Environmental Chemistry for a Sustainable World 67

Vineet Kumar · Praveen Guleria
Shivendu Ranjan · Nandita Dasgupta
Eric Lichtfouse *Editors*

Nanotoxicology and Nanoecotoxicology Vol. 2

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Environmental Chemistry for a Sustainable World

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Preface

Nanoparticles have unique properties that make the application of nanotechnology in diverse fields possible. Nanotechnology suffers a major drawback in the form of uncertainty associated with the toxicity behaviour of nanoparticles. Nanoparticles can affect plant growth and enter the food chain. The bioaccumulation, biomagnification and biotransformation abilities of nanoparticle have further added to the problem. Once nanoparticles enter the food material, they can affect human health by interacting with human oral microflora and organs (Fig. 1).

Nanotoxicity testing methods, procedures and strategies need to be modified keeping in mind the unique properties of nanomaterials. This book has 12 chapters focusing on different aspects of nanotoxicity testing and management.

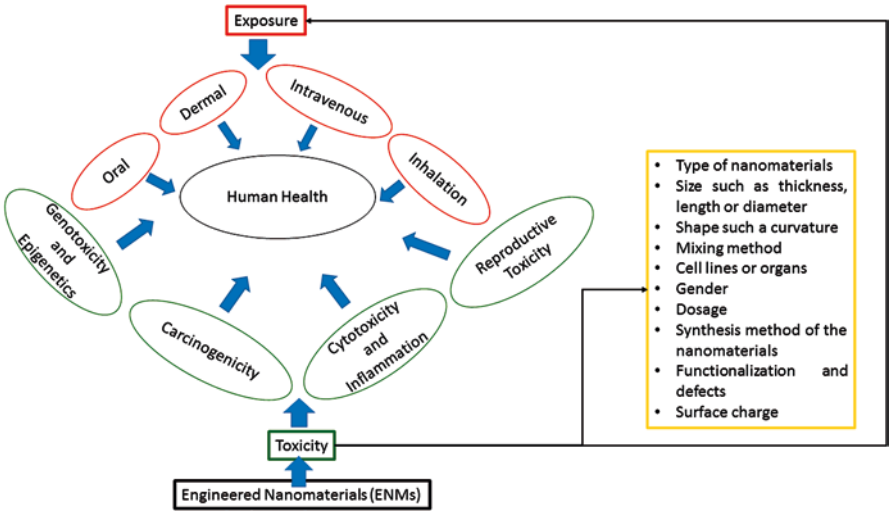

Fig. 1 Summary showing factors that should be taken into account when considering the toxicity of engineered nanomaterials. (From Chap. 7 by Abedin et al.)

Singh et al. discuss in Chap. 1 the various applications of nanosensors in the field of food, medicine, agriculture and nanotoxicology. Chapter 2 describes in detail the detection of food adulterants using nanosensors. Mallakpour et al. describe the use of metal oxides and biopolymer/metal oxides based bionanocomposites as green nanomaterials for heavy metals removal in Chap. 3. In Chap. 4, Chowdary et al. discuss the impact of nanomaterials on the components of the food chain. Shende in Chap. 5 reviews the safety aspects of nanomaterials. Asmatulu discusses in detail the accumulation, biomagnification and biotransformation of nanomaterials Chap. 6. Abedin et al. review the overall effect of nanomaterials on human health in Chap. 7. Pokrowiecki in Chap. 8 discusses the applications and challenges of nanomaterials in the field of dentistry. The effect of nanomaterials on the normal and diseased human is discussed along with other nanosafety approaches in Chap. 9 by Shende et al. Pokrowiecki et al. in Chap. 10 review the effect of nanoparticles on the oral microflora. The focus of the chapter is on the prevention and treatment of human oral infections. Asmatulu et al. discuss in Chap. 11 the methods of in vitro nanotoxicity and genotoxicity testing. Different techniques, methods, procedures and protocols of nanotoxicity testing are discussed in detail by Ilangovan et al. in Chap. 12. Overall, this book emphasizes on the procedures, methods and strategies of nanotoxicity testing.

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Chapter 1
Nanosensors Applications in Food, Medicine, Agriculture and Nanotoxicology

Namita Ashish Singh and Pradeep Kumar

Abstract Nanosensors are being used globally due to its specific features over the traditional methods. In this chapter we have emphasized on the need of nanosensors and their applications in various fields i.e. food industry, medicine and agriculture. In the food sector we have highlighted the use of nanomaterials in food packaging, food processing and food quality as well as safety. The next section describes the applications of nanosensors in the medicine for the detection of diseases namely diabetes, asthama, cancer etc. and targeted drug delivery. In agriculture nanosensors/nanoparticles are used for crop protection against various plant pathogens and pesticide detection. Further, in nanotoxicology section we have discussed the various routes through which nanomaterials enter into environment. Toxicity of nanomaterials on various organs i.e. respiratory system, gastrointestinal system, cardiovascular system, central nervous system and skin are also discussed in the last section.

Keywords Nanosenors · Nanomaterials · Nanotoxicology · Food · Medicine · Agriculture

1.1 Introduction

Nanotechnology is the use of nanomaterials in different areas for the human benefit. Nanomaterials are used for the novel sensing and monitoring size and unique properties at nanoscale (Singh 2017). The main aim of nanosensors is to measure any chemical, mechanical and physical changes which are related to an indicator of

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interest. On the basis of signal production nanosensors can be classified as optical, electrical and mechanical (Nguyen and El-Safty 2011). Nanosensors can be divided into two major categories, first sensors are those that are at nanoscale and second sensors are those that are used to measure nanoscale properties. The first type of nanosensors requires low material cost, reduced weight and less power consumption. The second type of nanosensors may have toxic effects of these engineered nanomaterials (Sadik et al. 2009). Nanosensors are widely used in the agriculture, food and medicine due to its selectivity, speed and sensitivity compared to traditional methods. Nanosensors can be used for monitoring of microbes, contaminants and pollutants (Joyner and Kumar 2015). Aptasensors are biosensors consisting of aptamers (the target-recognition element) and nanomaterial (the signal transducers). Aptamers are single stranded nucleic acid or peptide having size less than 25 kDa. They are highly specific towards their target compound i.e. proteins, toxins, microbes, viruses etc. due to their 3D structures. There are a wide variety of nanomaterials, which can be used in aptasensors i.e. metal nanoparticles & nanoclusters, carbon nanoparticles, magnetic nanoparticles etc. for the detection of various analytes (Sharma et al. 2015).

1.2 Nanosensors Applications

The nanosensors are cost effective, rapid, portable and ultrasensitive bioassays for monitoring various analytes. The nanobiosensors are used for detection of glucose, food pathogens, contaminants, biomedicine, agriculture etc. (Wang 2008; Palchetti and Mascini 2008; Prieto-Simon et al. 2008).

1.2.1 Nanosensors in Food

In the food production, the nanosensors are involved in the food packaging and transport. Food packaging prevents sensory exposure from the foods directly so the consumers have to trust on the expiry dates provided by producers. If the transport or storage conditions are fails to meet the terms then the quality of food may be declined which consumers may not know unless the food packet is opened, or consumed (Joyner and Kumar 2015). Nanosensors can detect the factors responsible for food deterioration in food packaging i.e. gasses, chemical contaminants, pathogens. Nanosensors improve food safety ensure and reduce the incidence of food-borne illnesses (Fig. 1.1).

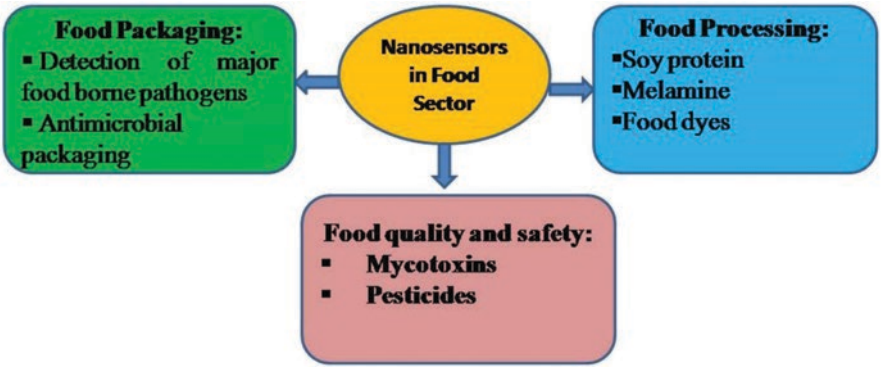

Fig. 1.1 Applications of nanosensors in food sector

1.2.1.1 Food Packaging

Food industry has major focus on food packaging as consumer's expectations are very high for safe food. The nanosensors interact with food components as well as environmental factors and generate a signal that indicates the status of the food. This information is helpful for consumers and producers to determine that which parameters should be taken care during production process (Mihindukulasuriya and Lim 2014).

Various nanosensors have been developed for the food industry to identify threats related to food poisoning or integrated into packaging. Shelf life of food products has been extended by the integration of clay nanoparticles into an ethylene-vinyl alcohol copolymer and polylactic acid biopolymer which improves the oxygen barrier properties of packets (Lagaron et al. 2005). Nanosensors based on copper nanoparticles protected by a 2–3 nm carbon coating, a silicon tenside have been developed to detect the presence of moisture content inside a food packet (Luechinger et al. 2007). Nanosensors are used in food packaging to detect the growth of microbes when a threshold level is achieved, so they can prevent food poisoning (Augustin and Sanguansri 2009). Some nanosensors used for identification of food borne pathogens are listed in Table 1.1.

Nanoparticles of silver or titanium dioxide can be used as antimicrobial in packaging as they are spread through the plastic and block oxygen, carbon dioxide as well as moisture subsequently prevent food spoilage. The nanoclay makes the plastic lighter, stronger and more heat-resistant. Thymol oil obtained from a group of aromatic plants (Thyme) is also used as antimicrobial in foods and packages. Different studies have demonstrated the effectivity of thymol oil against the food pathogens such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli, Shigella dysenteriae, Bacillus cereus,* and *Staphylococcus aureus* (Nabavi et al. 2015). Nanosensors and nanoscale coatings are helpful in preventing corrosion in comparison to thicker, wasteful polymer coatings. Nanosensors are used for the

Table 1.1 Nanosensors for identification of food borne pathogens

Nanosensors mechanism	Targeted pathogen	References
Poly(dimethylsiloxane) chips with fluid bilayer membrane and specific antibodies to the toxin	*Staphylococcus* sp. enterotoxin B	Dong et al. (2006)
G- liposomal nanovesicles based immune-magnetic bead	*E. coli* O157:H7, *Salmonella* sp., and *Listeria monocytogenes*	Rivas et al. (2007)
Immunosorbent assay using universal protein G-liposomal nanovesicles	Pathogenic microorganisms	Chen and Durst (2006)
Fluorescent sandwich immunoassay using quantum dots functionalized with high affinity antibodies	*Clostridium botulinum* neurotoxin serotype A	Warner et al. (2009)
A waveguide-based immunosensor using quantum dots as the fluorescent reporters	*Bacillus anthracis*	Mukundan et al. (2010)
A protective antigen-specific peptide onto a multi-wall carbon nanotubes with limit of detection 0.4pM	*Bacillus anthracis*	Huan et al. (2011)
Mass spectrometry-based immune-sensors using antibody modified gold nanoparticles	*E. coli*, *Staphylococcus aureus* and *Salmonella enteric*	Tseng et al. (2012)
Amperometric immunosensor with gold nanoparticles having very low limit of detection i.e. 10 CFU mL^{-1}	*Bacillus cereus*	Kang et al. (2013)
Gentamicin-modified fluorescent magnetic nanoparticles with Fe_3O_4 cores and fluorescent silica shells having limit of detection (1×10^7 CFU mL^{-1} from 10 mL of solution) within 20 min	*E. coli*	Chen et al. (2013)
Colorimetric immuno-sensor with cotton swab and nanoparticles with sensitivity of 10–10^8cfu/ml	*Salmonella typhimurium, Salmonella enteritidis, Staphylococcus aureus and campylobacter jejuni*	Alamer et al. (2017)
pH-responsive polymer nanobrushes embedded with platinum nanoparticles	*Listeria monocytogenes*	Oliveira et al. (2019)

detection of microbes and toxic contaminants through the food processing (Prasad et al. 2014; Lu and Bowles 2013).

1.2.1.2 Food Processing

Allergens found in food are a major concern for both industries and consumers as they can cause skin, respiratory and digestive disorders. Allergens are special proteins and they are not affected by cooking or other treatments and they are able to cause various human health problem related to skin, respiration and digestion (Simons et al. 2011). For screening and detections of allergens nanobiosensors are the best tool. Soy protein, a major food allergen found in soymilk, soy yogurt, fruit and soy juice has been detected using silica nanoparticles doped with nile blue with

sensitivity upto 0.05 mg/L and 2 h incubation (Godoy-Navajas et al. 2011). Egg-white protein (ovalbumin) added to the milk can be detected through silver dendrites using surface enhanced raman scattering up to 1 ppm within 20 min (He et al. 2011).

Melamine is an adulterant which is used to increase the protein content of infant food and raw milk artificially. Surface enhanced raman scattering based detection of melamine from contaminated noodles, wheat gluten, cakes and chicken feed has been done using gold nano-structures up to 2 ppm (Lin et al. 2008). Melamine can be detected by gold nanoparticles functionalized with cyanuric acid groups upto 2.5 ppb (Ai et al. 2009). A ultrasensitive colorimetric method has been developed using carbon quantum dots -silver nanocomposite in food sample with a sensitivity of 62.6 pM (Wang et al. 2018).

Ponceau 4R and Sudan I color or dye are carcinogenic adulterants although banned but being used in various food products (Duncan 2011). Carbon nanotubes based colorimetric assays have been developed for monitoring of these food colorants which are often used in soft drinks and beverages (Zhang et al. 2010). Sudan IV dye found in ketchup and chili powder was analyzed by graphene-modified carbon electrodes with sensitivity up to 2×10^{-7} M to 8×10^{-5} M (Mo et al. 2010). Multiwalled carbon nanotubes in integration with liquid nanocomposites have been used for monitoring of tartrazine and sunset yellow dyes which are used in food and beverages (Majidi et al. 2013).

An electrochemical sensor has been developed for the detection of tartrazine based on titanium oxide–reduced graphene oxide composite modified glassy carbon electrodes with sensitivity of 8.0×10^{-9} mol/L in carbonated beverage samples He et al. (2018). Shah (2020) has designed a novel electrochemical nanosensor for the simultaneous analysis of two toxic food dyes namely metanil yellow and fast green in real water and juice samples. The nanosensors was based on the modification of glassy carbon electrode with calixarene and gold nanoparticles with detection limit 9.8 and 19.7 nM for metanil yellow and fast green respectively.

1.2.1.3 Food Quality and Safety

Some nanosensors are used for detection of gases released from the food items and responsible for spoilage (Valdes et al., 2009). Various nanosensors are available for the detection of non microbial contaminants i.e. mycotoxins, pesticides etc. Mycotoxins are secondary metabolites produced by the fungi like *Fusarium, Aspergillus* and *Penicillium* (Li et al. 2012). They can contaminate many food items namely cereals, milk, nuts, coffee, oil seeds, beans, fruits etc. and cause threat to animals as well as humans due to their carcinogenicity. Jin et al. (2009) developed a piezoelectric immunosensor based on gold nanoparticles for the detection of aflatoxin B1 in contaminated milk with detection limit 0.01 ng/mL. An impedimetric DNA biosensor based on gold nanoparticles has been developed for the monitoring of aflatoxinM1 in milk and dairy products (Dinckaya et al. 2011). A fast indirect competitive-based biosensor with gold nanoparticles as a signal amplifier has been

developed for the detection of ochratoxin A in red wine (Karczmarczyk 2017). Kasoju et al. (2020) developed a colorimetric microfluidic paper based method using unmodified gold nanoparticles for the monitoring of aflatoxin M1 in milk samples with sensitivity of 10 nM.

Pesticides are being used in agriculture, food, industries and households to control various pests, subsequently shielding the human health and environment. Pesticides can be been classified as organophosphorous, organochlorine, carbamates, pyrethroids etc. (Tehri et al. 2020). Hu et al. (2019) developed a colorimetric sensor based on the inhibition of peroxidise mimicking the activity of gold naoparticles for monitoring dimethoate in tomato, cucumber and cabbage juice with limit of detection 4.7 $\mu g L^{-1}$.

1.2.2 Nanosensors in Medicine

Oral administration of drugs is more common compared to injection due to convenience of the patients. However, drug bioavailability through oral route is limited because of physiological barriers of the gastrointestinal tract. However, some new drug delivery systems are being developed due to the improvement of nanocapsule transportation. e.g. polymeric nanoparticles (Pridgen et al. 2014) which prevents drug from inactivation and degradation through acid and enzymatic barriers of gastrointestinal tract. Various studies reported the benefits of nanoparticles, such as the reversal of nonsteroidal anti-inflammatory drug-induced gastrointestinal injury (Fricker et al. 2010) and radioprotection from cancer radiotherapy (Pamujula et al. 2008). Cerium oxide nanoparticles have been also reported to protect gastrointestinal epithelium from the reactive oxygen damage (Colon et al. 2010).

1.2.2.1 Monitoring Glucose in Diabetes

Diabetes is a most common disease which can have side effects such as heart disease, stroke, kidney disease, eye problems, nerve damage etc. Although there is no treatment for diabetes but patients can reduce disease-associated problems through the control of blood glucose. Nanotechnology has been integrated into glucose sensors using two approaches. In first approcah, sensors can be designed using microscale components such as electrodes, membranes etc. These modified systems have several advantages, including higher surface areas (yielding larger currents and quicker responses) and enhanced catalytic activities. These sensors have the same drawbacks as current sensors, including sensor fouling and decreased sensor life (Cash and Clark 2010).

Secondly, nanofabrication techniques can generate glucose sensors that are nanoscale completely. These sensors offer some advantages over conventional

sensors for continuous monitoring. Due to the small size of these sensors, they could avoid the foreign body response of the immune system subsequently have longer lives. Nanomaterial-based sensors can be used to monitor glucose through changes in pH or charge, through a field effect transistor. These devices measure conductance which is affected by charges near the surface of the sensor or the pH of the solvent. As the concentration of glucose changes, the charge near the surface or the pH changes either as a result of an enzymatic reaction or competitive binding. Zenkl et al. (2008) have developed polymeric nanosensors incorporating phenylboronic acid derivative to recognize glucose. In the absence of sugar due to the small nanospheres fluorophores are close subsequently allow efficient forster resonance energy transfer while in presence of sugar, the polymer swells resulting into increased distance between the fluorophores which decreases forster resonance energy transfer.

Fluorescence-based sensors have the advantage to optically monitor the sensors through the skin in vivo. In this approach, sensors would be rooted into the skin of the patient as regular/smart tattoo. The sensors would change fluorescence properties in response to blood glucose, which we can read optically. In this method, there is no need to take blood samples from patients which subsequently reduces the chances of infection (Mou et al. 2010).

1.2.2.2 Asthama Detection

A nitric oxide nanosensors has been develoed which can detect asthma by breath analysis (Gouma and Kalyanasundaram 2008). Tallury et al. (2010) have developed a nanobiosenosr based on single-walled carbon nanotubes and gold electrodes on silicon oxide substrate and for detecting asthama attacks before 3 weeks of its occurence by testing the nitric oxide in pateints breath.

1.2.2.3 Cancer Detection and Drug Delivery

Carbon magnetic nanoparticles (40–50 nm) have been developed for drug delivery and targeted cell destruction in cancer (Pathak and Katiyar 2007). Various nanoparticles have been used as specific magnetic resonance imaging (MRI) contrast agents for cancer screening. These nano-contrast agents are able to recognize unique cell surface markers, long-lasting blood circulation half-life and better MRI properties (Cheng et al. 2013). A polymer nanovesicle platform has been developed for the first time to deliver and inhibit aurora-A kinase in cancer cells which is a potent oncogene and also targeted for cancer therapy. These polysaccharide nanovesicles (<200 nm) made from dextran were used as nanocarriers to carry the drug in their intermembrane space which is released by the action of esterase enzyme (Inchanalkar et al. 2018).

1.2.2.4 Alzheimer's and Parkinson's Disease Detection

Functionalized carbon nanotubes and gold nanoparticles based nanosensors have been developed for identifying the Alzheimer's disease and Parkinson's disease from alveolar breath (Tisch et al. 2013).

1.2.3 Nanosensors/Nanoparticles in Agriculture

1.2.3.1 Nanofertilizers

Balanced fertilizer, irrigation and quality seeds can increase the agricultural production by 35–40%. Nanofertilizers have unique qualities like ultrahigh absorption, increase in production, rise in photosynthesis etc (Fig. 1.2). The use of nanofertilizer reduces the toxicity of the soil, increases efficiency of the elements as well as reduces the frequency of application of fertilizers (Naderi and Danesh-Shahraki 2013). In zinc fertilizers, the solubility of zinc plays a significant role in the effectiveness of the fertilizer. Zinc oxide nanoparticles dissolve faster than bulk zinc oxide particles.

Nanoformulated fertilizers significantly increase the productivity of different crops with less toxicity. For example, Carbon nanoparticles with fertilizer can increase the grain yields of rice (10.29%), spring maize (10.93%), soybean (16.74%), winter wheat (28.81%) and vegetables (12.34–19.76%) (Liu et al. 2009a, b). Sulfur nanoparticles at the concentration of 300 ppm enhanced tomato's growth by increasing root and shoot lengths (Salem et al. 2016). Silver nanoparticles at 25 ppm concentration showed significant growth promoting activities on wheat growth and yield (Ghidan and Antary 2019). Silver nanoparticles improved the

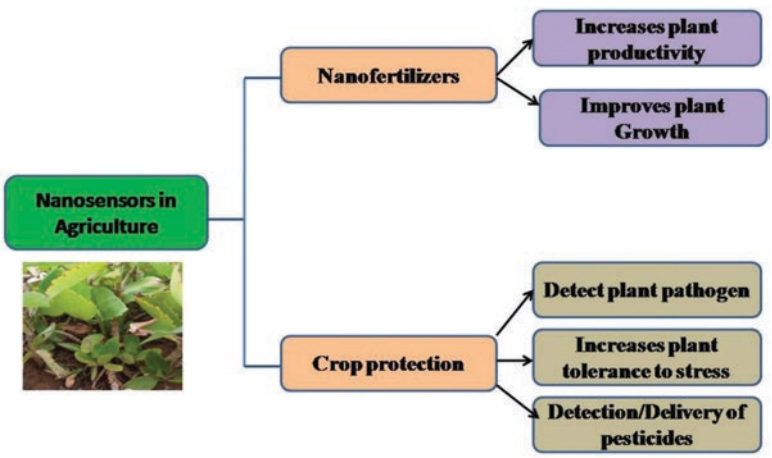

Fig. 1.2 Role of nanosensors in Agriculture

growth and tolerance to heat in *Triticum aestivum* crop at 50 mg/L and 75 mg/L concentration (Iqbal et al. 2019). Nanocapsules and nanoparticles are helpful for the nutrients absorption by plants and the delivery of nutrients (Milani et al. 2012).

1.2.3.2 Crop Protection

Etefagh et al. (2013) have developed fluorescent silica nanoparticles conjugated with antibodies for detecting plant pathogen such as *Xanthomonas axonopodis pv. vesicatoria* which causes bacterial spot disease in tomatoes and peppers. Silver-based nanoparticles, are used for detecting contaminants and microbial pathogens in the soil. So, the nanosensors are helpful in plant disease forecasting and disease management (Bogue 2008). A gold electrode modified with copper nanoparticles was developed to monitor salicylic acid in oilseed rape infected with the fungal pathogen *Sclerotinia sclerotiorum* (Wang et al. 2010). Silver nanoparticles inhibit sun hemp rosette virus on bean leaves (Jain and Kothari 2014). Yüksel et al. (2015) reported the detection of plant pathogen *Phytophthora ramorum* from actual samples using surface enhanced raman scattering.

Silicon, titanium dioxide, alumina and copper nanoparticles are involved in controlling agricultural pests, increasing plant tolerance to various abiotic and biotic stresses, and improving the plant growth (Gogos et al. 2012; Kah and Hofmann 2014; Mondal and Mani 2012; Norman and Chen 2011; Paret et al. 2013). Nano titanium dioxide at the concentration of 0.01, 0.02, and 0.03% increases drought tolerance by increasing growth, yield, gluten and starch content of wheat (*Triticum aestivum* L.) (Jaberzadeh et al. 2013). Mustafa et al. (2015) studied the affect of aluminum oxide nanoparticles at concentration of 50 ppm on soybean (*Glycine max L.*) during flood stress. They found that the nanoparticles have reduced the generation of cytotoxic byproducts of glycolysis, increased stress-related proteins and promote the growth of soybean.

Pesticides are used to prevent insects, fungi and herbs but simultaneously contaminate the environment. The contamination of pesticide residues and their metabolites in food, water and soil has become an alarming issue of research (Mostafa 2010). Amperometric and optical transducers are used for the monitoring of herbicides like phenyl urea and triazines (Cock et al. 2009). A liposome based nanobiosensors has been developed for detection of organophosphorus pesticides namely dichlorvos and paraoxon at 10^{-10} M levels in drinking water samples (Vamvakaki and Chaniotakis 2007). Copper based nanoparticles were found more useful against *Phytophthora infestans* compared to available non-nano copper formulations without any detrimental effect (Giannousi et al. (2013).

Nanoemulsion (31.03 nm) composed of neem oil (*Azadirachta indica*) and non-ionic surfactant Tween 20 was found to be an effective larvicidal agent against *Culex quinquefasciatus* (Anjali et al. 2012). Eucalyptus oil nanoemulsion consisting of eucalyptus oil, tween 80 and water prepared by ultrasonication showed higher activity compared to bulk emulsion against *Culex quinquefasciatus* (Sugumar et al. 2014). Ghidan et al. (2018) has done green synthesis of copper oxide, zinc oxide,

magnesium hydroxide and magnesium oxide using aqueous extracts of various plants and found that magnesium hydroxide bionanoparticles were best to control the green peach aphid, *M. persicae*. Nano-microcapsules or nanospheres have been prepared using light-sensitive, thermo-sensitive, enzyme and soil pH-sensitive polymer materials for the delivery of pesticides. Controlled release of nanomaterials and targeted delivery can get better pesticide utilization and reduce pollution as well (Huang et al. 2018).

1.3 Nanotoxicology

As we have discussed that with the fast development of nanotechnology, currently industries are using nanomaterials in variety of products. Nanotoxicology is the study of the toxicological activities of nanoparticles and their products on the environment, human and other biological systems. It also deals with the evaluation of the nanotoxic effects on exposure of the organisms. Nanotoxicological studies will be helpful for designing safe nanomaterials and nanoproducts, for their direct uses in the medicine. In drug delivery system, it is also essential to know the toxic properties of nanoparticles. The US Food and Drug Administration have raised the issue of identifying the toxicity related with nanoparticles products (Bahadar et al. 2016). Toxicity of nanoparticles to various systems was described in Table 1.2.

Genotoxicity of nanomaterials was evaluated by the single cell gel electrophoresis assay in which carbon nanotubes induced a dose-dependent increase in DNA damage at all treatment times even at the lowest dose. Graphite nano fibres cause DNA damage at all doses in the 24 h treatment, at two doses i.e. 40 and 100 μg/cm^2 in the 48-h treatment and at four doses i.e. lowest 10 μg/cm^2 in the 72-h treatment (Lindberg et al. 2009).

Injectable anti-inflammatory drug ibuprofen sodium was loaded with gelatin nanoparticles modified with polyethylene glycol of approximately 200 nm size in vitro. The developed nanomedicine has proved to be nontoxic, hemocompatible and non-immunogenic at the dose which is required for effective drug delivery (1 mg/ml), which was also confirmed by assessment of in vivo inflammatory cytokine levels as well as histological analysis of liver and kidney (Narayanan et al. 2013).

Marquez et al. (2018) demonstrated that *S. cerevisiae* strains S288C that lack genes involved in transmembrane, membrane transport, cellular ion homeostasis and cell wall organization showed the highest sensitivity against engineered zinc oxide nanoparticles. In contrast, strains which lack genes involved in transcription, RNA processing, cellular respiration, endocytosis and vesicular transport showed the highest sensitivity against engineered silver nanoparticles.

Kerfahi et al. 2015 studied the effect of raw and functionalized multi-walled carbon nanotubes on soil bacterial diversity. They found that at higher concentration functionalized multi-walled carbon nanotubes affect dominant bacterial phyla i.e. Acidobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi. Sohn et al. 2015 studied The single-walled carbon nanotubes toxicity on a micro

Table 1.2 Nanoparticles and their toxicity

Nanoparticles	Size	Toxicity	References
Quantam dots modified CdSe	10, 20 nM	Showed decreases in cell viability 20% and 30% respectively	Tang et al. (2008)
Aluminium oxide nanoparticles	<50 nm	Cause genotoxic effects in the form of DNA damage without any mutagenic effects in mouse lymphoma cells line	Kim et al. (2009)
Iron oxide Nanoparticles (chitosan-coated)	13.8 nm (conc. of 123.52 µg/mL)	Showed 10% cell viability after 12 h exposure	Ge et al. (2009)
Iron oxide Nanoparticles (1-hydroxy-ethylidene-1,1-bisphosphonic acid coated)	20 nm, (conc.0.1 mg/mL)	Showned 70% cell viability after exposing rat's mesenchymal stem cells for 2 days	Delcroix et al. (2009)
Titanium dioxide nanoparticles	<100 nm	Induces oxidative stress and form DNA adducts	Bhattacharya et al. (2009)
Titanium dioxide nanoparticles	5–200 nm	Causes toxic effects on immune system, liver, kidney, spleen, glucose and lipids homeostasis in experimental animals	Liu et al. (2009a, b, 2010)
CdTe-gelatinized/ nongelatinized	1–100 nM	1 nM don't have any harmful effects but 100 nM resulted in the death of all cells	Prasad (2010)
Silica naoparticles	–	Decreases cell viability of human keratinocytes at 30–300 µg/mL concentration	Park et al. (2010)
Copper oxide nanoparticles	–	Induce oxidative stress by depletion of glutathione, induction of lipid peroxidation, catalase and superoxide dismutase in human lung epithelial cells (A549)	Ahamed et al. (2010)
Zinc oxide nanoparticles	–	Lead to cellular morphological modifications, mitochondrial damage and cause reduction of superoxide dismutase, depletion of glutathione and DNA damage in human hepatocytes cell line (HEK 293)	Guan et al. (2012)
Gold nanoparticles	–	Induce autophagy with oxidative stress in MRC-5 human lung fibroblasts	Li et al. (2010)
Gold Nanoparticles (citrate capped)	15 nm	It's ingestion of 12 µg/g per day in *Drosophila melanogaster* causes reduction of life span and fertility, DNA fragmentation as well as over-expression of the stress proteins	Pompa et al. (2011)

(continued)

Table 1.2 (continued)

Nanoparticles	Size	Toxicity	References
Gold Nanoparticles	5 nm and 15 nm	Disruption of actin cytoskeleton in vitro on Balb/3 T3 mouse fibroblasts.	Coradeghini et al. (2013)
Silver nanoparticles	20 nm	Triggers significant oxidative stress insides macrophages that reduces cell viability	Haase et al. (2011)
Silver Nanoparticles (polyvinyl-Pyrrolidone-coated)	35 nm	Toxicity against bacteria, fungi, viruses, protists, animals and humans at inhibitory concentration of 10^{1} µg/mL	Vazquez-Munoza et al. (2017)

crustacean (*Daphnia magna*), freshwater microalgae (*Raphidocelis subcapitata* and *Chlorella vulgaris*) and a fish (*Oryzias latipes*). They revealed that single-walled carbon nanotubes inhibited the growth of the algae *C. vulgaris* and *R. subcapitata* at concentration 30.96 mg/mL and 29.99 m/L respectively.

1.3.1 Routes of Exposure in the Aquatic Environment

Due to the significant growth of nanotechnology, various nanoparticles are released into the aquatic environment. Aquatic ecosystems are susceptible to environmental contamination from production processes, wastewater treatment plants as well as accidental exposure during the transport. Baun et al. (2008) showed that nanoparticles may penetrate into algae which then are consumed by animals present in water and subsequently transferred to higher trophic levels.

In the aquatic environment, nanoparticles may aggregate thus failing their direct uptake by aquatic organisms. However, aggregated nanoparticles may settle into sediment and subsequently create a threat to benthic organisms (Klaine et al. 2008). Nanomaterials are able to penetrate biological barriers due to their small size. In aquatic organisms, the major routes of entry are via ingestion or direct passage across the gill and other external surface epithelia (Moore 1990). Nanoparticles taken up via ingestion may accumulate in the hepatopancreas which is responsible for metabolism, detoxification and intracellular lysosomal digestion of food by endocytosis (Lee 2001). Nanosize particles may enter into the liver of fish. Endocytosis (<100 nm) and phagocytosis (100–100,000 nm) represent the two processes by which nanoparticles can be absorbed into eukaryotic cells.

1.3.2 Toxicity of Nanoparticles to Organs

Toxicity of nanoparticles varies when they enter into different organs. Human skin, intestinal tract and respiratory tract have direct contact with the environmental nanoparticles. Nanoparticles invade into the human body through these exposure routes. The predominant routes of nanoparticles exposure, uptake and potential risks are depicted in Fig. 1.1.

1.3.2.1 Respiratory System

Nowadays the air we breathe in is not pure due to pollutants of industrial processes. So, the toxicity research on nanoparticles has been performed on respiratory system of mammalian in vivo. Nanoparticles accumulate throughout the entire respiratory tract; the deposition fractions show significant variation depending on the size of inhaled nanoparticles. For instance, 90% of inhaled 1 nm particles are deposited in the nasopharyngeal, only 10% in the tracheobronchial region and none in the alveolar region. On the other hand, if 5 nm particles are inhaled then there is equal accumulation of 30% of the inhaled particles in all three regions; 20 nm particles have the highest deposition in the alveolar region (50%) while in tracheobronchial and nasopharyngeal regions these particles deposits with 15% efficiency (ICRP 1994). The deposition of nanoparticles in the alveolar regions of the lung leads to a scattered chemoattractant signal, resulting in lower alveolar macrophage responses (Kreyling et al. 2002).

Jia et al. (2005) demostrated that both single walled and multi walled carbon nanotube induce the alteration of cell structures. The macrophage cell exhibited condensed folds, when exposed to 5 μg/mL single walled carbon nanotubes, while the nucleus degenerated and the nuclear matrix reduced during treatment with multi walled carbon nanotubes. Cells exposed to single walled carbon nanotubes at a higher dose i.e. 20 μg/mL, became swelled and presented phagosomes clearly; while those that were exposed to multi walled carbon nanotubes the chromatin was concentrated and vacuole in cytosol were presented. All the above changes show signs of cell apoptosis. The translocation of nanoparticles from lung to other organs such as bone marrow, lymph node, brain or heart can cause more severe diseases including alzheimer, parkinson or cardiac cancers (Janrao et al. 2014; Arora et al. 2012).

The iron oxide (magnetite) is a major human made pollutant which is derived primarily from industrial sources. The effects of four different size fractions of magnetite on signaling pathways, free radical generation, cytotoxicity, and genotoxicity was evaluated in human alveolar epithelial-like type-II cells (A549). It was observed that iron oxide causes activation of c-Jun N-terminal kinases without increased nuclear factor kappa-B and increased reactive oxygen species (Konczol et al. 2011).

Quantum dots are luminescent nanoparticles having potential in medicines but there are safety concerns also. Choi et al. (2012) studied the cytotoxic and genotoxic

effects of quantum dots with a cadmium selenide/zinc sulfide core/shell and found that they cause apoptotic and necrotic cell death (Fig. 1.2).

1.3.2.2 Gastrointestinal System

Nanoparticles can be ingested directly via food, water, drugs or drug delivery systems. Nanoparticles cleared from the respiratory tract can further enter into the gastrointestinal tract. Chen et al. (2006) compared the oral toxicity of copper nanoparticles and micro particles in mice. On exposure with 17 μm particles only few mice have side effects while by nano-copper treatment (23.5 nm) all mice showed symptoms of alimentary canal function disorder such as loss of appetite, diarrhea and vomiting, etc. Pathological examination showed serious injuries on kidney, liver and spleen in mice which was not found in mice exposed to micro-copper particles (Fig. 1.3).

Toxicity of zinc oxide nanoparticles were analyzed on rats of different body weight i.e. 50, 300, 1000 and 2000 mg/Kg through oral route. Clotting time was effected in all the male groups except in 1000 mg/kg body weight. The microscopic

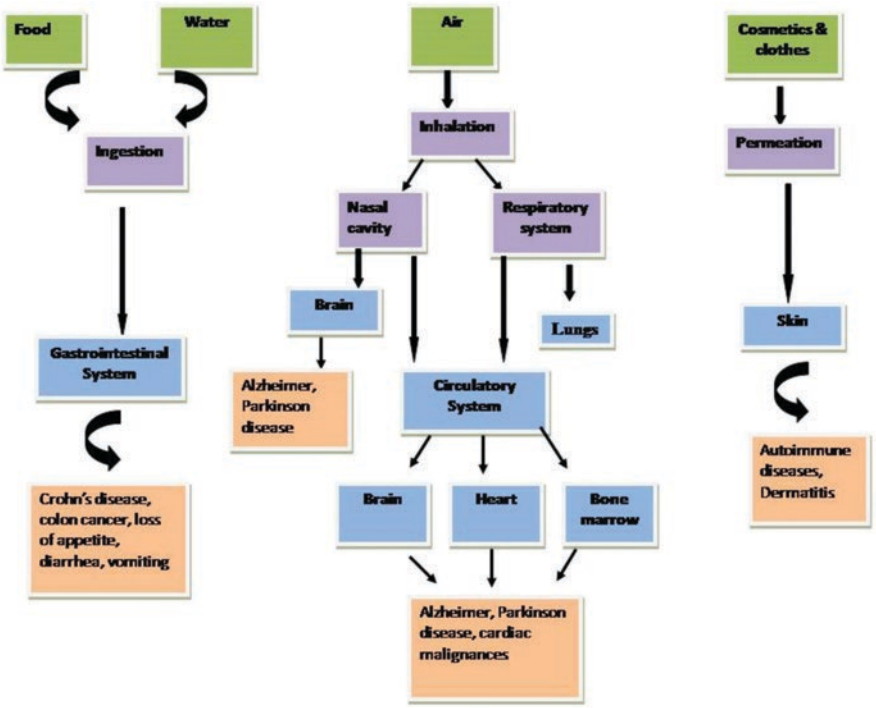

Fig. 1.3 The predominant routes of nanoparticles exposure and potential risks

lesions in liver, pancreas, heart and stomach were higher in lower doses of zinc oxide nanoparticles compared to higher dose (Pasupuleti et al. 2012).

Lin et al. (2014) showed that carbon nanorods induce growth inhibition, decrease body weight and delay calcification on oral exposure in zebrafish larvae. Titanium oxide ingestion resulted into oxidative stress which leads to digestive gland cell membrane injuries. Apart from epithelium, liver and kidney injuries, Crohn's disease and colon cancer are also associated with gastrointestinal absorption (Arora et al. 2012; Valant et al. 2012).

1.3.2.3 Cardiovascular System

The cardiovascular system consists of the heart, blood vessels, the cells and plasma that make up the blood. The main function of the heart is to pump blood continuously around the cardiovascular system. The mice exposed to single walled carbon nano tubes (10 and 40 μg/mouse) causes cardiovascular diseases such as mitochondrial DNA damage, elevation of mitochondrial glutathione and protein levels (Li et al. 2007).

Chen et al. (2008) developed a sealed plexiglas exposure chamber to study the age-related difference (young, adult, old rats) in cardiovascular responses against silver oxide nanoparticles. They analyzed the changes in serum biomarkers, hemorheologic, heart injury, as well as in the pathology. They found that on inhalation these nanoparticles caused severe myocardial problem, elevation of blood viscosity and fibrinogen concentration in old rats while less change in young and adult rats. The study showed that old rats are more sensitive to nanoparticle exposure compared to the young and adult rats.

Mitra Korani et al. (2013) analyzed the toxicity of silver nanoparticles in guinea pigs organs i.e. kidney, heart and bone at different concentrations. They found that the exposure with 0.1 mg/kg of silver nanoparticles may result in slight kidney, heart and bone damage.

1.3.2.4 Central Nervous System

The central nervous system consists of the brain and spinal cord which is responsible for receiving, interpreting and sending signals from the peripheral nervous system. The inhaled ultrafine carbon (35 nm) and manganese oxide nanoparticles (30 nm) can transport in the brain via the olfactory neuronal pathway (Oberdörster et al. 2004; Elder et al. 2006). Some studies showed that inhaled ultrafine particle results into proinflammatory response in the nervous tissue e.g. inhaled ultrafine carbon black (14 nm) can cause inflammatory changes in the brain olfactory bulb (Tin-Tin-Win-Shwe et al. 2006).

Zebra fish embryos were exposed to 50 μg/mL concentration of 1.5 nm gold nanoparticles having functional groups with different surface charges. The negatively charged 2-mercaptoethanesulfonic acid or positively charged

trimethylammoniumethanethiol 10 μg/mL of the gold nanoparticles having different functional group affects larval behavior, along with behavioral effects which will continue into adulthood (Truong et al. 2012).

Superparamagnetic iron-oxide nanoparticles in murine neural stem cells cause oxidative stress due to imbalance in the reactive oxygen species formation along with the antioxidant cell defense system. Superparamagnetic iron-oxide nanoparticles results in reduction of intracellular glutathione levels, altered activities of superoxide dismutase and glutathione peroxidase, hyperpolarization of the mitochondrial membrane, reduced cell membrane potential as well as increased DNA damage (Pongrac et al. 2016).

1.3.2.5 Skin

The human skin is having three layers; epidermis, dermis and subcutaneous which protect the body against the environment with a surface area of approximately 18,000 cm^2. Nanomaterials can penetrate the uppermost stratum corneum layer of skin and gain entry to the epidermis resulting toxicity in the lower cell layers. Some studies in rodent, pig, or human skin have showed that titanium oxide nanoparticles remain on the skin surface had not penetrated into the living skin (Pflücker et al. 2001; Schulz et al. 2002). However, some studies evidenced that the nanoparticles may penetrate into the epidermis or dermis.

Bennat and Mueller-Goymann (2000) tried titanium oxide nanoparticles to human skin as an aqueous suspension as well as oil-in-water emulsion and evaluated skin penetration. They found that these nanoparticles penetrated deeper into human skin when applied as an oil-in-water emulsion and that penetration was higher to hairy skin which shows that TiO_2 nanoparticles penetrate through hair follicles.

Emzaloid, a type of emulsion particle such as liposomes and non-ionic surfactant vesicles (niosomes) having diameter of 50 nm-1micron were found in the epidermis of human skin (Verma et al. 2003). Nanoparticles less than 4 nm can penetrate into skin, nanoparticles size between 4 and 20 nm can permeate intact and damaged skin, nanoparticles size between 21 and 45 nm can penetrate only through damaged skin while nanoparticles greater than 45 nm cannot penetrate into the skin. Metal nanoparticles can cause local and systemic effects, sensitization and create aggregates (Larese et al. 2015).

The toxicity of iron oxide nanoparticles of size 65 nm has been evaluated on human dermal fibroblasts and cells of the squamous tumor cell line. These nanoparticles induced oxidative stress via generation of reactive oxygen species and subsequent initiation of lipid peroxidation (Alili et al. 2015).

1.4 Conclusion

Nanomaterials are widely used in various fields such as food sector, medical and agriculture due to their specific properties. Nanosensors increases the quality and safety of food by decreasing the time for monitoring of various analytes i.e. pathogen detection, adulterants, chemical as well as biological contaminants. In medicine and agriculture nanosensors provide better management against various diseases. The nanomaterials reveal new green revolution with reduced agriculture risks. Increased use of nanoparticles resulted into significant nanoparticle exposure to environment as well as humans. The toxicity of nanoparticles depends mainly on their size, concentration, metals and the surface functional groups. Although we have covered toxicity of major nanomaterials but still there is less data regarding bioaccumulation of nanomaterials in tissues of aquatic animals and the ecotoxicity of different nanomaterials. Subsequently, further research is needed to reveal the interaction of nanosensors with human and environment. We can conclude that the nanomaterials found currently have many benefits and their side effects are limited which can be altered by the modifications in nanomaterial.

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Chapter 2
Nanosensors for the Detection of Chemical Food Adulterants

Namita Ashish Singh, Nitish Rai, and Avinash Marwal

Abstract Food adulteration is a major problem all across the globe and needs to be handled with the highest priority. Growing awareness about food safety and quality leads to the development of tools and techniques for the detection of food adulterants. With the advent of nanotechnology, it is now possible to detect the food adulterants using nanomaterials with enhanced sensitivity and low detection limits. In this chapter, several chemical food adulterants with their worldwide adulteration incidences and hazardous effect on human life have been discussed. Further, for each adulterant, novel nanosensors are described for their detection in various food samples along with the detection limit and mode of action. It was found that several major food adulterants exist like preservatives, melamine, urea, antibiotics, synthetic food dyes, dioxins, sucrose, starch, etc. Some of them possess a hazardous effect on human health. Several kinds of nanosensors exist for their detection in a variety of food samples like beverages, fish, vegetables, namkeen, sauces, milk, and milk products. Though the area of nanosensors based detection of food adulterants is growing swiftly, it has a long way to go since there are many adulterants for which no nanosensors are available. So, further research studies are needed to develop nanosensors for common food adulterants and explore the possibility of designing the novel nanosensors that could improve the detection sensitivity and specificity of the existing ones. With a tool as powerful as nanosensors, we will be better equipped to combat future scenarios of adulteration scandals.

Keywords Adulterants · Chemicals · Incidence · Nanosensors · Food safety

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2.1 Introduction

Food safety and quality are the most important features of healthy food and naturally becomes an essential aspect of human life enticing wide public attention. With growing awareness and easily accessible information, there is an increasing demand for food products that are free from hazardous contaminants and adulteration. Adulteration usually refers to the mixing of other substances of inferior quality with food or drink and subsequently, the product fails to meet the standards. The undesirable substance which is used to add in the food product for obtaining more profit is called an adulterant. As a result of adulteration, food becomes impure and unfit for human consumption. Food adulteration is a major problem, particularly in developing countries as approximately, 22% of foods are manually adulterated. Globally, 57% of people have developed health problems due to the consumption of adulterated and contaminated foods. In rural India, about 8–13% of milk is adulterated whereas in cities around 60–68% of milk supply is adulterated. Milk adulteration is uncontrolled in many developing countries such as China, Bangladesh, India, and Pakistan (Devrani and Pal 2018).

The U.S. Centre for Disease Control and Prevention has estimated that 48 million Americans get sick because of contaminated food, 1,28,000 were hospitalized and 3000 die due to food borne diseases (CDC 2010).The Food and Drug Administration has banned the transportation of adulterated foods, drugs, and cosmetics as per act and released some standards for food and milk products. Prevention of Food Adulteration 1954 act prohibits the manufacture, sales, and distributions of adulterated food as well as food contaminated with microbes, toxicants. The Food Safety and Standards Authority of India (FSSAI) in 2011 have developed some standards for food and to regulate as well as monitor the manufacture, processing, storage, distribution, sale and import of food to ensure the availability of safe food for human consumption.

Due to the rising interest in nanotechnology, the possibilities in various sectors are endless and the food industry is not an exception. Nanotechnology has revolutionized the food industry with a swift rate in a number of area such as packaging, preservation, nutrient delivery and most important nanosensing (Sekhon 2010). Nanosensors refer to nanoscale devices having a biological, chemical, or surgical sensory point to measure physical quantities and transmit information for detection and analysis (Kuswandi et al. 2017; Singh 2017). Nanosensors utilize different kinds of nanomaterials to detect toxic food adulterants with high sensitivity and specificity and this area is expanding quickly with new knowledge being added almost every day. So, in this chapter, chemical food adulterants, their worldwide incidence and hazardous effect on human life is discussed with the focus on their detection by nanosensors. Each adulterant has been described and their available nanosensors are discussed in detail with the mode of detection and components.

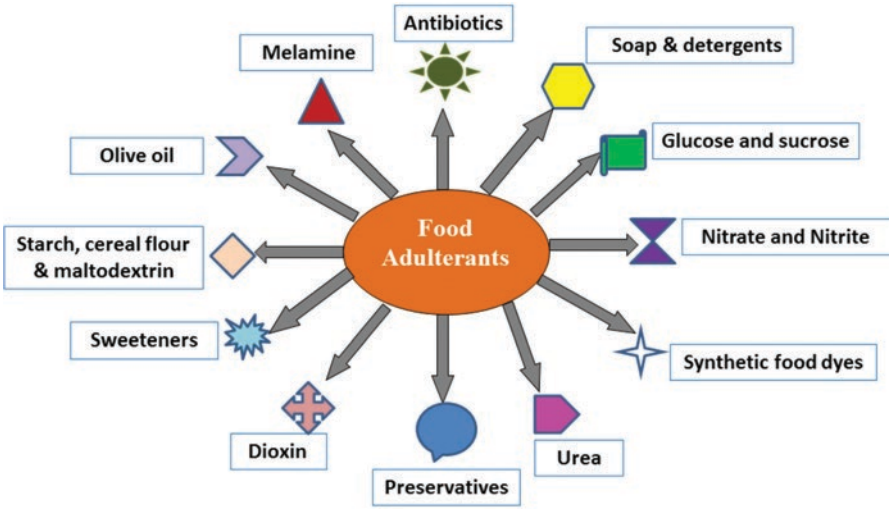

Fig. 2.1 Different types of adulterants in food

2.2 Food Adulterants

The food is spoiled due to adulteration with many harmful chemicals such as preservatives, melamine, sugar, starch, detergents, synthetic dyes, olive oil, antibiotics, urea, etc. depicted in Fig. 2.1.The worldwide incidence of food adulteration is shown in Table 2.1.

2.2.1 Food Preservatives

Food is a good nutrient medium for the growth of microbes. Bacteria present in food/milk converts lactose sugar into lactic acid subsequently spoil the product. Preservatives or neutralizers are added to prevent curdling and increase the shelf life of milk. Nowadays the use of preservatives is more common due to the growing demand for processed food. Sodium carbonate/bicarbonate, benzoic acid, boric acid, hydrogen peroxide, and formalin are commonly used preservatives (Das et al. 2016).

2.2.1.1 Hydrogen Peroxide

Hydrogen peroxide is widely used as a preservative in milk and milk products due to its potential to inhibit microbes and prevent milk spoilage, subsequently increasing shelf life. Hydrogen peroxide has natural bactericidal as well as sporicidal

Table 2.1 Incidence of food adulteration worldwide

S.No.	Sample	Adulterant	Incidence (%)	Locality	References
1	Fish	Dioxins	100	Italy	Scortichini et al. (2004)
2	Milk	Boric acid	8	Sindh, Pakistan	Barham et al. (2014)
		Detergent	32		
		Caustic soda	20		
		Rice flour	17		
		Hydrogen peroxide	13		
		Starch	12		
		Formalin	11		
		Urea and vegetable oil	10		
		Glucose	5		
3	Raw buffalo's milk (street vendors)	Formalin	10	Sohag Governorate, Egypt	Shaker et al. (2015)
		Hydrogen peroxide	3.3		
		Boric acid and borax	30		
4	Buffalo milk (Packed & Loose)	Glucose	30	Gandhinagar, Gujarat, India	Makadiya and Pandey (2015)
		NaCl	46		
		Sucrose	50		
		Ammonium Sulphate	96		
		Urea	100		
5	Market milk	Formalin	75	Hyderabad, Andhra Pradesh, India	Shaikh et al. (2016)
		Cane Sugar	60		
6	Chilli and curry	Sudan dye	62.50	Mumbai, Maharashtra, India	Singh et al. (2017)
		Rhodamine B	68.75		
7	Turmeric	Metanil yellow	48	Delhi-NCR, India	Purba et al. (2015)
8	Namkeen		44		
9	Jelly	Malachite green	48		
10	Green peas		40		
11	Milk	Starch	40	Lucknow, U.P. India	Maurya et al. (2017)
		Detergent	50		
		Formalin	60		
12	Paneer	Starch	50		
		Detergent	10		
13	Khoa	Starch	80		
		Detergent	30		
		Formalin	10		

(continued)

Table 2.1 (continued)

S.No.	Sample	Adulterant	Incidence (%)	Locality	References
14	Milk	Urea	22	Jabalpur, M.P. India	Pouranik et al. (2017)
		Neutralizer	62.96		
		Detergents	44		
		NaCl	59.25		
		Glucose	11.11		
		Nitrate	25.92		
		Ammonium Sulphate	22		
15	Raw milk from co-operative society	NaCl	16	Coimbatore Tamilnadu, India	Brindha et al. (2017)
		Neutralizer	13		
16	Meat	Melamine	100	New York, United State	Zhu and Kannan (2019)
17	Fish/seafood		82		
18	Cereal products		100		
19	Vegetables		100		
20	Cooking oil		80		
21	Beverages		100		
22	Dairy products		100		
23	Nutritional supplements	Melamine	47	South Africa	Gabriels et al. (2015)
24	Vegetables	Nitrate	30	Ljubljana, Slovenia	Kmecl et al. (2019)
		Nitrite	50		
25	Raw milk	Antibiotic residues	9.9	Niger	Madougou et al. (2019)
26	Milk	Tetracycline	5.7	Kermanshah, Iran	Bahmani et al. (2019)

properties and is often used to clean bottles, transporting and packing instruments in the food industry. As per the US Food and Drug Administration regulations, it is generally recognized as a safe antimicrobial oxidizing agent and the permissible limit of hydrogen peroxide is 0.05%. Hydrogen peroxide is also used for the inactivation of aflatoxins in contaminated raw whole milk followed by the addition of catalase enzyme to inactivate the residual hydrogen peroxide. The effect of hydrogen peroxide on the nutritive value of milk depends on its concentration. A low concentration of H_2O_2 (0.01%) results in increased casein proteolysis with rennin (Abbas et al. 2010). Side effects of hydrogen peroxide on humans include irritation of the gastrointestinal tract, nausea, vomiting, lethargy, sleep disorder, cardiac arrest, coma and shock finally resulting in death (Watt et al. 2004; Silva et al. 2012).

2.2.1.2 Benzoic Acid

Benzoic acid and its salts have been used as preservatives in the food industry for many years to control various bacteria, yeasts and fungi were responsible for food poisoning/spoilage, such as *Escherichia coli, Listeria monocytogenes, Aspergillus spp.* and *Penicillium spp.*(Wang et al. 2013). China, the USA, India, and the European Union have banned its addition to milk. However, it is considered generally recognized as a safe category with a maximum limit of 0.1% in the USA, although its concentration varies from0.15% to 0.25% in other countries (Chipley 2010).

As per the Codex Alimentarius Commission, the maximum recommended level of benzoates including benzoic acid is 300 mg/kg in dairy-based desserts and 1000 mg/kg in dairy fat spreads, but it is not allowed in raw milk (Codex 1995). Side effects of benzoic acid include asthma, hyperactivity in children, genotoxicity, mutagenicity and pseudo allergy in human beings (Tfouni and Toledo 2002; Mutlu 2010).

2.2.1.3 Boric Acid

Boric acid is frequently used preservative by small level industries in different food processing products namely noodles, seafood (fish ball), dairy products and meat products. Sometimes it is added in food products to control starch gelatinization and to enhance the colour, texture as well as flavour of the food. It may cause nausea, vomiting, bloody diarrhoea, renal failure, delirium, headache followed by weakness and coma (See et al. 2010). Yiu et al. (2008) analysed five kinds of noodles and a type of fish ball from Bintulu, Sarawak, Malaysia by the curcumin-acetic acid method. They found that the yellow noodles contained the highest concentration of boric acid with a mean of 2.034. Boric acid has been declared unsafe by FAO/WHO Expert Committee as a food additive and many countries, including the United Kingdom, Thailand, China, and Malaysia have banned it but still, manufacturers are using it.

2.2.1.4 Sodium Carbonate/Bicarbonate

Sodium carbonate/bicarbonates are mainly used to avoid the spoilage of milk by neutralizing the natural as well as developed acidity of milk by bacteria (Malame et al. 2014). Sodium bicarbonate is a generally recognized as a safe category and can be used in food up to 2% (Federal regulations, 1990). It is being used as a resource of carbon dioxide for leavening of baked items and the control of pH, taste, as well as texture (Singh et al. 2013). It is used as a stabilizer in condensed, evaporated and powdered milk at a concentration less than 0.3% (Cerdan et al. 1992). Carbonates/bicarbonates can cause gastrointestinal problems, including gastric

ulcer, diarrhoea, and colon ulcer along with electrolyte disturbance (Barham et al. 2014).

2.2.1.5 Formaldehyde/Formalin

Formalin is a 40% solution of formaldehyde, which is intentionally added as a preservative in food products namely raw milk, noodles, fish, chicken, etc. to increase its shelf life. Suwanaruang (2018) analysed the formalin contamination in seafood and frozen meat samples in Kalasin, Thailand and found the highest contamination in white shrimp, second highest in shrimp and dolly fish while the third was frozen chicken. Formalin lowers the microbial count of raw milk but does not have an effect on fat and increases its heat stability which affects the quality of milk products in different ways (Sharma et al. 2011).

Formalin is extremely, and by the International Agency for Research on Cancer has classified formaldehyde as a carcinogen which is very toxic to humans even in small concentrations (IARC 2004). According to the US Environmental Protection Agency, the oral reference dose is 0.2 mg/kg (US EPA 1999). European Food Safety Authority has recommended an oral exposure limit i.e. 100 mg formaldehyde per day, corresponding to 1.7 and 1.4 mg kg^{-1} body weight per day for 60 kg and 70 kg respectively in humans (EFSA 2014). However, the Food Safety and Standards Authority of India, 2011 does not allow formaldehyde for the preservation of any food product. It ingestion results into vomiting, diarrhoea, abdominal pain, decreased body temperature, memory loss, insomnia, mood swings, blindness, renal failure, associated cancer and tumor development (Wooster et al. 2005; Afzal et al. 2011). Inhalation of formalin causes respiratory symptoms, irritation of eyes, nose along with throat (Zhang et al. 2008).

2.2.2 Melamine

Melamine is widely recognized as one of the major food adulterants across the globe. It is a heterocyclic organic compound with a 1,3,5-triazine-2,4,6-triamine structure. Melamine appears as a white crystalline solid exhibit partial solubility of 13.4 g/l at 50 °C. Melamine has diverse industrial usage in resins, flame retardants dinnerware, glue, laminates, adhesives, moulding compounds and coatings (Singh and Kumar 2009). Melamine was utilized as a cheap alternative of protein in pet and ruminant feed due to the presence of large nitrogen (66%) content. However, the practice was discouraged later mainly because of slow melamine metabolism and the release of toxic ammonia as a by-product. Past scandals of melamine adulteration have shown that it is added illegally in food items to falsely inflate their apparent protein content. These apparent 'protein-rich foods' give false-positive values in standard protein assays which are utilized by the majority of industries (Field and Field 2010). Though melamine adulteration was practiced for quite a long time, it

became obvious in 2008, after the illness of around 3 lakh infants and death of 6 others in China. In the Chinese scandal, the infant formula, milk, and milk-derived products were found to be tainted with melamine. In 2007, illegal adulteration of pet food in the U.S. caused illness and death of dogs and cats due to the deposition of melamine– cyanuric acid crystals in the kidney leading to kidney failure. Other incidences across the globe have demonstrated melamine toxicity and therefore, melamine is found to be a highly hazardous addition in foodstuff (Rai et al. 2014). Nonetheless, the chance of recurrence of melamine adulteration has still not subsided, especially in the developing countries (Rai and Banerjee 2017). The World Health Organization (WHO) has limited daily intake of melamine to 0.2 mg/kg body weight per day (WHO 1989) whereas the US Food and Drug Administration promulgated the allowed limit of melamine of 1 mg/kg in infant formula and 2.5 mg/kg for other dairy products (Mauer et al. 2009).

2.2.3 Antibiotics

Antibiotics revolutionized the health industry by providing the treatment of a wide range of infectious diseases. They are used to check and/or prevent microbial growth in several pathological conditions. However, their unnecessary and excessive use has led to the development of resistant microorganisms who have acquired the ability to rescue themselves from particular or set of antibiotics. Also, there is an inappropriate use of antibiotics such as in animal feed, which increases the chance of antibiotic resistance and possesses serious side effects. To improve the growth and production of animals, antibiotics are heavily used in animal husbandry which contaminates the derived food products (Mungroo and Neethirajan 2014). Moreover, the antibiotics are excreted unaltered in the urine and faeces of animals due to limited degradation, and thereby contaminating soil and natural water resources (Carvalho and Santos 2016). These harmful effects of antibiotic usage in animal feed have led to a strict ban on the usage of a certain antibiotic such as enrofloxacin. However, how much these preventive measures are working is still debatable. To meet the growing demand for the product and gain more profit, some farms may still illegally use antibiotics causing the production of a contaminated food product like milk and meat. For this reason, the level of antibiotics in the products of farm animals must be monitored strictly with sensitive and specific assays for the assurance of food safety.

2.2.4 Urea

Urea is another commonly available chemical that is illegally added mainly in milk and dairy products. It is used as an adulterant due to low cost, wide availability most importantly rich in nitrogen. So it is used to enrich milk with non-protein nitrogen

and decrease the overall nutritive value of the milk. Although urea is normally present in the milk, however, its typical concentration in milk varies around 18–40 mg/dL (Kohn 2000). The upper limit for urea concentration in milk is normally accepted at 70 mg/dL (Trivedi et al. 2009). Urea is especially added to milk to increase the shelf life, taste consistency, appearance and for the apparent fat content reduction in the milk (Renny et al. 2005). The milk adulterated with high levels of urea is quite hazardous for human consumption especially to children and during pregnancy. It can also develop toxicity and several conditions in healthy individuals like ulcers, osteoporosis, indigestion, acidity, cancer and kidney failure. It can speed up the process of attaining puberty in young girls (Tariq 2001). The presence of urea above the upper limit in milk can cause severe health problems, including indigestion, acidity, ulcers, cancer, malfunctioning of kidneys, and osteoporosis (Nikoleli et al. 2010; Sadat et al. 2006). During milk processing at a higher temperature, the excess urea decomposes into ammonia, carbonic acid, and acetic acid causing the partial fermentation mediated formation of urinary bladder calculi. Further, there can be the formation of biuret which is known to cause hypotension and irritation while urinating (Prout 2003). Due to such a harmful effect on humans, the level of urea must be strictly regulated and tested from time to time to ensure the presence of permitted levels.

2.2.5 Synthetic Food Dyes

Synthetic food dyes/colours are the chemicals which are fabricated and do not exist in nature. The synthetic food dyes/colours are being universally used in various processed foods namely chilli products, sauces, ice creams, soft drinks, green peas, jelly, and juices, etc. to make the products more attractive. Food Adulteration Act, 1954 have permitted eight food colours namely carmoisine, ponceau 4R, erythrosine, sunset yellow-FCF, tartrazine, brilliant blue FCF, indigo carmine and fast green FCF. However some unpermitted colours such as metanil yellow, rhodamine B, orange G, pararosaniline, auramine O, Sudan dyes, blue VRS, etc. are also added in some foods as adulterants (Singh et al. 2017). The dye sunset yellow is being used globally in malachite green in frozen peas and tur dal beyond the permitted level. However, malachite green is completely banned and sunset yellow FCF can be used upto 100 ppm (FSSAI 2012). The dye metanil yellow is a mutagen and responsible for cancer development as well as cause damage to gastric mucin (Gupta et al. 2003; Saxena and Sharma 2015). These dyes have side effects like gastroenteritis with vomiting, diarrhoea, cancer of liver and kidney.

2.2.6 Dioxins

The polychlorinated dibenzodioxins and polychlorinated dibenzofurans are considered as dioxin. Several incidences of dioxin contamination in food and feed occurred all over the globe especially in Europe in the years 1997 and 2010. Due to their hazardous contamination in fatty rich food like milk, dairy products, meat products, and fatty fish, etc., it becomes a major problematic chemical with significant human exposure. They are very harmful and potential carcinogens due to their high stability and reactivity. They can cause severe bioaccumulation by depositing in the fatty tissue of an organism after entering the food chain either intentionally or unintentionally (Chobtang et al. 2011). The reaction between the aryl hydrocarbon of dioxins and the xenobiotic-responsive element is the main cause of toxicity leading to genetic manipulation, malignant formation and immune pathology (Kasai et al. 2006). It is because of this hazardous nature, the necessity of establishing food and feed monitoring programs for dioxins is of utmost importance.

2.2.7 Glucose and Sucrose

Glucose and sucrose (cane sugar) may be added to cover the addition of water in milk and sucrose is added to enhance its taste. Sugar is being added into milk to increase the specific gravity of the milk which was diluted by the addition of water (Sharma et al. 2011). The addition of glucose and sucrose in milk enhances milk density which is much higher compared to other adulterants namely urea (Goswami and Gupta 2008). The increased sugar concentration in condensed milk lowers the water activity and subsequently prevents the growth of pathogenic as well as spoilage microbes.

2.2.8 Nitrate and Nitrite

Nitrates and nitrites are an innate part of our ecosystem and exist naturally in our environment. The atmospheric nitrogen is converted to nitrate and nitrite as a part of the nitrogen cycle. They also form an important part of plant-based food. As far as human health is concerned, nitrate and nitrites have quite a diverse effect. While a moderate amount is extremely important for survival, excess amounts may cause cancer and nitrosative stress that can impair biomolecules like membrane proteins and DNA. To humans, the primary source of nitrate is vegetables which are rich in nitrates. These ions are extensively used as food additives for protection against chemical degradation, microbial spoilage and preserving food quality. These ions are also heavily used in processed meats during the curing process. The extensive use of nitrates and nitrites in meat processing has been discouraged due to their

potential ability to convert into nitrosamines that have carcinogenic potential. In the same line, the precise levels for nitrites and nitrates in meat and other food products have been set by the European Food Safety Authority recommendations and WHO. So it important to screen the nitrate and nitrite content in food items which are potential sources for humans.

2.2.9 Soaps and Detergents

Soap is sodium and potassium salt of fats or fatty acids having high molecular weight while detergent is a surfactant that has foaming properties. Surfactants can be divided into four major groups namely anionic, cationic, non-ionic, and amphoteric based on their nature of the hydrophilicity. Soaps and detergents are important constituents for the construction of synthetic milk (Kamthania et al. 2014). Synthetic milk is not real milk, it is prepared by adding approximately 1.2 g/l of detergent in water along with detergent, urea, melamine, sodium chloride, sodium bicarbonate, vegetable oil, and water. The adulteration of synthetic milk is usually done at the rate of 5–10% in natural milk. Synthetic milk is prepared with low cost and resembles pure milk in color as well as thickness (Sadat et al. 2006; Santos et al. 2013; Mudgil and Barak 2013).

Strong detergents may result in odd odour and taste to the milk, simultaneously decreases the nutritive value of the milk. Adulteration of detergents may cause respiratory irritation, hypotension, food poisoning and gastrointestinal complications such as abdominal pain and vomiting (Trivedi et al. 2009; Singuluri and Sukumaran 2014). Detergents have octylphenol and nonylphenol content which results in breast cancer in women as well as decreases sperm production in men (Afzal et al. 2011).

2.2.10 Olive Oil

Olive oil is the commonly adulterated vegetable oil globally due to its high demand and low production. The main components of olive oil are triacylglycerols and fatty acids. Olive oil is having high nutritional value due to antioxidants including vitamin A comparative to other vegetable oils (sunflower, soybean, canola, etc.). In the United States, the highest cases of food scam were found related to olive oil adulteration (Johnson 2014). Usually, olive oil is adulterated with low-quality oil having the same color as well as texture e.g. mixing other edible or non-food grade oils with olive oil. Adulteration of olive oil results in adverse effects on humans.

2.2.11 Starch, Cereal Flours and Maltodextrin

Starch is a polysaccharide being broadly used as a thickening and gelling agent in foods mainly in milk-based sweets, jellies, sauces, custards, and desserts. Starch is a mixture of amylase and amylopectin. Wheat flour, arrowroot, rice flour, starch, dextrin etc. is added in milk to increase the solids not fat content as well as thickness and to prevent the detection of additional water (Barham et al. 2014). In humans, consumption of starch adulterated milk causes diarrhoea and its collection in the body is lethal to diabetic persons (Afzal et al. 2011).

Maltodextrin is a gluten-free polysaccharide that can be produced from a different variety of starches namely corn, potato, rice or wheat starch by partial hydrolysis. Food safety and standards authority of India has not permitted maltodextrin as an additive. However, it is used as a food additive in different processed foods namely beer, dairy products, salad dressings, and sauces. The maltodextrin is soluble in water comparative to native starch. Milk distributors add maltodextrin in raw milk to increase its total solid content and it replaces the fat in milk. Maltodextrin at the rate of 3% acts as a fat replacer, stops oxidation and enhances the flavour of the product (Divya et al. 2012).

2.2.12 Sweeteners

Sweeteners are chemical substances which are sucrose alternative that is used to impart a sweet taste and increase the appetite with or without calorie addition. Sweeteners are synthesized either through plant extracts or processed by chemical synthesis. They are used in canned foods, carbonated and powdered drinks, breakfast cereals, jam, baked and canned foods, candies, etc. (Mortensen 2006). The sweeteners are of two types- intense and bulk- based on the use and sweetness value to sucrose. Generally, intense sweeteners are many times sweeter than the sucrose (e.g. aspartame and saccharin) mainly used as a low-cost alternative while bulk sweeteners have low sweetness than sucrose (e.g. sugar alcohol such as sorbitol) used as bulking ingredients to provide a texture of the product (Kuswandi et al. 2017). Sweeteners, in excess, may cause several health complications such as high blood sugar, cancer, cardiovascular disease, obesity and dental caries (Findikli and Turkoglu 2014). So it is quite necessary to analyze the sweetener concentration in various foodstuffs.

2.3 Nanosensors for Detection of Adulterants

Detection of these adulterants is very important nowadays for improving the food quality. Many conventional methods are available for the detection of various adulterants such as gas chromatography, mass spectrometry, high-performance liquid chromatography (HPLC), fluorimetry, other biosensors, etc.(Ngamchana and Surareungchai 2004; Pena et al. 2005; Abbas et al. 2010; Yeh et al. 2013; Rai et al. 2014). These methods have higher sensitivity and selectivity with qualitative as well as quantitative detection. However, the above methods have some disadvantages such as high cost, tiresome sample preparation, complicated instruments, more time consumption and requirement of skilled personnel.

Nanosensors are nanotechnology-based sensors having either the size of the sensor or its sensitivity at the nano level. The sensitivity and specificity of biosensors could be improved by using nanomaterials known as nanobiosensors. Nanobiosensors are widely used in food packaging, processing and agriculture (Singh et al. 2017). Nanoparticles have an active role in the adsorption of biomolecules due to their high surface ratio and free energy. Different studies report that as their optical, mechanical and transport properties change due to their nanoscale properties (Vastarella and Nicastri 2005).

Nanosensors can be classified based on different aspect as shown in the Fig. 2.2. Optical nanosensors produce optical signals such as absorbance, fluorescence, luminescence, surface-enhanced Raman scattering, etc. to convert the biological or chemical signal. The electrochemical biosensors are based on the use of electrochemical transducers for capturing the signals generated in the presence or absence of analyte. They can be further divided into amperometric, conductometric,

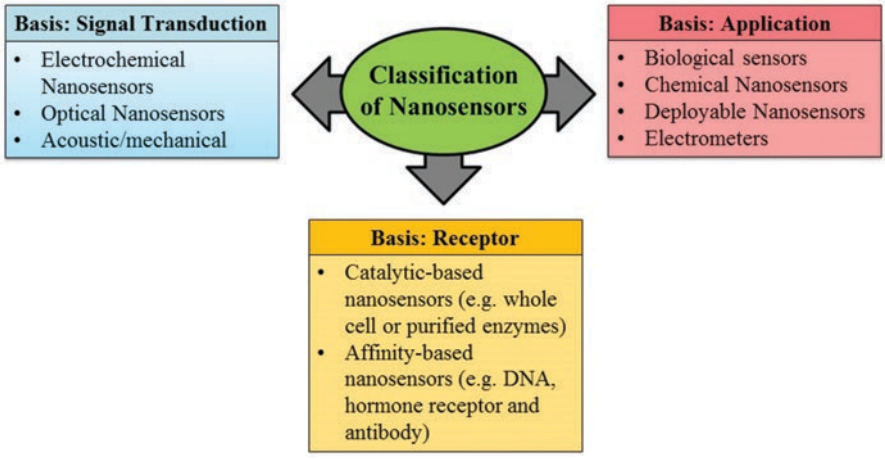

Fig. 2.2 Broad classification of nanosensors on the basis of signal transduction, receptors used and application

potentiometric. Nanomaterials like carbon nanotubes and nanowires are used as electrochemical sensing components (Sarkar et al. 2017).

