in the initial stage of utilizing these nanoproducts in the food industry and so not much data are available on toxicity profiling. Cumulatively, when studying nanomaterials there are many

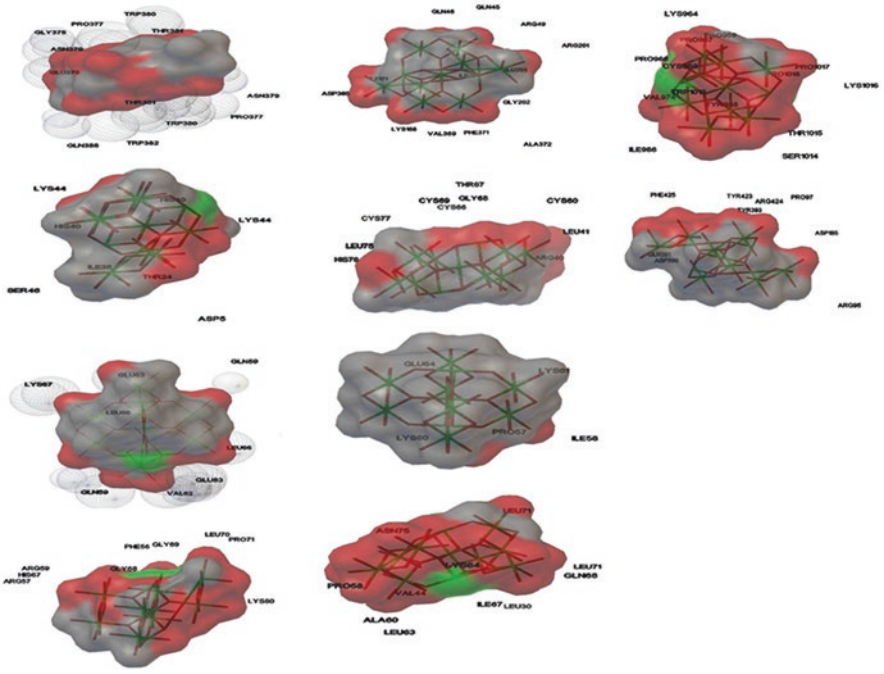

Fig. 4.15 Docking results from interaction between titanium dioxide nanoparticles and different toxicology related cellular proteins. Docking energy data shows titanium dioxide nanoparticle has significantly higher affinity towards intercellular adhesion molecule-1, p38 mitogen-activated protein kinases, placental growth factor, and nuclear factor kappa-light-chain-enhancer of activated B cell proteins (NF-kappaB) (Ranjan et al. 2015).

factors, both intrinsic and extrinsic, which give rise to the genotoxic discrepancies. Thus great care has to be given while relying only on few reports.

Because of the situation of incomplete toxicity profiling, extensive and inclusive sampling of products on the market has to be considered for toxicity evaluation (EFSA Scientific Committee 2011). Today we are not having a common platform having unbiased toxicity information. With the help of government agencies and active industrial involvement, it is possible to raise a repository of nanotoxic information which could be freely accessible to all. With the advent of computational big data analysis, by combining all the food toxicity data available it is possible to view efficiently the overall picture, thus harvesting the scientific advancement in improving the quality food available for us. In this direction, Titanium dioxide nanoparticle-protein interaction studies were attempted in computational *in silico* docking analysis utilizing publicly available PDB file of cellular proteins for which toxic interaction has been reported earlier (Ranjan et al. 2015). (Fig. 4.15) Likewise, Quantitative Nanostructure-Toxicity Relationship (QNTR) could be applied in line with Quantitative structure-activity relationship (QSAR). Here like drug toxicity prediction, modeling the biological effects of nanomaterials could be attempted

without using any tedious, time-consuming, and expensive animal testing (Kumar et al. 2014). These molecular simulation approaches not only help predicting the toxicological responses, they could also support validating the existing data and thus quicken the market introduction of engineered products.

4.5 Reported Genotoxic Trends from Food Bound Nanomaterials

A variety of food industry based engineered nanoparticles with different size, shape, and surface properties have been shown to induce genotoxicity, cytotoxicity, and oxidative stress in different cellular models, and some of which are explained here briefly. Regulatory agencies like FDA in USA or even other important developers like Japan and China are not having proper nanotechnology specific regulations. Regular risk assessment procedures followed by the companies are not sufficient, and the sensitivity and validity of currently existing test systems followed by them are also disputable. This kind of situation is because of the discrepancies like usage of only a selected type and size of nanoparticles, exposing the test animals with high concentrations under artificial conditions, inability to extrapolate the existing data from one type of nanomaterial to another or from one size to another, and limited availability of datasets (Chau et al. 2007). Thus until the risk assessment information based on cytotoxicity or genotoxicity on food industry is strong enough, the usage of such product has to be restricted.

4.5.1 Silver Nanoparticles and Genotoxicity

Currently nanoscale silver is commonly used in various consumer products, medicinal applications, and bioengineered devices mainly because of its potential nutraceutical and therapeutic values. For instance, silver nanoparticles are used in wound dressing due to their anti-microbial properties. To the extent the silver nanoparticles are proposed for food industry applications, currently their complete toxic profile are not studied in detail (Dasgupta et al. 2015b).

When the safety of silver nanoparticles proposed for medical applications were tested for their genotoxicity profiling, there were oxidative stress induced chromosomal aberrations in the bone marrow cells of albino rats. Additionally hepatic histopathological lesions were observed when albino rats were intraperitonially introduced with various doses of silver nanoparticles and thus confirming the presence of both hepatocytotoxic and genotoxic stresses (El Mahdy et al. 2015). Supportively there was cell specific genotoxicity among liver and colon cells (*viz*, HepG2 and Caco2 cells) in *in vitro* model where liver derived HepG2 cells are more susceptible to food-related nanosilver. Because of this cell specific nature in

genotoxicity, fungal and bacterial targeting role of silver nanoparticles in food, food-contact materials, dietary supplements and cosmetics has to be considered with serious caution (Sahu et al. 2014).

Similar to this, a report on *in vivo* clastogenicity and mutagenicity of silver nanoparticles with different sizes and coatings (polyvinylpyrrolidone, and silicon-coated silver nanoparticles) confirmed the cytotoxic and genotoxic potential of them. When evaluated by mouse micronucleus (MN) assay, phosphatidyl-inositol glycan, Class A (‘*pig-a*’) gene mutation assay and Comet assays, it was demonstrated that the AgNPs could reach mouse bone marrow and liver, and selectively generate cytotoxicity to the reticulocytes and cause oxidative DNA damage to the liver (Li et al. 2013b). Consolidated studies using both plant and animal derived systems (*Allium cepa, Nicotiana tabacum* and Swiss albino male mice) showed impairment of nuclear DNA and chromosomal aberrations upon treatment with Ag-nanoparticles, thus raising the concern about the safety issues associated with its food applications (Ghosh et al. 2012).

In primary cells raised from Syrian hamster embryo also, silver nanoparticles significantly increased dose-dependent micronucleation frequency in addition to cytotoxicity, thus confirming the widespread genotoxic nature of silver nanoparticles (Li et al. 2013a). Using high-throughput methods to compare the nanotoxicity in intact animals (using *Caenorhabditis elegans*), it was reported that metal based engineered nanomaterials like nano-Ag were highly toxic to *C.elegans* because of their intrinsically toxic nature (Jung et al. 2015). In fact, nano silver materials were comparatively more toxic than TiO_2 nanomaterials in bacteria, algae, crustaceans, and fish (Kahru and Dubourguier 2010). Thus considering the wide spread genotoxicity among different systems, it is not safe to consider silver nanoparticles even after taking into consideration their anti- fungal and anti-bacterial capabilities.

4.5.2 *Genotoxicity from Clays, Clay Minerals and SiO_2 Nanoparticles*

Clays and clay minerals such as kaolinite, montmorillonite and sepiolite are served in food packaging industry. Clay nanocomposites help extending the shelf life of beer from 6-months to 18 months by preventing the escape of oxygen, and make plastic bottles shatter-proof for handling (Fig. 4.16) (Garber 2006). Usually clay based nanomaterials are mixed with other minerals while being utilized in these applications. Although reports suggest involvement of dose dependent cytotoxic role through necrosis, apoptosis, oxidative stress, and genotoxicity from mineral components, there is not much information available on toxicity biomarkers with respect to immune-modulation and genetic expression (Maisanaba et al. 2015). Similarly when pyrogenic (NM-202 and 203) and two precipitated (NM-200 and -201) nanosized synthetic amorphous silica were administered orally in Male Sprague Dawley rats, irrespective of the dose and the organ investigated there was

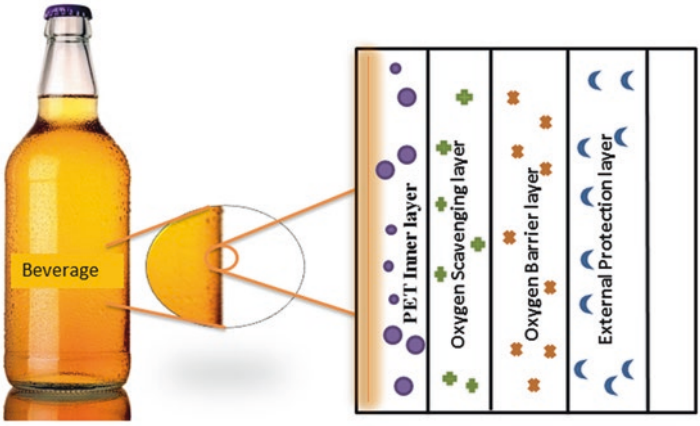

Fig. 4.16 Multilayered beverage packaging system consisting of nanoengineered oxygen scavenging layer, oxygen barrier layer and external protection layer for enhancing gas permeation and so in quality enhancement (Ramos et al. 2015)

not any conclusive DNA damage with the Comet assay. However only a mild, insignificant increment in micronucleated cells were reported in the colon even at the lower dose (Tarantini et al. 2014). Same compound also when administered through another introductory route, i.e. through intra-tracheal instillation and intravenous injection of SAS, there was no significant increase in DNA damage or micronucleus frequency. Absence of genotoxicity despite acute hepatotoxicity, thrombocytopenia, and even animal death like obvious toxic effects only raises the need for more research data. These differences, i.e. toxicity without genetic damage suggest the need for further additional scrutiny with respect to acute and chronic toxicity and the role of agglomeration/aggregation of synthetic amorphous silica nanomaterials and their relative uptake by different cell types (Tarantini et al. 2014).

Other than in the cosmetics, foodstuffs, and biomedical devices, SiO_2 nanoparticles are currently being applied in various industries like chemical-mechanical polishing, varnishes, and printer toners. When using high-throughput methods to study nanotoxicity using live *Caenorhabditis elegans* as a model and when automated analysis system for toxicity was utilized, various degrees of toxicity were detected among differing forms of fumed SiO_2 nanoparticles, graphene, carbon black, Ag, and carbon nanotubes. Though SiO_2 nanoparticles were toxic in this organism, simultaneously other nanomaterials tested like fullerene nanoparticles (nC60), fullerol, TiO_2, and CeO_2 were relatively safer. In this high-throughput study toxicity was studied only using parameters like body length, locomotion speed, and lifespan, and which not include any genotoxicity profiling (Jung et al. 2015).

When genotoxic effects of SiO_2 were studied using bacterial mutation assay, *in vitro* chromosomal aberration test, *in vivo* comet assay, and *in vivo* micronucleus test by following OECD guidelines, there were no genotoxicity reported both in *in vitro* and in *in vivo* systems (Kwon et al. 2014a). In the absence of OECD guidelines, the same group reported previously the genotoxic effects on the same

compound (Kim et al. 2014). Thus within the same laboratory based on the evaluation methods, variations could occur. This highlights the insufficiency of risk assessment standards and requirement of consensus evaluation guidelines, like the one proposed by OECD, among the monitory agencies worldwide.

4.5.3 Reports on TiO_2 Nanoparticles on Genotoxicity

With a wide range of applications, titanium dioxide (TiO_2) nanoparticles are manufactured worldwide in large quantities. Because of its inert, insoluble and persistent nature, it is already used as European Union approved food additive (E171) and also in paints, cosmetics, and personal care products.

Using comet and micronucleus assays TiO_2 nanoparticles were proven to induce DNA damage and a corresponding increase in micronucleus frequency. This genotoxic nature of TiO_2 nanoparticles is possibly due to the observed increase in the level of oxidative stress and ROS generation. Because of the oxidative stress there were consequent DNA double strand breaks and cell cycle arrest in G2/M phase. Even though approved as food additive agent, the authors suggested for the careful evaluation before going for the regular, wide scale applications in the food industry (Kansara et al. 2015).

In Drosophila, when TiO_2 nanoparticles as well as their bulk format were tested for routine genotoxicity using comet assay, there was DNA damage highly influenced by the physico-chemical properties of nanoparticulate TiO_2 than its corresponding bulk form (Carmona et al. 2015). Interestingly even when tested using the plant cellular system, in *Allium cepa*, there was dose dependent uptake/internalization of TiO_2 nanoparticles and reactive oxygen species based oxidative stress have been established. This stress was a major contributor towards the genotoxic effects observed (Xu et al. 2013). Thus even after having consensus existence of genotoxicity using different experimental study systems (Drosophila, *Allium cepa*), TiO_2 nanoparticles also shows some conflicting genotoxic results. That is, having greater number of positive toxicity results when checked *in vitro* systems than when checked using *in vivo* systems.

Also there was no correlation between DNA/chromosome damage and gene mutation, i.e. having produced more chromosomal damage than the extent of functional loss through gene mutation. This kind of variation might be due to the presence of corrective machinery present in the whole animal system than at the cellular *in vitro* level (Chen et al. 2014a). Results like least toxicity in the *in vivo* system than the *in vitro* system should not be taken as positive sign for confident applications, as acute toxicity having serious effects on organs like brain, lung, spleen, liver, and kidney along with animal death were reported after high dose intravenous injection of TiO_2 nanoparticles in mice. Acutely toxic nature of TiO_2 nanoparticles without any genotoxicity and hematological toxicity raises the serious concerns on their safeties to humans (Xu et al. 2013).

4.5.4 Genotoxicity Effects from Modified ZnO Nanoparticles

Zinc as Zinc Oxide (ZnO) nanoparticles has unique properties like biocompatible properties, semiconducting, piezoelectric and pyroelectric properties, which separates them from other metal nanoparticles. Thus worldwide, huge quantity of ZnO nanoparticles are produced and utilized in personal care products, pharmaceutical industries, transparent electronics, piezoelectric devices, UV emitters, chemical sensors, and in spin electronics based industries. Similar to titanium oxide, zinc oxide are applied in many sunscreen products to prevent the sunburns. With their antimicrobial properties, ZnO nanoparticles have wide application in food industries for microbial-free packaging, smart packaging and quantification, and for nutritional supplementation purposes. They are also used in environmental remediation during water or air treatment processes. To the extent they are applied in varying consumer products, their genotoxic profiles are not clearly revealed. ZnO nanoparticles usually show a systemic distribution pattern in the *in vivo* studies after administered through different routes like respiratory tract, digestive system and parenteral routes (Vandebriel and De Jong 2012). Thus they have the potential to reach any organ and tissue and involve a risk for human health.

Utilizing the test guidelines provided by Organization for Economic Cooperation and Development (OECD), when surface-modified ZnO nanoparticles (positively or negatively charged particles of 20 nm and 70 nm size) were evaluated for genotoxicity they do not induce toxicity in both *in vitro* or *in vivo* test systems (Kwon et al. 2014b). But when the Cu-Zn alloy nanoparticles (ANPs) were evaluated for genetic modification using micronucleus, comet and γ-H2AX foci counts in human lung epithelial cells (BEAS-2B), there were significantly increased chromosomal damage alongside the single and double stranded DNA damages (Kumbıçak et al. 2014).

The surface modification might be necessary in case of some nanoparticles made from Zinc. In such cases they exhibit altered toxic behavior. When variously sized and surface charge modified (using citrate, L-serine, or L-arginine) ZnO and SiO_2 nanoparticles were characterized for genotoxic behavior, no mutagenic potential including chromosomal aberrations, and micronucleus formation were observed in mice bone marrow cells. Even the repeated dose 90-day toxicity study did not cause DNA damage in the bone marrow cells, liver, and stomach (Park and Meang 2014). The organic coating might have provided the necessary safeguarding against inflammatory conditions and might prevent any toxic role exerted by these nanoparticles. Further analysis on these toxicity reduction claims has to be checked with other group of nanoparticles while attempting to reduce the toxic nature of synthesized nanomaterials.

Interestingly oil from rice bran, soybean or sunflower seed were attempted as lipid-core nanocapsules for drug delivery systems in controlled delivery measures. These lipid-core nanocapsules were genotoxically safe when tested with *in vivo* protocol (*Allium cepa* test) supporting the organic masking role (Rigo et al. 2014).

4.5.5 *Genotoxicological Impact of Cadmium from Food Sensors*

Though not directly used as the food constituent, Cadmium is applied as the biosensors in the food industry. In a plant assay system, when 3-Mercaptopropanoic acid-CdSe/ZnS quantum dots were analyzed for toxicity, there was accumulation of these quantum dots in their cytosol and nucleus. With these cellular accumulations even the concentration as low as 10 nm could induce cytotoxicity and genotoxicity from increased reactive oxygen species synthesis. Although the tested concentration is not lethal to the plant cellular system, any practical application utilizing the nanodevices made of this MPA-CdSe/ZnS quantum dots, and the carryover effect into the human or animal food cycle has to be completely evaluated before (Santos et al. 2013).

Similarly cadmium sulphide nanoparticles when checked in *Danio rerio* zebrafish there was Cadmium accumulation in fish brain and muscles tissues after 60 days of dietary exposure. Here the sizes of 8 and 50 nm were fed with low concentrations, 100 or 40 ng CdSNPs/day/g body weight for 36 or 60 days respectively. RAPD-PCR genotoxicity test could show some genomic alteration. This non-lethal nature and only the mild genomic alterations have to be carefully analyzed before any conclusion (Ladhar et al. 2014).

Thus there are many peer reviewed studies stating about the genotoxic implications of varying types of nanomaterials, and there has to be a platform or repository which has to be maintained for international debate among all the agencies, government, food scientists, nano engineers, and the common public. This could provide the comprehensive knowledge about the adaptability and also will assist in taking further alternative measures before introducing any nanomaterials for human use.

4.6 Conclusion

Nanotechnology based applications are commonly adapted in food industry for multiple applications like enhancement of shelf life, sensing and eliminating contamination, improving food storage, tracking, tracing and brand protection. These engineered nanomaterials could be entering into the food chain through air, water, sediment, and soil media during their manufacturing, use, and disposal. Also unintentional release or formation might occur from the combustion of fossil fuels in motor vehicles and industries. Toxically it is been accepted that oxidative stress and lipid peroxidation due to entry of nanoparticles in cellular system plays an important role in DNA damage, cell membrane disruption and cell death. Currently more research studies are going on in the food biotechnology field to increase the awareness and knowledge on the genotoxic and cytotoxic potentials of nanoparticles.

It is clear that nanoparticles reported are exhibiting some form of genotoxic effects, atleast from mildest range to severest extent. Despite the benefits associated

and money invested on research and innovation, compared with other nanotechnological innovations, nanofoods are not getting the publicity, interest, their promotion even among the market leaders in food industry like Nestle, Altria, H.J. Heinz, Unilever and other smaller companies. Incomplete information on risks associated with nanotechnology, and the debate over nanofood safety and regulations has slowed the interest and large-scale market introduction of nanofood products (Garber 2006). Regardless of various concerns about nanomaterial toxicity, they still have various beneficial effects in food and healthcare industry.

At this juncture it is important to understand toxic mechanisms well, so that by utilizing further research on toxicity-reducing measures and by applying special techniques like organic coating based neutralization, the industry could bring back the lost interest towards food based nanotechnology. Using well-characterized nanomaterials in well-defined validated assay systems, complete toxicological data has to be created for wide range of food materials. Data derived can be studied using computational data analysis programs, and from the resulting evaluation, required modification can be attempted in the robust manner. Also more understanding of naturally occurring nanostructures like nano-hairs, which exist in lotus leaves to makes them water repellent, and in gecko, a reptile which has the keratinecious nanosized hairs in maintaining the surface energy for climbing, could help in our deeper understanding about nanotechnology and safer use of them like the natural ones to the mankind. Thus as reviewed by Kumar et al. (2014), the need of the hour are (a) developing combination of different analytical methods considering size, shape, surface properties, and morphology in different environmental media, (b) conducting toxicity studies using environmentally relevant exposure conditions, (c) predictive data analysis using quantitative nanostructure-toxicity relationships (QNTR), and (d) developing guidelines for regulating exposure of nanomaterials in the environment.

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Chapter 5
Reverse Micelles for Nanoparticle Synthesis and Biomolecule Separation

Ram Saran Chaurasiya and H. Umesh Hebbar

Abstract Reverse micelles are used for the selective separation and purification of biomolecules, and for the synthesis of nanoparticles. Reverse micelles are nanometer-size droplets of aqueous phase, stabilized by surfactants in an organic phase. Reverse micellar systems have been developed using various organic and aqueous phases and surfactants. Nanometer-sized aqueous systems are used to carry out specific reactions for the development of materials of controlled size and shape. The size of reverse micelles is one of the parameters for controlling the size of nanomaterial during nanomaterial synthesis. The size of reverse micelles increases with an increase in water content, which results in larger nanoparticle. Reverse micelles are also used as nanoreactors for nanoparticle synthesis because they increase the reaction rate and the distribution of nanoparticles is more uniform. Here we review fundamental principles of formation of reverse micelles; the effects of reverse micellar system components on the size of reverse micelles; the effect of process parameters on selectivity and yield; the synthesis of nanoparticles using reverse micelles. The chapter also discusses the effect of process parameters such as type of surfactant and solvent, use of ionic liquids and temperature on the synthesis as well as properties of nanoparticles.

Keywords Reverse micelles • Nanoparticle • Nanoreactor • Biomolecule • Surfactant • Solvent • Ionic liquid • Synthesis • Particle size • Separation

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5.1 Introduction

The separation and purification of many biomolecules from the mixture is still performed by batch mode small-scale processes such as salt and solvent precipitation, column chromatography and electrophoresis for which scale-up poses substantial problems, making them uneconomical unless the product is of high value. Hence, the current research in the area of downstream processing is directed towards efficient and scalable alternative bioseparation process with potential for continuous operation. In the recent past, liquid-liquid extraction has been identified and proved as an alternative technique that can overcome some of the problems associated with conventional downstream processing. The liquid-liquid extraction technique is based on the partitioning of biomolecules between two phases. However, reduced solubility of the large protein molecules in typical organic solvents limits the range of solvents available for use in such a separation process. In general liquid-liquid extraction, high solvent content also affect the bioactivity of many biomolecules. Liquid-liquid extraction using reverse micelles is now becoming a focus of many researchers since it helps to retain the bioactivity of biomolecules.

Reverse micellar extraction is a cost effective method and can easily be scaled up and also has the potential to be operated in continuous mode. Reverse micelles, also known as water-in-oil microemulsions, are a transparent and stable dispersion of aqueous (generally water) droplets in immiscible organic medium, with the help of surfactant present at the interphase. Surfactants are bipolar in nature, which arranges themselves according the solvent nature and properties, in the mixture of aqueous and the organic phase (Kadam 1986). Some biomolecules can be solubilized in the polar cores of the reverse micelles, preventing the biomolecule from coming in contact with hostile organic solvent and getting denatured (Kilikian et al. 2000). Separation and purification of biomolecules by reverse micellar extraction is mostly carried out in two stages. The first stage, called as forward extraction, involves selective solubilization of target biomolecule into reverse micelles. This is followed by the release of a biomolecule from reverse micelles into a fresh aqueous phase that is known as back extraction. In the synthesis of nanoparticles, aqueous core of the reverse micelles works as a nanoreactor for the reaction of synthesis, and size of particle directly depends on size of reverse micelles (O'Connor et al. 2001; Pileni et al. 1985; Carpenter et al. 1999).

Reverse micelles have wide range of applications and can be used as reaction systems for enzymatic catalysis (Franqueville et al. 2002; Hong et al. 2015; Thudi et al. 2012), nanomaterial synthesis (Carpenter et al. 1999; Lisiecki and Pileni 2003; Hieda et al. 2008; Esmaeili et al. 2011; Li et al. 2006), as membrane systems for separation of biomolecules (Bhavya et al. 2012), separation and purification of biomolecules (Hebbar and Raghavarao 2007; Setapar et al. 2008; Leser and Luisi 1990), as compatible micro surrounding for proteins to retain structure (Luisi et al. 1988), protein refolding (Sakono et al. 2000; Singh and Panda 2005; Goto et al. 2000; Hashimoto et al. 1998) and as drug delivery systems (Moniruzzaman et al. 2010; Lv et al. 2005).

5.2 Formation of Reverse Micelles

Reverse micelles are formed when aqueous and organic phases are mixed in the presence of a surface active agent. The stability of reverse micelles formed and process efficiency mainly depends on properties of surfactant as well as other components used, and processing conditions employed.

Critical micellar concentration is an arbitrary concentration within a narrow range, of the surfactant above which micelles, reverse micelles or aggregates of surfactants are formed (Dominguez et al. 1997; Kilikian et al. 2000). Critical micellar concentration depends on the nature, structure and concentration of components of reverse micelles, operating pressure and temperature (Kadam 1986). The driving forces responsible for the solubilization of solute into reverse micelle are (i) Electrostatic interaction between charged surfactant head group and the biomolecules (ii) Hydrophobic interaction between the hydrophobic tail of surfactant, biomolecules, and solvent and (iii) Steric interactions, wherein the size of the solute plays a major role in the selection or rejection of biomolecules (Hong et al. 2000). Reverse micellar extraction can be carried out by three methods, namely, phase transfer, dry addition and injection. In the phase transfer method, organic phase containing dissolved surfactant is mixed with the aqueous phase containing biomolecules. The interactions, such as electrostatic interaction between biomolecules and surfactant leads to solubilization of biomolecule in the core of surfactant aggregates (Fig. 5.1). Phase transfer is most widely used in hydrophilic biomolecule separation and purification from crude aqueous phase, and also in nanoparticle synthesis. The

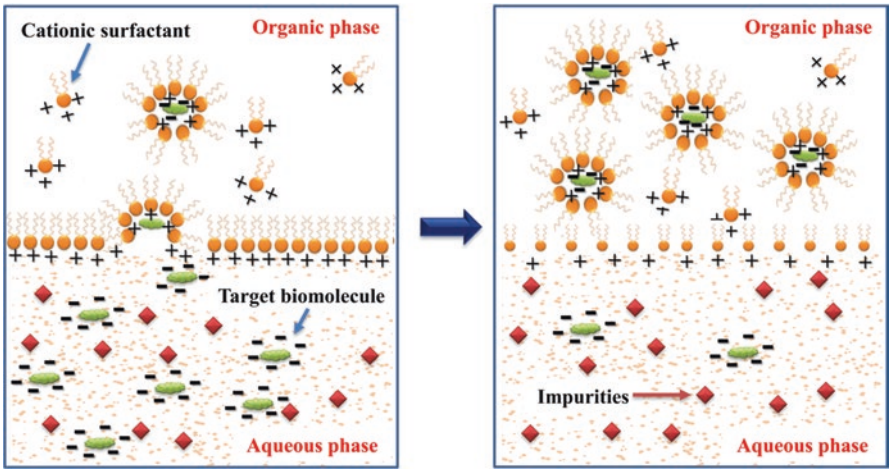

Fig. 5.1 Pictorial representation of reverse micelles formation during forward extraction upon mixing the crude aqueous phase containing target biomolecule with the organic phase consisting dissolved surfactant, selective solubilization of target biomolecule takes place due to electrostatic interaction between biomolecule and surfactant

other two methods are well suited for hydrophobic biomolecules, enzymatic reactions, protein refolding and drug delivery system.

5.2.1 Factors Affecting Forward Extraction

The efficiency of forward extraction mainly depends on many factors such as aqueous phase pH, surfactant type and concentration, ionic strength, type and concentration of salts (Gaikaiwari et al. 2012; Hemavathi et al. 2007; Kilikian et al. 2000; Chaurasiya and Hebbar 2013; Kinugasa et al. 2003; Streitner et al. 2007). The forward extraction efficiency can be increased by appropriately selecting components of reverse micelles and processing conditions (Yu et al. 2003; Ono et al. 1996; Wolbert et al. 1989). Apart from this, the surfactant dependent parameters such as size of reverse micelles and energy required to enlarge the reverse micelles may also influence the biomolecule distribution (Bu et al. 2012; Hebbar et al. 2011; Hebbar et al. 2008; Nandini and Rastogi 2009; He et al. 2013; Haghtalab and Osfouri 2004; Sakono et al. 2004; Krei et al. 1995; Sun et al. 2008; Krishna et al. 2002). By controlling the ionic strength, it is possible to attain a selectable biomolecule transfer based on size exclusion effect (Shin et al. 2003; Nandini and Rastogi 2009; Wei et al. 2011; Dhaneshwar et al. 2014).

5.2.2 Factors Affecting Back Extraction

A successful reverse micellar extraction should have efficient forward and back extraction steps. Normally, it has been assumed that the conditions which are not favorable for forward extraction can be applied for back-extraction. However, many studies have reported that such an approach has failed to achieve a successful back transfer (Lee et al. 2001; Lee et al. 2004a; Dhaneshwar et al. 2014; Hemavathi et al. 2007; Shin et al. 2003; Mitra and Paul 2005a).

The problems associated with back extraction could be overcome by altering the process parameters (Lee et al. 2004b; Hong et al. 2000; Hong et al. 1997). Difference in ionic strength of water pool in reverse micelles and that of stripping aqueous phase also plays a crucial role (Chaurasiya and Hebbar 2013; Hebbar et al. 2011; Fileti et al. 2009; Zhang et al. 2002; Peng et al. 2012; Porto et al. 2005; Gaikaiwari et al. 2012; Nishiki et al. 1995; Ono et al. 1996). Apart from biomolecule-surfactant interactions, micelle-micelle interaction plays a significant role in the back transfer of biomolecules (Mathew and Juang 2005; Hemavathi et al. 2007). Addition of isopropyl alcohol or silica gel also helps in improved biomolecule recovery (Carlson and Nagarajan 1992; Hemavathi et al. 2007; Lee et al. 2001, 2004a; Leser et al. 1993) but it may also affect the enzyme activity (Mathew and Juang 2007, 2005). The efficiency of back extraction can be improved by combination approach such as altering pH of the aqueous phase and ionic strength of aqueous phase and addition

of alcohol (Ono et al. 1996). Effect of other parameters such as temperature (Dekker 1990) and micro size glass beads (Dhaneshwar et al. 2014) have been also reported to improve the back extraction efficiency.

5.2.3 *Effect of Reverse Micellar Components on Size of Reverse Micelles*

Reverse micelles size is mainly dependent on the molecular ratio of [water]/[surfactant] rather than these factors individually. This ratio known as water content (W_0) is widely used for the estimation of the size of reverse micelles. The size of reverse micelles is one of the key parameters used for the selective separation of biomolecules from a mixture containing more than one biomolecule (Luisi 1985). In few cases, biomolecules can also create new reverse micelles around itself to fit into the core (Wolf and Luisi 1979). This strategy can be used for solubilization of bigger size biomolecules such as high molecular weight proteins and plasmids.

The size of reverse micelles depends on the ionic strength of the aqueous solution and as the ionic strength increases reverse micelles size decreases. The literature reports suggest that effect of surfactant on reverse micelles size is not uniform for all kind of surfactants. It depends on several other parameters such as the type of surfactant, type of biomolecules and operating parameters (Shrestha et al. 2010). There are few reports which showed that, as the bis(2-ethylhexyl) sulfosuccinate sodium salt concentration increases the number of reverse micelles of constant size increases (Table 5.1; (Lemyre and Ritcey 2010; Shrestha et al. 2010), where as Sun et al. (2008) observed an increase in size of reverse micelles on increasing concentration of bis(2-ethylhexyl) sulfosuccinate sodium salt. The concentration of surfactant and salt affects the size of the reverse micelles. Apart from that the other parameters such as presence of ionic liquid, temperature and location of biomolecule in reverse micellar core also plays a major role in deciding the size of reverse micelles (Falcone et al. 2009; Paul and Panda 2014; Gao et al. 2009; Shrestha et al. 2010; Rao et al. 2012, 2013; Pileni et al. 1985).

