Chapter 2
Nanoparticles Types, Classification, Characterization, Fabrication Methods and Drug Delivery Applications

Abstract The most emerging branch in pharmaceutical sciences known as “Pharmaceutical nanotechnology” presents new tools, opportunities and scope, which are expected to have significant applications in disease diagnostics and therapeutics. Recently nano-pharmaceuticals reveal enormous potential in drug delivery as carrier for spatial and temporal delivery of bioactive and diagnostics. Additionally it also provides smart materials for tissue engineering. This discipline is now well-established for drug delivery, diagnostics, prognostic and treatment of diseases through its nanoengineered tools. Some nanotech based products and delivery systems are already in market. Pharmaceutical nanotechnology comprised of nano-sized products which can be transformed in numerous ways to improve their characteristics. Drugs that are transformed in to nano range offer some unique features which can lead to prolonged circulation, improved drug localization, enhanced drug efficacy etc. Various pharmaceutical nanotechnology based systems which can be termed as nanopharmaceuticals like polymeric nanoparticles, magnetic nanoparticles, liposomes, carbon nanotubes, quantum dots, dendrimers, metallic nanoparticles, polymeric nanoparticles, etc. have brought about revolutionary changes in drug delivery as well as the total medical service system. With the aid of nanopharmaceuticals, Pharmaceutical nanotechnology could have a profound influence on disease prevention to provide better insights into the molecular basis of disease. However some recently found health risk evidences limits their utilization in pharmaceutical industry. Some concerning issues like safety, bioethical issues, toxicity hazards, physiological and pharmaceutical challenges get to be resolved by the scientists. Current researchers are still lacking sufficient data and guidelines regarding safe use of these nanotechnology based devices and materials. Therefore pharmaceutical nanotechnology is still in infancy. The present chapter summarizes the types of nanopharmaceuticals with the most important applications and nanoparticles associated health risk related information available till present.

Keywords Nanotechnology • Nanoparticles • Types • Applications • Fabrication
2.1 Introduction

Delivering therapeutic compound to the desirable site is a major problem in treatment of many diseases. Conventional utilization of drugs is characterized by poor biodistribution, limited effectiveness, undesirable side effects, and lack of selectivity. Strategies like controlling drug delivery can potentially overcome these limitations by transporting drug to the place of action. Moreover drug delivery system provides protection against rapid degradation or clearance. It also enhances drug concentration in target tissues; therefore, lower doses of drug are required. Such type of therapy is required when there is a discrepancy between a dose or concentration of a drug and its therapeutic results or toxic effects. Targeting cell or specific tissue by the means individually designed carriers that are attached to drugs is a more reliable approach in drug delivery system. Such approach is known as cell or tissue specific targeting. Size reduction of targeted formulation and designing its pathways for suitable drug delivery system is a more fundamental and successful approach that forms the basis of nanotechnology. Recent advancement in nanotechnology has proven that nanoparticles acquire a great potential as drug carriers. Size reduction methods and technologies yields different types of nanostructures that exhibit unique physicochemical and biological properties. These methods make the nanostructures favorable material for biomedical applications and thus acquire the significance importance in pharmaceutical sciences. In addition these methods help in reducing toxicity, enhancing release, improving solubility and bioavailability and provide better formulation opportunities for drugs. Nanotechnology offers drugs in the nanometer size range which enhances the performance in a variety of dosage forms. Various advantages of nano sizing are mentioned below:

- Decreased fed/fasted variability
- Decreased patient-to-patient variability
- Enhanced solubility
- Increased oral bioavailability
- Increased rate of dissolution
- Increased surface area
- Less amount of dose required
- More rapid onset of therapeutic action

Nano word is originated from Latin word, which means dwarf. Ideal size range offered by nanotechnology refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a meter (i.e. 1 nm = 10^{-9} m). The branch nanotechnology is the science that particularly deals with the processes that occur at molecular level and of nano length scale size. Nanotechnology is now become an allied science which is most commonly used in other fields of science like electronic, physics and engineering since many decades. Recent exploration of nanotechnology in biomedical and pharmaceutical science results in successful improvement of conventional means of drug delivery system. This multidisciplinary science also covers several applications in other disciplines such as biophysics, molecular biology, and
bioengineering. Nanotechnology has created potential impact in various fields like medicine including immunology, cardiology, endocrinology, ophthalmology, oncology, pulmology etc. In addition it’s highly utilized in specialized areas like brain targeting, tumor targeting, and gene delivery. Nanotechnology also provides significant systems, devices and materials for better pharmaceutical applications.