Tripathy et al. (2019) have developed a smartphone-based miniaturized milk adulteration detection system. This system measures the level of acidity in milk using an indicator paper. They have prepared paper-like material that is made up of nanosized (~10^{-9} m diameter) fibres of nylon by electrospinning process. This material is loaded with a combination of three dyes that changes colour in response to changes in acidity. They have also developed algorithms that can be incorporated into a smartphone for accurate detection of the change in acidity. The sensor exhibit three color-signatures related to pure (pH between 6.6–6.9), acidic (pH < 6.6), and basic (pH > 6.9) milk samples facilitating a colorimetric detection. The colour changes on the sensor strips were captured using a smartphone camera which subsequently assigned to one of the three pH ranges using an image-based classifier.

2.3.1 Nanosensors for Food Preservatives

2.3.1.1 Hydrogen Peroxide

Annamalai et al. (2012) developed an amperometric sensor based on the multi-walled carbon nanotube and chitosan film coated glassy carbon electrode for the detection of hydrogen peroxide in milk detection of limit 0.463 AM^{-1} cm^{-1}. A novel amperometric sensor has been developed for the monitoring of hydrogen peroxide-based on Nafion/exfoliated graphene oxide-cobalt oxide nanocomposite with the detection limit of 0.3 μmol L(−1) for analysing milk samples (Ensafi et al. 2013). An amperometric sensor was developed using nano iron oxide and carbon nanotubes for monitoring hydrogen peroxide with a detection limit of 3.7 nM. Nano iron oxide was manufactured using the thermal co-precipitation technique further the electrochemical studies were performed using cyclic voltammetry and amperometry (Thandavan et al. 2015).

2.3.1.2 Benzoic Acid

Shan et al. (2007) developed a novel sensitive amperometric biosensor based on a polyaniline-polyacrylonitrile composite matrix for the monitoring of food preservative, benzoic acid. This biosensor was based on the inhibition of benzoic acid on the biocatalytic activity of the polyphenol oxidase enzyme to its substrate (catechol) with the detection of a limit of 2×10(−7) M for real-time monitoring of benzoic acid in milk, yoghurt, sprite and cola samples. A highly sensitive amperometric biosensor was developed based on the immobilization of tyrosinase enzyme by calcium carbonate nanomaterials for the detection of benzoic acid. This biosensor worked on the detection of benzoic acid was performed via its inhibition of benzoic acid on

the tyrosinase /nano-CaCO3 modified glassy carbon electrode with the detection of limit 1061.4 mA M^{-1} cm^{-2} (Shan et al. 2008).

An amperometric biosensor based on the inhibition of benzoic acid on the activity of tyrosinase and polyphenol oxidase was reported for the detection of benzoic acid with detection limit 0.03 μM in beverage samples. In this biosensor, tyrosinase is entrapped in titania gel modified with multiwalled carbon nanotubes and Nafion (Kochana et al. 2012).

2.3.1.3 Boric Acid

A localized surface plasmon resonance sensor has been developed using gold nanoparticles for the monitoring of boric acid. The plasmonic responses of gold nanoparticles were found linear with the increased boric acid (Morsin et al. 2012). A localized surface plasmon resonance sensor was reported based on gold nanoplates which were prepared by the seed-mediated method. This sensor can detect boric acid in water with a limit of detection of 0.01 mM (Morsin et al. 2017).

2.3.1.4 Formaldehyde/Formalin

An amperometric biosensor has been developed based on the doping of nanogold in a poly-2-hydroxy ethyl methacrylate membrane. In this biosensor, 1.0% ferrocene mediator and alcohol oxidase were used as biocatalysts which were further deposited on a carbon screen-printed electrode. The entrapment of nanogold particles increases the electrochemical response as minute nanogold particles ease the electron transfer in the formaldehyde. The designed biosensor is used to monitor formaldehyde in fresh foodstuffs namely tauhu (bean curd), meatballs, dried and wet fish with a limit of detection 0.007 mM (Sundari et al. 2012). An electrochemical sensor was developed based on gold nanoparticles and chitosan deposition on a glassy carbon electrode for the detection of formaldehyde in fish samples with detection of limit 0.1 ppm (Aini et al. 2016). A numerical surface plasmon resonance biosensor has been developed based on graphene-M_OS_2 with titanium oxide-silicon dioxide hybrid structure for the monitoring of formalin. The detection is based on the attenuated total reflection method by monitoring the change of surface plasmon resonance angle versus the change of minimum reflectance (Hossain et al. 2019).

2.3.2 Nanosensors for Melamine

Nanotechnology-based determination of melamine involves nanorods, nanotubes, nanocomposites, nanowires, carbon and quantum dots, nanocrystals and nanoclusters. A study reported bis(8-quinolinolato)zinc(II) complex nanorod array mediated melamine sensing which showed melamine assay linearity from 39.6 to 238 nM

making it suitable melamine detection tool (Li et al. 2010). Another study determined melamine through surface-enhanced Raman scattering (SERS) using ZnO/Au nanoneedle arrays and detected 10 nM to 100 μM concentration (Chen et al. 2010). Nanocomposites have also been successfully detected melamine in several studies. In a study by Chen and Liu 2011, a silver-nanoparticle-decorated composite silver/carbon nanospheres facilitated SERS-based detection of melamine with a limit of detection (LOD) 50 nM (Chen and Liu 2011). Another study reported melamine detection using composites of silver nanoparticles and glutathione capped zinc selenide quantum dots which detected melamine with the LOD of 872 pM (Cao et al. 2014). Wang et al. (2017) utilised a microfluidic chip system based on silver-gold nanocomposite modified indium tin oxide support for the SERS-based melamine detection with a LOD of 10 nM. A real-time screening approach in food sample using carbon quantum dots (CQDs)-silver nanocomposite was utilised by Wang and co-workers for ultrasensitive colorimetric determination of melamine with a LOD of 62.6 pM (Wang et al. 2018). In a recent study, fluorescence resonance energy transfer effect was exploited for melamine discrimination using polydopamine-glutathione nanoparticles and silver nanoparticles with a LOD of 23 nM (Tang et al. 2018). Carbon dots (C-dots) are also an attractive option for melamine detection due to their nano size, low toxicity and biocompatibility (Jaleel and Pramod 2018). A study showed the utility of C-dots and anti-quenching ability of Hg^{2+} for fluorescence mediated real-time melamine determination with a LOD of 0.3 μM (Lei et al. 2016).

The quantum dots have also proved helpful in melamine determination in several studies. A study showed room-temperature phosphorescenc -based detection of melamine from dairy products using L-cysteine-capped Mn-doped zinc sulfide quantum dots with a LOD of 47 nM (Demirhan et al. 2015). A study by Xu and Lu (2015) showed that the molecularly-imprinted CdTe quantum dots, synthesized in a single step, successfully performed ratio-metric discrimination of melamine with a LOD of 38 nM. Another study by Zhang and Chen (2018) showed a technique of visual detection of melamine in milk samples via a ratio-metric fluorescent probe. The assay utilised a CdTe quantum dots with a molecularly-imprinted polymer for melamine recognition and showed a LOD of 103 nM.

The studies using metal nanoclusters for the detection of melamine are also increasing. In a particular study, tiopronin-stabilized gold nanoclusters were developed which could discriminate melamine through fluorescence quenching with a LOD of 32 nM (Yang et al. 2016). Another study reported colorimetric melamine detection of melamine using horse-radish peroxidase (HRP) functionalized gold nanoclusters (HRP-Au NCs) based on the reversal of the mercury (II) mediated inhibition (Cao et al. 2016).

Kalaiyarasan et al. (2017) reported glutathione-protected gold nanoclusters mediated melamine detection in cow milk and infant formulas by fluorescence-based ratio-metric assay with a LOD of 28.2 μM. A recent study by Lin et al. (2018) reported fluorescence mediated sensing of melamine using egg-white protected gold nanoclusters by microwave technique with a LOD of 0.46 μM. Another study exploited the lipoic acid-stabilized silver nanoclusters and quantified melamine via

metallic interaction with a LOD of 174 nM (Ren et al. 2018). Similarly, Hou et al. (2018) reported SERS based melamine detection through silver nanocluster arrays over a large-area silica nanosphere template with a LOD of 100 nM.

2.3.3 Nanosensors for Antibiotics

The aptamer-based nano biosensing methods are quite commonly used for antibiotic detection, especially for kanamycin. In the same line, Li et al. (2014) described a method for the detection of kanamycin by ultrasensitive fluorescence resonance energy transfer (FRET) aptasensor, using oleic acid modified up conversion nanoparticles. The sensor showed a cross-reaction with other antibiotics and good kanamycin detection ability with a detection limit of 9 pM. A particular study used fluorescence-labelled single-stranded DNA (ssDNA) functionalized graphene oxide sensor in an aptamer-based assay for the simultaneous detection of multiple chemical food contaminants. This is usually a complicated process to detect multiple contaminants simultaneously due to the need for different microenvironment in different assays. However, Zhang et al. (2015) designed a low-cost paper-based microfluidic device for detecting aminoglycoside antibiotics, silver (I) ion (Ag(+) and mercury (II) ion (Hg(2+) residues simultaneously in food samples.

In another study, colorimetric aptasensors with fluorescence quenching ability was designed for streptomycin detection using aqueous gold nanoparticles and double-stranded DNA. The reaction involves aptamer binding to streptomycin and quenching of fluorescence by gold nanoparticles. The assay was used successfully in milk and serum and showed good selectivity with a limit of detections of 47.6 nM (Emrani et al. 2015).

Alternatively, immunosensors can also be used to determine antibiotics. In one such work, an enantio selective and sensitive electrochemical immunosensor was designed to determine chiral antibiotic ofloxacin. The analysis utilised multienzyme-antibody functionalized gold nanoflowers electrochemical sensor and antigen immobilized multiwall carbon nanotubes-poly(L-lysine) (He et al. 2015). Similarly, another study used silver nanoparticles based electrochemical immunosensing methods for the simultaneous electrochemical determination of tetracycline and chloramphenicol (Liu et al. 2014).

Further, a method of a competitive chemiluminescent immunoassay was described using the new luminol functionalized silver nanoparticles for the detection of chloramphenicol. The method reported a simple, fast, sensitive and selective technique applicable in foodstuffs like milk and honey with a low detection limit of 7.6×10^{-9} g/mL (Yu et al. 2014). Also, there are studies utilising phospholipid liposomes based new self-signalling sensory system with R6G dyes on the surface to detect neomycin. Upon neomycin recognition, the r6G dye displaces from the liposome surface to turn on the fluorescence signal (Seo et al. 2015). Besides, studies are utilizing molecularly imprinted sensors using different types of nanomaterials such as magnetic nanomaterials, graphene oxide, and metal nanoparticles for

electrochemical sensing of antibiotic residues (Lihua et al. 2013; Liu et al. 2012; Yola et al. 2014).

2.3.4 Nanosensors for Urea

In a study, a urease immobilised nanoporous alumina membranes were used to develop a novel piezoelectric sensor for urea that measures the change in the conductivity of immobilized urease/urea reaction with separated porous alumina/urease electrode has been developed through measuring the conductivity change of immobilized urease/urea reaction. The urea analysis was rapid, stable, and highly selective with a lower detection limit of 0.2 μM (Yang et al. 2007).Similarly, another study showed that the urease immobilized on modified fullerene nanomaterial may catalyse the urea hydrolysis in solution. This bioconjugate was deposited on potentiometric non-plasticized poly(n-butyl acrylate) electrode to screen urea with significant sensitivity and stability (Saeedfar et al. 2013). Alqasaimeh et al. (2014) showed urease immobilized, amine-modified silica-gel nanospheres for urea sensor development with a detection limit of 10 mM. The analysis determined the urea level through colorimetric change by the enzymatic urea hydrolysis. In another study, the urease enzyme nanoparticles were prepared and found to be more active and stable than the native enzyme. The enzyme nanoparticles were immobilized nitrocellulose membrane which was preactivated with chitosan. The membrane was then mounted on a pH meter connected electrode for potentiometric urea screening. The analysis showed a detection limit of 1 μM/L with a working range of 2–80 μM/L (Jakhar and Pundir 2018). In a recent study, a nanodevice combining enzymatic receptor and a signalling subunit is introduced for the design of fluorometric urea detection. A urea nanosensor was designed using urease-functionalized Janus gold-mesoporous-silica nanoparticles (Au-MSNPs) and the urea hydrolysis mediated release of Alexa-Fluor-647-labeled oligonucleotide from Au-MSNPs. This urea nanosensor was found to be suitable for the identification of urea adulterated milk samples (Llopis-Lorente et al. 2018).

2.3.5 Nanosensors for Synthetic Food Dyes

Wang et al. (2015) developed an electrochemical sensor for the detection of sunset yellow based on gold nanoparticles/graphene electrode deposition on glassy carbon electrode with detection of limit 2 nM. The fluorescent carbon quantum dots (diameter 5 nm) which release bright yellow photoluminescence were prepared for the detection of tartrazine in food samples. These carbon dots have a high fluorescent quantum yield and low toxicity (Xu et al. 2015). A novel sensor was developed for the monitoring of erythrosine using glucose modified carbon paste in food samples with detection of limit 21.6 nM (Nayak and Shetti 2016). An electrochemical sensor

has been developed based on graphene oxide and multi-walled carbon nanotubes for the monitoring of synthetic food dye i.e. sunset yellow and tartrazine in orange juice with a limit of detection 0.025 μM and 0.01 μM respectively (Qiu et al. 2016).

He et al. (2018) developed an electrochemical sensor for the monitoring of tartrazine based on titanium oxide–reduced graphene oxide composite modified glassy carbon electrodes. This composite increased the electrochemical response in the presence of tartrazine with a limit of detection 8.0×10^{-9} mol/L in carbonated beverage samples. A novel electrochemical nanosensor has been designed for the concurrent analysis of two toxic food dyes namely metanil yellow and fast green based on nanosensors in real water and juice samples. The surface of a glassy carbon electrode was modified with calixarene and gold nanoparticles which significantly increase the signals of the selected food dyes in comparison to the bare glassy carbon electrode. The developed nanosensor has detection limit 9.8 and 19.7 nM for metanil yellow and fast green respectively (Shah 2020).

2.3.6 Nanosensorsfor Dioxins

Mascini et al. (2004) designed an analytical system for dioxins using a pentapeptide coupled piezoelectric sensor where peptides, designed through molecular modelling, work as dioxin selective biomimetic receptors. The analysis of 2,3,7,8-tetrachlorinated dibenzo-p-dioxin and a mixture of dioxins was done using a quartz crystal microbalance sensors array functionalised by two terminal cysteine residues. The polypeptide layer on the sensor facilitated the electrostatic interactions between the amino acids and the dioxins with a detection limit of 1 ppb (Mascini et al. 2004, 2005). Soh et al. (2003) described a method utilising surface plasmon resonance (SPR) for monitoring of a known dioxin precursor 2,4-dichlorophenol through indirect competitive immunoassay. The anti-2,4-dichlorophenol antibody was immobilized on a gold-thin layer of SPR sensor chip which measured the 2,4-dichlorophenol with good sensitivity and detection limit of 20 ppb Park et al. (2006) utilized quartz crystal microbalance immunosensor for determining 2,3,7,8-tetrachloro-p-dibenzodioxin using monoclonal antibodies which showed an excellent capacity for dioxin detection. Tsutsumi et al. (2008) determined toxic equivalent concentrations of dioxin-like polychlorinated biphenyls in retail fish samples through rapid biosensor immunoassay using surface plasmon resonance technique with a quantitative limit of 1 ng/gm of the tested sample.

2.3.7 Nanosensors for Glucose

An amperometric biosensor has been developed based on the activity of glucose dehydrogenase and diaphorase co-immobilized with NAD (+) into a carbon nanotube paste electrode modified with an osmium functionalized polymer. This sensor

can detect glucose up to 10 micromol L^{-1} in alcoholic beverages (Antiochia and Gorton 2007).

2.3.8 Nanosensors for Nitrite

Afkhami et al. (2014) designed gold nanoparticles/multi-walled carbon nanotube/carbon paste electrodes using electrodeposition for a highly sensitive and selective voltammetric determination of nitrite in foodstuffs with the detection limit as low as 1×10^{-2} µmol per L of nitrite. A nanosensor was developed for the detection of nitrite using the direct electrochemistry of myoglobin on a reduced graphene oxide multi-walled carbon nanotubes -platinum nanoparticles nanocomposite (Mani et al. 2014). Canbay et al. (2015) developed an electrocatalytic analyser based on immobilized myoglobin on the surface of the multi-walled carbon nanotube -Nafion-cysteamine modified gold electrode. It may detect nitrite at a concentration of 52.0 ± 2.1 µM in a water sample. In a recent study, a titanium nitride nanoparticles decorated multi-walled carbon nanotube nanocomposite was used for nitrite sensing. The analysis showed good reproducibility with a detection limit of 0.0014 µM (Haldorai et al. 2016). A recent study utilised a novel reduced graphene oxide/molybdenum disulfide/poly (3, 4-ethylene dioxythiophene) (rGO/MoS2/PEDOT) nanocomposite electrode for non-enzymatic detection of nitrite ion. The electrode performed with good sensitivity (874.19 µA/µM/cm^2) and a low detection limit (LOD) (0.059 µM, S/N = 3) and found suitable for nitrite ion sensing in milk and water (Madhuvilakku et al. 2020).

2.3.9 Nanosensors for Detergents

A simple, user-friendly, cost-effective gold nanoparticles based method has been developed by Kumar et al. (2019) for detection of anionic detergents sodium dodecyl-benzene sulfonate and anionic detergents having a limit of detection i.e. 23 µg/ml and 92 µg/ml respectively. Gold nanoparticles were synthesized by chemical reduction of chloroauric acid using trisodium citrate. The principle of this assay includes the prevention of salt-induced aggregation of gold nanoparticles in the presence of anionic detergents, where anionic detergents protect the gold nanoparticles andcause a red shift in surface plasmon resonance absorption. On addition of inducer (NaCl) to the gold nanoparticles causes aggregation which is reflected in color change of the solution from red to purple while in presence of anionic detergent spiked sample inhibition of gold nanoparticles aggregation was observed. The developed method is specific for anionic detergent detection and is free from interfering with other adulterants such as sodium hydroxide (1 mg/ml), sodium

bicarbonate (1 mg/ml), urea (60 mM), sodium azide (1% w/v) hydrogen peroxide (100 ppm), potassium (1.74 mg/ml) and lactose (4.7% w/v).

2.3.10 Nanosensors for Olive Oil

An electrochemical sensor has been developed to differentiate between different types of vegetable oils e.g. olive oil, sunflower oil, and corn oil (Apetrei et al. 2005). An optical nanosensor based on graphene quantum dots was developed for the analysis of phenol in olive oil with detection limit 0.12 mg L^{-1} (Benitez-Martinez and Valcarcel 2014).

2.3.11 Nanosensors for Sweeteners

The level of sweetener in food items has been detected using nanosensors in several studies. A study by Nikolelis et al. (2001) reported that a bilayer lipid membranes based nanosensor detected the levels of potassium acesulfame, saccharin, and cyclamate through electrochemical transduction. A sensitive method of voltammetric determination of neohesperidindihydrochalcone using single-walled carbon nanotubes modified glassy carbon electrode as described (Yang et al. 2014). A direct quantification method for the determination of saccharinate ion was developed based on a polypyrrole-doped selective membrane with the quantification limits found between 5×10^{-4} mol L^{-1} and over 1 mol L^{-1}(Alvarez-Romero et al. 2010).

2.4 Conclusion

A rapidly emerging field of nanotechnology has widened the application of nanosensors in diverse areas such as medicine, industry, pharmacy, agriculture, etc. Nanosensors are made up of diverse varieties of nanomaterials which by working in conjunction with chemical and/or biological components signals for the detection of food adulterants. Nanosensors are widely used in the area of food safety and quality assurance and it is a major tool in combating problems like food contamination and adulteration. It is mandatory to emphasize the huge difference these nanotechnology-based tools are making in everyday life. In this chapter, several incidences of food adulteration, chemical adulterants, and their specific nanosensors have been discussed in detail. Several nanosensors are described for each adulterant by briefly explaining their mechanistic action for the possible detection.

Through our discussion, it can be stated undoubtedly that nanosensors have a major role in the detection of chemical food adulterants such as preservatives, melamine, antibiotics, urea, dyes, dioxins, nitrite, etc. In this context, different

nanosensors equipped with advanced technologies will continue to explore the area of food safety and quality assurance in times to come. However, still, this area of study has a long way to go since there are many adulterants for which no nanosensors were available like starch, cereal flours maltodextrin while few are available for detection of glucose. So, further research studies are needed to ensure the rapid nanosensing of these common food adulterants and explore the possibility of designing the novel nanosensors that could improve the detection sensitivity and specificity of the existing ones. Such improvised nanosensors will enable us to take necessary measures in the future if needed.

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Conflict of Interest The authors declare no conflict of interest.

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Chapter 3
Metal Oxides and Biopolymer/Metal Oxides Bionanocomposites as Green Nanomaterials for Heavy Metal Ions Removal

Shadpour Mallakpour and Farbod Tabesh

Abstract Regarding issues like increase in world population, industrialization, global warming, and irregular consuming of the underground water sources led to less accessibility of clean water for daily needs and drinking. On the other hand, these less available water sources are exposing to hazardous and harmful pollutants like heavy metal ions. Therefore, purification of water sources became a huge concern. Many efforts have been made in this regard using numerous methods and materials. Some of them are expensive, complicated, and time-consuming. One of these materials, which are less expensive and simple, is metal oxides and their nanocomposite with polymers. Metal oxides owing to enormous capacity, high energy density, and rich resources are widely used and favorable, while metal oxides have some intrinsic defects like poor electrical conductivity, aggregation, and large volume change. A highly efficient to overcome these lacks is combining metal oxides with polymers to make polymeric nanocomposites. In this chapter, an endeavor has been made to demonstrate the superiority of metal oxides over other materials in water treatment as well as cover recent works based on the preparation of metal oxides and polymer/metal oxide nanocomposites and their capability in water treatment.

Keywords Green nanotechnology · Polymer/metal oxide nanocomposite · Adsorption · Water remediation · Thermodynamics · Isotherm · Kinetic · Adsorption mechanism · Free energy

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3.1 Introduction

Serious problems are created in the human body due to the existence of heavy metal ions in aqueous media and food resources. Heavy metal ions would bring several diseases like memory loss, digestive problems, and so on. These toxic and hazardous metal ions are released in the environment from several industries like a battery, pesticides, fertilizer, paint and pigment, steel, electronics, automobiles, etc. in different ways. Opposing dyes, which are biodegradable, heavy metal ions, are not, they can be deposited in the human body and disorder the function of biomolecules by replacing the metal ion in the biological molecules of the human body. Although some of the heavy metal ions are necessary for the human body like Mg, Cu, Zn, Cu, etc., they should not exceed their limits.

Thus, removing or detoxification of the water sources are a new, vital, and inevitable subject. Several methods are employed for this aim like adsorption, reverse osmosis, chemical precipitation, etc. and it is reported that the adsorption is the most favored method owing to its significant advantages. Among numerous materials for the elimination of poisonous and hazardous heavy metal ions from aqueous media, metal oxide nanoparticles and especially their polymeric nanocomposites because of excellent features have been center of attention. This chapter will present a short survey of the synthesis method of metal oxide nanoparticles, advantageous of the metal oxide nanoparticles, and their nanocomposite over other used materials and the applied methods for detoxification of water sources.

3.2 Toxicity, Sources, and Consequence of Heavy Metal Ions on the Human Body

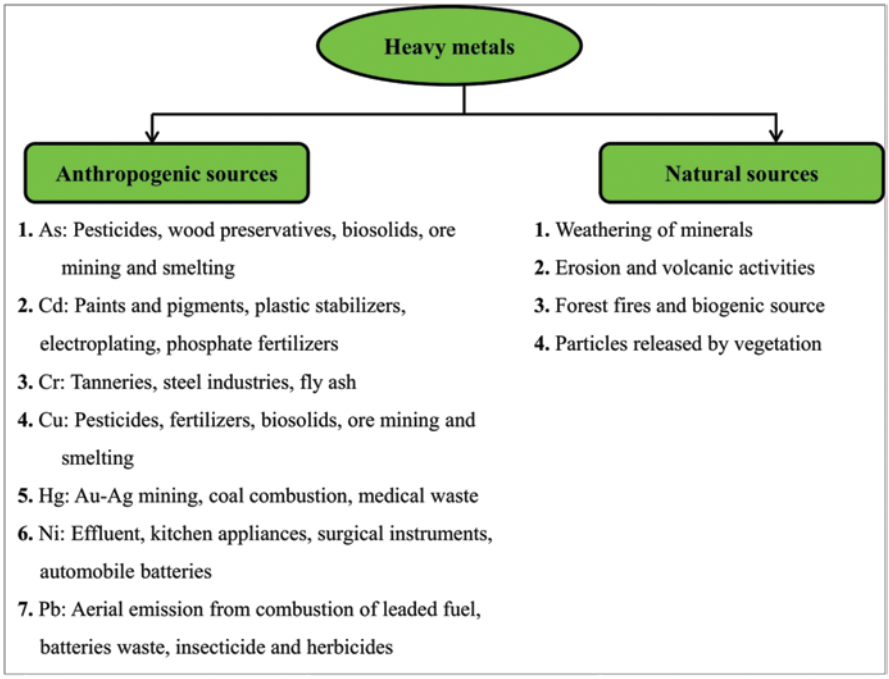
Heavy metal ions owing to their toxicity cause severe problems to human and living creature health like immunological, pulmonary, neurological, cardiovascular, endocrine disorders, and cancers (Krstić et al. 2018; Zhou et al. 2018). Heavy metal ions prevent biological groups of the biomolecules like proteins and enzymes to function regularly by replacing metal ions in the biomolecules (Krstić et al. 2018). Carboxyl, sulfide, and amino groups are the most common electron donor to heavy metal ions. Table 3.1 shows the side effects of these ions on the human body (Barakat and Kumar 2014).

Generally, heavy metal ions are released from their polluting source to the ecosystem in different ways (Li et al. 2018b). These hazardous pollutants are mostly used in the industries such as leather tanning, battery factories, electronics, mining operations, fertilizer, metal plating, power generations, pharmaceuticals, mining, etc. (Guo et al. 2018; Krstić et al. 2018; Zhou et al. 2018). Scheme 3.1 illustrates the sources for several metal ions in the ecosystem (Dixit et al. 2015).

Table 3.1 Permissible limits and health effects of various toxic heavy (Barakat and Kumar 2014)

Heavy metal	WHO[a], permissible limit for portable water (ppm)	Toxic effect
As	0.01	Skin manifestations, visceral cancers, vascular disease
Cd	0.003	Kidney damage, renal disorder, human carcinogen
Cr	0.05	Headache, diarrhea, nausea, vomiting, carcinogenic
Cu	2.0	Liver damage, Wilson disease, insomnia
Hg	0.001	Rheumatoid arthritis, and diseases of the kidneys, circulatory system and nervous system
Ni	0.02	Dermatitis, nausea, chronic asthma, coughing, human carcinogen
Pb	0.01	Damage the fetal brain, diseases of the kidneys, circulatory system and nervous system
Zn	3.0	Depression, lethargy, neurological signs and increased thirst

[a]World Health Organization



Scheme. 3.1 Sources of heavy metals in the environment. (Dixit et al. 2015)

3.3 Detoxification Methods and Used Materials

The limit of metal concentrations in food, water, and any other sources, which are related to the human, is lowered every year. Therefore, the process of elimination of heavy metal ions are done using several methods like chemical precipitation, biological systems, adsorption, ion-exchange, reverse osmosis, ultrafiltration, membrane process, electrochemical process, solvent extraction, photocatalytic degradation, etc. (Krstić et al. 2018; Lu and Astruc 2018; Mallakpour et al. 2018; Mallakpour and Rashidimoghadam 2019; Saravanan et al. 2016). Choosing a suitable process depends on some determining parameters like concentration of the metal, pH, temperature, solubility of the pollutant, and costs. Among these methods, adsorption seems the best one due to its advantages such as cheap, fast, more efficacy, convenience, and so on (Mallakpour et al. 2018). Table 3.2 provides a comparison among common methods (physical or chemical processes) to remove heavy metal ions (Barakat and Kumar 2014).

For this aim, many materials have been applied as adsorbents for the adsorption process like activated carbon, biosorbents, resins, polymeric fibers, nanomaterials,

Table 3.2 Current treatment technologies for heavy metals removal involving physical and/or chemical processes (Barakat and Kumar 2014)

Method	Advantage	Disadvantage
Oxidation	Rapid process for toxic pollutants removal	High energy costs and formation of by-products
Ion exchange	Good removal of a wide range of heavy metals	Absorbent requires regeneration or disposal
Membrane filtration	Good removal of heavy metals	Concentrated sludge production, expensive
Adsorption	Flexibility and simplicity of design, ease of operation and insensitivity to toxic pollutants	Adsorbents require regeneration
Coagulation/ flocculation	Economically feasible	High sludge production and formation of large particles
Electrochemical treatment	Rapid process and effective for certain metal ions	High energy costs and formation of by-products
Ozonation	Applied in gaseous state: alteration of volume	Short half-life
Photochemical	No sludge production	Formation of by-products
Irradiation	Effective at lab scale	Requires a lot of dissolved O_2
Electrokinetic coagulation	Economically feasible	High sludge production
Fenton's reagents	Effective and capable of treating variety of wastes and no energy input necessary to activate hydrogen peroxide	Sludge generation
Biochemical treatment	Feasible in removing some metals	Technology yet to be established and commercialized

and metal oxide nanoparticles (Gupta et al. 2015, 2018; Gupta and Saleh 2013; Hussain and Mishra 2018; Kobielska et al. 2018; Rajendran et al. 2016; Vikrant and Kim 2019; Zhao et al. 2018). Regardless of some advantages of these materials, some defects such as costs, regeneration, and the operation have limited the use of them (Krstić et al. 2018). Among these materials, metal oxide nanoparticles have been focused by researchers owing to its extraordinary feature. Some advantageous and disadvantageous of metal oxide nanoparticles are as follows (Wu et al. 2012):

A. **Advantages:**

Huge capacity/capacitance, high packing and energy density, abundant resources, and so on.

B. **Disadvantages:**

Poor electrical conductivity, massive volume change, sever aggregation/agglomeration, large irreversible capacity, low initial coulombic efficiency, and poor rate capability.

3.4 Methods of Synthesis of Metal Oxide Nanoparticles

Generally, two main groups of ways exist for the synthesis of the metal oxide nanoparticles based on the nature of the mechanism, which are as follows (Fernández-Garcia and Rodriguez 2007):

3.4.1 Liquid-Solid

This is the most common method in which the control of the morphological properties is more convenient. This category is divided into several subgroups as follows:

3.4.1.1 Co-precipitation

In which the salts of precursor-like chloride, nitrate, etc. are hydrated in the aqueous in the presence of a base to form oxo-hydroxide. To enhance the control of the size and chemical homogeneity which are difficult, using sonochemical methods, surfactants, etc. would be useful approaches to overcome this defect (Fernández-Garcia and Rodriguez 2007; Rao et al. 2018).

3.4.1.2 Sol-Gel

In this method, the precursors (usually alkoxides) are hydrolyzed in alcoholic solutions to create oxo-hydroxides. Losing water and condensation of the molecules creates the metal oxide network in this way where the polymerization by condensation happens for hydroxyl species to form a gel. Drying and calcination lead to excellent the metal oxides (Fernández-Garcia and Rodriguez 2007; Salinas et al. 2018).

3.4.1.3 Microemulsion

In this method, a ternary system containing water, oil, and surfactant create micro/nano-reaction vessels. Precipitation of metal oxide precursor as oxo-hydroxides in water which are limited by surfactant-hydroxide contact (Fernández-Garcia and Rodriguez 2007; Osseo-Asare 2018).

3.4.1.4 Solvothermal

Thermally decomposition of metal complexes (using an autoclave) is the basis of this manner. Surfactants can be added to control the particle size and avoid aggregations (Fernández-Garcia and Rodriguez 2007; Wu et al. 2018).

3.4.2 Gas-Solid

This widely used manner which is suitable for the synthesis of ultrafine metal oxide powders consists of two methods, chemical vapor deposition and pulsed laser deposition (Fernández-Garcia and Rodriguez 2007).

3.4.2.1 Chemical Vapor Deposition

Production of uniform and pure nanoparticles and films are the advantage of the method while the experimental parameters need to be set up carefully. Among several processes for chemical vapor deposition, classical (activated/pyrolytic), plasma-assisted, photo pulsed laser deposition, and metalorganic is the most used processes (Fernández-Garcia and Rodriguez 2007).

3.4.2.2 Pulsed Laser Deposition

During this route, the sample is heated up to 4000 K and quick evaporation, ionization, and decomposition followed by mixing of wanted atoms occur. The radiation energy from pulses causes the gaseous moieties to obtain the power to be deposited in a heated substrate to allow crystalline growth (Fernández-Garcia and Rodriguez 2007).

3.5 Application of Metal Oxides in the Elimination of Heavy Metal Ions

Here, several works about utilization of the most used metal oxides in the elimination of heavy metal ions are reported. It was tried to collect different metal oxide structure including nanoparticles, nanorods, nanotubes, etc.

3.5.1 Iron Oxide Nanostructures

Hao et al. (2019) removed Cu^{2+} and Sb^{3+} using core-shell nanoparticles containing Fe_3O_4. First, they used the co-precipitation method to prepare the Fe_3O_4 and subsequently, they modified the as-prepared NP to obtain the NCs. By calculating the isotherm models, the adsorption was compatible with the Langmuir model. For understanding the mechanism of this procedure, kinetic models were applied, and results fit with the pseudo-second-order model. In the removal of Cu^{2+}, the best pH was 6 and for Sb^{3+} was 2. According to the Langmuir isotherm, the $q_{e,\,max}$ was 55.56 and 51.07 mg/g for Cu^{2+} and Sb^{3+}, respectively. Figure 3.1 shows the impact of time on the removal of Cu^{2+} and Sb^{3+}.

(Ge et al. 2012) investigated isotherm, kinetics, and thermodynamics of removal of Cd^{2+}, Zn^{2+}, Pb^{2+}, and Cu^{2+} using modified Fe_3O_4 which was prepared through co-precipitation method. As reported the optimum pH was 5.5 (Fig. 3.2), the mechanism of sorption was explained by pseudo-second-order model (and therefore, chemisorption), the Langmuir isotherm model fit well with the results, and through thermodynamic calculations, the adsorption process reported as an endothermic process. The value of $q_{e,\,max}$ for Cd^{2+}, Zn^{2+}, Pb^{2+}, and Cu^{2+} reported as 29.6, 43.4, 166.1, and 126.9 mg/g, respectively.

(Campos et al. 2019) used a novel adsorbent containing core-shell magnetic nanoparticles, $CoFe_2O_4$@γ-Fe_2O_3, to adsorb Cr^{6+} from the water. According to the outcomes, the optimum pH was 2.5, because in pH range of 1–6 the Cr^{6+} is the $HCrO_4^-$ and is favorable with the positively-charged surface of the adsorbent. The rate of the adsorption at the first minutes was fast and the equilibrium reached within 20 min, and pseudo-second-order model was fitted kinetic model for the removal of

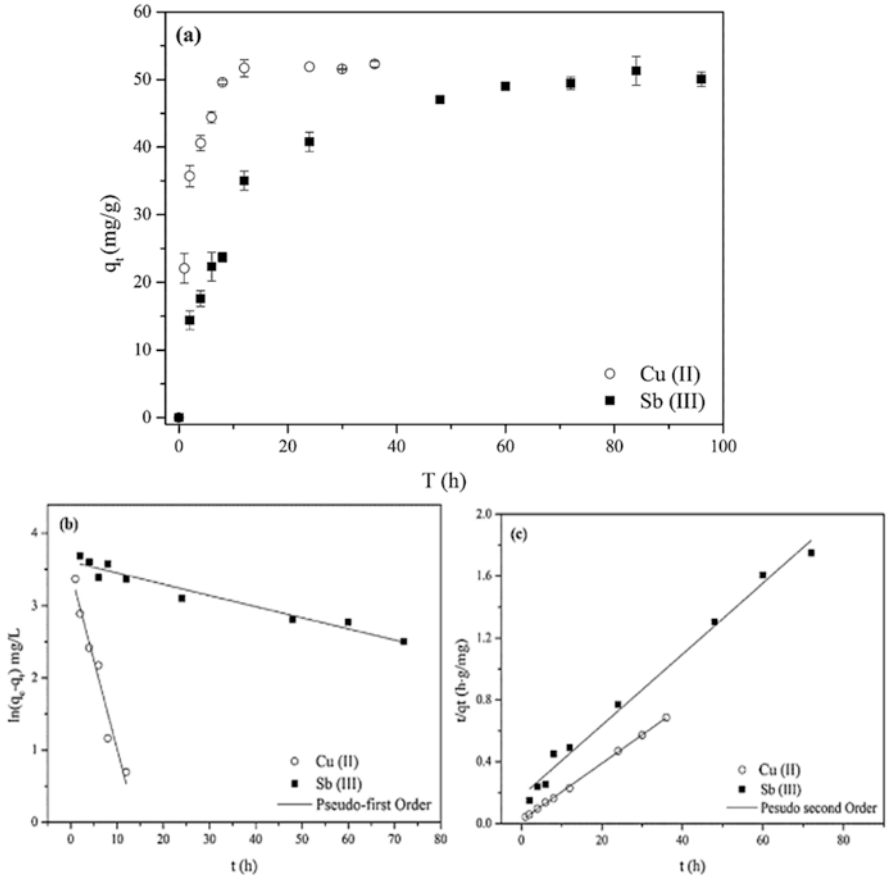


Fig. 3.1 Effect of contact time on Cu (II) and Sb (III) sorption onto NH_2-Fe_3O_4-NTA (**a**). Initial metal concentration, 50 mg/L; sorbent dose, 0.5 g/L; pH, 5.5 ± 0.2 (for Cu(II)), 4.6 ± 0.2 (for Sb(III)) and temperature, 25 ± 1 °C. Fitting with the pseudo-first-order adsorption kinetic model (**b**); Fitting with the pseudo-second-order adsorption kinetic model (**c**). (Hao et al. 2019)

Cr^{6+} onto the $CoFe_2O_4$@γ-Fe_2O_3. Also, the Freundlich isotherm model was the suitable isotherm model which suggesting multilayer adsorption (Fig. 3.3). Based on the thermodynamic equations, the adsorption is spontaneous because of the negative value of the Gibbs free energy; and increasing the temperature led to the increase in the adsorption, because the amount of the Gibbs free energy became more negative. This means that the adsorption is endothermic which is proved by the positive value of the enthalpy. Therefore, it is expected that the randomness increases and the positive amount of the entropy proved that. The amount of $q_{e,\,max}$ for the removal of Cr^{6+} reported being 15.6 mg/g. These results obtained using nonlinear isotherm forms. It could have been better if they had used linear forms and made a comparison among them to find a better one.

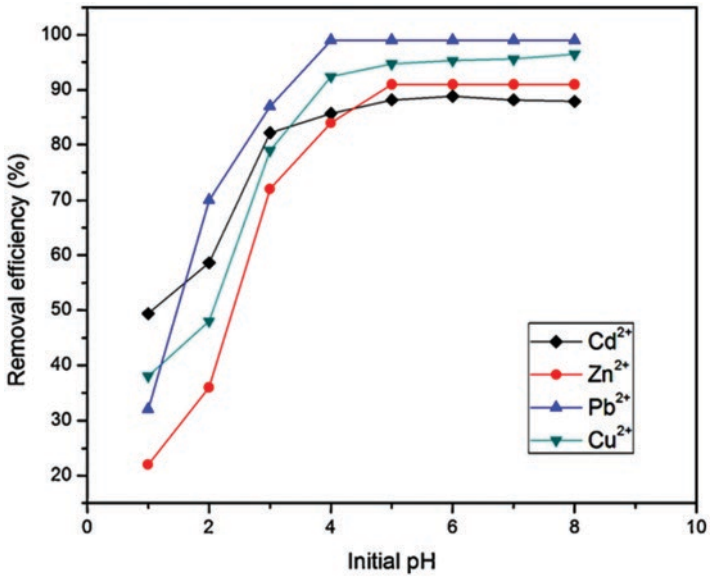


Fig. 3.2 Effect of pH on the adsorption of metal ions; adsorbent: 0.05 g, concentration of initial metal ions: 100 mg L^{-1}, volume of metal ions solution: 50 ml, time: 2 h, at 298 K. (Ge et al. 2012)

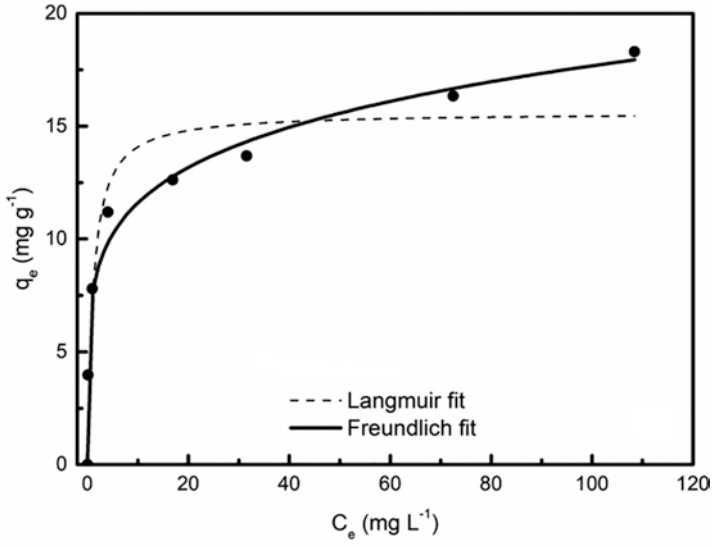


Fig. 3.3 Fitting of equilibrium adsorption data using Langmuir and Freundlich models for sample MNA-S (Campos et al. 2019)

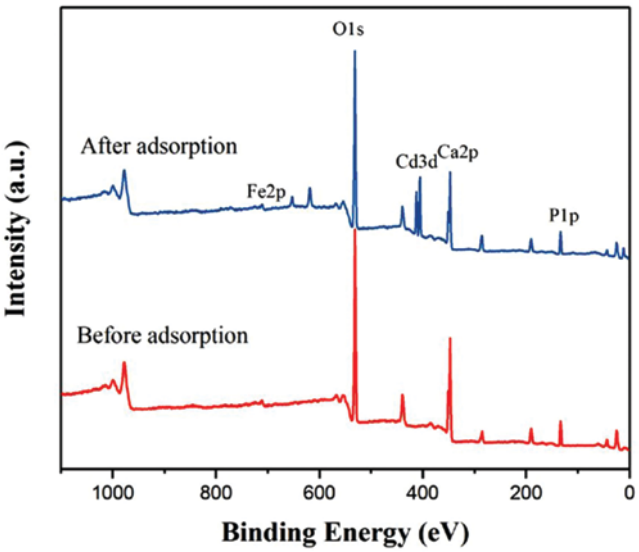


Fig. 3.4 XPS survey spectra of the γ-Fe_2O_3/Fe-doped HAP before and after Cd(II) adsorption. (Xiao et al. 2018)

Magnetic γ-Fe_2O_3/Fe-doped hydroxyapatite (HAP) was used for the adsorption of Cd^{2+} from water by Xiao et al. (2018). The adsorbent was synthesized through co-precipitation method, and then the adsorption took place. It was mentioned that at pH above 8 the Cd^{2+} is precipitated as hydroxylated form. In pH range of 5–8, the adsorption was steady. The Freundlich isotherm model was the proper model for the removal of Cd^{2+} also, according to the kinetic models, pseudo-second-order was a better model for this process. The $q_{e,\,max}$ calculated to be 257.9 mg/g. To prove the adsorption of Cd^{2+} onto the adsorption, the X-ray photoelectron spectroscopy image was shown in Fig. 3.4 and appearing the binding energy around 406 eV corresponded to the Cd3d photoelectron, which proved the adsorption of the Cd^{2+}.

3.5.2 Titanium Oxide Nanostructures

(Debnath and Ghosh 2011) used TiO_2 nanoparticles to remove Cd^{2+} and Cu^{2+}. The NPs were prepared through co-precipitation method using $TiCl_4$ as a precursor, and the adsorption of metal ions was done using the adsorption. They examined the morphology of the synthesized TiO_2 nanoparticles using scanning electron microscopy and transmission electron microscopy to show they reached nanosized TiO_2 (Fig. 3.5).

Influences of pH, temperature, time, and on concentration on the removal process were studied, and the kinetics, isotherms, and thermodynamics were

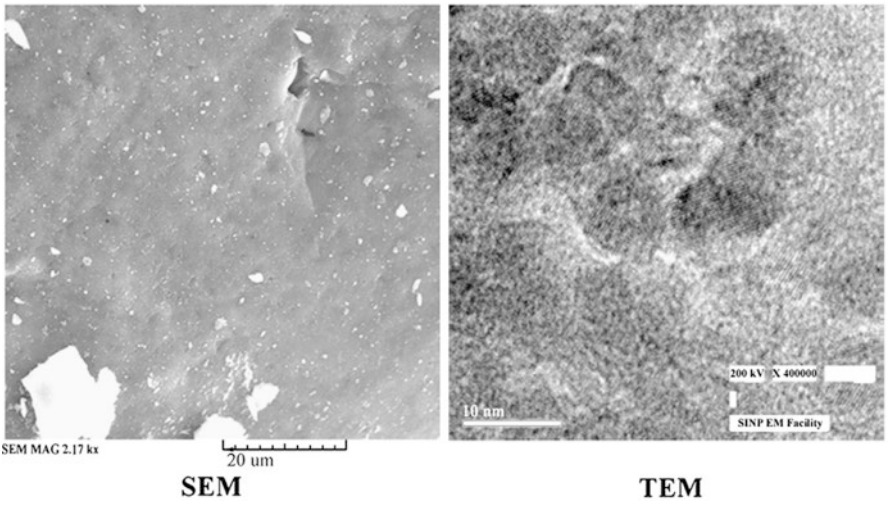


Fig. 3.5 Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images of NHTO (*NHTO* Nano-particles agglomerate of hydrous Ti^{4+} oxide). (Debnath and Ghosh 2011)

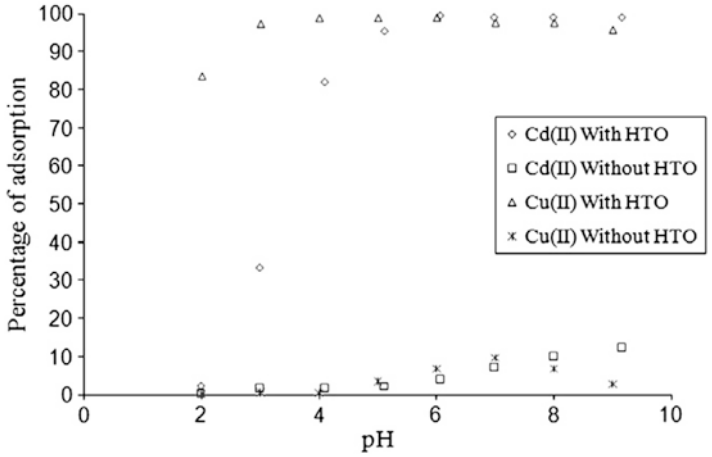


Fig. 3.6 Variation of adsorption percentages of Cd(II) and Cu(II) on NHTO versus initial solution pH at 30 °C. (Debnath and Ghosh 2011)

calculated. They examined the pH from 2 up to 9 and found that the adsorption was increased with increasing the pH up to 6, in where the adsorption maintained constant up to the pH = 9. It is reported that after pH = 6, the formation of the hydroxyl form of ions prevents further adsorption. Therefore, the author selected pH = 5 as an optimum pH (Fig. 3.6). In order to examin the effect of temperature on the process, they investigated the adsorption capability in the temperature range of 15–60 °C. They

found that the adsorption was increased with increasing the temperature and reported the endothermic nature of the adsorption. The kinetics study revealed that the adsorption obeys pseudo-second-order model. Calculations of the isotherms proved that the adsorption matched with Langmuir isotherm model, which suggested the creation of a monolayer. It was reported the $q_{e,\,max}$ for adsorption of Cd^{2+} was 60.24 mg/g and for Cu^{2+} was 52.63 mg/g.

3.5.3 Manganese Oxide Nanostructures

(Li et al. 2018a) reported the elimination of Tl^{+} by a MnO_2 and magnetic pyrite cinder based composite. The adsorbent was prepared through the hydrothermal process. In this work, the influence of altering pH, adsorbent dosage, time, and concentration were investigated. The outcomes showed that in basic pH, removal efficiency is more due to the negatively-charged surface of the composite, which attracts the positive metal ions. Increasing in the amount of the adsorbent increased the adsorption of Tl^{+} (Fig. 3.7). Also, increasing the dosage decreased the equilibrium time and it can be concluded that an increase in dosage increased the rate of adsorption. Studies of isotherm models revealed that the adsorption mechanism follows Langmuir isotherm model and the maximum adsorption capacity obtained 290 mg/g at room temperature. Regarding the kinetic models, the adsorption was fit with the pseudo-second-order model better, which suggests chemical interaction between adsorbent and adsorbate. $q_{e,\,max}$ was obtained 320.1 mg/g for adsorption of Tl^{+}.

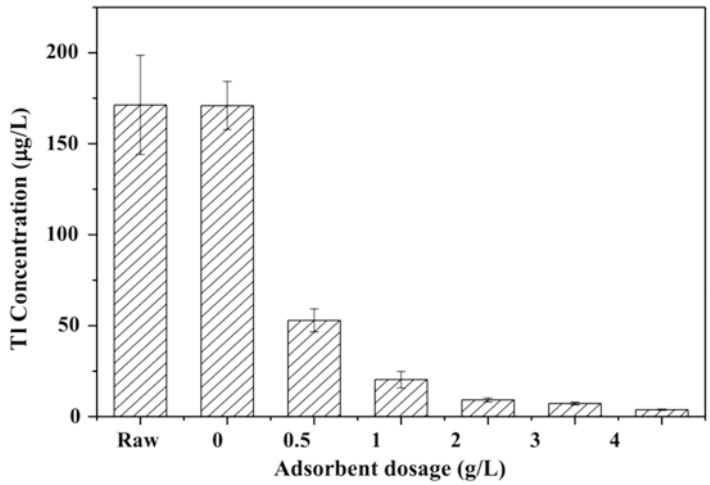


Fig. 3.7 Application of the MnO_2@pyrite cinder for the treatment of zinc oxide wastewater (Reaction temperature 298 K, reaction pH 10, reaction time 30 min). (Li et al. 2018a)

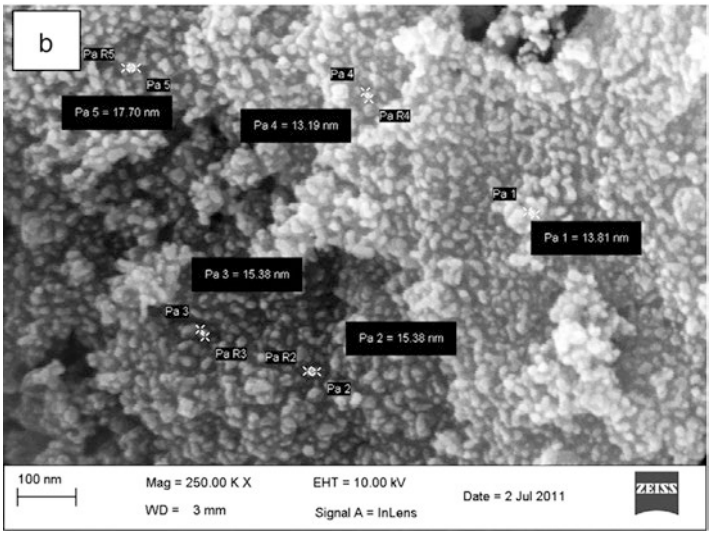


Fig. 3.8 SEM micrographs of SnO_2 nanoparticles. (Kumar et al. 2013)

3.5.4 Tin Oxide Nanostructures

(Kumar et al. 2013) synthesized SnO_2 nanoparticles using the co-precipitation method and used it to remove Cr^{6+} from the water. Nanosized structure of SnO_2 nanoparticles was proved using scanning electron microscopy images which is given Fig. 3.8.

Several effecting parameters on the adsorption are investigated which the results are as follow. By increasing in the concentration of the adsorbate, the adsorption decreased, and it could be owing to lowering in the obtainability of the adsorptive active sites on the adsorbent surface. Study of the contact time showed that increasing in time led to increasing the adsorption, therefore, 40 min was enough to reach adsorption equilibrium. When the amount of adsorbent increased, the adsorption increased too. This could be said that the amount of obtainable active sites increases. By investigating the effect of pH, it was revealed that in lower pH the metal oxide are protonated, and there is a tendency to the ions (which are present as CrO_4^{2-} or $Cr_2O_7^{2-}$), while in higher pH the surface of the metal oxide becomes deprotonated and shows no trend to the ions. Pseudo-second-order was in accordance with the kinetic results, which indicates that chemisorption is the primary interaction of the process. By studying the impact of temperature, it was revealed that increasing the temperature caused a reduction in the adsorption, this might be owing to the exothermic nature of the adsorption, which was proved by the thermodynamic calculations. These calculations proved that the nature of this process is exothermic through the negative value of the enthalpy; also, the negative value of the ΔG° shows that the adsorption is spontaneous. This could also be proved by the positive value of the entropy. Table 3.3 shows the obtained parameters from thermodynamic calculations, also, the $q_{e,\,max}$ was 3.09 mg/g for the adsorption of Cr^{6+}.

Table 3.3 Thermodynamic parameters for the adsorption of Cr on metal oxides (Kumar et al. 2013)

Parameters	Temperature	Cr^{6+} on SnO_2
ΔG° (kJ/mol)	30 °C	−3.02
	40 °C	−4.19
	50 °C	−5.50
ΔH° (kJ/mol)		−35.33
ΔS° (J/K mol)		0.123

3.5.5 Zinc Oxide Nanostructures

ZnO NPs was used to remove Zn^{2+}, Cd^{2+}, and Hg^{2+} by (Sheela et al. 2012). They studied several parameters found that for all heavy metal ions, Langmuir isotherm model was fit well with the data, as well as a pseudo-second-order kinetic model, which was fit more for those metals. Changing in metal ion concentrations revealed that increasing the concentration of the metal ions the adsorption was increased, while at higher concentrations owing to the lack of free active sites the adsorption decreased. The authors investigated the influence of pH on the process, and the optimal point was at pH = 5.5 for all metals. It is justified that in the pH lower than the optimal, the surface of the ZnO becomes positive (protonated) and the adsorption is low, instead, at pH higher than the optimal the surface becomes negative and is desired for the adsorption but, the metals start precipitating as hydroxylated form. For the adsorption of Zn^{2+}, Cd^{2+}, and Hg^{2+} the $q_{e,\,max}$ were reported as 357, 384, and 714 mg/g, respectively. Figures 3.9 and 3.10 show the energy-dispersive X-ray spectroscopy spectra of the adsorbent before and after the adsorption.

3.6 Application of Polymer/Metal Oxide NCs in the Removal of Heavy Metal Ions

As mentioned earlier, metal oxides have some defects like weak electrical conductivity, massive volume change, sever aggregation/agglomeration, etc. An effective, useful, and the best approach to overcome those weaknesses is to combine them with polymeric matrix to produce a less-incomplete adsorbent. In the following, some of these perfect adsorbents are covered.

3.6.1 Polymer/Iron Oxide NCs

(Sun et al. 2018) synthesized magnetic adsorbent beads based on alginate, Fe_3O_4 nanoparticles, and MgAl-layered double hydroxide (LDH) for the adsorption of Cu^{2+}, Cd^{2+}, and Pb^{2+} through adsorption method. The nanoparticles were prepared

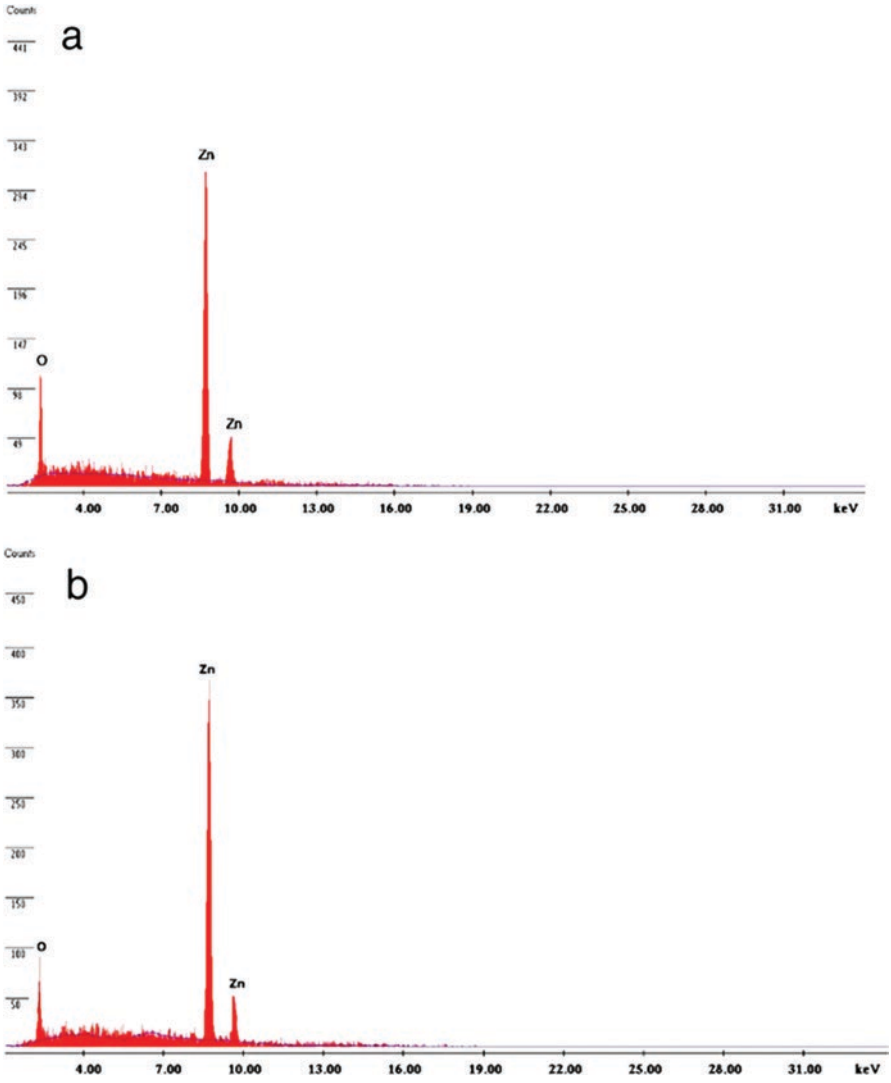


Fig. 3.9 EDX pattern of zinc oxide before and after adsorption of metal ions. (**a**) Unloaded ZnO, (**b**) after adsorption of Zn(II). (Sheela et al. 2012)

by the solvothermal method. They optimized several parameters like Fe_3O_4/LDH nanoparticles amount, adsorbent dosage, time, and pH. The obtained results are as follow: 3% m/v for the amount of NPs, 0.05 g of adsorbent, and pH 5–6 were the optimized parameters. The study of kinetics revealed that this process fit pseudo-second-order, which suggests chemisorption. It is noteworthy that both Langmuir and Freundlich isotherm models fit the removal mechanism. The maximum adsorption capacity ($q_{e,\,max}$) was stated to be 64.66, 74.06, and 266.6 mg/g for Cu^{2+}, Cd^{2+},

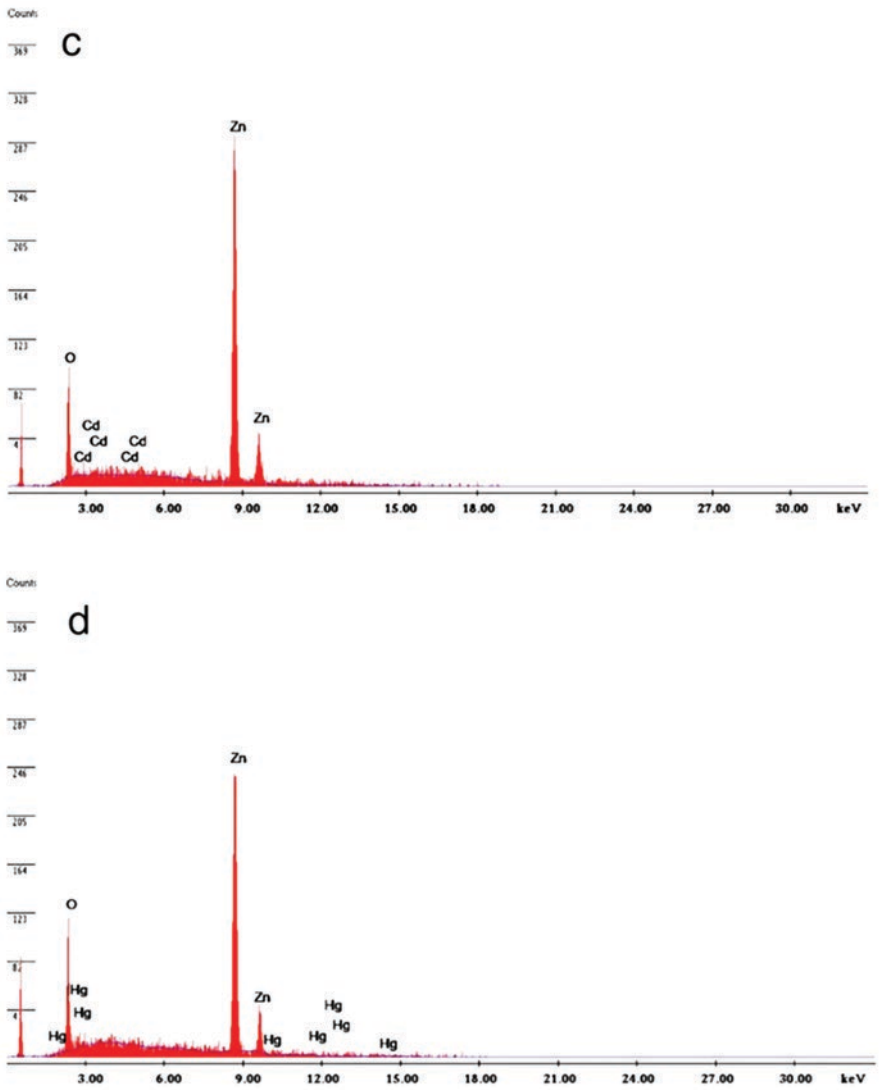


Fig. 3.10 EDX pattern of zinc oxide before and after adsorption of metal ions. (**c**) after adsorption of Cd(II) and (**d**) after adsorption of Hg(II). (Sheela et al. 2012)

and Pb^{2+}, correspondingly. The mechanism for the removal of these heavy metal ions was claimed using X-ray photoelectron spectroscopy which is shown in Fig. 3.11. It was mentioned that based on the X-RAY PHOTOELECTRON SPECTROSCOPY image, heavy metal ions are coordinated with hydroxyl and carboxylate groups of the adsorbent. Furthermore, the precipitation of the studied heavy metal ions as carbonated forms was also seen. The carbonate anions came from interlayer space of the used LDH.

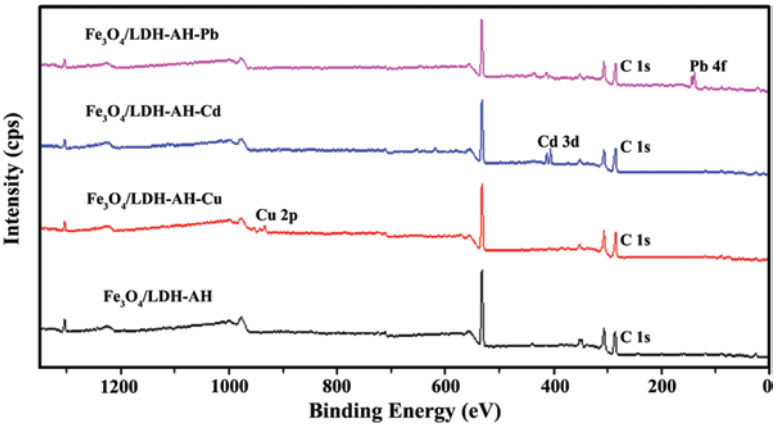


Fig. 3.11 XPS spectra of the Fe_3O_4/LDH-AM after Cu^{2+}, Cd^{2+}, and Pb^{2+} adsorption. (Sun et al. 2018)

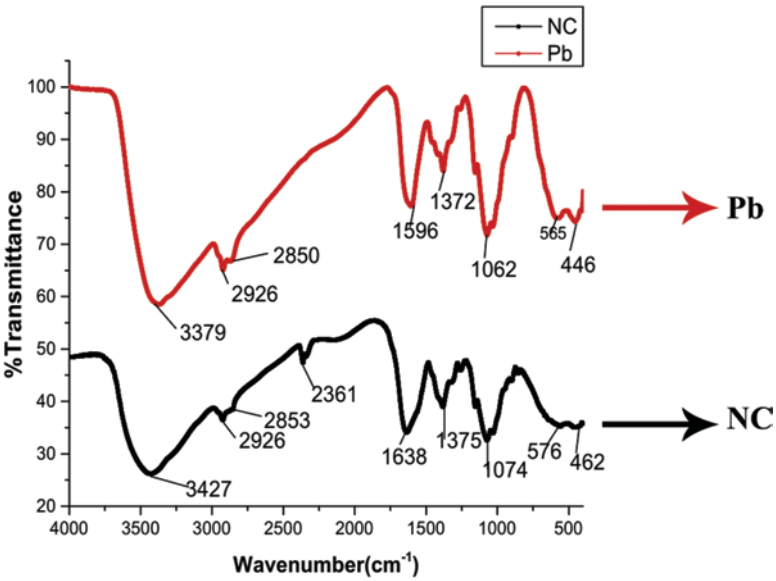


Fig. 3.12 FTIR spectra of (**a**) nanocomposite (NC) and (**b**) Pb(II) loaded nanocomposite. (Ahmad and Mirza 2018)

A nanocomposite consisting of chitosan and Fe_2O_3 NPs was prepared to remove Pb^{2+} and Cd^{2+}. Fe_2O_3 nanoparticles were synthesized *in-situ* in the solution of chitosan in lemon juice (Ahmad and Mirza 2018). This nanocomposite was used as an adsorbent of $Pb^{2+,}$ and the Furrier transform infrared spectra of the NC before and after the process are shown in Fig. 3.12. As seen, disappearing a peak in 2361 cm^{-1} and shifting the other peaks are evidence of the presence of Pb^{2+} onto the NC

surface. Influencing parameters like pH, time, the concentration of adsorbates, and temperature were investigated to find the optimal condition. Several pHs were tested (1–7) and the optimal pH for both metals obtained at pH 5. It was mentioned that at pH below 5, a competition between H^+ and M^{2+} is observed, while in pH upper than optimal pH, M^{2+} is precipitated as $M(OH)^+$ and $M(OH)_2$. Several times (5–360 min) were chosen to study the effect of time, and it was found that after 180 min, the process reached the equilibrium. Based on the results, the suitable kinetic model was pseudo-second-order. In order to examine the impact of the concentration of adsorbates, a range of concentration (10–100 mg/L) was selected. This investigation showed a coherent increase in both adsorption and concentration. Hence, isotherm models were applied to investigate the process more in-depth, and the results indicated that adsorption for both metals obeys Langmuir model. Study of the temperature of the adsorption system and thermodynamics shows that increasing the temperature increased the adsorption, which reveals an endothermic process. This was also proved through the positive value of the enthalpy.

Fe_3O_4 nanoparticles were used to prepare an nanocomposite of chitosan which the nanoparticles were synthesized through a solvothermal procedure (Chen et al. 2019). The obtained nanocomposite was functionalized with ethylenediaminetetraacetic acid disodium salt. Afterward, this nanocomposite was used to remove Pb^{2+} and $Cu^{2+,}$ and several factors of adsorption were studied. As an essential factor, pH of the metal ion solution was adjusted in the range of 1–6 (Fig. 3.13). It was found for both metals the adsorption is too low in pH below 2, which is mainly due to the impulsive interaction among the positive sites of the adsorbent (protonated adsorptive groups). As the pH is increased, the adsorption increases, because in higher pH

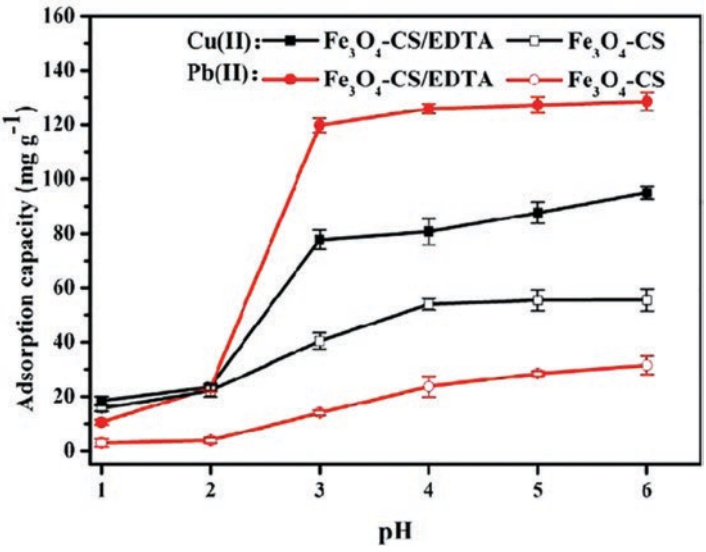


Fig. 3.13 Effect of solution pH on sorption of Fe_3O_4-CS and Fe_3O_4-CS/EDTA toward heavy metals. (Chen et al. 2019)

deprotonation occurs and interaction (chelation and electrostatic) between the adsorbent and adsorbate increases.

Study of the impact of the time revealed that the process reached the equilibrium in 60 min. Using the obtained results, kinetic models were compared, and it was revealed that the pseudo-second-order kinetic model was the better and suitable model. Langmuir and Freundlich isotherm models were employed to investigate the nature of the interaction between the adsorbate and adsorbent, and the consequences proved that the Langmuir model was better fitted. Spontaneous and endothermic behavior of the adsorption obtained through calculations of the thermodynamic equations.

Another nanocomposite consisting of Fe_3O_4 nanoparticles was prepared with chitosan-(D-glucosimine methyl)benzaldehyde (Shahraki and Delarami 2018). The preparation procedure was shown in Scheme 3.2.

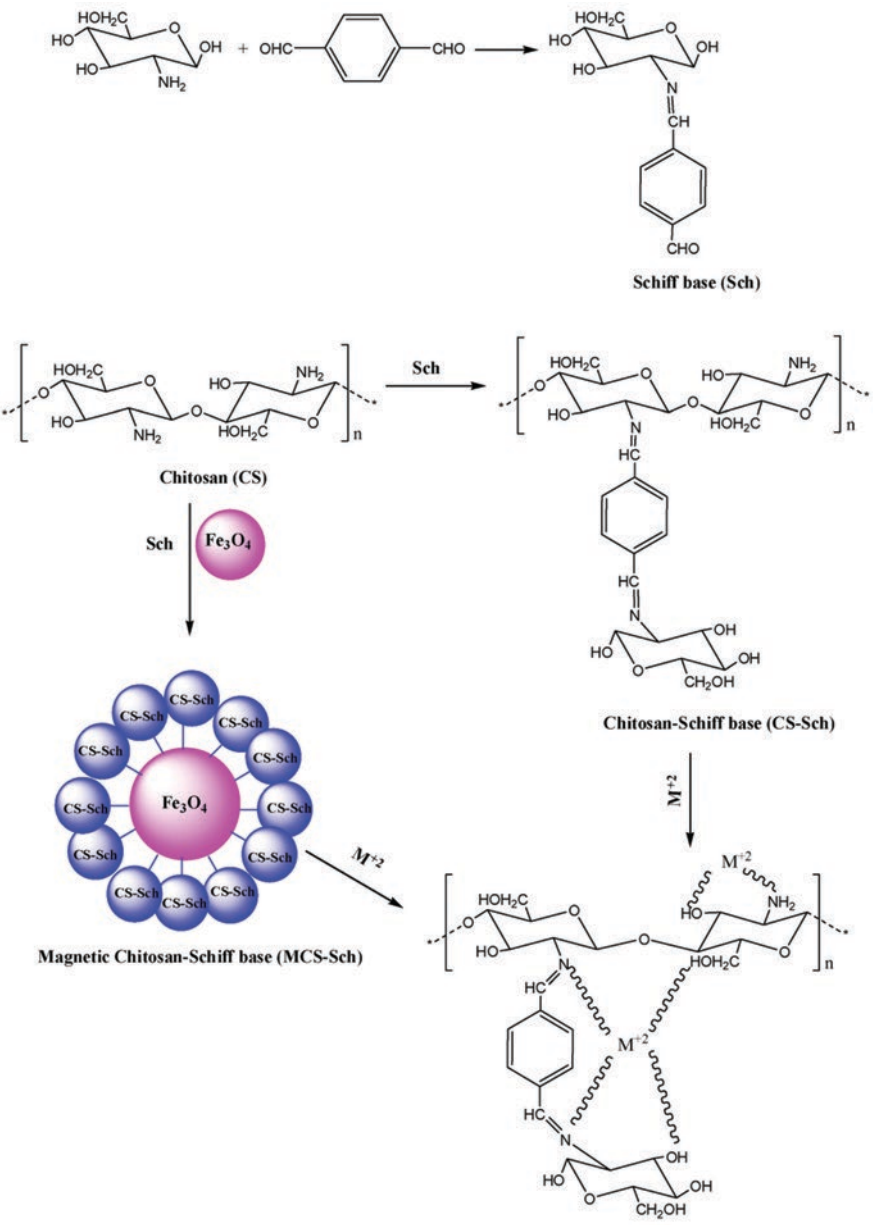
In this work, Pb^{2+} was aimed to be removed from the water. Several solutions of Pb^{2+} with different pH (3–7) were prepared to study the impact of pH on the removal process. It was observed that pH = 5 is the best pH for this process (Fig. 3.14a). By conducting the adsorption at different times, the equilibrium of adsorption was gained in 90 min (Fig. 3.14b).