Several empirical models (Eqs. 5.1, 5.2, 5.3, 5.4, 5.5, and 5.6) showing a relationship between water content (W_0) and the hydraulic core radius (R_0) have been reported.

$$R_0 = 0.175W_0 \quad (\text{Bru et al. } 1989) \tag{5.1}$$

$$R_0 = 0.164W_0 \quad (\text{Gaikar and Kulkarni } 2001) \tag{5.2}$$

$$R_0 = 0.150W_0 \quad (\text{Motlekar and Bhagwat } 2001) \tag{5.3}$$

$$R_0 = 0.145W_0 + 0.57 \quad (\text{Kinugasa et al. } 2003) \tag{5.4}$$

Table 5.1 Combination of different concentrations (per ml of cyclohexane) of bis(2-ethylhexyl) sulfosuccinate sodium salt and water volume resulting in same size of reverse micelles

Reverse micelles size (nm)	Surfactant mass (mg)	Water volume (ul)
5	75	6.7
	96	16.7
	110	33.3
	128	50
	138	66.7
6	62	6.7
	80	16.7
	94	33.3
	111	50
	119	66.7
7.7	47	6.7
	62	16.7
	74	33.3
	91	50
	96	66.7
10	36	6.7
	47	16.7
	58	33.3
	73	50
	76	66.7

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$$R_0 = 0.138W_0 \quad \text{for unfilled reverse micelles} \quad (\text{Hebbar et al. } 2011) \tag{5.5}$$

$$R_0 = 0.136W_0 \quad \text{for filled reverse micelles} \quad (\text{Hebbar et al. } 2011) \tag{5.6}$$

Comparison of different models for measuring the size of filled and unfilled reverse micelles has been reported in Table 5.2 (Hebbar et al. 2011).

5.3 Synthesis of Nanoparticles Using Reverse Micelles

The advantage of reverse micelles in material synthesis is the size of the nanostructures can be controlled by altering the process parameters to change the size of reverse micelles. Another advantage of reverse micelles in nanoparticle synthesis is that nucleation can be started at a number of locations, simultaneously. Surfactant molecules keep nucleation sites isolated from each other and acts as a stabilizer for particle formed. Uniform nano-droplet structure and specific intermicellar interactions during

Table 5.2 Comparison of different models for measuring the size of filled and unfilled reverse micelles, when surfactant concentration increased from 50 mM to 150 mM an increase in size of filled reverse micelles was observed compared to unfilled. Whereas, further increase in surfactant concentration resulted in smaller size of filled reverse micelles than that of unfilled. This results indicates that increasing the surfactant concentration above the optimum level destabilizes the reverse micelles

CTAB concentration (mmol/L)	W_0		Radius of the reverse micelle (nm)							
			Gaikar and Kulkarni (2001)		Kinugasa et al. (2003)		Motlekar and Bhagwat (2001)		Bru et al. (1989)	
	UF	F	UF	F	UF	F	UF	F	UF	F
50	53	68	8.7	11.1	8.3	10.4	7.9	10.2	9.3	11.9
100	55	66	9.0	10.8	8.5	10.1	8.2	9.9	9.6	11.5
150	52	64	8.5	10.5	8.1	9.8	7.8	9.6	9.1	11.2
200	51	48	8.4	7.9	8.0	7.5	7.6	7.2	8.9	8.4

W_0 = [water]/[surfactant]; UF-Unfilled; F-Filled; CTAB-cetyltrimethylammonium bromide
Hebbar HU, Hemavathi AB, Sumana B, Raghavarao KSMS (2011) Reverse Micellar Extraction of Bromelain from Pineapple (*Ananas comosus* L. Merryl) Waste: Scale-up, Reverse Micelles Characterization and Mass Transfer Studies. Separation science and technology 46 (10):1656–1664. reprinted by permission of the publisher (Taylor & Francis Ltd., http://www.tandfonline.com

nanomaterial synthesis in reverse micelles keeps the nucleation and growth stages totally separated, which results in the formation of monodisperse particles. Reverse micelles have narrow droplet size distribution because of the interactions between polar head groups and nonpolar tails of the different surfactant molecules results in aggregates of very specific size (Uskoković and Drofenik 2005).

A few reports available on the development of alloys/coated nanoparticles using different types of reverse micellar system. It is reported that the magnetic properties of a single metal particle can be increased by development of alloys of two or more metals. Carpenter et al. (1999) observed an increased blocking temperature and higher coercive force for cobalt/platinum alloys and gold-coated cobalt/platinum nanoparticles compared to cobalt alone, synthesized in reverse micelles of cetyltrimethylammonium bromide. Later, Teruoki Tago et al. (2002) also reported an increase in the coercivity of the SiO_2-coated ferrite nanoparticles synthesized in polyoxyethylene(15) cetyl ether/cyclohexene reverse micellar system (Tables 5.3 and 5.4).

Fluorescent silica nanoparticle with a double layer was synthesized first time by Yoo and Pak (2013) using Brij35-reverse micellar system. In this study, first NH_4OH was added to tetraethylorthosilicate for hydrolysis followed by polymerization after the addition of fluorescein (an organic fluorophore) to generate fluorescent silica nanoparticle. To form the second layer 3-(Aminopropyl) triethoxysilane was added to the reaction mixture, which reacted on the surface of silica nanoparticle and formed a shell on the surface of pre-synthesized silica nanoparticle. The second layer can protect dye in the silica matrix. Polyoxyethylene (15) cetyl ether/cyclohexane system have been used for synthesis of different types of nanoparticles

Table 5.3 Nanoparticles synthesized using different types of reverse micelles

S. No.	Nanoparticles synthesized	Reverse micellar system used	Important findings	References
1	Cobalt, cobalt/platinum alloys, and gold-coated cobalt/platinum	Cetyltrimethylammonium bromide	Coated nanoparticles exhibit an increased magnetic properties such as greater blocking temperature and larger coercive force	Carpenter et al. (1999)
2	$KMnF_3$	Cetyltrimethylammonium bromide/1-butanol/octane	self-assembled cubic anti-ferromagnetic $KMnF_3$ nanocrystals were formed	Agnoli et al. (2001)
3	Pd and Pt bimetallic nanoparticles	bis(2-ethylhexyl) sulfosuccinate sodium salt/isooctane	The nucleation rate of Pd was much faster than that of Pt	Wu et al. (2001a)
4	Platinum nanoparticles	Poly(ethylene glycol) monododecyl ethers ($C_{12}E_4$, $C_{12}E_5$, $C_{12}E_6$) /n-heptane	The reaction rate was higher in all the microemulsions based on either of the alcohol ethoxylates than the microemulsions based on bis(2-ethylhexyl) sulfosuccinate sodium salt.	Ingelsten et al. (2001)
		bis(2-ethylhexyl) sulfosuccinate sodium salt/n-heptane		
		Mixtures of the alcohol ethoxylates and bis(2-ethylhexyl) sulfosuccinate sodium salt/n-heptane		
5	Triangular CdS Nanocrystals	Cd(bis(2-ethylhexyl) sulfosuccinate sodium salt)$_2$/isooctane	Crystallized hexagonal (wurtzite) nanocrystals of around 5 nm were formed.	Pinna et al. (2001)
6	Silica-coated rhodium nanoparticles	Polyoxyethylene (15) cetyl ether/cyclohexane	SiO2-coated Rh nanoparticle exhibited an extremely high thermal stability	Tago et al. (2002)

(continued)

Table 5.3 (continued)

S. No.	Nanoparticles synthesized	Reverse micellar system used	Important findings	References
7	Gold–silver bimetallic	bis(2-ethylhexyl) sulfosuccinate sodium salt/ isooctane	Formation of Au nanoparticles was found to be much faster than that of Ag nanoparticles.	Chen and Chen (2002)
8	Silica (SiO_2)-coated ceria (CeO_2)	Polyoxyethylen(15) cetylether/cyclohexene	CeO_2 nanoparticles were spherically coated with SiO_2 when using oxalic acid ($(COOH)_2$) as a particle forming agent of CeO_2	Tago et al. (2003)
9	Zinc selenium quantum dots	poly(ethylene oxide)-poly-(propylene oxide)-poly(ethylene oxide) or PEO-PPO-PEO/n-heptane	The ZnSe nanocrystals exhibit size-dependent luminescence and excellent photostability	Karanikolos et al. (2004)
10	Yttrium fluoride	polyoxyethylene isooctylphenyl ether/ cyclohexane	Monodisperse amorphous sphererical nanoparticles of diameters between 6 and 50 nm	Lemyre and Ritcey (2005)
11	Copper nanoparticles	bis(2-ethylhexyl) sulfosuccinate sodium salt/ isooctane or compressed propane	Presence of chloride ions during synthesis affects the growth rate, geometry, and structure of nanoparticle.	Kitchens et al. (2005)
12	Copper–nickel bimetallic alloy	SDS/n-butanol/n-heptane	Narrow distribution of Cu–Ni nanoparticles	Feng and Zhang (2006)

(continued)

Table 5.3 (continued)

S. No.	Nanoparticles synthesized	Reverse micellar system used	Important findings	References
13	Platinum–copper bimetallic alloy	Cetyltrimethylammonium bromide/ isooctane/n-butanol	XPS illustrates that both elements in the nanoparticles are in zero-valence and possess the characteristic metallic binding energy	Weihua et al. (2006)
14	GeO_2	Cetyltrimethylammonium bromide /heptane	capsules of GeO_2 can be prepared by using octane as the oil phase	Wu et al. (2006)
15	Cadmium selenide (CdSe)	bis(2-ethylhexyl) sulfosuccinate sodium salt/n-heptane	hexagonal CdSe nanorods were synthesized at 100 °C	Xi and Lam (2007)
16	Gold nanoparticles	bis(2-ethylhexyl) sulfosuccinate sodium salt/ Dodecane	Size of gold nanoparticles formed inside the water droplets was regulated by the size of reverse micelles	Hieda et al. (2008)
17	Highly fluorescent double-layered silica nanoparticles	Surfactant (Brij35), co-surfactant, organic solvent, water, and fluorescein as an organic fluorophore	Second layer effectively protected the fluorescein dye within the silica matrix	Yoo and Pak (2013)

including ferrite, and silica coated cobalt ferrite, ceria and rhodium (Teruoki Tago et al. 2002; Tago et al. 2003; Tago et al. 2002).

Karanikolos et al. (2004) reported a gas contacting technique for the synthesis of ZnSe nanocrystals. In this technique microemulsion of formamide/poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)/n-heptane was bubbled by hydrogen selenide gas, which diffused into reverse micelles core and reacted with the diethylzinc to produce ZnSe. This method could eliminate the micelle-micelle coalescence and provided good control on particle size. Highly stable single luminescent ZnSe nanocrystals were formed in each reverse micelle.

Recently the use of reverse micelles have been proved to be a potential drug delivery system apart from synthesis of nanomaterials in the presence of biologically active compounds (Lv et al. 2005). Most of the studies are for small molecule drugs using bis(2-ethylhexyl) sulfosuccinate sodium salt-reverse micellar system because of its ability to solubilize more water compared to other surfactants. Only

Table 5.4 List of the ionic liquids used in reverse micellar system

Ionic liquid used	Other components of reverse micelles	References
1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF4]	Triton X-100 /cyclohexane	Gao et al. (2004)
	Triton X-100/ cyclohexane	Eastoe et al. (2005)
	Triton X-100/ p-xylene	Gao et al. (2006)
	Triton X-100/Benzene	Gao et al. (2007)
	Triton X-100/Toluene	Li et al. (2007)
	Triton X-100/Benzene	Falcone et al. (2009)
	Benzyl-n-hexadecyldimethylammonium chloride/Benzene	Falcone et al. (2009)
1-pentyl-3-methyl-imidazolium tetra-flouroborate ([pmim][BF4])	Triton X-100/Benzene	Adhikari et al. (2007)
1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (bmimTf2N)	Triton X-100/Benzene	Falcone et al. (2009)
	Benzyl-n-hexadecyldimethylammonium chloride/Benzene	Falcone et al. (2009)
Dimethylimidazolium dimethylphosphate	Polyoxyethylene sorbitan monooleate (Tween-80) and sorbitan laurate (Span-20), isopropyl myristate	Moniruzzaman et al. (2010)
Ethylene glycol	bis(2-ethylhexyl) sulfosuccinate sodium salt/Isooctane	Setua et al. (2010)
Room-temperature ionic liquid, N-methyl-N-propylpyrrolidinium bis(trifluoromethanesulfonyl) imide ([P13][Tf2N])	Triton X-100/Benzene	Pramanik et al. (2011)
1-ethyl-3-methylimidazolium n-butylsulfate	Polyoxyethylene sorbitan monooleate (Tween-80) and sorbitan laurate (Span-20) isopropyl myristate	Mandal et al. (2013)
1-butyl-3-methylimidazolium dodecylsulfate ([Bmim][DodSO4]), and room temperature ionic liquid 1-ethyl-3-methylimidazolium ethylsulfate ([Emim][EtSO4])	Toluene	Rojas et al. (2013)
benzyl-n-hexadecyldimethylammonium 1,4-bis-2-ethylhexylsulfosuccinate and cetyltrimethylammonium 1,4-bis-2-ethylhexylsulfosuccinate	Bis(2-ethylhexyl) sulfosuccinate sodium salt/Benzene	Villa et al. (2014)
1-butyl-3-methylimidazolium chloride (bmimCl)	Benzyl-n-hexadecyldimethylammonium chloride/Toluene	Blach et al. (2014)

(continued)

Table 5.4 (continued)

Ionic liquid used	Other components of reverse micelles	References
1-butyl-3-methylimidazolium tetrafluoroborate (bmimBF4)	Benzylhexadecyldimethylammonium ([BHD]+) chloride ([Cl]−)/ Benzene	Shi et al. (2014)
1-Butyl-4-methyl pyridinium tetrafluoroborate ([b4mpy][BF4]	(Tween 20 + n-pentanol)/n-heptane	Paul and Panda (2014)
1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim][PF6]	p-(1,1,3,3-tetramethylbutyl) phenoxypolyoxyethyleneglycol (Triton X-100)/Ethylene glycol	Chen and Zhao (2015)

hydrophilic drugs can be solubilized in reverse micelles core, is the major limitation of this system.

Solubility problem of non-hydrophilic drugs into reverse micelles can be overcome by using suitable cosolvent of hydrophilic nature with high dielectric constant to achieve miscibility with water and low solubility in organic solvents. Some of the solvents having these characteristics and being used as co-solvents are ethylene glycol, propylene glycol, glycerol, lower alcohols, 3-hydroxyquinolinones, dimethylformamide, formamide, dimethylsulfoxide and dimethylacetamide (Durantini et al. 2009; Moniruzzaman et al. 2006). From the above-listed co-solvents, dimethylsulfoxide is one of the best option to solubilize the poorly soluble bioactive compounds in reverse micelles (Bürglová et al. 2015; Elles and Levinger 2000).

Incorporation of co-solvents helps in the formation of spherical reverse micelles and forms bigger reverse micelles compared to water alone (Falcone et al. 2009; Durantini et al. 2009, 2011). The interaction of co-solvents with water and surfactant and their partitioning at either side of the interface depends on the chemical characteristics of co-solvents. In general, co-solvents interacts with a polar head group of surfactant molecules in different ways and alters the reactivity of water within the reverse micelles and the associated nucleation and growth of the nanomaterial. From the literature report, it can be concluded that, by changing the chemical composition of co-solvents, properties of the reverse micelles can be altered as per requirement. The exact mechanism of action of these solvents are still not very clear; detailed studies are required to explore the mechanism and proper role of the co-solvents.

Controlling of the nanoparticle size and size distribution is very complex, and it depends on the properties of reverse micelles and also on the processing conditions. In particular, nanoparticle size and size distribution depends on several factors such as size of reverse micelles, number of reverse micelles, core material of reverse micelles, interface behavior, temperature and kinetics of material exchange during collision (Arriagada and Osseo-Asare 1995).

5.4 Mechanism of Nanoparticle Synthesis in Reverse Micelles

Even though there are many reports available on use of reverse micelles for nanoparticle synthesis of well controlled particle size (Liz-Marzán and Lado-Tourino 1996; Tojo et al. 1997; Sakai et al. 1998; Lemyre and Ritcey 2005; Tojo et al. 2014) but their growth mechanism is still not very clear.

Three different periods for the growth of iron oxalate nanorods in reverse micelles of cetyltrimethylammonium bromide/1-butanol/isooctane have been observed by using the combination of dynamic light scattering, fluorescence correlation spectroscopy, and transmission electron microscopy (Sharma et al. 2012). The above growth process includes nucleation-dominant growth that results in an average size nanoparticles of ~53 nm followed by the formation of nanorods and final elongation of nanorods and growth of nanoparticles. The first process could be considered as the growth-limiting step because of the time taken more than the other two stages individually (Fig. 5.2).

Lemyre et al. (2011) have demonstrated the two mechanisms for the synthesis of YF^3 nanoparticles in reverse micelles (Fig. 5.3). Two methods differ at the nucleation stage. In the case of addition of F^- as an emulsion to Y^{3+} containing nonionic reverse micelles, nucleation starts because of the intermicellar collisions and conditions of comparable Y^{3+} and F^- concentration (Fig. 5.3a). Whereas, in case of direct addition of F^- dispersed in aqueous solution to nonionic reverse micelles containing Y^{3+}, nucleation occurs by the interaction of reverse micelles with aqueous droplets of F^- at higher concentration of F^- (Fig. 5.3b). The first method yields stable nuclei that are amorphous and smaller, whereas, the second method generates wider size distribution of unstable nuclei and crystalline nanoparticles.

5.5 Techniques to Characterize Nanoparticle and Reverse Micelles

To monitor certain static and dynamic characteristics of nanosize reverse micelles in an organic medium, indirect techniques are being used because of the difficulty in measuring these properties by direct methods of reverse micelles. Several indirect methods have been reported for the measurement of the phase diagram of microemulsions, but conductivity measurements, transparency measurement, and interfacial tension (between surfactant and solvent) measurement are the most widely used methods.

Scattering techniques for characterization of reverse micelles and nanoparticles have become more popular because of its accuracy, reliability, and nondestructive nature. Different scattering techniques such as dynamic light scattering, static light scattering, small-angle X-ray scattering, fourier transform infrared spectroscopy, ultraviolet-visible spectroscopy, freeze-fracturing electron microscopy, ^{1}H–nuclear

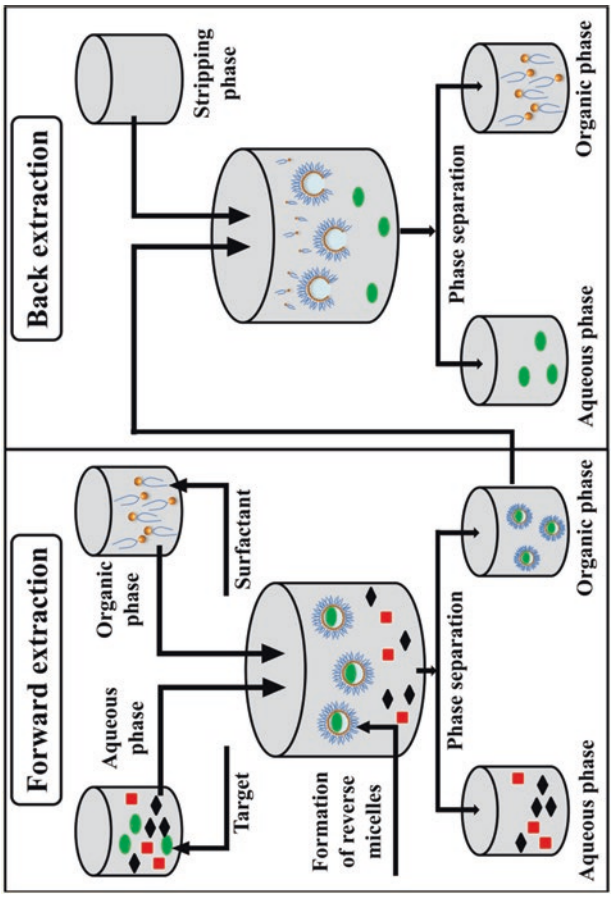

Fig. 5.2 Reverse micellar extraction of biomolecules involves two stages: (i) Forward extraction: when crude extract contaning target biomolecule along with other contaminants is mixed with organic phase consisting surfactant molecules formation of reverse micelles takes place and also selective solubilization of targeted biomolecule into reverse micelles core, (ii) Back-extraction: destabilization of reverse micelles for recovery of target biomolecule into striping phase i.e. fresh aqueous phase of slightly higher ionic strength and opposite pH compared to crude aqueous phase

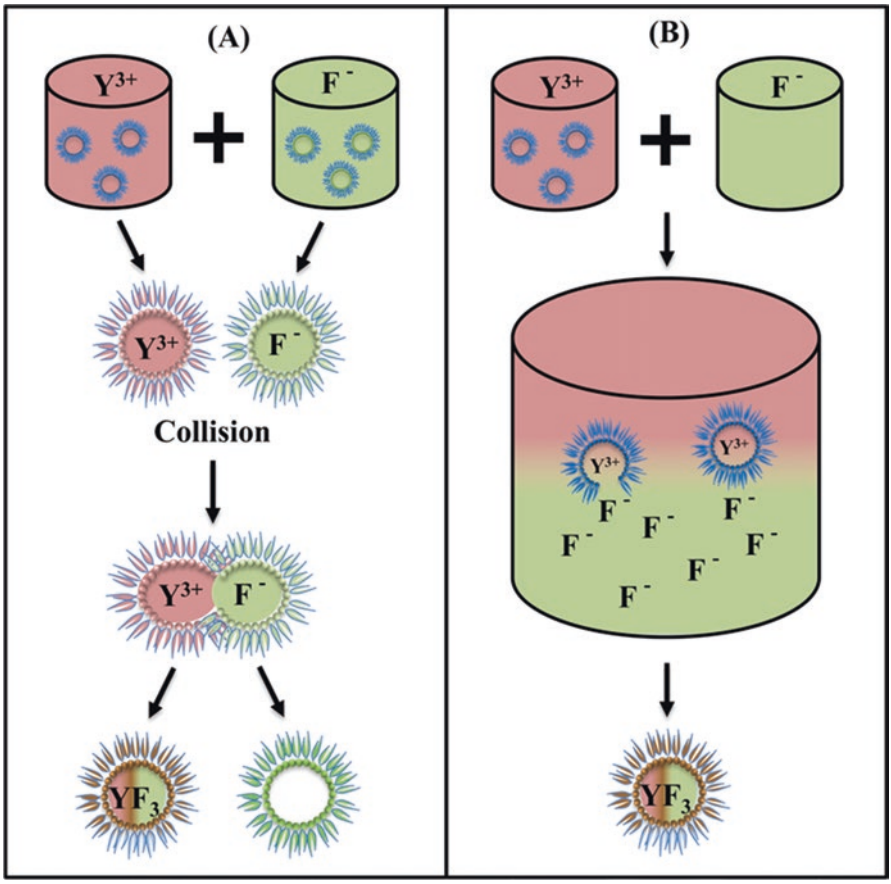

Fig. 5.3 Mechanism of nanoparticle synthesis (**a**) Collision method: nucleation starts with the collision of reverse micelles after mixing of two microemulsions containing Y^{3+} and F^{-} separately; (**b**) Direct interaction: nucleation starts after mixing the reverse micelles containing Y^{3+} with dispersed F^{-} (Modified after Lemyre et al. (2011))

magnetic resonance, pulsed-field gradient spin-echo nuclear magnetic resonance and fluorescence resonance energy transfer have been used to study the behavior of water entrapped in two different catanionic reverse micelles formed by ionic liquid-like surfactant (Subramanian et al. 2001; Gao et al. 2004; Pramanik et al. 2011; Mandal et al. 2013; Villa et al. 2014).

The kinetics of the reagent is mixing, and particle growth within the reverse micelles core can be monitored by 19F nuclear magnetic resonance (19F nuclear magnetic resonance) and transmission electron microscopy (Lemyre et al. 2011). Li et al. (2003) have used transmission electron microscopy and X-ray diffraction techniques for the characterization of the nanoparticles synthesized in cetyltrimethylammonium bromide/n-Octane reverse micellar system. The dynamic light scattering technique provides the useful information about the size of microemulsions

and the size distribution of droplets (Pramanik et al. 2011; Agazzi et al. 2014). These techniques have been used for the in-depth study of the characteristics of biomolecule inside the reverse micelles. For example, structural analysis of the formamide solubilized inside the bis(2-ethylhexyl) sulfosuccinate sodium salt-reverse micellar system, and the interaction between formamide and bis(2-ethylhexyl) sulfosuccinate sodium salt have been studied using fourier transform infrared spectroscopy and ^{1}H–nuclear magnetic resonance technique, respectively (Correa et al. 2005).

Apart from these well-known instruments techniques several other non-conventional techniques have been also used for the characterization of reverse micelles (Setua et al. 2012). Recently solvatochromic behavior of 1-methyl-8-oxyquinolinium betaine (QB) and 6-propionyl-2-(N,N-dimethyl)aminonaphthalene, PRODAN have been used, to monitor the interface properties and sequestrated water structure of water/Benzyl-n-hexadecyldimethylammonium chloride/n-heptane:benzene reverse micelles (Agazzi et al. 2013; Agazzi et al. 2014). The PRODAN is a useful probe to study the effect of external solvent composition on reverse micelles interface properties.

Fan et al. (2015) have used flow-mixing microcalorimetry and isothermal titration microcalorimetry technique to calculate the enthalpies of mixing water/bis(2-ethylhexyl) sulfosuccinate sodium salt/n-alkane reverse micellar system with different water concentration (W_0) and chain length of n-alkane. Curcumin was used as the molecular probe with fluorescence and absorption spectroscopy to monitor the state of ionic liquid in the reverse micelles (Paul and Panda 2014). To study the effect of dimethylsulfoxide as co-solvent on the properties of reverse micelles, fourier transform infrared spectroscopy and fluorescence spectroscopy were used (Moniruzzaman et al. 2006).

Using molecular simulation, Shi et al. (2014) have studies the structural and dynamic properties of ionic liquid based reverse micelles upon absorption of CO_2. The simulated and experimental results showed that CO_2 molecules are absorbed in the four various regions of the ionic liquids based reverse micelles, that is (i) inner core of ionic liquids, (ii) [BHD] + surfactant cation layer, (iii) interface between the [BHD] + surfactant cation layer and benzene solvent, and (iv) benzene solvent. The CO_2 permeability and diffusivity decreased in the following order: benzene solvent > interface between the [BHD] + surfactant cation layer and benzene solvent > [BHD] + surfactant cation layer > inner core of ionic liquids. Whereas the solubility decreased in the order: [BHD] + surfactant cation layer > interface between the [BHD] + surfactant cation layer and benzene solvent ~ benzene solvent > inner core of ionic liquids (Fig. 5.4).

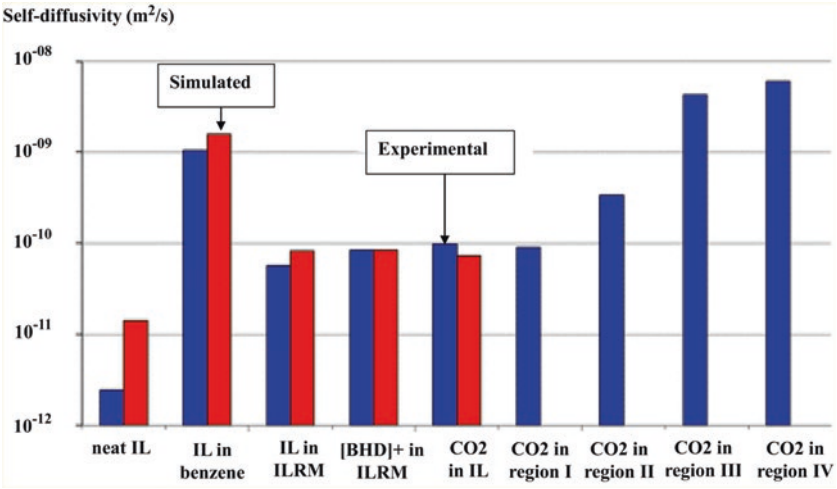

Fig. 5.4 Summary of simulated and experimental self-diffusivity coefficients for different systems at 298 K and 1 bar. Self-diffusivity of CO_2 was maximum in benzene region. IL: Ionic liquid, ILRM: Ionic liquid based reverse micelles (Reprinted with permission from Shi W, Hong L, Damodaran K, Nulwala HB, Luebke DR (2014) Molecular Simulation and Experimental Study of CO_2 Absorption in Ionic Liquid Reverse Micelle. The Journal of Physical Chemistry B 118 (48):13870–13881. Copyright (2015) American Chemical Society)

5.6 Effect of Process Parameters on Synthesis and Properties of Nanoparticles

5.6.1 Salt and Surfactant

Use of reverse micelles for nanoparticle synthesis is very popular nowadays because of it is exceptionally small size of the polar core in most of the organic solvents. In past few years, this reverse micellar system has gained the attention of many researchers for the synthesis of nanomaterial inside the core. Because the size of these reverse micelles can be easily changed by varying the processing conditions, thus the size of nanoparticle synthesized inside the core also changes.

The size of the nanoparticle increases with increase in polar core size and aqueous phase volume. At constant W_0, an increase in surfactant concentration increases the number of nanoparticles rather than size. Particle size polydispersity increases with increase in salt concentration and polar core size, but the average particle size decreases as the function of salt concentration (Natarajan et al. 1996). The critical nanomaterial nucleation number and polar core size of reverse micelles are the major factors that influence the final particle size.

Surfactant polar head and the solvent properties also play a significant role after incorporating cyclodextrins into reverse micelles. These assemblies of reverse micelles act as powerful nanoreactors because of the two active sites: the chiral

hydrophobic cavity of cyclodextrin and, of course, the aqueous core of reverse micelles. These reverse micelles are reported as supramolecular assemblies because of its dual active nature (Silva et al. 2014). By using a cationic surfactant, Cavallaro et al. (2015) have developed an hydrophobically modified halloysite nanotubes (as nanobioreactor) with the hydrophilic cavity and hydrophobic shell, to generate tubular nanostructure. This nanobioreactor of reverse micelles could be considered as eco-friendly with a bipolar interface that might be useful for selective synthesis of organic compounds and suitable for many other biological applications. Nanoparticle size can be controlled by using acids such as HCl or by altering the W_0 (Yoo and Pak 2013).

Unlike many conventional nanoparticle synthesis techniques, reverse micelles not only synthesizes uniform size nanoparticle, but the synthesized particles might also be well dispersed (Yener and Giesche 2001). Enhancement of reaction rate is another advantage of synthesis in reverse micelles, compared to other techniques (Natarajan et al. 1996; Ghosh 2001).

Natarajan et al. (1996) have reported Eqs. (5.7 and 5.8) for the calculation of particle size by assuming packing fraction for a specified lattice and spherical particles.

$$V_p = \frac{1}{\phi} z \; V_{met} \tag{5.7}$$

$$r_p = \left(\frac{3}{4\pi} V_p \right)^{\frac{1}{3}} \tag{5.8}$$

V_p solid volume of metal particle, Φ packing fraction of the solid metal particle, z variable denoting the number of metal atoms inside a RM, V_{met} atomic volume of metal in liquid phase, r_p size (radius) of metal particle formed in a RM.

5.6.2 Ionic Liquids

There has been an increased interest of researchers on use of nonaqueous microemulsions by replacing the water core with nonaqueous solvents like methanol, glycol, acetonitrile, ethylene glycol and ionic liquids (Correa et al. 2005; Setua et al. 2007; Setua et al. 2010; Gao et al. 2004; Eastoe et al. 2005). Ionic liquids are organic salts with many advantageous characteristics such as environment-friendly, wide electrochemical window, negligible vapor pressure, high thermal stability, nonflammability and wide liquid range (Ranke et al. 2007). Nature and structure of the microemulsion formed by the ionic liquid directly depends on the ratio between ionic liquid and oil phase (Fig. 5.5) (Rojas et al. 2013; Chen and Zhao 2015; Rao et al. 2013).

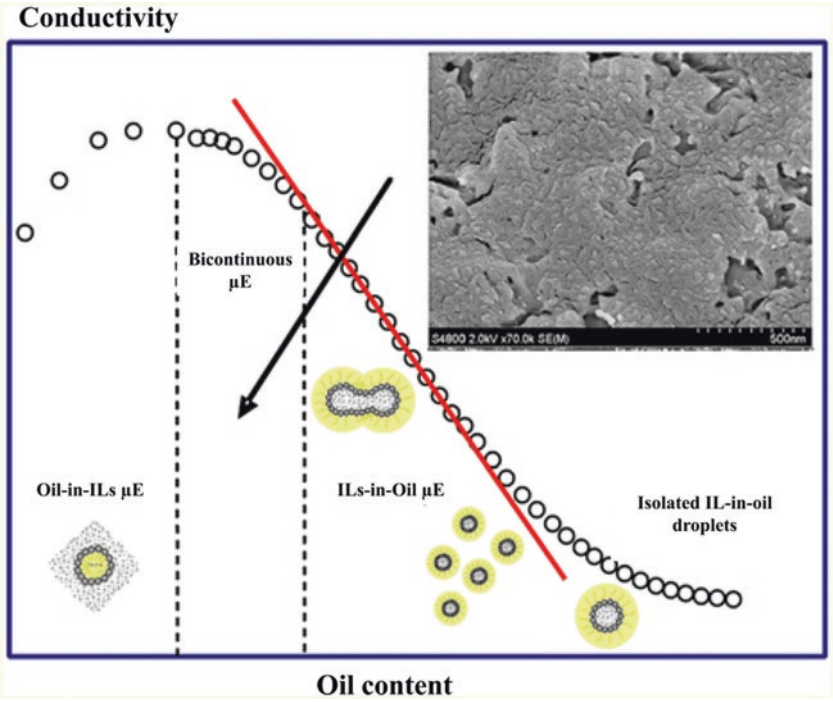

Fig. 5.5 Through measurement of conductivity of solution, Rojas et al. (2013) explained type of emulsion formed (oil-in-IL, bicontinuous, and IL-in-oil) in combinations of IL and oil phases in presence of surfactant (Reprinted with permission from Rojas O, Tiersch B, Rabe C, Stehle R, Hoell A, Arlt B, Koetz J (2013) Nonaqueous microemulsions based on N, N′-alkylimidazolium alkylsulfate ionic liquids. Langmuir 29 (23):6833–6839. Copyright (2015) American Chemical Society)

There are a few reports available on use of ionic liquids to form nonaqueous reverse micelles for different applications such as production of metallic or semiconductor nanomaterials, and in biological extractions or as solvents for enzymatic reactions (Li et al. 2007; Falcone et al. 2009; Pramanik et al. 2011). Moniruzzaman et al. (2010) reported that the use of ionic liquid improved the solubility of sparingly soluble or less soluble drugs and enhanced their topical and transdermal delivery (Fig. 5.6).

Rojas et al. (2013) demonstrated the formation of small size (≤ 10 nm) nanoreactor using ionic liquid in toluene. It is also reported that the ionic liquid surfactant 1-butyl-3-methylimidazolium dodecyl sulfate ([Bmim][$DodSO_4$]) forms reverse micelles in toluene which swells after the addition of room temperature ionic liquid 1-ethyl-3-methylimidazolium ethyl sulfate ([Emim][$EtSO_4$]). The use of imidazolium based ionic liquid in reverse micelles is very popular but the use of pyridinium based ionic liquid reverse micelle can still enhance the reverse micelles efficiency because of its superior properties than that of imidazolium based ionic liquids (Paul and Panda 2014).

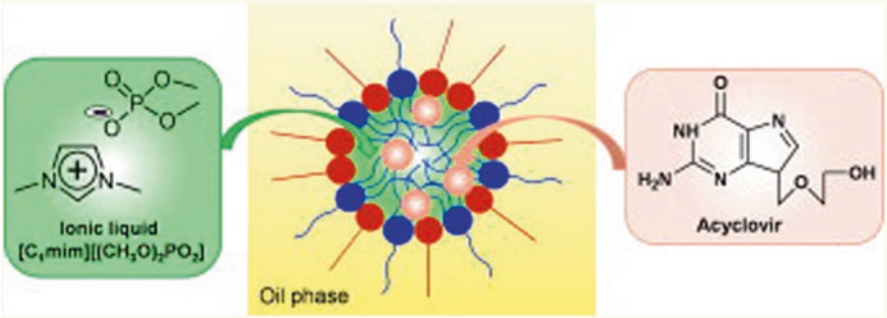

Fig. 5.6 Increase in the solubility of sparingly soluble drug (acyclovir) after addition of IL [C1mim] [$(CH_3O)_2PO_2$] (dimethylimidazolium dimethylphosphate) in reverse micelles formed by polyoxyethylene sorbitan monooleate (Tween-80) and sorbitan laurate (Span-20) (3:1 ratio) as surfactant and isopropyl myristate (IPM) as an oil phase (Reprinted from International journal of pharmaceutics 400 (1) Moniruzzaman M, Tamura M, Tahara Y, Kamiya N, Goto M. Ionic liquid-in-oil microemulsion as a potential carrier of sparingly soluble drug: Characterization and cytotoxicity evaluation: 243–250 (2010). Copyright (2015) with permission from Elsevier)

5.6.3 Reducing Agent

The size distribution of the nanoparticles is highly affected by the concentration of reducing agent (Fig. 5.7). A higher concentration of reducing agent destroys the reverse micelles and results in reduced size distribution of the nanoparticle. However, reverse micelles works as nanoreactors at a lower concentration of reducing agent, which helps in the rise of ~29% in nanoparticle size distribution (Lisiecki and Pileni 2003). Excess of the reducing agents affects both, the growth and nucleation of the nanoparticle as well as chemical and structural integrity of the reverse micelles, which results in the decrease in size of the nanoparticle and lower stability of reverse micelles.

The nature of reducing agent also affects the size of nanoparticles by altering the size of reverse micelles core. In a study of two different reducing agents glucose and sodium borohydride, Setua et al. (2012) observed that glucose resulted in small size Ag + nanoparticles than the sodium borohydride, under the same conditions (Fig. 5.8). In the solvation dynamics and rotational relaxation measurement, it was confirmed that larger size nanoparticles were causing larger perturbation than the smaller size nanoparticles. There are a few reports which says that increasing the concentration of reducing agent upto an optimum level increases the nanoparticle size (Karanikolos et al. 2004; Xi and Lam 2007; Magno et al. 2010; Tojo et al. 2009).

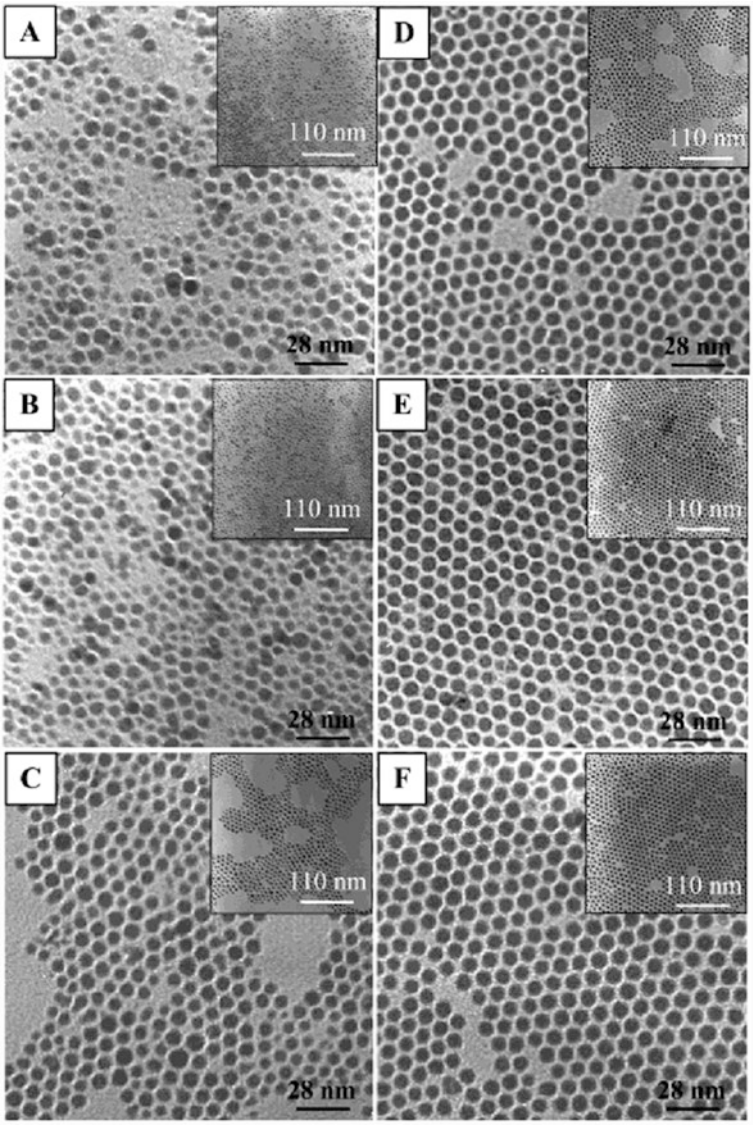

Fig. 5.7 Transmission electron microscopy images showing the effect of various sodium tetrahydroboride concentrations R R = $[NaBH_4]/[Co(bis(2\text{-ethylhexyl})\ sulfosuccinate\ sodium\ salt)_2]$: (**a**) R = 0.5, (**b**) R = 1, (**c**) R = 2, (**d**) R = 4, (**e**) R = 6, and (**f**) R = 8 on cobalt nanocrystals formation bis(2-ethylhexyl) sulfosuccinate sodium salt-reverse micellar system. As the concentration of sodium tetrahydroboride increases size distribution of the nanoparticles decreases while the average diameter of the particles remains almost constant. At R = 1, well dispersed and highly stable nanoparticles were obtained. (Reprinted with permission from Lisiecki I, Pileni M (2003) Synthesis of well-defined and low size distribution cobalt nanocrystals: the limited influence of reverse micelles. Langmuir 19 (22):9486–9489. Copyright (2015) American Chemical Society)

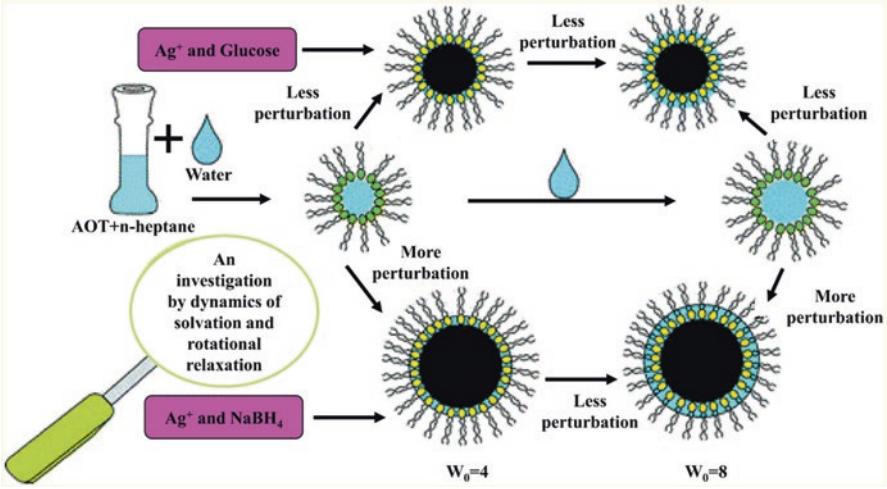

Fig. 5.8 Effect of the different reducing agents (glucose and sodium tetrahydroboride) on the size of silver nanoparticle synthesized in bis(2-ethylhexyl) sulfosuccinate sodium salt/n-heptane system. Sodium tetrahydroboride thus produces bigger size of silver nanoparticles, which causes more perturbation to reverse micelles (Reprinted with permission from Setua P, Ghatak C, Rao VG, Das S, Sarkar N (2012) Dynamics of Solvation and Rotational Relaxation of Coumarin 480 in Pure Aqueous- bis(2-ethylhexyl) sulfosuccinate sodium salt Reverse Micelle and Reverse Micelle Containing Different-Sized Silver Nanoparticles Inside Its Core: A Comparative Study. The Journal of Physical Chemistry B 116 (12):3704–3712. Copyright (2015) American Chemical Society)

5.6.4 Miscellaneous

The type of particle forming agent also affects the morphology of synthesized nanoparticles. The well dispersed spherical coated (with SiO_2) CeO_2 nanoparticles were observed when oxalic acid ($(COOH)_2$) was used as a particle forming agent of CeO_2, whereas ammonium hydroxide (NH_4OH) resulted in non-uniform size, surface adhered CeO_2 nanoparticle on SiO_2 interface (Fig. 5.9) (Tago et al. 2003). Size dependency of bimetallic nanoparticles on composition of individual metals have been reported by many researchers (Chen and Chen 2002; Yonezawa and Toshima 1995; Esumi et al. 1991; Wu et al. 2001b, a). Feng and Zhang (2006) have observed that the size and composition of Cu-Ni alloy nanoparticle depends on several factors which includes W_0 value of reverse micelles, mole ratio and method of addition of individual metal ions in the initial precursor solution.

Tojo and Vila-Romeu (2014) have predicted by simulation modeling that the shell formation in a bimetallic nanoparticle can influences merely by changing the concentration of reactants, even though there is a difference in reduction potential (about 0.15 V) of both metals. AT higher concentration, a core-shell structure can be formed, whereas at lower concentration a pure core with mixed surrounding shell can be formed. In previous study Tojo et al. (2009) concluded that the reduction rate

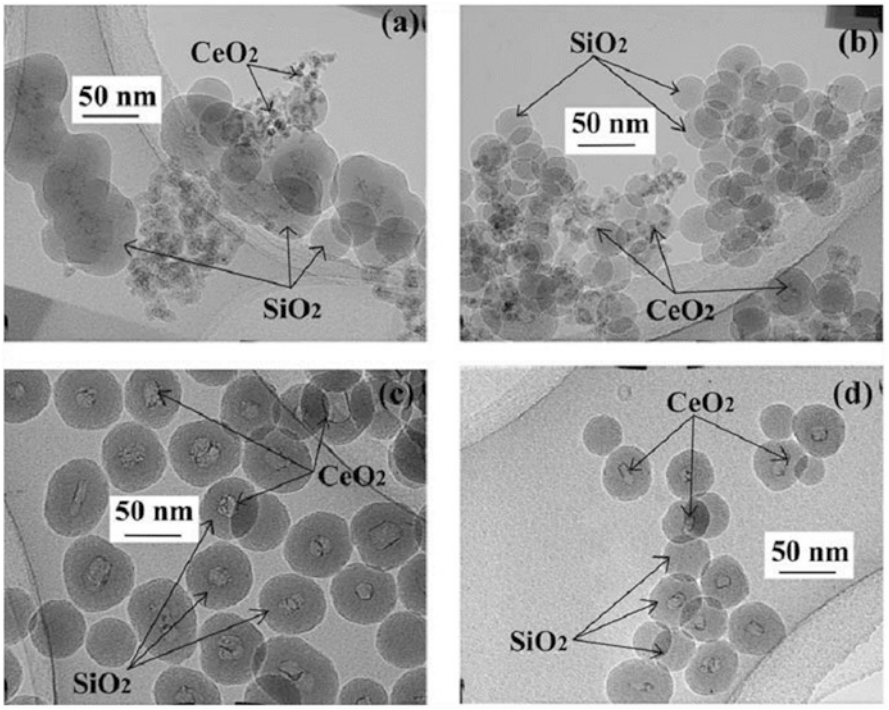

Fig. 5.9 Transmission electron microscopy photographs showing the effect of particle-forming agent [NH_4OH and $(COOH)_2$] on the morphology of the SiO_2 coated CeO_2 nanoparticles. When NH_4OH aq. was used (**a**, **b**), the aggregation of the CeO_2 nanoparticles, which seemed to be dispersed on the surface of the SiO_2 particles and not to be coated with SiO_2, was observed. Whereas $COOH)_2$ (**c**, **d**) resulted in larger size of spherical SiO_2 particles containing CeO_2 at center of void. (Reproduced from Journal of Nanoparticle Research (2003) 5 (1–2):55–60, Synthesis and optical properties of SiO_2-coated CeO_2 nanoparticles. Tago T, Tashiro S, Hashimoto Y, Wakabayashi K, Kishida M; (Fig. 5.1) with kind permission from Springer Science and Business Media)

difference affects the nanoparticle structure only in two cases: (i) when both reactions take place at same rate an alloy is obtained, (ii) when both reactions have very different rate a core-shell structure is formed.

The size of reverse micelles is one of the parameters for controlling the size of nanomaterial during nanomaterial synthesis. The size of reverse micelles increases with an increase in W_0, which results in larger nanoparticle formation within, and vice versa (Falcone et al. 2009; Lemyre and Ritcey 2005). The dynamic interactions between reverse micelles have been reported as the most important factor, which affects the properties and morphologies of nanomaterial (Natarajan et al. 1996). It is reported that the particle size can be controlled by varying the particle growth rate and the nucleation site size, which are known as the material exchange rate between micelles and the reverse micelles size, respectively.

5.7 Conclusion

Reverse micellar extraction technique could be successfully applied for the separation and purification of biomolecules. The studies revealed that the reverse micellar extraction process is usually controlled by numerous factors including charge on the protein, concentration and type of surfactant, pH and ionic strength of the aqueous phase, concentration of salts, temperature, water content and volume ratio of organic to aqueous phases. The preparation of nanoparticles involves performing simple reactions of synthesis in the aqueous "pools" of reverse micelles that act as nanoreactors. The sizes of microcrystals of the product are directly determined by the sizes of these pools. The size of the nanoparticle synthesized inside reverse micelles can be varied by altering the polar core size and aqueous phase volume. The processing conditions such as concentration of surfactant, ionic strength, pH, use of ionic liquids as polar core and addition of reducing agent plays significant role in controlling the size of nanoparticles. Unlike many conventional nanoparticle synthesis techniques, reverse micelles not only synthesize uniform size particle, but the synthesized particles might also be well dispersed. Enhancement of reaction rate is another advantage of synthesis in reverse micelles, compared to other techniques. The nanoparticles formed using reverse micelles will have advantages like the absence of aggregates of particles, metastable or amorphous crystal phase, and unique structure, leading to substantial changes in their properties.

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Chapter 6
Nanotechnology Delivery Systems of Coenzyme Q10: Pharmacokinetic and Clinical Implications

Shweta Paroha, Arvind K. Singh Chandel, and Ravindra Dhar Dubey

Abstract Coenzyme Q10 is an antioxidant essential for biochemical reactions in the human body. The deficiency of the coenzyme Q10 in the body leads to several disorders including neurological degeneration, ageing, and cancer. In cell mitochondria, coenzyme Q10 is a cofactor as electron transport system and is responsible for the synthesis of adenosine triphosphate (ATP), a major source of energy. Clinical trials reported the role of coenzyme Q10 in as the drug or dietary supplement. The major issue concerning coenzyme Q10 delivery is its high molecular weight and poor water solubility. This limitation ultimately leads to its poor oral bioavailability. Traditional approaches has been made to overcome poor water solubility, such as size reduction and ionization. New drug delivery carriers include nanoparticles, solid dispersions, liposomes, nanoemulsions, self-emulsifying drug delivery system, nanostructured lipid carrier, cyclodextrins and nanocapsules. These nanocarriers facilitate absorption of coenzyme Q10 from gastrointestinal tract and increase oral bioavailability. Here we review nanotechnology-based drug delivery system for coenzyme Q10 with special emphasis on pharmacokinetic perspective and clinical relevance.

Keywords Coenzyme Q10 • Nanotechnology • Pharmacokinetic • Bioavailability • Delivery Systems • Dietary Supplement • Antioxidant

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6.1 Introduction

Coenzyme Q10 is an antioxidant and essential molecule for proper growth of every cell in the body. Our diet has 3–5 mg every day intake of coenzyme Q10 (Safar and Dellimore 2007). It plays the main role in mitochondria and an essential component of energy production in the body (Santos et al. 2009). Red meat, fish, and poultry are the main sources of coenzyme Q10 (Santos et al. 2009). It is a very hydrophobic drug resulting in poor bioavailability and poor delivery significance (Balakrishnan et al. 2009). The main problem in the delivery of the coenzyme Q10 is that it is very insoluble molecule in the water. It is significantly absorbed when taken with the lipid diet (Santos et al. 2009).

The low intake of dietary coenzyme Q10 resulted in various pathophysiological disorders and aging. Therefore, to enhance uptake of coenzyme Q10 in the brain and peripheral tissue the plasma concentration must be at its therapeutic window (Miles 2007). The low uptake causes several serious disorders. Therefore to improve solubility and oral bioavailability of the coenzyme Q10 is essential for the proper uptake in the body. The chemical structure of the coenzyme Q10 composed of a benzoquinone ring with isoprenoid side chain which is lipophilic in nature. The length of the lipophilic isoprenoid side chain varies among different organisms. In the human being, the isoprenoid side chain contains 10 trans-isoprenoid units. The chemical structure of coenzyme Q10 and process of nano-encapsulation has been depicted in Fig. 6.1.

The oral route for drug delivery has been the major route due to the better patient compliance. However, the 50% of the drug absorption was hampered due to the high lipophilicity. Several new chemical entities do not enter in the clinical trial due to the poor aqueous solubility. The oral delivery of poorly water-soluble drugs has a major challenge due to its low water solubility (Gursoy and Benita 2004). To improve the solubility of the poorly aqueous soluble drug one of the conventional approach is modification of the physicochemical properties of the salt formation

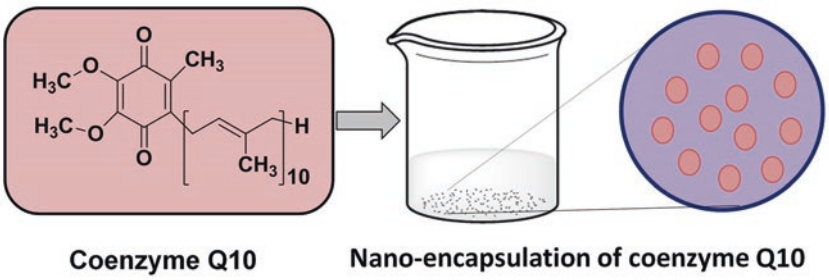

Fig. 6.1 Chemical structure of coenzyme Q10 and the process of nano-encapsulation in the carriers. Due to lipophilic nature of coenzyme Q10, the oral bioavailability is very low. So, to improve the oral bioavailability the drug was firstly dissolved in organic solvent and then encapsulated within the suitable nano-carrier

Fig. 6.2 Advantages of coenzyme Q10 nano-delivery system. The coenzyme Q10 which is an essential molecule for proper growth of every cell in the body showed several advantages when administered through nano delivery system. The major advantage of coenzyme Q10 nano delivery system is improvement of overall therapeutic index of the native drug

and reduction in particle size (Wadke et al. 1989). However, these techniques have their own limitations like the salt formation of the weak acid and weak base compound not much practical as these salt convert back to its original state (Serajuddin 1999). Further, the particle size reduction technique is not suitable for situations of handling difficulties and poor wettability (Serajuddin 1999). To overcome these limitations various nanotechnology-based approaches has been explored. The advantages of coenzyme Q10 nano-delivery system is depicted in the Fig. 6.2.

6.2 Nanotechnology in Delivery System of Coenzyme Q10

Nanotechnology-based approaches has been explored in several field including agriculture (Speiser 2008; Lai et al. 2006), in fiber and textiles (Perelshtein et al. 2008), electronics (Huang et al. 2003), space (Liu et al. 2007), forensic science (Choi et al. 2008) and in therapeutics (Bender et al. 1996; Bonduelle et al. 1992; des Rieux et al. 2006; Kawashima et al. 2000). Moreover, Nanotechnology proved to be emerging field in the delivery of small molecule (Cho et al. 2011; Choi et al. 2010; Peer et al. 2007). There are several nanotechnology-based formulations has been prepared for the effective delivery of the coenzyme Q10. The main advantage of these nano-delivery systems is modulation of the pharmacokinetic properties of the coenzyme Q10 to enhance its bioavailability. The nano-formulation that is explored in the delivery of the coenzyme Q10 has been shown in the Fig. 6.3.

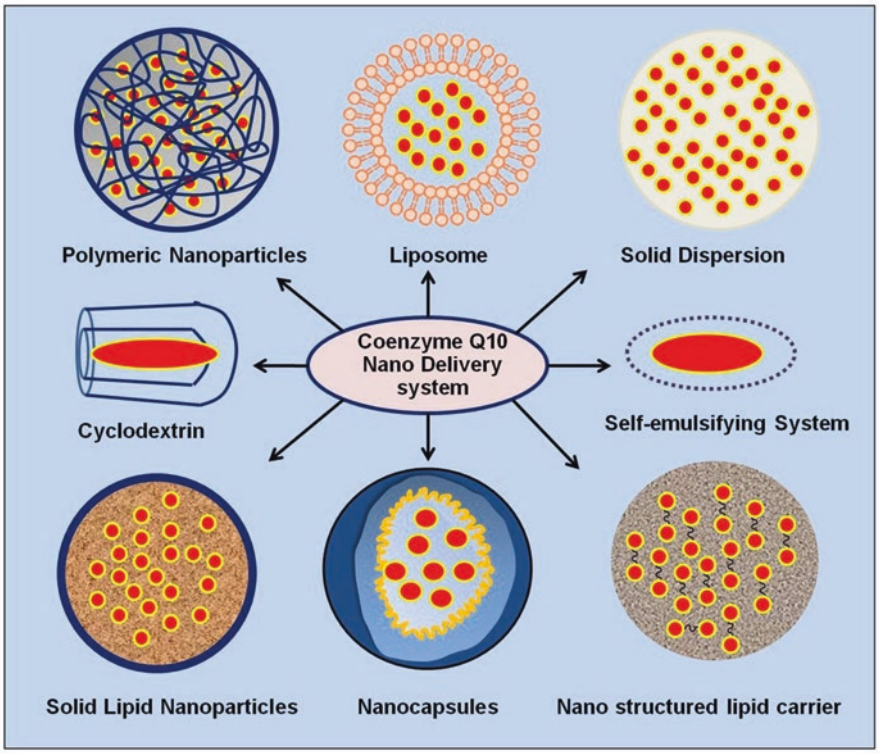

Fig. 6.3 Different formulations of coenzyme Q10 nano-delivery system. The coenzyme Q10 can be encapsulated into lipid as well as polymeric formulations. The nanoformulations feature of the main feature of these nano formulations of coenzyme Q10 is modulation of the pharmacokinetic properties of the coenzyme Q10 to enhance its bioavailability

6.2.1 Nanoparticles

Nanoparticles are nano-sized carrier ranging between 10 and 1000 nm and the drug is dissolved or encapsulated in the polymeric matrix depending on the method of preparation (Soppimath et al. 2001). Nanoparticles are used in drug delivery as it exhibited targeting properties, better bioavailability and controlled release of the drug. There are various types of a polymer including poly(lactic-co-glycolic) acid (PLGA), polylactic acid (PLA), chitosan, gelatin, polycaprolactone and poly-alkyl-cyanoacrylates which are widely used in the field of drug delivery (Kumari et al. 2010). Jain et al. designed polymeric nanoparticles of coenzyme Q10 and investigated for their oral bioavailability and therapeutic efficacy. Emulsion diffusion evaporation method and the size were reported to be less than the 100 nm. The prepared coenzyme Q10 nanoparticles showed 4.28 times enhancement in the oral bioavailability as compared to that of free coenzyme Q10. The nanoparticles also

offer high anti-inflammatory properties and hepatoprotective activity as compared to that of free coenzyme Q10 (Swarnakar et al. 2011).

Piao et al. described loaded lipid nanoparticles as well as nanocrystals and stated that high oral absorption of the coenzyme Q10 was achieved as compared to that of coarse suspension of coenzyme Q10 (Piao et al. 2011). The improved oral absorption of the lipid nanoparticles, as well as nanocrystals, was due to the enhance dissolution profile of the coenzyme Q10. Hsu et al. designed nanoparticles in which coenzyme Q10 had been incorporated using a simple and potentially scalable method and demonstrated that the nanoparticles have been able to control the release of the coenzyme Q10 (Hsu et al. 2003). The nanoparticles are able to enhance the oral absorption of the coenzyme Q10 mainly due to its size in nanometer range which facilitates its absorption.

Swarnakar et al. reported enhanced oral anticancer activity and safety of doxorubicin liquid crystalline nanoparticles in combination with coenzyme Q10 liquid crystalline nanoparticles. The drug-loaded nanoparticles were solvent diffusion evaporation method and provide prophylactic anticancer activity. It was reported that the combination of both the drug significantly reduces the dose of the drug due to higher cellular uptake and nuclear localization. It can be concluded that the quality of life of the cancer patient can be improved by oral administration of the combined nanoparticles in which the coenzyme Q10 potentiate the effect of the doxorubicin nanoparticles (Swarnakar et al. 2014).

6.2.2 Liposomes

Liposomes are spherical vesicles having hydrophilic core and a hydrophobic outer layer which can carry both hydrophilic and hydrophobic drugs (Yang et al. 2011). The liposomes exhibited most promising delivery system among the nanomedicine due to their biodegradability, biocompatibility and non-immunogenic nature (Muthu and Singh 2009). Liposomal delivery posses one of the promising approaches to improving the drug delivery. The similarities of the liposomal cells with biological membrane allow its easy uptake with less toxicity (Duzgune and Nir 1999).

Verma et al. reported liposomes containing coenzyme Q10 prepared by using four different technique including lipid film hydration, ethanol dissolution, reverse phase evaporation and detergent dialysis. It was reported that the liposomal formulation of the coenzyme Q10 showed a low risk of Myocardial infarction as compared to that of plain coenzyme Q10 in rabbits (Verma et al. 2007). Lee et al. prepared coenzyme Q10 containing composed of soybean phosphatidylcholine and α-tocopherol and demonstrated that the liposomes enhanced its accumulation into the in rats skin as compared to that of an unencapsulated suspension (Lee and Tsai 2010). Several other reports also suggesting that the liposomal coenzyme Q10 prepared by different technique provide the improved effect as compared to free drug (Niibori et al. 1998; Xia et al. 2012; Xu et al. 2015).

Recently, Shao et al. reported TPGS liposome coated with the chitosan for improvement of bioavailability of coenzyme Q10. It was demonstrated that the liposomes containing coenzyme Q10 showed 30 times greater cellular uptake in Caco-2 cells as compared to that of the powder coenzyme Q10. The oral pharmacokinetic experiment indicated that the liposomal coenzyme Q10 increases 3.4 folds systemic exposure of the drug as compare to that of the powder coenzyme Q10. Additionally, it was seen that the TPGS liposomes enhanced apoptosis and reduce the intracellular reactive oxygen species (ROS) generation. Conclusively, liposomes delivery system proved to be an effective delivery platform for the oral delivery of the coenzyme Q10 (Shao et al. 2015).

6.2.3 Nanocapsules

Nanocapsules are nano-sized shells composed of a non-toxic polymer. It is a vesicular system which is made by a polymeric membrane and encapsulated an inner liquid core containing the drugs. The major advantage of these encapsulation methods are the protection of encapsulated drug, controlled release of encapsulated drug, and for targeted drug delivery (Ezhilarasi et al. 2012).