Also, some adsorbent dosages were chosen to peruse the effect of this parameter. With increasing in the amount of adsorbent, the adsorption increases till its equilibrium. It was said the key factor in this phenomenon is the aggregation of adsorbent, which leads to a deficiency in the adsorption. Therefore, 0.015 g of the adsorption had the best result (Fig. 3.14C). By applying Langmuir and Freundlich isotherm models, the results were found to be in accordance with the Langmuir model.

Sodium alginate has high bead-formation ability, thus, is used widely to prepare nanocomposites. In a work, a combination of Fe_3O_4 nanoparticles and graphene oxide was used as a filler for alginate beads. Graphene oxide was obtained from oxidation of graphite and then was coated by *in-situ* formation of Fe_3O_4 nanoparticles (Sharif et al. 2018). Nanocomposite beads were prepared by dropwise addition of the mixture of alginate and nanofiller into calcium chloride solution (Scheme 3.3).

Surface morphology of the micro-bead of Alg-MGO was studied using field emission scanning electron microscopy and the corresponding image is shown in Fig. 3.15.

The authors investigated the influences of pH, amount of adsorbent, the salinity of the solution, time, concentration of adsorbate, and temperature on the removal of Pb^{2+} and Cu^{2+}. The optimal conditions are as follows: pH: 5–6, adsorbent dosage: 40 mg, and time: 180. The concentration of NaCl in the solution up to 5% did not affect the adsorption of ions, but higher than that, adsorption decreases. The main reason is an increase in the amount of Na^+ which can chelate to the adsorptive functional groups. Using the obtained results from different ion concentrations, it was revealed that for both ions, Langmuir isotherm model fit the consequences. The ions are physically linked onto the surface of the adsorbent. This claim was proved through fitting the kinetics models that pseudo-first-order was a better model. By increasing the temperature of the system, the adsorption of both metals is increased. This shows the endothermic nature of the adsorption which was proved through the



Scheme 3.2 Complete scheme for the synthesis of Schiff base ligand (Sch) and magnetic chitosan nanocomposite (MCS-Sch). (Shahraki and Delarami 2018)

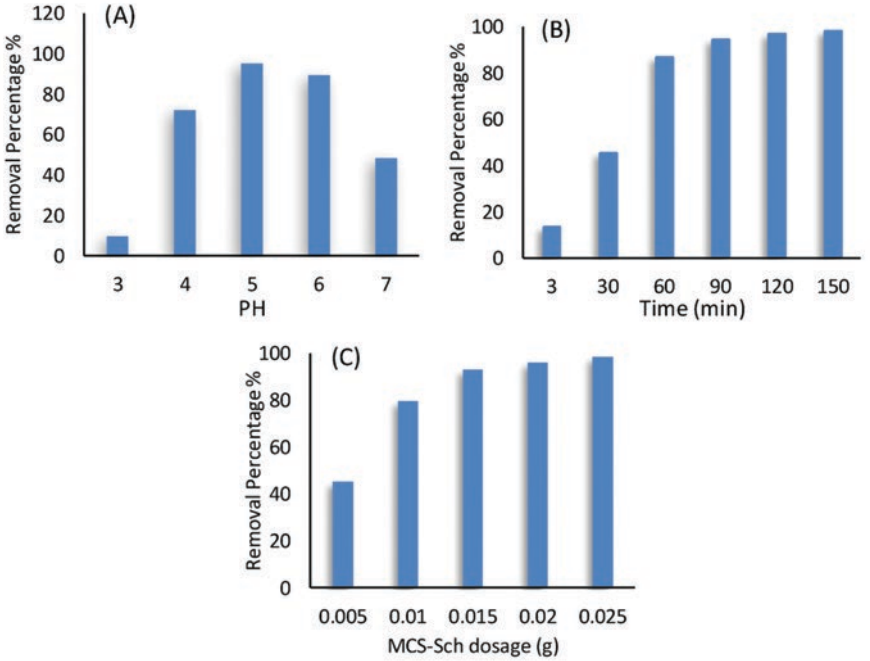
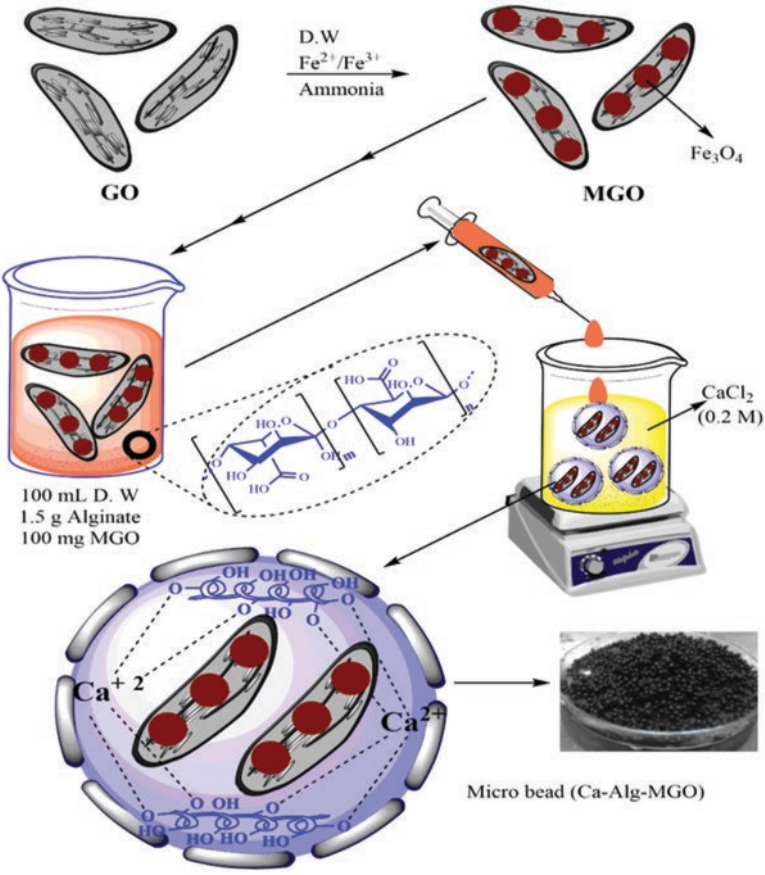


Fig. 3.14 Effect of (**a**) pH (**b**) contact time and (**c**) MCS-Sch dosage on the adsorption of Pb(II) ion by MCS-Sch (*Sch* Schiff base ligand, *MCS* magnetic chitosan nanocomposite). (Shahraki and Delarami 2018)

positive value of the enthalpy. Also, the negative value of the ΔG^O emphasizes the spontaneity of the adsorption. Although it was not mentioned in the article, values of ΔG^O <20 and ΔH^O <40 kJ/mol express physical adsorption.

SiO_2-coated Fe_3O_4 nanoparticles were applied to reinforce polyamidoamine, and then the obtained nanocomposite was applied to adsorb Cu^{2+}, Pb^{2+}, and Cd^{2+}. The Fe_3O_4 nanoparticles and SiO_2-coated Fe_3O_4 nanoparticles were prepared using co-precipitation and sol-gel methods, respectively (Zarei and Saedi 2018). Scheme 3.4 shows the procedure for preparation of the nanocomposite. For the adsorption process, pH 7 was chosen based on some theoretical and literature survey. Effect of ion concentration was studied with only 2 concentrations, 16 and 48 mg/L. It was observed that increasing the concentration, cause and increase in the adsorption efficiency, except Cd^{2+} (95.57 mg/g for 16 mg/L to 96.51 mg/g for 48 mg/L). Figure 3.16 shows the efficiencies of the adsorbents for heavy metal removal.

An NC of poly(trithiocyanuric acid) (poly($C_3N_3S_3$)) and Fe_3O_4 nanoparticles was successfully made as an adsorbent for Pb^{2+} and Hg^{2+} (Fu and Huang 2018). An *in-situ* procedure was conducted for the synthesis of magnetic nanoparticles, and the polymeric matrix was prepared through polymerization (disulfide linkages). Scheme 3.5 shows a schematic preparation of the nanocomposite.



Scheme 3.3 Schematic routes for the fabrication of the micro-bead of Alg-MGO. (Sharif et al. 2018)

pH range of 2–7 was selected to examine the impact of pH on the removal process. The outcomes revealed that the best pH for removal both Pb^{2+} and Hg^{2+} from water was 6. It was stated that in lower pH, strong competition between H^+ and the metal ions prevent effective removal of metal ions, while in higher pH, precipitation of metal ions decreases the removal efficiency. Also, using kinetic equations, for both metal ions pseudo-second-order model fit better the results and it was concluded that the adsorption is chemically controlled. Investigating the isotherm models revealed that the Langmuir model could be more match with the outcomes.

A dithiocarbamate-decorated poly(vinylamine)-based nanocomposite was synthesized using *in-situ* synthesized Fe_3O_4 nanoparticles (DTC-Fe_3O_4@PVAM) and radical polymerization of the desired monomer which is shown in Scheme 3.6 (Wang et al. 2018).

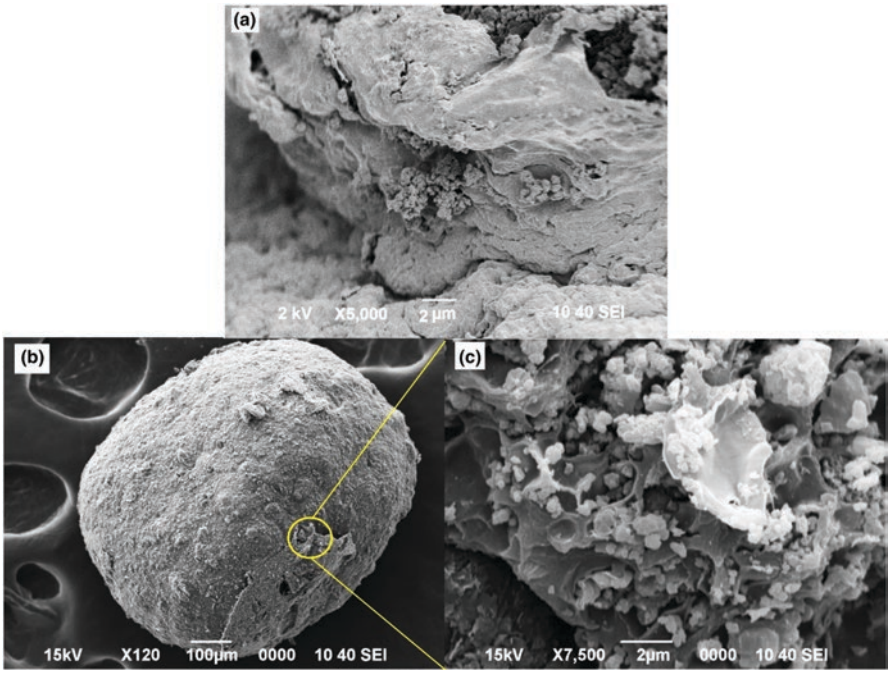
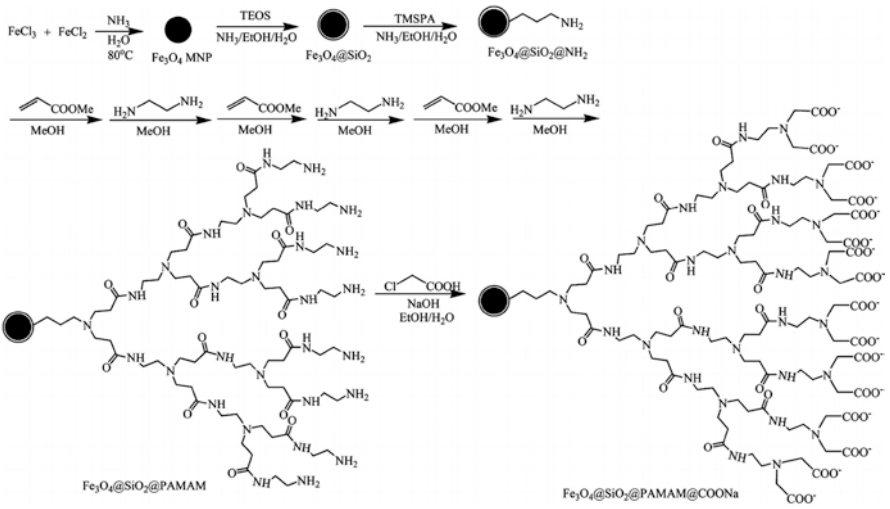


Fig. 3.15 FESEM image of (**a**) magnetic alginate X5000 (2 µm), (**b**) micro-bead of magnetic graphene oxide-alginate X120 (100 µm) and (**c**) high magnification of magnetic graphene oxide-alginate X7500 (2 µm). (Sharif et al. 2018)

As pH is a significant factor, the range of 1–6 was selected to find the best pH. Results showed pH 5 is the optimal pH for adsorption of Cd^{2+}, Pb^{2+}, and Cu^{2+}. Influence of existence other metal ions was investigated in the presence of Fe^{3+}, Mg^{2+}, Ca^{2+}, K^{+}, and Na^{+}. It was mentioned that Mg^{2+}, Ca^{2+}, K^{+}, and Na^{+} had no preventative impact on the adsorption, while in the case of Fe^{3+}, a decrease in adsorption was observed. Through calculation of kinetic equations, the chemisorption process was observed for all metal ions, due to high compatibility of the pseudo-second-order with the results. Adsorption isotherm models were applied to predict the nature of the adsorption. Thus, the Langmuir and Freundlich models were applied, and given results were in accordance with the Langmuir model for three metal ions adsorption (Fig. 3.17).

Also, through the Dubinin-Radushkevich model, free energy was calculated for Cu^{2+}, Pb^{2+}, and Cd^{2+} to be 12.35, 11.56, and 11.17 kJ/mol which shows chemisorption adsorption. A bright and shiny point of this work is that both linear and nonlinear isotherm equations were applied and then the results have been reported.

A combination of gelatin and poly(vinyl alcohol) (gel/PVA) was used as a matrix for Fe_3O_4 nanoparticles (Dolgormaa et al. 2018). NPs were prepared using the co-precipitation method and obtained super-paramagnetic iron oxide gel/PVA (SPIONs/gel/PVA) nanocomposite was applied as an adsorbent for the removal of Cu^{2+} and



Scheme 3.4 Schematic illustration of the synthesis of Fe_3O_4@SiO_2@Carboxyl-terminated PAMAM dendrimer nanocomposite. (Zarei and Saedi 2018)

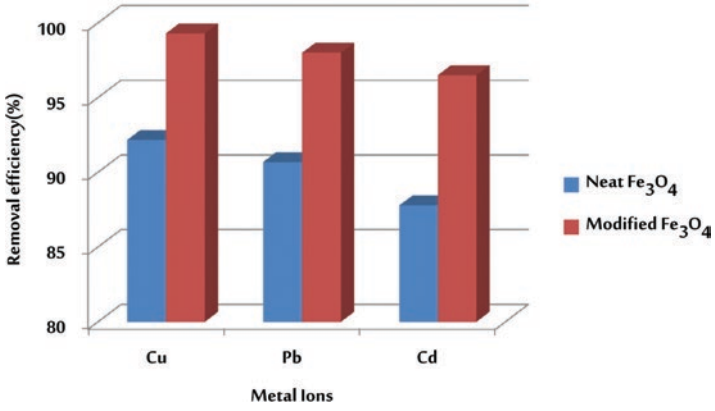
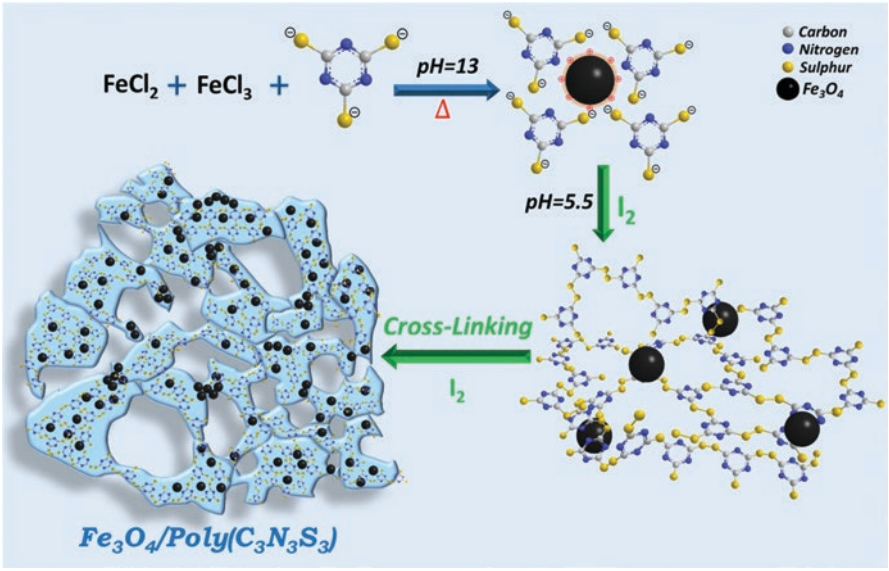


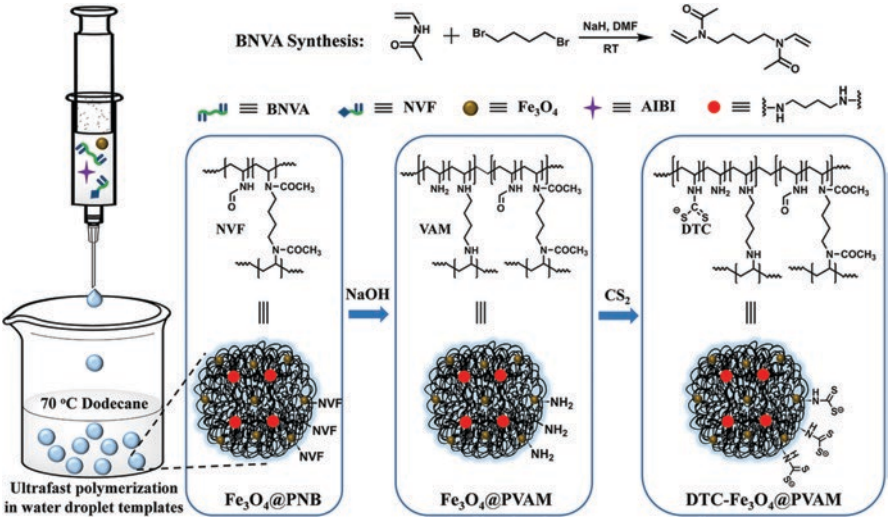
Fig. 3.16 Removal efficiency of modified and neat Fe_3O_4 for removal of Cu^{2+}, Pb^{2+}, and Cd^{2+}. (Zarei and Saedi 2018)

Zn^{2+}. The NC and NPs were fabricated *in-situ* and in one pot. The impact of of pH on the removal of Cu^{2+} and Zn^{2+} was studied and shown in Fig. 3.18. As seen, along with an increment in pH, the removal was increased until the equilibrium (pH 5–6).

It was observed that the adsorption rate was fast in the first 5 min and reaches the equilibrium at 60 min. By using kinetic equations, pseudo-second-order found to be a better model for both metals. In this work, three isotherm models were used: Langmuir, Freundlich, and Sips. After evaluation of the isotherms, the authors claimed that all three isotherms were suitable to describe the adsorption for both



Scheme 3.5 Synthesis process of 2D porous Fe_3O_4/poly($C_3N_3S_3$) network and its chemical structure. (Fu and Huang 2018)



Scheme 3.6 Illustration for the fabrication of DTC-Fe_3O_4@PVAM hydrogel beads adsorbent through ultrafast water droplet templating polymerization and functionalization. (Wang et al. 2018)

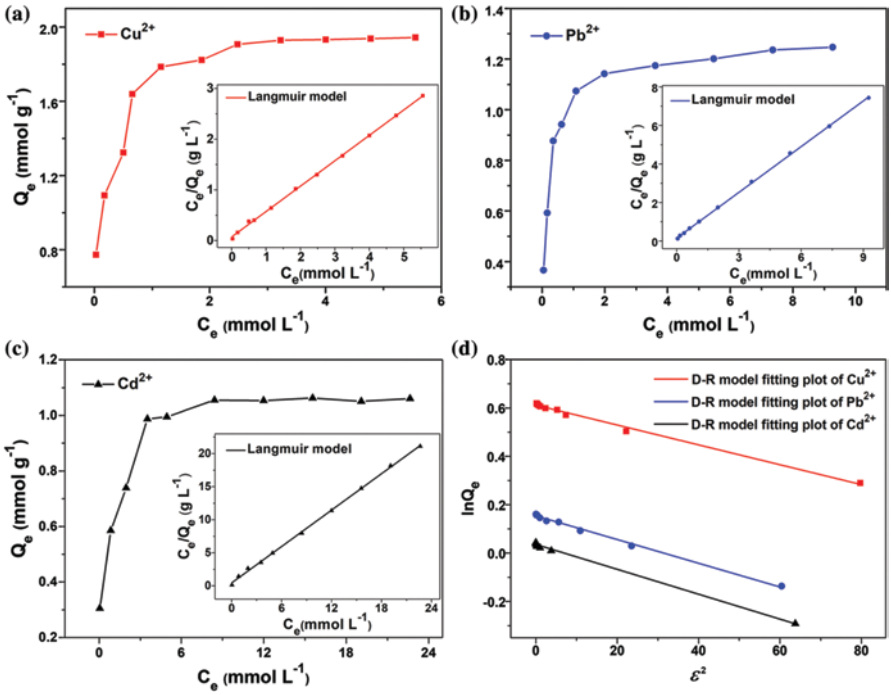


Fig. 3.17 Adsorption isotherms of Cu^{2+}, Pb^{2+}, and Cd^{2+} at 298 K and the corresponding fitting plots for Langmuir model (**a**–**c**). The D–R model fitting plots for adsorption isotherms of metal ions (**d**). (Wang et al. 2018)

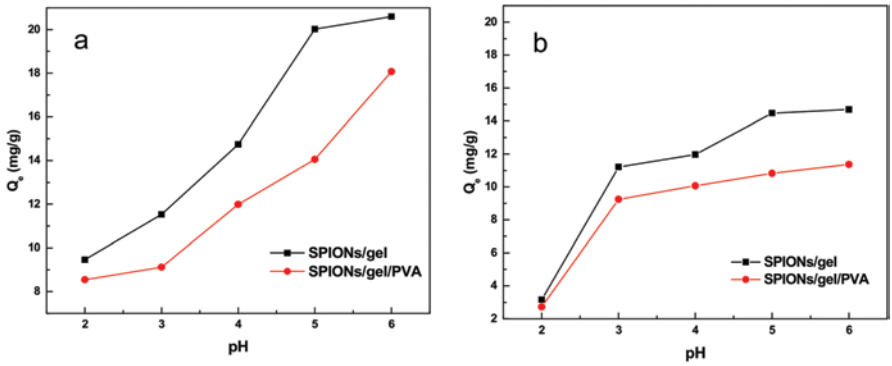


Fig. 3.18 Effect of pH on Cu(II) (**a**) and Zn(II) (**b**) ions adsorption by SPIONs/gel and SPIONs/gel/PVA. (Dolgormaa et al. 2018)

metals, but Sips and Langmuir are a little bit better than Freundlich. Thus, monolayer adsorption is the best description. This conclusion came from only correlation coefficients (R^2), while, considering just one parameter to say which isotherm model is better or all of them are suitable is not correct. It was better to use some error functions, and after determining these, a conclusion is made. However, it was mentioned that the adsorption of both metals on the adsorbent is chemically (using kinetic models) and monolayer adsorption happens (due to isotherm models).

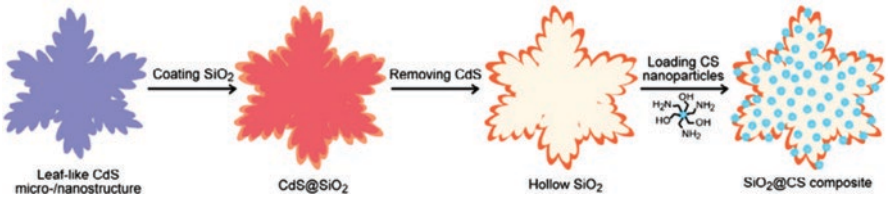
3.6.2 Polymer/Silicon Oxide NCs

A chitosan/SiO_2 NC was fabricated to remove As^{5+} and Hg^{2+}. For the preparation of this NC, at first, CdS was prepared hydrothermally and then SiO_2 was coated on it (Liu et al. 2019). Then the CdS was removed, and subsequently, chitosan (CS) was loaded onto the hollow SiO_2 (Scheme 3.7). Fig. 3.19 depicts the field emission scanning electron microscopy images of the structure of obtained SiO_2@CS, which clearly proves the leaf-like structure of SiO_2@CS.

To remove metal ions, kinetic models were applied; first of all, it was cleared that the adsorption reaches the equilibrium at 30 min. Then, using the obtained results, pseudo-second-order fit better the results. Also, isotherm models were used and for removal of As^{5+} Freundlich isotherm model was in accordance while, for Hg^{2+}, Langmuir model corresponded.

A nanocomposite consisting of polyethyleneimine (PEI) and SiO_2 nanoparticles was fabricated in one pot (Choi et al. 2018). The nanoparticles were produced through the *in-situ* manner in the solution of PEI. As a control, SiO_2 NPs were prepared by calcinating the PEI-SiO_2 NC. Afterward, the produced adsorbent was used to uptake Cr^{6+}. In this project, only three pHs were tested (2, 3, and 4) for the adsorption. At first, both adsorbents were tested, and it was found that despite the high surface area of the SiO_2 NPs, PEI-SiO_2 nanocomposite has higher adsorption performance. Along with the impact of pH, the influence of metal ion concentration at different pHs was investigated (Fig. 3.20). It was reported that Langmuir isotherm was matched with the results and maximum adsorption capacity obtained 120.7, 138.2, and 183.7 mg/g for pH 2, 3, and 4 respectively. Taking the importance of the adsorption process into the account, it could be more informative if the authors studied other determining factors on the adsorption, as well as other isotherm models. In this study only, nonlinear forms of isotherms were used and linear forms were not employed.

Hayati et al. used amino functional poly(propylene imine) (PPI) as a polymeric matrix to prepare an nanocomposite with SiO_2 nanoparticles (Hayati et al. 2017). Afterward, the nanocomposite was used to eliminate Pb^{2+}, Ni^{2+}, Cu^{2+}, and Co^{2+} from the water. At lower pH, owing to the protonation of amine groups in PPI, adsorption is low and with increasing the pH, adsorption increases. It was found that pH 7 is the optimal pH (Fig. 3.21). The same results were observed for investigation of a number of adsorbents and the suitable adsorbent dose obtained 0.25 g/L. But the



Scheme 3.7 Illustration for the preparation of the SiO_2@CS composite with a biomimetic leaf-like structure. (Liu et al. 2019)

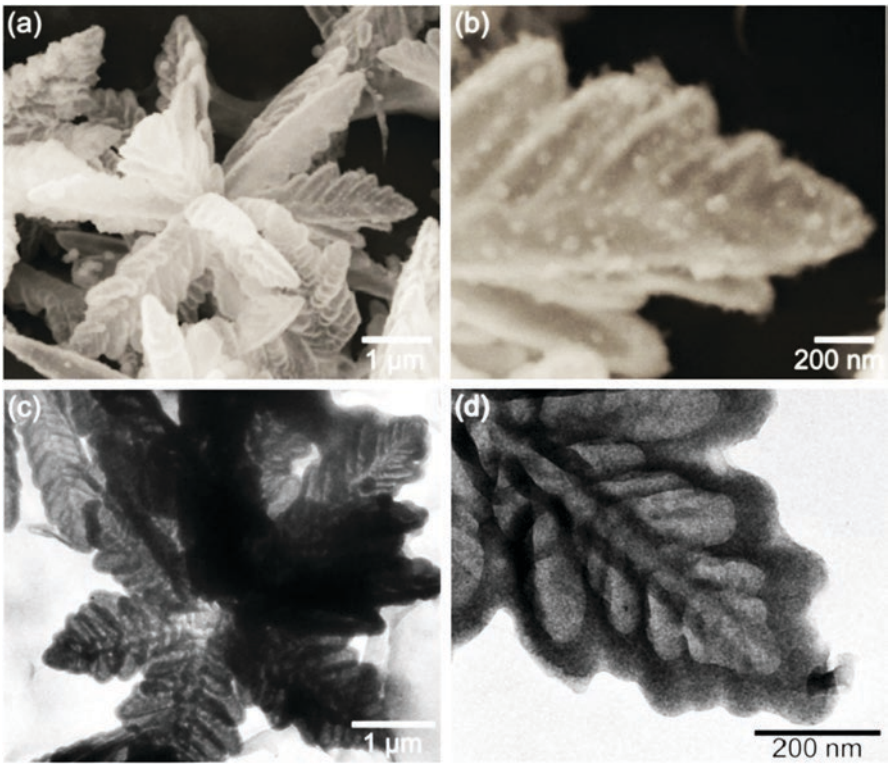


Fig. 3.19 (**a**), (**b**) FESEM and (**c**), (**d**) TEM images of the leaf-like SiO_2@CS composite. (Liu et al. 2019)

reverse process has been recorded when the concentration of metal ions increased and study of contact time showed that after 20 min, the adsorption reaches equilibrium. Using isotherm models and thermodynamic calculations, the Langmuir model, proved to be suitable isotherm for all four metal ions as well as the spontaneity of the process. Likewise, the positive amount of the enthalpy is the evidence of the endothermic nature of the adsorption.

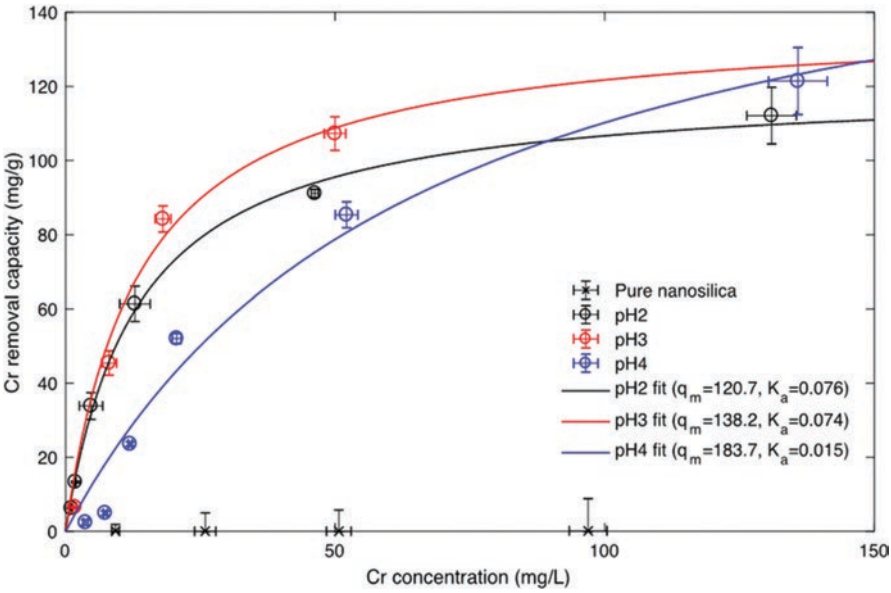


Fig. 3.20 Cr adsorption isotherm of pure nanosilica and PEI-silica nanoparticle under pH 2, 3, and 4 and its fitting with the Langmuir, adsorption model. (Choi et al. 2018)

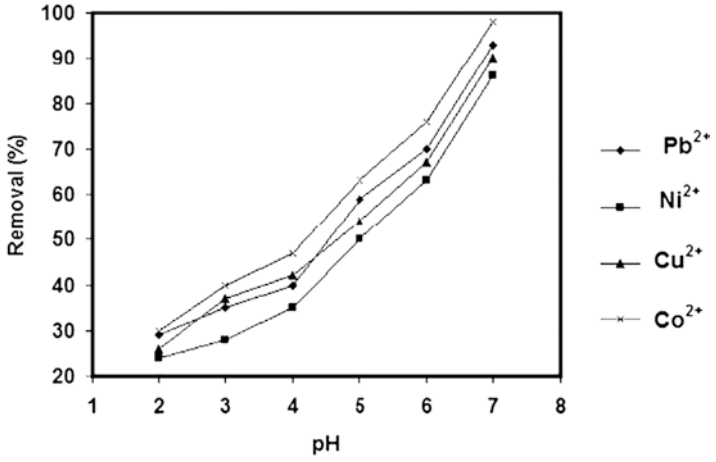


Fig. 3.21 Effect of pH on Pb^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+} removal by PPI/SiO_2 nanohybrid. (C_0 = 100 mg/L, m = 0.2 g/L, T = 298 K). (Hayati et al. 2017)

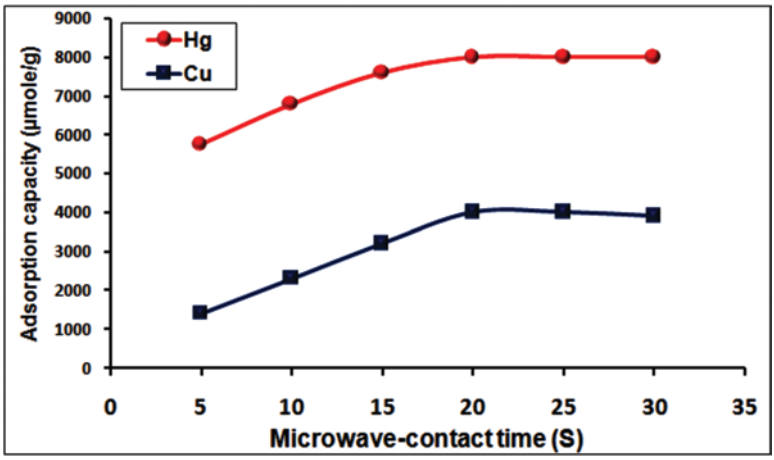


Fig. 3.22 Effect of Microwave-contact time (s) on mercury and copper capacity values. (Mahmoud et al. 2018)

In a work, a nanocomposite of CS and two nanofillers, SiO_2 and TiO_2 nanoparticles, was produced by crosslinking them with glutaraldehyde (Mahmoud et al. 2018). At first, CS was crosslinked, dried, and grinded. Then, was suspended in water and activated NPs were added into the suspension along with the addition of glutaraldehyde. The NC was further used as an adsorbent of Hg^{2+} and Cu^{2+}. First of all, the influence of irradiative time with microwave on the removal was investigated. As seen in Fig. 3.22, increasing in exposing time increases the adsorption for both metal ions until 20 s. Examining the solution pH showed that pH 6 in the best pH for both metals, because, a lower pH, lone pair electrons of oxygen and nitrogen are deactivated (protonated) and adsorption is not effective. At pH 6, those are deprotonated and are useful in adsorption of metal ions. Mass of adsorbent is another factor, which was studied, and it was found that raising the mass of NC enhanced the adsorption. This might be said that the surface area (or contact surface) increases. The author concluded the best adsorption results obtained with nanocomposite mass of 50 mg. Increasing the mass more than the optimal led to a reduction in the removal which could be owing to aggregation of the adsorbents.

To find the nature of the interaction between nanocomposite and metal ions, thermodynamic calculations were employed and based on the negative value of the ΔG^o the adsorption said to be spontaneous. Also, increasing in ΔG^o along with temperature is a sign of exothermic adsorption. It is mentioned the negative ΔH^o for both metals (−41.35 and − 10.46 kJ/mol for Hg^{2+} and Cu^{2+}, correspondingly) reveals exothermic and chemically adsorption. Referring to the literature, if ΔH^o<-80 kJ/mol the adsorption is physical, while chemically adsorption ranging in −80 up to −450 kJ/mol (Liu and Liu 2008; Piccin et al. 2017). Therefore, it seems that for adsorption of Hg^{2+} and Cu^{2+}, physical adsorption happens. Using isotherm equations, Langmuir isotherm model was reported to be suitable for both metal ions. Calculated free energy from Dubinin-Radushkevich obtained to be 0.244 and

1.62 kJ/mol, which was reported the mechanisms of the adsorption are physical, chemical, and ion-exchange. According to the literature, free energy lower than 8 kJ/mol indicated physical interactions, and for ion-exchange, the energy should be between 8–16 kJ/mol (Mallakpour and Behranvand 2017). Hence, the nature of the adsorption for both Hg^{2+} and Cu^{2+} is a kind of physical adsorption, not chemical and ion-exchange.

It is worth to mention that a comprehensive survey on the polymer/SiO_2 nanocomposites has been recently published (Mallakpour and Naghdi 2018).

3.6.3 Polymer/Titanium Oxide NCs

A nanocomposite of polyaniline/TiO_2 (PANi/TiO_2) was made through oxidative polymerization of aniline in the existence of pre-synthesized TiO_2 (via sol-gel method) and was applied to adsorb Pb^{2+}, Zn^{2+}, and Cu^{2+} (Chen et al. 2018a). Investigation of the influence of pH on the removal showed that an increment in pH increases the adsorption. Thus, the optimal pH was 5 (Fig. 3.23). By investigating the kinetics and isotherms, pseudo-second-order and Langmuir models found to be the more compatible model with the results for all metal ions. Also, to find the affinity of the adsorbent to metal ions, a multi-component adsorption system was designed and was shown that the adsorption selectivity of ions is in order of $Zn^{2+} > Pb^{2+} >> Cu^{2+}$.

Performance of another nanocomposite of TiO_2, with polythiophene (PTh) toward metal ions (Pb^{2+}, Zn^{2+}, and Cu^{2+}) was investigated (Chen et al. 2018b). The

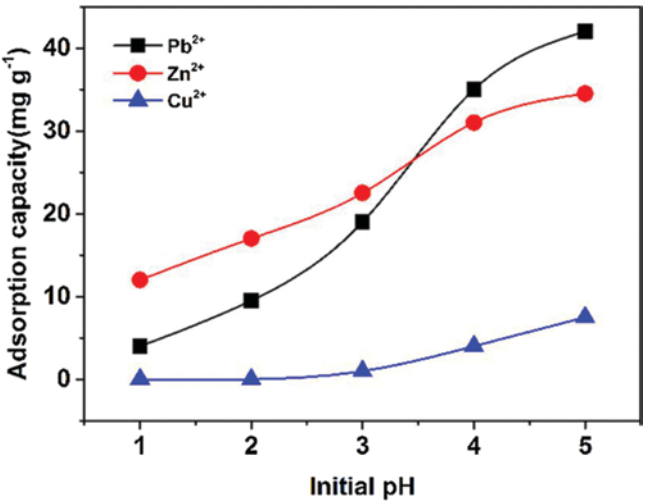


Fig. 3.23 The adsorption capacities of the PANi(ES^+)/TiO_2(O^-) composite for Pb^{2+}, Zn^{2+}, and Cu^{2+} in different initial solution pH. (Chen et al. 2018a)

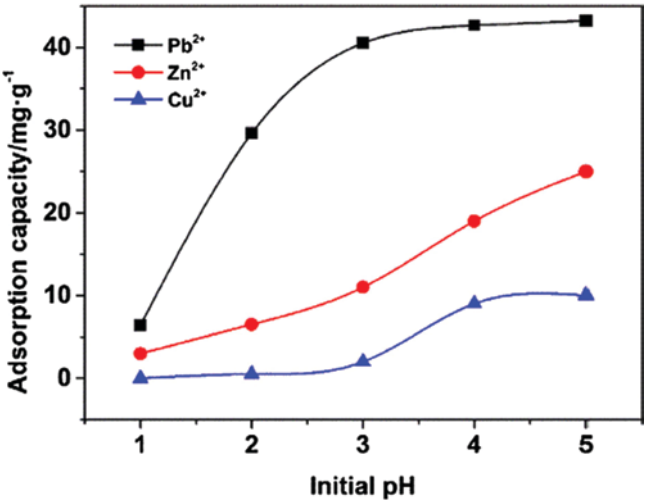


Fig. 3.24 Influence of initial solution pH on the adsorption capacities of PTh/TiO_2 composite for Pb^{2+}, Zn^{2+}, and Cu^{2+}. (Chen et al. 2018b)

NC was prepared through polymerization of thiophene in a suspension of TiO_2 (which was synthesized in the solution of HNO_3). In this project, it was cleared that pseudo-second-order is in more accordance with the results. Both single- and multi-component system were used to find the selectivity of the adsorbent toward the adsorbates. In this regard, it was observed that for both systems, Langmuir isotherm model was the best model. The adsorbent has the affinity to collect Zn^{2+} more than other metal ions. Impact of the solution pH revealed that the optimal pH is 5, according to Fig. 3.24.

3.6.4 Polymer/Manganese Oxide Nanocomposites

The NC of PVA reinforced with modified α-MnO_2 nanorods was used to remove Pb^{2+} from water solution (Mallakpour and Motirasoul 2017a). The nanoparticles were fabricated via hydrothermal manner and subsequently was modified with γ-aminopropyltriethoxy silane (KH550) in order to obtain a good dispersal of modified nanoparticles in the PVA. Transmission electron microscopy images of the obtained NC shows α-MnO_2 nanorods (Fig. 3.25).

At first, the influence of the adsorbent amount upon the removal was examined, and the obtained results declared that increasing in the amount of the adsorbent, increased the adsorption. Further examination on the isotherms proved that both Langmuir and Freundlich isotherms are adopted with the results, which was concluded that both monolayer and multilayer adsorption happens simultaneously. Fig. 3.26 shows the fitting of Langmuir and Freundlich isotherm models with the

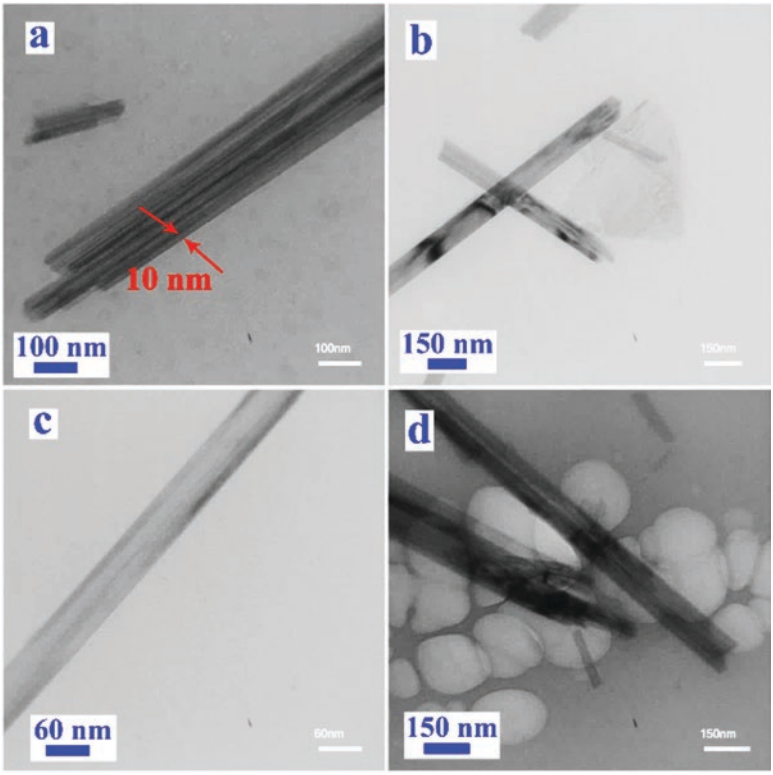


Fig. 3.25 TEM images of PVA/α-MnO_2-KH550 NC 5 wt% with different scales (**a**) 100 nm, (**b**) 150 nm, (**c**) 60 nm, and (**d**) 150 nm. (Mallakpour and Motirasoul 2017a)

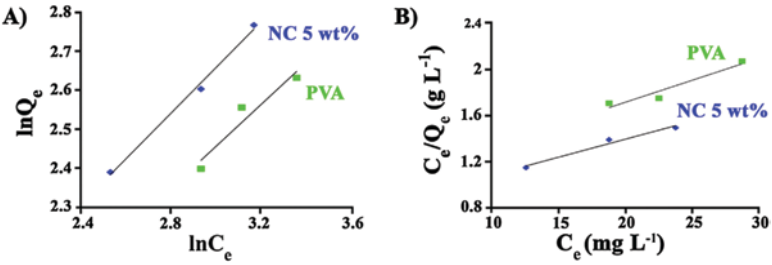


Fig. 3.26 The Freundlich adsorption isotherm (**a**) and Langmuir adsorption isotherm (**b**) of PVA/α-MnO_2-KH550 NC 5 wt% and pure PVA. (Mallakpour and Motirasoul 2017a)

obtained results. The author used only linear forms of isotherms, while, nonlinear forms could provide useful information.

A co-precipitation method was used to synthesize MnO_2 nanoparticles, and the as-prepared nanoparticles were embedded in polyethersulfone (PES) to make an adsorbent of Cd^{2+} with different amount of MnO_2 nanoparticles (Lai et al. 2017).

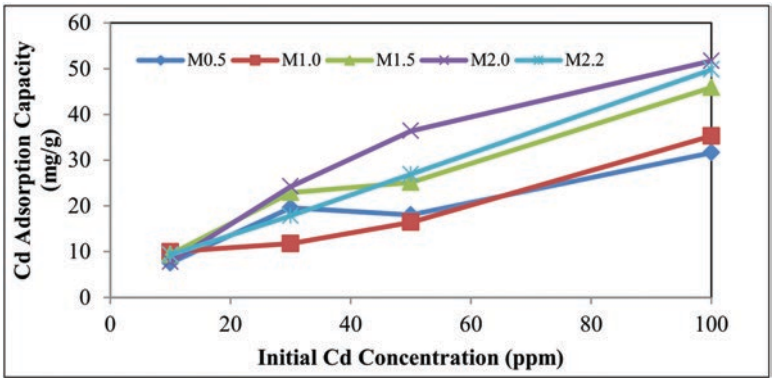
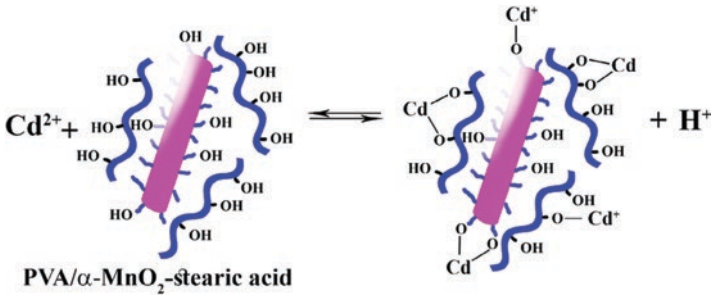


Fig. 3.27 Adsorption isotherm of Cd using membranes with different MnO_2/PES ratios. (Operating Conditions: pH = 9, membrane dose = 1 g/L, temperature = 25 °C, contact time = 48 h). (Lai et al. 2017)



Scheme 3.8 The mechanism of Cd^{2+} ion adsorption onto PVA/α-MnO_2-stearic acid NC. (Mallakpour and Motirasoul 2017b).

Solution pH was adjusted at 9 to investigate the effect of the ratio of the nanofiller/PES and ion concentration on the removal. Figure 3.27 shows the impact of the ratio of nanofiller/PES on the removal in which with an increase in the amount of nanofiller, adsorption increased. The same happening was observed when metal ion increased.

Different pH solutions were prepared to find whether solution pH affect the adsorption or not. It was found that raising the pH from 2 to 11 increases the adsorption owing to an increase in the possibility of electrostatic interactions between Cd^{2+} and the adsorbent.

A hydrothermal route was employed to produce α-MnO_2 nanorod from suitable precursors, which has been further modified with stearic acid (SA). As-prepared modified nanofiller was fixed in the PVA and obtained nanocomposite was used in the adsorption of Cd^{2+} (Mallakpour and Motirasoul 2017b). Investigation of the impact of the metal ion concentration on the removal showed that increasing the metal ion concentration caused a reduction in the adsorption. The main reason for this was reported to be a deriving force of the concentration gradient. Hence,

isotherm modes were utilized, and it was concluded that both Langmuir and Freundlich isotherm models were suitable. The author studied the kinetics and found pseudo-second-order to be more appropriate. Scheme 3.8 illustrated the suggested mechanism for the removal of Cd^{2+} onto the adsorbent, which is ion exchange and chelation.

Blending a polymeric matrix with another polymer remarkably affect the adsorption ability of the obtained nanocomposite. For example, poly(vinyl pyrrolidone) (PVP) was used to prepare a blend nanocomposite with PVA which was reinforced with stearic acid-modified α-MnO_2 (Mallakpour and Motirasoul 2018). α-MnO_2 was synthesized through the hydrothermal method and modified with stearic acid. The ratio of PVP/PVA was chosen 50:50 and the nanocomposite was prepared via solution casting. Adsorption performance of this NC was studied toward Cd^{2+}. Along with increasing in Cd^{2+} concentration, the adsorption was decreased which corresponds to the deriving force of ion concentration. It was mentioned that both Langmuir and Freundlich isotherm models were fit with the results. The author reported that this nanocomposite (PVP/PVA/MnO_2-SA) has more adsorption capability than that of without PVP (PVP/PVA/MnO_2-SA).

Wu et al. (2020) attempted to remove Cd^{2+} and Pb^{2+} from aqueous solution using an adsorbent based on MnO_2, biochar, and poly(acrylamide). They employed isotherm and kinetic equations for this process. The outcomes revealed that this process obeys pseudo-second-order model, which is indicative of chemical interactions. Comparing isotherm models, it was found that Freundlich model was the suitable model that indicates a multilayer adsorption. Although the adsorption process did not fit with Langmuir model, the reported values for adsorption capacity for Cd^{2+} and Pb^{2+} were 85 and 71 mg/g, respectively.

Since science and technology are being changed, to use the latest issues and knowledge and to be up-to-date, searching and literature survey are of vital tasks and should be done routinely. Some researchers used linear isotherm and kinetic models and some used nonlinear. It would have been much better if researchers had made a comparison between linear and nonlinear models to find out a suitable, precise, and accurate model using several error functions (like sum of squared errors, root mean square error, coefficient of determination, Chi-squared, and so on) which it could definitely be closer to the real results. Also, recently researchers have developed isotherm models like Langmuir and modified them which would be useful to obtain more accurate information about the adsorption (Azizian et al. 2018).

Table 3.4 summarized performances ($q_{e,\ max}$) of the adsorbent against several heavy metal ions which have been extensively discussed.

3.7 Conclusions and Future Scope

Today world demand on using purified water made researchers in the region of chemistry, ecosystem, energy, and so on to prepare suitable purifying agents. One of the harmful types of contaminants released in water sources is heavy metal in where

Table 3.4 Comparison of $q_{e,\,max}$ of studied adsorbents against heavy metal ions

Heavy metal	Adsorbent	$q_{e,\,max}$ (mg/g)	Reference
Pb^{2+}	Fe_3O_4@APS@AA-co-CA MNPs	166.1	Ge et al. (2012)
	Fe_3O_4/LDH-AM	266.6	Sun et al. (2018)
	Fe_3O_4-CS/EDTA	220.0	Chen et al. (2019)
	MCS-Sch	121.9	Shahraki and Delarami (2018)
	Alg-MGO	322.6	Sharif et al. (2018)
	Fe_3O_4@SiO_2@Carboxyl-terminated PAMAM	117.0	Zarei and Saedi (2018)
	Fe_3O_4/poly($C_3N_3S_3$)	232.6	Fu and Huang (2018)
	DTC-Fe_3O_4@PVAM	261.1	Wang et al. (2018)
	PANi/TiO_2	95.4	Chen et al. (2018a)
	PTh/TiO_2	163.7	Chen et al. (2018b)
	PVA/α-MnO_2-KH550	32.4	Mallakpour and Motirasoul (2017a)
	PPI/SiO_2	471.0	Hayati et al. (2017)
	MBCG	71	Wu et al. (2020)
Cu^{2+}	NH_2-Fe_3O_4-NTA	55.6	Hao et al. (2019)
	Fe_3O_4@APS@AA-co-CA MNPs	126.9	Ge et al. (2012)
	NHTO	47.2	Debnath and Ghosh (2011)
	Fe_3O_4/LDH-AM	64.7	Sun et al. (2018)
	Fe_3O_4-CS/EDTA	225.0	Chen et al. (2019)
	Alg-MGO	294.1	Sharif et al. (2018)
	Fe_3O_4@SiO_2@Carboxyl-terminated PAMAM	119.1	Zarei and Saedi (2018)
	DTC-Fe_3O_4@PVAM	131.5	Wang et al. (2018)
	SPIONs/gel/PVA	56.0	Dolgormaa et al. (2018)
	PANi/TiO_2	9.0	Chen et al. (2018a)
	PTh/TiO_2	9.0	Chen et al. (2018b)
	PPI/SiO_2	460	Hayati et al. (2017)
	Nano-SiO_2-crosslinked chitosan-nano-TiO_2	333.3	Mahmoud et al. (2018)
Cd^{2+}	Fe_3O_4@APS@AA-co-CA MNPs	29.6	Ge et al. (2012)
	γ-Fe_2O_3/Fe-doped HAP	257.9	Xiao et al. (2018)
	NHTO	53.5	Debnath and Ghosh (2011)
	ZnO NPs	384.0	Sheela et al. (2012)
	Fe_3O_4/LDH-AM	74.0	Sun et al. (2018)
	Fe_3O_4@SiO_2@carboxyl-terminated PAMAM	115.8	Zarei and Saedi (2018)
	DTC-Fe_3O_4@PVAM	122.5	Wang et al. (2018)
	MnO_2/PES	51.7	Lai et al. (2017)
	MBCG	85	Wu et al. (2020)
	PVA/α-MnO_2-SA	15.5	Mallakpour and Motirasoul (2017b)
	PVP/PVA/MnO_2-SA	47.0	Mallakpour and Motirasoul (2018)

(continued)

Table 3.4 (continued)

Heavy metal	Adsorbent	$q_{e,\,max}$ (mg/g)	Reference
Zn^{2+}	Fe_3O_4@APS@AA-co-CA MNPs	43.4	Ge et al. (2012)
	ZnO NPs	357.0	Sheela et al. (2012)
	SPIONs/gel/PVA	40.9	Dolgormaa et al. (2018)
	PANi/TiO_2	38.6	Chen et al. (2018a)
	PTh/TiO_2	74.4	Chen et al. (2018b)
Hg^{2+}	ZnO NPs	714.0	Sheela et al. (2012)
	Fe_3O_4/poly($C_3N_3S_3$)	344.8	Fu and Huang (2018)
	SiO_2@CS	204.1	Liu et al. (2019)
	Nano-SiO_2-crosslinked chitosan-nano-TiO_2	1515.2	Mahmoud et al. (2018)
Cr^{6+}	$CoFe_2O_4$@γ-Fe_2O_3	15.6	Campos et al. (2019)
	SnO_2	3.1	Kumar et al. (2013)
	PEI-SiO_2	183.7	Choi et al. (2018)
Ni^{2+}	PPI/SiO_2	438.0	Hayati et al. (2017)
Co^{2+}	PPI/SiO_2	503.0	Hayati et al. (2017)
Tl^{+}	MnO_2@pyrite	290.0	Li, H. et al. (2018)
As^{5+}	SiO_2@CS	198.6	Liu et al. (2019)
Sb^{3+}	NH_2-Fe_3O_4-NTA	51.1	Hao et al. (2019)

the pollution caused by them can bring several and uncurable diseases for the living-creature body. Metal oxides and their polymeric nanocomposite have been extensively applied in field of water treatment especially removal of heavy metal ions due to their advantageous over other adsorbents like large capacity, high surface area, rich resources, reusability, poor aggregation, high efficiency, etc. and the preparation methods for MO and polymer/MO NCs are green, safe, and simple. After surviving numerous published researches, the capability and efficiency of the MO and polymer/MO NCs were highlighted and finally summarized tabularly. Combining MO with polymers, even blending the polymeric matrix with another polymer, enhanced the efficiency of the obtained NC in heavy metal removal. Altogether, polymer/MO NCs could be appropriate adsorbents in the area of water treatment in comparison to other adsorbents, which may have petrol-based nature. The NCs can also be useful in the field of energy.

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Chapter 4
Impact of Nanomaterials on the Food Chain

A. Rajani Chowdary and Santosh Kumar Sanivada

Abstract Nanoscale materials are a set of substances having atleast one critical dimension less than approximately 100 nanometers. The application of nanotechnology offer lot of benefits in various sectors like medical, pharmaceutical, industrial, agriculture and food sectors etc. As an efficient alternative to the traditional food processing systems, nanotechnologies are more idealistic to ensure the quality and safety of food products, creating a healthy food culture. Nanoparticles are classified into two types: incidental and engineered nanoparticles. Due to the presence of unique chemical and physical properties, engineered nanomaterials are formulated to be attractive for various applications in various environments and biological systems. In agricultural sector, nanomaterials are found to decrease the volume of pesticide usage. Much of the research efforts of nanotechnology are going into the food packaging section of food sector. Despite of the novel properties, the increasing industrial production of nanomaterials had created a great concern regarding potential health hazards for human health. The usage of nanomaterials at higher levels may cause direct or indirect exposure to humans. For example the engineered nanoscale materials can interact with different organisms at both lower and higher trophic levels in various types of food chains. Recently there is a progress on the assessment of bioaccumulation, and on the trophic transfer of engineered nanomaterials. The use of the synthetic nanomaterials is harmful to environment, human beings, animals, and plants. Lack of awareness regarding the impact of nanomaterials on human health is a primary hindrance for the implementation of nanotechnology. These impacts can be reduced by the judicious usage of bionanomaterials instead of synthetic nanomaterials which can be synthesized through biosynthesis or by green methods.

Keywords Nanotechnology · Nanoemulsions · Engineered nanoparticles · Nanometers · Health hazards · Pharmaceutical · Bionanomaterials

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4.1 Introduction

Nanotechnology and nanomaterials in a broad sense forms an innate part of food processing and traditional foods, since the credited properties of numerous foods depend on nanometer sized components like foams and nanoemulsions (Ravishankar Rai and Jamuna Bai 2018). Nevertheless the current technological implementations pave the way to synthesize and produce nanoparticles which are to be added to different food items (Cushen et al. 2012). These nanomaterials are well divided forms of accessible ingredients, or entirely new chemical structures. The implementations in the nanotechnology field are felicitous to bring an array of properties to the entire food chain regarding the development of novel technologies, materials used, advanced technologies for the production of more efficient foods with minimal usage of agrochemicals, fats, salts, preservatives, enhancing the taste, consistency as well as texture of food items and promote easy digestion of nutrients and supplements, and modern packaging apprehensions (Sekhon 2010). Incorporation of nanotechnology in food chain is a new approach with few qualms in its applications considering about its safety measurements (Pal 2017). Nanoscale materials are a group of substances having no less than one critical dimension lesser than approximately 100 nanometers (Darrell et al. 2015). Nanomaterials at this scale are of great significance because of their unique emerging optical, magnetic, electrical, and other properties. These nanomaterials of emergent properties play a crucial and potential role in the field of electronics, medicine, food, pharmaceutical fields etc. (Fakruddin et al. 2012). Some of the nanomaterials exist naturally examples of this category are food proteins with globular structure ranging between 10–100 nm, most lipids and polysaccharides are linear polymers of 2 nm thickness and some of meticulous concern are engineered nanomaterials (EN), which are intended for, and already being used in many commercial products and processes (Wen et al. 2005). Currently they are involved in the production of stain-resistant clothing, cosmetics, sporting goods, electronic equipments and in medical field (for diagnosis, imaging, drug delivery etc). In addition two dimensional nanostructures like stabilized foams or emulsions of one molecule thickness are produced when food biopolymers accumulate into fibrous networks. Despite of significant possible benefits to both the consumers and industrial sector, nanomaterials and technologies may also initiate potential risks for human health and the environment (Viswanath and Kim 2016).

Despite of nanomaterials being synthesized and produced in higher quantities, less literature is available in regard to their biological effects, including their ability to biomagnify as they travel up through food chains (Burton 2008). They may not significantly get accumulated in organisms at the higher trophic levels. There are different kinds of nanomaterials, organisms, food chains and environments. More awareness should be there about the health risks perhaps allied with various types of nanomaterials; the nanomaterials toxicity data should be produced to the government for their appropriate and regulatory usage (Giese et al. 2018). Biomagnification of depends on the properties and concentration of various nanomaterials in food chain and is also influenced by the ability of the organisms to breakdown various nanomaterials.

Nanomaterials should be used safely. The impending environmental risk of nanomaterials, together with their impact on aquatic organisms, is the key central arguments for regulating the nanotechnology segment (Borm et al. 2006). Research carried by many investigators had suggested that engineered nanomaterials can be moved from single celled organisms in the lower level of the food chain, into the higher, multi-celled organisms. Though they are water-soluble, stable environmentally and biologically; their long-term impact on environment is yet to be identified (Luo et al. 2016).

4.2 Current Scenario of Nanotechnology in Food Market

Nanomaterials (NMs) or nanoparticles (NPs) are the new chemical structures equipped with well dispensed forms of affable ingredients. Nanoparticles are categorized as natural and anthropogenic, which later, are classifed into two general types: incidental and engineered nanoparticles (ENPs) (Von der Kammer et al. 2014). Incidental nanoparticles are the resultant products of human activities and have very poorly controlled shape, sizes, and may be composed with a mixture of different elements whereas engineered nanoparticles (polymeric- organic and inorganic) are unique, specially designed and purposefully synthesized by human beings (Contado 2015). Countless possibilities exist for the exploitation of the benefits of nanotechnologies in various phases of the food chain with an aim to improve animal nutrition and health, promote new food products and improve the microbial quality of foods during production and processing and storage (Srinivas et al. 2010). Focus on nanomaterials in various consumer products has revolutionized specifically in industrial sectors; few of them are very close to our daily life activities which include agriculture, medicine, pharmacy, food safety, cosmetics, and personal care (Fakruddin et al. 2012). Besides their potential applications in various fields their extensive utilization constitutes an important and integral part of food processing units involved in making of nano food products like nanoemulsions and foams. Their colossal applications have guided for the expanding growth of research and developmental activities and produced entirely a novel class of materials which finds application in various fields of optics, electronics and in medical ground as potential carriers in drug delivery and gene delivery systems or as diargnostic tools and contrast agents (Jariwala et al. 2014; De Jong and Borm 2008). Despite of many novel properties, increased industrial production of nanomaterials had created a great concern regarding potential health hazards for human health. Hence it is fundamental to study the cellular consequences of direct exposure of engineered nanomaterials to human cells and it is essential for their safe and successful use in many applications in various fields (Drasler et al. 2017). Specifically regarding their use in food sector i.e., before launching any nano foods into market, they are thoroughly checked and appraised for their manufacturing details because some of the processes may alter the composition of food and make the food novel, change the nutritional status, and topple out the contaminants of both chemical and

microbiological origin which may cause allergic manifestations (Fakhroueian et al. 2018). The assessment of any nano food should obey the guidelines prescribed by food safety regulating units.

At present, the USA is investing 3.7 billion USD through its National Nanotechnology Initiative (NNI) from the past 4 years whereas Japan spends 750 million and Europe spends 1.2 billion per year (Ketaki et al. 2012). Relatively the level of funding in developing countries was lower; however this has not reduced the impact of some countries on the global stage. A research conducted by Helmuth Kaiser Consultancy had forecasted that the nano food market has taken a climb from 2.6 billion USD to 20.4 billion USD by 2010. Their findings also revealed that, Asia contributes nearly 50% of the world Nanofood market in the year 2010 (Ketaki et al. 2012). A research by technical market and industry analysis had found that, the nanotechnology market was 7.6 billion USD in 2003 and 1 trillion USD in 2011. Not less than 400 companies all over the world today are actively involved in the nanotechnology research and development and this figure is expected to increase to more than 1000 within the next 10 years. Based on the findings of Lux Research, the nanotechnology industry has developed to US$ 2.6 trillion in manufactured products by the year 2014 (Taylor 2008). Besides that, U.S. Department of Agriculture (USDA) had announced that by the year 2015, the impact of nanoproducts globally will be US$ 1 trillion annually. A presumption connecting to the expansion of nanotechnology in foods and drinks show that it might reach US$ 3.2 billions in the year 2015. Currently near about 276 nanomaterials are available in the market; in which nano-encapsulated titanium dioxide and silver achieved the highest number of records in the Nano Inventory, food additives and food contact materials are the most frequent applications (Roopan and Madhumitha 2018). The future implications of nanotechnology on foods is of great concern, it is expected that a potential shift from inorganic nanomaterials like silver to organic materials like nanoencapsulates and nanocomposites may occur in the very near future. This implies that application of the above materials in novel foods, feed additives, biocides and pesticides have been so far used only at a R&D stage (Gallocchioa et al. 2015). There was an inadequate data available regarding their safety and potential impact on consumers' health despite of their wide usage in the food market. Nanoparticles are generally unstable and aggregate based on their varied chemical properties like density, size, shape, surface chemistry etc. (Guo et al. 2014). Subsequently it is highly fundamental to know how nanoparticles and biological parameters can influence in vitro and in vivo toxicity profile. Due to deficiency of standardized and consistent methods developed for assessing nanotoxicity, confusing and inconsistent data causes the hindering of development of nano particles risk assessment strategies (Gallocchioa et al. 2015). In addition deficiency of extensive and comprehensive toxicological data is also because of actual complexity to detect, characterize, and measure nanoparticles alone and in complex matrices like food, feed and biological samples. Mutually these aspects and insufficiency of reference materials for a huge variety of food and particle combinations make the development and validation of analytical methods to spot nano particles in food and feed still a tough and ongoing issue. All these above aspects have emerged in the EFSA opinion

"The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety" published in 2009 and are still a current issue. In India promising and feasible application of nanotechnology has also been initiated. It was observed that, early investments made by the Indian Government through various grants and schemes have resulted in early-level inventions. From the available online data, it was showed that 3 projects are on-going in India and 3 patents IN2008DE01339, WO200963508 and IN2005MU01196 were found to be at various stages of patent prosecution processes in the Indian Patent Office since 2005. These findings have revealed the current interests and thrust of Indian researchers in this field. However, the investments in nanotechnology in India are relatively less when compared with those at the global levels. In 2008, both private and public sectors worldwide have invested about $20 billion. Market forecasts represent $1880 billion investments in nanotechnology-related sales across all sectors by 2015.

4.3 Nanomaterials and Food Chain

Developments in the field of nanotechnology are propitious to fetch an array of assets to the entire food chain concerning to novel technologies, materials used, approaches for the production of more efficient foods, usage of lesser amount of agrochemicals, maintaining hygienic conditions in food processing units, upgrading the taste of food items and their textures, reducing the usage of salt, preservatives and fats, boost up the digestion of nutrients and supplements; and modern packaging perceptions (Pradhan et al. 2015). Though nanotechnology is an interesting field of science it has an impact on the whole food chain in terms of risk assessment, toxicological aspects, consumer perception, regulatory issues and communication (EFSA 2009). Most consumers are unwilling to agree the novel technologies in the food chain, specifically in terms of the information related to the deficient areas of risk assessment. As it is an emerging science, development of novel food products and processes, would require approval under the 'Novel Foods Regulation' (Regulation (EC) No 258/97) to ensure the safety of the products.

The present chapter will discuss the potential implications of the use of engineered nanomaterials in various food products for consumer safety and the regulations governing such developments. A number of recent research reports have recognized the current and short-term applications of nanotechnologies for food and its related sectors (Chaudhry et al. 2010, 2018). The main driving phenomenon behind all these developments seems to be intended at enhancing the uptake and bioavailability of nano-sized nutrients and supplements, and improving taste, stability, texture and consistency, of food products (Chaudhry and Castle 2011).

To prove whether nanomaterials are accumulated or moved up the food chain, an experimental research had been conducted by National Institute of Standards and Technology using two types of nanomaterials – carboxylated and biotinylated quantum dots prepared from selenium, zinc, cadmium, and sulfate (Burton 2008). They tested it in an aqueous environment in which *Escherichia coli* served as the food

source for the ciliate *Tetrahymena pyriformis*, which in turn was the food source for the rotifer. *E.coli* did not accumulate the quantum dots, whereas the ciliates accumulated it. Their findings also showed that, aquatic organisms present at higher levels in the food chains may potentially be exposed to nanomaterials through their diet. However, the quantity transferred was relatively very low and by which the authors suggest that they do not pose a significant threat in nature. These results were utilized by various policy makers dealing with the budding areas of nanotechnology, water pollution and ecosystem health. The findings also revealed that quantum dots accumulation in aquatic invertebrate food chains may not produce a considerable risk. However additional research was suggested to study the risks associated with their usage and to know the interaction of the nanoparticles with other organic particles in the natural environment (Burton 2008).

4.4 Applications of Nanotechnology in Foods: Overview of Potential Benefits and Risks

The potential applications and benefits of nanotechnology offer lot of possibilities in various sectors like medical- drug delivery and diagnosis, pharmaceutical, industrial, agriculture and food sectors etc. For instance they find application in sunscreen preparation, biomedical imaging, cosmetics, sporting goods, clothing, tires, electronics, ground water remediation etc. In agricultural sector, nanomaterials are found to decrease the volume of pesticide usage (Aithal and Shubhrajyotsna 2016). By the incorporation of nano-silver particles, pesticides are potentized into more valuable forms for targeting the pests with a reduced pesticide volume. Nano–metal oxides are applied to soil to kill target soil plant pathogens (Duhan et al. 2017). Addition of nano-silicon to soil will amplify the water uptake efficiency in plants (Jatav and De 2013); developing a DNA-based nanobio-sensor in a polymer to coat fertilizers, would discharge only as much fertilizer as “demanded” by plants root ionic signals. Much of the research efforts of nanotechnology are going into the food packaging section of food sector. Some of the newly synthesized nanoparticle-polymer composites are proved to bring vast improvements in mechanical performance of the packaging material and in the functional properties like antimicrobial activity to safeguard the packaged food products (Armentano et al. 2018). Most of the implications of the nanotechnology in the form of nano-textured materials in the food and the beverage sector are done at research and development stage (Chaudhry 2010) and many related products are available currently in the market. This majorily involves processing food stuffs to form nano structures and stable emulsions to increase the texture, taste and consistency of various food stuffs.

Nano-textured food stuffs permits the minimal usage of fat thereby making the products healthier to the consumer while still retaining the same taste of the original product (Sekhon 2010). Currently nano-textured foods like ice-creams, coloring agents, flavouring agents, preservatives, nutrient supplements, vitamins,

anti-oxidizing agents, wheat flour, rice flour, spices, herbal products, medicines are produced using nanotechnology (Sekhon 2010).

The word nanotechnology does not mean that the food is atomically modified or food is produced by nanomachines but instead nanotechnology tools are generally used during crop growing, transport, production, processing, or packaging of the food. As an efficient alternative to the existing food processing systems, nanotechnologists are more idealistic about the possible changes that can be made to ensure the quality and safety of food products, creating a healthy food culture. Nanotechnology also finds application in enhancing the nutritional quality of foods by incorporating selective additives and upgradings in the way by which body digests and absorbs the nutritional components (Kadam and Amanpreet 2018). For instance numerous nanocapsules containing food additives, would remain quiescent in the food and can be released only when they are taken up by the consumer. (Ketaki et al. 2012).

Nano-carrier systems are based on the encapsulation of the nutrients and supplements in the form of liposomes, micells or protein based carriers to mask the objectionable taste of some of the food additives, supplements and in few instances to protect them from degradation during processing (Huang 2012). These nanoencapsulated materials are challenged for their antimicrobial activity, enhanced bioavailability, and other health advantages.

Nanosized amorphous silica particles claimed large-scale applications in many consumer goods used in daily life. They exist as single, fused or agglomerated forms with spherical, tubular, and irregular shapes. These include nanotubes, fullerenes, quantum dots and dendrimers (Contado 2015).