Zhou et al. designed lecithin nanocapsules for improvement of the oral bioavailability of coenzyme Q10. The nanocapsules were prepared through high-pressure homogenization technique and composed of lecithin, GTCC, glycerol aqueous solution. The size of the nanocapsules was less than the 100 nm with negative zeta potential. It was reported that the coenzyme Q10 encapsulated nanocapsules showed higher as compared to that of the tablet after 24 h (Zhou et al. 2013b). Further, Cheuk et al. nano-encapsulated coenzyme Q10 in octenyl succinic anhydride modified starch and stated that the stability of the native coenzyme Q10 was increased by such encapsulation (Cheuk et al. 2015).

6.2.4 Nanostructured Lipid Carriers

Nanostructured lipid carriers (NLC) are nano-sized lipid body typically having a particle size in the range from 150 to 250 nm (Schwarz et al. 2013). There are various literature available which developed and extensively characterized coenzyme Q10 loaded NLC (Baisaeng 2013; Baisaeng et al. 2011, 2012). These NLC showed small size and high drug loading capacity than the conventional coenzyme Q10 loaded lipid nanoparticles (Baisaeng 2013).

Schwarz et al. developed ultra-small NLC loaded with coenzyme Q10 for improved dermal delivery. It was reported that decrease in particles size (80 nm) directly influence in the skin permeation and physicochemical stability of the NLC (Schwarz et al. 2013). In an another study Bruge et al. prepared a coenzyme Q10 loaded NLC and studied its effect on human dermal fibroblasts in normal as well as

UVA-mediated oxidative conditions. It was demonstrated that the NLC are able to efficiently revert UVA-associated mitochondrial depolarization indicating its potential role in anti-ageing cosmetological preparations (Bruge et al. 2013).

6.2.5 Solid Dispersion

Solid dispersion showed an excellent technique for improvement in the bioavailability of hydrophobic drugs by means of enhancement of solubility and dissolution rate (Chiou and Riegelman 1971; Leuner and Dressman 2000). The solid dispersion the size of the particles was reduced resulting in the increase in surface area and enhancement of the dissolution (Craig 2002). In addition, no energy is required for breakdown of the crystal lattice of the drug because of the drug present in the amorphous form (Taylor and Zografi 1997).

Bhandari et al. prepared a solid dispersion of coenzyme Q10 containing poloxamer-188 as a carrier and demonstrated that the prepared solid dispersion has little impact on the dissolution (Bhandari et al. 2007). Nepal et al. screened various solid dispersion formulation of coenzyme Q10 in which using poloxamer-407 with melting method for enhanced of solubility to a greater extent. To improve further solubility of the coenzyme Q10 Aerosil® 200 was incorporated with the poloxamer-407 and reported that the solid dispersion-enhanced the solubility as well the stability of the coenzyme Q10 (Nepal et al. 2010a).

6.2.6 Self Emulsifying Drug Delivery System (SEDDS)

Self emulsifying drug delivery system (SEDDS) are isotropic mixtures of oils and surfactants and co-solvents which after mild agitation inside in the gastro-intestinal tract emulsify into a very small globule. Hydrophobic drugs are often dissolved in oil phase for encapsulation into the SEDDS. Generally the SEDDS formulation was used for the improvement in the oral bioavailability of the poorly water-soluble drugs by enhance absorption in gastro-intestinal tract (Pouton 1997). Solid SEDDS are composed of liquid SEDDS on a solid support (Tang et al. 2008). The solid support may be Neusilin, Aerosil-200 which provide additional stability and flow properties.

There are several hydrophobic drugs available in the pharmaceutical market as SEDDS formulation e.g. Sandimmune® and Sandimmune Neoral (cyclosporinA), Fortovase (saquinavir) and Norvir (ritonavir) etc. There are several report are available for SEDDS formulation of coenzyme Q10 for solubility as well as bioavailability enhancement (Kommuru et al. 2001; Balakrishnan et al. 2009; Nepal et al. 2010b; Onoue et al. 2012). These SEDDS formulation marked increase in the bioavailability of the coenzyme Q10 after oral administration.

6.2.7 Cyclodextrins

Cyclodextrins consists of sugar molecule bound together in cyclic oligosaccharides ring which is produced by starch by enzymatic degradation. They are used in food, drug delivery, and pharmaceutical and chemical industries (Menuel et al. 2007; Thatiparti et al. 2010). Terao et al. designed γ-cyclodextrin complexation of coenzyme Q10 for improvement of oral bioavailability. It was demonstrated that after 6–8 h of dosing in healthy adults there was a significant increase in plasma of coenzyme observed as compared to that of a mixture of coenzyme Q10 and microcrystalline cellulose (Terao et al. 2006).

6.2.8 Nanoemulsions

Nanoemulsions are a colloidal dispersion which acts as the carrier for the bioactive molecules. Their size of the nanoemulsions varies from 10 to 1000 nm. Various methods are reported for the preparation of the coenzyme Q10 nanoemulsions. Zhou et al. designed D-alpha-tocopheryl polyethylene glycol 1000 succinate-based nanoemulsions by hot high-pressure homogenization method for targeted delivery of coenzyme Q10 to the heart tissues and demonstrated that the nanoemulsions effectively deliver the drug to the targeted site (Zhou et al. 2013a). Belhaj et al. prepared polyunsaturated fatty acids nanoemulsions containing coenzyme Q10 and reported that bioavailability was increased by two fold as compared to the native coenzyme Q10 tested in the male Wistar rats after oral administration of the three formulations (Belhaj et al. 2012). In continuation of this, many other approaches as applied for the preparation of the nanoemulsions which showed improved oral bioavailability either in rat or in healthy human volunteers (Trauschke et al. 2009; Hatanaka et al. 2008).

Cho et al. demonstrated that the droplet size of the nanoemulsions greatly influences the bioavailability of coenzyme Q10 and long chain fatty acids. It was reported that the smallest size nanoemulsions were absorbed in greater extend from the gastrointestinal tract (Cho et al. 2014). In an another application, Stratulat et al. designed a stable nutraceutical formulation of coenzyme Q10 which consists of calcium lecithin, flax seed oil with caseinate and demonstrated its application in cheese manufacturing (Stratulat et al. 2013). The different nanotechnology based drug delivery systems are summarized in Table 6.1.

Table 6.1 Nanotechnology-based formulations of coenzyme Q10

Drug formulation	Main excipients	Outcome	Route	Study type	References
Nanoparticles	PLGA	Oral delivery on rats shows 4.28 fold ↑ in bioavailability as compared to free CoQ10	Oral	Dawley rats	Swarnakar et al. (2011)
lipid nanoparticles	Witepsol® W35, MCT	Oral delivery on rats shows 3-fold ↑ in bioavailability	Oral	Wistar rats	Piao et al. (2011)
Nanoparticles	Emulsifying wax, Brij 78, and/or Tween 20	In vitro drug release shows controlled release of CoQ10	–	In vitro	Hsu et al. (2003)
Nanocapsules	Lecithin	The bioavailability of CoQ10-LNCs improved to 176.6% as compared to CoQ10 tablet	Oral	Mice	Zhou et al. (2013b)
Liposomes	PC, CH, PEG2000-DSPE, DOTAP	Prevent myocardial infarction	IC	Rabbits	Verma et al. (2007)
Liposomes	SDS, α-tocopherol	Enhance skin permeation after topical application	Dermal	In vitro	Lee and Tsai (2010)
Liposomes	Chitosan, TPGS-1000	Enhanced oral bioavailability	Oral	Dawley rats	Shao et al. (2015)
Nanostructured lipid carriers	Cetyl palmitate, Cetiol® OE and Span®	Improved dermal delivery	–	In vitro	Schwarz et al. (2013)
Nanostructured lipid carriers	Precirol© ATO Miglyol© 812	Reduced oxidative stress	–	In vitro	Bruge et al. (2013)
Solid dispersion	Poloxamer 407 and Aerosil® 200	75% release & ↑ in dissolution rate	–	Dissolution	Nepal et al. (2010a)
SEDDS	Oils (Myvacet 9–45 and Captex- 200), emulsifiers and cosurfactant	2 fold ↑ in bioavailability	Oral	dogs	Kommuru et al. (2001)

(continued)

Table 6.1 (continued)

Drug formulation	Main excipients	Outcome	Route	Study type	References
SEDDS	Oil (Labrafil M1944 & Labrafil M2125), surfactant	2 fold ↑ in bioavailability	Oral	Dawley rats	Balakrishnan et al. (2009)
SNEDDS	Witepsol® H35, Solutol® HS15 & Lauroglycol® FCC	4.6 folds ↑ in bioavailability	Oral	Dawley rats	Nepal et al. (2010b)
Solid-SEDDS	Triglyceride, fatty acid & HPC	5 fold ↑ in bioavailability	Oral	Dawley rats	Onoue et al. (2012)
γ-cyclodextrin complexation	γ-cyclodextrin and microcrystalline cellulose	Bioavailability significantly enhanced	Oral	Healthy human volunteer	Terao et al. (2006)
Nanoemulsion	TPGS, lecithin	2.5 fold higher concentration of TPGS-NE than LC-NE in heart	IV	Dawley rats	Zhou et al. (2013a)
PUFA's nanoemulsions	Salmon oil, salmon lecithin & water	2 fold ↑ in bioavailability than conventional oily formulations	Oral	Wistar rats	Belhaj et al. (2012)
Emulsion (emulsified CoQ10)	Coconut oil, skim milk, CSL	Bioavailability was slightly greater than commercial CoQ10 product (HJB Coenzyme Q10 EX)	Oral	Healthy human volunteer	Trauschke et al. (2009)
Liquid nanoemulsion & cyclodextrin	Triglyceride, surfactants, lecithin	1.7-fold ↑ in bioavailability of nanoemulsion than powder	Oral	Dawley rats	Hatanaka et al. (2008)

Abbreviation: ↑ Increase, *PC* phosphatidy lcholine, *Ch* cholesterol, PEG2000-DSPE distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy (polyethyleneglycol)-2000, *DOTAP* 1,2-dioleoyl 3-trimethyl-ammonium-propane, *SDS* sodium dodecyl sulfate, *IC* intracoronary, *PLGA* poly(lactic-co-glycolic acid), *CSL* calcium stearoyl-2-lactate, *CoQ10* coenzyme Q10, *SEDDS* self-emulsifying drug delivery system, *MCT* medium chain triglycerides, *HPC* hydroxy propyl cellulose, *IV* intravenous

6.3 Therapeutic Application of Coenzyme Q10 Supplementation

Coenzyme Q10 has been implicated as a potential clinical application in a large number of diseases, especially in the mitochondrial dysfunction disease. The coenzyme Q10 used in cardiovascular diseases including chronic heart failure, arteriosclerosis, hypertension cardiomyopathy, arrhythmias and ischemic heart disease. There are several articles published which indicated that the potential benefits of the coenzyme Q10 in the cardiovascular diseases (Kumar et al. 2009; Littarru and Tiano 2010; Wyman et al. 2010). The coenzyme Q10 also plays a potential role in cancer. There is some reported work available which indicates its role in cancer. Study indicated that survival of the patient increased who suffered from different cancer like breast, lungs, ovaries, kidneys, brain, esophagus, stomach, pancreas etc. (Chai et al. 2010; Hertz and Lister 2009; Nicolson and Conklin 2008; Sachdanandam 2008; Zhao et al. 2006). It is also beneficial in the reproductive disease as the fertility is affected by the stress condition.

There are various reports available which indicate its beneficial role in the reproductive disorder (Balercia et al. 2009; Roland et al. 2010; Safarinejad 2009; Teran et al. 2003). The several study also demonstrated the role of coenzyme Q10 in neurodegenerative disorders, especially in which the mitochondrial dysfunction involved (Spindler et al. 2009; Hyson et al. 2010; Littarru and Tiano 2010). Littarru and Tiano described that the oxidative stress related to the Parkinson's disease and the coenzyme Q10 has a direct benefit in on the disease (Littarru and Tiano 2010). Coenzyme Q10 showed several other therapeutic application which is depicted in the Fig. 6.4.

6.4 Conclusion and Future Perspective

Low levels of coenzyme Q10 have been caused high levels of oxidative stress produced during the ageing process and different diseases condition. Oral supplementation of the coenzyme Q10 provides the benefits in the ageing and cardiovascular disease. According to the webpage clinicaltrials.gov, there are several coenzyme Q10 formulation are available in a clinical trial I, II and III for various disease. The main limitation for coenzyme Q10 is its low bioavailability after oral administration. The Scientific community actively involved the development of a bioavailable formulation of coenzyme Q10 for the treatment of diseases like cardiovascular disorder, cancer, ageing and skin disorders. The nanotechnology plays a very important role in the development of the formulations. The nano-formulations like nanoparticles, liposomes, solid dispersion, nanostructured lipid carriers, nanocapsules, γ-cyclodextrin, Self-emulsifying drug delivery system etc. showed promising approaches for the enhanced solubilization and bioavailability of the coenzyme Q10. Although the chemical modified form of the coenzyme Q10 also available

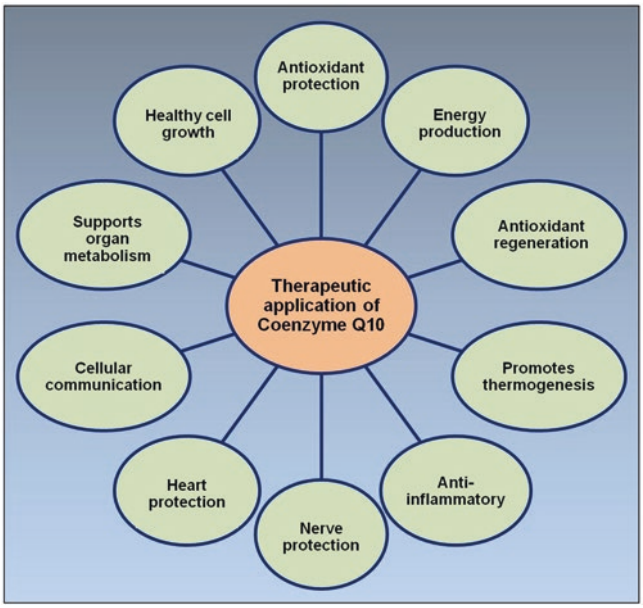

Fig. 6.4 Therapeutic application of coenzyme Q10 in the different disease condition. The coenzyme Q10 is an anti-oxidant and a major source of energy in the body. The coenzyme Q10 is used clinically as prophylaxis as well as therapeutic application for different disease

with enhanced water solubility but the nano-formulations may be a better choice for the clinical practice.

While considering the pharmaceutical aspect, one of the major challenges in novel drug delivery system is the transformation of formulations from the laboratory to industrial scale. There are several reasons that hampered commercialization of nanocarriers based formulations including regulatory status, reproducibility, the cost of materials, etc. The ultimate goal of research should be the commercialization of formulations to consume by the patient. Therefore, there is a need to design and develop reproducible and cost-effective novel commercial formulations of coenzyme Q10.

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Chapter 7
Enzymatic Nanobiosensors in the Agricultural and Food Industry

Madan L. Verma

Abstract Detection of the environmental contaminants in the agricultural and food industries is a major challenge. Indeed, the widespread contamination of food by pesticides and other pollutants has raised concerns of the public. Fast, cheap and sensitive sensors are thus needed. The technology of enzymatic nanobiosensor offers a quick and cost-effective solution to the current concerns of agri-food industry. This article reviews recent trends in enzymatic nanobiosensor technology employed in agri-food industries, in particular the design of a bioconjugation strategy. Nanobiosensors offer ultrasensitivity and quick detection time for various pesticides and food-borne contaminants. The minimal detection limit of contaminant in soil samples by an enzymatic nanobiosensor is in the range of 50 picogram per litre, while the minimal contaminant detection limit in food samples is 1.6 nanomolar.

Keywords Nanomaterials • Enzymes • Nano-Bioconjugation • Molecular-Modeling • Environmental Monitoring • Pesticide • Food-Quality • Food-Safety

7.1 Introduction

Nanotechnology holds a great prospect that includes applications in the agriculture and food sectors (Dasgupta et al. 2015; Sekhon 2014; Putzbach and Ronkainenen 2013; Verma et al. 2012). It is a multidisciplinary domain that encompasses synthesis, characterisation and applications of the materials at the nanoscale level (Verma et al. 2013a, b, c, d, 2016; Pal et al. 2014, Cao et al. 2012). The nanomaterials possess one or more dimensions lower than 100 nm (Auffan et al. 2009). The behaviour of the materials at the nanoscale show unique optical, electrical, thermal and catalytic properties (Dasgupta et al. 2015; Pavlidis et al. 2014; Verma et al. 2013a, b, c).

Its application to the agri-food industry is relatively recent compared to biomedical application (Sharma et al. 2015; Ranjan et al. 2014; Garcia et al. 2010).

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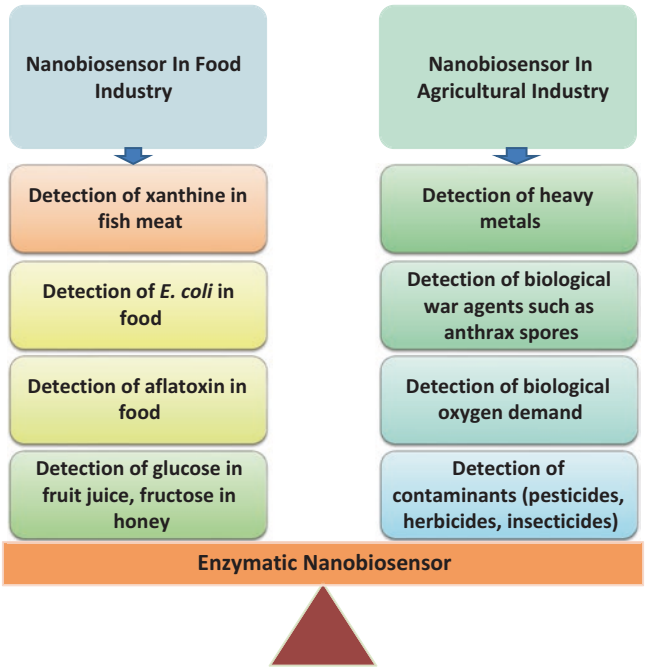

Fig. 7.1 A bird's eye view of nanobiosensor technology in the food and agricultural industry. Nanobiosensor endows with high order enzyme specificity and unique nanomaterial property. Nanobiosensor provides rapid and ultrasensitive detection of various contaminants. The excellent performance of nanobiosensor provides a plethora of applications in agri-food industry

Nanotechnology has become a new system for delivery agrichemicals to crop plants. Agrichemicals delivery to crop plants through nanotechnology intervention have used an effective alternative to traditional surfactant for penetrating plant surfaces as the nanomaterials delivered chemicals effectively, caused less damage to crop plants, required less water and resulted in lower environmental impacts (Nadiminti et al. 2013). The potential applications of nanotechnology in food industry include food safety examinations and pathogen detection (Ranjan et al. 2014; Ghormade et al. 2011). The usages of nanomaterials for improving biosensors technology hold a great promise to circumvent the agri-food industry issues (Fig. 7.1).

In order to circumvent the environmental issues thorough biosensor technology, enzymatic nanobiosensor offers a quick and cost-effective solution due to the commercial availability of the genetically modified enzymes and the ease of nanomaterial synthesis for construction of such biosensors (Ghormade et al. 2011; Luong et al. 2008; Tothill 2001). Enzymatic nanobiosenors utilise the affinity and selectivity of the enzyme towards the target molecules such as substrate, co-substrate, co-factor, and inhibitor. Nanobiosensor is typically an analytical device that includes a biological recognition molecule immobilised on the surface of a transducer (Fig. 7.2; Jain et al. 2010; Verma et al. 2010). The reaction between the analyte and the

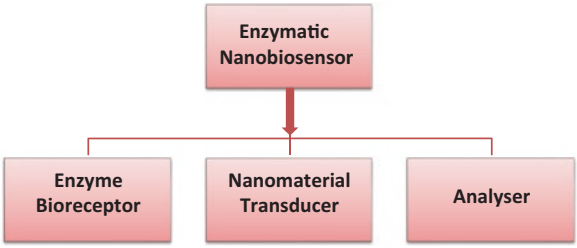

Fig. 7.2 Components of an enzymatic biosensor. The nanobiosensor assembly arranged in the following order: (1) bioreceptor includes biomolecules such as enzyme which demonstrates high order specificity for the pesticides and food-borne pathogens; (2) nanomaterial-transducer produces signal conversion achieved via enzymatic conversion of a substrate to a product; and (3) detector/analyser which generates reproducible and rapid response

biological recognition molecule is largely depends on the nature of the biosensing interface. Recent advances in controlled manipulation of the materials at the nanoscale level have profoundly improved the analytical quality of the biosensors (Zhang et al. 2014). The application of high aspect ratio materials such as nanoparticles, quantum dots, nanowires, nanorods, nanotubes, graphenes, has dramatically enhanced the biosensing output with higher sensitivity and shorter responses time (Sharma et al. 2015; Ranjan et al. 2014; Putzbach and Ronkqinen 2013).

Advancement in the enzyme based nanobiosensing system is primarily correlated with the degree of improvement in sensitivity and stability towards target molecules detection. Nanomaterials owe to their unique optical, and electrical properties impart a special distinction in the methodology of robust nanobiosensor development (Ghormade et al. 2011).

The present article is focussed on the recent advances in enzyme based nanobiosensor in agri-food industry. The bioconjugation steps required to design and develop a nanobiosensor is critically reviewed and discussed. Nanobiosensor application in the agricultural and food industries is separately discussed. The future direction and implication is also discussed.

7.2 Nano-Bioconjugation Strategies for Enzymatic Biosensor Development

Biorecognition part of the biosensor is the heart of biosensor technology. It works on the interaction of the biomolecules and the material in a very specific manner; it requires an insight of the biomolecules functionality at the nano-bio interface (Verma et al. 2016; Pavlidis et al. 2014). Enzymatic nanobiosensor has enzyme bioreceptor that possesses the high degree of substrate specificity. Selection of enzyme depends on the critical parameters such as thermal stability, and high turnover number. In such scenario, enzymes sourced from thermophilic microorganisms

and genetically modified enzymes is the best candidate for selection in biosensor development (Verma and Kanwar 2012). Biorecognition part of the biosensor regulate the sensitivity, selectivity and stability of the biosensor. Hence biorecognition part of the biosensor requires a critical understanding for improving the biosensor performance (Sassolas et al. 2012).

The biomolecules immobilisation on the surface of the transducer is a crucial step towards biosensor development. The biomolecules immobilisation is an enabling technology that improves the performance of the nanobiocatalytic system (Perez-Lopez and Merkoci 2011; Verma et al. 2008a, b, 2009, 2011; Verma and Kanwar 2008, 2010; Luong et al. 2008). The bioconjugation techniques are classified based on the nature of interaction between the biomolecule and the materials. The nano-bioconjugation technique is commonly employed in biosensor development such as adsorption, covalent, cross-linking, entrapment and affinity (Verma et al. 2012, 2013a, 2016; Verma 2009; Kanwar et al. 2008a, b). The pros and cons of each bioconjugation method are listed in Table 7.1.

Recently, few comprehensive research articles have been reviewed on nano-bioconjugation technology (Verma et al. 2008a, 2013a, 2016; Verma and Barrow 2015; Putzbach and Ronkainenen 2013; Kanwar and Verma 2010; Mateo et al. 2007; Kanwar et al. 2007). Each immobilisation method has a specific advantages and disadvantages. That is why a combination of two or more immobilised method is used to circumvent their limitations. The criterion for selection of bioconjugation method has relied on the critical factors such as the enzyme nature, transducer and detection mode (Sassolas et al. 2012). Retention of the biomolecules conformational and functionality is the topmost priority in all these enzyme nanobioconjuation methods. Sometimes, the poor selection of the immobilised method can cause modification in the active-site conformation of the enzyme that lead to the poor sensitivity of the biosensor. Hence such pitfalls can be overcome to the larger extent by employing a suitable bioconjugation methodology that preserve the active confirmation of the enzyme and avoid protein denaturation (Bello-Gil et al. 2014; Ley et al. 2011).

To sum up, a comprehensive understanding of biomolecules stability and functionality at the nano-bio interface is required for the design of ultrasensitive enzymatic nanobiosensor. This can be possible to a greater extend by the selection of best bioconjugation strategy.

7.3 Enzymatic Nanobiosensor Versus Conventional Biosensor

Recently nanomaterials are in the focus of biosensing research due to their great promise in industrial biotechnology and biomedical applications (Verma et al. 2016; Verma and Barrow 2015; Pavlidis et al. 2014). The unique structural features and exceptional chemical, electrical, and mechanical properties of the nanomaterials

Table 7.1 Nano-bioconjugation strategies for enzymatic nanobiosensors development. Nanobioconjugation methods such as physical (adsorption, entrapment), covalent (covalent binding, cross-linking) and bio-affinity are commonly employed for the design of robust nanobiosensor

Nano-bioconjugation method	Nature-of-interaction	Advantages	Disadvantages	References
Adsorption method	Non-covalent interaction	Simple and easy method, matrix regeneration possible, chances of enzyme denaturation is minimum	Enzyme leakage	Verma et al. (2008b, 2009, 2011, 2013d), Verma and Kanwar (2008), and Kanwar et al. (2007)
Covalent method	Chemical binding between the functional groups of the enzyme and the nanomaterial	Robust method, Induced high stability,	Matrix regeneration is not possible, Blockage of enzyme active site is possible	Verma et al. (2012, 2013b, c, 2016) and Kumar et al. (2014)
Cross-linking method	Matrix free method, enzyme-enzyme binding through a cross-linker	Simple immobilisation step, and high enzyme activity	Enzyme activity loss	Mateo et al. (2007)
Entrapment method	No direct bonding between the enzyme and nanomaterial but enzyme confinement within the nano-environment of a polymer	No chemical reaction involved, Co-immobilisation of many enzymes is feasible	Enzyme leakage and diffusion barrier	Ramanathan et al. (2009) and Vamvakaki and Chaniotakis (2007)
Affinity method	Strong affinity bonds between affinity tag of an enzyme and functional group on the support	Well controlled and oriented method with fully viable enzyme	Applicable to genetically modified enzyme having affinity tag e.g. biotin	Bello-Gil et al. (2014) and Ley et al. (2011)

and their ability to affect the nanoenvironment of biomolecules makes an ideal platform for the synthesis of ultrasensitive bioconjugates, for various applications in food and agriculture industries (Verma et al. 2016; Sharma et al. 2015; Pavlidis et al. 2014). For example, polymeric nanomaterials are most suitable for agrochemical delivery due to controlled release of ingredients. Metal nanoparticles exhibit size dependent properties such as magnetic, fluorescence, and photocatalytic that have tremendous aid in biosensing applications (Putzbach and Ronkainenen 2013).

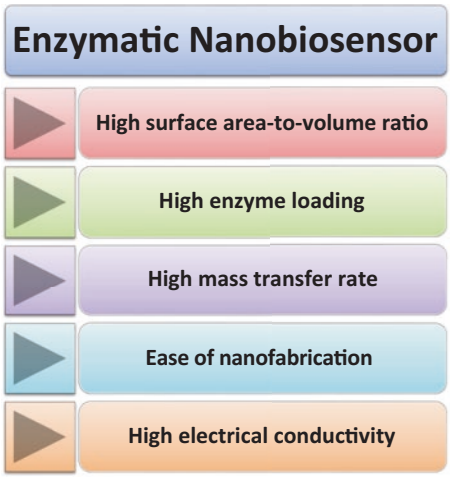

Fig. 7.3 Salient features of an enzymatic nanobiosensor. Nanomaterials improve the capabilities of nanobiosensor technology. Advanced functional materials possess the following inherent properties: (1) Higher aspect ratios and low mass transfer resistance render high biomolecules loading and quick response time; (2) Ease of nanomaterial's surface modification extends the versatility towards diverse bioconjugation methodologies for nanobiosensor technology; (3) Nanomaterials possess excellent inherent properties such as nanoparticles for good catalytic, quantam dots for fluorescence and carbon nanotubes as well as nanowires for better electrical communication

Nanotechnology has provided a significant impact to traditional enzyme immobilised technology (Verma et al. 2013a, 2016). Biosensing capabilities have improved tremendously with inclusion of the nanomaterial support in the biosensor development (Sassolas et al. 2012). The top-down and bottom-up approaches for nanomaterial synthesis is well documented and even easy availability of the nanomaterials at the various commercial centres (Puri et al. 2013). Various nanomaterial forms such as nanoparticles, nanofibres, nanocomposite, graphene and nanotubes, have been used effectively in recent years in enzymatic nanobiosensor development (Singh et al. 2014; Verma et al. 2012, 2013a, b, c, 2016).

The salient properties of nanomaterials as compared to the conventional bulk materials such as higher surface area-to-volume ratios, higher biomolecule loading, biocompatibility, amenability of surface modifications, lower mass transfer resistance, ease of separation and ocst-effective operation cost have a stronger advantageous parameters for robust nanobiosensor development (Fig. 7.3; Verma et al. 2012, 2013a, b, c, 2016; Pavlidis et al. 2014; Cao et al. 2012).

Thus the amalgamation of the inherent properties of both nanomaterial and enzyme has improved the sensitivity as well as stability of the enzymatic nanobiosensor as compared to the conventional biosensor.

7.4 Enzymatic Nanobiosensors for the Agriculture Industry

In the current scenario, the most common problem in the agricultural sector is the indiscriminate usage of pesticides. In addition to soil pollution, such consequences have disturbed the soil biodiversity to a significant level (Ghormade et al. 2011). Such problems can be minimised to a greater extent by nanotechnology interventions. Nanotechnology based biosensors can detect traces of pesticides residues as well as plant-pathogen that is very useful for on-site environment monitoring and suitability of the soil for agricultural usages (Table 7.2). The pesticides, in particular to organophosphorus compounds, have a strong affinity for acetylcholinesterase enzyme (Periasamy et al. 2009). The cholinesterase enzyme is catalysed a hydrolytic reaction that regulates the function of neurotransmitter acetylcholine. The organophosphorus compound cause irreversible inhibition of the acetylcholinesterase. Thus, nanobiosensors based on the recognition part of the acetylcholinesterase have been exploited for the detection of the pesticides residues. The application of nanobiosensor technology for early detection of pesticide residues in context to agricultural industry in this section is discussed.

Enzymatic nanobiosensor was developed for discriminative detection of organophosphorus and non-organophosphorus pesticides (Zhang et al. 2015). Researchers reported for the first time a novel bi-enzyme biosensing system and used novel layer-by-layer assembled multi-enzyme/carbon nanotube methodology for nanobiosensor design. Nanobiosensor used electrostatically bound enzyme-nanotube-pesticide and nanotube-enzyme along with a set of cushioning bilayers comprising of multiwalled carbon nanotube-polyethyleneimine and multiwalled carbon nanotube complex. Nanofabrication of layer-by-layer interfaces and conductivity properties of nanobiosensors were characterised by using surface plasmon resonance and electrochemical impedance spectroscopy. Higher sensitivity of the nanobiosensor for organophosphorus pesticide paraoxon (0.5 μM) and 1 μM for non- organophosphorus pesticide carbaryl was reported. The nanobiosensor sensitivity was also validated using apple samples.

Nanobiosensor based on atomic force microscopy (AFM) tips was developed for detecting agrochemicals (Da Silva et al. 2014). Nanobiosensors used specific interactions between an enzyme and herbicides. The AFM tip was functionalised with acetolactate synthase to detect the acetolactate synthase-inhibitor herbicides metsulfuron-methyl and imazaquin. The binding force in the nanobiosensor was calculated using atomic force spectroscopy. The specific biorecognition force improved the sensitivity of the nanobiosensor.

Enzymatic nanobiosensor was developed by using molecular modeling techniques (Franca et al. 2011). This nanobiosensor was fabricated using functionalisation of atomic force microscopy tips with acetyl co-enzyme A carboxylase. The detection was monitored by measuring the forces between the immobilised enzyme and the herbicide analyte. The molecular modeling technique was employed to optimise the enzyme adsorption studies on the tips of the atomic force microscopy and the level of detection. The molecular dynamics simulations studies revealed that

Table 7.2 Enzymatic nanobiosensors for the agricultural industry

Nanomaterials	Nano-bioconjugation approach	Application	References
Carbon nanotube	Layer-by-layer electrostatic assembly of multi-enzyme/carbon nanotube on the surface of transducer	Organophosphorus pesticide paraoxon and non-organophosphorus pesticide carbaryl detection at the limit of 0.5–1 μM	Zhang et al. (2015)
Tip of atomic force microscopy	Physical adsorption of acetolactate synthase on the surface of transducer	Detection of higher adhesion forces in the order of 160–250% for herbicides (metsulfuron-methyl and imazaquin)	Da Silva et al. (2014)
Tip of atomic force microscopy	Physical adsorption of acetyl co-enzyme A carboxylase on the surface of transducer	Detection of the interactive force for enzyme-diclofop herbicide at the limit of 1.6 nN per enzyme	Amarante et al. (2014)
Tip of atomic force microscopy	Physical adsorption of acetyl co-enzyme A carboxylase on the surface of transducer	Herbicides (diclofop and atrazine) detection at the limit of 0.168–44.11 μM	Franca et al. (2011)
Nanocomposite of magnetic nanoparticle, carbon nanotube, and zirconium nanoparticle	Covalent immobilisation of acetylcholinesterase on the surface of transducer	Ultrasensitive detection of dimethoate pesticide at the limit of 50 pg l^{-1}	Gan et al. (2010)
Silica nanoparticle	Entrapment of organophosphate hydrolase in silica on the surface of transducer	Detection for organophosphate paraoxon at the limit of 34 μM	Ramanathan et al. (2009)
Nanoliposome	Entrapment of acetylcholinesterase in liposome on the surface of transducer	Ultrasensitive detection of pesticides paraoxon and dichlorvos at the limit of 1 nM	Vamvakaki and Chaniotakis (2007)
Carbon nanotube	Covalent immobilisation of acetylcholinesterase on the surface of transducer	Detection of pesticides (carbaryl, malathion, dimethoate and monocrotophos) at the limit of 0.96–4.28 μM	Du et al. (2007)
Carbon nanotube	Layer-by-layer electrostatic self-assembly of acetylcholinesterase on the surface of transducer	Ultrasensitive detection of organophosphorus pesticides at the limit of 0.4 pM	Liu and Lin (2006)
Carbon nanotube	Physical adsorption of acetylcholinesterase on the surface of transducer	Ultrasensitive detection of paraoxon pesticide at the limit of 0.5 nM	Joshi et al. (2005)

Note the trends of ultra-sensitivity of enzyme based nanobiosensors for the detection of agriculture contaminant. The minimal detection limit of contaminant in the soil sample was reported in the range of 50 pg l^{-1}

dimeric form as compared to monomeric form in an aqueous solution was more suitable form for enzyme immobilisation due to small structural fluctuations. The best orientation for the analytes (herbicides diclofop and atrazine) with the enzyme active site was estimated by using molecular docking calculations. The inhibition coefficient for diclofop and atrazine was 0.168 μM and 44.11 μM respectively. This nanobiosensor validated the modeling predictions and showed high selectivity for two herbicides.

Recently, nanobiosensor was developed by using modeling the coverage of an atomic force microscopy tip with acetyl co-enzyme A carboxylase and its application was done for detection of low limit of herbicides (Amarante et al. 2014). The interaction energy for enzyme-analyte was calculated by using Steered Molecular Dynamics. Enzyme arrangement over the surface of atomic force microscopy tip and the interaction of biomolecule-analyte was studied at the molecular level. The experiments validated the theoretical modeling results. Such studies have paved the way to optimise the biomolecules interaction on the transducer surface and improved the efficiency of the nanobiosensor.

Nanobiosensor based nanocomposite immobilised acetylcholinesterase was employed for the detection of the organophosphorus pesticide (Gan et al. 2010). The enzyme coated magnetic nanoparticles was bound to carbon nanotubes and zirconium nanoparticles composite on the screen printed electrode surface. The nanobiosensor was used for the detection of pesticide dimethoate. High sensitivity of the nanobiosnesor was achieved at the limit of 50 pgl^{-1}. Nanobiosensor based silica nanoparticle encapsulated organophosphate hydrolase was reported for paraoxon detection (Ramanathan et al. 2009). The enzyme was co-encapsulated with a pH-responsive fluorophore in the silica nanoparticles. The nanobiosensor was stable up to two months and show detection limit of 34 μM for paraoxon.

Nanobiosensor was developed by entrapment of enzyme acetylcholinesterase in the liposome (Vamvakaki and Chaniotakis 2007). Nanobiosensor was successfully employed for the detection of two organophosphorous pesticides paraoxon and dichlorvos. The internal nano-environment of the liposomes stabilised the enzyme and a pH sensitive fluorescent indicator pyranine. The optical transduction of the enzymatic activity was measured by counting the strength of fluorescent signal of the pH indicator. Higher concentrations of the pesticides residues have inhibited the enzyme hydrolysis of the acetylcholine that rendered low fluorescent signal of the pH indicator. This nanobiosensor has also been applied to monitor the quality of the drinking water.

Nanobiosensor based multiwall carbon nanotube/chitosan immobilised acetylcholinesterase was developed for the detection of four pesticides (Du et al. 2007). The modified nanocomposite was used for covalent immobilisation of the enzymes using glutaraldehyde cross-linker. Four pesticides of carbaryl, malathion, dimethoate and monocrotophos were investigated for inhibition efficiencies towards acetylcholinesterase. The sensitivity of the nanobiosensor was in the range of 0.96 μM to 4.28 μM, respectively.

Nanobiosensor was developed by modified the glassy carbon electrode with multiwall carbon nanotubes immobilised acetylcholinesterase (Liu and Lin 2006). Researchers employed layer-by-layer electrostatic self-assembly of enzyme on carbon nanotubes. Self-assembly was controlled by positively charged poly(diallyldimethylammonium chloride) and negatively charged carbon nanotubes and enzymes. This sandwich self-assembly approach protected the enzyme leakage and retained the catalytic activity. The robust nanobiosensor was sensitive as low as 0.4 pM residues of pesticides.

Nanobiosensor based multiwall carbon nanotubes immobilised acetylcholinesterase enzyme was developed for early pesticide detection (Joshi et al. 2005). The acetylcholinesterase was immobilised onto carbon nanotubes by adsorption method. The screen-printed electrode was modified by casting a film of nanotubes. The resulted nanobiosensor have exhibited higher sensitivity limit of nM pesticide residues than the unmodified screen-printed electrode. High surface area and catalytic behaviour of nanomaterials immobilised enzymes improved stability and reproducibility.

To sum up from the above nanobiosensing studies of recent past, enzymatic nanobiosensor improves the sensitivity for early detection of the pesticides residues in the agriculture sector.

The ultrasensitive detection of the pesticide contaminant in the soil sample by the enzymatic nanobiosensor was reported in the range of 50 picogram per litre. A new trend of ultrasensitive detection of agriculture contaminant by the enzymatic nanobiosensor has advanced the biosensor technology.

7.5 Enzymatic Nanobiosensors for the Food Industry

Detection of the pathogenic microorganisms (*Salmonella*, *Pseudomonas aeruginosa*, and *E. coli*) in the food samples through nanotechnology intervention have been reported (Dasgupta et al. 2015; Ranjan et al. 2014; Perez-Lopez and Merkoci 2011). The application of optical biosensing methods such as fluorescence and surface plasmon resonance have been most commonly employed for detection of pathogens. These techniques are straightway monitoring the changes in the optical signal due to the interactions between the nanomaterials and pathogenic microorganisms (Comparelli et al. 2007). Flourescense emission of organic dyes as well as protein based fluorophores has been used for pathogen detection in fluorescence based sensing detection (Simpson-Stroot et al. 2008; Ko and Grant 2006).

Recently integration of the nanomaterial in the biosensor assembly for faster electron transfer of enzymes as well as higher specificity for analyte has shown great promises in the food industry. Various forms of nanomaterials immobilised with enzymes such as gold and quantum dot nanoparticles, single and multiwalled carbon nanotubes has been employed for the checking the quality of food samples (Perez-Lopez and Merkoci 2011; Ozdemir et al. 2010; Li et al. 2009; Prakash-Deo et al. 2005). Nanobiosensor has developed for the detection of biomolecules in the

food analysis (Ozdemir et al. 2010). The real time monitoring of the sugars in the food is required to regulate the process of fermentation. The industrial enzymes such as glucose oxidase, β-galactosidase, pyranose oxidase and fructose dehydrogenase is being used as biorecognition molecules for checking carbohydrate molecules such as glucose, lactose/lactulose and fructose in the food samples (Ozdemir et al. 2010; Serna-Cock et al. 2009). Some examples of nanobiosensor application in food industry are discussed in the following section (Table 7.3).

Table 7.3 Enzymatic nanobiosensors for the food industry

Nanomaterials	Nano-bioconjugation approach	Application	References
Nanorod film of zinc oxide	Covalent co-immobilisation of choline oxidase and peroxidase on the surface of transducer	Detection of choline in milk at the limit of 0.5 μM	Pal et al. (2014)
Nanocomposite of ZnO nanoparticle, chitosan, carbon nanotube, and polyaniline	Covalent immobilisation of xanthine oxidase on the surface of transducer	Detection of xanthine in fish meat at the limit of 0.1 μM	Devi et al. (2012)
Gold nanoparticle	Physical adsorption of β-galactosidase on the surface of transducer	Colorimetric detection of *E. coli* at the limit of 1×10^2 bacteria/millilitre	Miranda et al. (2011)
Carbon nanotube	Covalent immobilisation of aflatoxin oxidase on the surface of transducer	Detection of aflatoxin in food at the limit of 1.6 nM	Li et al. (2011)
Gold nanoparticle	Covalent immobilisation of pyranose oxidase on the surface of transducer	Detection of glucose at the limit of 50 μM	Ozdemir et al. 2010
Carbon nanotube	Covalent immobilisation of β-galactosidase on the surface of transducer	Colorimetric detection of *E. coli* at the limit of 10 colony forming unit per millilitre	Cheng et al. (2008)
Nanoclusters of ZnO and Co	Covalent immobilisation of glucose oxidase on the surface of transducer	Detection of glucose at the limit of 20 μM	Zhao et al. (2007)
Carbon nanotube	Physical adsorption of fructose dehydrogenase on the surface of transducer	Detection of fructose in honey at the limit of 1 μM	Antiochia et al. (2004)

Note the trends of ultra-sensitivity of nanobiosensors for the detection of food contaminants. The minimal detection limit of contaminant in the food sample was reported in the range of 1.6 nM

Nanobiosensor based on chemiluminescence choline has been developed using aligned zinc oxide nanorod films (Pal et al. 2014). The covalent co-immobilisation of enzymes choline oxidase and peroxidase was achieved using a 16-phosphonohexadecanoic acid cross-linker. The optimised cross-linker concentration rendered significant stability to nanorod immobilised enzyme resulting in improved shelf life as well as storage stability. Enzymatic nanobiosensor demonstrated higher sensitivity. The limit of detection for choline in milk sample was in the wide range of 0.5 μM to 2 mM.

Nanobiosensor based nanocomposite immobilised xanthine oxidase was developed for the determination of xanthine in fish meat (Devi et al. 2012). Researchers used a nanocomposite film of zinc oxide nanoparticle, chitosan, carboxylated multiwalled carbon nanotube, and polyaniline electrodeposited over the surface of a platinum electrode. Nanobiosensor exhibited optimum response within 4 s and detection limit of 0.1 μM xanthine in fish meat during storage. Nanobiosensor was stable and retained 70% of its initial activity after 80 uses over one month.

Nanobiosensor was developed for detection of microbial contamination (Miranda et al. 2011). An enzyme β-galactosidase was adsorbed on the gold nanoparticles by electrostatic interactions. Nanoparticle immobilised enzyme catalysed the hydrolysis of chlorophenol red β-D-galactopyranoside in the vicinity of *E. coli* and detection was done using colour changes.

Nanobiosensor based multiwall carbon nanotubes immobilised aflatoxin oxidase was employed for the detection of aflatoxin B_1 (Li et al. 2011). The covalent immobilisation technique employed for enzyme binding to nanotube was very robust that retained the full activity of the enzyme. Nanobiosensor showed high sensitivity with low detection limit of 1.6 nM.

Nanobiosensor based gold nanoparticles immobilised pyranose oxidase has been developed by Ozdemir et al. (2010) and employed for the detection of glucose in various fruit juices such as orange, peach, pomegranate and mixed fruit. The nanobiosensor showed excellent substrate kinetics for glucose indicating the protection of the enzyme molecules. In addition to fast and sensitive response for analyte, biocompatible gold nanoparticle with ease of functionalisation enhanced the operational stability.

Nanobiosensors based multiwall carbon nanotubes immobilised β-galactosidase was employed for the detection of pathogenic bacterium *Escherichia coli* (Cheng et al. 2008). The amount of current produced was proportional to the degree of the substrates hydrolysis that was considered as indicator of *E. coli* contamination. The electrochemical and mechanical properties of the carbon nanomaterials as well as high selectivity and affinity of the enzyme has improved the detection limit for *E. coli* population in the bacterial solution. Nanobiosensors have shown low limit of detection (10 colony forming units/millilitre) with a time span of five hours.

Nanobiosensors based nanoclusters immobilised glucose oxidase has employed for the detection of glucose (Zhao et al. 2007). Nanocluster (ZnO:Co) was synthesised by nanocluster-beam deposition method. Such nanocomposites have increased

the electroactive area of the electrode for higher glucose oxidase loading. This has led to increased sensitivity for the detection of glucose. The lowest detection limit was 20 μM; the apparent K_M was 21 mM, indicating the high affinity of the immobilised enzyme to glucose.

Nanobiosensor based carbon nanotube immobilised fructose dehydrogenase has employed for detection of fructose in honey (Antiochia et al. 2004). The nanomaterial was modified with an electropolymerised film of 3,4-dihydroxybenzaldehyde. The enzyme fructose dehydrogenase was immobilised onto the membrane placed on the top of the electrode surface. The nanobiosensor was higher sensitive with the detection limit of 1×10^{-6} mol/L and good reproducibility for the detection of fructose in real samples. The nanobiosensor have shown superior biosensing limit than the conventional solid electrodes.

It is evident from the above discussed application of enzymatic nanobiosensor in the food industry that the sensing limit for the detection of food biomacromolecules and contaminants has been improved significantly. The minimal detection limit of contaminants in the food sample by the enzymatic nanobiosensor was in the range of 1.6 nanomolar. Thus enzymatic nanobiosensor opens up new trends for estimating the food quality in an ultrasensitive way.

7.6 Conclusion

There have been significant advances in the fields of biotechnology and nanotechnology. Firstly, the usage of genetically engineered enzyme has improved the sensing limit of the enzymatic nanobiosensor for the detection of the environmental contaminants in agri-food industry. The utilisation of genetically modified enzymes offers excellent storage and thermal stability, the most important requirement for a robust enzymatic nanobiosensor development. Mostly, these enzymes are available commercially at low cost. Secondly, nanotechnology has provided many advanced functional nanomaterials. Nanomaterials have become the pivotal part of current biosensoring system. The unique structural and exceptional thermal, chemical and catalytic properties of the nanomaterials have improved the sensing limit of the nanobiosensors tremendously. As a result, there have been considerable improvements in the performance of enzymatic nanobiosensors. Enzymatic nanobiosensor offers a cost effective platform for monitoring the environment contaminant at the early stage. The performance of enzymatic nanobiosensor can surpass to any other costly bioanalytical system. To sum up, enzymatic nanobiosensors holds a great potential for the agri-food industry.

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Chapter 8
Transformation of Natural Products into Synthetic Copolymers

Mukesh K. Pandey, Virinder S. Parmar, and Arthur C. Watterson

Abstract Natural products are attractive synthetic substrates to design new and diverse polymeric nanomaterials. However, due to the absence of mechanical properties, natural products remain unexplored in the field of materials sciences. Copolymerization of natural products using macromeres can lead to nanomaterials with diverse properties and applications. For instance natural substances such as curcumin and coumarin can be copolymerized with polyethylene glycol and polydimethylsiloxane to produce novel polymeric materials using *Candida antarctica* lipase as a catalyst in solvent-less conditions. The resultant copolymers are amphiphilic in nature and self-assemble to form nano-micelles in aqueous medium, which broaden their applications. More generally, nanomaterials can be used for determination of trace quantities of metal impurities in drinking water and food, nano formulation of active pharmaceutical ingredients and active agriculture ingredients, sensing of trace quantities of explosives, preparation of flame retardant materials and manufacturing of dye sensitized solar cells. We briefly review here recent developments of natural product-based polymeric materials and their applications.

Keywords Transformation • Natural product • Copolymers • Novozym • Nanomaterial • Structural modification • Drug delivery • Agricultural applications

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8.1 Introduction

Natural products have always been source of inspiration to develop new therapeutics (Harvey 2008; Jain et al. 2005; Jain et al. 2006; Marcaurelle and Johannes 2008; Newman and Cragg 2007). Majority of existing drugs are either derived from natural products or inspired from their molecular achitecture (Butler 2004, 2008; Butler and Buss 2006; Butler et al. 2014; Kancheva et al. 2010; Li and Vederas 2009; Tietze et al. 2003). These natural products have shown considerable interest in medical science either by retaining their original natural product structure or as a synthetic analog of their parent compounds to improve their therapeutic efficacy (Jantan et al. 2015; Kontogiorgis et al. 2012; Lee 1999; Schumacher et al. 2011). Small molecular weight natural products have not been thoroughly explored as a component of a polymeric material or to transform them into co-polymers *via* copolymerization with other macromolecules like polyethylene glycol (PEG), pluronics or polydimethylsiloxane. However, some of the natural products have potential to improve the existing material property of a copolymer or moreover, provide completely new application to the copolymer if copolymerized with a suitable natural product (by virtue of their structural uniqueness and diversity) (Kumar et al. 2010; Pandey et al. 2010a).

For example, when curcumin was copolymerized with polyethylene glycol, it improved the therapeutic efficacy of curcumin by enhancing its bioavailability (Pandey et al. 2011a) and aqueous solubility and when it was copolymerized with polydimethylsiloxane, it detected extremely small quantities of nitro aromatics (explosives) (Pandey et al. 2014). This kind of diversity is uncommon because scientists usually limit natural products or natural polymers to biomedical applications such as biodegradable scaffolds, hydrogels in tissue engineering and drug delivery only (Pandey et al. 2010b; Singh and Peppas, 2014; Tyagi et al. 2008; Wurch et al. 2012; Yunus Basha et al. 2015).

On the other hand material scientists have mainly centered on synthetic and inorganic materials for the development of solar cells, sensors or even for flame retardant applications. So, attempts are yet to be undertaken to bridge this gap. Furthermore, natural products have unique and diversified molecular architecture, which is difficult to achieve synthetically, their structural uniqueness lends a unique property (Sen and Samanta 2015; Trindade et al. 2015). Therefore, this manuscript provides an overview of work carried out by incorporation of natural products into polymer back bone to enhance and diversify their material, and therapeutic properties.

Due to amphiphilic nature of the PEGylated coumarin and curcumin copolymers they do intent to form Nano-sized micelles in aqueous medium (Chittigori et al. 2014; Pandey et al. 2007a). Many of their potential agricultural applications like formulation of poorly aqueous soluble pesticides/insecticides/rodenticides as an encapsulated active agriculture ingredient can be directly attributed to their self-assembling nature in aqueous medium. Nano-formulation of active agriculture ingredients, detection and removal of toxic metal contaminants, synthesis

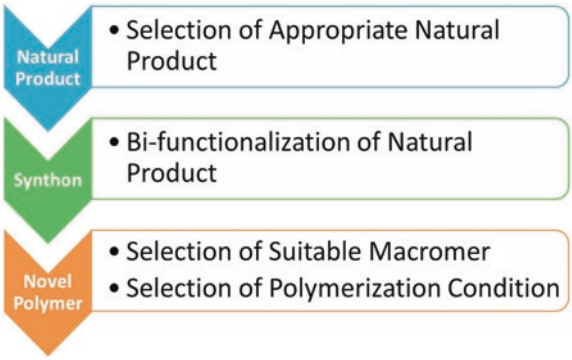

Fig. 8.1 Steps involved in design and synthesis of natural product based polymeric materials

of non-toxic flame retardant polymeric materials to protect crops and grain storage areas are a few of the many unexplored applications of natural product based copolymers. Furthermore, this is a simple, biocompatible and eco-friendly approach of copolymer synthesis that offers solution to many of the daunting problems of agriculture and food sciences. Present manuscript also provides a stepwise guidance of how to choose a suitable natural product and the critical factors involved in natural product based polymer design. The overview of natural product based polymer design and synthesis are depicted in Fig. 8.1.

8.2 Selection of a Natural Product

Selection of an appropriate natural product is a hallmark of this natural product based polymer design. Selection of a natural product is highly dependent upon its final application(s) but still following criteria can be applied to choose most suitable natural product.

1. Natural product should have already been widely used either in traditional medicine or in day-to-day applications.
2. It should have minimum toxicity.
3. It should be fairly stable at ambient conditions.
4. It should have multiple functional groups; it is even better if functional groups are located at symmetrical positions.
5. Preferably it should be ultraviolet or fluorescence active with some rigid aromaticity in its structure.
6. Additional functional groups should be located in positions to be easily accessible even after co-polymerization for potential interactions with other targets.

Incorporation of an appropriate natural product into the polymer backbone augments the application of resultant copolymer. For example, depending upon selected natural product, it can add optical property, structural rigidity and different functional groups on the copolymer for additional interactions and thus newer applications.

Therefore, this fusion of natural product and macromer potentiates and expands the usefulness of both natural product and macromer, which could not be otherwise easily achieved.

8.3 Design of an Appropriate Synthon

Design of appropriate synthon is a critical step in natural product based polymer synthesis because success of a polymer synthesis depends on appropriate design of the synthon. An appropriate bi-functionalization of a natural product is dependent upon the type of the catalyst chosen to do the polymerization reaction. For example, in case of condensation polymerization using Novozym 435 (*Candida antartica* lipase), the bi-functionalized group should be a methyl or ethyl ester of the carboxylic moiety, which should be at far most distance from each other to minimize the steric hindrance during the process of polymerization. Besides, there should not be any competitive functional groups which can possibly interact with any of the reactants or even by the synthon through inter/intramolecular reactions. In case, there is any such group present on the natural product then that functional group should be properly protected. Protecting group should be chosen in a way that even the protecting group should not cause steric hindrance or interact with any part of the molecule or even to the catalyst during the process of polymerization.

Additionally, for oxidative or ring opening polymerizations, the design of a synthon will differ from what we described for the condensation polymerization. Therefore, appropriate design of synthon can vary based on chosen natural product and polymerization condition.

8.4 Selection of a Macromer

Selection of a copolymerizing macromer depends on the type of property desired from the final copolymer. If the intention is to improve the therapeutic efficacy of a natural product *via* improving its aqueous solubility and bioavailability then polyethylene glycol is a choice of the macromer. Polyethylene glycol increases aqueous solubility and depending upon the selected size of polyethylene glycol chain, its longevity increases in blood circulation and thereby enhancing its bioavailability (Fishburn 2008). The size of polyethylene glycol chain should be chosen by considering the molecular weight or size of the natural product (Pandey et al. 2011a). There is no strict rule but a large chain of polyethylene glycol will definitely decrease the therapeutic efficacy of the natural product as overall effective dose will be decreased drastically due to large polyethylene glycol chain. Therefore, size of the polyethylene glycol chain should be between 2 and 5 times of the molecular weight of the targeted natural product for better efficacy.

Similarly, if natural product based polymers are considered for sensing applications then it should be copolymerized with a material which should not be soluble in water and protect the chromophore molecule from fluorescence quenching and aggregation during the thin film formation (Kumar et al. 2010). In this case polydimethylsiloxane would be a choice of macromer (Kumar et al. 2010). Polydimethylsiloxane is extremely hydrophobic, has low glass transition temperature and renders diffusion of analyte molecule across the thin film due to its viscous nature (Kumar et al. 2010; Pandey et al. 2014).

Design and development of novel natural product based polymeric materials should not be limited by two of the aforementioned macromers, many other macromers like pluronics, polyethyleneimine (linear/branched), chitosan, polysorbate and many more can be explored. Property of the final copolymer can also be tweaked by varying the size of the macromer and degree of copolymerization (Pandey et al. 2008b, c, 2010a, b, 2011b). Additionally, property of the final polymer can also be modulated by addition of more than one macromer, however in such a situation it may result into a random copolymer (Pandey et al. 2008b, c, 2010b).

8.5 Design and Optimization of Polymerization Condition

Once the natural product and macromer are selected, it is important to find a suitable polymerization condition which should retain the inherent property of the parent molecules by protecting their conformation and functionalities. There are different polymerizing conditions documented in the literature to explore for an optimum reaction condition. Overall, these conditions can be broadly classified as (i) chemical polymerization and (ii) biocatalytic polymerization.

Chemical polymerizations are typically aggressive in nature as they often require high temperature, use of acid/base or metal complexes as catalyst, longer reaction time and cumbersome work-up procedures. Besides, in many instances chemical polymerizations are not stereo/regio selective. On the contrary, bio-catalytic polymerizations are regio/chemo selective and milder in nature. Additionally, biochemical workup procedures are straight forward and simple but biocatalysts are a bit expensive. So depending upon the nature of the selected natural product and macromer, a suitable reaction condition can be adopted for copolymerization. Herein, we would like to discuss two distinct cases of curcumin (Fig. 8.2a) and 4-methylcoumarin copolymerization (Fig. 8.2b) with polyethylene glycol and polydimethylsiloxane, respectively using *Candida antartica* lipase (Novozym 435) under solvent less condition to get four different series of copolymers (Kumar et al. 2010; Pandey et al. 2007b, 2008a, 2010a, 2011a, 2014).

Curcumin is a natural product isolated from Turmeric (*Curcuma longa*) plant, it is a polyphenol, a strong chromophore and has 1, 3-di keto group which can undergo keto-enol tautomerism (Pandey et al. 2011a, 2014). One of the two keto groups predominantly exists in the enolic form. This keto-enol functionality acts as the active site for interaction with other targets and coordinates with various metal ions

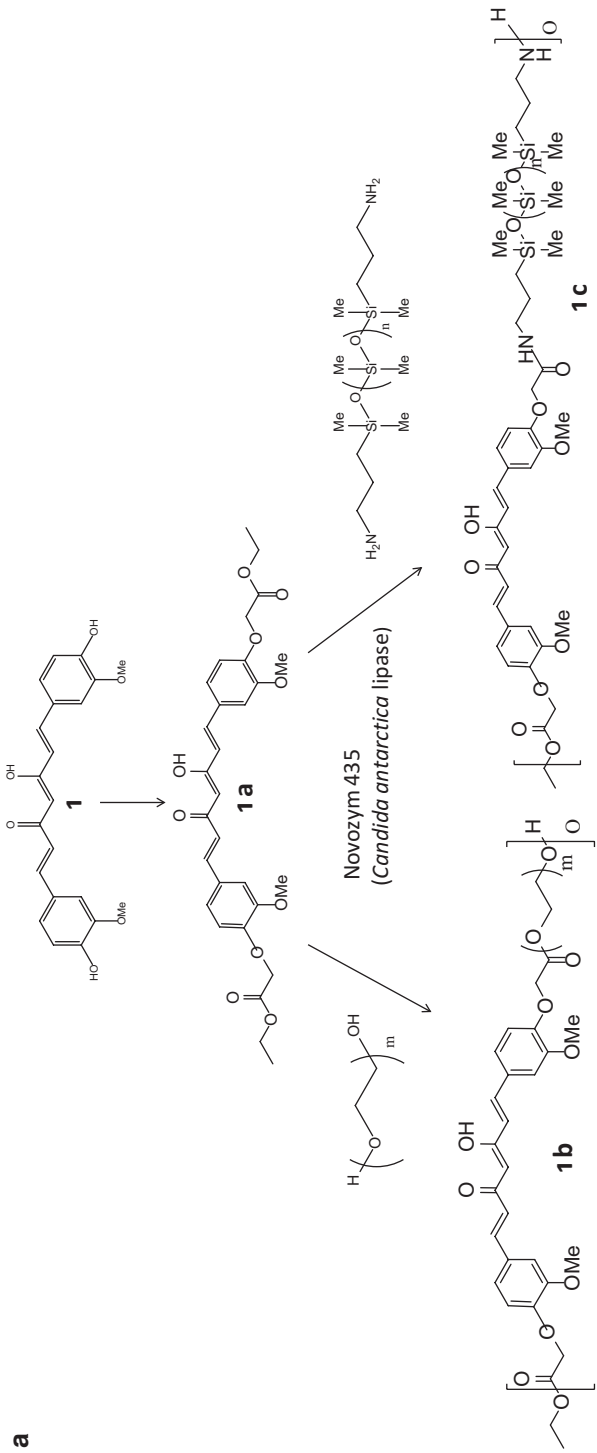

Fig. 8.2a Biocatalytic synthesis of PEGylated and siloxonated curcumin copolymers

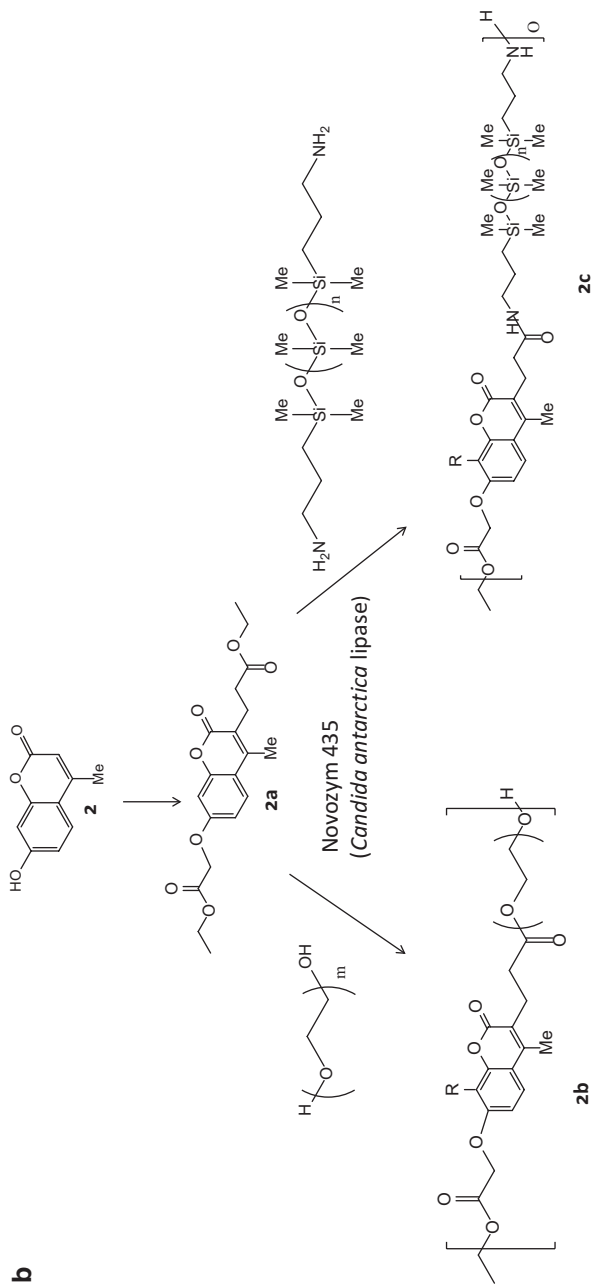

Fig. 8.2b Biocatalytic synthesis of PEGylated and siloxonated 4-methylcoumarin copolymers

to form metal complexes (Chittigori et al. 2014). Curcumin is a symmetrical molecule and contains two free phenolic hydroxyls on each of the aromatic nucleus making it a perfect candidate molecule for polymerization (Pandey et al. 2011a).