4.4.1 Risks

The risks associated by the presence of nanoparticles in foods may be due to the direct contact of food components with the nanopackaging material or due to migration of nanoparticles from nanopackaging materials into food (He and Hwang 2016). After ingestion of engineered nanomaterials by humans they pass into intestine and later enter into circulation (Martirosyan and Schneider 2014). The two major organs liver and the spleen are involved in the distribution of nanoparticles. Few past studies revealed that nanostructured materials which enter into the gastrointestinal tract get rid of from the intestine immediately (Buzea et al. 2007). Converse to ingestion mode there are other routes of nanoparticles entry, which include inhalation and skin exposure. Magnesium oxide nanoparticles will enter through inhalation into olfactory bundle beneath the forebrain through the axons of olfactory nerves in the nose and finally will reach other parts of the brain (Sharma et al. 2017). ZnO nanoparticles have exhibited genotoxic potential in epidermal cells of humans. Lung tumors have also been reported subsequently afte chronic inhalation of very high doses (10 mg/m^3) of nano-TiO2 (Belal and El-Ramady 2016). However there is no convincing data available about the dissemination of

nanoparticles through human skin and results were controversial. Few studies have shown that nanostructured particles could not penetrate healthy, intact skin. The impact of nanomaterials on human body also relies on the properties of nanomaterials. Nano particles which enter into bloodstream may influence the blood vessel lining or function and support blood clot formation or may be linked with cardiovascular effects connected to inhaling ambient ultrafine particle and also induce platelet aggregation and vascular thrombosis (Frohlich 2016). As they affect the blood flow they can possibily induce negative effects in any organ of the body. From the available literature, it was revealed that some nanoparticles can cross the blood brain barrier, and enter into the cells and organs and interact with metabolism or can even migrate into the foetus (Jong and Borm 2008). The toxicity of engineered nanomaterials depends on oxidative stress, although the literal mechanism is not known about the induction and generation of reactive oxygen species (ROS) from engineered nanomaterials (Manke et al. 2013). Activation of oxidative stress-responsive transcription factors stimulates chronic inflammation. Chronic inflammation and oxidative stress are important to cause a number of particle-specific effects such as genotoxicity, fibrosis, and cancer caused by secondary mutations (Manke et al. 2013). Some studies have shown that, there were many signs of toxicity only when there was a relatively high dose of nanomaterials consumed orally. Inorder to assess the toxicity of engineered nanomaterials there are several factors taken into consideration like physicochemical characteristics of nanomaterials, complex process involving material source, surface chemistry, agglomeration state, preparation method and storage.

4.5 Risk Assessment and Toxicological Effects of Nanomaterials Related to Public Health and Public Perception

Due to the presence of unique chemical and physical properties, engineered nanomaterials are formulated to be attractive for various applications in various environments and biological systems. The bulk industrial production of nanomaterials and nanoproducts presently and the predicted increase of their usage in the very near future may produce an increasing appearance in the environment, chance of human exposure through inhalation, skin contact, or ingestion (Jeevanandam et al. 2018). The possible environmental risks involve their influence on aquatic organisms; have been a major argument for monitoring the production sector of nanotechnology. The current research on nanotechnology had found that engineered nanomaterial particles can be transmitted from lower level unicellular organisms in the food chain, to higher level multicellular organisms (Luo et al. 2016). Nanomaterials are minute, biologically and environmentally stable and water-soluble, however, their long-term environmental impact is yet to be known. The findings of David Holbrook and his

colleagues at the U.S. National Institute of Standards and Technology had reported that in simplified food web carboxylated and biotinylated quantum dots can be transferred to higher trophic organisms (rotifers) through the dietary intake of ciliated protozoans (Burton 2008). Accumulation of quantum dots from the immediate environment (bioconcentration) takes place in the ciliates and no quantum dots enhancement (biomagnification) was identified in the rotifers. They concluded that, dietary intake of nanomaterials must also be considered for higher trophic level aquatic organisms. They also found that, aquatic organisms at higher levels in food chains may also be potentially exposed to nanomaterials through their diet. Nevertheless, limited bioconcentration and lack of biomagnification may hamper the recognition of nanomaterials in invertebrate organisms (Burton 2008).

Nanotechnology is used to produce packages with improved mechanical and thermal properties, while nanosensors may be integrated in the food packaging systems to alert consumers if the food products are no longer safe for consumption (Fuertes et al. 2016). There are many views over the consequences of using nanoparticles on environmental and human health. There are several different compounds in engineered nanomaterials existing in several forms and sizes with assorted surface coatings (Simko and Mattsson 2014). The health appraisal issues of using such diversified compounds involving complex process requires a more validated and promising analytical method for their characterization and certification. There are variety of routes by which nanomaterials can penetrate the body and these include inhalation, ingestion or by dermal penetration (Sharma et al. 2017). In addition to the above nanotechnology-based medical devices, drug injections and implants may also serve as the way for nanoparticles entrance (Buzea et al. 2007). Basing on the fact that air is the major route for the entry of nanoparticles into human body, it is equally important to assess the quantity and quality of nanomaterials in the air at work place along with the determination of their presence and concentration in bulk samples. The usage of nanomaterials at higher levels may cause direct or indirect exposure to humans (Pattan and Kaul 2012). In the field of medicine, nanoparticles are intentionally injected into the body. For imaging and drug delivery, nanomaterials are often purposefully coated over biomolecules such as DNA, protein, and monoclonal antibodies to target particular cells (Lewinski et al. 2008; Nalwa 2014). The novel physicochemical properties of the engineered nanomaterials may commence new mechanism of injury and toxicological impacts due to the harsh interactions of nanomaterials with the biological systems and the environment (Ray et al. 2009; Yan et al. 2015). Chen et al. (2006) reported the acute toxicity of copper particles (bulk) and nanocopper in mice and revealed that nano copper was several folds fatal than bulk copper. Nanocopper was also reported to source pathological damage to spleen, liver and kidney. Many features have been identified that are significant towards the interpretation of nanoparticles ingestion studies and these studies include physicochemical characterization of nanoparticles, interactions with the gut microbiota and reporting of metadata from in the invivo studies.

4.6 Trends in Nanotechnology to Enhance Biopackaged Food, Food Quality and Safety

The appraisal of any nanofood or its ingredients before launching into market comprises the evaluation of the details of its manufacturing process because certain processes may have an impact in altering the composition and nutritional value of foods making them novel thereby altering the intended purpose, toxicity and its metabolism in the biological system (Hansen and Baun 2012). The evaluation of nanofoods will be carried by following the guidelines issued by Food Safety authorities. Many previous studies have reported that the term nanotechnology was not recognizable by the public. The two terms related to labeling of nanofoods which establish the individual view on nanotechnology in foods were nano-outside (e.g., packaging) and nano-inside (e.g., foods) (Ravichandran 2010). In general the consumers can readily accept the innovations associated with packaging than those which are related to foods. Hence nanotechnology packaging seemed to be less problematic from the public view (Ravichandran 2010). Nanotechnology packaging is alleged as being more advantageous and represents lesser health risks than nanotechnology involved into foods (Baltic Milan et al. 2013). Lack of awareness regarding the impact of nanomaterials on human health is a primary hindrance for the implementation of nanotechnology. This requires close and secure collaborations between the product developers, nanoparticle, researchers, risk assessors and regulators. Much of the nanotechnology implementation in food business is restricted to packaging sector (Hwang et al. 2012). These packages will make the products retain quality and make them safer by maintaining the foods in the very best possible state with prolonged shelf-life. Incorporation of nanocomposites in food packing systems not only protect food but also improves the shelf-life and resolves environmental issues decreasing the usage of plastics. In general the packaging materials are either non-degradable or biodegradable (Honarvar et al. 2016). Biodegradable films have poor mechanical and barrier properties. Hence we need to rectify these properties before the films can replace traditional plastics and assist to handle worldwide waste problem (Sorrentino et al. 2007).

Currently large number of nanoclay polymers existing on the market have good commercial applications in various fields, for instance they are involved in the manufacture of wine and soft drink bottles and thermoformed containers (Muller et al. 2017). The active packaging often involves the incorporation of substances that can release or absorb substances present either in the food package or in the air that is in contact with food. Active packaging was mainly designed for its antimicrobial properties and also for ethylene elimination, CO2 absorption /emission, capturing oxygen, protection from malodours and steam resistance, discharge of preservatives, antioxidants, additives or flavours etc. (Muller et al. 2017).

Nanotechnology applications are also involved in coating or in labels over packaging providing information associated to traceability and tracking of outside as well as inside product conditions throughout the entire food chain. Currently it is used to detect leaks in foodstuffs which are packed under vacuum or inert

atmosphere (Ranjan et al. 2016) and to identify temperature and humidity variations all through the product shelf-life. The main common points which are raised by all companies related to commercializing nanotechnology includes towering processing costs, problems with the scalability of research and development for prototype and commercial industrial production and concern about public perception of health, environment, and safety issues (Servin and White 2016). Besides, research need to be done on novel and diverse applications of nanotechnology for developing reliable and reproducible instrumental techniques for identification, characterization and quantification of new materials in environment, human and food samples. At the same time it is essential to analyse various absorption pathways, exposure levels, metabolism, acute and chronic toxicity and its short or long term bioaccumulation. The knowledge acquired in all these areas is important to draw a practical and efficient nanotechnology regulatory framework.

4.7 Intentional and Incidental Use of Nanomaterials in Food Industry

The research and development activities in nanotechnology especially in food sector are vigorous and deep in all the stages of food processing, food packaging and distribution. The food products which are enriched by nano particles will improve the nutritional value, texture, bioactive delivery systems, flavor encapsulation and control the microbiological growth. In the food processing and packaging area nanoparticles are used either as antimicrobial compounds or as highly responsive biosensors for identifying microbial contaminants, allergens, degradants and pathogens which may produce disagreeable changes in food quality and safety (Lu et al. 2015). Due to the positive results of these applications in many food products, some traditional food products containing naturally occurring nano particles which are consumed from centuries are now enriched by intentionally adding nanoparticles by using engineered nanomaterials in agriculture, where nanoformulations are employed to boost up the production (Sekhon 2014). From the consumer safety point of view, the engineered nanoparticles are generally incorporated into foods at very low levels. Only in some instances, direct analysis of food samples can be carried out with some kind of sample preparation, but in majority of cases the analysis procedures require nanoparticles be extracted from their native environment, or that the environment be destroyed, digested, or seriously altered as a result the nanoparticles are in a state that can be measured (Contado 2015). However this leads to two issues that can negotiate the value of the analytical results. First, sample preparation protocols are usually not standardized, which results in the difficulties to differentiate the results from one laboratory to another with confidence. Second, very less information is known regarding how the sample preparation shows impact on the nano particle characteristics; hence it is a complicated phenomenon to know whether samples that have been prepared following a definite protocol produces a realistic

data of engineered nanomaterials in their native environments (Szakal et al. 2014; Wagner et al. 2015). This perspective demands for analytical procedures that require less destructive sampling methods (Noonan et al. 2014).

4.8 Experimental Strategies for Analysis of Nanomaterials in Complex Biological Matrices of Food Chain

The synthetic nanomaterials are synthesized using different physical and chemical methods by using many weak and strong chemical reducing agents and protective agents (alcohols, sodium borohydride and sodium citrate). The use of these synthetic nanomaterials is harmful to environment, human beings, animals, and plants. These impacts can be reduced by the usage of bionanomaterials instead of synthetic nanomaterials which can be synthesized through biosynthesis or by green methods (Iravani et al. 2014). For the synthesis of bionanoparticles microorganisms, plants, animal products like egg and animal by-products are used. It is a known fact that, many microbes have inorganic materials either intracellularly or extracellularly (Velusamy et al. 2016). For example, unicellular magnetotactic bacteria secretes magnetite nanoparticles and diatoms produce siliceous materials. Bioreduction methods are employed for the synthesis of bionanomaterials where reducing sugar, proteins, enzymes and phenolic compounds are used to initiate the reduction process (Velusamy et al. 2016). These bionanoparticles play a crucial role in the field of medicine, food, agriculture and various other industrial sectors. The microbiological methods for the production of nanoparticles is less efficient than using plants because with microorganisms the nanomaterials are produced at a relatively slower rate than that observed when plant extracts are used. The bionano materials produced by plant extracts have a potential medicinal value, safer to handle and have a broad variability of metabolites that may aid in reduction. Jianrong et al. (2004) investigation found that, silver or gold nanoparticles produced extracellularly by using *Fusarium oxysporum,* can be integrated in various materials like clothes. These silver impregnated clothes can be useful in hospitals as they are sterile to prevent or to minimize the infection caused by pathogenic bacteria like *Staphylococcus aureus*. Fayaz et al. *(*2010) had demonstrated that, the silver nanomaterials synthesized by *Trichoderma viridae* showed a synergistic effect with antibiotics. The delivery of small interfering RNA (siRNA) can be performed by a novel method based on nano device that combines unmodified siRNA with semiconductor quantum dots as multicolor biological probes. Magnetosome particles isolated from magnetotactic bacteria have been used as a carrier for the immobilizing bioactive substances including enzymes, DNA, RNA and antibodies (Mohanpuria et al. 2007). Gold nanoparticles that are synthesized using *Escherichia coli* has been used for analyzing the direct electrochemistry of haemoglobin (Du et al. 2007). Bionanomaterials are less risky than engineered nanomaterials, however their over exploitation in various sectors is increasing the release of bionanomaterials into the environment thereby demanding for quick and reliable assessment of their health hazard potential.

4.9 Identification of Link of Nanomaterials in Food Chain

The available literature and patents on nanotechnology, helps to evaluate and identify the appropriate research tools (Daim et al. 2006; Kostoff et al. 2007; Panpatte et al. 2016), which can be applied into the food sector applications. Thus, an attempt was made to recognize and map areas of nanoresearch with potential applications for enhancing food sector and food supply chain. These include food processing, quality control, packaging, nutraceutical delivery and functional food. Nanoresearch areas were varied ranging from formulations like particles, gels, to emulsions and devices. Applications of numerous technologies were broad and cover a multitude of areas. Majority of these were incremental and supplementing gaps in the existing level of knowledge for enhancing delivery and shelf life quality of the products (Kalpana Sastry et al. 2013).

Many advantages of using nanomaterials in biomedical, diagnostic, industrial and technical sectors were reported and these may be due to their unique optical and physical properties associated to their small size and large specific surface area (Ray et al. 2009). Despite of many advantages immediate attention is required about the manufactured nanoparticles which possess a significant risk to the environment making them less effective. Now-a-days the commercial applications of nanoparticles in many domestic products are taking hike rapidly for example in making detergents, cosmetics, food, and dental products etc. resulting in the rapid rise of the concentration of very potent nanoparticles into the environment thus producing undesirable changes (Contado 2015). This particularly creates a great concern in relation to nanoparticle effects in freshwater and marine ecosystems because several products containing nanoparticles will end up there through sewage systems (Ray et al. 2009). The toxicological examination of fish had revealed that, the investigated substances are observed to be present directly in the medium surrounding the organism (Skjolding et al. 2016). This results in the uptake of these substances through the skin or show direct effect on the gills and their function. Nanoparticles will form complex with substances present in nature which is taken up as a food by the fish in a natural pathway. Their studies have proved that polystyrene nanoparticles are transferred along with the aquatic food chain starting from algae; to zooplankton to fish and then the fat metabolism of fish, especially the mobilization of fat reserves, is severely affected by nanoparticles (Skjolding et al. 2016). Some studies also proved that, nanoparticles increase in concentration, or biomagnify, as they transfer from organism to organism in the food chain. Bertsch's group had studied and found that tobacco plants grown in a solution containing gold nanoparticles accumulated tiny materials in its leaves and then imparted them to feeding caterpillars (Kucukvar and Samadi 2015). Another study conducted by Patricia and Santa Barbara, observed the movement of cadmium selenide quantum dots from *Pseudomonas aeruginosa* bacteria into predator protozoa. The innards of both the protozoa and caterpillars contained high concentration of the nanomaterials than those organisms they ingested (Werlin et al. 2011).

By ocular observations, Cedervall et al. (2012) studied and found that, test fish were slow downed in their movements while compared to control fish and unable to hunt the zooplankton for their food. The data indicates that there was a strong change in the behaviour of the fish after eating food containing nanoparticles. The human plasma contain several apolipoproteins and one such is apoA-I which have the capacity to bind to polystyrene nanoparticles. The nano-sized particles like fullerenes, titanium dioxide and single walled carbon nanotubes, are known to produce biochemical changes in the brain of the fish, which causes behavioural changes. Three noticeable differences in their metabolic features were observed between test and control fish: distribution of cholesterol between liver and muscle, weight loss, and the cholesterol: triglycerides ratio in blood serum.

These findings strongly indicate that there was a disturbance in the lipid metabolism as a result of nanoparticle intake. Another parallel experiment conducted to know the transport of nanoparticles in the food chain using 28 nm polystyrene nanoparticles with encapsulated fluorescent molecules by Cedervall et al. (2012) had found that after 24 hours test algae showed a striking fluorescence and at the same time there was no fluorescence observed in control algae. The control and test algae were given as feed to Daphnia and kept in observation for 24 h. After 24 h in a test Daphnia, a huge number of nanoparticles were evidently visible as discrete fluorescent points, whereas diffused and much weaker auto-fluorescence was found in control Daphnia. When Daphnia were imaged on the net before washing, a considerable portion of fluorescent nanoparticles were identified in the surrounding liquid and they were cleared during the washing step. Their findings have concluded that, nanoparticles are moved through the entire food chain and are delivered to the top consumer, fish, through their food. The polystyrene nanoparticles upon transport along with the food chain produced a devastating effect on the lipid metabolism of top consumers, which in this experiment is fish. They produced behavioural changes in the fish followed by creating potential effects on ecosystem functioning. This was the first report that the authors had described and proved a link between the protein corona and an effect on the behaviour and metabolism of an organism and its function at the ecosystem level. They also reported that nanoparticles used in daily products may influence strongly the top-consumers both metabolically and behaviorally. They also described a protocol on how to test the nanomaterials, so that manufacturing of nanoparticles can be optimized in order to avoid future issues related to health care and environmental disasters (Cedervall et al. 2012).

Very few studies have been attempted concerning the effect of nanomaterials on human health, and also concerns regarding environmental impacts on areas like environmental chemistry, ecotoxicology, behaviour and fate (Zhu et al. 2008). According to available literature, nanoparticles will interact with complex networks of immune cells present within and beneath the epithelial surfaces and work as allergens during the neonatal period stimulating the immune system to produce allergic inflammatory manifestations in the later stages of life (Sly and Schuepp 2012). Cardiovascular damages were also reported due to nanoparticles exposure

in an epidemiological study (Liou et al. 2012). Smulders et al. (2014) had showed that, there is a strong possibility of assimilation of nanoparticles into the body through the lungs, skin and gastrointestinal tract. Nanomaterials can invade the gastrointestinal tract through ingestion of food, water, cosmetics, drugs and drug delivery devices or after mucociliary clearance from the respiratory tract via nasal region. As the nanoparticles are cleared from the respiratory tract through the mucociliary escalator and they can be ingested into the gastrointestinal tract. Hence gastrointestinal tract is said to be as critical target for nanoparticles contact (Liu et al. 2015). Besides these, upgrading the usage of nanoparticles may aggrevate environmental pollution and accidental ingestion via food, water, fish or animals (Bergin and Witzmann 2013). Absorption, accumulation of nanoparticles in extraintestinal organs and their potential to alter gut microbial flora and the effect of this perturbation on the host are the common features taken into consideration while evaluating nanoparticles (Bergin and Witzmann 2013). Many studies were reported on the toxicity of nanomaterials after oral ingestion, however inadequate work has been done till now on gastrointestinal tract exposure. Russel Jones (2000) customized the handling of biodegradable nanoparticles in the oral vaccines delivery for antigens which are susceptible to proteolysis. Due to rapid expansion of nanotechnology industries, there is an improper disposal of nanosized products thereby introducing a new pollution source to the aquatic environment, thus posing a potential threat to the aquatic organisms. Different nano-sized products will be thrown into the sewage system and end up in fresh water and marine habitats. They are not immediately water soluble but subjected to modifications and sorption to organics, such as humic acids or phytoplanktons which could greatly increase the concentration of nanoparticles in the water. Exposure dose and physicochemical form of nanoparticles are crucial for their toxicity to aquatic organisms. Fish is the primary trophic components of aquatic ecosystems, and it provides a primary exposure route for nanoparticles uptake and bioaccumulation in humans. Some effects induced by nanoparticles in fish may reflect their possible hazards in other vertebrates including human beings (Das et al. 2014). Trophic transfer of nanoparticles is now gaining a lot of research interest. Different species like bacteria, phytoplankton and fish in an aquatic system are necessary to be investigated inorder to clarify this issue. The possible biomagnifications of nanoparticles in an aquatic food chain may finally cause the potential threat to human beings. Based on simulative experiments using a simplified model of a fresh water food chain including both low and high trophic level organisms, Chakraborty et al. (2016) found that nanoscale TiO2 particles could be transferred from Daphnia to Zebra fish. Fish models have been extensively used for toxicity assessment of environmental pollutants which may be translated to be risk on human health. Genotoxicity induced by nanoparticles in fish model may have implications for the potential effects not only on aquatic species, but also on human beings (Fig. 4.1).

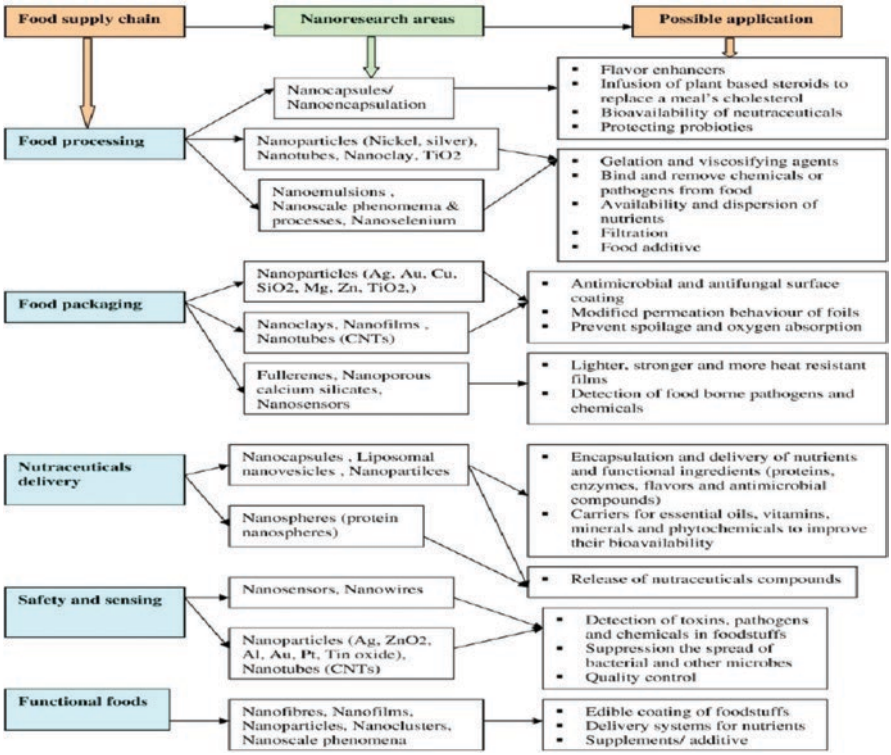

Fig. 4.1 Current applications of nanotechnology in food industry

4.10 Conclusion

Over the past few years the status of the use of structures on the nanometer dimension in the food sector is mounting, as a result, interest and activities in this research area have deeply focused. The chapter has focused on the countless possibilities for the exploitation of the benefits of nanotechnologies in various phases of the food chain with an aim to improve animal nutrition and health, promote new food products. Their applications in the areas of food preservation, food safety and food packaging are well established. Various Commercial applications of nanomaterials will persist to impact the food industry due of their novel and unique properties. Hence human exposure to nanomaterials, will continue to increase with time. Therefore, the impact of nanomaterials in food on the organisms in the food chain is of great public interest and concern. The chapter has discussed on the release of engineered nanomaterial into the environment and their accumulation in different trophic level organisms in food chain. The impacts of synthetic nanomaterials can be reduced by the usage of bionanomaterials which can be synthesized through biosynthesis or by

green methods. The biomagnification of the nanomaterials can be very unsafe to the ecosystems, consequently it is an urgent requirement to test the biomagnification of engineered nanomaterials in various organisms in the food chain.

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Chapter 5
Phytotoxic Impact of Nanomaterials for Nanosafety

Pravin Shende and Anjali Takke

Abstract Nanotechnology opens an indiscriminate extent of innovative utilization in the fields of plant biotechnology and agriculture. Environmental exposure to nanomaterials is imminent as nanomaterials moved toward and becoming part of our routine life, their bioavailability and toxicity are, key features for their enormous employment. Therefore, nanophytotoxicity research has attracted much attention. Phytonanotechnology has the potential to alter conventional plant production using nanomaterials. Exposure of designing nanomaterials on plant development and nanomaterials impacts on morphological, physiological and biochemical attributes which are the subject of intensive research because plants comprise a very important part to the biological system. The major points are the studies on the carbon-based, metal-based nanomaterials, nanofertilizers and their impacts on plants, including mechanism of phytotoxicity and regulatory perspectives. From the toxicological research to date, certain types of nanomaterials provide positive or negative impacts on plant growth in different developmental stages. Relationship between physicochemical properties of nanoparticles and induction of oxidative stress as a projecting paradigm for phytotoxicity. Stringent regulations by government organizations are necessary to detect toxic nanomaterials and their usage in living systems.

Keywords Nanomaterials · Phytotoxicity · Nanofertilizers · Nanosafety · Phytonanotechnology

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5.1 Introduction

Nanotechnology is a rapid-developing industry and impacting generous effects on environment economy and society. It is responsible for reduction in input cost, increase in production and improvement in quality of the life (Iavicoli et al. 2014; Takke and Shende 2019). With the rapid advancement of nanotechnology, expanding measures of nanomaterials will be definitely transferred into the environment, which represent an impedance to environmental division (Shah et al. 2017). Nanomaterials can be grouped into various type such as carbon nanotubes, fullerene, metal-based nanomaterials like quantum dots, gold, zinc, cerium, aluminum, titanium, nickel and other metal oxides like silicon dioxide, iron oxide, cerium oxide nanoparticles etc. as illustrated in Fig. 5.1. As a recent contaminant, the environmental implication and biological impacts of nanomaterials acquire utmost importance (Klaine et al. 2008). Most studies of the toxicity of nanomaterials showed on their impact on living things, however generally in substantial consideration is on plants (Miralles et al. 2012). The interactions amongst plants and nanomaterials revealed on insight into the natural outcomes of nanotechnology (Chichiriccò and Poma 2015). The majority of the accessible observations on nanophytotoxicity have concentrated for the most part on lethality effects on plants and moderately few investigation on the uptake of nanomaterials, phytotoxicity, accumulation, translocation and transmission (Lin et al. 2009). An area of nanotechnology is extremely broad and they can be distributed into various distinctive compound classes. Thus, it is crucial to have an efficient survey of the distributed investigates in this field. The following discussions will be based on the types of nanomaterials and their positive or negative impacts on plants, mechanism of phytotoxicity and effect on ecosystem with regulatory perspectives.

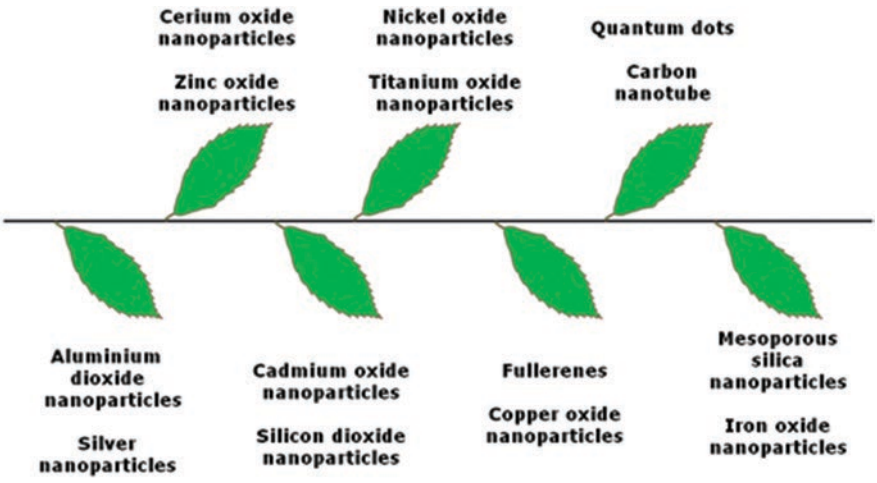

Fig. 5.1 Types of nanomaterials

5.2 Impacts of Carbon-Based Nanomaterials on Plants

Fullerenes and carbon nanotubes (CNTs) are considered as carbon-based nanomaterials. CNTs are of two types single-walled nanotubes (SWNTs) and multi walled-nanotubes (MWNTs). Carbon-based nanomaterials produces positive as well as negative impacts on plants (Morales-Díaz et al. 2017).

Cañas et al. examined the impact of SWNTs and modified SWNTs (3-aminobenzene sulfonic acid) on seedlings of *D. carota, B. oleracea, L. esculentum, C. sativus, L. sativa* and *A. cepa*. The first SWNTs inhibit root elongation in *L. esculentum* but enhance it in *C. sativus* and *A. cepa* whereas the modified SWNTs only inhibit the root elongation of *L. sativa* (Cañas et al. 2008). Exposure of MWNTs does not affect plant growth or germination in *Phaseolus mungo*, but build up elongation and biomass production in *Brassica juncea* (Chichiriccò and Poma 2015).

Hypersensitive response also gives an information of superoxidase activities and reduced dry cell weights chlorophyll in Arabidopsis T87 cells in suspension. It was also found that the exposure to MWNTs accelerates growth of *L. esculentum* plant. This effect may be related to the increased water uptake in exposed seeds (Khodakovskaya et al. 2011).

CNTs enhance root elongation in *Allium cepa* and *Cucumis sativus*. Cabbage and carrot showed impact of nanotubes. MWNTs show no negative effects on root elongation and seed germination in *zucchini* plants whereas a decrease in the biomass of plants during further growth in the presence of SWCNTs (Begum et al. 2014). The effects of different types of carbon-based nanomaterials on plants is presented in Table 5.1.

5.3 Impacts of Metal-Based Nanomaterials on Plants

Metal-based nanoparticles play crucial part because of their applications in the group of Nanoparticles and include metals such as Zn, Ag, Au, Cu, Fe, Mn and their oxides (Abdullaeva 2017). Nair et al. (2010) found that cadmium selenide (CdSe) QDs inhibit germination in rice seeds (Nair et al. 2010). Interaction of seeds of several plants such as *Z. mays*, *G.max* and *Cajanus cajan* with ZnO nanoparticles produce positive effect on germination (Adhikari et al. 2016). ZnO nanoparticles readily dissolve in soil and uptake by plant and TiO_2 nanoparticles concentrate in soil and interact with the cell wall of wheat plant. Both types of nanoparticles reduce biomass production of wheat plant (Du et al. 2011). Impact of Ag, magnetite and Au nanoparticles on lettuce and cucumber plants produces lesser or no toxic effects (Barrena et al. 2009). Gold nanoparticles implicate the physiological and genetic responses on *Arabidopsis thaliana* that it reduces root length by 75% at 100 mg L^{-1} concentration (Yanga et al. 2017). Effect of Cu nanoparticles in low concentrations (<50 ppm) on the germination of lettuce (*L. sativa*) seeds in a water medium shows

Table 5.1 The effects of different types of nanomaterials on plants

Nanomaterial	Plant	Size/ Concentration	Observations	References
ZnO nanoparticles	*Vigna radiata*	22.4 ± 1.8 nm 10 mg L^{-1}	Increase in root length and stem height	Raliya et al. (2016)
Cu nanoparticles	*Solanum lycopersicum*	Less than 100 nm 15–150 mg L^{-1}	Improve plant growth and quality	Juarez-Maldonado et al. (2016)
Fe_2O_3 nanoparticles	*Arachis hypogaea*	20 nm 2–1000 mg L^{-1}	Increase in root length, plant height and biomass production	Rui et al. (2016)
Iron oxide ($Fe_2O_{3)}$ nanoparticles	*Spinacea oleracea*	50–150 nm 200 mg L^{-1}	Increase in root and stem length, magnetic properties	Jeyasubramanian et al. (2016)
Iron oxide ($Fe_2O_{3)}$ nanoparticles	*Glycine max*	6 nm 500–1000 mg L^{-1}	Positive effect on root elongation, enhance photosynthesis	Rui et al. (2016)
Mn nanoparticles	*Vigna radiata*	50–1000 mg L^{-1}	Increase plant metabolism, uptake of nitrogen	Pradhan et al. (2014)
ZnO nanoparticles	*Zea mays, Oryza sativa*	50 nm 500–2000 mg L^{-1}	Inhibit root elongation	Yang et al. (2015)
ZnO nanoparticles	*Triticum aestivum*	Less than 100 nm 500 mg L^{-1}	Negative effect Reduce root growth, decreases chlorophyll content	Servin et al. (2015)
ZnO nanoparticles	*Zea mays*	370–410 nm 20 mg L^{-1}	Entry in the root epidermis, present in xylem vessels	Ruttkay-Nedecky et al. (2017)
Cu nanoparticles	*Cucumis sativus*	40 nm 10, 20 mg L^{-1}	Negative effect Accumulate in roots, decrease in root length	Wang et al. (2016)
CuO nanoparticles	*Elsholtzia splendens*	34–52 nm 100–2000 mg L^{-1}	Negative effect Reduce root length Accumulate in root and leaf cells	Shi et al. (2014)
Ag nanoparticles	*Linum usitatissimum, Hordeum vulgare*	10–100 mg L^{-1}	Negative effect Inhibit shoot length	El-Temsah and Joner (2012)
CeO_2 nanoparticles	*Brassica napus*	Less than 10 nm 2000 mg L^{-1}	Negative effect Inhibit root elongation	Ma et al. (2010)
Gd_2O_3 nanoparticles Gadolinium dioxide	*Cucumis sativus Lactuca sativa*	Less than 30 nm 2000 mg L^{-1}	Negative effect Inhibit root elongation	Ma et al. (2010)

(continued)

Table 5.1 (continued)

Nanomaterial	Plant	Size/ Concentration	Observations	References
Carbon nanotubes	*Brassica juncea*	Less than 200 nm 10–40 mg L^{-1}	Enhance root and shoot elongation	Ghodake et al. (2010)
SiO_2 nanoparticles	*Oryza sativa*	4–30 nm	Improve shoot biomass	Liu et al. (2009)
TiO_2 nanoparticles	*Zea mays*	Less than 40 nm 300–1000 mg L^{-1}	Root elongation not affected Reduce transpiration	Ghodake et al. (2011)
TiO_2 nanoparticles	*Triticum aestivum*	20–100 nm	Negative effect Decrease biomass	Frenk et al. (2013)
ZnO nanoparticles	*Allium cepa*	50–100 nm 5–20 mg L^{-1}	Negative effect Inhibit root elongation	Ghodake et al. (2011)
ZnO nanoparticles	*Lolium perenne*	Less than 30 nm	Entry of Nanoparticles in epidermis and vascular cylinder of the roots	Lin and Xing (2008)
CeO_2	*Zea mays*	37 nm	Agglomerate found Adsorbed on leaf surfaces	Birbaum et al. (2010)
SWNT	*Cucumis sativus*	Less than 10 nm	Adsorbed on root surfaces	Cañas et al. (2008)
MWNT	*Triticum aestivum*	Less than 200 nm	Adsorbed on root surface and entry on cell wall of roots	Wild and Jones (2009)
MWNT	*Nicotiana tabacum*	20 nm 100 mg L^{-1}	Genotoxicity Aquaporins production	Khodakovskaya et al. (2011)
Iron oxide ($Fe_2O_{3)}$ nanoparticles	*Helianthus annuus*	20–100 nm 50–100 mg L^{-1}	No effect on plant biomass production	Martínez-Fernández et al. (2016)
Carbon nanotubes	*Cicer arientinum*	Less than 50 nm	Absorption of water increase by 50% through xylem	Tripathi et al. (2011)
SiO_2 nanoparticles	*Nicotiana tabacum*	100–200 nm	Deliver genetic material and chemical to plant cell	Torney et al. (2007)
ZnO and TiO_2 nanoparticles	*Oryza sativa*	NA	Negative effect Reduce root length No significant reduction with TiO2	Boonyanitipong et al. (2011)
Au nanoparticles	*Oryza sativa*	25 nm	Negative effect Damage of root cell wall and deposition of au through xylem	Yan and Chen (2019)
Fullerene	*Oryza sativa*	1.2 nm	Plant uptake and accumulation	Lin et al. (2009)

(continued)

Table 5.1 (continued)

Nanomaterial	Plant	Size/ Concentration	Observations	References
QDs	*Arabidopsis Thaliana*	Sphere 18–53 nm	Plant uptake and translocation	Koo et al. (2015)
Carbon nanotubes	*Catharanthus roseus*	4 nm – 1 μm	Penetrate subcellular membranes and targets specific subcellular structure	Serag et al. (2011)
Al_2O_3 nanoparticles	*Nicotiana tabacum*	NA	Increase in root length and biomass production	Burklew et al. (2012)

more toxicity than Cu^{2+}ions and decreases seed germination and root elongation (Ruttkay-Nedecky et al. 2017).

γ-Fe_2O_3 nanoparticles produce less toxicity but stimulate root elongation of *L. sativa* seeds in a water medium (Sidhu et al. 2017). The impacts of Al_2O_3 nanoparticles and modified Al_2O_3 nanoparticles with carboxylate ligand coating (100 nm in size) on plants *(Phaseolus vulgaris)* and rye grass *(Lolium perenne)* produce no effect on the growth of the plants. Phytotoxicity of alumina nanoparticles on corn, soyabean, carrot, cabbage and cucumber reduces root elongation (Yanık and Vardar 2015). Nanoparticles of Au, CuO and TiO_2 produce higher germination rate in senescent seeds and suggest that the nanoparticles increase water permeability of the seed coat (Da Costa and Sharma 2016). Increased accumulation of antioxidants in plants has been reported in interaction of nanomaterials of CeO_2. Impact of Ag nanoparticles on *Pennisetum glaucum* improve germination initially but later it produce negative impact on seedling growth (El-Temsah and Joner 2012). The effects of different types of carbon-based nanomaterials on plants is presented in Table 5.1.

5.4 Impacts of Nanofertilizers on Plants

Extensive research on the use of nanofertilizers on plants has been reported, with plant species and the nanomaterials used. Enormous amount of chemical fertilizers and pesticide are used for plant development and disease control (Solanki et al. 2015). Nanoparticles used as nanofertilizers can be absorbed by the root, the apoplastic and symplastic pathways to the xylem, crossing the endodermis and then moving to the rest of the plant through the vascular bundles. This type of pathway observed for mesoporous silica nanoparticles and SiO_2 nanoparticles (Le et al. 2014). To reduce the uncontrolled release of nanofertilizers in the environment, they are coated with thin polymer films, which aggregate the fertilizers in complexes with metal nanoparticles (Liu 2006). Several studies are available on the application of nanoparticles and nanomaterials in the form of nanofertilizers to plants. Impacts of nanofertilizers at certain concentrations are generally positive, increase the plant

tolerance to abiotic stresses, pathogens and pests and the rate of certain metabolic reactions. In plants, if the level of oxidative stress does not exceed the toxic threshold that would lead to cell death, then a defense induction phenomenon is induced that include the accumulation of proteins and defensive metabolites and the expression of resistance genes (Jiang et al. 2012; Morales-Díaz et al. 2017).

5.5 Mechanism of Nanomaterials Toxicity to Plants

It is important to investigate intactness of nanomaterials and can be taken up by plants and transported to other plant tissues. Few studies indicate direct uptake, translocation, transmission and localization of nanomaterials in plants. *In vitro* studies on the regulatory mechanisms of endocytosis using protoplasts have revealed that plant cells, uses endocytic pathways for nanomaterials trafficking. (Kam et al. 2006; Mu et al. 2009). The germination of *Arabidopsis thaliana* (Thale cress) was not affected by Al_2O_3 nanoparticles or SiO_2 nanoparticles, but smaller ZnO nanoparticles interrupted germination. Nanomaterials may also alter the seed coat and create new pores, by regulating the gating of aquaporins, (Lee et al. 2010) and the membrane proteins that transport water to other cellular membranes (Abu-Hamdah et al. 2004).

Transportation of nanomaterials may take place via various routes such as cell wall pores, transport on the surface between the cell wall and the plasma membrane and through plasmodesmata (Nair 2016; Nair et al. 2010). Fullerene C70 enters the roots of *O. sativa* and is translocated to the stem and leaves. Effect of ZnO nanoparticles in *L. perenne,* TiO_2 nanoparticles in *A. thaliana* and CeO_2 nanoparticles in *Z. mays* observed no translocation to the shoots (Birbaum et al. 2010; Jiang et al. 2012; Lin and Xing 2008). Uptake of nanoparticles takes place via induction of new cell pores and enlargement of pores upon interaction with nanoparticles which in turn enhance nanoparticle uptake (Lammel et al. 2019). Depending on the type of metal ions and plant species the uptake and translocation of nanoparticles across root cells in which several active and passive transport processes are involved (Uzu et al. 2010; Zhang et al. 2019). For uptake and translocation, nanoparticles pass through chemical and physiological barriers, which control the size exclusion limits (SELs). Nanoparticles can also enter the vascular system at the root tip meristem, where the *Casparian strip* as not yet fully formed, or the sites of lateral root formation, where the *Casparian strip* is broken (Lazar et al. 2003). Exposure of magnetite (Fe_3O_4) nanoparticles on pumpkin plant (*Cucurbita maxima*) show that nanoparticles get absorbed, translocated and accumulated in plant tissues (Corredor et al. 2009; González-Melendi et al. 2008) (Fig. 5.2).

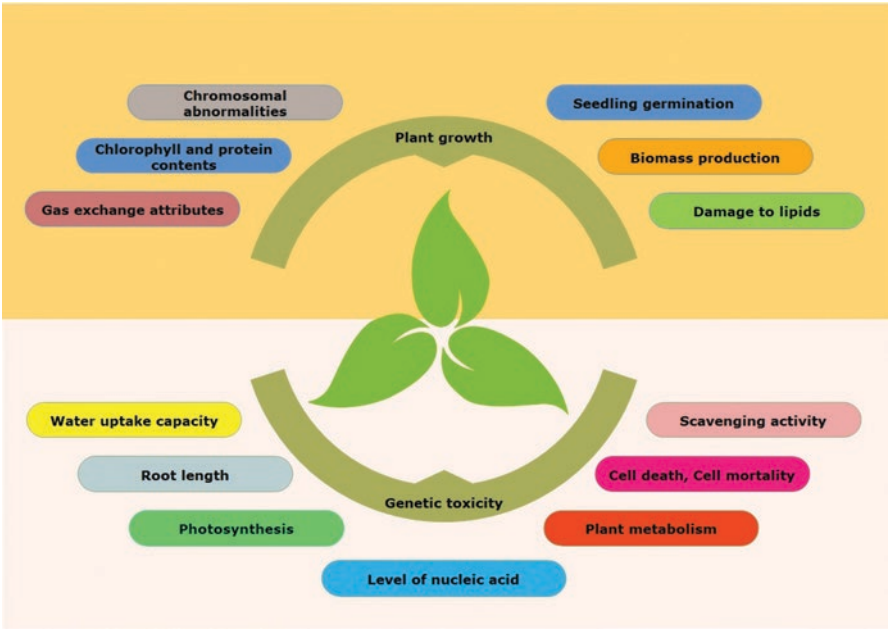

Fig. 5.2 Impact of nanomaterials on plant growth and genetic toxicity

5.6 Nanomaterials Induced Oxidative Stress in Plants

Nanomaterials of varying compound classes such as carbon-based, metal-based induce oxidative stress. Several studies demonstrated the significance of antioxidant defense response in presence of nanomaterials. The increase in production levels of ROS in *Oryza sativa* when exposed to Ag nanoparticles (Mirzajani et al. 2013). Induction of specific ROS such as superoxide radicals and hydrogen peroxide (H_2O_2) was studied by Panda et al., result indicates that it increases levels of these reactive species in the presence of Ag nanoparticles in *Allium cepa* (Onion). The increase in antioxidant enzyme indirectly indicates the increasing values of ROS in cells. Phenols and phenolic acids protect the cellular components from damage caused by oxidative stress because of the presence of nanoparticles in plants (Król et al. 2014; Stuper-Szablewska and Perkowski 2019). In response to oxidative damage, plants have developed a defense system that involves triggering antioxidative defense mechanisms with enzymatic as well as non-enzymatic components. These enzymes (SOD (Superoxide dismutase), POX including guaiacol peroxidase (GPOX), ascorbate peroxidase (APOX), glutathione reductase, dehydroascorbate reductase) scavenge or detoxify the ROS-generated plants exposed to nanoparticles (Khare et al. 2015). Cellular and molecular mechanisms of nanomaterials induce oxidative stress in plants and is utmost important to develop novel strategies to reduce the toxicity of nanomaterials.

5.7 Impact of Nanomaterials on Ecosystem and Affluence on Plants

Considering the phenomenon of nanoparticles and nanomaterials toxicities in plants from a plant community or ecosystem perspective, many possible interactions as well as emergent properties inevitably result from the larger scaled system, as well as greater difficulty in predicting outcomes due to their complexity (Bernhardt et al. 2010). The experiments performed in the laboratory under controlled conditions, with only one to a few plant species, hardly reflect the impacts on natural ecosystems. The reason is natural ecosystems, the space volume, exposure time and interactions between organisms and environment or between nanomaterials being studied and the natural chemical or biological environment may radically modify their structural and surface properties, which leads to different responses observed in the laboratory as found in corn, exhibits a decrease in nanoparticle toxicity of ZnO due to the presence of fungal mycorrhizae (Seneff et al. 2015). Unless the plant is grown under completely aseptic conditions, both the internal and the external environment will contain a microbial community that, combined with the abiotic environmental factors present, will shape the plant phenotype. There is very little research done on the impact of nanoparticles and nanomaterials on microbial populations associated with plants. This is because in general, the experiments reported are those that measured the response variables of plants or microorganisms, as opposed to whole plants and microorganisms (Chichiriccò and Poma 2015). Application of Ag nanoparticles in maize plant resulting high plant biomass and few changes in the plant-associated microbial communities (Lin et al. 2009). Nanomaterials released into water and soil considered as a determining factor for modifying both biodiversity and the relative impact of certain microorganisms found in the soil (Colman et al. 2013), the rhizosphere and the interior of plant tissues.

5.8 Regulatory Perspectives

Nanomaterials production and expenditure have increased the risk of environmental exposure but there is inadequate information available for nanomaterial and plant interaction to accurately characterize hazard or risk (Cañas et al. 2008). The European Food Safety Authority (EFSA) published a guideline emphasizing the potential toxicity of nanomaterials in 2009 (Servin et al. 2015). Unexpectedly, US FDA exists a similar and specific regulatory guidance for nanomaterials use in food sector. Nanomaterials in agriculture is also not exempted from registration under registration, evaluation, authorisation and restriction of chemicals (REACH) ("REACH – Chemicals – Environment – European Commission" 2019). Different avenues are being followed in Organisation for Economic Co-operation and Development (OECD) and non-OECD countries in regulating nanotechnology in

agriculture sector (Amenta et al. 2015). Only EU and Switzerland implemented nano-specific legislative provisions particularly for agriculture, food and feed sector whereas, other non-EU countries have non-mandatory frameworks binding with guidance for industry (Mishra et al. 2017). Exchange of information on regulatory guidelines among other countries and to ensure safe use of nanomaterials for plants as well as for humans which will be beneficial to develop new products and their existence in global market.

5.9 Conclusion and Future Prospects

Phytonanotechnology is an emerging area in plant science and has introduced many novel applications in agriculture. Several research groups have found toxic effects of nanomaterials, the causes for the toxicity are mostly unknown. If it is possible to have a distribution of properly functionalized nanomaterials throughout the plant vascular system and guide them to targeted sites; then these nanomaterials can be successfully used to incorporate fungicides, insecticides, plant hormones, elicitors, nucleic acids into localized areas of plant tissues. In a study of phytotoxic impacts of nanomaterials, scientists have made some consensus on the environmental behavior and ecological effects of nanomaterials; but there are still a lot of controversies and problems that need to be further studied. In this context, the development of quantitative structure-activity relationship (QSAR) studies related to properties of nanomaterials (nano-QSAR) to illuminate the relations between the characteristics of nanoparticles and their impacts on plants is essentially important. Development of nanosensors to identify nutrient efficiency, toxicity, diseases of plants and health of the plant. This will support the phytotoxic impact of nanomaterials for nano-safety. Still, there is a need of research related to safe use of nanomaterials on plant growth as well as to improve the physiological processes in plants.

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Chapter 6
Review of Bioaccumulation, Biomagnification, and Biotransformation of Engineered Nanomaterials

Md. Nizam Uddin, Fenil Desai, and Eylem Asmatulu

Abstract Engineered nanomaterial manufacturing and utilization has been increasing in both consumer and commercial products. As stated by the Project on Emerging Nanotechnologies, there are 1814 nanotechnology consumer products available in the market as of Mach 2015. In Project on Emerging Nanotechnologies' consumer product inventory list, nanoproducts are categorized into eight main categories; house appliances, automotive, cross-cutting, electronics and computers, food and beverage, goods for children, health, and fitness, and home and garden. Nanomaterials provide numerous advantages over conventional materials, even though their small size, shape and related properties may likewise expand their toxicity levels. The bioaccumulation of nanomaterials begins with nanoparticle accumulation in the organism, and then biomagnification follows the toxins accumulated by the predatory organism. Bioconcentration is the last stage, whereby the chemical concentration of toxins in the organism exceeds that in the environment. Here, we have reviewed the interaction of nanomaterials with biological substances focusing on bioaccumulation, biomagnification, and bioconcentration, in order to determine the effect of each nanomaterial on the microorganism as well as the environment from beginning to end. It has been observed that the effects of nanomaterials begin at the bottom of the food chain and move all the way through the human body.

Keywords Bioaccumulation · Biomagnification · Bioconcentration · Nanoparticles · Toxicity · Microorganisms · Silver · Gold · Quantum dots · Carbon nanotube · Titanium · Nanotoxicity · Ecotoxicity

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6.1 Introduction

The rapid development and expansion of nanotechnology industries have ultimately led to the mass production of a wide variety of engineered nanoparticles or engineered nanomaterials, which inevitably increase the possibility of release into the environment and exposure to ecosystems or even humans. These novel engineered nanoparticles exhibit extraordinary performance in mechanical, electric, electronic, thermal, and optical applications due to their unique properties, which traditional or bulk counterpart materials cannot begin to match (Peng et al. 2017). The use of engineered nanoparticles in the products have been drastically increasing. Engineered nanoparticles are defined as matter in the range of 1–100 nm that exhibits physical and chemical properties different from that of their bulk materials. Nanoparticles are used in consumer products, industries, and the remediation of pollutants. Their increasing use is due to their novel physical and chemical properties, which vary from that of their bulk forms. The real issue with engineered nanoparticle is their double activity, including both their synthetic character and their physical properties. For soluble chemicals, the chemical identity has been the parameter controlling ecotoxicological endpoints (such as;, toxicity and bioaccumulation). To study the inherent properties of engineered nanoparticles and how they influence bioaccumulation is a key issue in the current environment. In 2005, the Woodrow Wilson International Center for Scholars initiated a database to map the number of consumer products on the market. Currently, 1814 entries describing category, origin, and nanomaterial are listed in this database (Vance et al. 2015). One of the main applications of engineered nanoparticles and nanotechnology is to diagnose and treat diseases in humans, animals, and aquatic organisms. Engineered nanoparticles have proven their value and novel approaches, especially for biomedical applications (Caruthers et al. 2007). The small sizes of nanoparticles imply that they could target a biological area of interest. Furthermore, metallic nanoparticles can be made to resonantly respond to a time-varying magnetic field, with advantageous results related to the transfer of energy to the particles (Pissuwan et al. 2006). Bioaccumulation is the gradual accumulation of substances such as nanoparticles in an organism. As an example; engineering nano particles can build up in air, soil, and water body and then accumulate in an organism that lives in these surroundings. Typically, bioaccumulation is defined as the increase in the concentration of contaminants in aquatic organisms following uptake from the ambient environmental medium (Wang 2016). Accumulation involves the temporal aspects of exposure and includes kinetic factors such as exposure concentration, exposure duration, clearance, biotransformation, and degradation. Thus, concentration is the central component of any bioaccumulation study, and its significance must be understood. The major focus of research these days is engineered nanoparticle take up and effect in organisms (Ryman-Rasmussen et al. 2006). For aquatic organisms, the different sources of uptake are water (waterborne uptake) and/or food particles (foodborne uptake).

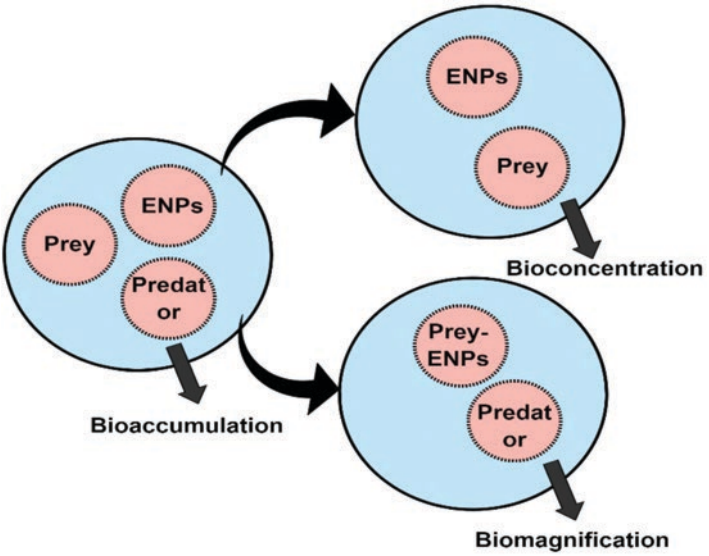

Fig. 6.1 Direct and indirect exposure paths of engineered nanoparticles in aquatic organisms to predator species from aqueous suspension and diet (prey) (Gupta et al. 2017)

Bioaccumulation investigates changes in the concentration of contaminants in organisms. Bioavailability is another concept, which is defined as the fraction of contaminants possibly available for uptake from the environment. Bioavailability explains the portion of contaminants in the environment that is possibly available for bioaccumulation. In ecotoxicological studies, bioaccumulation and bioavailability are considered together. Therefore, both are considered herein along with the usage of bioaccumulation in biomonitoring (Wang 2016).

The rate of engineered nanoparticles uptake and adverse effects especially rely on the routes of exposure as well as internalization in the cell and organism (Gupta et al. 2017). As shown in Fig. 6.1, there are three main types of uptake, based on the exposure route: bioconcentration, bioaccumulation, and biomagnification. However, biomagnification is the most dangerous to the environment and human health. The following sections concentrate on the principles and effects of various engineered nanoparticles due to bioaccumulation, biomagnification, and biotransformation in food, aquatics, and humans.

6.2 Bioaccumulation of Nanomaterials

Bioaccumulation is the immediate connection between contaminants in environments for living beings. Primary danger is showed after bioaccumulation happens. Bioaccumulation straightforwardly connects chemistry/processes along with

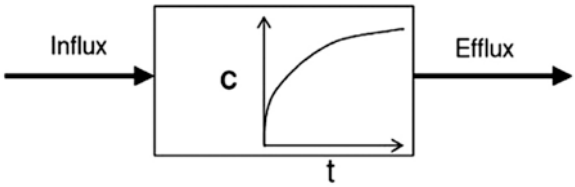

Fig. 6.2 Simple illustration of bioaccumulation in an organism, where C is the net accumulated concentration in the organism, and t is the time of exposure (Wang 2016)

organism physiology/biochemistry and in this manner can be viewed as an interface between chemistry and biology (Wang 2016).

6.2.1 Principles of Bioaccumulation

Bioaccumulation in any system can be illustrated with the simple example in Fig. 6.2, showing the amount of contaminant in a closed system by its any internal transference from influx to efflux. The net accumulated contaminants are considered the bioaccumulation in an organism. Both influx and efflux are an important part of nanoparticle bioaccumulation, and kinetics plays a significant role in the accumulation in various systems. The amount of nanoparticles that accumulate through influx and efflux is considered to be the net bioaccumulation in the system. As shown here, net accumulation depends on influx: if the influx is lower, then there will be fewer containments and less effect on organisms. Net containments and concentration in the organism will remain unaffected in the steady-state condition.

6.2.2 Effect of Bioaccumulation of Engineered Nanoparticles

The use of engineered nanoparticles and nanomaterials are increasing rapidly due to their novel characteristics in consumer products (Asmatulu 2013). Table 6.1 shows the amount of engineered nanoparticles produced by various countries. Information about how they can affect nature, and how they accumulate in the human body and the environment is relatively unknown (Asmatulu et al. 2012). Different patrons are progressively keen on the potential harmfulness as well as different dangers related to the nanomaterials all through the various phases of a product's lifecycle (e.g., advancement, manufacturing, use, transfer, and storage) (Jain et al. 2018).

Risk assessment methods and tools developed and applied to chemicals and other materials cannot be directly applied to engineered nanoparticles because they vary in size and shape. Engineered nanoparticles are nano-sized, which is an advantage in various applications; however, the effect of size varies. Therefore, the

Table 6.1 Quantities of production and utilization of engineered nanoparticles (tons/year) (Piccinno et al. 2012; Hendren et al. 2011)

Country	Silicon dioxide	Titanium dioxide	Zinc oxide	Carbon nanotube	Silver
South Korea	4779.6	3902.2	12.5	3.0	0.3
Switzerland	75	435	70	1	3.1
USA		7800–38,000		55–1101	2.8–20
Europe	55–55,000	55–3000	5.5–28,000	180–550	0.6–55
Worldwide	55–55,000	550–5500	55–550	55–550	5.5–550

developed risk assessment and models to alleviate damage to the environment may not work for engineered nanoparticles (Tervonen et al. 2009).

Nanoparticles may be accidentally or intentionally released into an aquatic system via industrial discharge, domestic waste, effluent disposal, indirect surface runoff from soils, and precipitation-carrying nanoparticles (Batley et al. 2012; Holden et al. 2012; Klaine et al. 2012). The uptake of nanoparticles by living organisms may have cumulative toxic effects, which organisms may counteract by either their storage or excretion in a benign form. Soil bacteria, which are abundant and versatile catalysts, can absorb and disperse engineered nanomaterial agglomerates (Horst et al. 2010). For some engineered nanomaterials, such as cadmium selenide, quantum dots, the bacterial membrane association generates damaging reactive oxygen species. Quantum dots can then enter and accumulate, causing further stress and inhibiting growth (Priester et al. 2009). Engineered nanomaterials could affect plant health and the food supply (Rico et al. 2011; Sardoiwala et al. 2018). X-ray synchrotron techniques identified zinc oxide engineered nanomaterial derivatives, and chemical quantification showed Zn translocation throughout hydroponic soybean and native desert plants; bioaccumulated Zn reduced root growth (López-Moreno et al. 2010; Dev et al. 2018).

The physical properties of engineered nanomaterials likely affect toxicity across the spectrum of the natural aquatic environment. Size variation causes different effects, and engineered nanoparticles will not behave as regularly manufactured materials and typical models that have been developed. Such as to mammalian cells and freshwater for zebrafish embryos, iron doping of zinc oxide nanoparticles is used (George et al. 2009; Xia et al. 2011). Environmental conditions may also impact toxicity, as was demonstrated with marine phytoplankton, the primary producers that support ocean food webs and are integral to the global carbon cycle. Experiments with coastal marine phytoplankton have shown no negative effects of titanium dioxide in tests conducted under standard test conditions with artificial lighting (Miller et al. 2010). Persistent hydrophobic chemicals may accumulate in aquatic organisms through different mechanisms: via the direct uptake from water by gills or skin (bioconcentration), the uptake of suspended particles (ingestion), and the consumption of contaminated food (biomagnification). Even without detectable acute or chronic effects in standard ecotoxicity tests, bioaccumulation should be regarded as a hazard criterion in itself, since some effects may only be recognized in a later phase of life, are multi-generation effects, or manifest only in higher

members of a food web, such as;, the impact of polychlorinated biphenyls on the hatching success of eggs (Tillitt et al. 1992). Bioaccumulation of chemicals in biota may be a prerequisite for adverse effects on ecosystems (Franke et al. 1994).

6.2.3 Bioaccumulation of Various Nanoparticles

6.2.3.1 Gold Nanoparticles

Gold nanoparticles, due to their consistency and synthesis, firmness, and properties of integrating with molecules such as peptides and proteins, make them especially useful for biomedical applications (Pissuwan et al. 2006). Kogan et al. (2006) and Olmedo et al. (2008) demonstrated the feasibility of using targeted graphine nanoplatelets for the remote removal of Alzheimer's amyloid deposits by using local heat dissipated by irradiation of the particles with weak microwaves. The molecule estimate subordinate organ conveyance of gold nanoparticles has been contemplated in vivo. Hillyer and Albertch (2001) showed that orally administrated gold nanoparticles appeared in various tissues in mice and that it concentrated in organisms inversely related to the engineered nanoparticles and its size.

Lasagna-Reeves et al. (2010) experimented with using gold nanoparticles in drug delivery, diagnosis, and treatment, and found that it is essential to characterize the bioaccumulation and toxicity associated with repeated administration of these molecules. Tissues were stained with hematoxylin/eosin as indicated in materials to assess for potential effects of gold nanoparticles treatment on the organ morphology and cellular damage. In all organs studied, there was a significant increase in gold levels after treatment, which was proportional to the dose administered. However, gold levels in the blood did not increase in proportion to the dose, indicating that gold nanoparticles are mostly taken up and accumulated by tissues. However, even assuming that no blood was removed from the organs, an estimation shows that from the quantity obtained in tissues, the contribution of blood was less than 6%, 2%, 3%, 1%, and 7.5%, for the values reported in brain, kidney, liver, spleen, and lung, respectively.

Particularly interesting is the case of the brain. Considering the relatively constant levels of gold in the blood after gold nanoparticles administration at different doses, the increased accumulation of gold in the brain suggests non-saturable uptake of gold nanoparticles across the blood-brain barrier. This is important for utilizing these nanoparticles for potential treatment and diagnosis of neurodegenerative disorders (Lasagna-Reeves et al. 2010). Longmire et al. (2008) concluded that gold nanoparticles of the size 12.5 nm could accumulate in the kidney, liver, and spleen. Also, gold nanoparticles can pass through a filtration barrier because of their small size. In a pioneering study on the uptake and localization of nanoparticles in invertebrates, a typical freshwater flea, *Daphnia magna*, was exposed to 17–23 nm of gold nanoparticles (Lasagna-Reeves et al. 2010). This study did not constitute a bioaccumulation study per se but rather a historical study to map the uptake of

nanoparticles and their localization in the animals. Using transmission electron microscopy, images showed that the gold nanoparticles were primarily located in the gut section. In the context of a single trophic level, the localization of nanoparticles greatly affects their potential for bioaccumulation (Xing et al. 2016; Kahlon et al. 2018). The pharmacokinetic, bioavailability, bioaccumulation, clearance, and toxicity of nanoparticles are likely dependent on the particle composition, size, and surface characteristics. These properties may be altered to reach the most appropriate balance for different applications. One factor regulating the pharmacological properties of nanoparticles may be the electrostatic state of the particle (Judy et al. 2012).

6.2.3.2 Titanium Dioxide Nanoparticles

Titanium dioxide nanoparticles are mainly used in industrial and household applications, and their use is increasing rapidly. The uptake, as well as accumulation of nanoparticles by living life forms may have total toxic impacts; nonetheless, life forms may neutralize these impacts either by storage or discharge of the nanoparticles in an amiable form (Nowack and Bucheli 2007; Shi et al. 2013; Tourinho et al. 2012). Concentrations of titanium dioxide nanoparticles in living beings rely upon their toxicokinetics, and life forms might be exposed to nanoparticles through number of pathways. Nanoparticles with a polycationic or anionic surface may tie to mucoproteins since bodily fluid can chelate cations. In fish body, macro size materials can enter the tissues by means of endocytosis over the gut, and diffusion of lipophilic nanoparticles via the cell membrane can't be precluded (Handy et al. 2008). The epidermis is ensured by mucous; in this manner, nanoparticles may not effectively infiltrate the skin of fish because of an absence of metal transporters in skin cells contrasted with gills (Menard et al. 2011; Hartmann et al. 2012; Schütz et al. 2012).

In humans, titanium dioxide nanoparticles collaborate with plasma proteins, coagulation variables, and platelets. Anatase-form titanium dioxide nanoparticles can penetrate red blood cells; this type of cellular uptake likely involves processes other than phagocytosis and endocytosis because erythrocytes do not have phagocytic receptors. Direct uptake via the skin is a possible route of exposure in soil organisms. The exposure routes and bioaccumulation patterns of titanium dioxide nanoparticles vary according to the organism, and titanium dioxide nanoparticles may bioaccumulate through trophic transfer.

6.2.3.3 Silver Nanoparticles

Silver nanoparticles are metal-based and mostly used in industrial applications. Examination of the impacts of silver nanoparticles on caudal fin regeneration in zebrafish (Yeo and Pak 2008) uncovered that silver nanoparticles had the option to enter fish organelles, including the mitochondria, nucleus, and veins. Absolute

silver levels in intestinal tissues of the zebrafish expanded during the exposure of silver nanoparticle. Levels of silver nanoparticles were most noteworthy in the gills and liver of perch as well as Japanese medaka, and were fundamentally adsorbed and gathered. Coating of silver nanoparticles can impact their conduct and transport at natural interfaces, such as fish gill epithelia; polyvinylpyrrolidone-covered silver nanoparticles basically ignored the multilayered gill multicellular epithelium, though citrate-covered silver nanoparticles inclined to be assimilated into singular cells (Fabrega et al. 2011; Thio et al. 2012). The degree to which the aquatic condition may improve the chemical properties of silver nanoparticles ought to likewise be think over when examining the bioaccumulation of nanoparticles. Bioaccumulation is a significant progression to comprehend when assessing the potential dangers presented by metal-based nanoparticles. Hazard evaluation requires thought of both exposure and bioaccumulation on the grounds that these are normally indicators of poisonous quality (Shi et al. 2013). Bioaccumulation is an immediate method to check the procedures that impact the bioavailability of nanoparticles crosswise over conceivable exposure pathways.

6.2.3.4 Cerium Oxide Nanoparticles

Cerium oxide nanoparticles have become the widely held nanomaterials in the past several years and are currently being used in numerous fields as catalysts, cell electrolytes, semiconductors, antioxidants, coatings, and polishing chemicals (Khan et al. 2011; Luo et al. 2006). Nano size of cerium oxide particles are more toxic than bulk form and may induce cell death, oxidative stress, as well as DNA damage (Arnold et al. 2013; Pulido-Reyes et al. 2015; Zhang et al. 2016). Zhao et al. (2017) constructed a freshwater ecosystem and examined the distribution, bioaccumulation, biomagnification, and impacts of cerium oxide nanoparticles through prolonge exposure. Their results shown that cerium was taken and accumulated through the tested biota and biodiluted in the structured food web, as expressed by a negative relationship between trophic levels and lipid-normalized cerium concentrations. Cerium oxide nanoparticles caused morphological variations in aquatic amphibian hydrophytes because of their chemical or physical properties, conceivably causing irreversible disruption in sustainability of the oceanic framework. These discoveries gave helpful data on the potential dangers from unexpected exposure of nanoparticles in an aquatic system.

6.2.3.5 Carbon Nanoparticles

Carbon nanomaterials, such as carbon nanoparticles, carbon quantum dots, fullerene, carbon nanotubes, and graphene have gained great research interest in the past decades (De Volder et al. 2013; LeCroy et al. 2016). Their unique structures and amazing properties make carbon nanomaterials right for biomedical applications. Particularly, carbon nanomaterials have been found to have great promise in

theranostics, involving bioimaging, diagnosis, drug delivery, gene therapy, photothermal therapy, etc. (Luo et al. 2014; Son et al. 2016). The accumulation and toxicity of carbon nanoparticles in mice were examined, where a carbon nanoparticle suspension injection was trapped in nature in many cases, and no apparent toxicity was detected. Carbon nanoparticle suspension injection accumulated dramatically in the liver and spleen after intravenous injection, but minor contamination was seen in the lungs. The mice behaved normally, and their body weight increases were not disturbed upon exposure to carbon nanoparticle suspension injection (Xie et al. 2017).

6.2.3.6 Zinc Oxide Nanoparticles

Zinc oxide nanoparticles are typical metal oxide nanoparticles broadly used in a range of products including sunscreens, cosmetics, paint, paper, plastics, ceramics, and building materials because of their high stability, anticorrosion, and photocatalytic properties (Osmond and McCall 2010). Zhu et al. (2009) established that zinc oxide nanoparticles had higher acute toxicity to zebrafish embryos than either titanium dioxide or aluminum oxide nanoparticles. The toxicity of zinc oxide nanoparticles (96 h LC50, 4.9 mg/L) to zebrafish was much higher than that of titanium dioxide nanoparticles (96 h LC50, 124.5 mg/L) (Xiong et al. 2011). Although there have been some findings on the potential toxicity of zinc oxide nanoparticles to aquatic ecosystems, that emerging research has mainly focused on acute toxicity or early developmental toxicity of aquatic organisms (Heinlaan et al. 2008). Hao et al. (2013) concluded that nano-zinc oxide exhibited much higher bioaccumulation and oxidative effects and more severe histopathological changes to the test fish than bulk-zinc oxide after a 30-day sub-acute exposure. However, the nano-zinc oxide was not realy contaminated, and the experiments were done with various size of nanoparticles (Bennett 2011).

6.3 Biomagnification of Nanomaterials

6.3.1 Principle of Biomagnification

Biomagnification is defined as the concentration of toxins in an organism by ingesting other plants or animals in which toxins are disbursed extensively throughout its body. Biomagnification can increase because of the following conditions:

- Persistence—where the substance could not decompose via environmental processes.
- Food chain dynamics—where the substance concentration rises as it goes up in the food chain.
- Substance Flow—where the low rate of inner degradation or flow of the substance mostly occurs through water insolubility.

Biological magnification begins with processes that include specific substances such as heavy metals and pesticides mixing with a body of water (such as;, river, lake, and ocean), moving into the food chain as water microorganisms that can be prey for fish, and traveling through the human body. Eventually, the substances are progressively gathered in tissues or inner organs as they progress through the food chain.

Bioaccumulants are substances that are expended in various life forms as they consume tainted air, water, or food, because engineered nanoparticles are used for various applications, and their daily use causes an increase in engineered nanoparticles contamination in nature (Landrum and Fisher 1999). For example, the use of the insecticide dichlorodiphenyltrichloroethane for insect control, was stopped in 1972 by the U.S. Environmental Protection Agency due to its high toxicity levels (NPIC 1999). The steadiness of dichlorodiphenyltrichloroethane in nature and its bioaccumulation and biomagnification has severely impacted numerous organisms. This insecticide has been associated with the occurrence of cancer, premature birth, infertility, and diabetes. It has more broadly been connected to the populace decay of bird species on the natural way of life; for example, the peregrine falcon and bald eagle may be linked to dichlorodiphenyltrichloroethane toxicity where the eggshell thickness has diminished (Olenick 2013). Figure 6.3 shows

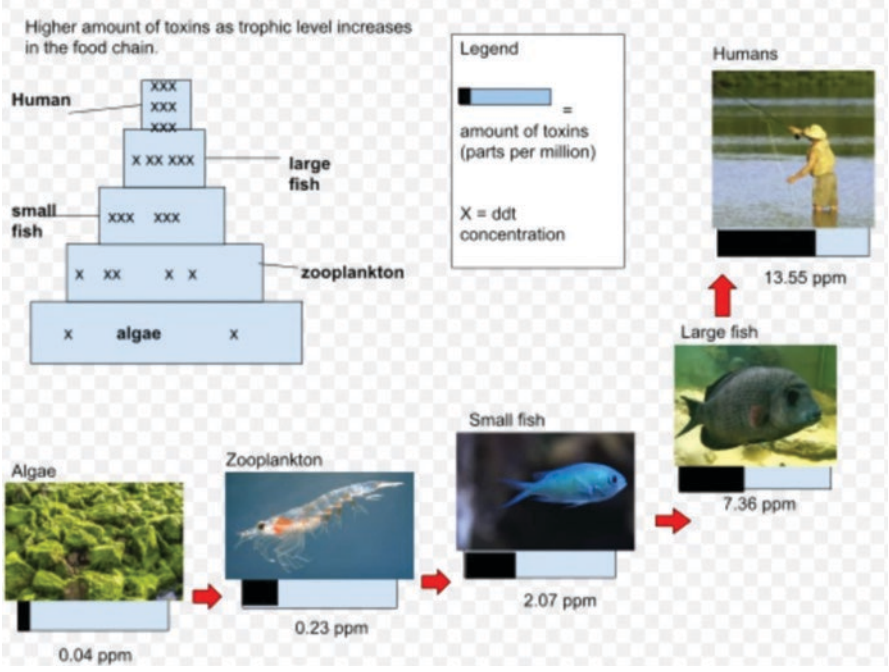

Fig. 6.3 Biomagnification of dichlorodiphenyltrichloroethane in food chain (concentrations in parts per million)

dichlorodiphenyltrichloroethane biomagnification movement in the food chain. As can be seen, the trophic level increases in the food chain, as does the amount of toxic accumulation. The accumulated toxin is higher in predators than in prey, since predators accumulate the toxin in their sensitive organs, tissues, and fat. For instance, tuna fish accumulate mercury and other toxins in their fat and other parts of the body; therefore, it is recommended that eating tuna be limited to twice a week. Since humans are at the highest level of the food chain, bioconcentration can be hazardous if biomagnification proceeds all the way through the body.