In order to polymerize curcumin either with polyethylene glycol or polydimethylsiloxane, it should be first converted to a suitable synthon or diester for the condensation polymerization. Two equivalents of ethyl α-bromoacetate were reacted with curcumin in the presence of potassium carbonate to make bi-functionalized diester of curcumin (Pandey et al. 2011a). Diester was then separately reacted with polyethylene glycol and polydimethylsiloxane in a solvent less condition (Pandey et al. 2011a, 2014). The polymerization reaction was catalyzed by *Candida antarctica* lipase (Novozym 435 or CAL-B). The enzyme *Candida antarctica* lipase is regio-selective and uses only primary hydroxyl and amine groups of the polyethylene glycol and polydimethylsiloxane for transesterification and amidation reactions, respectively (Gupta et al. 2010; Pandey et al. 2007b, 2008a, 2014). The beauty of *Candida antarctica* lipase enzyme lies in the fact that it does not interact either with secondary hydroxyl or amine group. So, the latter remains unaffected during polymerization even though there is a secondary hydroxyl (enolic) group present on the curcumin nucleus (Pandey et al. 2011a). This is a distinct case of how to choose a suitable natural product and polymerization condition by retaining its structural integrity and property even after the polymerization.

Similarly, coumarin was copolymerized with polyethylene glycol and polydimethylsiloxane in identical conditions to get two new series of copolymers. Different sizes of polyethylene glycol (MW 600–2000 Da) were used for copolymerization and to tweak the therapeutic efficacies of both curcumin and coumarin.

8.6 Characterization of Polymers

Spectroscopic characterization of polymeric materials is a daunting task because of the peak masking effect caused by the repeating units of the macromers. To avoid masking effect, product characterization should be carried out with extreme caution and also validated by cross characterization using different spectroscopic techniques. Typically, nuclear magnetic resonance (NMR) and gel permeation chromatographic (GPC) techniques are essential tools for appropriate characterization of the polymers. Ultraviolet (UV) and infrared (IR) could be instrumental in characterization of functional group inter conversions. In case of the condensation copolymers developed in our Laboratories, molecular weight determination using end group analysis by integral comparison of protons of end group ester bond to transester bond (repeating unit) of the copolymer using proton NMR was used (Pandey et al. 2011b). For material property evaluation, polymers can be analyzed by various techniques for various applications. For drug encapsulation and micelle size determination, UV, NMR and dynamic light scattering can be utilized. For sensing and flame retardant applications, fluorescence spectroscopy, thermogravimetric analysis (TGA) and pyrolysis-combustion flow calorimeter (PCFC) techniques are useful.

For surface characterization, atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) can be utilized.

8.7 Natural Product Based Copolymers for Diverse Applications

Due to inherent properties of natural product and macromer, the developed natural product based copolymers have additional attributes. In this section, we are highlighting how a natural product and macromer complement each other to enhance the property of the resultant copolymer. Four different series of copolymers (1a–b and 2a–b) are discussed individually to demonstrate various applications along with their potential applications in agriculture and food sciences.

8.7.1 PEGylated Curcumin Copolymer (1a)

Curcumin is a well-known antioxidant but its therapeutic application is limited because of its poor aqueous solubility and low bioavailability. Various attempts have been made including encapsulation of curcumin in drug delivery vehicles, oil based formulations and more polar synthetic analogs to address this problem but none of them has resolved the issue (Pandey and Rangarajan 2012). However, meticulous design of PEGylated curcumin has enhanced the aqueous solubility of the resultant copolymer by 10^6 fold compared to the free curcumin (Pandey et al. 2011a). This dramatic enhancement in aqueous solubility was attributed to polyethylene glycol. In this study various sizes: 600 Da, 1000 Da, 1500 Da and 2000 Da of polyethylene glycol were copolymerized with curcumin using *Candida antartica* lipase to evaluate and fine-tune the therapeutic efficacy of curcumin. The resultant copolymers were evaluated as nuclear factor (erythroid factor)-like 2 (NRf2) activator (antioxidant) and found out that all the synthesized PEGylated copolymers are strong NRf2 activators but the copolymer with 600 Da polyethylene glycol enhanced the efficacy more than three folds compared to the free curcumin (Pandey et al. 2011a). This approach evidently demonstrated that how a simple PEGylation of a natural product can lead to a more potent and easily formulable analog. Almost at same time other researchers found that PEGylated curcumin can be useful in cancer therapy (Li et al. 2009; Tang et al. 2010a; Tang et al. 2010b; Wichitnithad et al. 2011). Recently, PEGylated curcumin and free curcumin have also been shown to be useful in two-photon fluorescence imaging (D'Aleo et al. 2014; Kumar et al. 2012; Tiburcio-Moreno et al. 2013; Zhang et al. 2014).

Recent study by Chittigori et al. demonstrated that PEGylated curcumin can effectively interact with metal ions like Al^{3+}, Cu^{2+}, Zn^{2+} and Hg^{2+} and showed a significant difference in PEGylated curcumin's fluorescence in purely aqueous medium

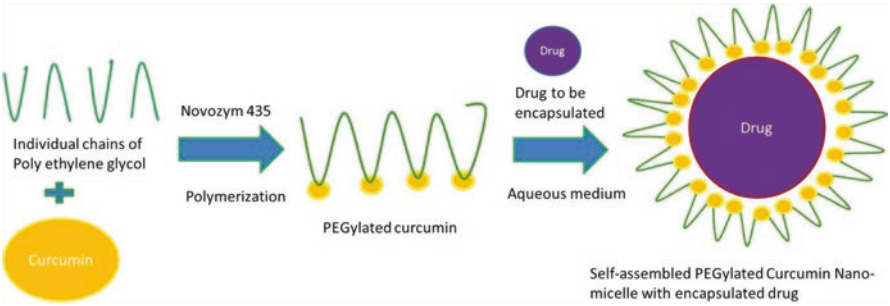

Fig. 8.3 Pictorial representation of synthesis of PEGylated curcumin, formation of nano-micelle and drug encapsulation

(Chittigori et al. 2014). This finding can be extended to estimate the presence of toxic metal ions in drinking water, agricultural water (irrigation) and food as a simple handy device to access the quality of water. Curcumin is also known for its antimicrobial activity (Krausz et al. 2015; Lee and Lee 2014), therefore, PEGylated curcumin could potentially be utilized as water soluble and biocompatible alternatives to exiting antimicrobial agents in food and grain preservation. Additionally, these PEGylated curcumin copolymers are amphiphilic in nature and they do self-assemble to form stable Nano-micelles in aqueous medium (Chittigori et al. 2014). Micellar behavior of PEGylated curcumin copolymers can be utilized to encapsulate many potent water insoluble pesticides, insecticides, molluscicides and rodenticides. This Nano-technological formulation approach will not only improve the aqueous solubility of these agricultural active ingredients but also minimize their overall consumption by potentiating their efficacy. Figure 8.3 shows how insoluble, active pharmaceutical/agriculture ingredients (drugs) can be encapsulated in PEGylated curcumin Nano micelles to improve solubility and bioavailability of the drug. However, Fig. 8.4 represents pictorially the multiple applications of natural product based polymeric materials.

8.7.2 Siloxanted Curcumin Copolymer (1b)

Siloxanated curcumin was synthesized on the same motif of copolymerizing curcumin with polydimethylsiloxane in the presence of *Candida antarctica* lipase (Pandey et al. 2014). In this case, polyethylene glycol macromer was replaced by polydimethylsiloxane and the resultant copolymer showed completely different properties. Siloxanated curcumin copolymer is found to be extremely hydrophobic in nature on the contrary to the PEGylated curcumin which is extremely hydrophilic in nature. The siloxanated curcumin was investigated for sensing of nitro aromatics (explosive) even at parts per billion levels (Pandey et al. 2014). This application is possible by merging the fluorescence property of curcumin, low glass transition

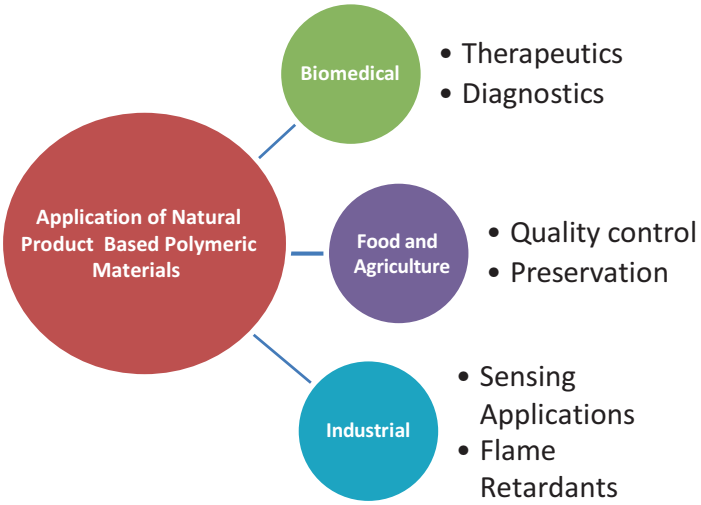

Fig. 8.4 Divers applications of natural product based copolymers

temperature and viscous nature of polydimethylsiloxane, which has allowed analyte molecules to diffuse into the resultant siloxanated curcumin copolymer and caused the fluorescence quenching. The remarkable change in material property of curcumin was only possible by selective use of macromers like polyethylene glycol and polydimethylsiloxane. Besides sensing application of siloxanated curcumin copolymer, it can potentially be useful as a fluorescent hydrophobic and antimicrobial paints. In fact, antimicrobial properties of curcumin based copolymers can be prudently utilized as a protective hydrophobic paint to protect agricultural storage areas.

8.7.3 PEGylated Coumarin Copolymer (2a)

Like curcumin, coumarins are well known natural products, reported for wide array of applications including anticoagulant (Lei et al. 2015; Marvig et al. 2015), perfumes (Wisneski 2001), dyes (Barooah et al. 2015) and sensitizers in photovoltaic devices (Soto-Rojo et al. 2015). Additionally, numerous coumarin analogs were also reported as agricultural fungicides, rodenticides and pesticides (Atta et al. 2010; Lawley et al. 2006; Li et al. 2010). These applications evidently demonstrate wide usefulness of coumarin analogs. Coumarin analogs are poorly soluble in aqueous medium thus limiting their aqueous formulation and applications. We copolymerized two of the coumarin analogs, 4-methylcoumarin and 4, 8-dimethylcoumarin with various sizes of polyethylene glycol using *Candida antarctica* lipase (Pandey et al. 2010a). The obtained copolymers were evaluated for nonsteroidal anti-inflammatory applications in comparison with the corresponding non-PEGylated

4-methylcoumarin and 4, 8-dimethylcoumarin. It has been found that PEGylated 4-methylcoumarin and 4, 8-dimethylcoumarin analogs have shown two–fourfold higher potency than their non-PEGylated analogs (Pandey et al. 2010a).

It is interesting to note how a simple PEGylation increases the therapeutic potency of 4-methylcoumarin and 4, 8-dimethylcoumarin analogs. The increased anti-inflammatory potency can be attributed to the expected increase in the aqueous solubility and bioavailability of the PEGylated analogs. These PEGylated 4-methylcoumarin and 4, 8-dimethylcoumarin analogs were not evaluated in agricultural applications. However, previous studies have already demonstrated fungicidal, rodenticidal and pesticidal effects of a few coumarin analogs. So these coumarin analogs can also be PEGylated to potentiate their agricultural applications. Besides, non-coumarin analogs can also be explored using the same motif to further advance the applications of these polymers in agriculture. Potent coumarin pesticide analogs can also be tethered to existing amphiphilic copolymers to prepare novel delivery vehicles for active agriculture ingredients delivery by exploiting copolymer's micellar behavior. Proposed nanoparticle based agricultural active ingredient delivery system can also be transformed to a dual delivery system, if same or other active agriculture ingredients are encapsulated in it, like it has been done with pharmaceuticals (Pandey et al. 2007a, 2008b, 2011b). This Nanoformulation approach will enhance the efficacy of agricultural active ingredients and minimize their overall consumption in more biocompatible manner. Additionally, due to antioxidant nature of 4-methycoumarins, these PEGylated coumarin copolymers can also be utilized in food preservation.

8.7.4 Siloxanted Coumarin Copolymer (2b)

Following the similar approach of copolymerization of natural products with polydimethylsiloxane, diesters of both 4-methylcoumarin and 4, 8-dimethylcoumarin were copolymerized with polydimethylsiloxane to yield the siloxanated coumarin copolymers again using *Candida antarctica* lipase as a catalyst (Pandey et al. 2008a). In this case, the resultant copolymers were evaluated for both flame retardant application and sensing of nitro aromatics. In terms of flame retardant application, the flammability properties like heat release capacity, total heat release and char yield were evaluated for both siloxanated 4-methylcoumarin and 4, 8-dimethylcoumarin analogs along with their decomposition temperatures. The heat release capacity was found to be 220 and 242 J/g K for siloxanated 4-methylcoumarin and 4, 8-dimethylcoumarins, respectively. However their decomposition temperatures were found to be 410 and 425 °C at 50% weight loss (Pandey et al. 2008a). These data indicated that the siloxanated analogs of 4-methylcoumarin and 4, 8-dimethylcoumarin are moderate flame retardant materials and can be useful in agricultural based applications to protect crop and grain storage areas from the fire.

Besides, siloxanated 4, 8-dimethylcoumarin was investigated for sensing application. The sensing of nitro aromatics like 2, 4-dinitrotoluene (DNT) and

2,4,6-trinitrotoluene (TNT) was possible as these nitro aromatics were able to quench the fluorescence property of coumarin based siloxanated copolymers even at parts per billion levels (Kumar et al. 2010). The low glass transition temperature of polydimethylsiloxane rendered the easy diffusion of analyte molecule across the thin film of the copolymer making them a decent copolymer for sensing applications (Kumar et al. 2010). This type of copolymers can be useful in identification of the potential explosion sites in post-mining areas open for agricultural use.

8.8 Conclusion

This perspective highlights the use of natural products in the design and development of smart polymers and Nano carriers for diverse applications. Two distinct examples of curcumin and coumarin have been discussed in terms of their environmentally benign synthesis and diverse applications ranging from biomedical to sensing and to agriculture. The conspectus of this perspective is that there are a wide array of natural products, which are unique in their structural design, nature and properties. If some of these natural products can be incorporated in to polymer design and synthesis then they can furnish unique material properties to the resultant copolymers. It is evident from the two examples of curcumin and coumarin discussed here, how their properties were tweaked and utilized by selective copolymerizing them with two different macromeres, polyethylene glycol and polydimethylsiloxane. This approach yielded four different series of copolymers having diverse applications ranging from theranostic (therapeutic and diagnostic) to industrial (sensing and flame retardant) and can be potentially extended to monitoring of water quality (estimation of metal contaminants) for drinking and agricultural applications. The present approach can be further extended in design and development of natural product based pesticides, fungicides and rodenticides to help and protect the crop. These natural product based copolymers can also be utilized effectively in Nano-formulation of both active pharmaceutical and active agricultural ingredients. Additionally, natural product based flame retardant copolymers can serve as an alternative to toxic halogenated flame retardant material to safely protect our agricultural storage areas.

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Chapter 9
Nanoparticles for Biofuels Production from Lignocellulosic Waste

Neha Srivastava, Manish Srivastava, P.K. Mishra, Pardeep Singh, Himanshu Pandey, and P.W. Ramteke

Abstract Lignocellulosic biomass is a sustainable alternative to current biofuels. The conversion of biomass-based sugars into biofuels, which emerged in 1970, is gaining more attention due to fossil fuel issues. Biohydrogen and bioethanol from cellulosic wastes is a sustainable and solves economic issues. This chapter reviews the use of nanoparticles for the bioconversion of biomass into biofuels.

Keywords Biofuels • Bioethanol • Biohydrogen • Cellulases • Lignocellulosic waste • Nanoparticles • Immobilization • Thermal stability

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9.1 Introduction

Biofuels are considered to be a potential option to replace fossil fuels for future scenario and are likely to be an advanced and novel research area to provide an alternative source in the form of renewable energy (Pandey et al. 2012; Srivastava and Jaiswal, 2016). Presently, Brazil and USA covers around 80% of global biofuels production and both of these countries produce mainly bioethanol (Hussein 2015). In the coming few decades, global demand for transport fuel is estimated to increase up to 55% by 2030 compared to 2004, and this will accelerate the demand of biofuels (Lund 2007; Srivastava et al. 2014a, b; Hussein 2015; <http://dektmk.org.tr/>). Numerous efforts are being made to enrich the cost-effective biofuels production, globally. From the point of cost economy, biofuels production is carried out using lignocellulosic biomass so that cost of the substrate can be reduced. Lignocellulosic biomass is the most potential and massive substrates found on earth for biofuels production (Srivastava et al. 2015b, c). Agriculture industries generate a vast amount of these biomasses as waste and after the commercial processing large residual biomass as waste is generally disposed-off (Sahaym and Norton 2008). Additionally, they compose high content of cellulose meanwhile, cheap and easily available raw materials for the biofuels production.

Besides biofuels production, lignocellulosics biomasses have potential, being convertible into the various values added products like chemicals (Chandra et al. 2010), cheap energy sources for the fermentation and improved animal feeds and nutrients. For the useful conversion of these biomasses into the biofuels, enzymatic hydrolysis is required, mainly. Cellulases are the key enzymes which are used for the bioconversion of cellulosics biomass in to useful products. It is well established that, in the process of biofuels production, cellulase production is the most expensive step and covers around 40% of the total cost of the overall process (Bhalla et al. 2013; Srivastava et al. 2015a). However, the main problems in the cellulase production using the lignocellulosics substrate are the insolubility, crystallinity and presence of lignin in the biomass (Sun and Cheng, 2002). Henceforth, treatments process facilitating disruption of lignin seal are supposed to increase the accessibility of cellulose to grow the cellulolytic organism. Apart from the pretreatments, the overall process of biofuels production is also affected by cellulase enzyme production efficiency, its enzymatic activity and stability of the enzyme at different pH and medium components, etc. (Yeoman et al. 2010).

Nanotechnology has gained much attention in the recent years because of wide range of engineering and technological applications (Hongliang et al. 2011; Singh et al. 2016). Nanomaterials have the potential for their commercial exploitation due to their versatile physicochemical properties. It is expected that the industrial market of nanomaterials will increase sharply in coming decade, and it is likely to increase from 2000 tons in 2004 to approximately 58,000 tons in 2011–2020 (Shi and Ma 2010, 2011; Olga et al. 2010). There is extensive use of nanoparticles in different fields including industries related to environmental remediation and biofuels production (Srivastava et al. 2014a, b).

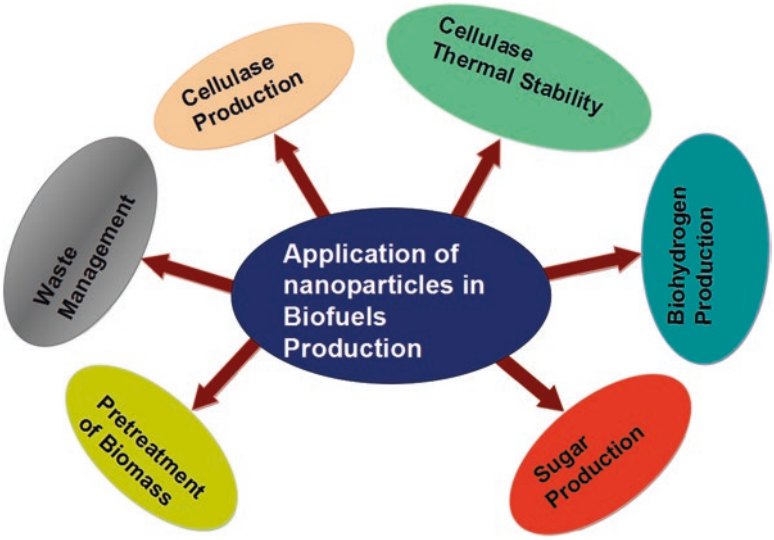

Fig. 9.1 Various applications of nanoparticles in biofuels production process

Nanomaterials can be used to improve the biofuels production; for example efficiency of biofuels such as biohydrogen/bioethanol can be enhanced using nanoparticles (Srivastava et al. 2015a). It has been reported that cellulase enzyme production, stability, and hydrolysis efficiency can also be improved using nanoparticles. Moreover, pretreatment of lignocellulosic biomass can also be improved in the presence of nanoparticles (Srivastava et al. 2015a). Figure 9.1 depicted various applications of nanoparticles in biofuels production process. Therefore, the present chapter deals with the application of nanoparticles in various biofuels production processes. Limitation of the process and possible approaches were also discussed.

9.2 From Biomass to Biofuel

Increasing rate of depletion of fossil resources and their harmful impact on the environment have necessitated the use of biomass derive compounds as a potential alternative to fossil fuels (Barnard et al. 2010; Barakat et al. 2012). Massive availability and high potential of these biomasses make them a suitable candidate for the production of fuels and chemicals (Alvira et al. 2010). The utilization of these biomass for the production of fuels has gained attention from the last decade, and till date more than 28,000 scientific research articles (according to the ISI Web of Knowledge database) along with 3,000 international patents (according to the Space net database) are available on this area, specifically (Chaker and Sillanpaa 2013). This enormous number of research publications and patent prove that the transformation of biomass into the biofuels is one of the most considerable areas for the research

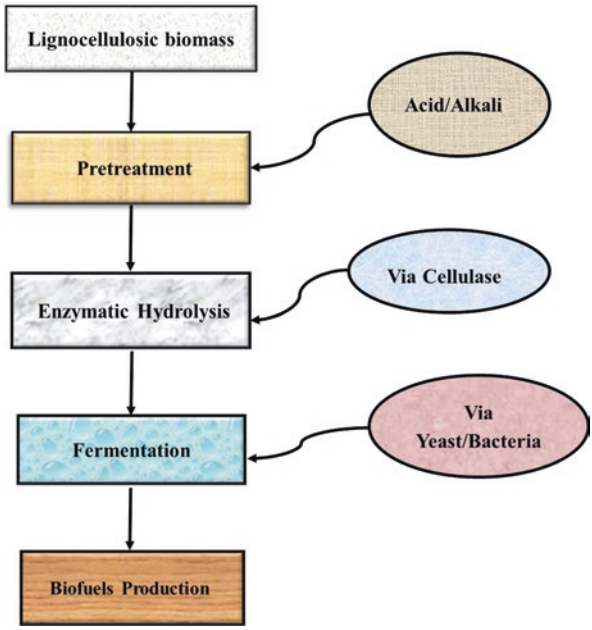

Fig. 9.2 Overview of biofuels production process from lignocellulosic biomass

community, and therefore, extensive efforts are being made for the enhancement of biofuels production.

Among, various approaches, application of nanomaterials is considered as one of the important approaches towards the improvement of biofuels production (Manzanera et al. 2008; Choedkiatsaku et al. 2011). In view of the facts as mentioned earlier, the aim of this chapter is to discuss the use of nanomaterials (nanoparticles/nanocomposite) for the enhancement of biofuels generation through different approaches (Kendry, 2002; Hahn et al. 2006), based on the available researches. The advantage to involve nanomaterials will give extra value to biofuel production process to become more sustainable by reducing the costs and positive environmental impacts. Figure 9.2 presents an overview of biofuels production process from lignocellulosic biomass. After the suitable pretreatment process the pretreated high cellulose content of biomass undergoes enzymatic hydrolysis using cellulase enzyme and the released sugar obtain from this enzyme hydrolysis are further process into bioethanol/biohydrogen via fermentative microbes through fermentation reaction.

9.3 Role of Nanoparticles in Biofuels Production

Nearly all biofuels production process suffers from certain limitations, due to which their commercialization process is hindered. The main challenges in the area of biofuels production are to develop such kind of biofuels which is highly efficient

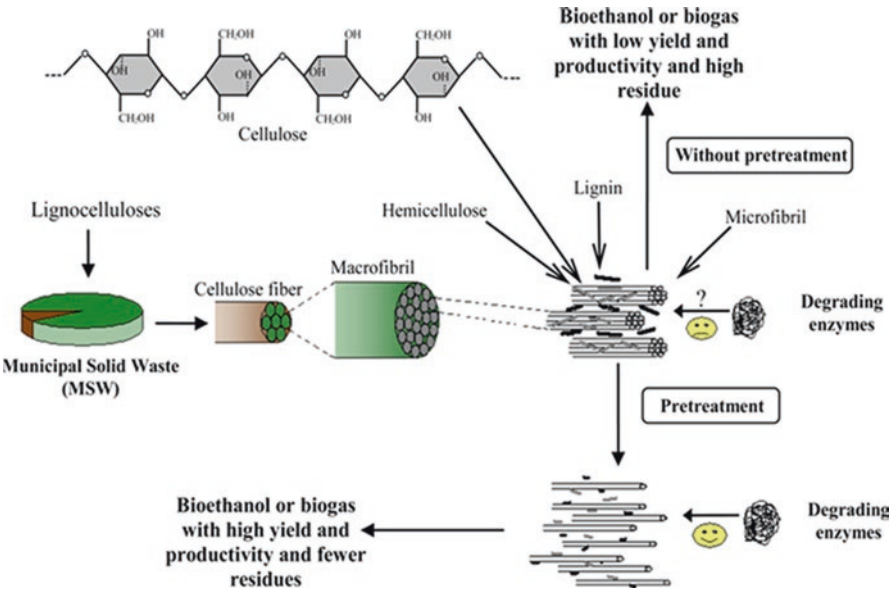

Fig. 9.3 Effect of pretreatment on accessibility of degrading enzymes. (Adopted from Taherzadeh and Karimi 2008)

regarding both cost effective and high energy yield. In this regard, utilization of nanoparticles may play a vital role in making this process economically feasible.

9.3.1 Application of Nanoparticles in Pretreatment of Lignocellulosic Biomass

Lignocellulose biomasses are the most abundant biopolymer having rich carbohydrate content. Cellulose and hemicellulose cover up to two-thirds of the lignocellulosic biomass and are the cheap sources for biofuels production (Hamelinck et al. 2005; Srivastava et al. 2014a, b; 2015a). For continuous assessment of these cellulosic and hemicellulosic fractions, pretreatments are being done earlier to enzymatic hydrolysis to open and released the lignin sheath (Alvira et al. 2010; Hendriks and Zeeman, 2009; Sun and Cheng, 2002). Different pretreatment methods are available for degradation of lignocellulosic biomass, among which chemical and biological pretreatment methods are more common (Cai et al. 2011; Chang and Yao 2011; Srivastava et al. 2015a).

Although pretreatment process is regarded as the steepest step in the conversion of cellulose, it has the potential to improve the efficiency significantly and reduce the overall production cost of the biofuels (Alvira et al. 2010; Srivastava et al. 2015a). Figure 9.3 shows the pretreatment technology involve in the production of

biofuels from the enzymatic hydrolysis of cellulosic biomass (Taherzadeh, et al. 2008). After the pretreatment, lignocellulose-deconstructing enzymes, cellulases and hemicellulases are used to release the fermentable sugars (Bhalla et al. 2013; Rawat et al. 2014). Therefore, more researches are focusing on enhancing the bioconversion efficiency of cellulosic biomass using the pretreatment process. In one of the very latest study by Wei et al. (2015) sugar production was observed to be enhanced in the presence of iron oxide nanoparticle by applying the acid pretreatment process on corn stover. Additionally, nanoparticle-acid pretreated substrate showed ~13–19% more glucose and xylose, when compared to control (acid pretreated substrate without nanoparticles). This study revealed that iron oxide nanoparticle can facilitate the pretreatment, and, therefore, experimental results showed better sugar production over the metallic iron. The experiments were carried out at 100 °C which suggests its suitability from the economical point of view, and the reported data reflected towards a positive correlation between the concentration of iron and released sugar during the pretreatment of biomass.

In one of the other study by Yang et al. (2015), hydrolysis of cellulosic materials were enhanced in the presence of reduced graphene oxide functionalized with Fe_3O_4 (magnetite) nanoparticles. The Fe_3O_4-reduced graphene oxide-SO_3H (Fe_3O_4-RGO-SO_3H) nanocomposite was successfully synthesized using the reduced graphene oxide (RGO), containing Fe_3O_4 nanoparticles and benzene sulfonic acid which were directly anchored on to the surface of reduced graphene oxide via C–C covalent bonds. The unique structure of Fe_3O_4-RGO-SO_3H nanocomposite along with high dispersion in water upholds the accessibility of cellulose to the active sites and improves the sugar production which can further be utilized for the biofuels production. Though these studies have opened a new avenue for the biofuels production using the nanomaterials, presently this area is at the very early stage. Therefore, much more efforts towards the economic viability are required.

9.3.2 Application of Nanoparticles in Cellulase Production and Stability

Lignocellulosic biomasses are used as the substrate for enzymatic hydrolysis via cellulase after the pretreatment process. For significant enzymatic hydrolysis, highly efficient cellulase is required, which do not lose its efficiency in harsh conditions. In addition, to different alternative approaches to enhancing the cellulase efficiency and its production industrially, uses of various cofactors such as metal ions have been reported in the number of studies (Srivastava et al. 2014a, b; Srivastava et al. 2015a). Nowadays, use of nanomaterials has emerged as a new area in the field of bioenergy generation to enhance the enzyme stability (Jordana et al. 2011; Srivastava et al. 2014a, b; Dutta et al. 2014; Singh et al. 2016). Very few but potential and promising studies have been reported in this area, recently. In the study by Dutta et al. (2014) an improved cellulase production was observed in the presence of hydroxyapatite nanoparticle using the bacterial strain. The enzyme used in this

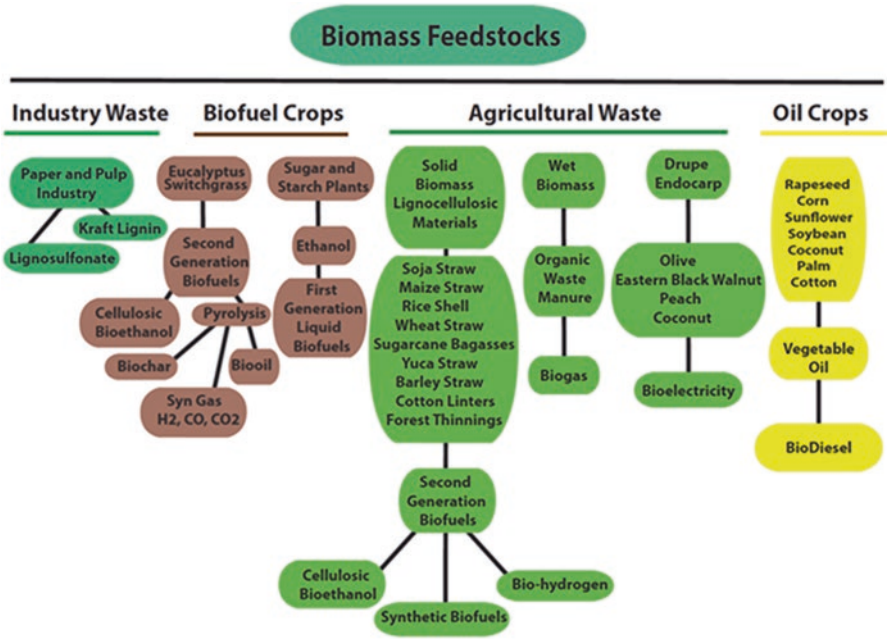

Fig. 9.4 Biomass feedstocks and their utilization in the production of biofuels, bioenergy and bioproducts. The figure includes the first- and second-generation feedstocks and their utilization for different bioproducts (liquid biofuels, biochar, bioelectricity and biogas). (Adopted from Welker et al. 2015)

study was highly thermostable and retained its half-life at 80 °C. Purified cellulase and xylanase enzymes obtained from bacteria were showed improved thermostability of ~35% in the presence of calcium hydroxyapatite nanoparticles.

Beside, enhanced thermal stability, an improvement in reducing sugars was also recorded by these authors using the rice husk and rice straw as substrates. Srivastava et al. (2015a), have reported, an improvement in the production of cellulase, its thermal stability and sugar productivity in the presence of Fe_3O_4/alginate nanocomposite. These authors have reported, a higher sugar productivity using thermotolarent fungal sp. *Aspergillus fumigatus* AA001 under the solid state fermentation in the presence of Fe_3O_4/alginate nanocomposite. Moreover, cellulase enzyme production along with its thermal stability was also improved in the presence of bare Fe_3O_4 nanoparticle as compared to control. The production of cellulase was increased by 35 and 40%, respectively in the presence of bare Fe_3O_4 nanoparticle and Fe_3O_4/alginate nanocomposite, when compared to control. Additionally, Fe_3O_4/alginate nanocomposite treated cellulase showed its thermal stability for 8 h at 70 °C by retaining its 56% of relative activity whereas; control (untreated cellulase) could retain only 19%. These studies clearly suggest that the nanoparticles may play a crucial role to alter the entire bioconversion process. Welker, et al. (2015) concluded in their study about the approximate availability of biomass and their conversion rate in biofuels production (Fig. 9.4).

It is expected that the immobilization of cellulase enzyme on to the nanoparticles was the main reason to enhance the thermal stability of cellulase enzyme. Additionally, an enhanced cellulase production, as well as thermal stability, has also been recorded in the presence of nickel cobaltite ($NiCo_2O_4$) nanoparticle via solid state fermentation by thermotolarent *Aspergillus fumigatus* NS (Class: Eurotiomycetes) (Srivastava et al. 2014a, b). An improvement of 40% was achieved in the production of cellulases whereas 49, 53 and 19.8% enhanced enzymatic activity was recorded in endoglucanases, β-glucosidase, and xylanase, respectively. Moreover, crude cellulase showed thermal stability for 7 h at 80 °C in the presence of $NiCo_2O_4$ nanoparticles, while control (untreated cellulase) was stable up to 4 h at the same temperature. Beside production of the enzyme, production time was also observed to be reduced in the presence of nanoparticles/nanocomposite in above studies. In this way, nanoparticles may prove their potential in biofuels production process in near future.

Besides above mentioned studies, there are other studies which have reported an improvement in cellulase production, thermostability as well as its hydrolysis efficiency in the occurrence of different types of materials for example iron oxide, zinc oxide nanoparticles etc. (Ansari and Husain 2012; Verma et al. 2013). In the study of Ansari and Husain (2012), the authors have discussed the immobilization of cellulase in the presence of iron oxide magnetic nanoparticles. These authors have discussed that the nanomaterials (nanoparticles/nanocomposite) may work as a carrier and provide not only the thermal stability but also develop the tolerance against the harsh pH and from inhibitor during the process of enzymatic hydrolysis. Verma et al. (2013) investigated that the thermostability of the β-glucosidase enzyme was improved in the presence of iron oxide magnetic nanoparticles and exhibited the half-life of the same enzyme at 70 °C. Figure 9.5 represents versatile use of nanomaterials and their influence to improve the biofuels production process. Though nanoparticles have opened the scope to improve the cellulase production and its thermal stability, their exact mechanism is still not well understood. Therefore, more study focusing on this particular area should be made for its economic and commercial viability at large scale.

9.3.3 Application of Nanoparticles in Hydrolysis of Lignocellulosic Biomass

Hydrolysis of lignocellulosic biomass to release sugars using cellulase enzyme is the next step after the pretreatment process. Enzymatic hydrolysis at temperature 45–50 °C makes the overall process slow, more prone to microbial contamination and often incomplete, resulting low yield of fermentable sugars and require higher enzyme loading. After the pretreatment step of lignocellulosic biomass, lignocellulosic degrading enzymes cellulases, apply to release reducing sugars via enzymatic hydrolysis Singhvi, et al. (2014) (Fig. 9.6). Generally, enzymatic hydrolysis of these

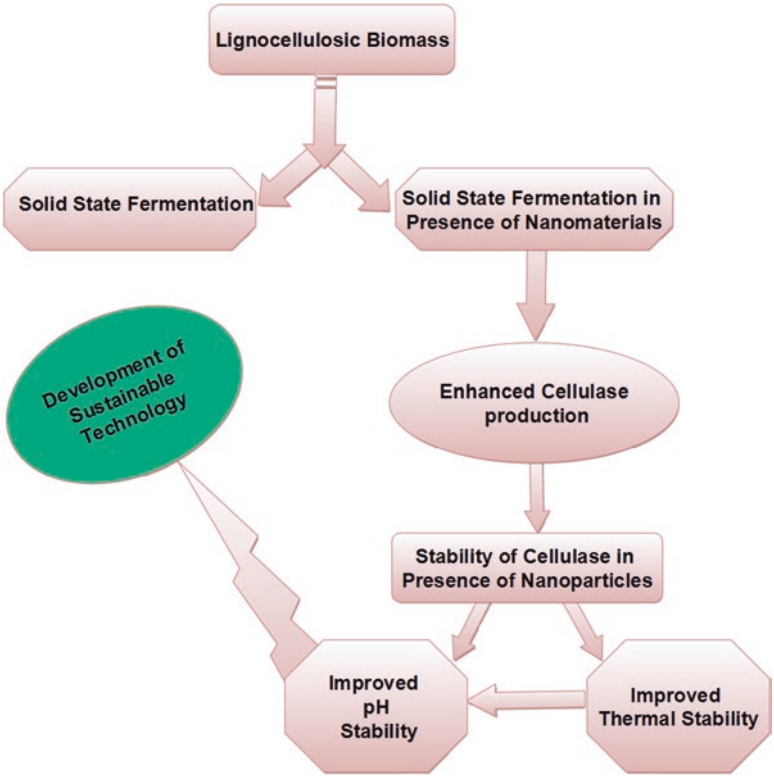

Fig. 9.5 Flow diagram shows effect of nanparticles on production and stability of celluases

biomasses is carried out at 45–50 °C, due to which hydrolysis rates becomes slow, low yields of sugars, incomplete hydrolysis, the need of high enzyme loading, and are very prone microbial contamination(Lu et al. 2006; Wang et al. 2010). Therefore, it has been recommended that these bottlenecks could be overcome using the thermophilic microorganisms and thermostable cellulase enzymes (Yeoman et al. 2010; Viikari et al. 2007). Cellulase which can withstand at higher temperatures can also sustain at elevated hydrolysis temperatures.

Thermal stability of cellulase can be improved in the presence of nanomaterials, and these thermostable cellulases can work efficiently at elevated hydrolysis temperatures. Some recent studies have reported high thermostability and hydrolysis efficiency at elevated temperatures (Dutta et al. 2014; Srivastava et al. 2015a). In the study by Dutta et al. (2014), an improved thermal stability of cellulase was gained with improved sugar production in enzymatic hydrolysis reaction, using the rice husk/rice straw as the substrates. In this study substrates were treated with xylanase and cellulase in the sequential order in the presence of calcium hydroxyapatite nanoparticles at 80 °C to obtain reducing sugars. When compared to control

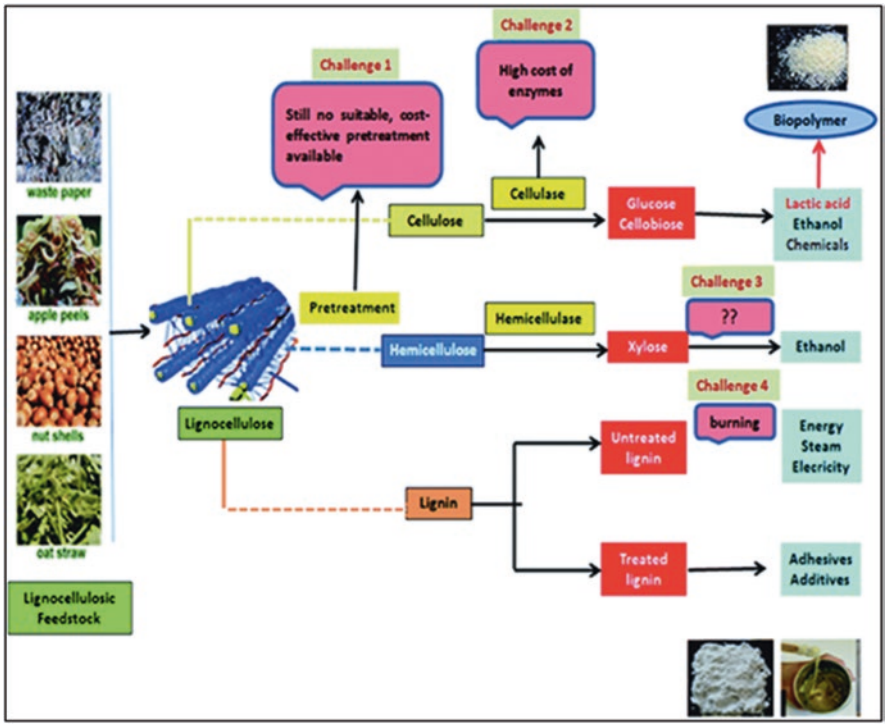

Fig. 9.6 The pretreatment and processing of biomass into fuels and chemicals. (Adopted from Singhvi et al. 2014)

(untreated cellulase), 5.71 fold higher D-xylose and 15 fold higher other reducing sugars were recorded in case of calcium hydroxyapatite nanoparticles treated cellulase and xylanase. In one of the recent study, by Srivastava et al. (2015c), an improvement in hydrolysis efficiency of cellulase and sugar productivity has been reported in the presence of Fe_3O_4/alginate nanocomposite at 70 °C. A higher sugar productivity using *Aspergillus fumigatus* AA001 under the solid state fermentation in the presence of Fe_3O_4/alginate nanocomposite was observed at elevated temperature. In the presence of Fe_3O_4/alginate nanocomposite, sugar productivity of cellulase was found to be 4.73 g/L/h whereas in case of untreated cellulase it was found to be 3.6 g/L/h. Although nanoparticles have tremendous potential to improve the hydrolysis efficiency of cellulase, their mechanism is not very clear; therefore emphasis should be done in this aspect for pilot scale study.

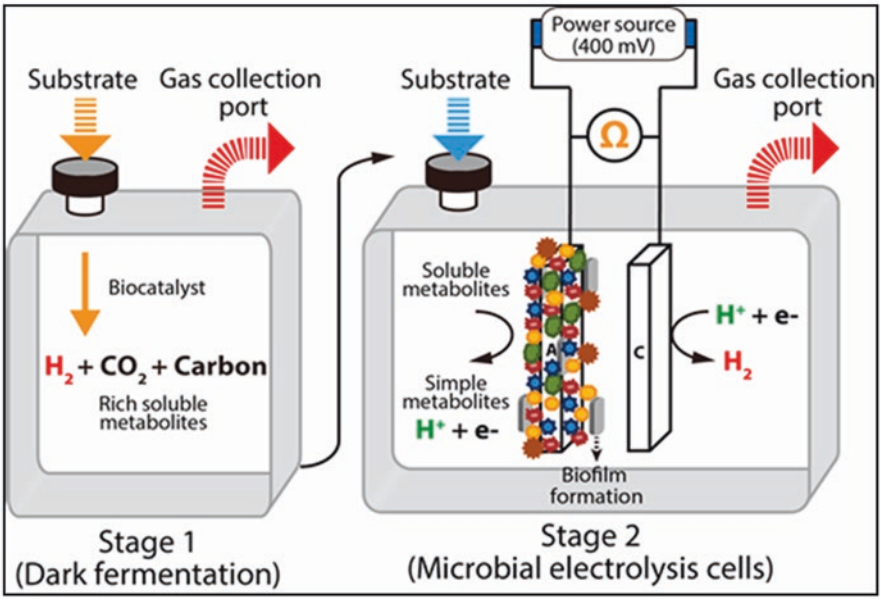

Fig. 9.7 Schematic illustration of microbial electrolysis cells (MECs) integrated with the dark fermentation process for higher H2 yield (A: anode; C: cathode; Biofilm: electrochemically active mixed microbial population). *Green, orange, brown*, and *blue* symbols represent a mixed microbial population. In stage 1, initially, complex substrates were used for H2 production in dark fermentation, and in stage 2, acid-rich effluents were used as substrates in MECs for further H2 production. http://dektmk.org.tr/. (Adopted from Chandrasekhar et al. 2015)

9.3.4 Application of Nanomaterials in Biohydrogen Production

The use of nanomaterials is also gaining momentum in the area of biohydrogen production (Ivanova et al. 2009; Zhang and Shen 2007). Biohydrogen production is very complicated process and depends on several factors such as nature of substrates, inorganic nutrients including metal ions, operational condition, etc. (Hallenbeck, 2005; Ferchichi et al. 2005; Wang and Wan 2008, 2009). For improved biohydrogen production it will be better if the appropriate experimental design is used to evaluate the effects of different factors on the process as suggested in the study of Chandrasekhar, et al. (2015) (Fig. 9.7). Apart from the experimental design, microorganisms play a significant role in biohydrogen production process. It has been predicted that microorganisms may be benefitted by nanoparticles in anaerobic conditions because electron transfer is more convenient to acceptors (Beckers et al. 2013). Use of some nanoparticles can improve the kinetics of bioprocess due the better competence to react fast with electron donors for improving the capacity of microorganisms as the biocatalysts (Xu et al. 2012). In the study by (Zhang and Shen 2007; Zhao et al. 2013), it has been reported that the addition of gold (Au)

Table 9.1 Summarizes the feasible applications of nanomaterials

Biofuels area	Feasible application of nanomaterials
Pretreatment of cellulosic biomass	Can improve the release of lignin and hemicellulose structure
Cellulase enzyme	Can improve the production, thermal and pH stability
Biohydrogen production	Can improve the production & stability of hydrogenase and nitrogenase enzyme
Bioethanol Production	Can improve the production and tolerance of microorganisms

nanoparticle (5 nm) can significantly improve the efficiency of biohydrogen fermentation. Moreover, in presence of gold (Au) nanoparticles, the hydrogen yield was increased up to 36.3% compared to the control.

Beside gold (Au), silver (Ag) nanoparticles may also improve the conversion efficiency of the substrate to biohydrogen via the fermentative route. In the study by Han et al. (2011), enhancement of biohydrogen production was observed in the presence of hematite nanoparticles. These authors have concluded that the immobilization of bacterial cells on the nanoparticles could be the reason for enhanced biohydrogen production. In one of the studies by Lower et al. (2001), strong affinity was identified in the range of few hundred nanometers distance between *Shewanella oneidensis*, (a dissimilatory metal-reducing bacterium) and goethite (α-FeOOH), which is increased by two to five times in anaerobic condition. It is concluded that though, number of efforts have been made to improve the biological hydrogen production using the variety of nanomaterials but, as compared to other biofuels production option this area of research is still in struggling phase for bench to pilot plant study. Additionally, more steps are required towards the large scale production of biohydrogen (fermentative or photofermentative) through the nanomaterials based approach (Table 9.1).

9.4 Future Prospects

Nanomaterials can play a significant role for the qualitative and quantitative production of biofuels. Various types of nanomaterials may alter the production process of biofuels via different ways such as improvement in stability of cellulases enzymes, improvement in chemical and biological digestion of cellulosic biomass and by enhancing the catalytic production of biohydrogen. However, different nanomaterial may influence the process due to distinct catalytic activity offered by their size, shape and structural morphology, and therefore, these characteristics of nanomaterial should be complimentary to the concerning process. Moreover, molecular investigations must be helpful to understand the basic mechanism that how nanomaterials are responsible for altering the production process and stability of related protein. Beside this, the cost involve in the synthesis of nanomaterial can also alter the overall production process of biofuels and therefore, for the cost effective

biofuels production, these points should be addressed. The cost effective synthesis of nanomaterial may make the overall biofuels production process, economically viable. Briefly, it is concluded for future strategies that this area is at the very early stage and several challenges are need to be overcome; (i)attention should be given to the synthesis of nanomaterials having controlled catalytic properties which should help to improve the production process, (ii) cost of the nanoparticles (iii) Compactibility rate among microorganism, involve enzymes and nanomaterials (iv) understanding of the mechanism of the interaction between the protein and the nanomaterials at the molecular level.

9.5 Conclusion

We reviewed the application of nanomaterials in the area of renewable energy. It is concluded that the nanomaterials can play a significant role and potentially contribute in this area by influencing various factors. Lignocellulosic biomass degradation, sugar production, improvement in thermal stability and hydrolysis efficiency of cellulase enzymes are the factors which have shown improvement in the presence of nanomaterials. Use of these nanomaterials in the production of biofuels may support to provide a cheap & clean energy source in near future and will become a strong global industry. Although biofuels industries can be benefited by using the nanoparticles in the process of biofuels production, much research is required to be the focus in this field. Besides, the number of published research articles, particularly in this field, is very limited. Therefore, more and focused study on the effect of nanoparticles on biofuels production is needed which may play a critical role towards the commercialization of biofuels.

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Chapter 10
Iron Oxide Nanoparticles to Remove Arsenic from Water

Prabhat Parida, Mayura Lolage, Ashwini Angal, and Debabrata Rautaray

Abstract Arsenic contamination in water is a widespread problem globally. Millions of people depend on arsenic-contaminated groundwater. Arsenic poisoning leads to fatal diseases such as skin and internal cancers. Hence, the current regulation of drinking water standard has become more stringent and requires arsenic content to be reduced to a few parts per billion. Therefore, effective and inexpensive technologies for arsenic removal are needed. Majority of communities affected by arsenic contamination could not justify the cost and maintenance of installing centralized arsenic treatment systems. Thus, there is a need to develop point-of-use water treatment devices. Here we review arsenic contamination, it's health effects, and available removal technologies. We then describe the development of a working prototype cartridge to remove arsenic from drinking water that meets international standard norms. For that we synthesized iron oxide nanoparticles using a chitosan biopolymer. Iron oxide originated from steel waste. Granules were thereafter packed in a column and evaluated for arsenic removal efficiency using simulated ground water compositions.

Keywords Arsenic • Arsenic removal technologies • Arsenic adsorption • Arsenic remediation • Water purification • Iron oxide • Drinking water • Point-of-use water treatment • Ground water contamination • Arsenic toxicology

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10.1 Introduction

Arsenic in drinking water is a major problem and the health effects have been observed in populations drinking arsenic-rich water over long periods of time in countries world-wide (Smith et al. 2000). Long-term exposure to arsenic via drinking-water causes cancer of the skin, lungs, urinary bladder and kidney infections. The toxic and carcinogenic effects of arsenic on living beings are well documented (Tchounwou et al. 2003). Arsenic is an odourless, colourless and tasteless semi metallic element, which is a naturally occurring element in the environment. Arsenic is introduced in the drinking water sources through geochemical reactions, industrial waste discharges, or agricultural use of arsenical pesticides.

Arsenic in soil or ground water is usually present either in the form of arsenite with +3 charges, or arsenate with a + 5 charges. Both arsenate (As–V) and arsenite (As–III) tend to combine with multiple oxygen atoms, each having −2 charges. Arsenite and arsenate compound in dissolved form tend to have an overall negative charge. Arsenite exists under anaerobic conditions, for example in water-logged soils. Under more oxidizing conditions, arsenite converts to arsenate. Arsenate naturally sorbs to soil minerals, particularly iron oxides and hydroxides. Arsenite tends to sorb less strongly than arsenate (Spayd et al. 2012). Arsenate is most common in surface waters while arsenite is more common in ground waters (Oremland and Stolz 2005). Arsenite is 10–60 times more toxic than arsenate. Concentrations of arsenite are particularly significant from a human health perspective, although arsenic levels are typically reported as total arsenic (Vu et al. 2003).

10.1.1 Arsenic Contamination of Ground Water

Groundwater resources have been the main source of exposure for millions of people in developing nations to high levels of inorganic arsenic (Nickson et al. 1998; Shankar et al. 2014). Although arsenic levels in natural waters do not usually exceed several parts per billion (ppb), drinking water in many regions of the world contain concentrations of total arsenic in excess of 100 ppb. World health organization (WHO) and U.S. environmental protection agency (USEPA) have recently revised the maximum contaminant level in drinking from 50 ppb to 10 ppb. If other safe water sources are unavailable, removal technologies are usually applied to lower arsenic concentration to the prevailing regulatory standard or to a level that poses the minimal threat to human health (Jadhav et al. 2015).

Arsenic contamination of drinking water poses a much greater challenge for developing nations. This is because water treatment required for arsenic mitigation often involves technologies that are relatively complex and costly. This problem is intensified in rural areas where access to safe drinking water is already limited. Arsenic being colourless and odourless poses a problem for rural communities with a perception that water is clean when it does not have any visible contaminants

(Smith et al. 2000). Another contributing factor to the large arsenic contamination problem for developing nations is the lack of proper nutrition. Those communities that are excessively poor and malnourished tend to experience more symptoms of arsenic poisoning such as arsenicosis and are more susceptible to develop chronic internal cancers (Saha et al. 1999).

10.1.2 Health Effect due to Exposure to Arsenic

Inorganic arsenic is considered the most potential human carcinogen and humans are exposed to it from soil, water, air and food. Chronic toxicity is observed from exposure to drinking water that contains ppb levels of inorganic arsenic. Acute poisoning has many metabolic effects, including stomach pain, diarrhoea, vomiting, bloody urine, anuria, shock, convulsions, coma and death (Singh et al. 2015). The consequences of exposure to arsenic for human health are potentially grave and may extend from general malaise to death. Chronic and systemic exposure to arsenic can lead to serious disorders, such as vascular diseases (black foot disease and hypertension) and irritations of the skin and mucous membranes as well as dermatitis, keratosis, and melanosis. The clinical manifestations of chronic arsenic intoxication are referred to as arsenicosis (hyperpigmentation and keratosis). It is, therefore, important to measure the arsenic levels in water resources (i.e. groundwater and surface water), in order to identify potential problems before they give rise to adverse health effects within the population (Sharma and Sohn 2009). Additional health effect of arsenic includes cancer of the bladder, lungs, skin, kidney, liver, prostrate and nasal passage (Yoshida et al. 2004 and Saha et al. 1999). Arsenic also harms the central and peripheral nervous systems and causes several skin disorders. Absorption of arsenic through the skin is minimal; therefore, hand washing or bathing with arsenic contaminated water does not pose human health risks.

10.1.3 Arsenic Removal Technologies

There are currently no simple and inexpensive effective technologies to mitigate such problems and as a result, arsenic mitigation approaches vary greatly from large, developed cities served by centralized water supplies to small rural communities in developing nations. There are number of arsenic removal methods that are suitable for the treatment of drinking water including anion exchange resins, porous ceramics, activated alumina and iron flocculation processes (Mohan and Pittman 2007 and Ramos et al. 2014 and Zaspalis et al. 2007). Conventional polymeric anion exchangers have a low selectivity for arsenate and at the same time the high concentration of sulfate in drinking water successfully competes with arsenate for available anion exchange sites resulting in a short operational life (Meng et al. 2000). While on the other hand, coagulation using iron flocculants as a treatment

method is highly effective in removing arsenic from water but generates large amounts of a ferric hydroxide flocculants which will require safe disposal in a landfill. Therefore, capital equipment requirements are generally high (Vaclavikova et al. 2008 and Tara et al. 2013).

Among various agents used in the purification and treatment of arsenic rich water, iron-based materials have gathered specific attention in view of their special qualities such as their earth-abundant and environmentally friendly nature (Thomas 2015). Water treatment method involving the use of low cost natural iron oxide minerals such as laterite for arsenic removal to meet drinking water standards has been well documented in the literature (Aredes et al. 2012). Various studies revealed that arsenate adsorption is related to the iron content of adsorbents, and adsorption rate increases in the following order: goethite < hematite < magnetite < zero valent iron (Pajany et al. 2011). It is also shown through desorption experiment; arsenic is strongly adsorbed onto hematite and zero valent iron. Among adsorbents, hematite appears to be better for removing arsenate in natural medium since it is effective over large ranges of pH and arsenic concentration (Pajany et al. 2009, 2011 and Grafe et al. 2001). Liu et al. have prepared iron oxide impregnated chitosan bead(s) using reverse phase suspension method to remove As (III) from water (Liu et al. 2010). Jiang et al. have shown chitosan-coated sand and iron–chitosan-coated sand for the removal of both As (V) and As (III) from aqueous systems. Various parameters including pH, equilibration time, initial arsenic concentration and adsorbent dosage have been optimized for maximum adsorption of arsenic (Jiang et al. 2013). Oxidation of As (III) to As (V) is also needed for effective removal of arsenic from groundwater by most treatment methods. Thus there is a need for a water purification composition which is effective in removing both arsenic species [As (V) and As (III)] from water. Additionally, the cost effectiveness of the arsenic purification system and safe disposal of the spent media are extremely important.

10.1.4 Use of Nanotechnology for Arsenic Removal

Properties of nanoparticles are exploited in fields such as catalysis, separation, sensor, biological, molecular isolation, chemical and physical adsorption applications. Recently nanoparticles are being increasingly used in adsorption processes for water purification as well mainly due to their available active high surface area and unusual unique properties. For example, both As (V) and As (III) can strongly adsorb onto metal oxide nanoparticles with high sorption capacities. A number of reports have shown that nano-sized sorbents such as elemental iron, titanium oxide and iron oxide are more effective compared to macro-sized particles (Mostafa and Hoinkis 2012). The high surface area to mass ratio, high surface reactivity and unique catalytic activity are the most important properties of a nanomaterial and led to increased efficiency as an adsorbent compare to macro-sized of the material. Nanoparticles of metal oxides such as Fe_2O_3, Fe_3O_4, TiO_2, Al_2O_3 etc. among others are promising for their large surface area and porous structure and have been used in water and wastewater purification processes as adsorbents (Mostafa and Hoinkis 2012).

Other nanostructures such as three-dimensional graphene-carbon nanotube-iron oxide nanostructures have been shown for absorption for arsenic from contaminated water, due to its high surface-to-volume ratio and the open pore network of graphene-carbon nanotube-iron oxide 3D nanostructures (Vadahanambi et al. 2013). CuO nanoparticles are shown to have effectively removing arsenic from groundwater. It was shown effectively removed both As (III) and As (V) between pH 6 and 10. In this study, the presence of sulfate and silicate in water did not inhibit adsorption of As (V) but only slightly inhibited adsorption of As (III) (Martinson and Reddy 2009). Multi-walled boron nitride nanotubes functionalized with Fe_3O_4 nanoparticles were also used for arsenic removal from water. The magnetite nanoparticles functionalized on multiwall boron nitride nanotubes led to a simple and rapid separation of magnetic metal-loaded adsorbents from the treated water under an external magnetic field (Chena et al. 2011).The Fe–Cu binary nano-oxide could also be a promising adsorbent for both As (V) and As (III) removal because of its excellent performance, facile and low-cost synthesis process, and easy regeneration (Zhanga et al. 2013). Magnetic nanoparticles modified simultaneously with amorphous Fe and Mn oxides were shown also to remove arsenite from water (Shan and Tong 2013).

10.1.5 Advantages of Nanostructured Iron Oxide for Arsenic Removal

Nanostructured iron oxide adsorbent used for the removal of arsenic uses the combined catalytic and adsorptive properties of iron oxide to breakdown arsenic into less toxic by-products while facilitating the filtration of these by-products out of the groundwater (Sylvester et al. 2007 and Mayo et al. 2007). Nanophase Fe_3O_4 and Fe_2O_3 were synthesized through precipitation method and were utilized for the removal of either arsenic (III) or (V) from aqueous solution as a possible method for drinking water treatment (Luther et al. 2012; Sharma et al. 2015). Alpha-Fe_2O_3 nanowires deposited onto diatomite was shown to remove efficiently As (III) and As (V). Parameters, such as adsorbent quantity, adsorption time, adsorption temperature, pH value, and initial As (III) or As (V) concentration, was shown to influence the As (III) or As (V) removal efficiency of the alpha Fe_2O_3 nanowires/diatomite sample (Du et al. 2013). Super paramagnetic iron oxide nanoparticles surface-coated with 3-mercaptopropanoic acid having an attribute of increased active adsorption sites were shown to remove arsenate from drinking water (Morilloa et al. 2015). Polymeric beads containing hydrous iron oxide nanoparticles (25% dry weight) was shown to effectively remove As (III) (Gang et al. 2010 and Katsoyiannis and Zouboulis 2003). Abid et al. have demonstrated iron oxide nanoparticles synthesized in large scale through gas-phase diffusion flame synthesis having smaller particle diameter and large surface area. These flame synthesised iron oxide nanoparticles has been shown significantly effective in removal of As (V) from water. Furthermore, the author have shown that by controlling Fe(III)/Fe(II) ratio,

the arsenic adsorption capacity can be increased multi-fold (Abid et al. 2013). Liu et al. have explained the probable mechanism that involves in the adsorption of arsenic onto iron oxide. As (III) and As (V) both form bidentate, bimolecular surface complexes with FeOH (or FeOOH or hydrous ferric oxide), as the primary species tightly immobilized on the iron surface. As (V) oxyanions (chemical moiety) are attracted to the iron-oxide-coated surfaces and bound with the active sites (A-OH groups), through weak intermolecular hydrogen bonding. Finally they are bound with the surfaces, eliminating water molecules (Maji et al. 2012). The primary factor make iron based adsorptive media treatment is attractive due to the fact that the system is low-cost and simple to operate. The affinity of iron media for arsenic is strong under natural pH conditions (Maji et al. 2012). This feature allows iron based sorbents to treat more bed volumes without the need for pH adjustment. Iron oxide (nano) systems are known to provide long operating cycles and low operating costs. Life expectancy is dependent on site-specific water quality and operating levels. Exhausted media is non-hazardous and can be disposed of using conventional methods.

10.1.6 Point of Use Water Purification

Point of use water purification devices are appropriate for removing contaminants that pose only an ingestion risk, as is the case with arsenic. Since only a small fraction of the total water supplied to a given household is ultimately treated and consumed, only that small fraction (the water intended solely for consumption-drinking and cooking) needs to be treated in order to reduce the risk. The primary advantage of using point of use treatment in a small system is a reduction in capital and treatment costs, relative to installing centralized treatment. Here, we demonstrate a working prototype cartridge that removes arsenic from water (without any side effects such as leaching) that meets appropriate standard protocols. In order to achieve this, we have used iron oxide nano powders obtained from a waste generated from a steel manufacturing plant. We have evaluated iron oxide fine powder for their chemical and physical properties, formulated an efficient composition, worked on a form-factor, customized a testing protocol plan against arsenic and studied the arsenic removal efficacy over a life 1000 l of water.

10.2 Experimental Section

The source of iron oxide fine powders used for the purpose of arsenic removal is obtained from a steel manufacturing plant (where it is generated as a waste), which also poses disposal problem in an eco-friendly manner. The iron oxide powder was thoroughly characterized using various techniques such as X-ray fluorescence (XRF) spectroscopy for compositional analysis, X-ray diffraction (XRD) for crystal

structure determination, thermo gravimetric analysis (TGA) for quantification, Brunauer–Emmett–Teller (BET) for surface area measurement, particle size measurement through dynamic light scattering method (PSD), Zeta potential for surface charge estimation and scanning electron microscopy (SEM) for particle size and morphology studies.

10.2.1 Formulation: Preparation of Granule of Chitosan Coated Iron Oxide Particles

About 500 g of citric acid is dissolved in 10 l of distilled water by stirring for 15 min in a vessel to obtain a solution. 200 g of chitosan is added to above solution under constant stirring for 1 h to facilitate mixing of chitosan thoroughly in the solution. 2000 g of iron oxide fine powder is added slowly under stirring to the solution obtained from the previous step followed by stirring for 1 h to facilitate mixing of iron oxide particles properly. 2 l of 10% (w/v) sodium hydroxide solution is added to the solution obtained from the previous step to facilitate precipitation of iron-chitosan matrix with the help of a master flex pump, such that the rate of addition of sodium hydroxide is 100 ml/min. Addition of sodium hydroxide solution is followed by stirring for 1 h. After said stirring, the contents of the vessel are transferred to a beaker of 10 l capacity. The precipitate is allowed to settle down. Separate the precipitate by decanting water from the beaker. The precipitate is then washed by adding 2 l of distilled water to the beaker followed by stirring for 5 min. The precipitate is then allowed to settle down and separated from the beaker again by decanting water from the beaker. The precipitate is washed again by repeating the step of washing. After washing the precipitate twice, the precipitate is transferred to a drying tray for drying at a temperature of 90–100 °C. Drying is carried out in a drying oven. The precipitate is removed from the oven while having 20–25% of moisture. This is then subjected to mild grinding to facilitate breaking of lumps followed by further drying at a temperature of 90–100 °C. The dried precipitate thus obtained is subjected to sieving through a nylon net having pore size of approximately 1 mm. The precipitate remaining on the top of the net is milled to facilitate breaking. The dried precipitate which passes through the net is collected and sieved through a mesh of pore size 150 μm to obtain granules. The granules are collected on the top of the mesh. The granules have a size in the range of 0.15–1 mm.