6.3.2 Biomagnification of Nanomaterials

Due to the invention of new applications in many disciplines (such as;, biomedical, mechanical, and electrical engineering), nanomaterial usage has increased expansively, which also means an increase in nanoparticles released into the environment. According to Keller and Lazareva (2013), some of the nanomaterials applications, in particular coatings, paints, cosmetics, and pigments, are the primary sources of their direct distribution into the environment. As an end of life fate, nanoproducts goes into waste disposal areas where release occurs and collects in water sources, such as wastewater treatment plants and aquatic environments. It has been assessed that the 69,200 and 189,200 metric tons of nanoparticles are released every year universally into water sources and landfills, respectively. Engineered nanomaterials can be released into different environments during their various life stages, including production, manufacturing, transportation, customer use, waste treatment plant, disposal, and landfilling (Gottschalk et al. 2009; Walser et al. 2012). As nanomaterials are released into the natural environment, they go through various stages and cooperate with chemical, biological, physical, and ecological elements that may inhibit their performance and transport in ecological networks where the danger starts (Gupta et al. 2017).

6.3.3 Biomagnification of Various Nanoparticles

6.3.3.1 Quantum Dots

The transmission of engineered nanoparticles is increasing and there is concern about its possible effect on ecological receptors as well as food webs. Nanoparticles differ in their chemistry, morphology, reactivity, and coating and biotic impacts. According to Stern et al. (2012), a wide range of metallic nanoparticles are associated with bacteria. For example, cerium oxide nanoparticles can be absorbed by *Escherichia coli* or can activate sludge, and cadmium selenide quantum dots can get

into a cell of *Pseudomonas aeruginosa* to affect the body function. nanoparticles such as titanium dioxide have been entering the environment at higher rates every year due to their high production level; therefore, the nanomaterials-bacteria relationship and mobilization in higher trophic levels have increased scientific and public concerns. Werlin et al. (2011) studied bare cadmium selenide quantum dots that accumulated in specific bacteria called *Pseudomonas aeruginosa*, which were biomagnified in the *Tetrahymena thermophila* protozoa by consumption. For example, the concentration of cadmium in protozoa as a predator is higher than in its bacterial prey. Because protozoa do not undergo lysis, they largely consume quantum dots to stay available at higher trophic levels. The detected biomagnification from bacterial prey is considerably high since they are at the center of the food chain.

6.3.3.2 Silver Nanoparticles

Toxicity of the bioaccumulation and biomagnification of silver nanoparticles is another area of study that has been examined in the model food chain (Yoo-iam et al. 2014). In this study, the toxicity effect of silver nanoparticles on *Chlorella sp*., *Chironomus spp*., *Moina macrocopa*, and *Barbonymus gonionotus* were examined. Based on the test results, toxicity order on all four organisms was that silver ions were more toxic than silver nanoparticles for some of the microorganisms. They found that the highest silver ion bioaccumulation factor was 101.84 L g^{-1} in *Chlorella sp*, and the least bioaccumulation factor of silver nanoparticles was 1.89 L g^{-1} in *B. gonionotus*, because the food chain transfer of silver nanoparticles happened only from *Chlorella sp*. to *Moina macrocopa*, and there was no sign of biomagnification from food sources to consumers in a basic tropical food chain (Yoo-iam et al. 2014). The authors' findings indicate that the biomagnification of heavy metals did not occur at a higher trophic level. In order for biomagnification to be considered at the trophic level, trace metal concentration should appear in at least two trophic levels. A lower concentration of heavy metal in the animal body indicates a lower level of heavy metal in the water body/source (Barwick and Maher 2003). Also, animals located in higher trophic levels might be more competent in removing heavy metals than organisms located in lower trophic levels. The antioxidant enzyme system in infected animals could also be in charge of the end of oxidative stress within the beginning time of the body's protective system (De La Torre-Roche et al. 2013; Thio et al. 2012).

Nanomaterials have been used in a broad range of applications, which has created concern since the direct and indirect effects of metal nanoparticles are unclear. Garcia-Reyero et al. (2014) investigated whether polyvinylpyrrolidone-coated silver nanoparticles (polyvinylpyrrolidone-silver nanoparticles) cause effects through the nanoparticles or the dissolved silver ions. The toxicity mechanism of fathead minnow fish (*Pimephales promelas*) was investigated by exposing it to either 4.8 μg/L of silver nitrate or 61.4 μg/L of polyvinylpyrrolidone-silver nanoparticles for 96 h. Microarray investigations were utilized to distinguish the affected receptors and danger pathways in liver and cerebrum tissues that were confirmed utilizing

as a part of vitro mammalian examines. The fish subjected to silver nitrate and polyvinylpyrrolidone-silver nanoparticle had normal and discrete effects that were constant with intact nanoparticles and dissolved silver-causing effects. Polyvinylpyrrolidone-silver nanoparticles and silver nitrate both affected pathways engaged with sodium, potassium, and hydrogen homeostasis and oxidative stress. In vivo effects were sustained via polyvinylpyrrolidone-silver nanoparticle actuation of five in vitro atomic receptor tests with a restraint of ligand binding to the dopamine receptor. Silver nitrate restrained the ligand binding to adrenergic receptors α1 and α2 and the cannabinoid receptor CB1, yet had no effect in atomic receptor examines. Also, polyvinylpyrrolidone-silver nanoparticles can possibly cause effects both through intact nanoparticles and metal particles, each associated with different starting occasions. Because the in vitro and in vivo measures considered here are generally utilized as a part of human and natural risk screening, this work recommends that ecological well-being evaluations ought to consider the effects of intact nanoparticles on top of the dissolved metals (Garcia-Reyero et al. 2014).

6.3.3.3 Titanium Dioxide Nanoparticles

Zhu et al. (2010) conducted a toxicity assessment to understand the potential ecotoxicity of nanoscale titanium dioxide to the aquatic organism *Daphnia magna. They performed a comprehensive study by modifing acute* (72 h) and chronic (21 days) toxicity tests along with titanium dioxide nanoparticles accumulation analysis. As a result, titanium dioxide nanoparticles applied minimal toxicity to *Daphnia* within 48 h exposure time and caused high toxicity when the exposure time was longer, such as 72 h. From this perspective, exposure duration might be the contributing factor in nanoparticle-mediated toxicity. Furthermore, upon chronic exposure to *t*itanium dioxide nanoparticles for 21 days, *Daphnia* exhibited critical growth delay as well as mortality, and reproductive defects. Captivatingly, an essential amount of *t*itanium dioxide nanoparticles were observed in *Daphnia*. Conversely, *Daphnia* showed difficulty in eliminating titanium dioxide nanoparticles from its body with the increased bioconcentration factor values. A high level of bioaccumulation may interfere with food intake and eventually distress the growth and reproduction of individuals as well as the population, thereby posing risks to aquatic ecosystems (Zhu et al. 2010).

Identifying the fate and effect of nanosized titanium dioxide on lower-trophic level organisms in the aquatic food chain is essential since this is the largest released engineered nanomaterial to the aquatic environment. Gupta et al. (2016) used Paramecium caudatum and Escherichia coli to assess the effects of titanium dioxide nanoparticle. The surface connection of titanium dioxide nanoparticles with Escherichia coli drastically improved with the addition of P. caudatum into the microcosm. This connection supported the hetero-agglomeration and co-sedimentation of titanium dioxide nanoparticles. The degree of titanium dioxide nanoparticles agglomeration under test conditions was as follows: joined Escherichia coli and Paramecium caudatum > Paramecium caudatum just > Escherichia coli just

> without Escherichia coli or Paramecium caudatum. An expansion in titanium dioxide nanoparticle disguise in the Paramecium caudatum cells was likewise seen in the nearness or nonattendance of Escherichia coli cells. These collaborations and titanium dioxide nanoparticle disguise in Paramecium caudatum cells prompted measurably critical ($p < 0.05$) impacts on development and the bacterial ingestion rate at 24 h. These discoveries provide insight into the destiny of titanium dioxide nanoparticles within sight of bacterial-ciliate collaborations in the oceanic condition (Gupta et al. 2016).

6.3.3.4 Gold Nanoparticles

Most nanomaterial-containing consumer products complete their service life and end up in waste streams. Many classes of nanomaterials accumulate in the sludge obtained from wastewater treatment and finally in soil resulting in land applications as bio-solids. In order to evaluate the impact of nanoparticles on terrestrial ecosystems, model organisms *Nicotiana tabacum* L. cv *Xanthi* and *Manduca sexta* (tobacco hornworms) were chosen to examine plant uptake and the potential trophic movement for 5-, 10-, and 15-nm-diameter gold nanoparticles. The outcome indicated a trophic movement and biomagnification of gold nanoparticles from a first producer to a first user through mean factors of 6.2, 11.6, and 9.6 for the 5-, 10-, and 15-nm treatments, respectively. This outcome is essential for the nanotechnology-related risks, involving the potential for human exposure (Judy et al. 2011).

6.4 Biotransformation of Nanomaterials

6.4.1 Principle of Biotransformation

Understanding the biotransformation (biochemical modification by living organisms) of nanomaterials and their cytotoxicity and potential environmental health issues involving their applications is inevitable. Knowing the interactions of nanomaterials with their environment are essential to assess their exposure, hazards, and risks. These interactions include biological, physical, and chemical transformations, which impact nanomaterial persistence, toxicity, bio-uptake, reactivity, etc. However, dissolution, sulfidation, aggregation, oxidation, and reduction reactions can typically occur with nanomaterials in biological systems.

The development and usage of nanomedicines in the medical sector validate the assessment of the fate of nanomaterials in vivo. Some acute effects of nanomaterials have been described, but their transformation by the biological environment is little investigated and therefore gaining a research interest these days. Nanoparticles can enter the human body through inhalation, ingestion and dermal penetration from contaminated environments and food (Sanchez et al. 2012). The interaction of

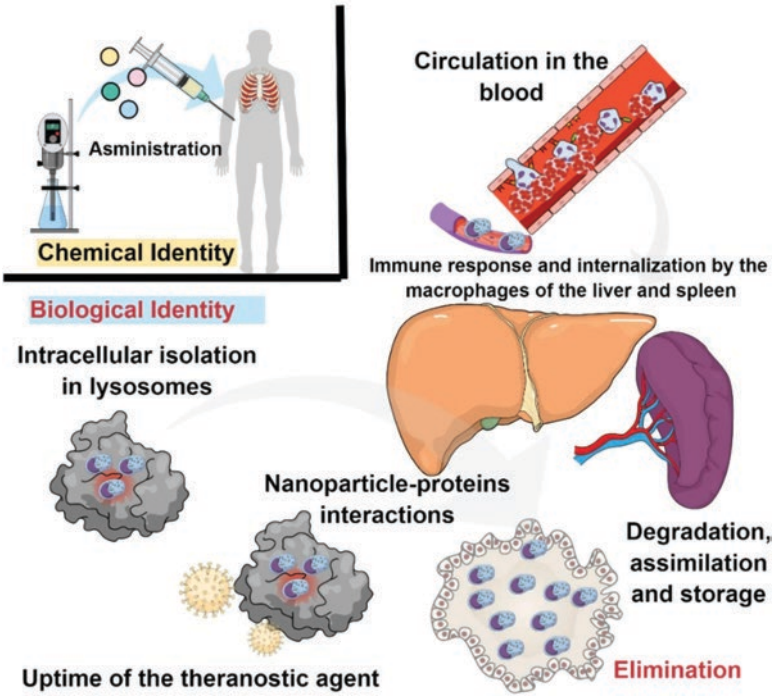

Fig. 6.4 Nanoparticle lifecycle after administration into veins (Kolosnjaj-Tabi et al. 2016)

nanomaterials with the biological environment, such as biological fluids, membranes, cell components, proteins, DNA, etc., remodel their structure, surface properties, biotransformation, enzymatic attack, toxicity, degradation, particle distribution, fate and bio-assimilation throughout the organism (Fig. 6.4) (Kolosnjaj-Tabi et al. 2016). This in turn leads to the transport of nanomaterials in physiological media, cellular internalization, and potential toxicity (Loeve et al. 2013). In addition, the length of time that nanomaterials are within an organism also prompts their fate. Although nanomaterials have a lengthy tenacity within the body, their transformation or transportation through kinetic processes are ultimately slower processes, which can evoke chronic inflammatory reactions. Hence, the lifecycle of nanomaterials within the body from initial exposure to complete elimination is a critical issue. Sometimes reactive, rapidly degradable nanomaterials are desired, whereas for persistence nanomaterials, be reside inactive in the organism. However, unexpected biological reactions with nanomaterials produce by-products that saturate lysosomal compartments and perturb degradative and autophagic pathways that are essential for cells to degrade proteins (Stern et al. 2012). It is necessary to explore the biological effects on the lifecycle of nanomaterials and methodologies to characterize its biotransformation in vivo over time from whole body level to the nanoscopic scale. Herein the biotransformation of different nanomaterials and

toxicity of pristine and bio-transformed nanomaterials and other health-related issues are discussed.

6.4.2 Biotransformation of Various Nanoparticles

6.4.2.1 Graphene-Related Materials

Graphene-related materials include graphene oxide, reduced graphene oxide, single and few-layer graphene, graphene nanoribbons, and graphene quantum dots (Bhuyan et al. 2016; Uddin et al. 2014). Due to having excellent characteristics and properties, these materials are extensively used in consumer products. Through inhalation, skin contact, or ingestion, graphene related materials can enter the human body; consequently, many in vitro and in vivo research studies have been carried out to assess their potential risk to humans (Ema et al. 2017). Once these materials enter the biological system, their physical-chemical properties may alter depending on the biological environment, such as temperature, pH, concentration, salts, and many other factors. Recent studies have shown that graphene related materials may be degraded by oxidase enzymes, such as horseradish peroxidase and human myeloperoxidase, both of which usually exist in physiological fluids (Gebel et al. 2014). One recent study was carried out to investigate the biotransformation, cytotoxicity, and inflammatory response of graphene oxide/few-layer graphene in the gastrointestinal tract (salivary, gastric, and intestinal) upon their in vitro digestion (Guarnieri et al. 2018).

A schematic diagram showing an in vitro digestion assay for the ingestion of graphene related materials is presented in Fig. 6.5. Graphene oxide and few-layer graphene exhibit typical D and G bands, and few-layer graphene also exhibits D′ and 2D bands. However, physiological juices exhibit two bands at 1450 and 2900 cm^{-1}, which are attributed to the bending and stretching vibrational modes of methylene or methyl respectively. Apparently, a change in defects was observed as the graphene oxide/few-layer graphene passes through the simulated digestive tract. Another study was carried out to investigate in vivo biotransformation of graphene oxide in two simulated lung fluids: Gamble's solution and artificial lysosomal fluid (Qi et al. 2018). The results show that graphene oxide significantly alters its physiochemical properties, morphology, and functionality in two simulated lung fluids. In particular, sheet-like graphene oxide was reduced to randomly wrinkled and stacked sheets, in comparison to Gamble's graphene oxide and artificial lysosomal fluid—graphene oxide. Also, after biotransformation, Gamble's graphene oxide and artificial lysosomal fluid—graphene oxide had increased functionalities compared to pristine graphene oxide. Physicochemical properties of the biotransformed graphene oxide are depicted in Table 6.2. The structural morphology of the biotransformed graphene oxide is shown in Fig. 6.6. As can be seen, the transmission electron microscopy images of pristine graphene oxide show a sheet-like structure with a smooth surface. However, the Gamble's graphene oxide and artificial

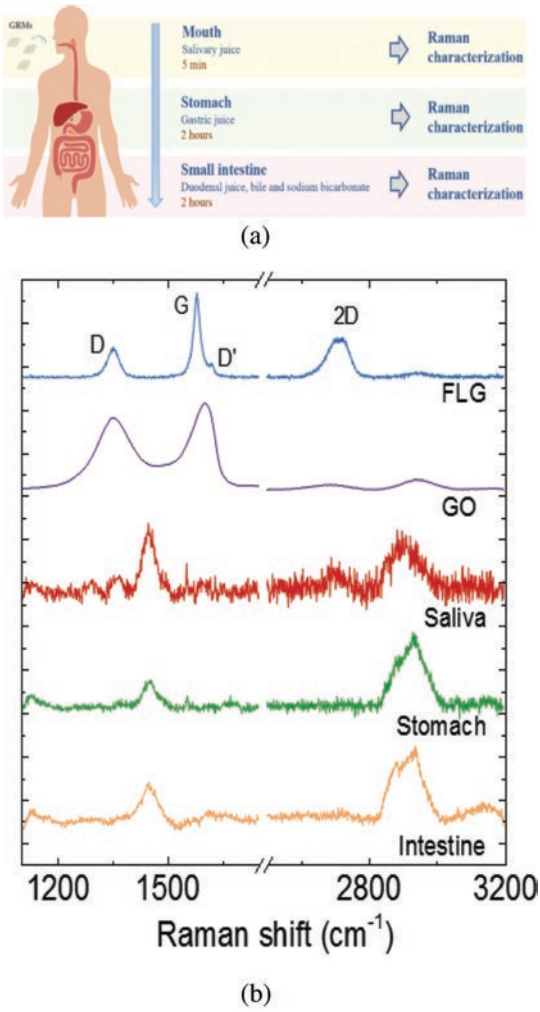

Fig. 6.5 (**a**) In vitro digestion assay for graphene oxide/few-layer graphene ingestion through different digestive sections, and (**b**) representative Raman spectroscopy. All appropriate factors in the digestion process, such as temperature, pH changes, transit times, relevant enzymes, and protein compositions, were considered (Guarnieri et al. 2018)

lysosomal fluid—graphene oxide are wrinkled structures with thicker flakes (Fig. 6.6b).

The scanning electron microscopy and atomic force microscopy images of pristine and biotransformed graphene oxide also reveal significant structural changes upon biotransformation, especially for Gamble's graphene oxide, likely due to the increase in van der Waals forces and hydrophobicity from reduction, resulting in a greater tendency of nanosheet aggregation. In addition, graphene oxide sheets are transformed by the blood plasma. However, little information is known about the effect of nanomaterial biotransformation in blood plasma. After 14 days of graphene oxide sheet exposure to human blood plasma, the graphene oxide sheets

Table 6.2 Physicochemical properties of pristine and biotransformed graphene oxide materials (Liu et al. 2018)

	C (wt%)										
	Aromatic rings	Epoxy/ hydroxyl	Carbonyl	Carboxyl	Total C (wt%)	Total O (wt%)	C/O ratio	pH	I_D/I_G	Hydrodynamic diameter (nm)	Polydispersity index
Graphene oxide	24.62	33.88	6.02	5.69	70.23	29.03	2.41	5.71	0.61	144.3 ± 6.1	0.27
Gamble's graphene oxide	29.04	28.61	1.92	8.7	68.27	28.07	2.43	6.45	0.82	345.7 ± 22.9	0.50
Artificial lysosomal fluid -graphene oxide	22.88	26.19	–	25.19	74.98	23.58	3.18	6.51	0.84	323.6 ± 15.2	0.45

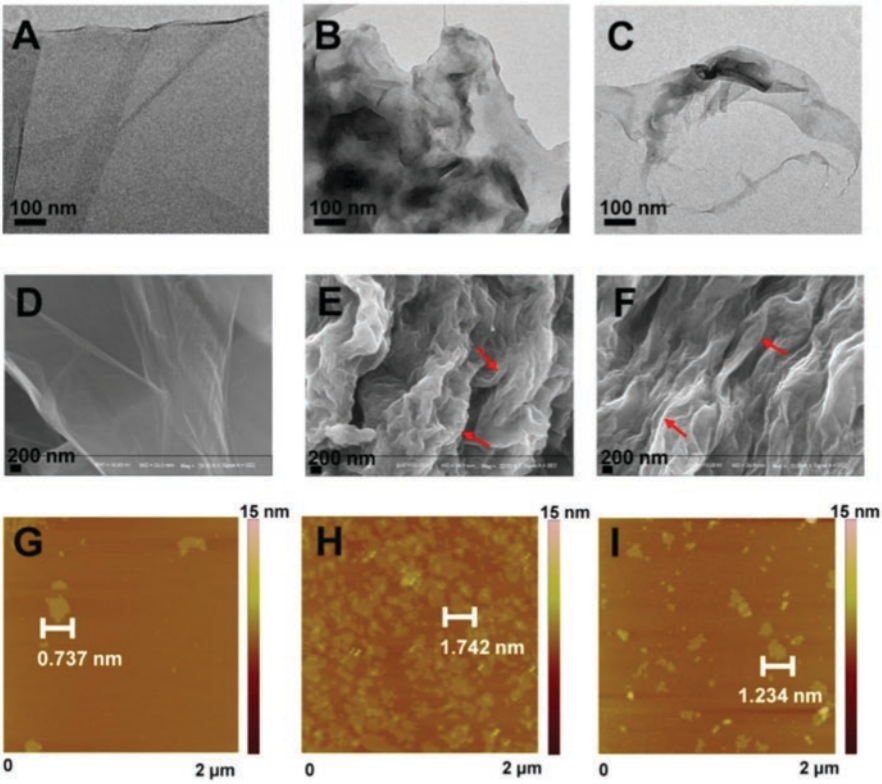

Fig. 6.6 Transmission electron microscopy, scanning electron microscopy, and atomic force microscopy images, respectively, of biotransformed graphene oxide materials: (**a**, **d**), and (**g**): pristine graphene oxide; (**b**, **e**), and (**h**): Gamble's graphene oxide; and (**c**, **f**), and (**i**): artificial lysosomal fluid—graphene oxide. Stacked graphene oxide sheets are shown with red arrows. ID/IG designates intensity ratio of the (**d**) and (**g**) bands (Liu et al. 2018)

degraded and formed biological corona on them (Wen et al. 2016). Notably, the biotransformation influenced the cytotoxicity induced by graphene oxide.

6.4.2.2 Magnetic Iron Oxide Nanoparticles

Magnetic iron oxide nanoparticles are favorable inorganic nanoparticles for diagnostic and therapeutic applications such as magnetic resonance imaging diagnosis. Magnetic resonance imaging is an intense and unrivaled technique to observe the long-term fate of magnetic iron oxide nanoparticles. In addition, electron paramagnetic resonance provides a particular and delicate quantification procedure for magnetic iron oxide nanoparticles. Iron oxide nanoparticles of various sizes, geometry, and coating (glucose-subsidiary, polyethylene glycol), and amphiphilic polymer) were studied more than 1 year after intravenous organization (2.5 mg/kg bw) using

multiscale techniques, followed up by magnetic resonance imaging, electron paramagnetic resonance quantification, and electron microscopy (Kolosnjaj-Tabi et al. 2016).

"Iron oxide nanoparticles can reach the liver and the spleen because of their coating". Also, electron paramagnetic resonance discloses the coating-dependent elimination of superparamagnetic iron from these organs, which occurs a long while after injection. Correspondingly, with the desertion of superparamagnetic iron, magnetic iron oxide nanoparticles dissipated in the liver and spleen, and transformed into nonmagnetic iron (Lartigue et al. 2013). Figure 6.7 illustrates the in vivo time-dependent biodistribution and transformation of magnetic iron oxide nanoparticles. It is expected that the particular metabolism that controls iron in the living being can likewise deal with magnetic iron oxide nanoparticles. The morphology and subcellular distribution of magnetic iron oxide nanoparticles have been studied by transmission electron microscopy (Fig. 6.7).

After 1 day of injection and at a later time period, the clusters of magnetic iron oxide nanoparticles within the lysosomes of splenic and hepatic macrophages shortened in the periphery and local degradation of magnetic iron oxide nanoparticles within the lysosomes. Another study by Levy et al. (2011) reported the

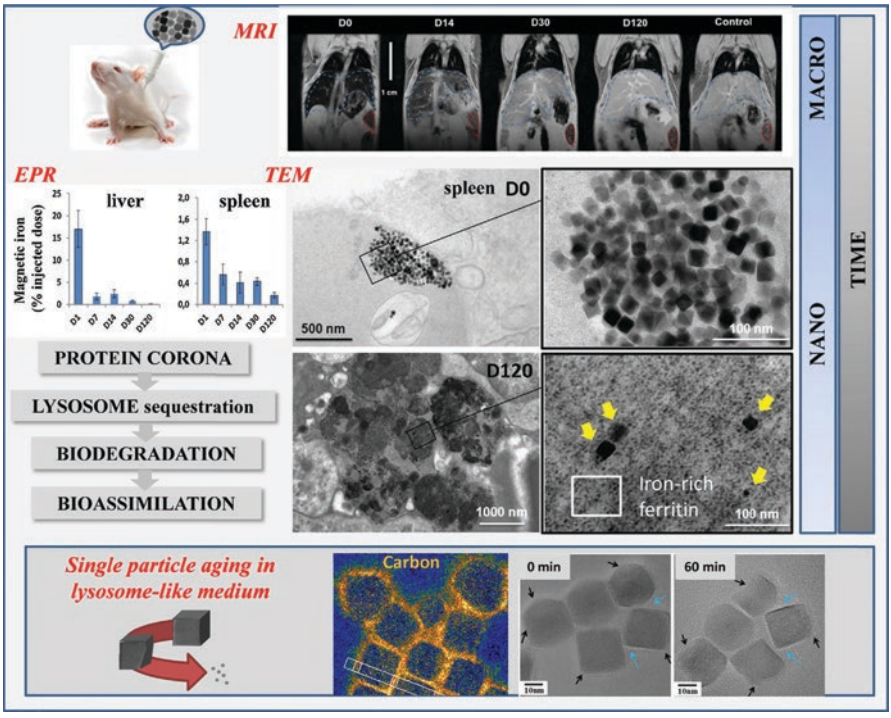

Fig. 6.7 In vivo biotransformation of magnetic iron oxide nanoparticles in mice using magnetic resonance imaging, ex vivo electron paramagnetic resonance quantification in organs, and associated transmission electron microscopy observations (Lartigue et al. 2013)

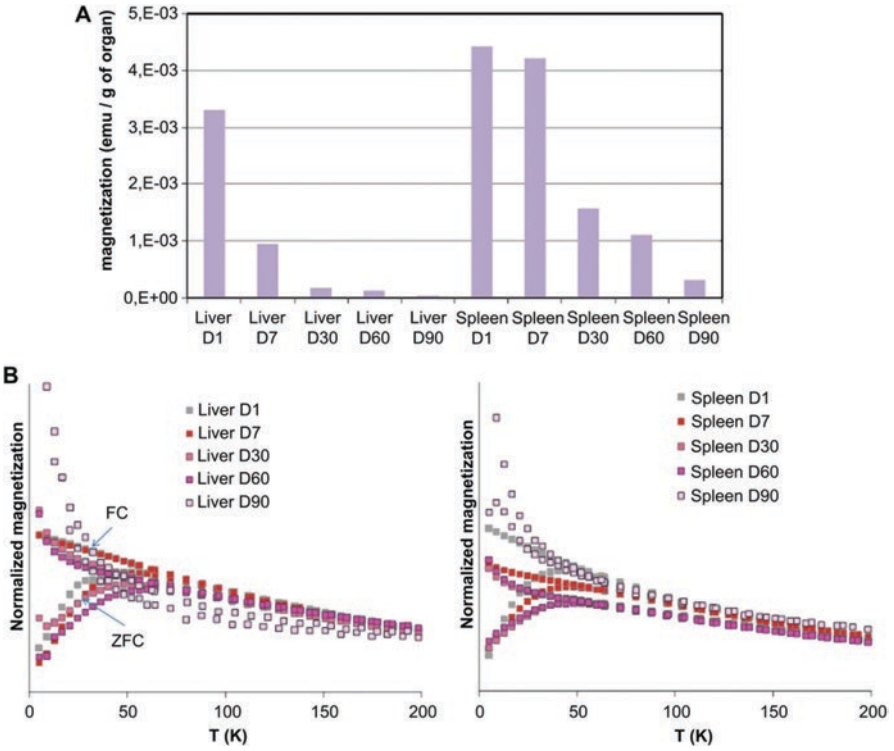

Fig. 6.8 (**a**) Saturation magnetization and (**b**) thermal dependence of magnetization in dried liver and spleen over 3-month time period after injection of magnetic iron oxide nanoparticles (Levy et al. 2011)

biotransformation of magnetic iron oxide nanoparticles in vivo over a long period of time. They used ferromagnetic resonance and inductively coupled plasma to observe magnetic iron oxide nanoparticles in mice tissue. The magnetizations of liver and spleen were estimated as a function of magnetic field strength (3×10^4 Gauss) and temperature of 5–300 K with a fixed magnetic field of 50 Gauss (Fig. 6.8a). Results show a reduction in magnetization of the liver or spleen over time after magnetic iron oxide nanoparticles are injected. The low-field magnetization for field-cooled and zero-field-cooled specimens of liver and spleen after injection exhibit similar results (Fig. 6.8b), revealing that superparamagnetic nanoparticles transformed to poorly magnetic iron species over a 3-month time period.

6.4.2.3 Carbon Nanotubes

Because of their extraordinary properties, potential uses, and a wide range of utilization (such as;, optical, electronic, biomedical), the production of carbon nanotubes has relentlessly increased, with an expected generation of more than 300 t/

year (Piccinno et al. 2012). Carbon nanotubes are extensively used in biomedical applications such as targeted drug delivery and remediation agents. As a result, public awareness of carbon nanotubes in the environment and related issues has gained much attention in the research community. However, assessing the effect of carbon nanotubes on human beings and the environment is a big challenge. Figure 6.9 presents a schematic illustration of biotransformed multi-walled carbon nanotubes by surface oxidation. Chouhan et al. (2016) investigated the biotransformation of multi-walled carbon nanotubes by bacteria identified as *Trabusiella guamensis*. The multi-walled carbon nanotubes and bacteria were allowed to interact in order to obtain insight into the biotransformation of their structure. Redox-enzyme activity and cell viability testing revealed that multi-walled carbon nanotubes are oxidized and biotransformed through the formation of C=O and COOH groups on the outer walls of nanotubes. Also, oxygen-containing functional groups on the surface of multi-walled carbon nanotubes increased. The morphological study showed that surface roughness and number of concentric walls of the multi-walled carbon nanotubes were also reduced. The biotransformation process is an oxidation and partial catalytic degradation process. The interaction between bacteria and multi-walled carbon nanotubes are presented in Fig. 6.10.

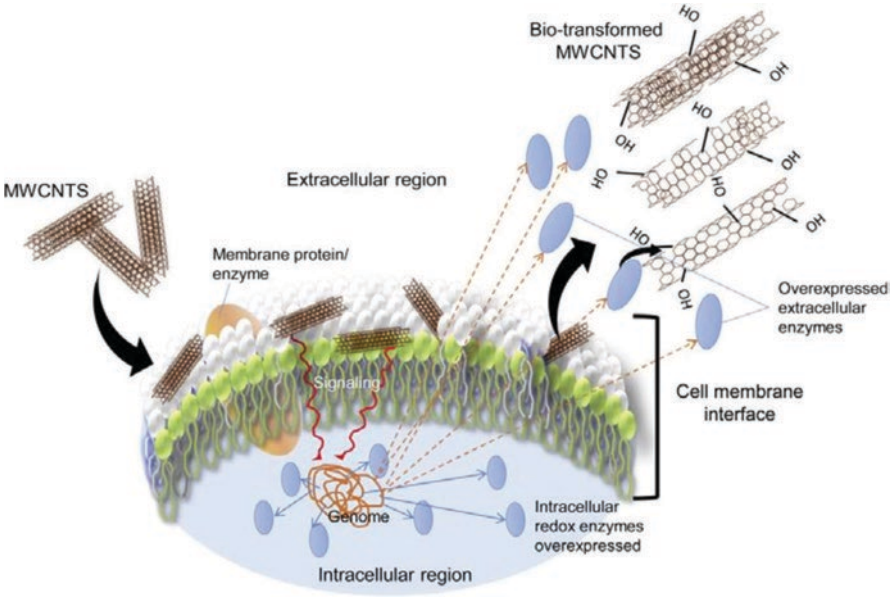

Fig. 6.9 Schematic of biotransformed multi-walled carbon nanotubes by surface oxidation nanotubes (Chouhan et al. 2016)

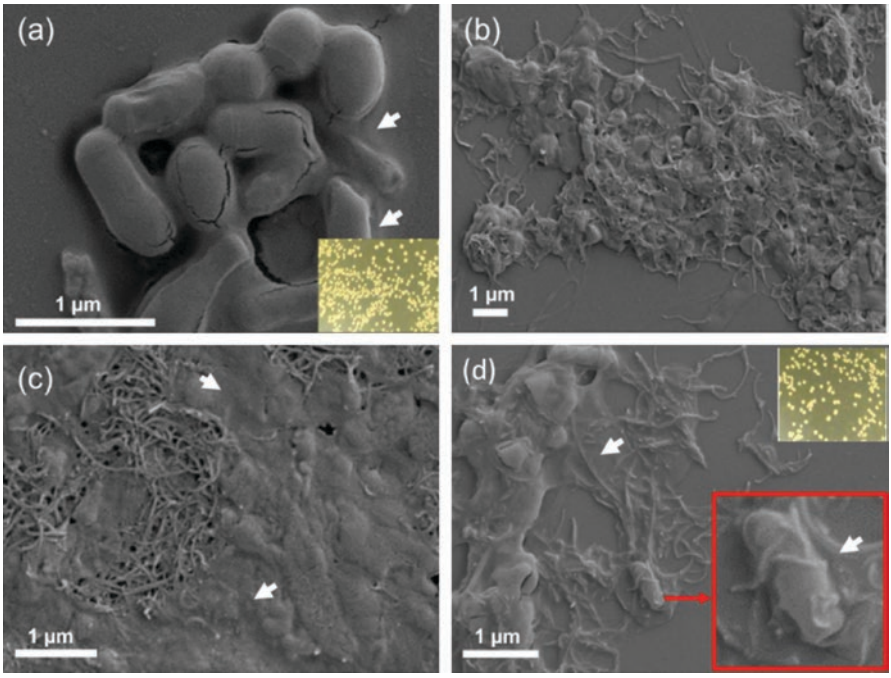

Fig. 6.10 Scanning electron microscopy images: (**a**) bacteria; (**b**, **c**) bacteria and multi-walled carbon nanotube bundles; and (**d**) bacteria wrapped by multi-walled carbon nanotubes (Chouhan et al. 2016)

6.4.2.4 Silver Nanoparticles

Because of their outstanding antibacterial and other properties, silver nanoparticles are extensively used in medical devices, cosmetics, food packaging materials, electronics, household appliances, and many other fields (Mallevre et al. 2016). These is one of the highest production nanomaterials across the globe. However, their production, transportation, and applications cause mean that these nanoparticles have unexpected exposure to the environment and humans. This nanomaterial can cause diverse toxicities because of its size, ion release, and ability to bind with various functional proteins. The potential routes of exposure of silver nanoparticles in humans are inhalation, dermal contact, and oral administration. In addition, they can gain access to the human body through coated contact lenses, bone cement and other implants, eye drops, nanosilver-coated medical catheters, cardiovascular implants, etc. (Ge et al. 2014). These particles can enter and gather in different tissues and organs such as the lung, spleen, heart, kidney, ovary, and brain.

In vivo, silver nanoparticles can be biotransformed into other forms of silver. Silver ion release is another biotransformation of silver nanoparticles (Liu and Hurt 2012). The release of silver ion is more toxic and greatly affects the toxicity of silver nanoparticles. Van der Zande et al. (2012) studied the biotransformation of silver

nanoparticles in vivo. Experimenting with rats, they found that the amount of free silver ions released in a silver nanoparticles suspension is directly related with the silver content in the main organs of the rat. Even after 28 days, the rat blood treated with silver nanoparticles exhibited approximately 7–10 times lower silver content than in the silver nitrate group. Another study on silver nanoparticle-containing paint additive reported that silver nanoparticles release ions and dissolve, crossing the air-blood barrier (Smulders et al. 2015). These particles can enter the blood circulation system and be transformed into an ion or precipitate into other silver-containing matter, thereby being distributed in the organs and parts of animal bodies. The possible biotransformation of silver nanoparticles includes silver sulfide and silver chloride by reactions with sulfur and chloride species, respectively. The silver nanoparticles exposed to mice lung of mice were dissolving oxidatively and transformed into thiol-containing molecules such as cysteine, glutathione, and others.

6.4.2.5 Cerium Oxide Nanoparticles

Cerium oxide nanoparticles are metal oxide nanoparticles and extensively used in various applications such as fuel-borne catalysts, polishing agents, cosmetics, semiconductors, etc. (Cassee et al. 2011). Cerium oxide nanoparticles improve fuel burning efficiency and thus are released into the environment. Numerous plants such as cucumber, corn, tomato, and soybean can store untransformed cerium oxide nanoparticles. In addition, cerium oxide nanoparticles have the potential to be used in a therapeutic strategy for cerium neurodegenerative diseases in humans (Kyosseva et al. 2013). Moreover, the cerium oxide nanoparticle nanoceria saturated elements of the reticuloendothelial system in the liver and spleen when exposed to high intravenous dose of cerium oxide nanoparticles. The cerium oxide nanoparticles retained in the hepatic phagolysosomes and release secondary plum while in the biotransformation of the mammalian system. Zhang et al. (2012) studied the biotransformation of cerium oxide nanoparticles in cucumber plants. They treated cucumber roots with 2000 mg/L cerium oxide nanoparticles. After 21 days of treatment, cerium phosphate was observed on the epidermis and intercellular spaces of cucumber roots. It was also observed that in the biotransformation and particle dissolution process, the reducing substances (ascorbic acids) and organic acids played a vital role. The biotransformation process includes cerium oxide nanoparticles being absorbed on the root surfaces and dissolved and released cerium (III) ions being precipitated on the root surfaces and in intercellular spaces with phosphate.

6.4.2.6 Copper Oxide Nanoparticles

Copper oxide nanoparticles is another type of metal oxide nanoparticle and has extensive applications in surfactants, sensors, antimicrobials, and catalysts, and an impact on the agricultural industry (Saison et al. 2010). In Asia and other parts of

the world, rice is the basic food and consumed heavily. It is of great concern to study the biotransformation of copper oxide nanoparticles in rice plants in order to be aware of metal contamination in the food chain. Up until now, very limited knowledge has been available on the possible translocation and biotransformation of copper oxide nanoparticles at tissue, organ, and cellular levels. One study was carried out to investigate the biotransformation of copper oxide nanoparticles in rice plants exposed to 100 mg/L copper oxide nanoparticles for 14 days (Peng et al. 2015). The experimental results revealed that copper oxide nanoparticles moved into the root exodermis, epidermis, and cortex, and ultimately reached the endodermis but could not easily pass the Casparian strip. In addition, copper oxide nanoparticles were transported from the roots to the leaves, and copper (II) combined with cysteine, citrate, and phosphate ligands and was even reduced to copper (I).

6.4.2.7 Zinc Oxide Nanoparticles

Rosa et al. (2011) investigated the toxicity and biotransformation of zinc oxide nanoparticles in three different desert plants at the germination stage: Prosopis juliflora-velutina (velvet mesquite), Salsola tragus (tumbleweed), and Parkinsonia florida (blue palo verde). Zinc oxide nanoparticles in concentrations between 0 and 4000 mg L^{-1} were used to treat the seeds of these plants, and the germination rate, root elongation, and zinc concentration in tissues were measured. Experimental results indicated that there was no significant effect on germination of the plants studied. However, there was an approximate 16% reduction in root elongation in the blue palo verde for nanoparticles concentrations of 4000 mg zinc oxide L^{-1}. For nanoparticles concentrations of 500 and 2000 mg L^{-1}, the tumbleweed root size was reduced by 14% and 16%, respectively. For any nanoparticle's concentrations studied here, the velvet mesquite root length was abridged. Moreover, zinc oxide nanoparticles biotransformed to zinc (II) within the root of the three different plants.

6.5 Designing Safer Nanoparticles

Nanoparticles are essentially potential transfer mechanisms for human health since humans sit at the highest point of the food chain, so they could be risky if biomagnification proceeded as far up the food chain possible. It is hard to generalize among nanoparticles, given their altogether different physical and chemical characteristics. Gold nanoparticles could be a good point to start since they are inert and stable; however, silver nanoparticles come with challenges because silver has many chemical forms. It is difficult to speculate how different plants or microbes might accumulate nanoparticles relative ethylene oxide concentrations in particular species. We may hope to see a variety of inclinations for taking up the study of nanoparticles, yet a case-by-case approach many not be vital. The intake of nanomaterials to organisms shows a range of different propensities, while a case-by-case approach may not

be required. Understanding the nanomaterial's accumulation and magnification for each species is still in its infancy.

Considering all three stages of nanoparticle movement in the food chain (bioaccumulation, biomagnification, and bioconcentration), manufacturers are playing a significant role regarding balancing the nanomaterial concentration. The characteristic of nanomaterials can be tailored to be safer if manufacturers design them carefully. For instance, a nanomaterial coating that would significantly reduce the concentration of nanomaterials in the environment could be used. Nanoparticle interactions that distract the membrane via free radical arrangement may be engaged while enabling particles to enter cells. Thus, coatings intended to decrease free radical development and make nanoparticles more secure may go far. Finding the balance point between safety and application is essential. Imagine planning a nanoparticle so environmentally friendly on the other hand that doesn't provide any market value over what is being done today, that is not useful for innovation. Likewise, imagine blending nanoparticles that are fabulous in items however amazingly lethal—that is no use for nature.

6.6 Conclusions

The manufacture and use of engineered nanomaterials have been increasing in both commercial and consumer products. Engineered nanomaterials have one-of-a-kind physical and chemical properties, which make them attractive materials for a broad range of applications but also contribute to their adverse behavior in biological systems including the environment and public health. Past investigations have demonstrated that engineered nanomaterials can be transmitted from prey to predator; however, the environmental effects of these nanostructured materials are undetermined. Specifically, it is not known whether these materials can be biomagnified, a procedure in which higher groupings of materials amass in living beings higher up in the life chain. Discovery of the balance point between safety and application is essential. It is very important to develop safe engineered nanoparticles and nano-enabled products so that they can benefit human health (such as;, targeted drug delivery and imaging), mitigate climate change (such as; fuel-saving vehicles), purify water (such as; nanosized membrane filters and selective sorbents), produce energy (such as; carbon capturing and solar cells), and be distributed and stored (such as; long-life batteries, fuel cells and catalysts for water splitting).

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Chapter 7
Nanomaterials and Human Health: An Overview

Farhana Abedin, Eylem Asmatulu, and Mohammad Nahid Andalib

Abstract With the advent of nanotechnology in commercial products, the risk of exposure of nanomaterials to humans and the environment is increasing at an accelerating rate. The impact of nanomaterials on humans is complex and not yet fully understood. A comprehensive understanding of the adverse effect of long-term exposure to nanomaterials on humans is warranted, and a balance between benefits and risks is required before nanomaterials are unleashed in large quantities as a part of commercial products. Most data on the consequences of nanomaterial exposure are obtained using *in vitro* and *in vivo* studies using animal models. The risk to human health is implied by these studies. In this chapter, the possible methods of exposure of humans to nanomaterials, the effect of some frequently used nanomaterials on human cells, and animal models are discussed. The primary methods of exposure to nanomaterials include oral, dermal, intravenous, and inhalation. The route of exposure can cause variation in the adverse effect on the human health. Nanomaterials elicit different negative effects/damage repair pathways depending on the type of cell, and the toxicity may vary vastly based on the type of nanomaterial. Also, the psychochemical parameters of nanomaterials such as size, shape, functionalization, and defects as well as the gender of the person can significantly alter the adverse effect on biological entities.

Keywords Human health · Toxicity · Nanomaterials · Risks · Exposure

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7.1 Introduction

Nanomaterials possess at least one dimension less than 100 nm. Nanotechnology is playing a vital role in innovation and the economy. Submicron-scale particles are ultrafine particles (UFPs) that are released into the environment by fossil fuel combustion or industrial emissions, whereas engineered nanomaterials are manufactured through controlled processes (Li et al. 2016). Both types of particles could have adverse impacts on humans, such as asthma, allergy, inflammation, DNA damage, and interference with signaling pathways. They could also adversely affect cardiovascular and respiratory systems (Li et al. 2016; Jain et al. 2018). Engineered nanomaterials have become attractive in various applications due to their unique properties imparted by the nano-scale size (Merwe and Pickrell 2018). Both commercial production and use of engineered nanomaterials are on the rise (Merwe and Pickrell 2018). It is predicted that titanium dioxide nanoparticle production could rise from 5000 tons in 2010 to 58,000 tons in 2020 (Smolkova et al. 2015). The market for graphene was estimated to be US$12 million in 2013 (Zurutuza and Marinelli 2014). In 2006, the production of synthetic amorphous silica was estimated to be one million tons per year (Fontana et al. 2017). According to the European consumer market, most engineered nanomaterial-containing products belong to the healthcare and fitness area (Mebert et al. 2017). With the rising use of engineered nanomaterials in consumer products, concerns have been raised regarding their impact on the human health and environment. Data is lacking regarding the production volume of engineered nanomaterials and their distribution in various products (Piccinno et al. 2012). Production volume, distribution, product life cycle, and product fate are an integral part of the risk assessment of engineered nanomaterials (Piccinno et al. 2012). Risk assessments to the environment and humans are very important as the use of engineered nanomaterials continues to rise since they can elicit toxicity. The risk/benefit assessment of nanomaterials before they are incorporated into consumer goods is also a very important issue and should be considered by industries (Fransman et al. 2017). Numerous studies have been carried out regarding the toxicity of engineered nanomaterials, but the fact that the toxicity of engineered nanomaterials can vary based on the size, psychochemical factors, route of administration, method of dispersion, etc., makes the investigation of impacts of engineered nanomaterials on human health complex. For example, despite numerous past studies on the cytotoxicity of engineered carbon nanomaterials, the results remain contentious (Yuan et al. 2019). Moreover, the lack of data and a comprehensive understanding of the mechanisms involved make it extremely challenging to develop regulations for engineered nanomaterial (Ganguly et al. 2018). Nanotoxicology is a new branch of the toxicology field which focuses on the understanding of toxicity of nanomaterials (Ganguly et al. 2018). The effect of nanomaterials on ecology and co-exposure of toxicant with engineered nanomaterials are also important to understand the risk posed by them (Merwe and Pickrell 2018). Developmental toxicity due to engineered nanomaterials, and the underlying mechanisms play an important role in the risk assessment of nanomaterials during

pregnancy (Dugershaw et al. 2020). Past studies have indicated that engineered nanomaterials can cause both direct and indirect developmental toxicity (Dugershaw et al. 2020).

Exposure of humans and the environment to nanoscale materials in quantities that may draw adverse biological response will continue to rise with increased use of nanomaterials in industries and consumer products (Merwe and Pickrell 2018). The method of exposure and possible impact on humans are discussed here. Most studies related to the toxicity of engineered nanomaterials have been carried out *in vitro* or *in vivo* using animal models. Therefore, most of the discussion on toxicity could be indirectly related to humans. The major focus here is on the impact of carbonaceous, silica, titanium dioxide, and silver nanomaterials on various cell lines and animal models.

7.2 Sources and Route of Engineered Nanomaterial Exposure to Humans

Due to their unique properties associated with their size, engineered nanomaterials have triggered an outburst of their exploitation in industrial applications. This has raised concerns about their safety and fate in the environment. The use of engineered nanomaterials is thriving in consumer as well as commercial/advanced products such as food, additives, supplements, feed, biocides, veterinary drugs, agriculture, water purification, soil cleaning, information technology, energy production, shampoo, and sunscreen (Martirosyan and Schneider 2014). Engineered nanomaterials are being considered for improving plant germination and growth, pesticides, pesticide/pathogen detection, fertilizer, etc. (Kah and Hofmann 2014; Khot et al. 2012; Parisi et al. 2015; Liu and Lal 2015). Although the majority of nanopesticides on the market exceeds the 100 nm upper size limit, as the research and nanotechnology field advances, it is possible that more and more agriculture-related products will fall into the nanoscale size range (<100 nm) (Kah 2015). This could lead to the trophic transfer of engineered nanomaterials to humans and the possibility of biomagnification (Lead et al. 2018; Judy et al. 2010). Moreover, engineered nanomaterials could end up in agricultural areas through their accumulation in sludge during wastewater treatment (Judy et al. 2010). A greener approach towards nanopesticides could be polymer-based nanoformulations (Kah and Hofmann 2014). Some studies have shown an enhanced germination rate and biomass in some plants in the presence of nanomaterials as well as their adverse impact (Khodakovskaya et al. 2009; Zheng et al. 2005; Rico et al. 2011). Hence, a greater understanding of the mechanisms involved in the use of engineered nanomaterials in agriculture causing beneficial and deleterious impacts is necessary.

Silica nanoparticles are found in processed food production and storage, and it was found that about 43% of amorphous silica is in the nanoscale range (Mebert et al. 2017). Silica is found in anticaking agents, antifoaming agents, and clarifying/

fining agents in food. Silica particles found in milk powder, instant soup, and spices may range from 50 to 200 nm in size (Mebert et al. 2017). Silica is also used as a nanofiller in food packaging, and may migrate when it comes in contact with food. Silica is extensively used in cosmetics, including hairstyling products, eyeliner, eyeshadow, lipstick, toothpaste, sunscreen, and antiperspirant commodities. Silica also paved its way in drug delivery and biomedical imaging. Silica nanoparticles can be advantageous in targeted drug delivery, imparting enhanced solubility and drug loading, whereas in the case of imaging, they can facilitate entrapment and functionalization of the imaging agents (Mebert et al. 2017). Therefore, dermal, oral, and intravenous exposure of humans to silica nanoparticles is inevitable. Workers in industries manufacturing these products are susceptible to exposure by inhalation as well.

Food-grade titanium dioxide may contain some particles in the nanosize range. Titanium dioxide nanocomposites are used as oxygen sensors in food packaging. Titanium dioxide and magnesium oxide nanoparticles are used as food preservatives and to facilitate the handling of food. The former is also used as an anticaking agent in powdered food products (Smolkova et al. 2015). Titanium dioxide nanoparticles are also used as a colorant in confectionery food items, non-dairy creamer, etc. They are used as photocatalysts in water treatment applications (Smolkova et al. 2015) and can be found in toothpaste, sunscreen, paints, and glazes (Weir et al. 2012), as well as photovoltaics, electrode material in lithium-ion batteries, and catalysts (Fröschl et al. 2012).

Silver nanoparticle coating is used in food as an antimicrobial agent as well as in cellulose pads that are often placed in packages of meat products (Smolkova et al. 2015). Silver nanoparticles are also used in bedding, water purifiers, toothpaste, nipples and nursing bottles, shampoos, fabrics, deodorants, kitchen utensils, etc. Aluminum nanoparticles are used in aluminum foil as an anti-adhesive agent (Smolkova et al. 2015).

Carbon nanotubes have applications in supercapacitors, metal composites, field emission displays, organic electrolytes, ionic liquids, and lithium batteries (Bianco et al. 2005; Zhang et al. 2013). Appropriately functionalized carbon nanotubes are also being considered for vaccine-delivery systems and protein transporters (Bianco et al. 2005). They have the potential to be used in nanoelectronic technology (Chen et al. 2016; Avouris et al. 2003). Graphene-based products that are already commercially available include tennis rackets, phone touchscreens, and battery straps (Zurutuza and Marinelli 2014). Graphene is being considered in applications such as metal alloys, filtration systems, printed electronics, flexible transparent conductors, polymer composites, multifunctional coatings, oil, etc. (Zurutuza and Marinelli 2014). Other carbon nanoparticles are found in caramelized sugar, bread, and corn flakes (Smolkova et al. 2015).

Therefore, it can be seen that humans can come in direct contact with engineered nanomaterials through food, cosmetics, household commodities, pharmaceuticals, water filtration, etc., leading to dermal, oral, and intravenous routes of exposure. Workers in engineered nanomaterial-related industries may be exposed directly through inhalation. Engineered nanomaterials in products such as composites,

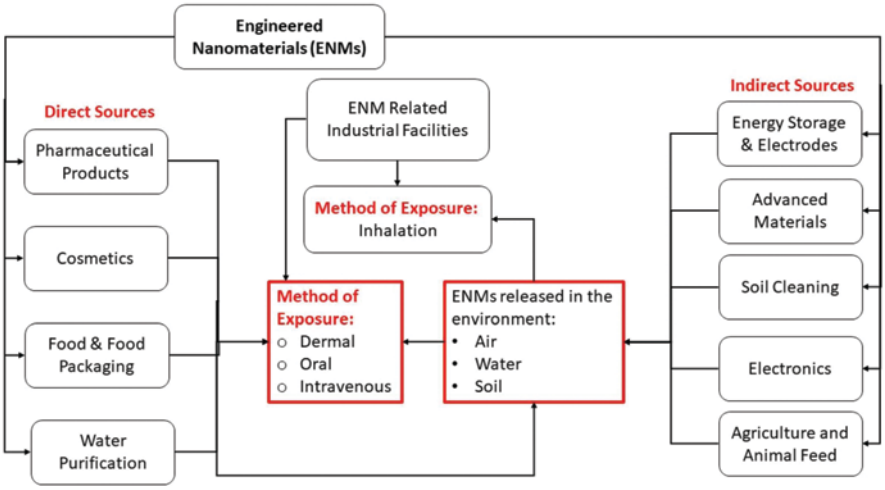

Fig. 7.1 Examples of possible sources of products containing engineered nanomaterials and methods of human exposure

batteries, electronics, paint, coatings, etc. could be released into the environment through their use and disposal, and are likely to end up in rivers, streams, air, and soil. Engineered nanomaterials released into the air could be inhaled by humans, and engineered nanomaterials released into the soil and water can enter the food chain, and eventually reach humans. Figure 7.1 shows a schematic for various sources and routes of exposure of engineered nanomaterials to humans.

7.3 Impact of Engineered Nanomaterials on Human Health

Direct data on how engineered nanomaterials influence human health is limited. There is also lack of data on the exposure of workers in industries handling nanomaterials. Most studies in this area were done on animal models. Risk factors in humans are governed by exposure level, routes of exposure and the type, size, reactivity, distribution and shape of the engineered nanomaterial (Aschberger et al. 2011). Figure 7.2 summarizes some of the different types of toxicities caused by nanomaterials and factors that may impact the type and level of toxicity. A suitable and well-established method to determine engineered nanomaterial exposure levels is very limited. Therefore, there are uncertainties and reliability issues relative to conclusions made about the health risk of engineered nanomaterials in humans (Aschberger et al. 2011). Based on the existing available database, Aschberger et al. reported the risk of four types of nanomaterials: fullerenes, carbon nanotubes, metals, and metal oxides (Aschberger et al. 2011). To assess the risk to human health, they used the indicative no-effect level (INEL), indicative no-effect

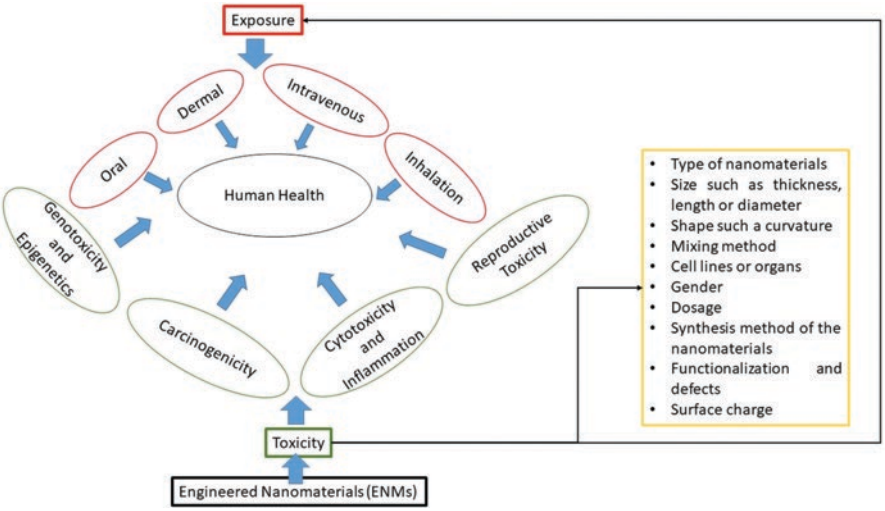

Fig. 7.2 Summary showing factors that should be taken into account when considering the toxicity of engineered nanomaterials

concentration (INEC), and predicted environmental concentration (PEC) (Aschberger et al. 2011).

For workers in situations of chronic inhalation exposure, it was observed that nanoscale titanium dioxide exhibited a higher indicative no-effect level (INEL) of 17 μg/m^3 followed by fullerenes. The impact of engineered nanomaterials taken in through respiration depends on their size, shape, and characteristics, as well as breathing rate, etc. Engineered nanomaterials in the size range of 10–100 nm accumulates in the alveolar region, whereas engineered nanomaterials smaller than 10 nm can accumulate in the thoracic region (Aschberger et al. 2011). For long multiwalled carbon nanotubes, the clearance mechanism from pleura may fail (Aschberger et al. 2011). Liao et al. monitored 124 engineered nanomaterial-handling workers and 77 unexposed workers for 6 months, and reported that workers with exposure to carbon nanotubes exhibited a change in antioxidant enzyme activities for glutathione peroxidase-1 and lung function (Liao et al. 2014). In the case of titanium dioxide nanomaterial, changes were observed in the antioxidant enzyme activity for copper-zinc superoxide dismutase and cardiovascular markers (Liao et al. 2014). Similar changes were observed for silver nanomaterials (Liao et al. 2014). This indicates that different types of engineered nanomaterials elicit variable adverse impacts on human health. This study showed a decreased level of serum CC16 and lung function in workers exposed to nanomaterials, which was consistent with past studies (Liao et al. 2014). The cardiovascular injury observed here was associated with the transfer of nanomaterials from respiratory epithelium to the circulatory system where they could elicit adverse changes in blood coagulation, cardiac frequency, and function. One interesting observation from this study was the lack of oxidative stress, which was contradictory to studies in the past.

According to the authors, this observation could be explained by the minimal level of worker exposure to the nanomaterials, and hence only a precursor response of the decrease in antioxidant enzyme activities was observed (Liao et al. 2014).

Studies have shown that nanomaterials could interfere with the epigenetic process, which involves modification in gene-expression levels without changes in the actual DNA itself through methylation, histone tail alteration, or microRNA mechanisms (Smolkova et al. 2015; Stoccoro et al. 2013). Epigenetic alteration has been associated with neurodegenerative diseases, cancers, cardiovascular complications, autoimmune disorders, behavioral disturbances, and psychiatric disorders (Stoccoro et al. 2013). Stoccoro et al. summarized the epigenetic impact of some nanomaterials, observing that silicon dioxide nanoparticles could lead to global DNA hypomethylation, PARP-1 hypermethylation, and PARP-1 mRNA suppression (Stoccoro et al. 2013). Quantum dots such as cadmium telluride (CdTe) could lead to global hypoacetylation and global changes in miRNAs expression, and multiwalled carbon nanotubes were observed to cause deregulation of miRNA expression (Stoccoro et al. 2013). Again, different nanomaterials exhibited different mechanisms to cause an alteration in the epigenetic process. PARP-1 initiates DNA repair by detecting defects in the chromosome, and hence its low expression is related to cancer. Some adverse effects caused by common engineered nanomaterials during *in vivo* and *in vitro* studies are discussed below.

7.3.1 Silver Nanoparticles

Due to their antimicrobial activity, silver nanoparticles have been employed in applications such as food packaging, deodorant, water purification, toothpaste, food and dietary supplements, etc., thereby leading to oral exposure. Moreover, they can be transferred through the food chain as antibiotic replacement in animal feed. The absorption of silver nanoparticles through the digestive system depends on size, surface reactivity, and hydrophobicity, and hence, agglomeration can ultimately reduce their absorption (Gaillet and Rouanet 2015). Past studies have shown that oral exposure to silver nanoparticles can lead to their transfer to various locations such as the liver, spleen, kidneys, lungs, bone marrow, brain, skin, eyes, muscles, blood, small intestine, stomach, prostate, tongue, teeth, thyroid, salivary gland, parathyroid, duodenum, heart, and pancreas. Studies on albino mice that were orally exposed to dose-dependent silver nanoparticles for 21 days exhibited weight loss, and negatively impacted microvilli and intestinal glands, leading to overall decreased absorption by the intestine (Gaillet and Rouanet 2015). Liver and kidney inflammation were observed in other studies with repeated oral administration of silver nanoparticles in the mice model (Gaillet and Rouanet 2015). An *in vivo* study with rats showed that silver nanoparticles could be transferred to offspring, and the oral administration of silver nanoparticles in doses higher than 100 mg/kg/BW/day could lead to oxidative stress in hepatic tissue during pregnancy. A dose of up to 1000 mg/kg/BW/day, revealed no toxicity related to the development of the

offspring. It was suggested that the oxidative stress caused by nanoparticles could play a dual role, as a consequence of toxicity and also as a modulator of inflammation (Gaillet and Rouanet 2015). It was also suggested that silver ions released from the silver nanoparticles were responsible for the impact observed in *in vivo* studies.

In the past, silver nanoparticles have shown a size-dependent toxicity in many cases (Miethling-Graff et al. 2014). It was demonstrated that exposure of LoVo cells to silver nanoparticles 10–100 nm in size elicited oxidative stress, thus leading to a high concentration of the reactive oxygen species (ROS). The ROS level was lower for silver nanoparticles 40–100 nm in size, compared to smaller-sized nanoparticles (Miethling-Graff et al. 2014). The mitochondrial activity of exposed cells decreased for silver nanoparticles 10 and 20 nm in size at 10 μg/ml, but for larger nanoparticles, the mitochondrial activities were observed to be similar to non-exposed cells (Miethling-Graff et al. 2014). The cell proliferation rate was observed to be size-independent and was adversely impacted by the presence of silver nanoparticles in a dose-dependent manner. It was demonstrated that the 39S ribosomal protein L50 was impacted by the 20-nm silver nanoparticles, whereas this was the 393 ribosomal protein L44 in the case of 100-nm particles (Miethling-Graff et al. 2014).

In another study, the genotoxicity and hepatotoxicity of silver nanoparticles were observed in female albino rats (El Mahdy et al. 2015). Exposure to 1 and 2 mg/kg led to hepatocellular necrosis and apoptosis. It was found that exposure to silver nanoparticles resulted in sinusoidal dilatation and leukocytosis for all *in vivo* models (El Mahdy et al. 2015). The investigators also observed chromosomal aberrations in the bone marrow metaphase cells. Both chromatid deletions and centromeric attenuations at significant levels were observed in rats exposed to silver nanoparticles at 2 and 4 mg/kg b.w. (El Mahdy et al. 2015).

Reproductive and developmental toxicity induced by silver nanoparticles were also studied. It was observed that silver nanoparticles could be passed on to the offspring, and kidneys, liver, lungs, and brain exhibited higher levels of silver nanoparticles when the parent rat orally ingested citrate-capped silver nanoparticles of approximately 7.9 nm at a concentration of 250 mg/kg/day (Ema et al. 2017). Silver nanoparticles were also observed in the maternal milk of female rats treated orally with labeled silver nanoparticles. Intravenous administration of silver nanoparticles led to their accumulation in high concentration in the maternal liver and spleen, but a nominal level was observed in the fetus (Ema et al. 2017). It was also seen that parent mice treated intraperitoneally with polyvinylpyrrolidone-coated silver nanoparticles led to silver nanoparticle accumulation in the embryo. Enhanced accumulation was observed at a lower dose than at a higher dose, thereby indicating that the higher dosage caused agglomeration, thus making it difficult to cross the placental barrier (Ema et al. 2017). In male rats, it was observed that silver nanoparticles adversely impacted Leydig cells, sperm quality, serum testosterone, and luteinizing hormone (LH) levels at 50 mg/kg a day and higher (Ema et al. 2017). Subcutaneous exposure of silver nanoparticles (average diameter 15 nm) at 1 or 5 mg/kg/day led to abnormal sperm and reduction of sperm concentration. On the other hand, intravenous exposure (average diameter 14 nm and 1 mg/kg/dose) did not lead to a significant impact on sperm concentration, fertility, and LH levels.

Intravenous administration of silver nanoparticles (average size 20 nm and 0.5 or 1 mg/kg) in female mice resulted in the reduction of follicle quantity in ovaries. At a dose level of 30 mg/kg/day, oral exposure in female rats caused apoptosis, inflammation, and degenerated follicles (Ema et al. 2017). It was also reported that fetal mortality was enhanced at a low-dose exposure to silver nanoparticles compared to a high-dose exposure, indicating that agglomeration at a higher dose prevented this adverse impact. For mice exposed subcutaneously to silver nanoparticles, the neurobehavioral development was more retarded in female offspring than in male offspring. Therefore, various factors intrinsic to nanomaterials (e.g., size and dosage) as well as factors not associated with nanomaterials (e.g., route of exposure, gender) may elicit different outcomes on health (Ema et al. 2017).

7.3.2 Carbon Nanotubes and Graphene

It has been observed that one of the primary routes of exposure to carbon nanotubes is through inhalation. Carbon nanotubes elicit a similar adverse impact as do asbestos, such as pulmonary inflammation, fibrosis, mesothelioma, and cancer. It has been reported that the toxicity imposed by carbon nanotubes depends on size, rigidity, impurities, method of dispersion, route, duration of exposure, and surface functionalization (Sharma et al. 2016; Orecchioni et al. 2014). Higher levels of the reactive oxygen species and low glutathione level in mice were observed for thin multiwalled carbon nanotubes. Several studies have reported that longer carbon nanotubes led to higher toxicity than shorter ones (Sharma et al. 2016). In mice, multiwalled carbon nanotubes 5–15 μm in length led to fibrosis, whereas shorter lengths in the range of 350–700 nm resulted in lower toxicity. Long multiwalled carbon nanotubes also led to genotoxicity and inflammation (Sharma et al. 2016). Van Berlo et al. investigated two different types of multiwalled carbon nanotubes, one that was longer in length and existed as rigid needle-shaped nanotubes and the other that was shorter in length and existed as entangled nanotubes (Van Berlo et al. 2014). It was demonstrated *in vitro* that rigid needle-shaped nanotubes induced cytotoxicity in RAW 246.7 cells. Exposure to both types of multiwalled carbon nanotubes led to the development of lesions consisting of nanotubes and macrophages in an animal model (mice), although rigid needle-shaped nanotubes resulted in a higher level of fibrosis. Long, thin, and rigid carbon nanotubes were able to reach bronchioles and alveoli, and were associated with impaired clearance due to the slow motility of the macrophage (Van Berlo et al. 2014). This slow motility is attributed to the intake of nanotubes in large quantities. In addition to fibrosis, alveolar inflammation and apoptosis in granuloma were also observed in both cases (Van Berlo et al. 2014).

There have been reports that the dispersion state and type of dispersant used for multiwalled carbon nanotubes impact the toxicity (Sharma et al. 2016). Higher cytotoxicity and genotoxicity were observed for multiwalled carbon nanotubes incorporating metal impurities such as iron, cobalt, etc. Functionalization of

multiwalled carbon nanotubes tends to reduce the toxicity (Sharma et al. 2016; Orecchioni et al. 2014). It was shown that carboxylate functionalized multiwalled carbon nanotubes did not stimulate an inflammatory response, whereas extensive cationic functionalization induced pulmonary fibrosis in a mouse model (Orecchioni et al. 2014). Functionalization of oxidized multiwalled carbon nanotubes with the ammonium group did not trigger the cytotoxic mechanism (Orecchioni et al. 2014). No impact on the proliferation of small airway epithelial cell (SAEC) was observed when exposed to multiwalled carbon nanotubes and nitrogen-doped multiwalled carbon nanotubes (Mihalchik et al. 2015). It was also observed that the nitrogen-doped multiwalled carbon nanotubes were less cytotoxic. Nitrogen-doped multiwalled carbon nanotubes were often shorter than the pristine ones, which could contribute to the lower cytotoxicity. Another reason for this observation could be due to the altered surface chemistry caused by nitrogen (Mihalchik et al. 2015).

Carcinogenic impacts of multiwalled carbon nanotubes were also investigated in the animal models. Multiwalled carbon nanotubes with different lengths, diameters, and curvatures were introduced to rat models by intraperitoneal injection, and these animal models were studied for 2 years (Rittinghausen et al. 2014). A high mortality rate and malignant mesothelioma were observed in all the animals exposed to multiwalled carbon nanotubes. Granulomas consisting of single fibers engulfed by macrophages and lymphocytes as well as thick connective tissue with granulomas around the liver and spleen were reported. Most of the malignant mesothelium was reported to be in the diaphragm, followed by the thoracic cavity. Sacromatoid type or biphasic (combination of sacromatoid and epithelioid types) mesothelium were more common in the multiwalled carbon naotube-treated rats. A possible pathway to mesothelioma could be associated with macrophages engulfing a large volume of nanotubes that were not able to be cleared. This could lead to their poor motility, and hence inducing chronic inflammation, oxidative stress and genotoxicity. As discussed above, longer carbon nanotubes pose greater toxicity, and nanotubes with more curvature elicit a lower toxic effect (Rittinghausen et al. 2014). It has been demonstrated that single-walled carbon nanotubes caused greater cytotoxicity and genotoxicity than multiwalled carbon nanotubes (Öner et al. 2018). In response to toxicity induced by nanotubes, epigenetic mechanisms such as hypomethylation or hypermethylation were also observed (Öner et al. 2018). Epigenetic alterations have been associated with many human diseases.

It was observed that graphite oxide nanosheets led to apoptosis, DNA fragmentation, and elevated levels of reactive oxygen species in spermatogonial stem cells at concentrations of 100 and 400 μg/ml (Hashemi et al. 2016). The method of oxidation to synthesize graphene oxide played an important role in the nanomaterial's toxicity response to lung epithelial cells (Chng and Pumera 2013). Graphene oxide with an increased oxygen content elicited lower cytotoxicity, and vice-versa. Since higher oxidation was achieved with permanganate compared to chlorate, the former could lead to reduced toxicity (Chng and Pumera 2013). At a graphene oxide concentration of 125 μg/ml obtained through various oxidation processes, the adverse impact on the viability of lung epithelial cells was observed, although there were conflicting results regarding the toxicity of graphene oxide (Chng and Pumera

2013). Carbon nanotubes and graphene oxide have sometimes exhibited enhanced cell proliferation. It is possible that carbon nanotubes can interfere with the mitotic spindle interaction, which may contribute to enhanced proliferation (Rittinghausen et al. 2014).

7.3.3 Silica Nanoparticles

Silica nanoparticles have shown comparatively lower toxicity than other nanomaterials although their toxicity is dependent upon size, dosage, chemical stability of the crystal structure, surface charge, and functionalization. It was observed that silica nanoparticles approximately 22.5 nm and 56.9 nm in diameter led to lower FE1 cell viability after 24 h of exposure compared to nanoparticles with average diameters of 237.5 nm and 2045.4 nm (Decan et al. 2016). Dose-dependent cytotoxicity to FE1 cells up to 250 μg/ml and synthesis of reactive oxygen species at a dosage of 12.5 μg/ml and 50 μg/ml were reported for silica nanoparticles (Decan et al. 2016). Silica nanoparticles are mostly cleared by lysosomal exocytosis, and their accumulation in the lysosome is size dependent (Decan et al. 2016). Pyrogenic silica nanoparticles are more cytotoxic than precipitated ones (Fontana et al. 2017). Past studies have shown that dermal exposure to silica nanoparticles did not induce skin damage and toxicity within internal organs (Trouiller et al. 2009; Fruijtier-Pölloth 2012). In animal models, silica nanoparticles when orally administered were able to cross the gastrointestinal tract and find a path to the circulatory system. Surface functionalization such as carboxyl- and amine-functionalized silica nanoparticles as well as smaller-sized particles exhibited enhanced transport through the gastrointestinal tract (Mebert et al. 2017).

In an animal model, food-grade silica nanoparticles at a single dose of 500-mg/kg were excreted with feces, although an increased concentration of silica particles was observed in the liver, spleen, and kidneys (Mebert et al. 2017). It could be suggested that silica nanoparticles were safer, based on studies that used a higher dose of silica nanoparticles than the allowed exposure levels for humans. The intratracheal administration of silica particles was mostly cleared from the lungs, lowering its possibility to induce an adverse effect on this organ. Silica nanoparticles were reported to cause epigenetic alterations such as hypermethylation of apoptosis-related genes in human bronchial epithelial cells and hypomethylation of keratinocyte cell lines when exposed to 15 nm silica particles (Mebert et al. 2017). On the other hand, weak chromosomal aberration or effects were observed *in vitro* and *in vivo* due to the exposure to silica nanoparticles, indicating limited mutagenicity and genotoxicity (Fruijtier-Pölloth 2012). A mutagenic response to silica nanoparticles 7.172 nm and 7.652 nm in size was reported for mouse lymphoma cell lines at 100 and 150 μg/ml (Demir and Castranova 2016). The genotoxicity and mutagenicity of silica nanoparticles are dependent on the type of cells, particle size, and other psychochemical parameters requiring more in-depth exploration of the effects for clarity and consistency. One *in vivo* study showed that the oral ingestion of silica

nanoparticles did not lead to a tumor in rats and mice, indicating that most likely, silica nanoparticles were not associated with carcinogenicity (Fruijtier-Pölloth 2012). Moreover, food-grade amorphous silica did not induce reproductive and developmental toxicity in rabbits and mice at 1600 mg/kg bw/day (Fruijtier-Pölloth 2012).

7.3.4 Titanium Dioxide Nanoparticles

Oral exposure of titanium dioxide nanoparticles to maternal mice caused enhanced DNA deletion in the fetus, indicating that it can be passed on to the offspring (Trouiller et al. 2009). It also led to single- and double-strand DNA breaks in mice and chromosomal damage, which was assessed by detecting micronuclei in erythrocytes (Trouiller et al. 2009). Enhanced micronuclei frequency at concentration levels of 500 mg/kg indicates that they are clastogenic in mice. Titanium dioxide nanoparticles also caused oxidative DNA damage in the liver of mice (Trouiller et al. 2009). DNA damage caused by exposure to titanium dioxide particles was also observed *in vitro* for A549 cells, in contrast to another study carried out on the same cell line (Karlsson et al. 2009; Hanot-Roy et al. 2016). Micron-sized particles exhibited higher levels of DNA damage than did particles in the nanoscale range (Karlsson et al. 2009). Titanium dioxide nanoparticles exhibited negligible cytotoxicity to A549 cells when exposed for 18 h at 40 μg/cm^2, and a similar observation was noted in another study (Karlsson et al. 2009; Hanot-Roy et al. 2016). The oral exposure of female mice to 25 nm and 80 nm of titanium dioxide nanoparticles at 5 g/kg resulted in a significantly higher inflammation in the liver compared to that in the male mice, and in this study, myocardial and kidney damage due to the nanoparticles was also reported (Wang et al. 2007).

The International Agency for Research on Cancer (IARC) suggests that experimental evidence supports the carcinogenicity of titanium dioxide particles in animal models, although it is inconclusive in the case of humans (Hanot-Roy et al. 2016). Relevant cell lines associated with lungs such as human pulmonary microvascular endothelial cells (HPMEC-ST1.6R), alveolar macrophage (THP-1), and alveolar epithelial cells (A549) have been investigated *in vitro* (Hanot-Roy et al. 2016). In all these cell lines, a significant increase in the reactive oxygen species generation was observed, but in the case of THP-1, the production was delayed. No significant cytotoxicity was observed for A549 and THP-1 cells, whereas HPMEC-ST1.6R cells exhibited cytotoxicity starting at 50 μg/ml (Hanot-Roy et al. 2016). The A549 cells also did not exhibit significant apoptosis, but the HPMEC-ST1.6R cells showed dose-dependent apoptosis (Hanot-Roy et al. 2016). After 24 h exposure to the nanoparticles at 200 μg/ml and 800 μg/ml levels, the A549 cells did not exhibit cell signaling in response to DNA damage, but for HPMEC-ST1.6R cells, phosphorylation of H2AX was observed (Hanot-Roy et al. 2016). In the case of THP-1 cells, along with H2AX, phosphorylation of both ATR and ATM proteins was noted. This study emphasizes that the response related to cytotoxicity and cell signaling

pathways for DNA damage may vary significantly based on the cell lines (Hanot-Roy et al. 2016). The repair kinetics for DNA damage in Caco-2 cells after exposure to titanium dioxide nanoparticles has also been studied (Zijno et al. 2015). Enhanced levels of OGG1 expression suggested that Caco-2 cells were successful in repairing the oxidative DNA damage when exposed to titanium dioxide nanoparticles for 6 h at 2.5 μg/cm^2 (Zijno et al. 2015). Humans have a higher chance of exposure to titanium dioxide nanoparticles since they are frequently used in food and cosmetics. Another study also observed DNA damage with titanium dioxide nanoparticles (21 nm and 50 nm) at 1000 μg/ml on human embryonic kidney cells (HEK293) and mouse embryonic fibroblast cells (NIH/3 T3), but no oxidative DNA damage was noted (Demir et al. 2015). The authors also reported similar results for both sizes of nanoparticles, thus indicating a size-independent effect (Demir et al. 2015).

7.4 Conclusion

It is difficult to understand the deleterious impact and risk posed by nanoparticles on humans since there is a lack of data regarding the volume of nanomaterials that are being produced or used in consumer goods. There is also a paucity of information on the quantification of nanomaterials released into the environment, making it further difficult to assess the risk of nanomaterials. The deleterious impact of nanomaterials on human health is mostly extrapolated from *in vitro* and *in vivo* studies using animal models. There are also contradictory reports in the literature regarding the toxicity of nanomaterials, most probably caused by the numerous factors that can impact the toxicity study, beginning with the type of cell line, type of nanomaterial, cell medium, dosage and size, method of mixing, functionalization, synthesis process of the nanomaterials, surface charge, shape, exposure method, gender, etc. This makes it very difficult to assess the risk of nanoparticles or to determine their effect in humans. Experimental designs using animal models and *in vitro* test settings in order to conduct a comprehensive study on the toxicity of a specific nanomaterial is complex and must take into account the interference of various external factors that may impact the results. With the increasing use of nanomaterials in peoples' lives and the higher frequency of their release into the environment, it may become essential to develop a comprehensive understanding of the impact of various nanomaterials on human health. Long-term exposure to nanomaterials will also become an important topic in the near future.

Already there is regulation imposed by the European Union on cosmetic manufacturers who are required to notify authorities if nanomaterials are being used in their processes. It is very important to consider a balance between the risks and advantages posed by engineered nanomaterials. As a result, a more robust method for detecting hazards and quantifying engineered nanomaterials and the life cycle analysis of engineered nanomaterial-contained consumer goods will be necessary in the near future, as different types and large volumes of nanomaterials transition towards commercialization.

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Chapter 8
Nanomaterials and Human Health: Nano-biomaterials in Dentistry

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Abstract Nanotechnology provides a new alternative and a possibly superior approach to diagnosis and treatment of oral pathologies. However, a vast majority of materials exposed to dynamic environment of oral cavity, mechanical preparation by dental burs may or other physical and chemical factors may release nanoparticles or their complexes into oral cavity and gastrointestinal tract. Moreover, preparation of nanomaterials or inappropriate handling may result in inhalation of nanoparticles into nasal cavity or eyes, increasing the risk of adverse, longitudinal toxic effects. The present article discusses the potential limitations and toxicological effects of dental nanomaterials to human organism.

Keywords Nanomaterial · Nanoparticles · Nanotechnology · Dental · Dentistry · Oral cavity · Toxic · Teeth · Saliva · Drug delivery

8.1 Introduction

Relentless and dynamic development of industry has allowed to design, manufacture and apply new materials at submicron scale which exhibit unique properties. Nanotechnology, originally regarded as a branch of science that deals with processes occurring at molecular level, contributed to extensive studies that significantly expanded the potentials of medical and related sciences: pharmacology, drug design and delivery, imaging and diagnostics tools, biomaterials, surgical equipment and many more (Webster 2006).

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Revolutionary approaches have change the face of medical market, but as such, they also must be constantly critically evaluated with a regard to human health and ecological consequences (Kuhlbusch et al. 2018; Priyadarsini et al. 2018). In this chapter, nanotechnological- based dental biomaterials will be analyzed with a regard to general human health and potential toxicology to human body.

8.2 Nanodentistry

Nanoparticles are defined as organic or inorganic parts of matter, 1–100 nm in size at least in one dimension. Therefore, definition of "nanoparticles" does not fully appreciate their spectrum available among the environment and industry. Moreover, it is usually not applied to individual molecules but rather to inorganic materials. From the point of view of the chemical structure, there are four classes of basic nanomaterials: carbon based, metal based, dendrimers and composites. Basing on dimensionality, nanoparticles can be categorized also in four groups: zero-dimension (nanopowders (ultra-dispersive) and nanoparticles), one-dimension (nanowires, nanorods, nanotubes), two-dimension (nanocoatings, nanofilms) and three-dimension (not confined to the nanoscale in any dimension: can contain dispersions of nanoparticles, bundles of nanowires, and nanotubes as well as multinanolayers).

The term "nanomedicine" is a branch of science utilized for medical market: drugs, devices, biomaterials and involves utilization of nanotechnology for the benefit to of human health in general. Consequently, by paraphrasing original definition of US NIH* (Martin 2006), "nanodentistry" should be regarded as highly specific medical intervention at the molecular scale for curing oral diseases, repairing or regenerating damaged tissues, such as teeth, alveolar bone, muscles, nerves or mucosa in oral cavity and related, anatomical structures.

*Nanomedicine refers to highly specific medical intervention at the molecular scale for curing diseases or repairing damaged tissues, such as bone, muscle, or nerve'

For such purpose, different nanoparticles and their formulations are required. As an example, teeth that need restoration due to dental carries will need completely different material than alveolar bone resorbed due to periapical inflammatory response. Dental nano-biomaterials are extremely diversified with a regard to chemical composition (organic, non-organic, metallic, non-metallic etc.), physico-chemical properties (ready to use, light- cured, chemically-set), fillers, medium, matrix, additive ingredients and chemical agents.

Nanofabrication and consequently nanodentistry assumes two approaches for materials manufacturing. *Bottom- up* which describes assembly of small components into compound structures and *top- down* which is a creation of small structures by using bigger ones in guiding their assembly (Bhardwaj et al. 2014a; Chandki et al. 2012).

According to dimensionality, dental materials based on nanotechnological solutions are mostly three-dimensional products that consist of bulk matrix filled with

consolidated or dispersed nanostructures or nanoparticles (Classification of Nanomaterials 2017).

8.3 Routes of Entry

As noticed by Kuhlbusch et al. (2018) and Landvik et al. (2018), human exposure to nanoparticles caused significant concerns of healthcare systems worldwide. It was mostly due to impossibility of identification of nanoparticles in the air, their quantification and most of all- differentiation between manufactured from natural or incidentally generated nano-sized materials (Kuhlbusch et al. 2018). Approaches to characterization and risk assessment of different nanomaterials have been made, however still little is known due to extreme diversity of nanomaterials with a regard to: physico-chemical properties, functionality, modes of action and interactions with biological environment (Landvik et al. 2018; Pokrowiecki et al. 2018).

Healthy human skin is a natural barrier, and it is known that nanoparticles are unable to penetrate it, as stratum corneum seemed insuperable barrier in the studies performed (Lemos et al. 2018; Labouta and Schneider 2013).

This however, is not the case with regard to other biological barriers and semi-permeable membranes such as oral mucosa which is of 4000 times greater permeability than skin (Roblegg et al. 2012). Nanomaterials used in dentistry may interact with following anatomical structures: teeth, oral mucosa, tongue and alveolar bone. Such interaction tissue: nanomaterial may be intentional, (e.g. nanostructured tooth filling: tooth) or unintentional (e.g. debris from nanostructured filling: semipermeable oral mucosa).

Physical and/or chemical factors may breach the structure of the nanomaterials and release the free nanoparticles which may interfere with routes of entry into the human body shown in Fig. 8.1. Table 8.1 shows endo- and exogenous physico-chemical factors that can interfere with dental-based nanomaterials in oral cavity.

Interaction of NPs with semipermeable membranes of human body is of high expectancy, especially as Landuyt et al. in 2014 proved that dental nanocomposites may be a source of significant amount of airborne nanosized dust particles when prepared with dental burs.

Dust may be inhaled by both, patient and general practitioner, interact with eye cornea and also disperse in patient mouth and interact with mucosa (Van Landuyt et al. 2014). In recent work, Hackenberg et al. (2017) showed that zinc-nanoparticles may induce some extent of genotoxicity effects in nasal mucosa when inhaled (Hackenberg et al. 2017). Moreover, nanoparticles that were unintentionally transported into the eye may be absorbed into its deeper parts through transcorneal permeation from the lacrimal fluid into the anterior chamber and noncorneal permeation across the conjunctiva and sclera into the anterior uvea (Joseph and Venkatraman 2017). An impact of unintentionally transmitted nanoparticles into the eye on vitreous humor, lens, retina, and other ocular tissues is unknown but potential

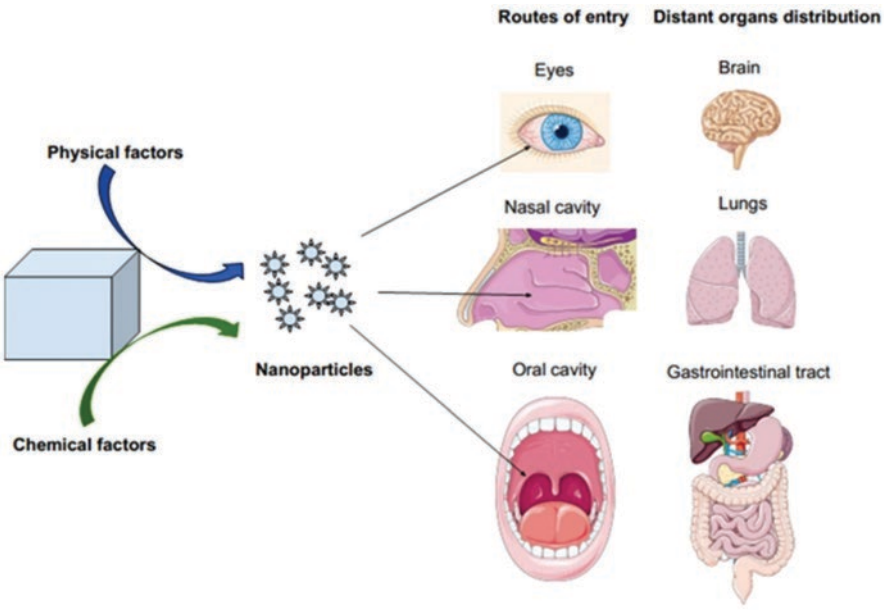


Fig. 8.1 A graph showing the impact of physical and chemical factors contributing to detachment of free nanoparticles from bulk nanomaterials and their further spread to vital organs through eyes, oral and nasal cavity (routes of entry)

complications such as tissue damage, infection and injury, and retinal hemorrhage cannot be excluded.

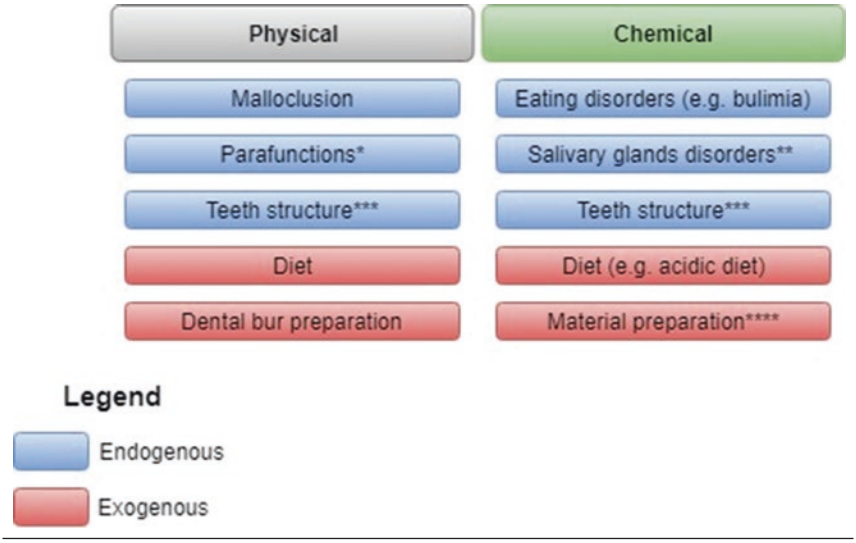
Undisputedly, application of nanomaterials designed for dentistry may be associated with local and distant complications. Local, are expected to occur in the near proximity of the material applied, whereas, the latter are associated with transport of NPs across the body by biological fluids (Corbo et al. 2016).

8.4 Oral Cavity and Vital Organs

Materials used for dental purposes may be divided into three groups, according to functionality: therapeutic, antimicrobial and reinforcemental (Priyadarsini et al. 2018).

Their application may result in biological response from hard and/or soft tissues of oral cavity such as teeth, gingiva, alveolar bone and lining mucosa. Such response may be an effect of beneficial action of the nanomaterial or appear as adverse effect caused by inappropriate application or most probably- not fully understood mechanisms of action of NMTs (Table 8.2). It is worth mentioning, there are no studies available which focus on potential local adverse effects of dental nanomaterials in oral cavity and distant organs (Solla et al. 2015).

Table 8.1 Example of common physical and chemical factors that may interfere in the structure of NP- based biomaterials and nanoparticles release into the human body



*Occlusal parafunctions cause excessive chewing forces that overload dental materials (e.g. dental filling) and induce cracking of the material and detachment of NPs

**Salivary glands disorders may result in sialopenia (diminished saliva production) and decrease buffering properties of saliva what exposes teeth and materials on acidic environment of oral bacteria

***Teeth structure disorders (local or general) are of genetic or acquired origin that decrease durability on physical and/or chemical factors. Such diseases may affect tooth: biomaterial bonding strength and material detachment

****Some materials are prepared by GP at chairside, before application in the oral cavity. When prepared inaccurately, they may exhibit different properties than designed by manufacturer and hence, be more susceptible to NPs release in the environment of oral cavity

Application of nanomaterials used for restoration of the outer structures of teeth (enamel, dentin) is expected to improve biomechanical properties of the materials, decrease polymerization shrinkage, provide remineralization of the enamel and antibacterial activity (Bhardwaj et al. 2014b; Mohamed Hamouda 2012; Shafiei et al. 2014). However, such materials may always exhibit cytotoxic effect to dental pulp and induce tissue irritation, inflammation, and in consequence, dental tissue necrosis due to vascular system compromise. Such scenario is plausible, especially as loose, smaller NP's could easily diffuse from the material through dentin tubules into near proximity of dental pulp. Up to now, there are no longitudinal comparative clinical studies on mechanical and esthetical behavior of nano- dental fillings in the oral cavity. Therefore, still is unknown whether these materials are more susceptible to discolorations, hypersensivity induction or marginal microleakage and secondary carries development after use of NMTs- based dental resins or fillings. Otherwise, first reports on nanoparticle- based root canal were promising, as iron oxide-nanoparticle irrigants were effective in eradication of bacteria in dentin tubules and

Table 8.2 Examples of desired (beneficial) and undesired (adverse) effects of NMTs designed for dental purposes

Tissues	Examples of beneficial effects of NMTs	Examples of adverse effects of NMTs
Enamel	Remineralization Restoration Anticariogenic effect	Demineralization Discolorations Hypersensivity
Dentin	Remineralization Restoration	Demineralization Discolorations Hypersensivity
Dental pulp and periodontium	Regeneration Angiogenic activity Antibacterial activity Anti-inflammatory effect	Irritation Antiangiogenic activity Cytotoxicity against dental pulp stem cells Inflammatory response Hypersensivity
Alveolar bone	Regeneration Antibacterial activity Anti-inflammatory effect	Resorption Osteolysis Ostesclerosis Demineralization
Alveolar gingiva, oral mucosa	Regeneration Antibacterial activity Anti-inflammatory effect	Recessions Irritation Inflammatory response Discolorations Cancerogenic effect

It can be seen that different nanomaterials may interact with different anatomical structures of oral cavity and hence, contribute to potential toxicity, allergic reactions, discolorations, tissue irritation and other

were non-toxic to soft tissues (Bukhari and Koo 2016). On the other hand, addition of nanoparticles to other root-canal treatment materials may decrease their sealing capacity and increase the risk of re-infection of the periapical tissues (Eskandarinezhad et al. 2017).

Local response of alveolar gingiva, bone and oral lining mucosa to dissolved nanoparticles from NMTs has not yet been investigated *in vivo* in the literature available. Notwithstanding the above, exiting *in vitro* studies indicate necessity for better evaluation NMTs being introduced into the market. Cicchetti et al. (2011) showed that single- wall carbon nanotubes which are increasingly produced, may induce significant genotoxicity in human gingival fibroblasts causing decrease of the cell proliferation, cell survival and apoptosis (Cicchetti et al. 2011). Inflammatory response, ulcerations, discolorations and even cancerogenic effect to exposed tissues must be taken into concern (Pokrowiecki and Pałka 2018).

More attention was given to the process of NPs diffusion through biological barriersm, such as oral mucosa. Nanoparticles were extensively evaluated as a carriers for mucosal drug delivery systems in order to protect drugs from degradation and deliver them to intended sites without contribution of the gastrointestinal tract (Ensign et al. 2012; Pridgen et al. 2014). Indeed, it is an efficient way of drug administration due to its semi-permeability. On the other hand such properties may

be a risk for potential adverse effect induced by nanoparticles used in dental materials. As not being inherently benign, nanomaterials may penetrate oral mucosa and travel throughout the body through blood circulatory or lymphatic system, deposit in organs, penetrate cell membranes, accumulate mitochondria, and trigger injurious responses (Feng et al. 2015).

Before any NPs- cell or organ interaction is discussed one must mention there are no free- floating NPs in the biological body fluids. Just after exposure of nanoparticles to the biological environment of the body such as blood, saliva, GIC fluids etc. nanoparticles interact with fluid's glycoproteins forming complexes described as protein coronas (PCs). First seconds after NPs exposure to biological fluids result in formation of soft coronas, which further form hard coronas when proteins of higher affinity to the given NPs appear. It is worth mentioning, physicochemical properties of such complexes may be extremely different when compared to NP's properties prior to their delivery to the fluids. The process of shift in physicochemical properties of NPs in the body fluids is described as "from synthetic identity to biological identity". These interactions are crucial in understanding NP interactions with living systems (Lundqvist et al. 2008; Bertoli et al. 2016; Lee et al. 2015; Albanese et al. 2014). Chemical composition of protein coronas depend on NPs properties, as well as, chemical composition of the given body fluid. There are limited studies that evaluated PC formation in the oral cavity so far, nor explaining their impact on oral mucosa and its permeability (Pokrowiecki et al. 2018). Cellular uptake of NPs or PCs is related to cell type, size of the NPs or PC as well as their chemical structure. In the study of Teubl et al. (2013, 2018) it was shown that when exposed to saliva, NPs may exhibit twofold lower cellular uptake capacity when compared to serum-free medium. Also, functionalized, positively and uncharged particles interact to a greater extent with the salivary components than uncharged NPs (Teubl et al. 2013, 2018). Cellular uptake of NPs or PC is generally based on three different mechanisms: clathrin- mediated endocytosis, interaction with membrane and transport through metal ions transporters or scavenger- mediated endocytosis (Pokrowiecki et al. 2018; Park et al. 2010). According to Cheng et al. (2015), the presence of PC may reduce toxicity of the NPs. Moreover, protein corona significantly decreases NPs uptake in a particle size- and cell type dependent manner. It shows significant inhibition on the uptake of large-sized NPs by phagocytic cell than small NPs by non- phagocytic cell (Cheng et al. 2015). On the other hand, specific "biological identity" of the given NPs may enhance cellular uptake and retention of nanoparticles contributing to toxicity and pathophysiology (Corbo et al. 2016; Albanese et al. 2014).

This is important from the pharmaceutical, as well as, toxicological point of view, as most of therapeutic NPs- based drugs or NMTs are designed for non-phagocytic cells, whereas removal of NPs from the body is mostly based on phagocytic cells of the reticuloendothelial system (Pokrowiecki et al. 2018; Park et al. 2010). Consequently, orally administered nanoparticles may reach distant organs through penetration of oral mucosa, blood stream or via gastrointestinal tract.

Oral mucosa is divided into masticatory mucosa (i.e., gingivae and the hard palate), specialized mucosa (i.e., the dorsum of the tongue), and lining mucosa (e.g.,

buccal mucosa, the floor of mouth), (Paderni et al. 2012). Lining mucosa constitutes 60% of the oral mucosa and is the most permeable to different substances due to relatively low grade of thickness and non- keratinization of the lining epithelium. Main barrier for substances lies in the top third region of the oral epithelium, with contribution of basal lamina to some extent as well (Teubl et al. 2014). Subepithelial connective tissue is not an effective barrier to the penetration of substances (Paderni et al. 2012) (Fig. 8.2).

Oral mucosa, as well as, the other parts of GIC are conditioned with protein rich mucus layer, acting as lubricant protecting the epithelial cell layers from pathogens, toxins and particles (Fröhlich and Roblegg 2012, 2016). In general, electrostatic repulsion from negatively charged sugar moieties favors the penetration of positively charged, hydrophilic molecules (Fröhlich and Roblegg 2012).

In the study of Robbleg et al. (2012) a system evaluating the behavior of nanoparticles in the buccal mucosa. Authors showed that positively charged 20 nm polystyrene NPs penetrated mucosa only to the extent of one-third stratum superficiale layer of the epithelium, whereas 200 nm NPs penetrated into deeper parts of the tissue. When temperature was decreased to 4 °C uptake was inhibited for 20 nm CP and 200 nm AP particles respectively, giving the confirmation that NPs cell internalization into the tissue is due to endocytotic mechanisms (Roblegg et al. 2012).

The importance of oral cavity as a potential route of NPs in the terms of toxicity was also held out by Teubl et al. (2014) Authors evaluated penetration ability of

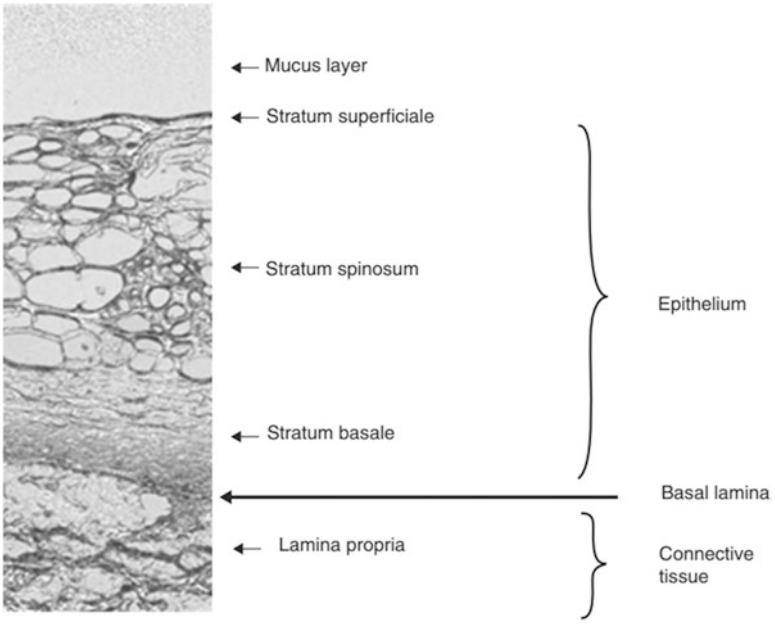


Fig. 8.2 Microscopic image of a cross-sectioned buccal mucosa. (Adapted from Robleg et al. 2012)

TiO_2 nanoparticles through buccal mucosa. They showed that investigated particles permeated the mucus layer and penetrated into the oral epithelium. Penetration depth was size dependent, similarly to the study of Robbleg et al. (2012). Larger particles reached the deepest parts of epithelium including basal lamina and connective tissue, whereas the smallest NPs (7 nm) were found only in the upper parts of epithelium. Authors described, when internalized by the cells, NPs induced inflammatory response by reactive oxygen species activation. When NPs breach the epithelial barrier they may remain in the oral epithelium about 14 days, until epithelial turnover is finished. Particles were found in close proximity to cellular membrane, collagen fibers of connective tissue and cellular cytoplasm (Teubl et al. 2014). It must be mentioned, that penetration of NPs through compromised/diseased tissue barriers may be increased even fivefold, as stated in the studies available (McGill and Smyth 2010).

Mucosa penetration by NPs was shown independent from particle's wettability. However, intracellular accumulation of nanoparticles in the epithelial cells relied on surface hydrophobicity. More hydrophobic NPs accumulated in vesicular structures of the cell, whilst hydrophilic in cellular cytoplasm. Also, hydrophobic nanoparticles were shown to initiate more toxic effects through reactive oxygen species mechanisms than hydrophilic NPs.

NPs may be swallowed with food and saliva and reach other parts of human body. Gastrointestinal tract (GIC) consists of esophagus, stomach, duodenum, small intestine and colon. Each part of GIC is also covered with protective mucus layer of different thickness and varying composition (Atuma et al. 2001). Due to environmental changes in each compartment of GIT (enzymes, pH), NPs may form new complexes and coronas changing their biological identity, physicochemical properties, permeation abilities and in consequence toxicity (Pokrowiecki and Pałka 2018). It was shown that orally administered NP' may induce abnormal mucus production in GIT, change the pH-dependent aggregation of mucins, as well as, impede mucin swelling and viscosity increase (Fröhlich and Roblegg 2012). Accumulation of NPs in GIC, stomach mucosa inflammation and damage of intestinal mucosa, weight loss were reported. Apart from local inflammatory response of mucosa, NPs may enter the circulatory blood or lymphatic system and be transported to vital organs such as liver, spleen, kidneys, pancreas and brain (Cha et al. 2008; Kim et al. 2008, 2010; Zhang et al. 2010; Bergin and Witzmann 2013).

Until now, many papers on accumulation and potential toxicity of different nanoparticles in vital organs have been report. However, there are only few studies on dental nanomaterials and their potential biodistribution across the living organism. As previously mentioned, nanoparticles detached from dental NMTs may be transported to circulatory blood or lymphatic system either by diffusion through mucosa in oral cavity or through mucosal barriers of gastrointestinal tract.

For example, titanium dioxide nanoparticles (n-TiO_2) which are considered a better dental implant surface may also exert negative effect on cells, even though titanium has been considered biologically inert so far. Some NPs may cross blood-brain-barrier (BBB) and accumulate inside the brain in the region of cortex and hippocampus (Priyadarsini et al. 2018; Feng et al. 2015). Moreover, it was shown

that NPs may be transported to brain not only through BBB but through cerebrospinal fluid, as well as, directly through nerves (Geraets et al. 2012; Kao et al. 2012). The latter is significant in case of aspirated nano-dust mentioned above by the patient or general practitioner. Nasal olfactory passage of NPs is frequently overlooked, but may result in transport of nanoparticles to brain through olfactory or trigeminal nerve systems. In the study of Garcia et al. (2015) authors concerned that there might be a correlation between air pollution caused by nanoparticles and human's neurodegenerative diseases such as Alzheimer's, Parkinson's or Huntington's diseases (Garcia et al. 2015). Metallic NPs are amongst the most potential neurotoxic NMTs, as by crossing the BBB barrier they may induce structural changes in the neuronal architecture and toxic effects on the brain vasculature. According to the study of Yamashita et al. (2011), complications with pregnancy in mice exposed to nano- silica were reported as NP's were found in placenta, fetal liver and brain. Authors, as well as, Okada et al. (2013) stated that exposure of pregnant mice to NP's may induce neurotoxic effect in offspring (Yamashita et al. 2011; Okada et al. 2013). Other way, proposed as a potential route of NP's transport into CNS was olfactory nerve pathway after intranasal administration of nanoparticles. Accumulation in the brain through axonal transport in humans is however, still unveiled.

8.5 Conclusions

Significant work has been undertaken in attempt to introduce nano-technological concepts into dental market. Nanomaterials offer new solutions for treatment of oral diseases. However, as not fully discovered interactions of NPs with cell's membranes, toxic effects to oral tissues, as well as, accumulation in vital organs must be taken into concern. First step in better understanding on how NMTs may induce harmful effect to human organism is discovering mechanisms of NP's interaction with human saliva and how does it influence their further physicochemical properties.

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Chapter 9
Nanotoxicological Approaches Towards Nanosafety

Sandip Pawar, Mrunmayi Sardesai, and Pravin Shende

Abstract The toxicity of nanoparticles has been an area of rigorous research for more than two decades. The nanotechnology is used extensively in medicines due to its numerous advantages such as small particle size, high surface area, increased solubility, etc. Nanotoxicology and nanosafety guidelines aim to prevent researchers from possible harmful effects of nanomaterials. The advancement of nano-drug delivery techniques in medical and pharmaceutical fields proved a useful alternative for therapeutics. Nanotechnology includes various drug delivery systems, such as nanoparticles, nanospheres, nanoneedles, nanoemulsion, nanosponges, etc. Moreover, nanomaterials synthesized with inert elements like gold, become active and used as contrast agents due to their nano dimension and high rate of accumulation. In this review, we highlighted and summarized the toxicity related to the different nanocarriers, such as magnetic nanoparticles, quantum dots, protein or therapeutic nanoparticles, diagnostic agents, etc. Nanotoxicity studies are very crucial to determine the extent of the threat posed by these nanosized drug delivery systems to humans and the environment, and to establish a level of nanosafety. Thus, there is a need for the research community to expand visions and predict the unforeseen problems in context with nanotoxicity and public health hazards arising due to the escalated use of nanomaterial in consumer products.

Keywords Nanotoxicology · Nanomaterials · Nanocarriers · Nanosafety

9.1 Introduction

Nanotoxicology is the sub-branch of toxicology which explores the potential toxicity related to nanomaterials. Nanotoxicology studies the probable noxious effects of nanoparticles on human and biological systems (Walters et al. 2016). The

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physicochemical properties of nanoparticles *viz,* size, surface chemistry, surface charge, shape and surface coating influence the extent and biodistribution of nanotoxicity. To estimate nanotoxicity levels, various *in vitro, in situ* and *in vivo* models have been utilized. The study of nanotoxicology addresses the toxicity of nanomaterials, such as neurological disorder, nephritis, bronchitis, emphysema, etc. (Aillon et al. 2009). Nanomaterials show peculiar attributes such as large surface area, natural infiltration into the cells and tissues, which induce an immune response. Nanomaterials translocate from solution to cells or tissues and even surpass the blood-brain barrier (BBB), which causes toxicity in the critical organs such as the brain, liver, kidney, etc. (Aguilar 2013). The infiltration of nanomaterials into cells and gradually into the unhealthy tissues extends their potential applications as delivery vehicles in various therapies. Nowadays, nanotechnology is also extensively used in medicines, health care, gene delivery and immunotherapy. But, nanomaterials of different sizes, surface topography and other characteristics need to be analyzed more precisely to establish effectiveness and safety for human consumption and use (Portney and Ozkan 2006; Aguilar 2013). The present review emphasized the possible mechanisms of nanotoxicity and undesirable effects on interaction with biological systems.

9.2 Mechanism of Nanotoxicity

The small size of nanoparticles helps them to escape the body's defense mechanism such as the mucous membrane of skin, lung epithelium, macrophages, etc.) to enter into the organisms and cells. Apart from nanoparticles properties, the presence and seriousness of any harmful effect strongly rely on different environmental conditions as well as cells and organisms' characteristics. Various nanotoxicity mechanisms have been studied in recent years and the reactive oxygen species (ROS) generation is of utmost significance. The overgeneration of ROS i.e. the by-product of cellular oxidative metabolism induces oxidative stress to result in the production of cells that fail to prolong the normal physiological redox-regulated functions which in turn causes DNA damage, unregulated signalling of cells, changes in cell movement, cytotoxicity, apoptosis and cancer initiation (Fu et al. 2014). This whole progression occurs in the powerhouse of the cell, i.e. mitochondria during ATP synthesis. Oxidative stress and ROS are correlated with many age-related degenerative diseases such as amyotrophic lateral sclerosis, arthritis, amyotrophic lateral sclerosis cardiovascular disease, inflammation, Parkinson's disease, diabetes, Alzheimer's disease and cancer. The ROS generation by nanomaterials plays a vital role in genotoxicity by oxidative DNA damage and causes mutagenesis, oncogenesis and aging-related diseases in humans (Khanna et al. 2015; Halliwell and Aruoma 1991). The various adverse effects of ROS are shown in Fig. 9.1.

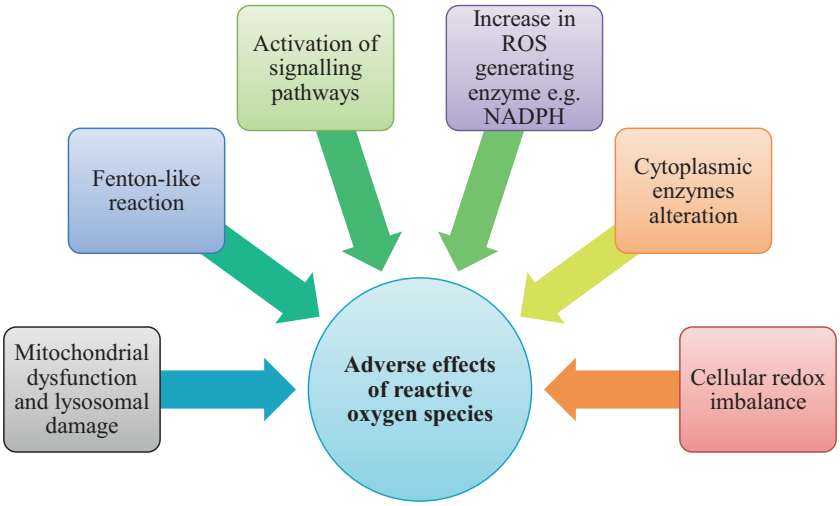

Fig. 9.1 The adverse effect of reactive oxygen species

9.3 Factors Affecting Nanotoxicity

The various factors affecting nanotoxicity are enlisted in Fig. 9.2.

9.3.1 Shape, Size and Surface Area

The tiny size of the nanoparticles favors their utilization in the field of medical applications and is often accompanied by improved biological reactivity. The size and shape of the nanoparticles play a significant role in determining their toxicity parameters since it affects the bio-distribution, protein adsorption and cell internalization. These factors, in turn, influence the accumulation and toxicity of the nanoparticles. The tremendous potential of nanoparticles to skip human defense mechanisms and accumulate at the diseased site further extends their potential in theranostic applications (Oberdorster et al. 1994; Ferin et al. 1992; Wilson et al. 2002). The size-dependent cytotoxic action of silver nanoparticles *in vitro* and *in vivo* has been experimentally verified. Moreover, titanium oxide (TiO_2) nanoparticles also showed size-dependent toxicity in rat lungs, whereas inhalational nanoparticles showed toxicity in the alveolar region and exert more detrimental action than larger sized nanoparticles due to their complexity in clearance mechanism. The various literature revealed that the shape of nanoparticles affects cellular uptake action, i.e. rod- and needle-like nanoparticles have higher uptake in comparison to cylindrical and other forms. Moreover, the rod shape of zinc oxide (ZnO) nanoparticles showed elevated toxicity in comparison to spherical shape. The inflammatory action of nanoparticles depends on the surface area, i.e. smaller nanoparticles have

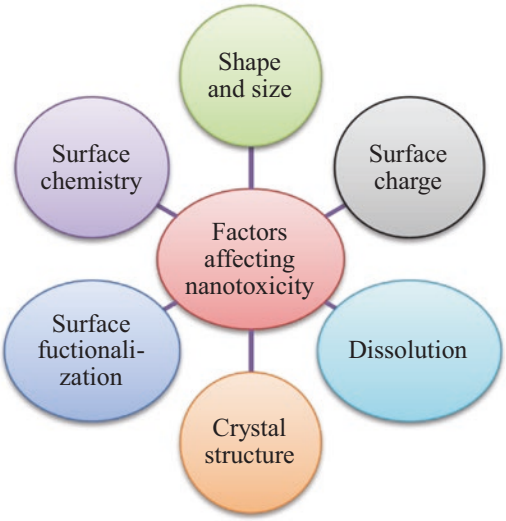

Fig. 9.2 Various factors affecting nanotoxicity

higher particle numbers per unit mass and surface area compared to larger particles. The higher surface area of nanoparticles caused increased reactivity, ROS generation and DNA destruction at a much higher rate than larger particles with the same mass dose (Vlastou et al. 2017).

9.3.2 Particle Chemistry and Crystal Structure

Particle chemistry is a more extensive and crucial factor than chemical composition to determine the toxic action of nanoparticles as it includes molecular chemistry of the cell and oxidative stress. Depending on their chemistry, nanoparticles exhibit different uptake by cells, potential to catalyze ROS production and subcellular localization. The crystal structure of nanoparticles also influences the cytotoxic action and mechanism of cell death (Xia et al. 2006). The crystal structures of TiO_2 nanoparticles showed different mechanisms for cell death, i.e. anatase form, in spite of size, induced cell necrosis, whereas the rutile form instigated apoptosis by overproduction of ROS (Braydich-Stolle et al. 2008; Love et al. 2012).

9.3.3 Dissolution

The dissolution of nanoparticles is also a vital parameter to determine their impact in the aquatic environment. Most nanoparticles remain insoluble in solution but form colloidal dispersions, which remain dispersed or aggregated. A recent study demonstrated the dependence of toxic action of silver nanoparticles on their

dissolution profile. The different parameters such as shape, temperature, size, pH, surface chemistry and ionic strength influence the physicochemical properties and induce precipitation, dissolution, or aggregation of silver nanoparticles besides the considerable impact on toxicity. The precipitation or aggregation process reduces the toxic effect of silver nanoparticles, whereas the dissolution process releases Ag^+ (silver) ions to result in elevated silver nanoparticles toxicity (Lee et al. 2018).

9.3.4 Surface Functionalization and Charge

Surface charge plays a significant role in affecting the toxicity of nanoparticles. The anionic charged plasma membrane showed the enhanced probability of cells with cationic charged nanoparticles. The different works of literature also revealed the greater toxic action of cationic nanoparticles in comparison to anionic or neutral nanoparticles. The particle surface plays an essential role in toxicity as it makes contact with biological material and cells. The use of surfactants alters the physicochemical properties of nanoparticles such as optical, electric, magnetic properties and chemical reactivity to cause cytotoxic action. Surface coatings possess the ability to convert harmful particles non-toxic and *vice versa*. The presence of oxygen radicals, ozone, oxygen and transition metals on nanoparticle surfaces results in ROS generation and oxidative stress. The particular cytotoxic action of silica is related to the incidence of surface radicals and ROS (Hoet et al. 2004; Janrao et al. 2014).

9.4 Different Routes for Administration of Nanoparticles

9.4.1 Oral

This route serves as a non-invasive for the delivery of the nanoparticulate system to the intended site. However, the first-pass effect in the hepatics significantly decreases oral bioavailability and, ultimately, the therapeutic action of actives (Yildirimer et al. 2011).

9.4.2 Pulmonary

This is a non-invasive route that not only provides greater surface area but also circumvents the first-pass effect in the hepatics. Since the pulmonary route offers local action, chances of local toxicity prevail (Yildirimer et al. 2011).

9.4.3 Transdermal

This is a non-invasive route for the delivery of nanoparticles with greater surface area and local action. Transdermal route causes local tissue irritation and the possibility to translocate into the systemic circulation (Yildirimer et al. 2011).

9.4.4 Intravenous

Parenteral route evades the first-pass effect in the hepatics to provide systemic action with improved bioavailability of actives. This route delivers active to the systemic circulation in a short period. However, nanoparticle administration through intravenous route causes systemic toxicity and are probably hepatotoxic (Yildirimer et al. 2011).

9.4.5 Intranasal

This route is a non-invasive methodology that offers direct delivery of actives from nose to the brain at a faster rate using extra- and intra-neuronal pathways in comparison to other conventional routes. Moreover, this route bypasses first-pass metabolism (Erdő et al. 2018).

9.4.6 Ophthalmic

This route is preferred to deliver drugs directly into the eye to treat ocular diseases. Various novel strategies, i.e. poly (lactic-co-glycolic acid) nanoparticles, liposomes, poly (methyl methacrylate) dendrimers, etc. are used as a promising drug delivery approach to target retina (Patel 2013).

9.4.7 Vaginal

This route is preferred to directly deliver drugs to the vagina for systemic as well as local action. The delivery of nanoparticles by this route also decreased the frequency of administration. Controlled-release nanoparticles showed zero-tolerance against microbial growth in the vagina due to their extended response to result in the improved activity of the active (Leyva-Gómez et al. 2018).

9.4.8 Buccal

Nanoparticles administered through this route are given in the buccal or oral cavity for a wide range of functions. They can be provided for dental purposes or as chewable entities for absorption into the systemic circulation. The infiltration of nanoparticles through the basement membranes and epithelium into the vital connective tissue demonstrated the possibility of oral transmucosal nanoparticle delivery for systemic action (Chinna Reddy et al. 2011).

9.5 Applications of Different Nanoparticles

Modern medicine uses nanoparticles for diverse applications *viz,* drug delivery, medical imaging, contrast agents and carriers for the delivery of genes to individual cells (Murthy 2007). Nanoparticles or zero-dimensional nanomaterials have the following applications in medicine such as:

9.5.1 Medical or Optical Imaging

The advanced applications of nanoparticles include optical imaging, contrast agents or magnetic resonance imaging. The early diagnosis of cancer is possible through the use of fluorescent nanoparticles. As drug delivery vehicles, nanoparticles demonstrate vast potential in the encapsulation of therapeutics to the target sites during biomedical imaging. Moreover, nanoparticles with multifunctional attributes provide multimodal imaging, with the possible advantages of a reduced dose of contrast agent and better targeting action. A potential role of nanoparticles as imaging agents includes diagnostics and therapeutics applications on the same stage, termed theranostics (Coll 2011; Han et al. 2018).

9.5.2 Drug and Gene Delivery

This segment is the most advanced use of nanoparticles, where various polymer and liposome-based delivery systems have been formulated for drugs and gene delivery. Nanoparticles are also used as carriers to deliver drugs, proteins, biomolecules, genetic material, etc. (Murthy 2007). Biodegradable or colloidal nanoparticles are also formulated for this purpose (Zhang and Saltzman 2013).

9.5.3 Cancer Therapy

Nanoparticles have the most significant impact on cancer mitigation and treatment, and various nanoparticle-based carrier systems have been used for drug delivery. Nanoparticles effectively target tumors in cancer therapy and promote the selective distribution of drugs to the target sites (Brigger et al. 2012). Metal nanoparticles showed significant and influential action in cancer therapy, providing better targeting, gene silencing and drug delivery. Surface functionalization of metal nanoparticles with ligands offer better control of energy deposition in the tumors. Apart from therapeutic advantages, metal nanoparticles also extend their potential action as a diagnostic tool for the imaging of cancer cells (Sharma et al. 2017).

9.5.4 Neurodegenerative Diseases

Nanoparticles improve the movement of actives across the blood-brain barrier (BBB) and deliver a payload to a large extent. The small dimensions of nanoparticles facilitate penetration across the BBB. Moreover, nanoparticles encapsulate drug molecules and enhance delivery into the BBB to treat various neurological disorders such as idiopathic Parkinsonism, stroke and Alzheimer's. The probable mechanism of nanoparticles to cross BBB includes transcellular or paracellular transport and carrier-mediated endocytosis (Saraiva et al. 2016).

9.5.5 Ocular Diseases

Nanoparticles encapsulates the drug to prolong the residence time of active in the eye. Nanoparticles possess enhanced penetration across the ocular membrane for the treatment of different diseases like glaucoma, corneal diseases like keratoplasty, corneal neovascularisation, viral keratitis and wounds of the corneal epithelium, uveitis, retinal diseases like macular degeneration and choroidal neovascularisation (Zhou et al. 2013).

9.5.6 HIV/AIDS

pH-sensitive nanoparticles deliver antivirals to disturb the HIV-1 replication cycle. Nanoparticles also target macrophages, primary HIV viral source, by using ligands such as fMLF peptides, tuftsin, galactose and mannose. Moreover, the targeted action of antivirals in a nanocarrier helps to treat viral reservoirs. HIV/AIDS vaccines prepared using nanotechnology show immense potential to target specific

cells with subsequent release of antigens in a sustained and controlled manner to extend their potential as a suitable replacement to viral vectors (Murthy 2007; Mamo et al. 2010).

9.5.7 Respiratory Diseases

Allergic, genetic and infectious diseases of the respiratory system are successfully treated using nanocarriers and include the utilization of biological materials, metals, polymers and ceramics to target pulmonary diseases with the advantages of improved bioavailability, enhanced absorption control, clearance and release of active. Nanocarriers further extend their potential in the treatment of severe pulmonary diseases by effective therapeutic action in the lung at lower doses, improve the delivery of lipophilic molecules and prevent compounds from degradation and clearance mechanism of lungs (Da Silva et al. 2013).

9.6 Toxicity Associated with Different Nanoparticles

Besides several applications of nanoparticles in the biomedical, electrical and industrial domain, there are specific toxicities that need to be addressed immediately. Nanoparticles stealthily penetrate the surroundings through air, soil and water to significantly impact the ecosystem and human health. Moreover, nanoparticles enter into the organisms through inhalation or ingestion and circulate to different tissues and organs to cause toxic effects. The possible repercussion of nanoparticles on the environment is likely to enhance in the near future (Khan et al. 2017). The various risks or toxicity issues related to different types of nanoparticulate systems are enlisted below:

9.6.1 Quantum Dots (QDs)

These are inorganic nanoparticles which are coated with an organic compound to make them biocompatible or bioactive. The inorganic core poses a risk upon the deterioration of the organic coating *in vivo*. The exposure of the inorganic-metal complexes used in QDs such as CdSe, ZnS, CdTe causes toxicity. Currently, luminescent colloidal QDs are used in biological investigations due to their peculiar size-dependent optical properties (Medintz et al. 2008). The two most extensively used metals in QD are cadmium (Cd) and selenium (Se). These two elements pose a significant risk to human health and environmental factors and cause chronic and acute toxic action in vertebrates. For instance, Cd with a half-life of 15–20 years in humans can bypass both BBB and placental barrier, with the highest toxic effect on

the kidney and liver. The Belews Lake, North Carolina band and Kesterson Reservoir, California, showed a noticeable impact on the local ecosystem resulted from eminent environmental concentrations of Se (Hardman 2006).

9.6.2 Metallic Nanoparticles

Iron oxide (Fe_2O_3)-based nanoparticles are extensively used as MRI contrast agents and various studies carried out on rats established their safety profile. On the other hand, gold nanoparticles used as X-ray contrast agents influence toxicity through their external morphology. The attachment of a cationic polymer monolayer onto the gold nanoparticles renders cytotoxic action. The toxic activity of metal nanoparticles include inflammation of tissues and increased the generation of ROS, to cause irregular function, cell destruction and death (Sukhanova et al. 2018). The clinical studies revealed that an important mechanism involved in the elimination of massive foreign particles from the lung includes macrophage clearance. The macrophage clearance mechanism is inefficient to remove inhaled nanoparticles because of nano size. Thus, remained inhaled nanoparticles in lung induce pulmonary damage and potentiate various disorders because of translocation to other tissues, including the brain (Lu et al. 2014).

9.6.3 Polymeric and Liposomal Nanoparticles

These nanoparticles are prepared from natural or biocompatible polymers like polyethylene glycol (PEG), poly-lactic-co-glycolic acid (PLGA) and are least challenging. Due to these properties, polymeric nanoparticles are frequently used as carriers for cytotoxic drugs. Synthetic polymers alter the rate of clearance of nanoparticles and lead to their accumulation in the spleen and liver. PEG-coated nanoparticles are preferred over the uncoated particles due to increased residence time, lower chance of accumulation and prevention of uptake by the reticuloendothelial system (Behzadi et al. 2017; Murthy 2007).

9.6.4 Magnetic Nanoparticles

These are a class of nanoparticles that respond to magnetic fields and usually contain a chemical component and a magnetic material such as iron, nickel or cobalt. The toxicity of these magnetic nanoparticles is based on their structural properties, dose and intended use. Cobalt ferrite nanoparticles ($CoFe_2O_4$ nanoparticles) possess tremendous paramagnetic properties and are currently utilized in catalysis, drug delivery and imaging. BALB/C mice demonstrated cardiac stress and DNA damage

in addition to procoagulatory action *in vivo* and *in vitro* at dose 0.4, 2 and 10 μg/kg following parenteral administration of ferric oxide nanoparticles. Fe_3O_4 nanoparticles at an LD_{50} dose of 163.60 mg/kg induced necrosis and denaturation in the heart muscles of ICR mice. Adult Wistar male rats revealed disproportion in the endocrine system, i.e. increased levels of T_3 thyroid hormone and decreased levels of thyrotropin following oral administration of ferric oxide nanoparticles (150 μg/kg) (Jiang et al. 2019).

9.6.5 Nanocapsules

These are nanodimensional structures made of a non-toxic polymer, which forms the outer shell. These are vesicular systems encapsulating an inner liquid core. Studies showed no significant toxicity at regular doses and when biodegradable polymers were used (Ostróżka-Cieślik and Sarecka-Hujar 2017).

9.6.6 Nanospheres

These are nanoparticles containing an encapsulated drug for targeted delivery. Nanospheres cause toxicity when the polymers are used in high concentrations (Ostróżka-Cieślik and Sarecka-Hujar 2017).

9.6.7 Nanosponges

These are targeted drug delivery systems that contain an encapsulated drug cross-linked in a nanosized sponge. The animal study showed no mortality following oral administration of nanosponges at doses up to 300–2000 mg/kg on the treated Wistar rats. At a concentration of 15 mg/ml, nanosponges showed excellent compatibility with blood and no hemolytic action after incubation for 90 min with human erythrocytes. Nanosponges synthesized from pyromellitic dianhydride showed less and more stability in basic and acidic solutions, respectively (Shende et al. 2015).

9.6.8 Dendrimers

These are branched structures widely used in therapeutic and diagnostic applications. The toxicity of dendrimers depends on its chemistry and the functional groups present and is mainly due to the contact of positive surface charge of dendrimers

with negative charged biological membranes *in vivo* which further results in membrane interference via membrane thinning, erosion and nanohole formation (Jain et al. 2010).

9.6.9 Silver Nanoparticles

These are nanocarriers that contain a large percent of silver oxide and extensively used in different fields of medicine. Silver nanoparticles, however, release silver ions on dissolution, which is the principal reason behind the toxic action. The researchers also found the concentration- and exposure-dependent toxicities of silver nanoparticles (Cho et al. 2018). The silver nanoparticles also showed size-dependent toxicity, i.e. silver nanoparticles of size 4 nm promoted IL-8 secretion from macrophage immune cells and generated higher levels of ROS in comparison to 20 and 70 nm silver nanoparticles (Zhang et al. 2016). Moreover, silver nanoparticles also showed concentration-dependent damage in the DNA of human cells (Cho et al. 2018).

9.6.10 Gold Nanoparticles

Gold nanoparticles contain colloidal gold to a large extent, and are used extensively in chemotherapy and biomedical science. Their toxicity mainly prevails due to the formation of ROS in the body on treating cancer cells. The *in vitro* studies showed that the gold nanoparticles induced ROS generation after entering the cells and cause oxidative stress-related cytotoxicities such as DNA damage, cell death (necrosis and apoptosis) and cell cycle arrest (Sun et al. 2018). Moreover, gold nanoparticles interfered with the normal functioning of the cell and showed size-dependent toxic action i.e. gold nanoparticles with mean particle size 45 nm were more noxious than 13 nm ones due to the more significant damaging effect on vacuoles and into the cytoplasm (Hoshyar et al. 2016).

9.7 Nanotoxicity in Various Populations

9.7.1 Pregnant Females and Neonates

The pregnant females showed wide range of manifestations following nanoparticle exposure such as miscarriage, lower gestational rates, dysfunction of placenta, etc. whereas, neonates showed retarded growth rate, mutated gene expression, renal abnormalities and increased susceptibility to cancer and other diseases. The

pregnant females are more vulnerable to nanoparticle exposure than normal females by twofold. The increased exposure might be due to the neuroendocrinal changes during pregnancy and the capability of nanoparticles to cross the placental barrier and enter the fetus to cause toxic action. Moreover, nanoparticles exposure induced oxidative stress to generate functional and structural deformity in the placenta and significantly decreased the gestational success rate. Nanoparticles enter the offspring from maternal circulation through different pathways such as lactation. Titanium dioxide (35 nm) and silica nanoparticles (70 nm) were traced in the placenta, fetal liver and brain after parenteral administration. Moreover, altered gene expressions, DNA damage in the liver and renal abnormalities were observed in descendants following exposure of carbon black nanoparticles to pregnant ICR mice (Li et al. 2014).

9.7.2 Diseased Populations

The intrinsic physicochemical attributes of nanoparticles and the reactions of biological systems to nanoparticle exposure collectively determine biocompatibility. The physiological response of nanoparticles to biological systems includes approachability to circulating nanoparticles, the potential to metabolize and expel them from the body. Moreover, BBB obstructs the entry of toxic nanoparticles to protect neuronal systems. The liver is the largest vulnerable organ and takes up most nanoparticles in circulation. In healthy individuals, these physiological functions work efficiently to protect the body and reduce toxicity caused by nanoparticle exposure, whereas, in diseased people, some of these functions are immobilized to result in toxicity following nanoparticle exposure (Longmire et al. 2008). The detrimental impact of nanoparticles on respiratory, circulatory and hepatitis-diseased patients are enlisted below:

9.7.2.1 Effect of Nanoparticles on Cardiovascular Disease

Nanoparticles stimulates cytoplasmic vacuolization, mitochondrial inflammation and ultimately apoptosis in human aortic endothelial cells (HAoEC). Fe_2O_3 Nanoparticles potentiates arterial sclerosis by inducing nitric oxide overproduction and better adherence of monocytes to HAoEC due to overexpression CD54 and interleukin-8. Various factors that influence arterial sclerosis include inflammation, oxidative stress and impairment in aortic mitochondrial DNA and endothelial cells of vessels. Nanoparticles such as soot, carbon nanotubes, or TiO_2 nanoparticles demonstrated size-dependent impaired coagulatory actions of lipopolysaccharide-treated ICR mice (Zhu et al. 2011; Li et al. 2014).

9.7.2.2 Effect of Nanoparticles on Chronic Respiratory Disease

A recent study revealed the exposure of nanoparticles might intensify respiratory diseases such as asthma, bronchitis, emphysema, etc. Particulate matter and ultrafine particles showed increased deposition in the lungs in comparison to healthy volunteers and cause inflammation. The clinical and laboratory results also revealed the potential action of inhaled microparticles on asthma symptoms (Chalupa et al. 2004; Takano et al. 2002; Li et al. 2014).

9.7.2.3 Effect of Nanoparticles on Hepatitis Patients

Nanoparticles mostly accumulate in the liver with subsequent circulation. A recent animal study showed severe action of gold nanorods on the liver such as allergic response and stress-induced cell death by triggering serum alanine aminotransferase (ALT) and hepatic macrophages levels whereas, showed no action on liver fibrosis in chronic hepatic injury. (Li et al. 2014; Bartneck et al. 2012).

9.7.3 Elderly Populations

Aging is responsible for decreasing the cellular and organic functions and result in increased susceptibility to ailments and toxicities. Animal studies also indicated that aged animals were more vulnerable to the adverse effects of nanoparticles compared to young and adult animals. Nanoparticles tend to readily induce severe pulmonary inflammation and cardiovascular disease in the aging population. Animal studies and human data revealed that inhalation of particulate matter led to impaired cardiac and pulmonary functions in the elderly individual. 20-months-old rats showed various side effects such as vascular damage, cardiac ischemia, blockage of cardiac valve, increased blood viscosity and fibrinogen concentration after exposure of silicon dioxide (SiO_2) nanoparticles (24.1 mg/m^3; 40 min/day) for 4 weeks, whereas, no side effects were observed in young rats (Chen et al. 2008a, b; Li et al. 2014).

9.8 Experimental Models Used for Nanotoxicology

The study of nanotoxicology determines the level of toxicity observed in humans due to the exposure of nanoparticles for a longer duration. However, using human volunteers to assess the toxic effects of nanoparticles is impractical. Thus animal models are used to mimic the same effects. The results of toxicology studies carried out on animal models (*in vivo* toxicology) or cell models (*in vitro* toxicology) can be extrapolated on humans (Baeza-Squiban et al. 2011).

9.8.1 In-Vivo *Models*

The different *in vivo* models used for nanotoxicological studies include mice, rats, rabbits, dogs, guinea pigs, pigs, monkeys, etc. and among them, mice and rats are most widely used. The assessment methods for *in vivo* toxicity include:

- Biodistribution studies detect nanoparticles in the living or killed animals through radiolabels and also inspect their route to the tissue or organ.
- The clearance of nanoparticles is impelled by analyzing the excretion and metabolism of nanoparticles at different intervals with subsequent exposure.
- Histopathology determines the toxicity caused by nanoparticles to visible tissues such as liver, lung, brain, eyes, spleen, heart and kidneys (Kumar et al. 2017; Greish et al. 2012).

The nanoparticles are visualized in the body using various imaging techniques like optical, confocal or electron microscopy. Nanoparticles like Fe_3O_4 with magnetic properties can be detected by nuclear magnetic resonance (NMR). External tracers are used sometimes for nanoparticles that are difficult to detect, e.g., fluorescent, radioactive, etc. However, the presence of the tracer should not alter the intrinsic behavior of the nanoparticles significantly and remain immobilized on the particles upon entering in the organism. The nanoparticles or its tracers are determined by chemical assay methods such as mass spectrometry, NMR, or optical ICP (inductively coupled plasma) (Kumar et al. 2017).

9.8.2 In-Vitro *Models*

In vitro methods are simple, less time-consuming and economical in comparison to *in vivo* studies. *In vitro* models fail to reproduce the exact intricacy of the organism, does not take into account the toxicokinetic phase and are used for short term studies (Baeza-Squiban et al. 2011; March et al. 2000; Nel et al. 2006). 3D *in vitro* models obtained using pluripotent stem cells deliver a genuine, patient-specific platform for toxicity evaluations, applications in drug selection and studying injuries of the biophysical framework at both cellular and tissue level (Handral et al. 2016).

The *in vitro/in vivo* toxicity of different types of nanoparticles are enlisted in Table 9.1.

9.8.3 *Mathematical Models for Nanotoxicology Study*

Many mathematical models have been developed to study nanotoxicity in animals. This dynamical model examines the potential systemic toxicity and homeostasis in a complex living biological system. Moreover, the dynamical model also extends their future applications in the field of nanotoxicology and determination of other factors such as kinetics, diagnosis, etc. (Han et al. 2009).

Table 9.1 *In vivo-in vitro* toxicity of various types of nanoparticles

Nanoparticles	Particle size (nm)	Cell culture/Animal model	Assay	Result	References
Copper oxide	55.8	HaCaT cell line (human skin keratinocyte cell line)	MTT LDH Lipid peroxidation assay	DNA damage Lipid peroxidation increase. Reduction in glutathione. Cell viability decreases.	Alarifi et al. (2013)
Copper oxide	20–95 and 50	A549 & THP-1 cells	MTT LDH	Lipid peroxidation increase. Autophagy increased in A549 cells. Cell viability decrease. LDH increase.	Ahamed et al. (2010)
Fullerenes	178	HEK 293, HELA & CHO	Single-cell gel electrophoresis assay, micronucleus test	Chromosomal damage. Breakage of DNA strand. Genotoxicity increased. LDH unaffected.	Dhawan et al. (2006) and Niwa and Iwai (2006)
Fullerenes	125	HEK 293	MTT	Behaviour disturbance and hematotoxicity (> 300 mg/kg or more). Leukopoies and thrombocytopoies suppressed at higher doses (>1200 mg/kg).	Prylutska et al. (2019)
Fullerenes	100–900	Embryonic zebrafish	Cellular death	Necrotic and apoptotic cellular death. Increase in pericardial edema, malformations, etc.	Usenko et al. (2007)
Quantum dots	100	4 T1 MRC-5	MTT Haemolysis test	No haemolysis. Cell viability decrease.	Helle et al. (2012)

Quantum dots	6.09	Marine mussel *Mytilus galloprovincial-is*	Micronucleus test Nuclear abnormalities test Comet test	DNA damage increase. Lysosomal membrane stability decreases. Chromosomal damage. Lipid peroxidation increased	Rocha et al. (2014)
Quantum dots	–	*Elliption complanata* (K-562 cells)	Olive's alkaline DNA precipitation assay	Oxidative stress increased. Edema and thickening of lamellae TBARS production increased DNA strands breakage increased.	Gagné et al. (2008)
Aluminium oxide	28	CHO-K1	MTT Sister chromatid exchange (SCE)	Lysosomal activity decreases. Genotoxic action increases. Cell viability decreases.	Di Virgilio et al. (2010)
Aluminium oxide	50	MLCL	Comet MTS	DNA damage.	Bahadar et al. (2016)
Aluminium oxide	8–12	HBMVECs	MTT DHE	Cell viability decrease. Oxidative stress increase. Alter proteins expression of the BBB.	Chen et al. (2008a, b)
Aluminium oxide	160	HMSC	MTT Annexin V-Cy3 assay	Cell viability decrease.	Alshatwi et al. (2012)
Aluminium oxide	50–80	L929 cells BJ cells	EZ4U	No cytotoxic action on mammalian cells.	Radziun et al. (2011)
Aluminium oxide	30–40	Rat blood cells	Comet Micronucleus test	Dose-dependent genotoxicity.	Balasubramanyam et al. (2009)

(continued)

Table 9.1 (continued)

Nanoparticles	Particle size (nm)	Cell culture/Animal model	Assay	Result	References
Titanium oxide	160	*In vivo*	Comet Micronucleus test LDH ALP	DNA damage. Genotoxicity. Dose-dependent inflammatory pulmonary lesions.	Liu et al. (2009)
Titanium oxide	20	CHO-K1	MTT SCE	Absence of metaphases. DNA damage increases.	Di Virgilio et al. (2010)
Titanium oxide	<100	IMR-90 and BEAS-2B cells (human lung epithelial cells)	ELISA Trypan blue DCFH-DA Comet	Oxidative DNA damage. Concentration-dependent generation of ROS. Cytotoxic action increases.	Bhattacharya et al. (2009)
Iron oxide	70–90	*Eisenia hortensis*	Comet test Micronucleus test	Genotoxicity. DNA lesions. Chromosomal damage.	Ciğerci et al. (2018)
Iron oxide	13.8	Human hepatocellular carcinoma cells	MTT	Cell viability decreases	Ge et al. (2009)
Iron oxide	30	Murine macrophage cells	MTT	Cell viability decreases.	Naqvi et al. (2010)
Iron oxide	75	MDA-MB-231	Cell viability study Prussian blue	Complete regression of tumor. Proliferation activity of tumor cells improved.	Kossatz et al. (2015)
Iron oxide	100–150	Human macrophages	MTS	Cell viability decreases.	Jeng and Swanson (2006)
Iron oxide	30–50	MCF7	MTT LDH Comet assay	ROS generation increases. Lipid peroxidation increases. Breakage of DNA strands.	Alarifi et al. (2014)

Zinc oxide	50–70	Human colon carcinoma cells	ELISA Flow-cytometry	Oxidative stress increases. Cell viability decreases. Inflammatory biomarkers.	De Berardis et al. (2010)
Zinc oxide	50	Human tongue cancer cell line	BCA protein assay kit JC-1 assay MTT	Increase in ROS generation. Decrease in mitochondria membrane potential. Inhibits autophagy.	Wang et al. (2018a, b)
Zinc oxide	215.8 (water) 30.9 (DMEM medium)	A431	MTT	Oxidative stress increases. Cell-cycle dependent cellular uptake. Genotoxic action in cancerous cells.	Patel et al. (2016)
Zinc oxide	307–419	Human cervix carcinoma cell line	MTT Comet	DNA damage. Cell viability decreases.	Osman et al. (2010)
Zinc oxide	50	HEK-293	MTT Comet	Cell viability decrease. Oxidative stress increase. Mitochondrial damage.	Guan et al. (2012)
Zinc oxide	4–10 nm	MCF-7	MTT DAP-1	Membrane disruption. Oxidative stress increase. Alter mRNA gene expression.	Kadhem et al. (2019)
Zinc oxide	20	Human bronchial epithelial cells	–	LDH release. Cell viability decrease. Oxidative stress increase.	Huang et al. (2010)
Silica oxide	15–46	Human bronchoalveola-r carcinoma cells	DCFH-DA Commercial kit	Increase in ROS. LDH increases. Malondialdehyde increase.	Cho et al. (2007)
Silica oxide	20	HT-29 (human intestine cell line)	Cytotoxicity study Flow cytometry	Cytotoxic effect increases. Genotoxic effect increases.	Sergent et al. (2012)

(continued)

Table 9.1 (continued)

Nanoparticles	Particle size (nm)	Cell culture/Animal model	Assay	Result	References
Silica oxide	13–17	SH-SY5Y	MTT Comet	Concentration- and time-dependent cytotoxic action. Increase in ROS leads to neurodegeneration. Cell viability reduces. DNA damage.	Kim et al. (2010)
Silica oxide	43	Hepatocellular carcinoma cells(HepG2)	DCFH-DA 5,5,6,6-tetraethyl-benzimidazo-lylcarbo-cyanide iodine	Increase in ROS. Mitochondrial damage Oxidative stress increases.	Sun et al. (2011)
Silica oxide	15 and 300	Balb/3 T3 cells	Colony forming efficiency Cell transformation assay	No morphological transformation. No genotoxicity. Cell viability significantly reduced (150–200 nm).	Uboldi et al. (2012)
Gold nanoparticle	15	Sprague Dawley rats	Pig-a gene micronucleus comet assay	Apoptosis through oxidative damage. DNA breakage and micronuclei formation.	Wang et al. (2018a, b)
Gold nanoparticle	15–20	MDA-MB 231 MCF-7	Alamar blue assay	Apoptotic body formation. Tumor cell viability decreases.	Moses et al. (2016)
Gold nanoparticle	16 and 49	MDA-MB 231	MTT Clonogenic assay	Cell arrest at G_2/M phase. Cytotoxic action increases.	Wang et al. (2013)
Gold nanoparticle	65–149	A549 B16F10 (human alveolar cell line)	MTT Cellular uptake study	Proliferation of tumor inhibited. Induces apoptosis of cancerous cell.	Mukherjee et al. (2016)

Gold nanoparticle	12.5	MDA-MB 231 MCF-7 (breast cancer cell line)	MTT Comet assay DAPI staining	Apoptosis in G_0/G_1 to S phase. DNA damage.	Uma Suganya et al. (2016)
Gold nanoparticle	3–4.5	MDA-MB 231 MCF-7	Cell viability assay Annexin-V FITC assay	Increase in apoptosis. Suppress EGFR signaling. Cytotoxicity increases.	Balakrishnan et al. (2017)
Silver nanoparticles	30	HaCaT (human skin keratinocyte cell line)	Comet assay Micronucleus assay	Cell viability decrease. Genetic damage. Cell proliferation decreased.	Bastos et al. (2016)
Silver nanoparticles	15–100	BRL3A	LDH MTT Glutathione DCFH-DA	ROS increases. LDH increases. Cell viability decrease.	Hussain et al. (2005)
Silver nanoparticles	5	Mouse	Pig-a assays comet assay	Oxidative DNA damage. Cytotoxic action to reticulocytes.	Li et al. (2014)
Silver nanoparticles	20–40	Human leukaemia cell line	WST-1 LDH	LDH increases. Cell viability decrease.	Haase et al. (2011)
Silver nanoparticles	10 and 100	CHO-K1 CHO-XRS5	Cell viability assay Clonogenic assay micronucleus test Comet assay	Greater genotoxic and cytotoxic action by 100 nm than 10 nm. Repair of damaged DNA. Induction of ROS to impair cell proliferation.	Souza et al. (2016)
Silver nanoparticles	30–50	Human alveolar cell line	MTT DCFH-DA	ROS increases. Cell viability decrease.	Foldbjerg et al. (2010)

(continued)

Table 9.1 (continued)

Nanoparticles	Particle size (nm)	Cell culture/Animal model	Assay	Result	References
MWCNT	30–50	Human bronchial epithelial cells (A549)	Comet Micronucleus test	DNA damage. Genotoxic action to BEAS-2B cells. Cell viability decreases.	Lindberg et al. (2009)
MWCNT	20	Lung cancer cells	MTT	Cell viability decrease.	Magrez et al. (2006)
SWCNT	800	HACECs NHBECs	Clonogenic	Cell death.	Herzog et al. (2007)
SWCNT	14	Mouse lung epithelial cells	Comet	Generation of ROS. Cell increase in G_1 phase. No damage to DNA.	Jacobsen et al. (2008)

Abbreviations: *HBMVECs* Human brain micro vascular endothelial cells, *HMSC* Human mesenchymal stem cells, *BBB* Blood- brain- barrier, *MLCL* Mouse lymphoma cells line, *LDH* Lactate dehydrogenase, *HACECs* Human alveolar carcinoma epithelial cell line, *NHBECs* Normal human bronchial epithelial cell line, *DHE* Dihydroethidium, *AST* Aspartae transaminase, *ALT* Alanine transaminase, *BRL 3A* Buffalo rat liver cells, *CHO* Chinese Hamster ovary cells, *HELA* Human epidermoid-like-carcinoma cells, *HEK* Human embryonic kidney cells, *ROS* Reactive oxygen species, *DCFH-DA* Dichlorodihydrofluorescein diacetate, *DNA* Deoxyribonucleic acid, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, *DMEM* Dulbecco's Modified Eagle Medium, *MWCNTs* Multi- walled carbon nano tubes, *SWCNTs* Single walled carbon nano tubes

9.8.4 In-Silico *Models for Nanotoxicology*

In-silico models use computational strategies to provide a new array for novel molecular formulations. *In-silico* models predict the cytotoxic effects of nanoparticles at the BBB and analyze the nanoparticle permeation, aggregation as well as interactions. *In-silico* methods help to understand the modes and routes of nanotoxicity in animals. Such computational models are essential in terms of risk assessment and involve extensive analysis in addition to quantitative structure-activity relationships (QSARs) (Shityakov et al. 2017; Richarz et al. 2015). *In-silico* models form an essential part of toxicology studies due to their potential to analyze, mimic, visualize and anticipate the toxicity of chemicals (Raies and Bajic 2016).