10.2.2 Prototype Designing to House Iron Oxide Granules for Arsenic Removal from Water

Iron oxide granules are accommodated in a cylindrical column with a screen at the bottom to protect iron fine leaching (Fig. 10.1). Appropriate flow rate is maintained using a knob attached outside the column. Photograph below illustrates the details

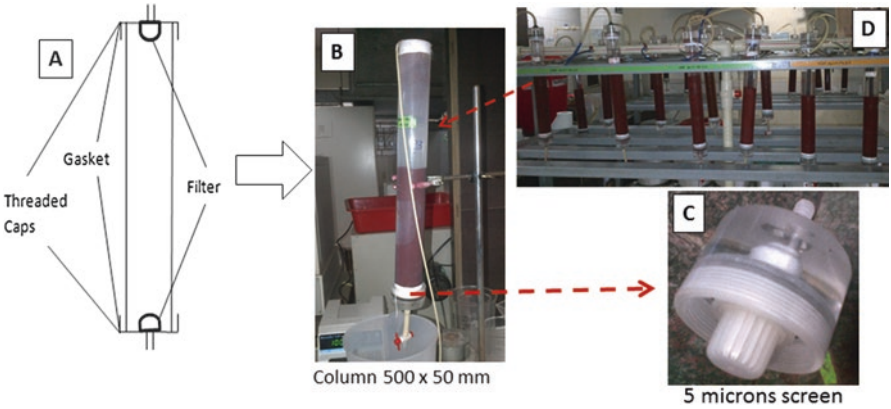

Fig. 10.1 (**a**) Basic drawing of the fabricated column; (**b**) Photograph of a cylindrical column packed with Iron oxide granules; (**c**) Photograph of a specially designed screen of 5 microns to restrict iron oxide leaching; and (**d**) Photograph of a water testing platform to evaluate performance of arsenic removal filters

of the column fabricated to house the iron oxide granules. The cylindrical column used is 500 mm in height and 50 mm in diameter and is fabricated with a distinctively designed screen of pore size ~5 microns. The screen is designed in a manner that restricts granules or iron fines to escape into the output water. The special design of the screen also helps overcoming any choking of the filter over its life. The columns are packed with 200 g of iron oxide granules containing granule size ranging 150 microns to 1 mm size.

Once granules are packed, approximately ~4 l of reverse osmosis (RO) water is passed through the granules to remove fine particles of iron oxide (if any) that passes through the screen. Washing is continued until clear water is obtained in the output water. Thereafter, washed packed columns are installed in the testing lines (Fig. 10.1d) to conduct trials as per standard testing protocols.

10.2.3 Evaluation Protocol for Arsenic Removal from Water: Guidelines for Water Purifier Against Arsenic (III and V –Influent and Effluent)

Based on our studies of various protocols [NSF/ANSI 53–2011, US-EPA and BIS –IS3025 (Part 37)], we have designed a simulated ground water conditions for input challenge water for spiking and quality assurance of output water. US-EPA and NSF have marginally different protocols for arsenic. However, NSF international body has prepared the US-EPA protocol for arsenic removal from water. NSF has also created a separate protocol for point of use unit for arsenic removal. Both the protocols says different ways of treating Arsenic III and Arsenic V species, however the

Table 10.1 Composition of input challenge test water for 50 ppb arsenic (III)

Source	Parameter	Addition of salt (mg/l)
Sodium silicate ($Na_2SiO_3.9H_2O$)	(silica) 20 mg/l	93 mg/l
Sodium nitrate ($NaNO_3$)	(Nitrate) 2.0 mg/l	12 mg/l
Magnesium sulphate ($MgSO_4.7H_2O$)	(magnesium) 12 mg/l	128 mg/l
Sodium fluoride (NaF)	(fluoride) 1 mg/l	2.2 mg/l
Sodium phosphate ($NaH_2PO_4.H_2O$)	(Phosphate) 0.04 mg/l	0.18 mg/l
Calcium chloride ($CaCl_2$)	(calcium) 40 mg/l	111 mg/l
Sodium bicarbonate($NaHCO_3$)	–	250 mg/l
Sodium arsenite ($NaAsO_2$.)	As(III) = 0.050 mg/l	0.087 mg/l
Temperature	20 °C ± 2.5 °C	–
Turbidity	< 1 Nephelometric turbidity unit (NTU)	–
pH	6.5 ± 0.25 and 8.5 ± 0.25	–
Dissolved Oxygen	<0.5 mg/l	–

input and output parameters have many similarities. Tables 10.1 and 10.2 below show the composition of input challenge test water used for arsenic (III and V) removal from the iron oxide granule filter.

As per protocol, to qualify for an arsenic reduction claim, a water treatment system shall pass the test for pentavalent arsenic reduction and shall pass a separate test for trivalent arsenic reduction at two different pH conditions (6.5 and 8.5). Claims may be made for pentavalent only and for arsenic reduction (As-III as well as As-V). A claim for only trivalent arsenic reduction shall not be made.

10.2.4 Toxicity Characteristic Leaching Procedure (TCLP)

This test is performed as per TCLP protocol US-EPA method 1311 to check the acid neutralization capacity of the experimental waste (arsenic loaded iron oxide media from the spent filter). Those with low acid neutralization capacity are extracted with TCLP solution no.1 (i.e. 0.1 M sodium acetate buffer, pH 4.93 ± 0.05) and those with high acid neutralization capacity are extracted with TCLP solution no.2 (i.e. 0.1 M acetic acid, pH 2.88 ± 0.05).

100 g of the dry media (arsenic-iron oxide-waste from the spent filter) was taken by sieving through a 9.5 mm sieve and transfer to an extraction bottle. Add 2 l of TCLP solution (No. 1 or 2 as determined by preliminary evaluation) and close the extraction bottle. Rotate the extraction bottle in an agitating apparatus at 30 rpm for 20 h at a temperature of 23 ± 2 °C. After completion of the agitation, filter it through a glass fibre filter (0.6–0.8 micrometre pore size). Collect the filtrate and record its pH. Take 3 aliquot samples from the filtrate for the determination of arsenic concentration. Immediately acidify each aliquot samples with nitric acid to a pH little less

Table 10.2 Composition of input challenge test water for 150 ppb arsenic (V)

Source	Parameter	Addition of salt (mg/l)
Sodium silicate ($Na_2SiO_3.9H_2O$)	(Silica) 20 mg/l	93 mg/l
Sodium nitrate (NaNO3)	(Nitrate) 2.0 mg/l	12 mg/l
Magnesium sulphate ($MgSO_4.7H_2O$)	(Magnesium) 12 mg/l	128 mg/l
Sodium fluoride (NaF)	(Fluoride) 1 mg/l	2.2 mg/l
Sodium phosphate ($NaH_2PO_4.H_2O$)	(Phosphate) 0.04 mg/l	0.18 mg/l
Calcium chloride ($CaCl_2$)	(Calcium) 40 mg/l	111 mg/l
Sodium hypochlorite (NaCIO)	(Free available chlorine) 0.5 mg/l	0.125 ml/l
Sodium bicarbonate($NaHCO_3$)	–	250 mg/l
Sodium arsenate ($NaHAsO_4.7H_2O$)	0.150 mg/l	0.54
Temperature	20 °C ± 2.5 °C	–
Turbidity	<1 Nephelometric turbidity unit (NTU)	–
pH	6.5 ± 0.25 and 8.5 ± 0.25	–

than 2. Analysis of arsenic in aliquot and filtrate is done by inductively coupled plasma (ICP-MS) spectroscopy measurements.

Arsenic concentration in the extracting solution during testing must be less than 5 mg/l in order to be considered a non-hazardous waste and safe for municipal solid waste landfill disposal.

10.3 Results and Discussion

The source of iron oxide fine powders used here for the purpose of arsenic removal is generated as a waste from steel manufacturing plant which also poses disposal problem in an eco-friendly manner. However, the use of fine powder of iron oxide for water filtration application could pose following difficulties such as, (a) flow water through a device that is densely packed with fine powders, (b) combining other porous medium like sand or carbon in conjunction with iron oxide powder will lead to less amount of active medium (iron oxide fines) thus, limiting the arsenic adsorption capacity for long term performance. Iron oxide powder used for the current application is characterized as shown in Table 10.3.

It is found that in the iron oxide obtained from the steel plant waste composed of 90–95% of iron oxide with a BET surface area of more than 50 m2/g and the particles are found to be ranging from nano size to submicron size (Table 10.3). Characterization of iron oxide particle using XRD reveals the x-ray peaks matching with standard diffraction pattern for alpha-Fe_2O_3 (Fig. 10.2).

Dynamic light scattering method was employed to measure the particle size distribution of iron oxide powder (Fig. 10.3a). The particle size analysis done in a

Table 10.3 Basic characterization of iron oxide powder

% Iron oxide (XRF)	Surface area (BET)	Tap density	Particle Size Distribution (DLS)
90–95% Iron oxide	56 m2/g	0.9 cc/g	200 to >1000 nm

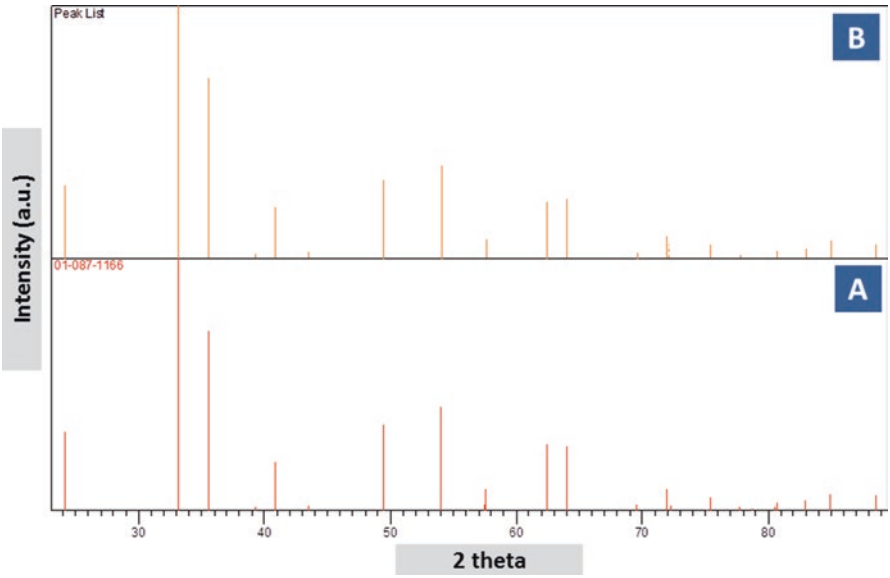

Fig. 10.2 X-ray diffraction measurement. (**a**) Standard diffraction pattern for alpha-Fe2O3 and (**b**) diffraction pattern of iron oxide powder obtained from a steel manufacturing waste

Malvern DLS instrument by dispersing 0.5% of iron oxide powder in distilled water and ultrasonicated for 5 min. Particle size distribution of iron oxide is found to be in the range of 200 nm to >1000 nm. Additionally, zeta potential measurement of iron oxide powder was done (Fig. 10.3b) in order to study the surface charge present on the particle. Zeta potential of 48.7 mV (±7.7) indicates high positive charge on the particle surface.

10.3.1 Details of Iron Oxide Granule Formation and Characterization

The use of fine powder of iron oxide can restrict arsenic removal application due to the fact that it is difficult to flow arsenic rich water through a device that is densely packed with fine powders. Therefore, to address this issue, a novel methodology was developed to synthesize granules of iron oxide fine powders using a biopolymer

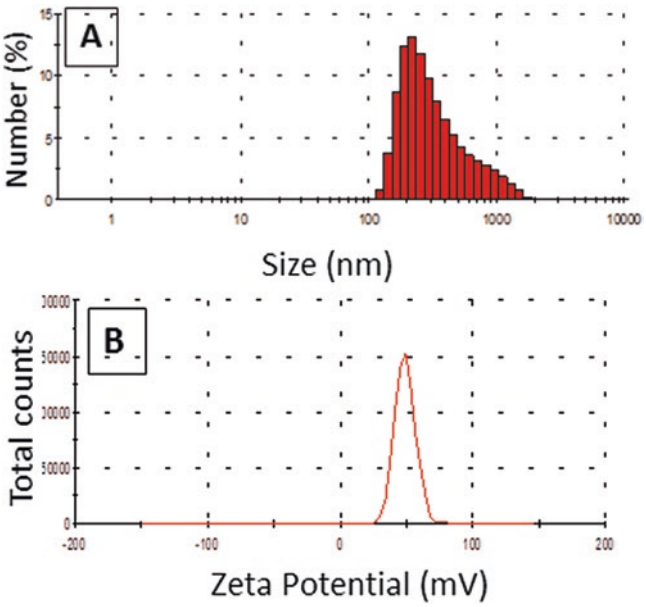

Fig. 10.3 (**a**) Particle size distribution of iron oxide particles; (**b**) Zeta potential measurement of iron oxide particles

–chitosan. The method of granule preparation is explained in the experimental section which follows various steps of first mixing iron oxide powders in chitosan solution dissolved in acidic pH water followed by polymerization of chitosan by increasing pH of the solution. This helps in binding individual particles of iron oxide in the presence of chitosan polymeric matrix (Gang et al. 2010; Jiang et al. 2013; Liu et al. 2010). With this, the individual iron oxide fine particles are glued together to form desired sized granules (Fig. 10.4).

These iron oxide-chitosan granules formed are porous and when come in contact with water, they swell indicating water entering the granules easily. The easy access of water to the granules will be helpful in arsenic ions in the water to come in contact with iron oxide particles for better adsorption. Analysis revealed that the iron oxide particle morphology and size remained intact even after granulation.

Scanning electron micrograph studies of iron oxide nanoparticles and granules (made out of this powder) was carried out and shown in Fig. 10.5. This characterization is essential in order to understand if there is any effect/change in particle size/morphology of the iron oxide powder during granule making process that may restrict/reduce granules performance vs. virgin iron oxide.

SEM micrograph of iron oxide fine powder at different magnification is shown in Fig. 10.5a, b. The SEM images indicate individual iron oxide nanoparticles distributed in a closed packed aggregate. SEM micrograph of iron oxide-chitosan granules at different magnification is shown in Fig. 10.5c, d. It is found from the SEM analysis that the particle morphology and size seems to be intact even after granulation.

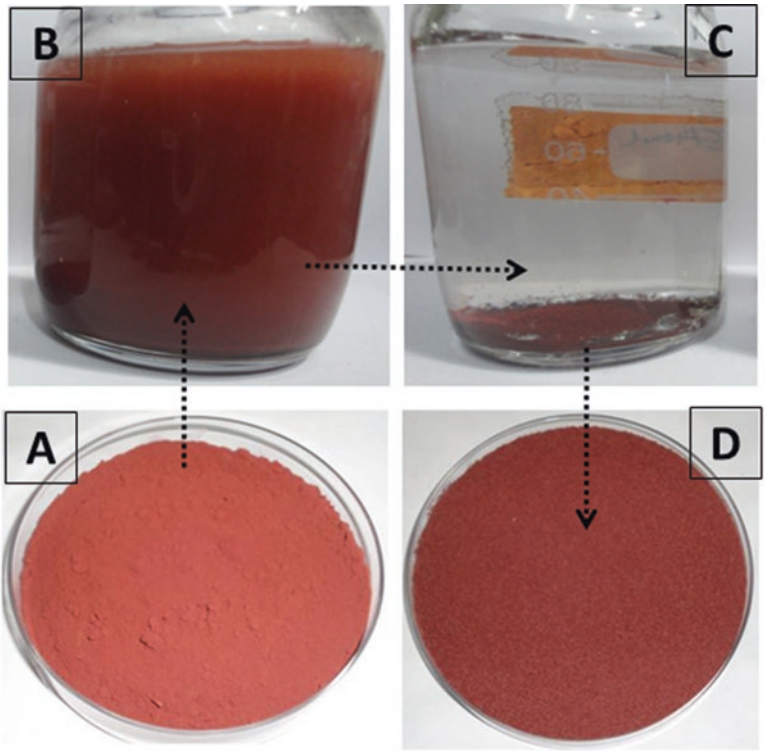

Fig. 10.4 (**a**) Photograph of iron oxide powder; (**b**) Photograph of Iron oxide-chitosan complex dispersed in water; (**c**) Iron oxide-chitosan complex controlled precipitation from reaction mixture to form granules; and (**d**) Photograph of dried iron oxide granules

Figure 10.6 illustrates thermo gravimetric analysis of the iron oxide particles and iron oxide-chitosan granules. Thermo gravimetric analysis of iron oxide particles indicates minimal weight loss. Whereas, thermo gravimetric analysis of the granules indicates weight loss of approximately 15%, this is attributed to the fact that the granules are composed of 85% (w/w) of iron oxide particles and 15% (w/w) of the chitosan.

The iron oxide granules are advantageous as large amount of iron oxide can be packed in a filtration column/ block thus avoids combining the use of other media such as carbon, and sand. Dried iron oxide granules is seen to swells when come in contact with water and gives a spongy feel, implies accessibility of water into the granules. The swelling property of granules is advantageous to pack them in a loose bed with appropriate mesh support. The biopolymer (chitosan) used for the granulation was carefully selected, also adsorbs arsenic thus playing synergistic effect with iron oxide for the arsenic removal (Gang et al. 2010; Jiang et al. 2013; Liu et al. 2010). Post granulation and separation of desired fractions (150 microns to 1 mm), these iron oxide granules are housed in a column (500 mm height × 50 mm diameter) with a specially designed mesh cap (5 microns pore) and is evaluated for arsenic removal efficacy using NSF-53 and US-EPA guidelines.

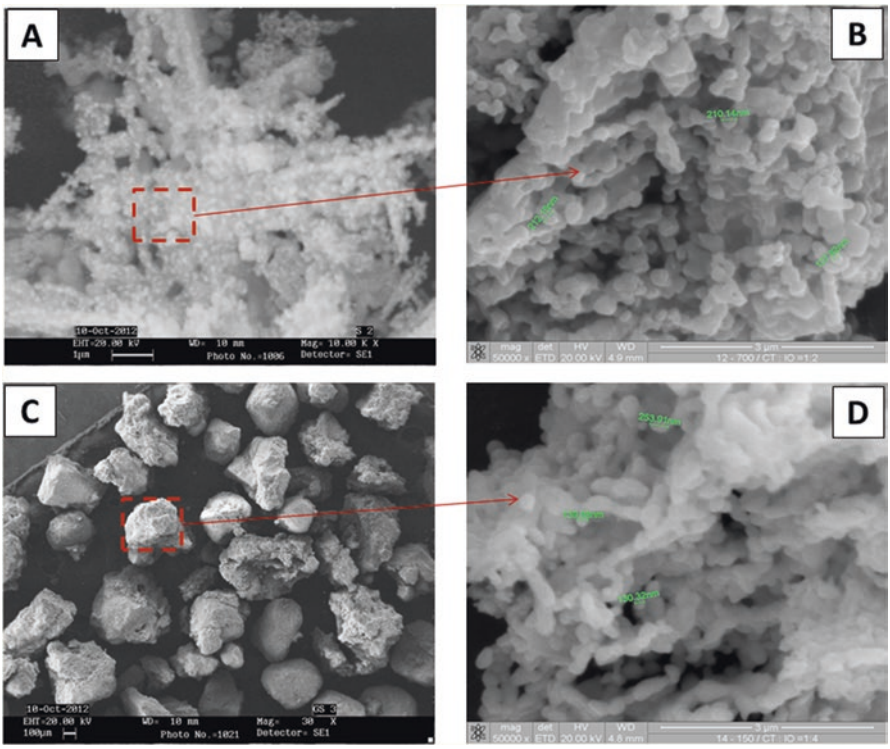

Fig. 10.5 (**a**, **b**) SEM micrographs of iron oxide fine powder at lower and higher magnifications; (**c**, **d**) SEM micrographs of iron oxide granules at lower and higher magnifications

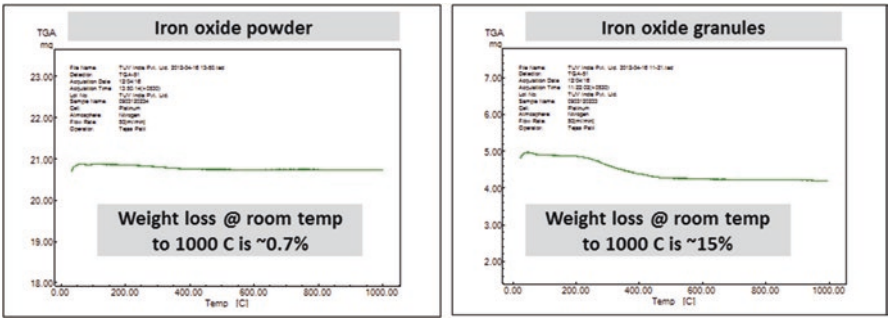

Fig. 10.6 Thermo gravimetric analysis of iron oxide powder and granules

Table 10.4 Iron leaching analysis from 2 numbers of random filters tested over filtration volume of 1000 l of water

Fe leaching- ICP MS measurements (Filter 1)		Fe leaching- ICP MS measurements (Filter 2)			
Sr. No.	Water passed in Liter	Fe in ppb	Sr. No.	Water passed in Liter	Fe in ppb
1	10	<10	1	20	<10
2	90	<10	2	110	13
3	180	<10	3	190	<10
4	310	<10	4	240	<10
5	420	<10	5	350	<10
6	560	<10	6	420	15
7	690	11	7	590	<10
8	810	12	8	710	15
9	920	14	9	890	12
10	1005	15	10	1012	14

10.3.2 Iron Leaching Test

In order to understand the leaching of iron oxide particles from the filtration column, tests were conducted by collecting output water from 2 random filters at different volume of water flow over a life of 1000 l. As per standard guidelines, minimum iron leaching in the output water allowed is ~1000 ppb (1 ppm). Table 10.4 below illustrates iron leaching analysis from 2 numbers of random filters tested over filtration of 1000 l. Inductively coupled plasma spectroscopy was employed to measure iron content in the output water. It is clear from the Table 10.4 that iron leaching is well below than the stipulated allowance limit.

10.3.3 Adsorption Efficiency of Iron Oxide Granules

For arsenic removal efficiency of the filtration device, USEPA advocates use of any input arsenic concentration of your choice based on local ground water contaminations level and reduce it to less than 10 ppb and claim the same conditions in the product (e.g. this product meets US EPA standard for arsenic with an input of say 200 ppb arsenic). Waters containing naturally occurring arsenic are preferable over synthetic water spiked with arsenic. US-EPA also suggests on using local ground water (arsenic contaminated) as the input source, however in case it is not available, one can choose appropriate input arsenic concentration and follow the standard protocol.

In this study, 12 numbers of columns containing iron oxide granules were fabricated and installed in the arsenic testing platform to evaluate arsenic removal ability of these filters as per NSF-53/US EPA testing guidelines (as explained in the experi-

mental section). Each cylindrical column is packed with 200 g of iron oxide granules containing granule size ranging 150 microns to 1 mm size. As per standard protocol, to qualify for an arsenic reduction claim, a water treatment system shall pass the test for pentavalent arsenic reduction and shall pass a separate test for trivalent arsenic reduction at two different pH conditions (i.e. 6.5 and 8.5). Claims may be made for pentavalent only or for total As reduction (As-III as well as As-V). A claim for only trivalent arsenic reduction shall not be made, suggests the guidelines.

For trivalent arsenic (As-III) reduction test, input challenge test water including 50 ppb of sodium arsenite was made as per composition given in Table 10.1 (experimental section). As per the test protocol, the above input water composition would be prepared at two different pH (6.5 and 8.5) conditions. Table 10.5 below shows the evaluation of 3 numbers columns containing 200 g each iron oxide granules passed through its life of ~1000 l of input challenge water comprising 50 ppb of sodium arsenite (As -III). The pH of the above water composition was maintained at pH 6.5.

Table 10.6 below shows the evaluation of 3 numbers columns containing 200 g each iron oxide granules passed through its life of ~1000 l of input challenge water comprising 50 ppb of sodium arsenite (As -III). The pH of the above water composition was maintained at pH 8.5.

For pentavalent arsenic (As-V) reduction test, input challenge test water including 150 ppb of sodium arsenate was made as per composition given in Table 10.2

Table 10.5 The performance evaluation of 3 numbers of iron oxide granule columns passed through its life of ~1000 l of input challenge water comprising 50 ppb of sodium arsenite, As (III) at pH 6.5

Filter no.	10 bed volume water passed in Liter.	As in ppb (Output water)	Water passed in Liter. (~25% Life)	As in ppb (Output water)	Water passed in Liter. (~50% Life)	As in ppb (Output water)	Water passed in Liter. (~75% Life)	As in ppb (Output water)	Water passed in Liter. (~100% Life)	As in ppb (Output water)
1	31	0	260	0–5	556	0–5	776	5–10	1030	5–10
2	35	0	252	0–5	527	5–10	735	5–10	1089	5–10
3	34	0	265	0	553	0–5	806	0–5	1062	5–10

Table 10.6 The performance evaluation of 3 numbers of iron oxide granule columns passed through its life of ~1000 l of input challenge water comprising 50 ppb of sodium arsenite, (As-III) at pH 8.5

Filter no.	10 bed volume water passed in Liter.	As in ppb (Output water)	Water passed in Liter. (~25% Life)	As in ppb (Output water)	Water passed in Liter. (~50% Life)	As in ppb (Output water)	Water passed in Liter. (~75% Life)	As in ppb (Output water)	Water passed in Liter. (~100% Life)	As in ppb (Output water)
4	38	0	257	0	492	5–10	724	5–10	1002	5–10
5	36	0	254	5–10	552	5–10	769	5–10	1032	5–10
6	30	0	251	0	559	5–10	756	0–5	1018	5–10

Table 10.7 The performance evaluation of 3 numbers of iron oxide granule columns passed through its life of ~1000 l of input challenge water comprising 150 ppb of sodium arsenate, As (V) at pH 6.5

Filter no.	10 bed volume water passed in Liter.	As in ppb (Output water)	Water passed in Liter. (~25% Life)	As in ppb (Output water)	Water passed in Liter. (~50% Life)	As in ppb (Output water)	Water passed in Liter. (~75% Life)	As in ppb (Output water)	Water passed in Liter. (~100% Life)	As in ppb (Output water)
7	33	0	230	0	527	5–10	748	0–5	1034	10–25
8	32	0	286	0–5	558	5–10	731	10–25	1009	10–25
9	39	0	272	0–5	545	10–15	786	5–10	1021	10–25

Table 10.8 The performance evaluation of 3 numbers of iron oxide granule columns passed through its life of ~1000 l of input challenge water comprising 150 ppb of sodium arsenate, As (V) at pH 8.5

Filter no.	10 bed volume water passed in Liter.	As in ppb (Output water)	Water passed in Liter. (~25% Life)	As in ppb (Output water)	Water passed in Liter. (~50% Life)	As in ppb (Output water)	Water passed in Liter. (~75% Life)	As in ppb (Output water)	Water passed in Liter. (~100% Life)	As in ppb (Output water)
10	36	0	251	5–10	494	5–10	767	5–10	1005	5–10
11	34	0	239	5–10	498	5–10	725	10–25	1049	10–25
12	29	0	244	5–10	516	5–10	751	5–10	1082	10–25

(experimental section). As per the test protocol, the above input water composition would be prepared at two different pH (6.5 and 8.5) conditions. Table 10.7 below shows the evaluation of 3 numbers columns containing 200 g each iron oxide granules passed through its life of ~1000 l of input challenge water comprising 150 ppb of sodium arsenate (As-V). The pH of the above water composition was maintained at pH 6.5.

Table 10.8 below shows the evaluation of 3 numbers columns containing 200 g each iron oxide granules passed through its life of ~1000 l of input challenge water comprising 150 ppb of sodium arsenate, As(V). The pH of the above water composition was maintained at pH 8.5.

Each data point presented in the performance evaluation chart (Tables 10.5, 10.6, 10.7, 10.8) is the average of 3 separate measurements done for arsenic quantification. As per the standard protocol, output water from the arsenic filtration device to be evaluated in 5 intervals of the filters total life. That means the arsenic removal efficacy to be tested after the filtration device passed through minimum 10 bed volumes of water and at its life of 25%, 50%, 75% and 100%. Performance evaluation (Tables 10.5, 10.6, 10.7, 10.8) of As (III) and As (V) at pH 6.5 and 8.5 over a volume of 1000 l filter life, we have demonstrated the ability of iron oxide-chitosan granules in successfully reducing arsenic from water and help making water potable.

The biopolymer (chitosan) not only helped in the iron oxide granulation but also helped in removal of arsenic from water. Chitosan presence was advantageous in many ways such as it acts as a binder for the granule formation, its water absorption capability helped making the granules soft thus avoid granule disintegration, it allowed water molecules to permeate through the granules thus allowing arsenic ions to come in contact with iron oxide particles and finally, it is also believed to have played a synergistic role of binding arsenic ions along with iron oxide nanoparticles. The affinity of iron oxide granules media for arsenic ions was shown to be strong under both the pH (6.5 and 8.5) conditions. This feature allows iron based sorbents to treat more bed volumes and considered to be the best media to filter arsenic from arsenic rich water (Gang et al. 2010; Jiang et al. 2013; Liu et al. 2010).

10.3.4 Safe Disposal of Spent Arsenic Media using TCLP

TCLP determines hazardous or non-hazardous nature of the spent arsenic filter media. As per US EPA TCLP guidelines, arsenic concentration in the extracting solution from the spent filter during testing must be less than 5 mg/L in order to be considered a non-hazardous waste and safe for municipal solid waste landfill disposal.

Leaching study of iron oxide granules recovered from arsenic filter columns after passing through ~1000 l of spiked arsenic water was done following USEPA TCLP method-1311. Random filters were selected for the TCLP studies. Spent iron oxide granules containing arsenic after 1000 l of water passed are analysed by ICP-MS to estimate the concentrations of arsenic present in TCLP extracts as per protocol explained by USEPA.

All the leaching studies conducted from the spent iron oxide granules containing arsenic were found to be well below the specified limit (Table 10.9). Therefore, we have successfully tested the mechanism for the safe disposal of exhausted /spent filters, which has passed the TCLP as per US EPA guidelines enabling it to be disposed of in a municipal solid waste landfill.

Table 10.9 TCLP studies of spent arsenic filtration media

Filter numbers	Quantification of Arsenic loaded in granules (ppm)	Quantification of Arsenic remained in granules after TCLP treatment (ppm)	Arsenic leached from the spent filter (TCLP extract) (ppb)
1	93.92	76.38	17.04
2	50.64	42.23	13.9
4	101.03	99.41	36.05
5	97.14	99.96	25.1
7	219.25	199.7	78.85
8	206.06	203.05	79.5
10	135.04	128.34	40.1
11	132.35	133.29	44.55

10.4 Conclusions

We have shown the development of a novel methodology to synthesize granules of iron oxide nanoparticles using a biopolymer. These granules are porous leads to easy access of water to the granules allowing effective binding of arsenic present in the water. The basic advantages with the granulation of iron oxide is that it allows large amount of active ingredient (~85% –iron oxide) that can be packed in a cartridge/ block thus avoid combining the use of any other media such as carbon and sand. The biopolymer used as an aid for the granulation also helps in adsorbing arsenic thus playing synergistic effect with iron oxide for the arsenic removal from drinking water. Post granulation and separation of desired iron oxide granule fractions, these granules are housed in a column with a specially designed mesh support and evaluated for arsenic removal efficacy using NSF-53 and US-EPA guidelines. 12 columns with granules have been tested with an input of 200 ppb arsenic (150 ppb As– V and 50 ppb As –III) at two different pH conditions (6.5 and 8.5). Over a volume of 1000 l of water passed through these columns containing iron oxide granules, a basic understanding of the performance of iron oxide granules in reducing arsenic was established. We believe that significant improvements in the output performance can further be achieved by emphasizing the process engineering and designing aspects of the device.

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Index

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