9.9 Nanosafety Regulations

A regulatory framework for responsible and synchronized strategy is required to assure the potential safety issues concerned with human health and the environment because of advancements in nanotechnology and their products. OECD is a global forum concerned with the safety issues of nanomaterials and deals with the analysis and evaluation of chemicals used in nanotechnology-based products. The objective of OECD is to implement an internationally harmonized standard for hazard and risk evaluation strategies as well as encourage assistance on the benefit of human health and ecosystem from manufactured nanomaterials (Soares et al. 2018 and www.oecd.org 2011).

9.10 Conclusion

The exponential growth of nanotechnology and nano-based products in biomedical and industrial applications caused an inevitable impact on the world. The unavoidable exposure to nanomaterials raised concerns regarding their implications on human health, safety and ecology. The review summarizes and highlights nanomaterials' associated toxicity and their impact on human health and the ecosystem. All nanoparticles, according to attributes and concentration, are related to toxic actions of different levels. The non-uniformity in experimental practices results in contradictory conclusions, which further create difficulty in understanding the precise mechanisms and features of nanomaterial related toxicity. In brief, the entire scientific community should scrutinize and establish innovative and reproductive procedures for toxicity assessment to determine the potential hazards and exposure arising from the use of nanomaterials and nanotechnology-based products.

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Chapter 10
Nanomaterials in the Treatment and Prevention of Oral Infections

Rafał Pokrowiecki, Krzysztof Pałka, Tomasz Zaręba, and Agnieszka Mielczarek

Abstract Oral biofilm- associated infections are common problem that is still actual, despite progress being made in the field of bioengineering and pharmacology in the last decade. This is due to diverse microbiology of oral biofilms, that are more frequently resistant to commonly prescribed antibiotics. Such infections may infiltrate deeper anatomical areas and eventually become a serious threat to patient's life. Nanotechnological approaches aim at designing antibacterial biomaterials and oral hygiene products that could provide prolonged, sustained activity preventing from disease. In this chapter extensive review of the current approaches to antibacterial nanomaterials for the purposes of dentistry and oral health will be discussed.

Keywords Nanodentistry · Nanomaterials · Nanotechnology · Biomaterials · Dental implants · Dentistry · Implantology · Nanodentistry · Silver

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10.1 Introduction

The vast majority of the infections in the area of the head and neck are caused by mixed anaerobic and aerobic bacteria which form bacterial biofilms (Cordesmeyer et al. 2017). It proceeds as a four-step process: (1) initial attachment of bacterial cells; (2) cell aggregation and accumulation in multiple cell layers; (3) biofilm maturation and (4) detachment of cells from the biofilm into a planktonic state to initiate a new cycle of biofilm formation elsewhere (Arciola et al. 2015; Elter et al. 2008; Zhao et al. 2014).

The head and neck have its unique, and extremely rich vascular system, which provides the vital organs with nutrients, oxygen and immune cells required for proper functioning. However, the distinct anatomy of head and neck may also contribute to the development of deep infections that may have serious threat on the life of patients, if not diagnosed or treated adequately (Cordesmeyer et al. 2017; Motahari et al. 2015). The vast majority of deep infections of the head and neck are of dental- associated diseases origin (dental carries, periodontal or peri- implant diseases) (Gujrathi et al. 2016) (Fig. 10.1).

Early diagnosis and appropriate treatment can save the patient's life and prevent from complications of disease. Oral cavity is an ecosystem that significantly differs

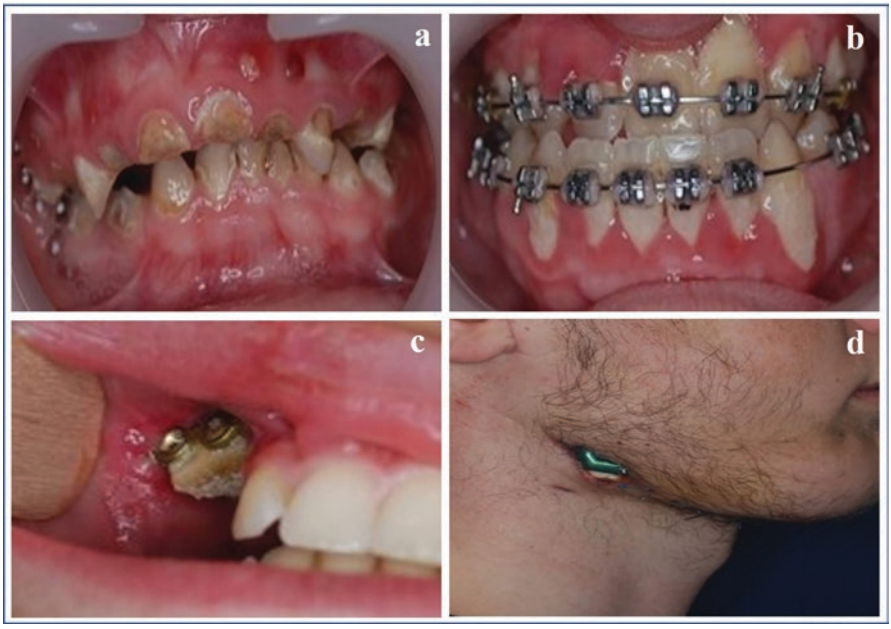


Fig. 10.1 Examples of different infections in the area of the head and neck: early childhood carries with periapical chronic periodontitis and gingival fistulas (**a**), plaque- induced gingivitis (**b**), infection of the stabilization plate and bone graft necrosis (**c**), surgical site infection and mandible reconstruction plate (**d**)

from all others, in the human body. This is due to the presence of protein and sugar rich saliva and bacteria which cover all the surfaces in the mouth that influence disease development, progression, as well as, pharmacokinetics of the drugs administered orally. Moreover, the environmental- specific biological fluids, dynamics of biofilm formation, its microbial diversity, as well as, virulence factors and increasing global resistance to commonly used antibiotics make it more challenging. Traditional biomaterials used for dental purposes, reconstructive surgery and prosthodontics in the area of the head neck, exhibit features which predispose them to bacterial biofilm formation and infection development.

Nanoparticles were recently proposed as a potential way to prevent and treat oral biofilm- based infections.

10.2 Antimicrobial Activity of Nanomaterials

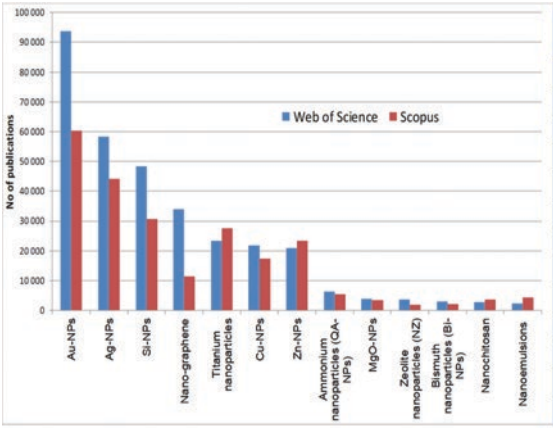
Several mechanisms of antibacterial activity were proposed depending on the nanoparticle size, shape, and surface charge, enhanced by the inherently large active surface area (Elbourne et al. 2017). Biocidal properties of different nanoparticles are based, but most probably not limited to: disruption of the bacterial cell membrane, inhibition of the active transport and metabolism of sugars, generation of reactive oxygen species (ROS), displacement of magnesium ions required for the enzymatic activity of oral biofilms, impairment of electron transport through bacterial membrane, and DNA replication inhibition (Abou Neel et al. 2015; Vimbela et al. 2017; Santos et al. 2013).

Nanomaterials may be used as targeted drug delivery carriers in the form of nanotubes, nanoliposomes, tubular nanostructures, nanosheets or nanomats.

Encapsulation of antibiotics in nano-carriers may provide better drug activity against bacteria and decrease the bacterial resistance. This was shown by Hadiya et al. (2018) who evaluated such approaches with levofloxacin encapsulated in chitosan nanoparticles (Hadiya et al. 2018). Therefore, nanomaterials may be applied as antibacterial agents and/or be used as novel drug delivery systems for already used biocides. Both approaches were recently evaluated with the use of different nanomaterials for the purposes of dentistry and head and neck surgery.

10.2.1 Selected Antimicrobial Nanomaterials for Dentistry

Nanomaterials may be composed of elements or their compounds (e.g. TiO_2). Through the last decade, a plethora of publications on nanoparticles and their applications has been published (Fig. 10.2). For the sake of this chapter, only nanoparticles and nanomaterials will be discussed, that were evaluated with a regard to antibacterial dental purposes.



Type of nanoparticles	Web of Science	Scopus
Gold (Au)	93 673	60 385
Silver (Ag)	58 328	44 128
Silica (Si)	48 470	30 751
Nano-graphene	33 916	11 520
Titanium nanoparticles	23 481	27 521
Copper (Cu)	21 822	17 435
Zinc (Zn)	20 946	23 384
Ammonium nanoparticles (QA)	6 293	5 432
Magnesium oxide (MgO)	3 822	3 453
Zeolite nanoparticles (NZ)	3 619	2 026
Bismuth nanoparticles (Bi)	3 065	2 037
Nanochitosan	2 733	3 604
Nanoemulsions	2 425	4 451

Fig. 10.2 Summary of number of publications about examples of nanoparticles and their compounds published within last 10 years according to Web of Science and Scopus

10.2.1.1 Titanium Nanoparticles

Titanium dioxide nanoparticles were shown to decompose organic compounds by the formation of hydroxyl radicals and superoxide ions when exposed to non-lethal ultraviolet light (Jesline et al. 2015). They have been proven to exhibit antibacterial activity against cariogenic *S. mutans in vitro* by Elsaka et al. (2011). Similar observations were confirmed further by Garcia-Contreras et al. (2015) who evaluated 3 and 5% wt. of nano- titanium added to the glass-ionomer dental cement. The addition of Titanium dioxide nanoparticles provided sufficient antibacterial activity >1 mm in disc diffusion tests) and better fracture toughness, flexural strength and compressive strength compared to the unmodified dental material giving the opportunity to use them in the areas of the increased load- bearing tooth area. Moreover, the addition of titanium nano- powder to dental cement did not affect bonding strength to dental tissues, nor decrease the materials'sfluoride release which is regarded as the most beneficial and important activity of glass- ionomer cements. However, the increase of titanium dioxide nanoparticles up to 7% wt. reduced the material's mechanical properties (Elsaka et al. 2011; Garcia-Contreras et al. 2015). The antibacterial effect on titanium dioxide nanoparticles is suggested through reactive oxygen species production, hydroxyl free radicals and peroxide (Wang et al. 2011). It can be reduced when the agglomeration of nano-powder occurs and active particles are conjugated and not uniformly incorporated between the filler particles and matrixes of the cement. In the study of Chambers et al. (2017), silver doped titanium dioxide nanoparticles were used as a filler to dental resins in order to provide antibacterial activity in the area of *"locus minoris resistentiae"* of dental fillings: the tooth- filling margin which always create a micro- gap that poses a favorable niche for bacterial leakage and secondary caries development. The authors showed, that silver-titanium dioxide nanoparticles incorporated into epoxy resin (<

2 wt%) may exhibit antibacterial activity of *S. mutans*, however it also decreased significantly their photocatalytic properties and therefore, antibacterial properties may be hampered (Chambers et al. 2017). Moreover, nanoparticles used in adhesive resins are prone to agglomeration, turning the material susceptible to physical failure (Garcia et al. 2016). More recently, Besinis et al. (2014) compared bactericidal properties of titanium dioxide nanoparticles, silver nanoparticles and silica nanoparticles with commonly used disinfectant in dentistry- chlorhexidine The antibacterial effect was conducted on *S. mutans* species, but both, silica nanoparticles, titanium dioxide nanoparticles showed limited activity. Silver nanoparticles were most efficient when compared to other nanoparticles and chlorhexidine (Besinis et al. 2014). On the contrary, Ahrari et al. (2015) showed that mouthwashes containing titanium dioxide nanoparticles can be potentially used clinically as they exhibited a better antibacterial effect than chlorhexidine or sodium fluoride rinses (Ahrari et al. 2015).

10.2.1.2 Silver Nanoparticles

Silver nanoparticles were most extensively evaluated among all nanoparticles and nanomaterials during the last decade. They possess a wide range of bactericidal properties against multitude microbes, among which, oral bacteria were also found significantly susceptible to (Pérez-Díaz et al. 2015). Silver nanoparticles may be relatively easily incorporated onto a biomaterial surface (Fordham et al. 2014) and because of that, they were incorporated into various formulations for dental bioengineering purposes so far (Monteiro et al. 2009; AlKahtani 2018).

Arash et al. (2016) described orthodontic brackets covered with silver nanoparticles which exhibited antibacterial effect against *S. mutans* for 30 days (Arash et al. 2016). The application of silver nanoparticles in orthodontics in order to decrease dental carries was also proposed by other authors (Prabha et al. 2016; Metin-Gürsoy et al. 2017). Nanoparticles were added to dental polymers which are used for dentures and dental filling preparation by Chambers et al. (2017). As silver nanoparticles were found efficient in the eradication of *Enterococcus feacalis,* as well as, smear layer removal capacity, some authors considered them as possible root canal-disinfectant during endodontic treatment as well (Chan et al. 2015; González-Luna et al. 2016) (Fig. 10.3).

Silver nanoparticles were also considered as an additive to bone regenerative-biomaterials. For example, González-Sánchez et al. (2015) described methacrylate hydrogels with nanosilver, that sufficiently inhibited *Staphylococcus epidermidis and staphylococcus aureus* (González-Sánchez et al. 2015).

10.2.1.3 Zinc Nanoparticles

Zinc oxide nanoparticles based materials have been used in general dentistry for many years due to their antibacterial and wound healing properties, however nanoscale- formulations seemed to expand their potential clinical applications

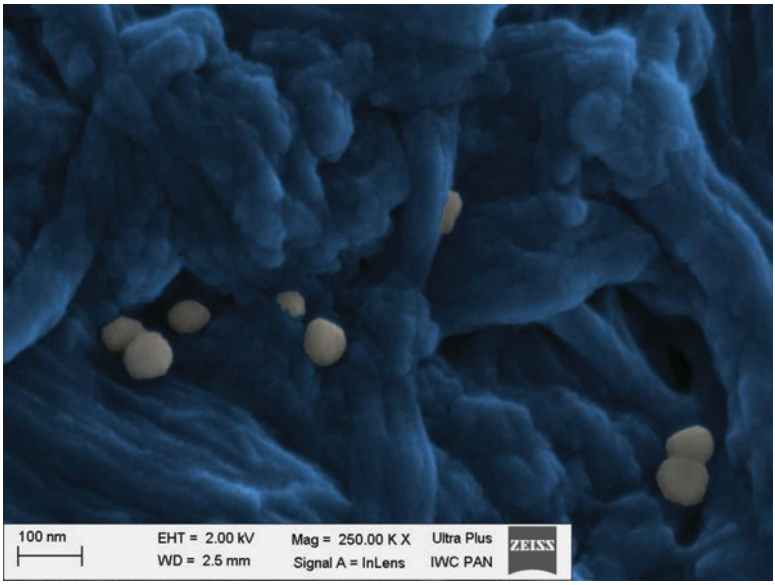


Fig. 10.3 Scanning electron microscopy (SEM) of **e**ndodontic paper point soaked with colloidal silver solution (yellow dots) (For the courtesy of Jacek Wojnarowicz. The Intitute of High Preasures, PAN, Warsaw, Poland) Digital colouring for the courtesy of Krzysztof Palka, Lublin University of Technology, Faculty of Mechanical Engineering

(Moezzi et al. 2012; Cierech et al. 2016; Wojnarowicz et al. 2018). The release of hydrogen peroxide is one of the possible mechanisms for antibacterial activity of zinc nanoparticles (Jesline et al. 2015) which was proven to exhibit sufficient activity against bacteria and fungi (Sawai and Yoshikawa 2004). In the study of Aguiar et al. (2015) it was shown that zinc nanoparticles were effective in the eradication of *Enterococcus feacalis* species which are involved in peri-apical dental infections. Antibacterial effect was achieved by mixing zinc oxide nanoparticles with calcium hydroxide paste which promoted greater initial alkalization of the medium. The antibacterial effect was even greater when ChX was used additionally (Aguiar et al. 2015). Kasraei et al. (2014) reported that zinc oxide nanoparticles added to dental composite resins provide antibacterial activity against *S. mutans* and *Lactobacillus acidophilus* species, commonly responsible for caries initiation (*S. mutans*) and progression (*L. acidophilus*) (Kasraei et al. 2014). On the other hand, in the of Aydin Sevinç and Hanley (2010) study zinc oxide nanoparticles blended at 10% (w/w) fraction into dental composites displayed some ability to decrease three-specie (*Streptococcus oralis, Streptococcus gordonii, Actinomyces naeslundii*) biofilm formation, but did not provide any antibacterial activity in the disc diffusion test. They concluded that such materials may decrease bacterial settlement by direct contact up to 3 days, but due to the insolubility of zinc oxide nanoparticles, the antibacterial effect may be insufficient (Aydin Sevinç and Hanley 2010). Similar observations were described in the study of Tavassoli Hojati et al. (2013) who evaluated

composites with zinc oxide nanoparticles blended at 1–5% weight (Tavassoli Hojati et al. 2013). Antibacterial effect of zinc oxide nanoparticles modified electro spun membranes was achieved in the study of Münchow et al. (2015). Disc diffusion test tests confirmed antimicrobial activity of such materials against *Porphyromonas gingivalis* and *Fusobacterium nucleatum, suggesting their materials could find practical applications in periodontal treatment* (Münchow et al. 2015).

In another study, mesoporous calcium-silicate nanoparticles (MCSNs) loaded with zinc oxide nanoparticles and silver nanoparticles were investigated with a regard to the flexural strength of dentin. Such modified MCSNs adhered well to the root canal walls and infiltrated into the dentinal tubules without affecting their mechanical properties. Moreover, the presence of zinc ions induced the release of silver ions into the medium, which could be beneficial for dental root canal fillers (Tamayo et al. 2016). On the other hand, the study of Samiei et al. (2017) showed that zeolite- zinc- silver nanoparticles added to Mineral Trioxide Aggregate (MTA), which is used endodontic treatment, provided an antibacterial effect but also decreased the material's compressive strength (Samiei et al. 2017). This implies that whether, the antibacterial effect is necessary, it must be weighed against the potential unfavorable effects with a regard to the biomechanical properties of the biomaterial. In the study of Abdulkareem et al. (2015), zinc oxide nanoparticles were mixed with nano-HaP and used as a dental implant coating, which proved to decrease human saliva- derived bacterial biofilm giving an opportunity to prevent implant associated infections (Abdulkareem et al. 2015).

10.2.1.4 Copper Nanoparticles

Although the antibacterial properties of other nanoparticles have been extensively studied, there are only a few reports on copper nanoparticles for their potential application in dentistry (Kruk et al. 2015). Aqueous solutions of copper nanoparticles were founded to exhibit sufficient antibacterial effects against *S. aureus, S. epidermidis* and *Candida* species in a range of 1,8–3.7 ppm (Kruk et al. 2015). Ramazanzadeh et al. (2015) evaluated antibacterial properties of orthodontic brackets covered either with copper nanoparticles, zinc oxide nanoparticles or both. The authors showed that materials covered with copper and a mixture of copper and zinc nanoparticles exhibited excellent antibacterial activity against *S. mutans.* This was not observed in the case of a control group (uncoated brackets) nor in the group covered only with zinc oxide nanoparticles (Ramazanzadeh et al. 2015). On the other hand, braces coated with zinc nanoparticles were found to decrease orthodontic friction forces, which could enhance teeth movement through the alveolar bone and shorten the treatment duration (Kachoei et al. 2013). Argueta-Figueroa et al. (2014) evaluated copper, nickel and bimetallic copper–nickel- nanoparticles against *S. aureus, E. coli* and *S. mutans* for their potential use in dental materials. They showed the concentration of 1000 ug/L of each nanoparticles was effective against all tested bacteria (Argueta-Figueroa et al. 2014). Preliminary studies confirmed copper nanoparticles as a potential antibacterial agent for dental purposes, but more data is required.

10.2.1.5 Gold Nanoparticles

Antibacterial properties of gold nanoparticles were not extensively evaluated with a regard to oral biomaterials so far. However, change in the membrane potential and ATPase activity prevention leading to cellular metabolism decrease, as well as, ribosome for transfer ribonucleic acid (tRNA) subunit binding inhibition were proposed as most probable mechanisms (Vimbela et al. 2017).

In the study of Hernández-Sierra et al. (2008), Au-NPs exhibited antibacterial activity against *S. mutans* but this effect was significantly decreased when compared to silver nanoparticles or zinc oxide nanoparticles (Hernández-Sierra et al. 2008). Their study confirmed, that gold nanoparticles exhibit a weak bactericidal effect which was stated in other researches. They may, however exhibit a beneficial impact on bone healing and therefore, were recently proposed as an additive to dental implants (Heo et al. 2016; Jadhav et al. 2018). Despite the lack of direct pathogen killing abilities, gold nanoparticles were shown to significantly decrease both, fungal and bacterial biofilm formation trough alteration of adhesion of the cells to the substrate, and stimulated immune response in the host's dental pulp cells in the study of Yu et al. (2016) (Hernández-Sierra et al. 2008).

10.2.1.6 Silica Nanoparticles

Silica nanoparticles are mostly used as dental materials fillers. Their antibacterial effect against oral bacteria was described as limited or absent in some studies available (Song and Ge 2019). However, despite being less effective than nanosilver or titanium dixide nanoparticles in bacteria eradication, silica NPs may require further investigation (Besinis et al. 2014). Cousins et al. (2007) showed that silica nanoparticles were found to inhibit bacteria adherence to oral biofilms. In another study, silica nanoparticles were functionalized with amphotericin B and incorporated into dental reins. Such biomaterials exhibited excellent antifungal properties against *C. albicans, C. glabrata, C. krusei* and *C. parapsilosis* without a toxic effect to mammalian cells. The antimicrobial activity of resins was more effective than resins with nanosilver (Lino et al. 2013). Author concluded, that such material may be used for development of prosthetic dentures, which are commonly settled by oral fungi. Similar observations were described in the further study performed by Hetrick et al. (2009) where Si-NPs immobilized with nitric oxide provided anti-biofilm activity against *P. aeruginosa*, *E. coli, S. aureus* and *S. epidermidis* as well as, *C. albicans.* This effect was achieved by the local release of nitric oxide into the medium (Hetrick et al. 2009). In the study of Botequim et al. (2012), silica nanoparticles were coated with a quaternary ammonium cationic surfactant, didodecyldimethylammonium bromide (DDAB) which exhibited antimicrobial properties against *S. aureus, E. coli* and *C. albicans.* This effect on the other hand, was obtained

not through active release of silica nor DDAB, but was rather described as antiadhesive coating which provided a long lasting repulsive effect (Botequim et al. 2012). Lu et al. (2017) described silica NPs decorated with CHX-n-silver complexes which exhibited a synergistic antibacterial effect against *S. aureus* and *E. coli,* and a higher biocompatibility than chlorhexidine or silver ions (Lu et al. 2017). In another study, Zhang et al. (2014) confirmed than mesphorus nanosilica could be sufficiently bound with chlorhexidine for manufacturing antibacterial dental composites. In conclusion, combing silica nanoparticles with silver and/or chlorheksidine is considered a promising approach for dental restorative materials, but before their clinical application, they should be evaluated in order to determine pH values, oral bacteria, and the volume and compositions of fluids which may differ between individuals (Zhang et al. 2014; Mehdawi et al. 2013).

10.2.1.7 Bismuth Nanoparticles

Bismuth has been recognized as an antimicrobial agent, but its application as nanoparticles as a potential antimicrobial agent in dentistry was evaluated only recently. Hernandez-Delgadillo et al. (2017) described MTA supplemented with bismuth lipophilic nanoparticles (BisBAL- NPs) which inhibited the growth of *Enterococcus faecalis, Escherichia coli,* and *Candida albicans* without compromising the material's physico-chemical properties (Hernandez-Delgadillo et al. 2017). Vega-Jiménez et al. (2017) evaluated 4–22 nm, polygonal Bi-NPs against periodontopathogenic *A. actinomycetemcomitans, C. gingivalis*, and *P. gingivalis* with satisfying results which justified the potential application of bismuth NPs as an antibacterial additive to dental materials and antiseptics (Vega-Jiménez et al. 2017).

10.2.1.8 Zeolite Nanoparticles

Zeolite is a crystalline hydrated aluminosilicate of alkaline metals such as sodium, calcium, potassium and magnesium (Shameli et al. 2011). Nano- Zeolite (NZ) has been recently successfully introduced into endodontic materials, due to its beneficial effect on the material's adhesion and penetration into dentin tubules, higher hardness, as well as, resistance to acidic environment. Zeolite nano- compounds with nanosilver were evaluated as a potential additive to acrylic denture base materials, endodontic sealers and other factors with proved antibacterial effect against *S. aureus, S. mutans, L. casei, C. albicans* (Shameli et al. 2011; Odabaş et al. 2011; Saengmee-Anupharb et al. 2013). However, it was shown that the addition of zeolite nanoparticles with more complex compounds such as silver nanoparticles with zinc oxide nanoparticles, may decrease the material's compressive strength (Samiei et al. 2017).

10.2.1.9 Magnesium Nanoparticles

Magnesium nanoparticles were proven to inhibit growth of bacteria such as *S. aureus, E. Coli, Salmonella Stanley*, and were proposed as an antibacterial agent to enhance food safety (Dizaj et al. 2014). There is insufficient data on the application of these -nanoparticles for dental purposes. In one study of Monzavi et al. (2015) who proved that 5 mg/L magnesium oxide nanoparticles aqueous solutions were more effective in root canal disinfection than sodium hypochlorite (NaOCl) (5.25%). Authors suggested that magnesium nanoparticles could be used as a root canal irrigants (Monzavi et al. 2015).

10.2.1.10 Quaternary Ammonium Nanoparticles

Quaternary ammonium compounds (QAC) are used as ingredients or oral mouthwashes due to their proven bactericidal properties. However, their nano- forms were investigated with a regard to root canal fillers additives. Kesler Shvero et al. (2013) showed that the addition of quaternary ammonium nanoparticles at 2% wt. to commonly used endodontic fillers provided a prolonged antibacterial effect (up to 4 weeks) against *E. Feacalis* (Kesler Shvero et al. 2013).

10.2.1.11 Nano-Graphene

Graphene is an allotrope of carbon, and its nano-forms were recently extensively evaluated for the purposes of industry and medicine due to their biocompatibility, mechanical properties and antibacterial properties. The potential application of nano- graphene oxide for dental purposes was evaluated by He et al. (2015) who showed that this material exhibited bactericidal properties against oral bacteria (He et al. 2015). Then, Bregnocchi et al. (2017) studied graphene oxide nanoplatelets added as filler to a dental adhesive and showed that the materials exhibited excellent antibiofilm properties on human teeth study model. Moreover, the addition of graphene did not hampered material's adhesive properties (Bregnocchi et al. 2017).

Lee et al. (2018) described dental polymers) incorporated with 2% wt. of graphene-oxide nanosheets. The obtained material exhibited a sustained antibacterial- antiadhesive effect for up to 28 days (Lee et al. 2018). In another study, Akbari et al. (2017) showed that nano-graphene can be sufficiently used with indocyanine green in root canal disinfections by photodynamic therapy (Akbari et al. 2017). The promising preliminary data with nano-forms of graphene indicates there is a possibility of obtaining antibacterial materials for prosthodontics, conservative dentistry and orthodontics without the addition of soluble drugs (Lee et al. 2018). However, the high costs of graphene and its manufacturing are still and obstacles for the large-scale application in dentistry.

10.2.1.12 Nanoemulsions and Nanosuspensions

Nanoemulsions were investigated for prevention of the contamination of dental unit waterlines, but recent studies indicated their potential application in therapeutics to the oral cavity (Karthikeyan et al. 2011; Ramalingam et al. 2013). Nanoemulsions are a class of disinfectants made by mixing a water immiscible oil phase into an aqueous phase under high shear forces (Karthikeyan et al. 2011). Nanoemulsions are found as a potential agent that can inhibit biofilm formation on teeth and hence, decrease the percentage of caries, periodontal diseases and candidiasis. Karthikeyan et al. (2011) described 72–99% *S. mutans* biofilm reduction from *the glass surface after coating it with nanoemulsions* (Karthikeyan et al. 2011). Soybean oil with water nanoemultion was found to inhibit cariogenic *S. mutans* and *L. casei* in the study of Lee et al. (2010). Kassem et al. (2016) and Fernández Campos et al. (2012) proved antifungal activity self-nanoemulsifying drug delivery systems with nystatin in the treatment of patients suffering from fungal- associated inflammation of oral mucosa (Kassem et al. 2016; Fernández Campos et al. 2012).

10.2.1.13 Nanochitosan

Chitosan [(1, 4)-2-amino-2-deoxy-D-glucan] is a polyaminosaccharide obtained by N-deacetylation of chitin (Ghadi et al. 2014). It was proved as a non-toxic material and some studies mentioned its antibacterial and anti-plaque properties against oral bacteria depending on molecular weights and degrees of deacetylation (Chávez de Paz et al. 2011; Sarasam et al. 2008). It was proposed, that low weight chitosan works through cellular level impairment of the bacterial cells due to its penetrating abilities, whereas, high- weight counterparts work as barrier for nutrients for bacteria (Sarasam et al. 2008; Husain et al. 2017).

Chitosan and its nano-formulations are likely to be used as local drug delivery carriers for bone and periodontal regeneration (D'Almeida et al. 2017; Husain et al. 2017). Antibiotics such as metronidazole, chlorhexidine or nystatin can be encapsulated in chitosan NPs and delivered directly to the inflamed gingiva or alveolar bone. In the study of Samprasit et al. (2015) mucoadhesive electro spun nanofiber mats made of chitosan nanofibers and thiolated chitosan were evaluated as antibacterial materials for dental carries prevention (Samprasit et al. 2015). In the work of D'almeida et al. (2017) and Norowski et al. (2011), nano-chitosan coatings on titanium implants were studied as a potential antibacterial additive to the surface in order to decrease the risk of peri- implant infections (D'Almeida et al. 2017; Norowski et al. 2011). Chitosan and its compounds are gaining particular interest in dentistry due to its fluoride release capacity and antibacterial, as well as, antifungal properties when used as carrier or local drug delivery system (Senthil Kumar et al. 2017; Ikono et al. 2019).

10.2.1.14 Other Experimental Nanomaterials for Dental Purposes

Leung et al. (2016) described novel Nano-Mix (Nano-CHX + Nano- *Scutellaria baicalensis*) on mono- and multispecies bacterial biofilms: *S. mutans*, *S. sobrinus*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans.* MIC concentrations were 50μg/mL and 12.5μg/mL for mono- and multispecies models respectively. The authors concluded that such nano-solutions could be effectively used as oral disinfectants and periodontal care (Leung et al. 2016). In another study, Wassel and Khattab (2017) proposed combining chitosan nanoparticles with natural products such as miswak and propolis with fluoride in order to provide antibacterial dental varnishes. Their materials exhibited antibacterial activity in a disc diffusion test against *S. mutans* and decreased teeth colonization and enamel demineralization (Wassel and Khattab 2017). In the study of Shvero et al. (2010) and Beyth et al. (2008) polyethyleneimine nanoparticles were added to dental cement (Shvero et al. 2010) and a resin composite (Beyth et al. 2008) which exhibited inhibiting properties against *S. mutans*, *E. faecalis, Pseudomonas aeruginosa* and *Escherichia Coli* just at 1–2% wt of nano- polyethyleneimine (Shvero et al. 2010; Beyth et al. 2008).

10.3 Conclusion

The main reason of why nanoparticles were considered as a reliable alternative to conventional antibiotics and chemotherapeutics was nanoparticles ability to kill a wide range of microbes and prevent from drug resistance, which is considered a serious threat to global public health (Wang et al. 2017; Qiu et al. 2012). Their high surface-to-volume ratio, as well as, chemical and physical properties provided several ways of interaction with bacterial cell structure, making it difficult to develop resistance mechanism. However, Espinosa-Cristóbal et al. (2012) showed that susceptibility and resistance to nanoparticles may be related to the specified serotype of bacterial species (Espinosa-Cristóbal et al. 2012).

Several nanoparticles, most of them based on silver nanoparticles were already introduced into the market with an enthusiasm, but extensive research and over-implementation of different nanoparticles or their mixtures into industry, may in time change the state of the art in this regard. As nanoparticles may interact with bacterial cells at several levels, they may overcome at least one of the common bacterial resistance mechanisms: plasmids, transposons, and integrons (Wang et al. 2017). While indeed, bacteria were most commonly unlike to develop resistance against NPs, the study published, by Qiu et al. (2012) revealed that nano – alumina promoted the horizontal transfer of antibiotic-resistance genes between bacteria (Qiu et al. 2012). In another study, Panáček et al. (2018) proved that *E. coli* bacteria may develop resistance to silver nanoparticles after repeated exposure, and this process is related to the production of the flagellin, aggregating nanoparticles and decreasing their antibacterial potential. They also described, that such a process cannot be overcome by the application of surfactants and other stabilizers that prevent from particle aggregation in the colloidal solution (Panáček et al. 2018). In the

earlier study, Graves et al. (2015) also described resistance development to 10 nm silver nanoparticles in *E. coli* (Graves et al. 2015) and stated that the production of sustainable nanomaterials in the industry is of great interest in order to decrease the possibility of potential bacterial resistance to such materials. One approach that contributes to this, is avoiding application of single type of nanoparticles as these require simple genomic changes to induce resistance development (Graves et al. 2017). It means, that in the near future, a combination of nano- based substances will be of great interest in medical and dental biomaterial engineering.

Despite proven, short term antibacterial activity, several obstacles remain unsolved, for nanoparticles incorporated, as well as, all drug- releasing materials. The first is related to the incorporation time of the nanoparticles into bulk material. If nanoparticles are incorporated before the material's crosslinking (hydrogels, composites) and sintering (metallic biomaterials). they are entrapped within the bulk structure of the material, and such state may decrease their bacterial activity. On the other hand, when nanoparticles are simply embedded onto the outer surface of the material, they may be rapidly washed- out by the biologic fluid flow, especially within the first minutes- days after material exposure. This phenomenon is known as "burst release" and contributes to fast dissolution of the drug/substance from the biomaterial's surface. Also, a decrease of the drug concentration at the implant-tissue interface which may induce drug resistance when it is at sub-inhibitory level or below (Pokrowiecki et al. 2018). Last but not least, the methodology of nanoparticles incorporation must be well evaluated, as discrete factors may affect surface structure. Pokrowiecki et al. (2017) showed the longer the silver is being incorporated on the titanium surface, the larger clusters of silver nanoparticles may form on the surface and in the end, form micro- particulate silver compounds of distinct physicochemical properties (Pokrowiecki et al. 2017) [Fig. 10.4].

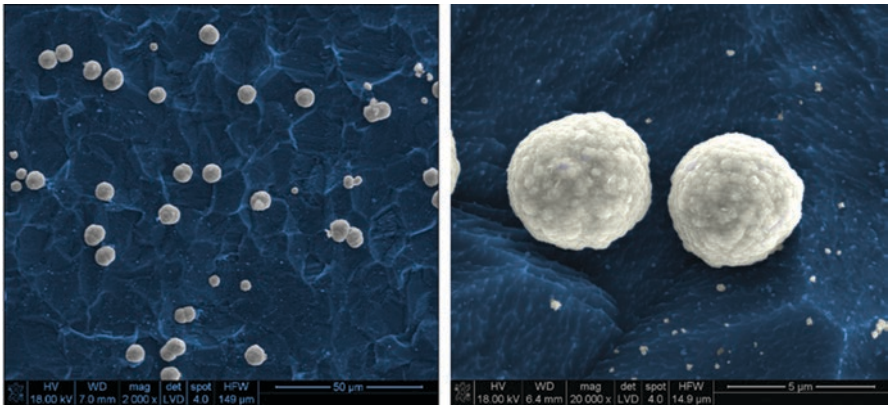


Fig. 10.4 Scanning electron microscopy at x2000 (**a**) and 20,000 (**b**) magnifications of titanium plate modified with nanosilver via chemical method (For the courtesy of Barbara Szaraniec, D. Eng, Faculty of Material Science and Ceramics, AGH University of Science and Technology, Cracow, Poland.). It can be seen that silver particles nucleated into large round micro-sized structures. Digital colouring for the courtesy of Krzysztof Palka, Lublin University of Technology, Faculty of Mechanical Engineering)

The application of nanomaterials in dental bio-engineering may significantly increase the concentration of free nanoparticles in saliva (Metin-Gürsoy et al. 2017) due to dissolution, share forces or the material's wear. Moreover, in some cases, metal nanoparticles may promote tissue toxicity in humans which remains an ongoing concern (Elbourne et al. 2017; Pokrowiecki et al. 2018). Nanostructured materials with antibacterial properties, also for dental purposes are of great desire. However, still little is known about the general response and prolonged effect of such biomaterials on human vital organs and oral microbiome.

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Chapter 11
Nanomaterials Causing Cellular Toxicity and Genotoxicity

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Abstract Nanotechnology has become one of the fastest developing fields of science and engineering in the World. Nanomaterials that play a crucial role in nanotechnology are increasingly used in a broad range of areas including automotive, biomedical, cosmetics, defense, energy, and electronics. Nanomaterials are used in a wide variety of products due to their unique chemical, biological and physical properties. The increase in the production and use of nanomaterials could lead in further exposure to humans, animals, and the environment. Therefore, understanding the toxicity of nanomaterials and their potential risks is urgently needed. Nanomaterial toxicity has been evaluated in various studies, but the adverse poisoning effects on target organs are still very limited. This chapter presents an overview of the applications of nanomaterials, including both metal-based and non-metal-based. Furthermore, it provides an overview of the mechanisms of cell toxicity and genotoxicity. Finally, the potential cell toxicity and genotoxicity associated with different types of nanomaterials are presented in detail.

Keywords Nanotechnology · Nanomaterials · Nanotoxicology · Cellular toxicity · Genotoxicity

11.1 Introduction

Nanotechnology has become one of the fastest developing fields of science and engineering in the World. Nanomaterials that play a crucial role in nanotechnology are increasingly used in a broad range of areas including automotive,

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biomedical, cosmetics, defense, energy, and electronics. The global nanotechnology market is expected to reach $90.5 billion by 2021 from $39.2 billion in 2016, with a compound annual growth rate of 18.2% from 2016 to 2021 (McWilliams 2016).

The nanomaterial is defined as a material with at least one external dimension that is less than 100 nanometers and include nanoparticles, nanofibers and nanotubes, nanocomposites, and nanostructured materials (Borm et al. 2006; Gonzalez et al. 2009; Kaphle et al. 2018). The unique chemical, biological and physical properties of nanomaterials including a high surface area to volume ratio, small size, and optical properties have led to their increasing use in many commercial products (Matthew Hull 2014; Sajid et al. 2015; Bahadar et al. 2016; Ray and Jana 2017; Wu and Tang 2018). For example, single- and multi-walled carbon nanotubes are extensively used in different applications, such as electronics devices, drug delivery, biotechnology, cosmetics, and aerospace engineering (Manke et al. 2013; Wong et al. 2013). Silver nanoparticles are used in consumer products including cosmetics, electronics, household appliances, textiles, and food products. They have also been used in medical applications like drug delivery, biosensing, and imaging (Fard et al. 2015). Titanium dioxide nanoparticles are frequently used in cosmetics, paints, pharmaceutical preparations and food additives (Weir et al. 2012; Yin et al. 2013). Zinc oxide nanoparticles are being used in paints, personal care products, cosmetics, wave filters, ultraviolet detectors, and food products (Huang et al. 2006, 2010; Senapati and Kumar 2018). The production and use of nanomaterials in various fields have grown exponentially in the last few decades, and this is expected to raise up to 58,000 tons by 2020 (Ganguly et al. 2018). The increase in the production and use of nanomaterials could lead to their enhanced exposure to humans, animals, and the environment. Therefore, understanding the toxicity of nanomaterials and their potential risks to humans, animals, and the environment is urgently needed. Several research studies on the toxicity of nanomaterials have carried out (Buzea et al. 2007; Park and Park 2009; Liu et al. 2013; Manke et al. 2013; Fu et al. 2014; Gatoo et al. 2014; Fard et al. 2015; He et al. 2015; Sahu and Hayes 2017; Jeevanandam et al. 2018; Mulenos et al. 2020). These researchers have reported that the overproduction of reactive oxygen species, which cause oxidative stress, inflammation, and deoxyribonucleic acid damage, is the most important mechanism of toxicity. The level of resolved oxidative stress generation induced by nanomaterials is dependent on physical and chemical properties of the nanomaterials (Fu et al. 2014). Physicochemical properties, such as size and shape, particle surface, chemical composition, aggregation/agglomeration, and morphology can influence the toxicity mechanisms of nanomaterials (Shvedova et al. 2012; Gatoo et al. 2014; Gliga et al. 2014; Magdolenova et al. 2014; Jeevanandam et al. 2018; Su et al. 2020). Silver nanoparticles, for example, were observed to induce size-dependent cytotoxicity in human lung cells due to the rate of intracellular silver release (Gliga et al. 2014). Nanorod-shaped zinc

oxide nanoparticles were proven to be more toxic to human lung epithelial cells than the corresponding spherical zinc oxide nanoparticles (Hsiao and Huang 2011). Ahamed et al. (2008) indicated that the coated and uncoated silver nanoparticles were distributed in the mammalien cells differently and caused different levels of deoxyribonucleic acid damage in mouse embryonic stem cells and mouse embryonic fibroblasts. It has been observed by Wick et al. (2007) that rope-like agglomerated carbon nanotubes induced more pronounced cytotoxic effects than well-dispersed carbon nanotubes.

Nano-sized materials can enter the human body through inhalation, ingestion, skin contact, and injection (Pujalté et al. 2011; Fu et al. 2014; Wu and Tang 2018), depending on the nanomaterials and the specific application (WHO 2013). These materials are able to cross many biological barriers and access the various organs such as lungs, kidney, heart, liver, and brain, thus causing deoxyribonucleic acid mutations, mitochondrial damage, and cell death (Tan et al. 2018). Guo et al. (2012) have shown that exposure of human lung alveolar epithelial cells to multi-walled carbon nanotubes induce inflammatory reactions, including inducible nitric oxide synthase expression and reactive nitrogen species generation, which result in DNA damage and contribute to carcinogenesis. Cui et al. (2005) investigated the effect of single-walled carbon nanotubes on human embryo kidney cells and the potential biochemistry mechanism. They observed that single-walled carbon nanotubes could inhibit human embryo kidney cells growth by inducing cell apoptosis and decreasing cellular adhesive ability. Ahamed et al. (2010a) reported that exposure to silver nanoparticles induces toxicity in a various organs, including the liver, lung, brain, reproductive organs, and vascular system. Another study reported that silver nanoparticles were found to lead to an increase in reactive oxygen species associated with deoxyribonucleic acid damage, apoptosis, and necrosis in human lung cancer cells (Foldbjerg et al. 2011). Titanium dioxide and zinc oxide nanoparticles are generally used in sunscreen products to minimize the unwanted skin whitening effect and to protect the skin by absorbing ultraviolet radiation, but they have been reported to cause toxic effects, including genotoxicity, inflammation, and oxidative stress (Trouiller et al. 2009; Stark et al. 2015). Ahamed et al. (2010b) demonstrated that copper nanoparticles have the potential to induce deoxyribonucleic acid damage in human lung epithelial cells, which could cause lipid peroxidation and oxidative stress. Numerous studies have been done to understand the potential toxicity and possible health effects of nanomaterials exposure. There is still a significant knowledge gap exists regarding the toxicity effects of nanomaterials exposures. This chapter presents an overview of applications of metal and non-metal-based nanomaterials. Moreover, it provides the most recent research studies associated with the possible toxic effects of different types of nanomaterials on humans and the environment.

11.2 Applications of Nanomaterials

Nanomaterials can be classified based on their chemical composition into four groups: carbon-based, metal-based, inorganic-based, and composite-based. Carbon-based nanomaterials consist of single-walled carbon nanotubes, multi-walled carbon nanotubes, fullerenes, graphene, carbon nanofibers, and carbon black (Bahadar et al. 2016). These nanomaterials have unique physicochemical properties such as lightweight, high tensile strength, chemical stability, and thermal conductivity (Lam et al. 2008; Lewinski et al. 2008). Carbon-based materials are mainly used in biomedical applications, batteries, fuel cell electrodes, supercapacitors, adhesives, aircraft, composites, sensors, and consumer electronics, aerospace, and automotive (Lam et al. 2008; Lewinski et al. 2008; Bahadar et al. 2016). Metals (titanium dioxide, zinc oxide, aluminum oxide, iron oxide, etc.) and metal oxides (gold, silver, aluminum, and iron) are categorized as metal-based materials (Srivastava et al. 2015). They have high reactivity and varied properties based on type, and some have photolytic properties and UV-blocking ability (EPA, U 2017). Among all nanomaterials, metal containing nanomaterials are the most common nanomaterials (Aitken et al. 2006; Fu et al. 2014), and are widely used in several applications such as cosmetics, coatings, paints, solar cells, packaging, and environmental remediation (EPA, U 2017). Organic-based nanoparticles are another group of nanomaterials that made from branched units capable of being tailored to perform specific chemical functions (EPA, U 2005). Organic-based nanomaterials such as polymeric micelles, liposomes, dendrimers, etc. are mainly used in therapeutic and imaging agent carrier and drug delivery (Farré and Barceló 2012; Sandeep 2013). Composite-based nanomaterials are a combination of carbon-carbon nanomaterials, carbon-inorganic nanomaterials, and inorganic-inorganic nanomaterials (Jeevanandam et al. 2018) and they can provide remarkable properties, including thermal, magnetic, catalytic, electrical, mechanical, and imaging features. They have applications in cancer detection and drug delivery, as well as in packaging and auto-parts materials. The physicochemical properties and applications of different nanomaterials are summarized in Table 11.1.

11.3 Mechanisms of Toxicity

11.3.1 Cell Toxicity

Toxicity that causes a destructive action on living healthy cells is known as cytotoxicity. Any material or process results in cell injury and cell death, which may contain snake venom and animal lymphocytes, is considered cytotoxic (Srikanth 2012).

Two types of cell death have been identified: necrosis and apoptosis. Necrosis can be defined as the death of cells as a result of infection, inflammation, chemicals,

Table 11.1 Applications of different nanomaterials

Nanomaterials		Physicochemical properties	Applications	References
Carbon-Based	SWCNTs	• High thermal and electrical conductivity	Biomedical applications, drug delivery, sensors and instruments, membranes and filters	Guo et al. (2012), Du et al. (2013), and Ma-Hock et al. (2013)
	MWCNTs	• High tensile strength and electrical conductivity	Catalysts, energy storage, solar cells, batteries, transistors	Guo et al. (2012) and Du et al. (2013)
	C60	• Anti-oxidants capacity • Radical scavenging	Drug delivery, energy applications, polymer electronics, cosmetics	Aschberger et al. (2010)
	Gr	• High surface area • High thermal and electrical conductivity • Impermeable to gas • High strength and elasticity • Easily functionalized • Chemically inert	Chemical and biosensors, energy storage, supercapacitors, batteries, transistors, components of high-strength machinery, coatings, display screens, biomedical applications	Ma-Hock et al. (2013) and Gurunathan and Kim (2016)
	Carbon Black	• Thermal and electrical conductivity • Resistance to UV radiation and antioxidation effect • Reinforcement effect	Reinforcing filler in rubber products, pigment in paints and inks, electric conductive components, coatings	Sahu et al. (2014) and Stark et al. (2015)
Metal-Based	Au	• Easily functionalized	Targeted drug delivery	Bahadar et al. (2016)
	Ag	• Superior anti-microbial activities	Medical products, including catheters, implants and other materials to prevent infection, cosmetics, textiles, water purification systems, electronics, household appliances, textiles, food products	Park et al. (2011), Lee et al. (2014), Fard et al. (2015), and Tan et al. (2018)

(continued)

Table 11.1 (continued)

Nanomaterials		Physicochemical properties	Applications	References
	Al_2O_3	• High mechanical strength • High hardness • High wear resistance • Very good electrical insulation • Chemical inertness with good biocompatibility	Coatings, paints, biomaterials, fuel cells, polymers, grinding media, polishing abrasives, textiles, batteries, adsorbent, catalysis	Srivastava et al. (2015) and Bahadar et al. (2016)
	CuO	• Super thermal conductivity • Photovoltaic properties • High stability • Antimicrobial activity	Semiconductors, antimicrobial reagents, heat transfer fluids, intrauterine contraceptive devices	Aruoja et al. (2009) and Tran and Nguyen (2014)
	Fe_2O_3	• Extraordinary magnetism	Magnetic resonance imaging, drug delivery, sensors, catalysis, separation of biomolecules, magnetic storage media, remediation of environment, cellular labeling	Srivastava et al. (2015) and Tan et al. (2018)
	TiO_2	• Stronger catalytic activity • Brightness • Very high refractive index • High stability • Anticorrosive • Photocatalytic	Food industries, medicines, waste water treatment, papers, paints, drug delivery agents, coatings, plastics, inks, pharmaceuticals, cosmetics	Weir et al. (2012), Shi et al. (2013), Fu et al. (2014), Fard et al. (2015), and Srivastava et al. (2015)
	ZnO	• Electronic • Optoelectronic	Industrial and commercial applications including paints, wave filters, pigments, coating, ceramic products, UV detectors, sensors, personal care products	(Huang et al. 2006; Huang et al. 2010; Fu et al. 2014)

(continued)

Table 11.1 (continued)

Nanomaterials		Physicochemical properties	Applications	References
Organic-Based	Dendrimers	• Hyperbranched, monodispersed, and star-shaped structures	Drug delivery, biomedical field, energy harvesting, biosensors/ diagnostics, gene delivery, catalysis	Sandeep (2013) and Azmi and Shad (2017)
	Polymers	• Hyperbranched, monodispersed, and star-shaped structures	Drug delivery, diagnostic imaging, sensors, modified electrodes, gene delivery,	Sahu and Hayes (2017)
Composites-Based	Quantum Dots	• Unique electrical and optical properties including high fluorescent quantum yield, broad absorption, narrow emission, high photostability	Cancer therapy and diagnostics, drug delivery, medical imaging, solar cells, lasers, quantum computing	Monteiro-Riviere and Zhang (2009), Fard et al. (2015), and Tan et al. (2018)
	Silica	• Large surface area, and pore volume • Size tunability • Ease of surface modification • Biodegradability	Additive in food and plastics, bioremediation, catalysis, energy storage, medical imaging, biosensors, fuel cells, drug delivery, carriers of enzymes, drugs, and DNA	Kumar et al. (2004) and Slowing et al. (2008)

C60 fullerene, *Gr* graphene, *MWCNT* multi-walled carbon nanotube, *SWCNT* single-walled carbon nanotube, *Ag* silver, Al_2O_3 aluminum oxide, *Au* gold, *CuO* copper oxide, Fe_2O_3 iron oxide, TiO_2 titanium dioxide, *ZnO* zinc oxide

toxins, injury, or lack of blood supply (Boulton et al. 2012). Cells undergoing necrosis show typical morphologic features, including loss of membrane integrity, rapid swelling, shut down metabolism, and release their contents into the environment (Dube et al. 2015). Cells that experience rapid necrosis in vitro do not have adequate time or energy to actuate apoptotic machinery and do not communicate apoptotic markers. During the apoptosis process, a change in the refractive index of the cell takes place first followed by cytoplasmic shrinkage and nuclear condensation. The cell membrane begins to show blebs or spikes, which are protrusions of cell membranes, depending on the type of cell. these blebs or spikes separate from the dying cell and form apoptotic bodies that are phagocytosed by neighboring cells. These cells likewise stop to keep up phospholipid asymmetry in the cell membrane. The mitochondrial external layer additionally experiences changes that incorporate loss of its electrochemical inclination. At that point adjoining cells or macrophages phagocytose. The test and their affecting area are membrane integrity (LDHe), metabolic activity (GLU), respiratory chain activity (XTT/MTT), total protein synthesis, DNA content (CVDE), and lysosomal activity (PAC, NR).

11.3.2 Genotoxicity

Nanoparticle-induced genotoxicity can be defined as the destructive effect on the genetic material of the cell by particles. Genotoxicity can be classified as either "primary" or "secondary."Primary mechanisms for genotoxicity is defined as destruction from the nanoparticles themselves in the absence of inflammation (Schins and Knaapen 2007). If nanoparticles are able to enter the cell nucleus, or free in the cytoplasm, they might directly interact with deoxyribonucleic acid during mitosis (Emam et al. 2014). Direct genotoxicity is caused by a physical interactions of the particles with the genomic deoxyribonucleic acid because small nanoparticles may reach the nucleus via transportation through the nuclear pore complexes (Nabiev et al. 2007).

Secondary mechanisms for genotoxicity can be the result of oxidative deoxyribonucleic acid damage via excessive generation of reactive oxygen species by activated phagocytes, including neutrophils and macrophages, through a chronic in vivo inflammatory response (Barabadi et al. 2019). Secondary genotoxicity is thought to be threshold level. This level is determined by the exposure concentration that will trigger inflammation and overwhelm antioxidant and deoxyribonucleic acid damage repair capacities in the lung (Schins and Knaapen 2007). Since the ability of particles to elicit inflammation has been shown to depend on various properties (particle solubility and surface reactivity) the threshold is expected to vary between different nanoparticles. The comet assay, also known as single cell gel electrophoresis assay, is a sensitive and rapid technique for measuring deoxyribonucleic acid damage in individual cells.

The mechanisms for nanomaterial-induced genotoxicity are shown in Fig. 11.1.

11.4 Nanomaterials and Their Toxicity

Nanotechnology has gained momentum over the last decades due to its wide applications in the fields of medicine, electronics, agriculture, business, environmental science, food, fuel cells, and chemical sensors. Exposure to nanomaterials is unavoidable because of their prevalent use in various industries and consumer products, and as a result, nanotoxicity becomes an intense growing research area. This review provides a summary of the recent research studies associated with the fate, behavior, and toxicity of a variety of nanomaterials in the environment. Since the material size is so small, its physicochemical properties can be different from their bulk forms.

Nanotechnology empowers us to make useful materials, devices, and frameworks by controlling matters at the atomic and sub-atomic scales, and to develop unique properties and phenomena. Nanoscale materials provide superior properties, which in turn create lightweight, high-strength, chemically reactive, and conductive products. It is possible to build molecular-size and microscopic devices using

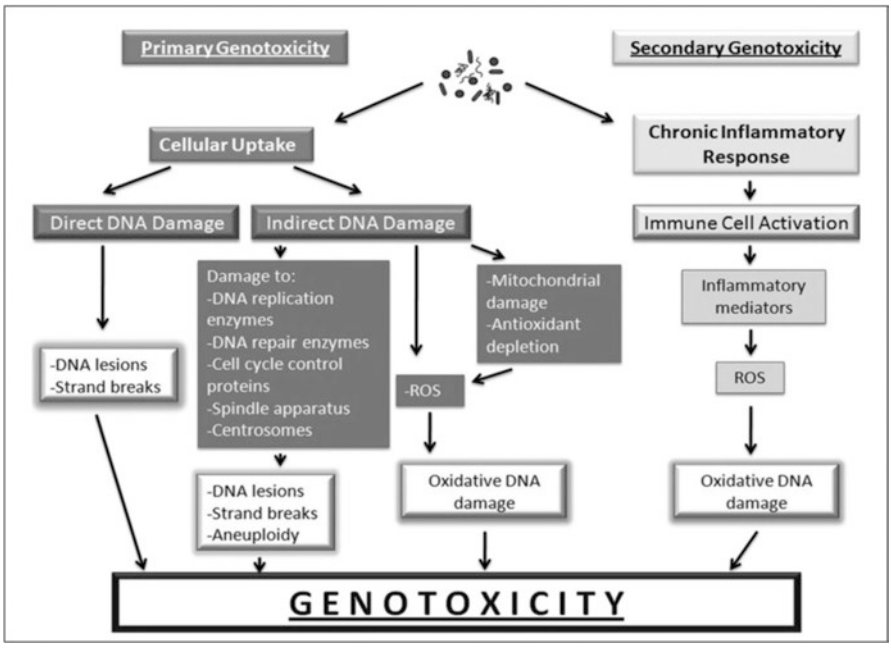

Fig. 11.1 Mechanisms for nanomaterial-induced genotoxicity. (Doak et al. 2017)

nanomaterials, which could serve in energy, space, defense, medical, and environmental applications, specifically sensors with higher sensitivity, transistors with the required resolution, and high-speed and lightweight computers. Nanoscience and nanotechnology are one of the quickest developing research and innovation fields. The President's 2019 budget provides almost $1.4 billion to the National Nanotechnology Initiative (NNI), a proceeded with an interest in essential research, thereby beginning a period of applied research and innovation efforts that will prompt the leap forward without boundaries.

In total, nearly $27 billion in funding has been budgeted from the beginning of the NNI in 2001 (counting the 2019 demand), which reflects the continued significance of ventures that propel a fundamental knowledge of and capacity to control matter at the nanoscale, and the interpretation of that information into technological achievements that serve American individuals. The NNI interests in 2017 and 2018, and those proposed for 2019, show a continued emphasis on broad, essential research in nanoscience to give a proceeding with a baseline of new disclosures that will empower future transformative business products and services (nano.gov 2018). Federal organizations that have the most significant investments for nanotechnology include the following:

- National Institutes of Health (NIH), nanotechnology involved in biomedical research.

- National Science Foundation (NSF), research and teaching covering all science and engineering fields.
- Department of Energy (DOE), research regarding new energy technologies.
- Department of Defense (DOD), research advancing defense systems
- National Institute of Standards and Technology (NIST), innovative work of estimation and creation devices, systematic techniques, metrology, and principles for nanotechnology.

There are many proposed and ongoing projects regarding nanotechnology and its products, which have been extensively used in various industries. Thus, research laboratories, producers, and manufacturers involve many people (e.g., students, faculty, workers, and engineers) who are exposed to engineered nanomaterials and its adverse effects. Nanoproduct consumers are exposed to nanomaterials toxicity as well. Much research is expected in order to comprehend the major effects of nanotechnology on health, and to decide proper introduction observing and control procedures.

11.4.1 Metal Nanoparticles

11.4.1.1 Gold Nanoparticle Toxicity

The bulk form of gold (Au) is considered to be chemically inert as well as biocompatible, while the nanoform of gold comes with unusual chemical and physical (mechanical, optical, electrical, and magnetic) properties (Thota and Crans 2018). The outcome about gold nanoparticle toxicity is as still contradictory, several demonstrating that these particles are biocompatible and have unimportant toxic levels, while others demonstrate that they are cytotoxic for the most part, due to their surface functionalization and structures. Based on the in vitro genotoxicity research, gold nanoparticles cause DNA damage in different cell lines. Threshold concentration and nanoparticle size play a significant role in gold nanoparticle toxicity. According to some researchers, if the gold nanoparticle size is 3–5 nm and the threshold concentration is fairly low, then it can be considered to be not cytotoxic, whereas smaller gold nanoparticles are cytotoxic because they create an irreversible binding with biomolecules and cell structures (Thota and Crans 2018).

Furthermore, short period exposure to gold nanoparticles may not be toxic to cell development; however, extended period of exposure might disturb the cellular metabolism and energy homeostasis. Cellular uptake can happen with gold nanoparticles also they can remain in endosomes/lysosomes or rely upon their surface functionalization into nuclei. Gold nanoparticle uptake depends on functionalization also type of the cell. If the gold nanoparticle is taken up via a healthy cell, then it will be killed inevitebly, while if it is taken up via a cancer cell, subsequently the outcome will be cell death.

11.4.1.2 Silver Nanoparticle Toxicity

Due to their antibacterial properties, silver nanoparticles have been employed in many applications, for instance wound dressings, paints, coatings of surgical instruments, and protheses (Chen and Schluesener 2008). They enter the body in many ways then store in the organs, passing the blood-brain barrier then finally accessing the brain. They were found to be transferred and found in many rat organs, including lung, kidney, liver, and brain, after their inhalation and also by skin injection (Tang et al. 2009). Evaluated by other nanosized metals, silver nanoparticles display greater toxicity concerning the production of resolved oxidative stress, lactate dehydrogenase (LDH), as well as cell viability. Each nanosilver coating comes with a different level of toxicity. Vazquez-Muñoz et al. (Vazquez-Muñoz et al. 2017) examined the toxic effect of polyvinyl-pyrrolidone-coated silver nanoparticles on bacteria, viruses, microalgae, fungi, and human and animal cells (including cancer cell lines) through a range of feasibility and toxicological assays. It was revealed that biological systems from different taxonomies were reduced through a concentration of silver nanoparticles at a same magnitude (Vazquez-Muñoz et al. 2017).

11.4.1.3 Cobalt Nanoparticle Toxicity

Chattopadhyay et al. (2015) investigated intracellular signaling transduction routes included in cobalt oxide (CoO) nanoparticles resolved oxidative stress in both *in vitro* as well as *in vivo* systems. As investigated for the in vivo system, cobalt oxide nanoparticles did not generate any extend of toxic effects on healthy cells. These nanoparticles release their excess Co^{++} ions, which may stimulate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and supports in resolved oxidative stress production. The outcome of the cobalt oxide nanoparticle study showed a significant ($p < 0.05$) level of resolved oxidative stress in lymphocytes. The in vivo and *in vitro* pretreatment along with N-acetylene cystine provided a protective function for lymphocyte death induced by cobalt oxide nanoparticles. Both *in vitro* and *in vivo results indicated a high level of tumor necrosis factor alpha (*TNF-α) after cobalt oxide nanoparticle treatment. This factor phosphorylated the p38 mitogen-initiated protein kinase taken after enactment of caspase 8 and caspase 3, which might possibly actuate cell death. This investigation demonstrated that cobalt oxide nanoparticles initiated oxidative stress/pressure along with started the signaling route of TNF-α-caspase-8-p38-caspase-3 to primary immune cells. This investigation also revealed that exposure to cobalt oxide nanoparticles creates a toxic effect on the human immune cell. Surface adjustment or else surface functionalization might open the door for additional utilization of cobalt oxide nanoparticles in various biomedical sciences as well as technologies (Chattopadhyay et al. 2015).

11.4.1.4 Aluminum Nanoparticle Toxicity

Aluminum oxide (Al_2O_3) nanoparticles have been employed in a wide variety of applications, such as polymers, nanocomposites, paintings, fuel cells, coatings, textiles, and biomaterials and devices. As reported by the Global Aluminum Oxide Market, aluminum-based nanoparticles donate to 20% of the entire nanoscale chemicals (Bahadar et al. 2016). In accordance with Chen et al. (2008), aluminum *nanoparticles display* adverse effects on cell viability including increased oxidative stress, altered mitochondrial function as well as tight junction protein articulation of the blood-brain barrier. Radziun et al. (2011) *also investigated different concentrations of* aluminum nanoparticle*s* (10, 50,100, 200, and 400 μg/mL) *and their toxic effects on the viability of mammalian cells; however, no toxic effect was* found. With respect to its broad range of usages in many fields and resulting human exposure, aluminum-based nanoparticles are incredibly appealing to study in order to determine other toxic impacts on individuals using standard procedures.

An expansion in the wide use aluminum nanoparticles in the food nourishment and farming divisions may create unpredictable danger as far as human wellbeing. Alshatwi et al. (2012) investigated the acute toxicity of aluminum nanoparticles in human mesenchymal stem cells (hMSCs) in vitro. The quantitative real-time polymerase chain reaction (qRT-PCR) was employed to assess cell viability, cellular uptake, morphology as well as gene expression. Test results show that the cytotoxicity effect of aluminum nanoparticles depends on the nanoparticle dose. Control cells demonstrated a trademark, homogeneous nuclear staining pattern, although aluminum nanoparticle-exposed cells indicated strange atomic morphological changes, including fragmentation and condensation.

The first attribute for apoptosis was seen in aluminum nanoparticle-treated cells. Advance affirmation of cell death in hMSCs was seen via the expanded articulation of picked signaling genes, and furthermore diminished articulation of Bcl-2 amid mitochondria-mediated cell death. Despite the fact that aluminum nanoparticles provide extraordinary points of interest in foodstuff and farming items, their constant and intense poisonous quality should still be evaluated methodically.

11.4.1.5 Titanium Nanoparticle Toxicity

Nanoscale particles, unlike micrometer-size particles are able to enter through the skin. Nanosize titanium dioxide particles exhibit greater photoreactivity than coarser ones, so nanoparticles may produce free radicals that can damage cells and their structures. Some manufacturers coat nanoparticles to prevent the formation of free radicals. Nanosize particles usually interact with the stratum corneum and deeper hair follicles, as well as some mother organs; however, micron-size particles cannot reach the epidermis and dermis layers.

According to the Department of Environmental Health and Safety at Massachusetts Institute of Technology, titanium dioxide nanoparticles between 14 and 40 nm in size generate lung cancer in rats in doses of mg/m^3, while

micron-sized particles generate cancer in higher doses, such as 250 mg/m^3. Thus, the National Institute of Occupational Safety and Health (NIOSH) defined an exposure level for titanium dioxide as 1.5 mg/m^3 for micron-size particles and 0.1 mg/m^3 for nanosize particles. Meanwhile, the International Agency for Research on Cancer (IARC) categorized titanium dioxide nanoparticles as 2B carcinogen materials, which may casue carcinogenic effects in humans. The titanium dioxide nanoparticles could move to the brain through olfactory neurons when inhaled. Once the transported particles reach the brain, they will trigger oxidative stress and neuronal degeneration in some areas like the hippocampus, which may casue short-term memory loss. Nanoscale titanium dioxide joins a few different nanomaterials (manganese oxide, nanocarbon, and some infections) that can enter the brain directly through the olfactory pathway from the nose (ehs.mit.edu 2016).

11.4.1.6 Platinum Nanoparticle Toxicity

Platinum (Pt) nanoparticles are biologically more inactive compared with gold and silver nanoparticles. Platinum has been used in anti-cancer drugs. It shows ferromagnetism, yet is very stable and relatively inactive. Pristine platinum nanoparticles about 5–10 nm in size can be internalized in cells and concentrated in phase-kind structures with a small effect on cellular processes. Platinum nanoparticles do not generate oxidative stress, release ions, or cause cell death. In a recent study, platinum nanoparticles facilitated DNA strand breakage during cell incubation, while internalized nanoparticles did not penetrate the nucleus. Considering the biological effects, platinum nanoparticles below 1 nm in size produced necrosis of tabular epithelial cells, kidney damage, and urinary casts. Also, there were no observed toxic effects in the spleen, heart, and lung. In vitro studies on renal cells exposed to 1-nm-size platinum nanoparticles exhibited substantial toxicity. Intraperitoneal injection of 1-nm-size platinum nanoparticles in mice twice a week for about 4 weeks developed in tubular atrophy, urinary casts, and inflammatory cell accumulation in the kidneys, while 8-nm-sized nanoparticles did not create toxic effects. Bimetallic nanoparticles made out of gold and platinum stabilized by citrate and pectin (CP-Au/Pt) about 4.7 nm in size were observed to have solid cancer prevention agent properties, quenching hydrogen peroxide and superoxide anion radicals. Platinum nanoparticles along with polyacrylate (PAA-Pt) were examined as a remedy for pulmonary inflammation in smoking mice. It was observed that platinum nanoparticles (estimated 2 ± 0.4-nm in size) exceed a function of cell reinforcement, repressing aspiratory aggravation actuated by cigarette smoking (Thota and Crans 2018).

11.4.1.7 Zinc Oxide Nanoparticle Toxicity

Ng et al. (2017) examined the toxicological outlines of zinc oxide (ZnO) nanoparticles in MRC5 human lung fibroblasts in in vitro along with in vivo models employing the fruit fly *Drosophila melanogaster*. A detailed research was performed to analyze the uptake, cytotoxicity, resolved oxidative stress formation, gene expression profiling, and genotoxicity induced by zinc oxide nanoparticles. Relative to in vitro toxicity, the outcomes demonstrated that there was a noteworthy discharge of extracellular lactate dehydrogenase and diminished cell viability in zinc oxide nanoparticle-treated MRC5 lung cells, indicating cell damage and cytotoxicity. Generation of resolved oxidative stress was seen to be found together with the critical articulation of DNA damage-inducible transcript (DDIT3) and endoplasmic reticulum (ER) to nucleus signaling 1 (ERN1) genes, which are stress-associated genes. Oxidative stress-induced DNA damage was characterized through the release of a DNA oxidation product, and 8-hydroxydeoxyguanosine (8-OHdG). Considering the in vivo study of the fruit fly *D. melanogaster*, considerable toxicity was found in F1 progenies rely on the intake of zinc oxide nanoparticles, which motivated remarkable diminishing in the egg-to-adult suitability of the flies. This research suggested that zinc oxide nanoparticles induce significant oxidative stress linked with cytotoxicity along with genotoxicity in human lung fibroblasts in vitro and in *D. melanogaster* in vivo. Further wide-ranging investigations would be required to identify safety matters relative to enhanced utilization of zinc oxide nanoparticles through users (Ng et al. 2017).

11.4.1.8 Iron Oxide Nanoparticle Toxicity

Drug delivery, biomedical, and diagnostic fields use the majority of iron oxide (Fe_2O_3) nanoparticles, which can bioaccumulate in the liver as well as reticuloendothelial system organs. In vivo examinations have demonstrated that iron oxide nanoparticles, once they enter the cells, remain in the cell organelles (endosomes/lysosomes) and then are released into the cytoplasm right after breaking down, thereby contributing to the cellular iron poll. Furthermore, magnetic iron oxide nanoparticles, after inhalation, store in the liver, spleen, lung as well as brain. Previous studies have confirmed that nanoparticles apply their toxic effect in the shape of cell lysis, inflammation, also disturbance of the blood coagulation system. Considering the in vitro studies, iron oxide nanoparticles show a toxic effect that is a form of reduced cell viability. Naqvi et al. (2010) analyzed the toxicity of surfactant Tween-coated super magnetic iron oxide nanoparticles on murine macrophage cells. In conclusion, at a low-level concentrations of iron oxide nanoparticles (25–200 μg/mL for a two-hour exposure) show higher cell toxicity than higher concentrations (300–500 μg/mL for a six-hour exposure). Nevertheless, after 7 days of incubation, dextran-coated iron oxide nanoparticles (100–150 nm, 0.1 mg/mL) decreased the cell viability in human macrophages around 20%. In addition, Jeng and Swanson (2006) conducted a research on the mouse neuroblastoma (Neuro-2A)

cell line, which indicated that iron oxide nanoparticles (25 nm) showed a less-toxic effect based on the shifting cell morphology, cell permeability, cell apoptosis, along with mitochondrial function. Chitosan-coated iron oxide nanoparticles (13.8 nm) at a concentration of 123.52 μg/mL exposure for 12 h to human hepatocellular carcinoma cells resulted in 10% cell viability. Conversely, 1-hydroxy-ethylidene-1,1-bisphosphonic acid-coated iron oxide nanoparticles (20 nm, 0.1 mg/mL) exhibited 70% cell viability after subject to rat mesenchymal stem cells for about 2 days. The MTS assay was utilized to identify cell viability (Delcroix et al. 2009; Naqvi et al. 2010; Bahadar et al. 2016).

11.4.2 Non-metal Nanoparticles

11.4.2.1 Silica Nanoparticle Toxicity

Silica and silicon dioxide (SiO_2) nanoparticles have been used for drug delivery systems. These nanoparticles comprise about 8% of all airborne nanoparticles. Earlier nano silica was defined as a vastly biocompatible material for use in drug delivery practices; however, based on the latest studies nano silica causes the occurrence of resolved oxidative stress and resulting oxidative stress. According to Lin et al. (2006), human bronchoalveolar carcinoma cells treated by silica nanoparticles (15–46 nm) at a dosage array of 10–100 μg/mL increased the level of resolved oxidative stress, lactate dehydrogenase, and malondialdehyde. In order to measure the resolved oxidative stress and lactate dehydrogenase level, the authors used 2′,7′-dichlorofluorescein diacetate and a commercial kit, respectively. In another study, silica nanoparticles caused the induction of inflammatory biomarkers (e.g., IL-1, IL-6, IL-8, TNF-α) as well as mitochondrial damage. Another invitro study studied the effect of silica-based nanoparticles on liver cells, and test results showed that silica nanoparticles modify biochemical parameters alongside hepatotoxic impacts (Lin et al. 2006; Bahadar et al. 2016).

11.4.2.2 Quantum Dots Toxicity

Quantum dots (QDs) are nanocrystals with distinctive optical properties that offer prolonged fluorescence, so they have been used in biomedical imaging applications. The optical fluorescent property of quantum dots can be conjugated by bioactive moieties to focus on specific biologic events and cellular forms, for instance labeling neoplastic cells, DNA as well as cell membrane receptors. The toxicity of quantum dots depends on various elements, primarily the outcome of individual quantum dot physicochemical properties combined with environmental settings such as size, concentration, charge, outer coating bioactivity. Additionally, oxidative, photolytic, and mechanical permanency appear to be key factors for quantum dot toxicity. In accordance with Zhang et al. (2008), skin penetration is one of the foremost ways of

exposure for quantum dots to enter a biological system. QD655 and QD565 covered with carboxylic acid were considered for 8 and 24 h in transfer via diffusion cells with flexed, tape-stripped and scuffed rat skin to decide whether these mechanical activities may affect penetration and disturb the barrier. The outcomes exhibited that QD655 and QD565 were restrained to the top stratum corneum layers of intact skin of the rat.

Another research study was conducted by Mortensen et al. (2008) on quantum dots skin penetration via an in vivo semiconductor quantum dots model system. Carboxylated quantum dots were connected to the skin of mice in a glycerol vehicle along with and with no *ultraviolet radiation (UVR)* exposure. The skin collection, as well as penetration forms, were assessed for 8 and 24 h after quantum dots treatment utilizing tissue histology, confocal microscopy, and transmission electron microscopy (TEM) with energy-dispersive X-ray spectroscopy (EDAX) investigation. Low levels of infiltration were observed in both the non-UVR and UVR-exposed mice. Subjectively larger amounts of penetration were obvious in the UVR-exposed mice.

Rouse et al. (2008) examined the effects of employed strain on the uptake of quantum dots through human epidermal keratinocytes (HEK). Quantum dots were connected with the concentration of 3 nM, and a 10% normal strain was linked to the cells. After 4 h of cyclic strain, the cells were analyzed for cell viability, quantum dot take-up, and cytokine generation. Outcomes demonstrated that adding the strain caused high-cytokine generation and quantum dot uptake, bringing about irritation and a destructive effect on cell viability (Rouse et al. 2008; Ray et al. 2009).

11.4.2.3 Fullerene Nanoparticle Toxicity

Fullerenes (C_{60}) are carbon-based nanomaterials that are extensively found in the environment and are the outcome of the combustion of fossil fuel. They are one of the smallest carbon molecules (1–2 nm) developed by humans. Non-functionalized fullerenes are extensively disseminated in all tissues, and indicate a long-lasting buildup, which has been noticed in bones, spleen, liver, and kidney. As demonstrated by in vitro studies, fullerenes may cause DNA strand breakage, chromosomal damage as well as micronucleus development in incubation fullerenes (1 ng/mL) with Chinese hamster ovary cells, human epidermoid-like carcinoma cells, and human embryonic kidney cells for 80 days (Dhawan et al. 2006; Niwa and Iwai 2006). However, in another study, fullerenes demonstrated insignificant effect on DNA strand fracture as defined by comet assay. These disparities between findings may occur due to experimental surroundings and selected materials and methods. The safe utilization of fullerene nanoparticles cannot be clearly answered because of the absence of a complete assessment of toxicity information (Jacobsen et al. 2008; Bahadar et al. 2016).

11.4.2.4 Polymeric Material Nanoparticle Toxicity

Polymeric nanoparticles have been utilized widely in biomedical uses for example targeted drug delivery in cancer and arteritis treatments, and encapsulation of peptides, nucleic acids, and proteins, in order to develop a new nanomedicine that provides continuous release and adequate biocompatibility along with cells and tissues (Panyam and Labhasetwar 2003). Furthermore, majority of biodegradable polymeric nanoparticles are considered to be non-toxic, non-inflammatory, and non-immunologic, and they do not stimulate neutrophils. For example, poly-(D, L-lactide-co-glycolide) has been utilized successfully for targeted drug delivery. To date this polymer-based nanosystem has the least toxicity because it experiences hydrolysis and generates biocompatible metabolites, lactic acid, and glycolic acid. Nonetheless, one report suggests that a surface covering activates the harmfulness of polymeric nanoparticles in the direction of human-like macrophages (Bahadar et al. 2016).

11.5 Conclusions

Progress in nanotechnology has been seen as one of the first direction in current technological progressions in numerous modern fields. In any case, the risk of debasing the earth and the possible antagonistic impacts on human wellbeing must be thought about. In synopsis, the conversation here has concentrated on the toxicity and conduct of different classes of nanomaterials in the earth. In light of past investigations, these nanomaterials were seen as toxic; be that as it may, the explanation behind their toxicity is uncertain. There is still an ambiguous link between nanomaterials and their environs. More investigations are expected in order to assess the strength of these interactions using a variety of tests to completely understand the potential for human exposure to nanoscale products and also future items. Yet to know which parts of nanomaterials ought to be estimated, including type, surface area or concentration of the mass, a combination of these, or something different entirely. Regardless of evolvement assessed here, numerous research barriers in the field must be addressed. In general, society must monitor the advancement of nanotechnology, along with its strenghts and weaknesses. Training in nanotechnology and distributing continuous knowledge to a more extensive crowd are critical future efforts.

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Chapter 12
Techniques, Methods, Procedures and Protocols in Nanotoxicology

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Abstract Nanotoxicology involves the study disclosing the toxicity of nanoparticles. An adverse effect caused by the manufacture either during processing or as the final product with nano materials may affect the human society either *in vitro* or *in vivo*. Determining the association of nanoparticles with cell mechanism, cell uptake, and subcellular confinement is necessary, as the novel physicochemical properties of nanomaterials may raise new toxicological concerns. It is mandatory to modify and organize the *in vitro* tests created to reflect the *in vivo* learning and make them suitable for all applications. Numerous assays are available for *in vitro* nanotoxicity assessment. This review summarizes the major techniques used in characterization and quantification of nanotoxicology studies. Various *in vitro* assays like cell viability assay, oxidative stress assay and inflammatory assay *etc.,* characterization and imaging techniques like optical imaging methods (optical microscopes), high – content screening method (electron microscopes) were discussed to reveal the impact of nanoparticles towards environment, human health and involves in examining the risk assessment of nanomaterials among the surrounding environment. This article will be beneficial for beginners and researchers in the field of nanotoxicology studies/research.

Keywords Nanotoxicology · Nanoparticles · Physiochemical properties · Characterization · Techniques · Imaging techniques · Risk assessment · Safety measures · Health and environmental impacts

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12.1 Introduction

Nanotechnology is a permissive technology which accords with 10^{-9} m sized matter. It is awaited that this field will be evolved at different phases namely nanomaterial and nano based systems (Fiehn 2002). The nanomaterial phase is the most leading phase in the current science and in the economical field (Murray et al. 2000). Nanotechnology also refers to an arriving area of science and technology which encompasses synthesis and progression of multiple nanomaterials (Hasan 2015). It is also expeditious to yield nano related products and as well as nanoparticles which have certain novelty when compared to that of large objects, as nanomaterials exist with different physico chemical properties (Kagan et al. 2005). This novelty in nanoparticles has paved the way for numerous applications in medicine, cloths, cosmetics, renewable energies, and other environmental remediation and biomedical devices (Mrinmoy et al. 2008; Ghosh Chaudhuri and Paria 2012). Previous studies have emphasized greatly on the size based physical and chemical characteristics of nanoparticles (Murray et al. 2000); but in the present scenario they have arrived a profitable analysis in numerous applications (Paull et al. 2003; Meesters et al. 2013).

The cells of the living organism are around 10 μm size, in sub-micron size and the proteins are even smaller than that of cells with 5×10^{-9} m (nm) which are correlated with the artificially produced nanoparticles. These contrasting concepts results in designing NP sized probe at the molecular level cellular machinery (Arora et al. 2012). The smaller size of nanoparticlesequivalent to the size of viruses in the range 10–100 nm makes the NPs to get adhere with biological body irrespective of switching their functions (Holsapple et al. 2005). Henceforth the suitable size of nanoparticlesfor biological applications ranges from 1 to 100 nm. Nanotechnology emerged by focussing on the biological systems on the nanoscale level which acts as an agenda (Whitesides 2003).

At present scenario, copper, zinc, titanium, magnesium, gold, alginate and silver are the commonly used precursors to produce metallic nanoparticles (Hasan 2015). These metallic nanoparticleshas numerous applications in life science and environmental sciences (Salata 2004; Monteiro-Riviere and Tran 2007; Zhang et al. 2007; Fan et al. 2011; Mottana and Marcelli 2013). The physic-chemical properties of metallic nanoparticles such as lower melting point, higher surface volume ratio, strengths and specific magnetizations show alluring industrial applications (Satoshi and Nick 2013).

The real concept of nanotechnology originated in the mid-twentieth century by Richard Feynman, Norio Taniguchi and Eric Drexler (Shirneshan 2013). Their significance was clearly explained in twenty-first century. The speedy flourish of their methodology and tools for characterization has been widely used for various cleaning purposes and in sporting apparatus. Nevertheless it safety ensures among scientists. Scientific associations in the air quality management scrutinize the properties of airborne nanoparticles to implement safety control measures. Mostly man-made

nanoparticles in the civic ambience are taken into consideration since it discloses air pollution and become a fate for managerial actions.

Nanoparticles originated from the marine or forest sources are taken into account only to set the civic situation (Shirneshan 2013). The firmly size related properties of nanoparticles offer uncountable open doors for shocking disclosures. The regularly unforeseen and phenomenal conduct of nanoparticles bears extraordinary potential for inventive mechanical applications, yet additionally postures incredible difficulties to the researchers. Nanoparticlescan be synthesized either biologically or chemically. Nanoparticlesmust be grown in controllable combination approaches to clarify the exploratory perceptions (Fortner et al. 2005).

In some crisis the nanoparticles persuades cytotoxicity due to size and certain nanoparticles displays toxicity during their synthesis (Federici et al. 2007; Li and Huang 2008) process. Few nanoparticlesare known to expose higher risks of toxicity than that of their respective bulky forms (Carragher et al. 2004; Yu et al. 2007; Dorota et al. 2009). Hence, particle size and surface characteristics are the key factors in distribution and accumulation of toxic nanoparticles in the body (Oberdörster et al. 2005b).

Figure 12.1 represents the categories of the mechanism and the factors of the toxicity of nanoparticle such as oxidative stress, genotoxicity, non-homeostatic effects, co-ordination effects and many more, which comes under the mechanism and then the size, dissolution, exposure, composition and shape comes under the factors.

Rapid advances in nanotechnology have paved the way to critically analyse the effects that each nanoparticles propagates on human health and surrounding. Still there exist difficulty and challenges in (1) understanding the performance of nanoparticles (2) Their metamorphosis and their circumstance in various biological materials (3) To appraise their toxicity and 4. to observe competent risk and threat appraisal. Since toxic chemicals are in the layers of the each chemical and materials,

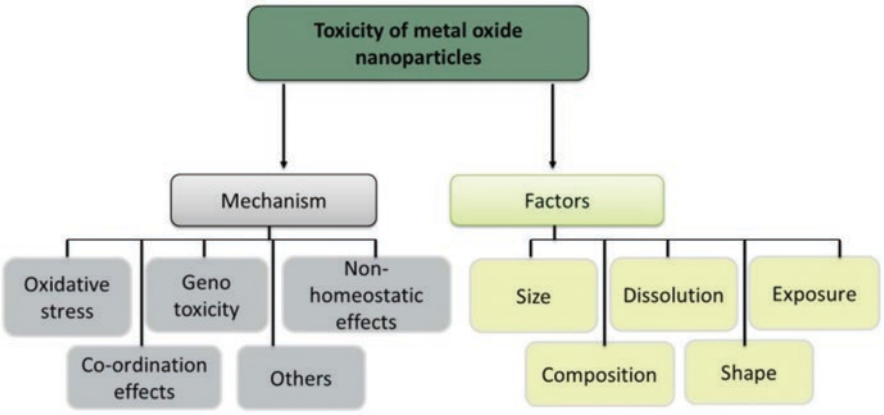

Fig. 12.1 Mechanism and factors of metal oxide NPs toxicity

certain conflicting effects have accompanied with nanoparticlesduring chemical synthesis (Marquis et al. 2009). Oberdorster defined nanotoxicology as "the investigation of the unfriendly impacts of built nanomaterials on living beings and the biological communities, including the counteractive action and enhancement of such antagonistic impacts" (Oberdorster 2009).

12.1.1 Nanotoxicology

Nanotoxicology is the study disclosing the toxicity of nanoparticles to address the competence of nanoparticlesand to fixate the harmful effects that are caused by nanoparticles. This may also be defined as the study of nature and mechanism of toxic effect of nanoscale particles. It is not only admissible to assess the risk of inadvertent nanomaterial vulnerability and also it will help in the establishment of nanomedicine which prevent from the side effects of pharmaceutical products which implies these nanoparticlesin drug delivery or other medical applications (Fig. 12.2).

It depicts the features of the nanotoxicology that were involved in various aspects of geno toxicity, immuno genecity, mechanism, biodistribution, exposure and many more.

Further, determining nanotoxicity is necessary in certain applications, for instance clay nanoparticles allows Miller Brewing to bottle its beer in plastic containers, toxicity of DNA- coated gold nanoparticles to identify the effects in significant proteins and important genes. As the production of nanomaterial inflated recent years, a sudden increase in nano toxicology and nano-risk became a challenging task for the scientist to investigate. Hence, Nanotoxicology not only explains about

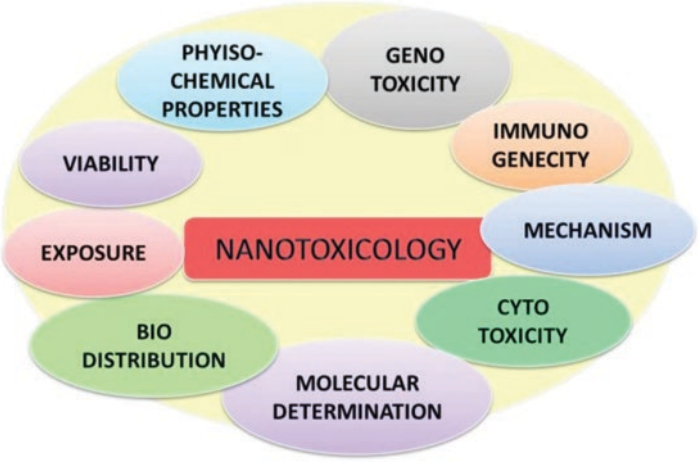

Fig. 12.2 Characteristic features in nanotoxicology

the harmful consequences of the manufactured nanomaterials in the biological environment but to give some contribution for the advancement of viable and safe nanotechnological applications.

In addition, Nanotoxicology is convoluted in offering sensible, flourishing and certain test procedures for nanomaterials towards human and their surrounding. Henceforth this field of study is hopefully for the advancement of a sustainable and safe life of the entire ecosystem. Interpretation of the toxicity caused by nanomaterials and their products is also essential for the civic acknowledgement.

12.2 Methods and Techniques

12.2.1 Methods

Rapid production and marketing of nanotechnology related products, its exposure to humans drastically increases and helps in crucial assessment of nanoparticlestoxicity (Takhar et al. 2011). An output as adverse effects caused during the production and application of nano materials may be either *in vitro* or *in vivo* (Ebbels et al. 2007; Schulze et al. 2011; Li et al. 2014; Meesters et al. 2013).

12.2.1.1 *In Vitro* Systems

In vitro studies aims in developing protocols for evaluating toxicity of nanoparticles. The key objective is to identify *in vitro* assay that precisely reduce the direct exposure of nanoparticles in people. However, the potential health impacts of these diverse array of nanoparticles (Stone et al. 2009) is still unknown. Further, the true effects of engineered nanoparticles on human health needsto be greatly considered during risk assessment. Mechanistic *in vivo* works suggested that particle size ssss and surface area (Salata 2004; Monteiro-Riviere and Tran 2007; Zhang et al. 2016) are the driving materials in inflammation and oxidative stress (Fan et al. 2011) following exposure *via* the lungs. Nonetheless, without more extensive *in vivo* dataand with the urgency in developing *in vitro* models, one proposal is to organize the *in vitro* tests to reflect the *in vivo* studies which are highly acceptable and accessible, in addition to *in vitro* inflammation and oxidative stress studies (*e.g*. genotoxicity).

As information accumulate for the toxicological effects of nanoparticles in diverse organism, researchers ought to have the capacity to develop a battery of *in vitro* tests that can be utilized as alternatives to animal testing. It is unlikely that one test will be adequate to survey toxicity for hazard assessment purpose (Stone et al. 2009). Further there is a need for advancement and approval of precise nanodevices and materials, which are suitable for predicting harmful and toxic responses (Takhar et al. 2011). There are various methods to assess the toxic impacts of nanoparticlessuch as epidemiology studies, human clinical studies and *in vitro* studies.

It is clear from previous literatures that the use of animal models can be substantially reduced with *in vitro* nanotoxicity studies, which results in identifying the starting dosage for *in vivo* studies, thereby reducing the toxic wastes.

12.2.1.2 General *In Vitro* Methods for Nanotoxicity Assessment

Cell Viability Assay

Proliferative Assay

Cell proliferation assay is an important metabolic assay which determines the cell viability. Initially a yellow tetrazolium salt, such as, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)- 2H- tetrazolium, internal salt) or MTT (3-(4, 5-dimethylthiazol-2-yl)- 2, 5-diphenyltetrazolium bromide), is added, along with different concentrations of drugs and the cells are treated for 2–4 h at 37 °C. During this procedure, an insoluble purple formazan product is formed by mitochondrial succinic dehydrogenase enzymes and it is solubilized by dimethyl sulfoxide and quantified using UV-Visible spectrophotometer. Based on the optical density compared to the control, information on the cell death are gathered. For example, the toxicity of Superparamagnetic nanoparticles (SPIONs) was determined with MTT dye reduction assay in our laboratory. The metallic nanoparticles, SPIONs exhibited non-toxic nature and only 50% cell death occured at maximum concentration of 500 μg/ml (Ilangovan and Sen 2016).

Alamar Blue Assay

This assay is used to study the cellular redox potential which is usually implemented in nanotoxicological studies. A cell reasonability marker has been used in alamar blue assay which has a characteristic of decreasing energy of living cells by changing into the fluorescent molecule resorufin from resazurin. The oxidation-reduction potential of Alamar Blue at pH 7.0, 25 °C is +380 mV (Mahmoudi et al. 2012). Kreft's dictromaticty index (DI) clearly shows that Resazurin solution is highly dichromatic . Viable cells constantly change over resazurin to resorufin, subsequently, creating a quantitative size of suitability and cytotoxicity. For example, Pesch and Simmert in 1929 demonstrated contamination in milk by bacteria or yeast using Resazurin (Kreft and Kreft 2009). The non-fluorescent blue dye Resazurin gets converted to highly fluorescent resorufin (Page et al. 1993).

Cologenic Assay

Interactions amongst nanomaterials and probe molecule can be determined altogether by using cologenic assay (Wang and Fan 2014), (Ghosh Chaudhuri and Paria 2012). The cologenic assay is identifies the efficiency of particular substances on the survival and proliferation of cells. Cells are plated and treated with materials of

interest (eg. Nanoparticles) and are flooded with crystal violet or nuclear stains and highly proliferating cells are counted manually under microscope. For example, carbon nanotubes (CNT) are exposed to S549 cells to determine the nanoparticle efficiency in depleting the availability of media components (Herzog et al. 2007; Takhar et al. 2011). Likewise, cytotoxicity of green synthesized platinum nanoparticles (ptNPs) were evaluated with different cancer cell lines by cologenic assay (Bendale et al. 2017).

Apoptosis Assay

Apoptosis, also known as programmed cell death determines the morphological changes occurring in a cellular material upon exposure to a toxic agent. It is the most common assay used in nanotoxicology (Takhar et al. 2011). Different types of apoptosis assays are summarized below.

DNA Laddering

DNA laddering is the common and oldest technique which involves degradation of DNA by caspase-activated DNase (CAD), resulting in fragments of DNA that are run in gel electrophoresis. The fragmented DNA appears as ladder pattern which are visualized with naked eye (Takhar et al. 2011).

Caspase Assay

Caspase (cysteine – aspartic proteases, cysteine aspartases or cysteine dependent asparate – directed proteases) plays a vital role in apoptosis, a programmed cell death mechanism, also pyroptosis and necroptosis and inflammation. The caspases will endure as an inactive zymogens in cells and it experiences a cascade of catalytic activation on the onset of apoptosis. The inhibition of apoptosis family for proteins were inhibited by activated caspases. For instance, when Nanoscale HAP hydroxy- was administered in human gastric cancer cells (SGC-7901) at 100 μg/ml, it caused release of cytochrome c and activation of caspases-3 and caspase-9 (Srinivasula et al. 2001; Oberdörster et al. 2005a; Park et al. 2008; Takhar et al. 2011). Further, reports convey that CeO_2 (5–40 μg/ml) and TiO_2 (5–40 μg/ml) nanoparticles in Beas-2B cells after 24 h of exposure activates caspase-3 (Shukla et al. 2005; Takhar and Mahant 2011; Takhar et al. 2011).

Annexin V

The assay is a simple and efficient method in the detection of apoptosis (Koopman et al. 1994; Takhar et al. 2011) with phosphatidylserine – (PS) in living cells (Trotter et al. 1995; Takhar et al. 2011). The PS gets translocated from the cytoplasmic phase of the plasma membrane to cell surface during apoptosis (Op den Kamp 1979; Fadok et al. 1992; Takhar et al. 2011). ANNEXIN V, has a Ca^{2+}- dependent activity which has affinity to PS and hence it can be used as a tool for disclosing apoptosis.

Apoptotic cells can be detected by using fluorescently labelled AnnexinV. Corroboration of annexin V to crosslinked iron oxide (CLIO) nanoparticles (Schellenberger et al. 2002) can also be utilized in detecting the apoptosis by enhancing the MRI imaging probe. This helps in visualizing even at lower concentration of particular magnetic substrate (Schellenberger et al. 2002).

Necrosis Assays

A feeble cationic supravital dye which is nothing but a neutral red dye which helps in binding and incorporating the viable cells based on their quantity. This particular dye instantly permits into the cell membrane by means of non-ionic diffusion. It also predominantly accumulates intracellularly in lysosomes, based on the lysosomal fragility and various modification were gradually which become irreversible. These necrosis assays are neutral red take –up cytotoxicity assays and works under the principle of cell viability assay (Mauzeroll et al. 2004; Takhar et al. 2011). For example, to assess the *in vitro* toxicity of the chemicals the neutral red uptake were injected into the NIH3T3 mouse fibroblasts and this is the only *in vitro* method for toxicity testing (Takhar et al. 2011) and has been unified into the REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances) (Monteiro-Riviere and Inman 2006; Takhar et al. 2011).

Trypan Blue Test

Cells are treated with trypan blue agent, trypsinized, and thusly recolored with trypan blue, a diazocolor, that was adsorbed inside dead cells, however avoided by live cells. Unstained cells mirror the aggregate number of recovered cells recouped from a given dish. This technique is profitable as it passes on the real number of viable cells, and increases or decreases in contrast with control and untreated cells. For example - Poly (lactic) acid nanoparticles (PLA) for gene delivery in human and bovine retinal pigment epithelial cells, do not reduce cell viability at concentrations up to 4 mg/ml PLA (Takhar et al. 2011).

Oxidative Stress Assay

The highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) either forms in excess or gets removed due to the aggravation in the oxidative balanced by engineered nanoparticles is considered to be Oxidative stress, ROS incorporate free radicals, for example, superoxide ($\bullet O_2^-$), hydroxyl (•OH), peroxyl ($\bullet RO_2$), hydroxyperoxyl ($\bullet HRO_2^-$), and, non-radical species, such as, hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl). RNS include free radicals like nitric oxide (•NO) and nitrogen dioxide ($\bullet NO_2$), and in addition, non-radicals, such as, peroxynitrite ($ONOO^-$), nitrousoxide (HNO2) and

alkyl peroxynitrates (RONOO), (Takhar et al. 2011). For instance, Nanoparticles are known to up-regulate the transcription of various pro-inflammatory genes, including tumor necrosis factor-α and IL (interleukins)-1, IL-6 and IL-8, by activating nuclear factor-kappa B (NF-κB) signalling. These sequential molecular and cellular events are known to cause oxidative stress, followed by severe cellular genotoxicity and then programmed cell death.

2, 7-Dichlorofluorescin (DCFH)

In this assay, the size and the color is acquired as the diacetate precursor gets cleaved due to high pH and develops non-fluorescent product DCFH which results in manufacturing of dye (Witold and Grzegorz 2013). The nearness of ROS changes over DCFH to a fluorescent product, 2, 7-dichlorofluorescein, which can be estimated with fluorimetry (Takhar et al. 2011). In a research, nanomaterial interaction with DCFH-DA was studied in relation to its nature and/or assay conditions (cell-based and time exposure) by incubating Rhodamine (Rhd)-labeled 25 nm and 50 nm silica (SiO_2), naked and oleic acid coated magnetite, (Fe_3O_4) and maghemite (Fe_2O_3) iron oxide, titanium dioxide (TiO2) and poly(ethylene oxide)-poly(lactide/glycolide) acid (PLGA-PEO) nanoparticles (NPs) with metabolically active rat hepatocytes for 4 and 24-h periods . These data indicate that despite the quenching effect of nanoparticles on DCFH-DA assay, it can be considered as a useful tool for quantitative measurement of NPs-induced oxidative stress by minor modifications of standardized protocols (Aranda et al. 2013).

Electroparamagnetic Resonance (EPR)

EPR is one of the most common technique to determine nanoparticle and its induced ROS generation. The utilization of specific spin traps or probes in combination with specific reagents can allow for the quantification, and additionally, specific identification of the free radical species generated (Takhar et al. 2011). For instance, Anticancer therapy with Nanoparticle-based drug delivery applies EPR effect. (Ngoune et al. 2016).

Lipid Peroxidation

Lipid peroxidation measures the presence of malondialdehyde or other thiobarbituric acid receptive where oxidative degradation of cell mechanisms occurs with the presence of ROS (Buege and Aust 1978; Ding et al. 1998; Levi et al. 2006). This assay has been utilized extensively to demonstrate the capacity of an assortment of nanomaterials to evoke lipid peroxidation in various cells, such as fullerenes in human dermal fibroblasts (HDF) and human liver carcinoma (HepG2) cells (Levi et al. 2006).

Inflammatory Assay

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is the most common biochemical assay used to distinguish the nearness of a counter acting agent or an antigen. In ELISA, an obscure size of antigen is appended to a surface, and afterward a particular counter acting agent is connected over the surface with the goal that it can bind to the antigen. This antibody is linked to an enzyme, and in the last stage a substance is conjugated, so that the enzyme can convert to some detectable signal, most commonly a colour change in a chemical substrate. The most commonly tested human and murine inflammatory markers are the chemokine Interleukin-8 (IL-8), trailed by TNF-α and IL-6 (Takhar et al. 2011). Gold nanoparticles were used to detect the antibody against the H9N2 subtype of avian influenza virus. Using 133 field chicken sera, the sensitivity of nano-ELISA compared to traditional ELISA was found to be 100%, whereas the specificity was observed to be 92% (Engvall 2005).

To examine or to identify the toxicity both *in vitro* and *in vivo* assessment can be done on the biological effects on nanoparticles which can either be an artificial or synthetic nanomaterials. In the current scenario for testing and examining the nanoparticle certain regulatory organisation were involved which were carried out through *in vitro* studies. For the assessment of nanoparticle this became more relevant and essential. To access the nanoparticle for diverse toxicological endpoints, to allow the development of mechanism- driven examination, and to provide refined information that like how these nanoparticles gets interacted with the human cells can be achieved by these *in vitro* model systems. When compared to the animal model these *in vitro* models were more fast and economical, hence these toxicity studies were implemented to select an apt animal model or human body is not a big issue in the *in vitro* system and those standardized cell lines of metabolic action were not characterised broadly. The nanotoxicological studies can use all the recent experimental method of cell lines (Mukhopadhyay 2014).

The method which helps in incorporating the lethality of nanomaterials (1) *in vitro* examination of cell sustainability *viz.,* ROS generation, apoptosis, necrosis, DNA damaging potential (2) microscopic evaluation of intracellular localization of biomaterials (3) gene expression analysis high-throughput systems (4) *in vitro* hemolysis and (5) genotoxicity and so on. Many nanomaterials were utilized for invite cytoxicity studies which contains different cell lines, incubation times and colorimetric assay. Only few literature survey were published resulting that the amount, types and applications of nanomaterials increase, to study the effect of their exposure and to report the potential toxicity (Li and Huang 2008). *In vitro* toxicity was not utilized to examine the engineered nanomaterials and to characterise the physical and chemical properties and also to predict the nanomaterial cytotoxicity which is indeed of requirement which should be related to matrix on the sensible cell lines and these *in vitro* assays results with the estimation of diverse cytotoxicity endpoints (Li and Huang 2008).

12.2.2 Techniques

12.2.2.1 Metabolomic Techniques

Metabolomics majorly focus on identification of early biomarkers. One advantage of utilizing a temporal report with a same animal is that it isn't necessary to understand the pharmacodynamics and pharmacokinetics of the toxic prior the biomarker investigation. This is especially beneficial in the investigation of toxicity caused by nanoparticles since little is known about their kinetics or mechanisms of action.

This metabolomics method can be carried by means of Fluid chromatography/ mass spectrometry (LC/MS) and Nuclear Magnetic resonance (NMR) spectroscopy. Some other technique also involved in this method such as gas chromatography coupled to mass spectrometry (GC/MS), thin-layer chromatography coupled to mass spectrometry (TLC/MS), matrix assisted laser desorption/ionization mass spectrometry (MALDI/ MS), and Fourier transform mass spectrometry (FTMS) (Holsapple et al. 2005; Hartmann et al. 2014). It is highly essential for assessing nanotoxicity with the aid of high – throughput screen methods that bolster to screen the nanomaterials, since those early practised method are balancing in some techniques such as for analysis of drugs, chemical or nanomaterials. This high- throughput screening method is carried out in omics technologies which involves transcriptomics, proteomics and metabolomics that can handover certain information about the endogeneous markers for both *in vivo* and *in vitro* systems (Sherborne and Nguyen 2015).

Omics approach mostly incorporates bioinformatics, transcriptomics, proteomics, metabolomics, genomics and epigenomics. Screening of toxic quality of engineered nanomaterials incorporates measurement and evaluation of physicochemical characteristics, dose metrics, *in vitro* and *in vivo* assays, eco toxicity assays and *in silico* modeling, *etc.,*

Metabolomics, particularly, permits rapid screening of biomarkers. For profiling the metabolic nanomaterials, metabolomics and metabonomics are applied. To define metabolomics, it refers to quantitative metabolite pool that exists inside a cell or a tissue based on the certain optimum environmental condition (Robertson 2005) whereas metabonomics, refers to that, they have measured a pathophysiological stimuli or genetic modification of metabolic response of living system which means the quantitative measurement of dynamic multiparameter. On the whole the pattern of biomolecule are identified and categorized on the basis of toxicity source, mainly by using a specified drug compound that causes adverse effect to the particular organ (Lindon et al. 2005; McCunney et al. 2011; Schnackenberg et al. 2012; Marlene et al. 2015).

Hence, a mechanistic intuition upon the nanotoxicity have been given by this metabolomics technique disclosure of nanomaterial. There is an inflammatory response and initiation of oxidative stress by means of ordinary response in both *in vitro and in vivo* method. This oxidative stress is carried out by means of interacting certain reactive species which were excited during inflammatory response; but this

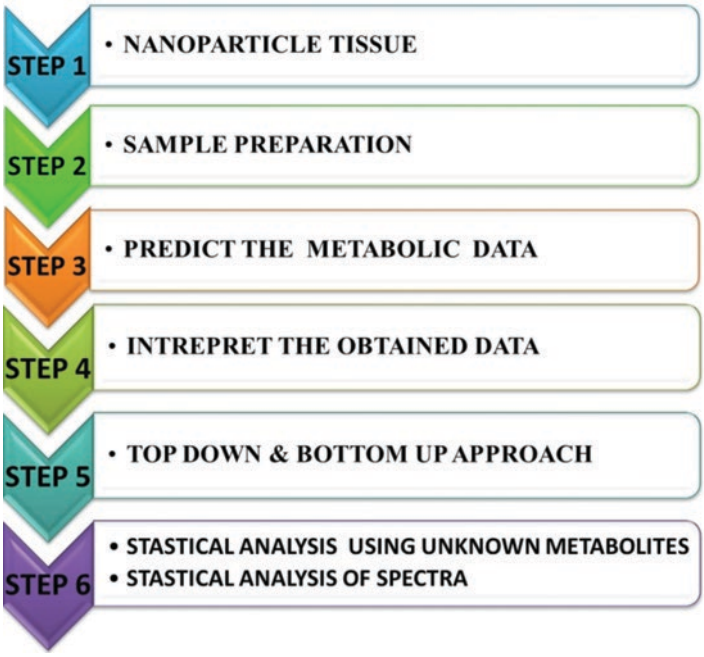

Fig. 12.3 Steps involved in metabolomic technique

can be possible only if those nanomaterial contain transition metal. For example: Fujita *et al* (Gao et al. 2016) undergone specific experiment using living tissue of rat which unmark C(60) fullerence over inhalation, done for gene expression profiling (Fig. 12.3).

12.2.2.2 Liquid- Chromatographic Technique (LC/MS)

In comparison with NMR, the LC/MS has a better sensitivity, even though it has many demerits such as it includes ion suppression, enhancement, lower responsibility, but it is not quantitative, and those ionization methods will be able to analyse the kinds of analytes examined. In order to inflate the application of MS in metabolic profiling studies, this MS instrumentation and technology will help in the enhanced reproducibility and high mass measurement with growth great accuracy (Matsuda et al. 2005; Zhang et al. 2016). To a certain pictogram, 10^{-12} m concentration to analyse the metabolites are reduced by means of the MS instrumentation which has a high sensitivity. It is not adequate for a single ionization technique to analyse almost any type of compound within the complex biological matrices in single analytical run.

There are some runs part from the above, MS instrumentation analysis which includes the effects of ion suppression and enhancement which will directly effects the quantitation and reproducibility of date. Since MS has a great data dimensionality, it is applied in imaging techniques (Ryan et al. 2007). Whereas, for imaging tissue sample, the matrix assisted lesser desorption /ionization (MALDI) can be widely used. This can also help in viewing certain information regarding peptides, proteins, lipids, metabolitics and other endogeneous compounds like nanoparticles in complex tissue samples.

12.2.2.3 Nuclear Magnetic Resonance Spectroscopy

The NMR has the ability to examine the presence of metabolites in tissues, urine and serum within a limited period of time. The nature of NMR is that it helps in evaluating each proton present within the particle and their reproducible pattern, chemical shifts and their quantative information and hence NMR is more advantageous than metabolomics techniques. This advancement made NMR to evaluate or examine all the metabolites and their instruments within few minutes. NMR also have some drawbacks, they have certain overlaps over some metabolites. Further, NMR renders low susceptibility which fails to differentiate those metabolites that have sub micro molar range. Thus the both the techniques (NMR or MS) helps in better understanding in explaining changes in the metabolome by means of nontoxicity. This combination defines the identification of biomarkers, pathway analysis and also illustration of toxic mechanism of a specific materials (Steuer et al. 2019).

12.2.2.4 Analysis of Metabolomic Data

A miscellaneous protocol ended up by the examination of those metabolomics information. A solid chemometrics plays a major role in eradicating the most difficult spectroscopic information. An omics dataset, to distinguish biomarkers and to analyse the ultimate characterisation test, the chemometrics plays a vital role (Deming 1986; Nastassja et al. 2008). The most essential size related properties of chemical system namely pressure, flow, temperature, infrared, Raman, NMR spectra and mass spectra were established as a model in order to examine the properties of their interest, is the main aim of this chemometrics (Ning et al. 2018).

In order to relate the dose-reaction information and to connect the dangerous reaction in numerous nano related species or strains that are similar to nano species this SMART analysis plays a vital role that helps in investigating this irrespective of research facility, physiological and phenotypical diversities. A best example for nanoparticles which acts a part of disease diagnosis, in the manufacturing and control of biological products (vaccines), infection identification, drug delivery, cancer therapy, and biosensors, Silica (SiO2) is used. Other techniques like GC/MS based metabolomics techniques that is used to study the size and dose response followed by exposure of SiO2 particles in mice.

12.2.2.5 Synchrotron Radiation (SR)

It is a high frequency high source with a frequency range from infrared to highest energy X rays. It has highest energy and pulsed light emission that these two properties highly enhance the signal to noise ratio, spatial and temporal resolution and reduce acquisition on time. The beam of synchrotron radiation has two ways for the interaction with the matter namely absorption and scattering based on this three techniques which were introduced as (1) Based on the absorption process, X-ray absorption fine Structure (XAFS), Fourier transformed infrared spectroscopy (FTIR), ultraviolet– visible spectroscopy (UV – Vis), soft/hard X-ray microscopy (scanning transmission X-ray microscopy, STXM; transmission X-beam microscopy, TXM), and X-beam computed tomography (X-CT), *etc.,* are produced; (2) Based on the scattering, X ray diffraction (XRD), protein X-ray crystal diffraction (PX), Circular dichroism (CD), – Small angle X ray scattering‖ (SAXS), diffuse scattering, elastic/inelastic scattering (Raman spectroscopy), and topography, and *etc.,* are developed; and, (3) Based on the detection of the emission of secondary particles, X-ray fluorescence spectrometry (XPS), and X-ray fluorescence spectrometry (XRF) are developed. In order to examine nanotoxicology various synchrotron radiation based procedures are established as it is an emerging field around the world (Wilson et al. 2005; Chen et al. 2013; Li et al. 2015).

12.2.2.6 Characterization of Nanomaterials

Before testing a toxicity of nanomaterials certain physical and chemical properties should be examined. For the perfect physicochemical characteristics of nanomaterials, transmission electron microscope (TEM), scanning electron microscope (SEM), atomic force microscope (AFM), Scanning tunnelling microscope (STM), *etc.,* are used (Deming 1986; Sommer and Golla-Schindler 2008; Rao and Biswas 2009; Li et al. 2015; Aditi et al. 2018). TEM and SEM are the benchmark electron magnifying lens based techniques to evaluate geometric size, estimate dissemination, and state of nanomaterials with the spatial determination of not exactly 0.1 nm (Williams and Carter 1996; De Graef 2003; Gupta and Tripathi 2011). The mechanism behind the synchrotron radiation based SAXS is to scatter the power of X beams by breaking down at the edge 0.1° and 5°, that helps in evaluating the shape and size and also separation of materials, pore size and some other information of nanoparticles which has spatial determination ≤ 5 nm (Lindon et al. 2005; Wang et al. 2007; Yan et al. 2016; Walenta 2018).

These mechanisms were already applied in investigation of *in vitro* or *in vivo* toxicological studies for accurate determining and affectability with less time period and estimating *in situ* mechanism involved in routinely episode photons for perfect nanomaterial characterisation done with the help of synchrotron radiation based XRD, XPS, SAXS, Raman spectroscopy, FTIR, miniaturized scale/nano-CT *etc.,* Certain nanobelts such as Sn_2O_3 or ZnO are characterized with these techniques to

characterize individual nanomaterials (Schütz et al. 1987; Schnackenberg et al. 2012; Mottana and Marcelli 2013).

To withstand, point by point data on the electronic structure on the surface of nanoparticles with high accuracy synchrotron radiation high determination synchrotron radiation XPS, Synchrotron radiation X-ray photoelectron spectroscopy (SR-XPS) procedures were used. A high intensity, tunability continuous energy should be distributed on a large energy region which are provided by SR-XRS, where as in convention XPS ideal excitation energy is used instead of fixed excitation energy. XMCD is the result of synchrotron radiation range from X ray to XAFS. These XCMD (X-ray Magnetc Circular Dichronism) is a tool to measure the absorption of both left and right circularly polarized light with strong magnetic field (Carragher et al. 2004). In order to characterise mangentic nanomaterials XMCD is used, as it provides specific data on magnetic material, it also provides information about specific element and quantify the amount and balance state of particular atom for this characterisation.

To evaluate the geometric nature, an electronic structure of matter acts as a synchrotron radiation source with high intensity and tunable X –beams can be used. XAFS is predominantly used for this examination where collected samples can either be in gas phase, solution or solid (Ryan et al. 2007; Dusinska et al. 2015). For instances, for photo catalytic application TiO_2 nanoparticles are widely used along with TiO_2, it also includes decomposition of organic compounds and production of H2using solar energy. A detailed survey was undertaken based on the surface and bulk structure of rutile and anatase. In order to understand the source of photo catalytic activities that were revealed by TiO_2 nanoparticles, it was analysed by XAFS.

Absorption, Distribution, Metabolism and Excretion of Nanomaterials

Pristine nanomaterials involves many physicochemical transformation in the biological environment as a major characterization of nanomaterials. To obtain, the nutrients to and excrete the waste products is simple and easy for unicellular organism where as to multicellular organism including human being it is not simple because, many cells present in them are indirectly have contact with the environment. To carry nutrients inside the cells and waste products from the cells and to carry chemical communicators, biological fluids acts as a medium or carrier. For example, artificial gastric juice when administered orally in mice, a rapid interaction takes place between copper nanoparticles and H^+ results in conversion into ionic copper that enhance toxicity (Lindon et al. 2005).

Along with *in vivo* studies, *in vitro* assays is vital in nanotoxicology since it is faster, costless and easy to control with low ethical concerns than before. Certain viability assays were performed such as proliferation assays, necrosis assays and apoptosis assays. For examples, carbonaceous nanotubes (CNTs) can be connected to microorganism (*Lactobacillus acidophilus, Bifidobacterium adolescentis, Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus*) that were normally experienced by human absorption system. These nanoparticles also involves in the efficiently up taking the cells by phagocytosis, macropinocytosis, clathrin-interceded endocytosis, clathrin-and caveolae-free endocytosis or caveolae

mediated endocytosis. TXM (Synchrotron Radiation Based Transmission X-Beam Microscopy) isan efficient method for studying biodistribution of nanoparticles in both 2D and 3D dimensions. It is a high delicate method to identify many elements present inside biological tissues, C, N, O for soft X-rays and Z elements for hard X-rays.

For long term effect and optimal relevant model system for further *in vitro* studies, *in vivo* toxicity assessment of nanoparticles can offer tissue localization, biodistribution and excretion by up taking complete living organism. Two level methodologies for *in vivo* nanotoxicity examination, level 1; to study about the appropriate human exposure like pulmonary, oral injection and dermal exposure. Level 2; to study the usage of susceptible models effects of multiple exposures deposition translocation and bio persistence studies, estimation of reproductive effects and mechanistics studies such as genomics, proteomics and metallomics (Oberdörster et al. 2005a; Sahu and Casciano 2009; Li et al. 2014).

12.2.2.7 Imaging Techniques

In traditional toxicology, it was difficult to visualize and examine the cellular uptake translocation, accumulation, and clearance of NMs in human (Oberdörster et al. 2005b; Nel et al. 2006; Zhao et al. 2008; Li et al. 2014; Yan et al. 2016). This leads to risk of human health and hence imaging techniques were introduced which is a powerful tool for the research of *in vivo* and *in vitro* study that helps in understanding simple pathological mechanism and also to examine NMs toxicity in biological system. In Imaging techniques, *in vitro* is used for characterising physical and chemical properties of NMs structural analysis of bio – nano interaction and visualization of NMs where as in *in vivo,* it helps in quantification of biodistribution, bioaccumulation, transformation of NMs. Compared to traditional imaging techniques, the advanced nuclear analytical related techniques in nanotoxicology research become more advantageous due to their absolute quantification, high sensitivity, excellent accuracy and precision, low matrix effects and non-destructiveness (Yan et al. 2016) (Fig. 12.4).

Optical Imaging Methods

Optical Microscope

The most traditional imaging technique for researcher are optical microscopy but it has more advantages like to visualize the dynamic process of live cells with fluorescent labels. For instance, in absence of transfection agents, the initiation and regulation of antigen specific immune response takes place, with the help of confocal microscope, a superparamagnetic iron oxide NPs encapsulated with a photonic ZnO shell that are found to be efficiently up taken by the dendritic cells (Cho et al. 2011; Yan et al. 2016). This is prodigious in selectively imaging photo luminescence or

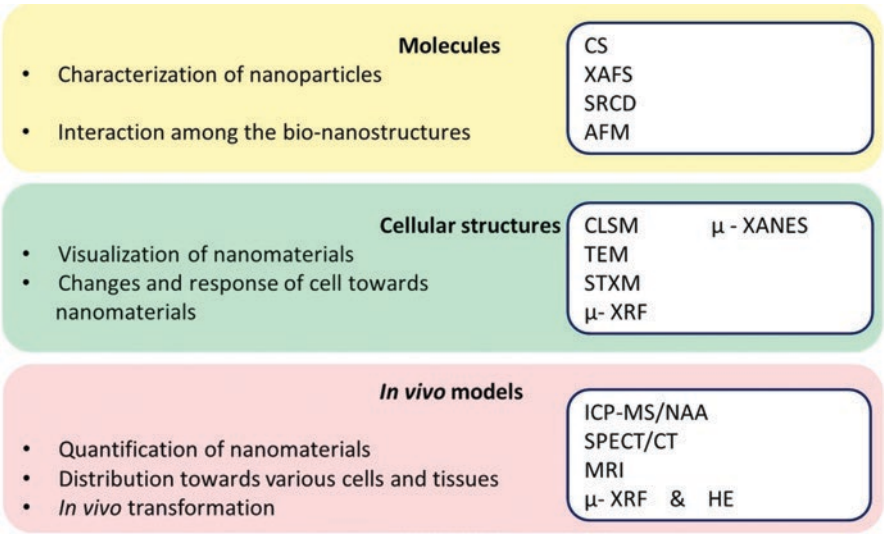

Fig. 12.4 Imaging techniques

fluorescence label NPs and certain subcellular components; occasionally multiple fluorophores can be widely used. Using Confocal Laser Scanning Microscope (CLSM) and Multi photon Fluorescence Microscope (MFM) (Lowry et al. 2012; Yan et al. 2016) 3D images can be reconstructed in 3D with the help of computers, range from single NP spectroscopy to label free live tissue imaging Dark field microscopy (DFM) is extensively used (Murphy et al. 2008; Lowry et al. 2012; Yan et al. 2016).

High-Content Screening Method

To integrate and to automate the quantitative fluorescence microscopy and image analysis, a modern advancement is introduced which is high screening method. The novelty of NMs is the rapid establishment that leads to an inclusive methodology for screening biological activity and cytotoxicity of NMs. In order to meet the future demand for nanotoxoicity studies high throughput cytotoxicity assay platform based on HCS technology were involved and HCS is the most efficient and powerful method to detect toxicity in nanoparticles (Zhang et al. 2006; Yan et al. 2016), was the first to evaluate the cytotoxicity of quantum dots (QDs) by using high-content image examination along with high-throughput examination. To examine cell counting, to estimate apoptotic and necrotic cell population and to produce cell cycle profiles, HCS method were used. They also involve in the investigation of the cellular and molecular impact of high doses of poly(ethylene glycol) silanized quantum spots (PEG-silane-QDs) in the human lung and skin epithelial cells and detailed PEG-silane-QDs instigated insignificant cytotoxicity even at high dosages (Jan et al. 2008; Yan et al. 2016).

Electron Microscopes

The intake of nanoparticles inside the cells and subcellular localisation of multiple nanoparticles, electron microscope is applied because, NPs have electron density of the element and the thickness of the particle (Carragher et al. 2004; Wang et al. 2010; Qu et al. 2011; Lowry et al. 2012; Yan et al. 2016).

Electron cryomicroscope (CryoTEM) is used to visualize biosamples (Oberdörster et al. 2005b; Powers et al. 2012; Yan et al. 2016). Modifying TEM into STEM (Scanning Transmission Electron Microscope) can be done with the combination of certain appropriate detector to obtain the required image (AshaRani et al. 2009).

Quantification of Nanomaterials

X-Ray Computed Tomography

This technology use computer assisted X ray analysis, in order to yield chromographic images by scanning a particular area in an object and allows the user to visualize inside without cutting the sample (Ryan et al. 2007; Yan et al. 2016). X-ray computed tomography (CT) is one of the most wide spread diagnostic imaging techniques because of their advantages such as rational high-contrast resolution, cost-effectiveness, facile image processing and unlimited penetration depth. This is involved in the following application like medical science, biology, and medical use (Yu and Watson 1999; Yan et al. 2016).

12.3 Characterisation

Some characteristic studies were involved in both medical pharmaceutical and in medical devices together with implementation of nanotechnology with the following examples

- Enhancement /alteration (modification) of solubility and ADME property.
- Development of formulation stability
- Escalation of excipient solubility and decreasing the consumption of excipient
- Lowering the toxicity
- Enhancement of surface properties of medical devices
- Modification of molecular targeting nanobranch therapy (Thomas et al. 2008; Hobson et al. 2016).

A biological effect of nanoparticle system is examined by means of particle characterization which is a major key factor and this environmental system is likely mystery to understand and analyse. Hence a complete examination should be undertaken upon the characterization of that particular matter, which is again an intermediating task. The two major problem were listed out; (i) measuring the properties

(ii) expressing the measurements, which expenses the properties of interest. The nanoparticle and the interacting particles are in same size, length and surface properties. High surface area for the finite particle is the most apparent surface property. When a certain nanoparticle enter into the biological setting, it suddenly changes the functional surface chemistry of nanoparticles, later rate the potential that rely on surrounding and also on particle surface and therefore the surface composition and properties should be characterized and understand.

12.3.1 Engineering Materials at Nanoscale for Biomedical Applications

The physicochemical parameters of nanomaterials either exclusively or helpfully can influence the underlying nano-bio interfacial connections, grip of nanomaterials on cell mechanism/ surface, their cell uptake or coordinate infiltration inside the cells. To conclude, nanomaterials correspondence with the cell parts; which at last translate into the bio- similarity or danger of these nanomaterials towards a particular natural substance prompting helpful or then gain adversative impacts (Nel et al. 2006) to form further understanding of nanomaterial properties (Nel et al. 2006).

For example, biological dyes are manufactured using nanoparticles by modifying the optical properties of the nanoparticles. Some of the properties like photocatalytic properties are involved in functions; namely in size and crystal structure. The properties like crystal structure along with size directly affects the physical-chemical properties and this is individual by anatase (Anatase is a polymorph with two other minerals) which is more effective than rutile which is a crystal structure and have the capacity to influence the factor which affects physical-chemical properties. Decrease in size helps in varying the thermal properties. A regulatory agenda were developed which is more important and vital by means of reliable characteristics of nanoparticles in air. Due to the deficiency of standard methodologies and applications tool it has been restricted to certain limit, but in recent years, a new advanced instruments have emerged to progress. Currently, it is very important to define the particle size range of each mode (Rose et al. 2012) (Fig. 12.5).

12.3.2 Nucleation Mode

Nucleation mode is a mixture of two or mutually by means of aerosol population and those particles were characterised based on the size that range from 1 to 30 nm (Lingard et al. 2006; Kumar et al. 2010). These aerosol are not present in the initial exhaust emission but they were formed by nucleation method. These were ordinarily liquid droplets which comprises of volatile components which were resulted from unburned fuel and lubricant oil (Charron and Harrison 2003; Kittelson et al. 2006; Kumar et al. 2010).

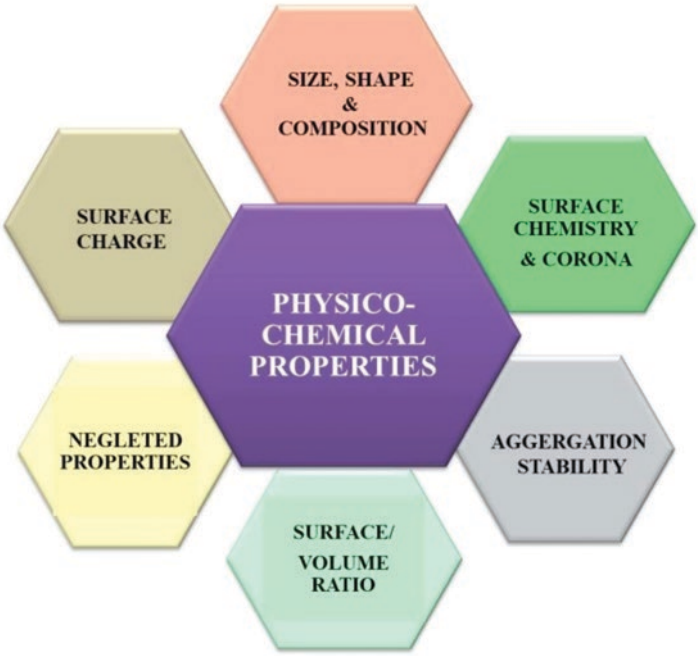

Fig. 12.5 The various properties of nanoparticles are given in the above figure listing the size, shape, aggregation stability, surface charge and aggregation stability

12.3.3 Aitken and Accumulation Modes

The overlapping functions of nucleation mode and accumulation mode of particles is the Aitken mode with the size ranging from 20 to 100 nm (Olsson and Benner 1999; Kumar et al. 2010). This mode cannot be distinctly clear in various ambient temperatures (Kumar et al. 2008a, b, c, 2010). For the growth and to coagulate the nucleation mode particles and also by producing the significant numbers by primary combustion sources, this Aitken mode of particles were used, for instance, vehicles (Kulmala et al. 2004). These particles comprises of soot/ash core which were absorbed by the volatile materials (Lingard et al. 2006).

In the nucleation, aitken, accumulation and warm modes, the lognormal distribution to the size distribution is the most appropriate one over the rest range of particle size. A specific characteristics, sources, chemical composition and sensitivity to particle dynamics were revealed by each mode. Nature based and anthropogenic based are the major source of ambient nanoparticles. When dealt with the impact on wealth and environment, have a substantiality on other nanoparticles then those who have low concentration ambient air and it also suggested that due to the advancement of physic-chemical characteristics, toxicity, release paths (intentional/unintensional) size, atmospheric life span and dependability of nanoparticle. The promotion of growth, enhancement and alteration of optical properties, due to the nanoparticles

which is considered to main pressure of large particles, this results affects the radioactive properties of the atom. Due to the alteration of nanoparticle size by means of global climate, are not recently demonstrated with high level of accuracy.

A best example for light extinction scattering and absorption properties were hygroscopic, or hydrophobic nature which are linked with the visible impairment. On basis of number, the regulatory agenda is found which acts as an essential role for these advancements. Along with those advancement, air quality monitoring program helps in including number of nanoparticles and their size distribution measurements by urban air quality monitoring networks.

Nanoparticles are covered with hard and soft coatings those weak proteins are bound around the hard protein surface which is the basic protocol of the coating of nanoparticle just simply called as corona (Hobson et al. 2016).

Based on the corona principle nanoparticle are extremely important, certain instruments or tools were used to examine or to estimate the biological activity either pharmacological and toxicological characteristics or safety measurement of engineer nanomaterials. The results should be pragmatic as it has certain issues and so it needs a vigilant observations for instance, chemical compound can be analysed with respect to their safety assessment their distinctive physical and chemical properties whereas nanoparticle cannot be treated, if treated it will produce misleading information data (Oberdörster et al. 2005b; Samberg and Monteiro-Riviere 2014). Nanoparticle also have an impact on health and nature which also have certain effects and causes on the environmental nanoparticle that results on health and urban visibility.

12.3.4 Health Impacts

Large amount of nanoparticles the range of ultrafine size is deposited in lung. These nanoparticles can penetrate into the body either through skin, or gastrointestinal track and from there it can be permitted inside epithelial cells and stored in lymph nodes (Nemmar et al. 2002; Nel et al. 2006; Kumar et al. 2010). Hence nanoparticle act as a carrier because it carriers certain amount of toxins that results in induction of inflammatory response by means of reactive oxygen species or different mechanism. This results in adverse effects on human health (as concentration of nanoparticle number is exposed which is adverse).

12.3.5 Visibility

As these nanoparticles gets suspended in the atmosphere it result in visibility impairment (Horvath 2008; Kumar et al. 2010). This increases the relative humidity and atmospheric pressure and decreases the temperature and wind speed (Tsai 2005; Kumar et al. 2010). This is much advantageous in urban areas than in rural areas

because the urban area has higher concentration of atmospheric particles with high relative humidity, size and chemical composition of the particle. Urban visibility is caused mainly due to change in their chemical and optical properties which is mainly due to nanoparticles as it play vital role in development of coarse particles (Kittelson 1998; Kumar et al. 2010).

12.3.6 Nanoparticles in Environmental and Occupational Hazard

The adverse effects of health impacts due to occupational and environmental settings which were because of advancement nanotechnological area. The significant product nanoparticle is a manufacturing of Carbonaceous nanomaterial and their composites which becomes world wide product (Maynard et al. 2006; De Volder et al. 2013). These nanomaterial have certain benefits and increase in usage of CNTs, a major toxicology information and risk assessment incorporated with the lifecycle perception which is literally needed for the advancement of safety designed nanomaterial and nano applications. Several factors where involved for the induction of cytotoxicity of nanoparticles. Since the nanoparticles are toxic at certain levels and there exists no clear mechanism based on particle distributed within the body, some particles may gets accumulated on specific part of the body, they are considered as some critical issues (Kitaura et al. 2002; Boundy et al. 2006; Kumar et al. 2010). If accumulated nanoparticle has improper excretion that may leads to incessant toxicity (Ju-Nam and Lead 2008; Kumar et al. 2010).

12.3.7 Measurements and Methodologies Important for Characterization of Nanomaterials

Various technologies were used to detect *in vivo* and to quantify ROS. Different methods were used to detect ROS *in vivo* utilizing synthetic probe, whereas detecting *in vitro* is easy because many experiments were already undertaken to rectify non-specific reactions (Bartosz 2006; Wardman 2007).

Certain ultrafine particle have health effects which were exposed by advanced toxicological and evident studies (Donaldson et al. 2005; Murr and Garza 2009) and epidemiological (Ibald-Mulli et al. 2002). Even though natural system were included, the toxic impacts is a representative to the particle number concentration that were proposed in the above studies, this is due to ultrafine particles as they have (i) significance suspensions of ultrafine particle in the atmosphere result in prolong residence time (Air Quality Expert Group 2005), (ii) huge probability of penetration and deposition in respiratory and cardio vascular system (Donaldson et al. 2005; ICRP 1994), and (iii) In order to observe natural intensifies high surface region with unit volume containing larger particles used by which literally cancer causing

substitution (Agency 2002; Donaldson et al. 2005). Hence to assess toxicology properties of nanomaterial, nanotoxicology is widely used.

12.4 Safety and Risk Assessment

12.4.1 Nanomaterials Effect on Human

Nanoparticles have an indistinguishable, dimension from biological molecule. In the current scenario, these nanoparticles are explained clearly and as a result of susceptibility along with toxic characteristics, and concerns have aroused insisting that these materials deceit the novel health risk for the purchasers, specialist and environment (Braakhuis et al. 2016; Viswanath and Kim 2017). For example, an organism response to a specific nanomaterial might be identified with the amount of dose given, or might be identified together with its physicochemical properties (Viswanath and Kim 2017).

12.4.2 Nanomaterials- Biological Effects on Ecosystem

Nanoecotoxicology is a sub-discipline of ecotoxicology and especially aims to distinguish and anticipate effects drawn by nano sized materials on biological system. To express the exposure of nanotoxicology behaviour that takes places during their entry routes and the fate of nanoparticles in both abiotic and biotic condition, which is the main objective evolved (Oughton et al. 2008; Viswanath and Kim 2017). It also clearly explains that the physical and chemical properties and their morphology and structure have a much impact on toxicity (Sigg et al. 2014; Viswanath and Kim 2017). The rigorous characteristics and the accumulation of nanoparticles involve environmental risk assessment of engineered nanoparticles.

The two major processes is combined together (*i.e*), the remediation of organic pollutants, which reinforce in immobilization of organic compounds and metals which are done by using carbon nanotubes and zero-valent iron oxides. Nanoparticles have more bioavailability and cell uptake mechanism becomes more efficient (Morrison 2007; Sigg et al. 2014; Viswanath and Kim 2017).

Terrestrials and aquatic ecosystems, have a major impact on the nanomaterials due to the biological effects that are distributed among the environment.

12.4.3 Environmental Fate of Nanomaterials in Air

Eventhough the small particles present in the air is of fate one, there are still certain problems undergoing respective of the launching the procedures that govern their conduct, transport and fate (An-Hui et al. 2007; Nguyen et al. 2015; Viswanath and Kim 2017). Air helps in exposing those engineered nanoparticles to expose towards

sunlight that too especially UV wavelength of light, which will be bigger in size when compared to other environmental parts (Mody et al. 2010; Viswanath and Kim 2017).

The physicochemical properties of nanoparticles is based upon the fate of nanomaterials present in the surrounding, which contains a medium, cooperate with nanomaterials and some other environmental pollutants.

Once in a while nanoparticles can likewise be absent from the climate by dry and wet testimony, both of which are fit for to a great degree little particles of normal starting point thus apparently additionally for built nanomaterials (Clarke et al. 2004; Viswanath and Kim 2017).

12.4.4 Environmental Fate of Nanomaterials in Water

Nanomaterials may affect the water system in different ways. For example, aggregation and disaggregation, dispersion, interaction between nanoparticles and characteristic water components, transformation, biotic and abiotic degradation and photoreaction (Vale et al. 2016). Available literature comprehends the colloidal nanoparticles to be the possible conduct of engineered nanomaterials to enter the water ecosystem. As of now, nanomaterials are widely suggested for wastewater medicines because of their extraordinary properties. A few examinations report the different points of interest of nanotechnology in the remediation of wastewaters, however lacking explore has been coordinated toward the fate and potential effects of the solid residues after the use of such innovations (Nogueira et al. 2015; Viswanath and Kim 2017).

Subsequently, in aquatic condition nanoparticles may collaborate with common natural issue, normal colloids and suspended particulate issue, bringing about accumulation and conceivably sedimentation from arrangement.

The nanoparticles in aquatic condition are bioaccumulated by deposit and filter feeding organisms. Research on such interactions have not been completely contemplated till date because of the way that robust and sensitive analytical techniques are not yet accessible for detecting and characterizing nanoparticles in complex environmental matrices, for example, common waters and soils (Dusinska et al. 2015; Viswanath and Kim 2017) yet may extensively influence nanoparticle fate and toxic quality.

12.4.5 Environmental Fate of Nanomaterials in Soil

Nanomaterials are the smallest particles that can easily go through soil pores (Murr and Garza 2009; Viswanath and Kim 2017). Further they gets attached to soil particles because of their high surface area and gets immobilized (Mitrano et al. 2015; Viswanath and Kim 2017). Whereas, nanoparticles that forms aggregates gets immobilized by sedimentation, filtration, or straining in smaller pores. IWhile harmfulness mechanism have not yet been completely elucidated for most

nanoparticles, plausible mechanism incorporate interruption of mechanisms or mechanism potential, oxidation of proteins, genotoxicity, interference of vitality transduction, arrangement of responsive oxygen species, and arrival of toxic constituents (Xiao et al. 1999).

12.4.6 Ecotoxicological Impacts of Nanomaterials

Nanomaterials can be created by normally happening procedures, for example, volcanic action, fire, and disintegration; in that capacity, living beings have for quite some time been presented to and have advanced with these materials. Fabricated nanoparticles can enter the earth accidentally through climatic emanations, local wastewater, agribusiness, and unplanned discharge amid produce/transport; or through deliberate discharges, for example, soil and water remediation efforts (Weixian and Elliott 2006; Li et al. 2015).

With the high splendor, wide recurrence run, short beat width, exceptionally captivated, tunable, collimated pillars, SR procedures could accomplish much enhanced flag to commotion proportion, lessened procurement times and enhanced spatial determination contrasted with regular photon sources. *In vivo* and *in vitro* methodologies were utilized in characterizing the NMs to examine the various parts involved by SR systems, this is also manufactured using natural resources like living being and some other common habitants. Electron magnifying mechanism, counting TEM and SEM are widely used high level resolution instruments with spatial determination by 0.1 nm, which were used to characterize the nanomaterials (Li et al. 2015). However, due to low infiltration ability of electrons, thin examples are required for the electron magnifying lens estimation. Further, X-rays are more penetrating than electrons, which make the estimation of tests in 2D and even 3D conceivable with a spatial determination at several nanometer scale utilizing SR X-beams (Li et al. 2015).

On the whole, SR methods with the current light sources and with the anticipated fourth era XEFLs make it conceivable to misuse a high spatial determination and a high worldly determination, to vitality determination and better affectability in nanotoxicology deliberates contrasted with customary light sources. However, raising mindfulness about the remarkable limits of SR methods is still important among nanotoxicologists, despite the fact that insufficient shaft time is accessible considering the boundless use of SR procedures in many other scientific fields (Li et al. 2015).

12.4.7 Nanomaterials-Global Strategies to Address Human Health and/or Environmental Safety

Globally, numerous associations initiated research projects or systems intended to report human health and/ or on the other hand environmental safety aspects of nanomaterials (Zharov et al. 2006). On the impact of nanotoxicity based on human health

and environment along with European Commission (EC), in order organize and fit arrangements, problem based on concern and universal issues, this is monitored by Organization for Economic Co- activity and Development (OECD) (Schulte et al. 2016).

In order to report about the health and health concerns caused due to engineered nanomaterials, it was initiated by National Institute for Occupational Safety and Health (NIOSH) that built up the Nanotechnology Research Center (NTRC) in 2004 (Rashidi et al. 2015). This leads to the advancement, implementation of business nanotechnology that plays a vital role based on planning and research, by open and private sector along with the United States and abroad (Brenner et al. 2016).

12.4.8 Development of Test Protocols for Nanomaterials

As the need for manufactured nanomaterial is increasing day by day, the assortment of these materials should be carried on for the safety of human health. In this manner, toxicological research here is of most extreme significance and reemphasizes the importance of toxicology all in all. The objective of this exceptional issue was to incorporate the most late perspectives and work of driving specialists in nanotoxicology.

The main audit outlines our knowledge on the toxicity of quantum dots (QDs) specific to its surface modifications (Hoshino et al. 2011). As QDs are predominant for marking biomolecules and additionally cells, for example, in cancer therapy, they became valuable devices for essential research and nanomedicine with care towards their toxicity (Gibson et al. 2011).

Labelling nanomaterials with radioisotopes ideally will take into consideration the proper quantification of nanomaterials in distinctive biological environment and make ready for the proper dosimetry of nanomaterials (Gibson et al. 2011). Also, the decision of model systems for risk appraisal is a progressing wrangle about. Naturally, *in vitro* systems are presently investigated because of their simplicity, higher throughput and as another option to animal experiments (Clift et al. 2011; Weiss and Diabaté 2011).

The so far known cellular targets also, signal transduction pathways influenced by nanomaterials are explored, and this is surely simply the initiation of an interesting voyage to unwind mechanisms of actions in nanotoxicology (Weiss and Diabaté 2011). The extent to which surface changes of purposefully produced nanomaterials influence their toxic quality isn't known (Oberdörster et al. 2005b; Drobne 2007).

Administrative offices, proficient societies, scholarly group, NGOs, and industry are included in creating and approving standard rules for toxicity testing methodologies of nanomaterials to seize and maintain a strategic distance from undesirable shocks from deliberate or then again unexpected exposures to nanomaterials (Wang et al. 2007; Sommer and Golla-Schindler 2008). These techniques must be opportune and cost– viable, need to give toxicological data to the variety of nanomaterials (Drobne 2007).

12.4.9 Human and Environmental Risk Assessment of Nanomaterials

To evaluate the scientific data that rely upon the hazardous characteristics, involves mixture of operators, the dose- response relationship, and the degree of exposure to the people and the environmental targets to those operators. To explain about the probability of humans who were exposed and the ecosystem that are harmful and to some extent it causes risk for the human and other biological system is the result obtained (Mazzola 2003; OECD 2007; Wang et al. 2007; Sommer and Golla-Schindler 2008; Viswanath and Kim 2017).

(a) The foremost step to survey the hazardous substance is done by either identification or characterisation of materials. The adequate variety and many-sided quality of nanomaterials makes substance recognizable proof, characteristics more troublesome than with other chemicals. A more extensive range of properties will be expected to adequately describe a given nanomaterial, assessing the harmness and evaluate the hazard.
(b) Basic properties regarding the ecological condition of nanomaterials are not well comprehended. Models used to evaluate the natural surrounding and to examine customary chemicals are definitely not pertinent to deliberately created nanomaterials. Contingent upon the pertinence of concoction (physical and chemical) properties or change, new models may must be created to give estimations to new materials. In any case, a certain size of dependable exploratory information must be obtained before the ecological condition, transport, furthermore, interactive media analysis of nanomaterials can be viably displayed.
(c) To identify the nanomaterials from the natural source to provoke to a great grade of small sized particles, so far by adding either of one physical structure and phyisco-substance characteristics. The mixture of physical and chemical properties is influenced by either extracting or examining the biological systems which helps in evaluating nanomaterials (Maynard et al. 2007).

In particular, examined the part of covering compose on the toxic quality and gathering of ZnO nanoparticles (NPs) to soil-developed greenpea (*Pisumsativum L.*). Imperatively, this work as led to break down the effect of introduction amid the full lifecycle of the plant and incorporated an immediate correlation of the nanoparticles against relating mass and particle controls (Marmiroli and White 2016).

However, among the nanoparticle types, overall phytotoxicity and seed healthful quality was affected by molecule covering. Examined the phytotoxicity and genotoxic impacts of CeO_2 and TiO_2 nanoparticle introduction on grain under hydroponic conditions (Marmiroli and White 2016).

As the nanoparticle has a vast study as does its applications, there are many applications some of that includes microorganism, plant tissue, magnetic responsive drug delivery, types of cancer, nano sensors, environmental factors, antimicrobial, photo imaging and so on as shown in the Fig. 12.6.

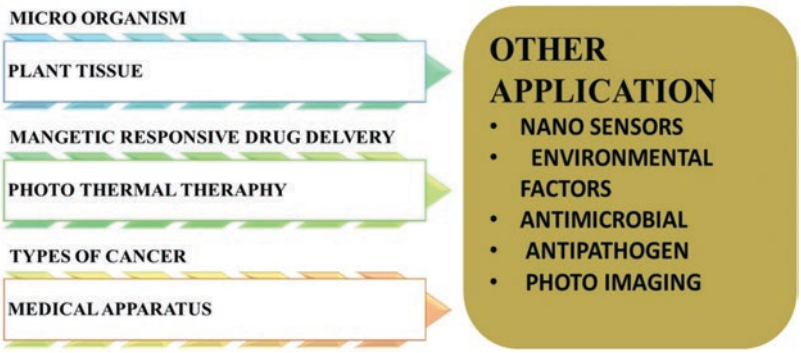

Fig. 12.6 Application of nanoparticals

12.5 Conclusion

In this review article, we summarized different imaging methods, which could be utilized for the physicochemical characteristics of NPs, basic examination of bio–nanorelation, representation of NPs *in vitro*, analysis of biodistribution, bioaccumulation, and change of NPs *in vivo*. Metabolomics strategies uncover tremendous potential to assess the impacts and toxic of nanomaterials. This synchrotron radiation procedures have been assuming an imperative part in the characterisation of NMs as made or in fortified natural structure in their *in vivo* and *in vitro* practices. Both analysis are generally simple to perform, take into consideration the discovery of worldwide DNA impairment and transformations at cellular and molecular level, and are additionally relevant in an inevitable resulting *in vivo* testing. Consequent examinations could include *in vitro* mammalian mutagenicity tests and possible *in vivo* genotoxicity testing. Nanomaterials because of their novel physical and substance trademark are broadly utilized as a part of research and pharmaceutical. Notwithstanding, these properties may now and again indicate disastrous impacts in people.

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