Nanotechnology is the science of material featuring between $10^{-9}$ and $10^{-7}$ of a meter [1]. Or in another words it’s the science of materials and devices whose structures and constituents demonstrate novel and considerably altered physical, chemical and biological phenomenon due to their nanoscale size. Thus nanotechnology is defined as the manipulation of matter on an atomic, molecular, and supramolecular scale involving the design, production, characterization and application of different nanoscale materials in different potential areas providing novel technological advances mainly in the field of medicine. This forms an independent branch of nanostructures, referred as nanomedicine which is specifically utilized for medicines. Nanomedicine involves utilization of nanotechnology for the benefit of human health and well being. Nanomedicine was defined by European Science Foundation as ‘the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body’ [1]. This definition was revised by the US NIH as: ‘Nanomedicine refers to highly specific medical intervention at the molecular scale for curing diseases or repairing damaged tissues, such as bone, muscle, or nerve’. The European Science Foundation specified five sub-disciplines of nanomedicines [1]:

- Analytical tools
- Nanoimaging tools
- Nanomaterials and nanodevices
- Clinical and toxicological issues
- Novel therapeutics and drug delivery systems

These specified disciplines are overlapping which in many ways. The use of nanotechnology in various sectors of therapeutics has revolutionized the field of medicine where nanoparticles are designed and used for therapeutics, diagnostics, and as biomedical tools for research. With the help of nanotechnology it’s now possible to provide therapy at a molecular level which may further help in treating and pathogenesis of disease. Major limitations of conventional drugs (such as non specificity of drug action) urgently requires the developed system of nanomaterials which can be easily used in the diagnosis and treatment of various diseases especially cancer (have major limitations such as poor sensitivity or specificity and drug toxicities). Recently various novel and advance methods of cancer detection based on nanoparticles are being developed. These designed nanostructures are used as fluorescent materials, contrast agents, drugs with targeting antibodies and for molecular research tools. Recent modifications of nanoparticulate systems such as paramagnetic nanoparticles, quantum dots, nanoshells and nanosomes are widely used for diagnostic purposes. Nanotechnology provides the better safety profile against drugs with high toxic potential and these nanoforms can be directed to act specifi-
cally at the target tissue by active as well as passive means. In addition other modalities of therapy such as heat induced ablation of cancer cells by nanoshells and gene therapy are also being developed. Optimization of nanoparticles based drug delivery approaches concerns the early detection of cancer cells and/or specific tumor biomarkers, and the enhancement of the efficacy of the treatments applied. Prominent applications of nanomaterials in biomedical sciences are demonstrated in Figs. 2.1 and 2.2. Potential of nanomedicines in cancer is dependent on passive targeting (due to the enhance of the permeability and retention effect promoted by angiogenic vessels) which can be reinforced by specific targeting (based on multifunctional nanomaterials that bypass the biological barriers and reach cancer cells). Nanoparticles based specific drug targeting and delivery platforms reduce toxicity and other side effects and also improve the therapeutic index of the targeted drug. In the primary objective of nanotechnology especially in cancer therapy is the development of suitable targeting delivery systems which has been taking the lead in what concerns overcoming the MDR problem. Such targeted delivery systems that are based ‘Nanosizing’ of drugs:

- Decrease drug resistance
- Decrease toxicity [2]
- Enhance oral bioavailability [3]
- Enhance rate of dissolution
- Enhance solubility [4]
- Increase the stability of drug and formulation [5]
- Increase drug targeting ability [6–8]
- Increase patient compliance [5]
- Increase surface area
- Reduce the dose needed [9]

![Fig. 2.1 Various nanoforms and their morphological features](image-url)
Such advantages lead to the development of most efficient targeted therapeutic nanoparticle which is the potential to revolutionize the drug development process and change the landscape of the pharmaceutical industry [10, 11]. Considering the unique physicochemical properties, nanoparticles have shown promise in delivering a range of molecules to desired sites in the body. These targeted nanomedicines may improve the therapeutic index of drugs by enhancing their efficacy and/or increasing their tolerability in the body. Nanotechnology is also efficient in improving the bioavailability of water-insoluble drugs, protect the therapeutic agents from physiological barriers, enable the development of novel classes of bioactive macromolecules as well as carry large payloads. In addition integration of imaging contrast agents within nanoparticles can allows the drug delivery site visible to us and examination of in vivo efficacy of the therapeutic agent [12]. So far various nanotechnology products have been approved by the US Food and Drug Administration (FDA) for clinical use, and many are under clinic and preclinical development [13]. Among these clinically approved products the first-generation nanotechnology products are liposomal drugs and polymer–drug conjugates, which are relatively simple and generally lack active targeting or controlled drug release components. While designing therapeutic nanoparticles the ultimate goal of nanotechnology is to develop safer and more effective therapeutic nanoparticles. Therefore the main focus of current researchers is to design novel multifunctional nanoparticle platforms for cell/tissue-specific targeting, sustained or triggered drug delivery, co-delivery of synergistic drug combinations, etc.
This chapter discusses the various platforms of nanotechnology that are being used in different aspects of medicine with special focus on targeted drug delivery systems and novel therapeutics based on nanotechnology. Here, we have also discussed the recent applications on nanoparticles as platforms for anticancer therapy, emphasizing strategies for targeted delivery for gene silencing focusing on the optimal pathways to test these therapeutics in vitro and in vivo. It’s very essential to report toxicological aspects of these nano materials. Therefore potential toxicities of the nanoparticles are also described in addition to the safety of nanomedicine is not fully defined yet. However, it is possible that nanomedicine in future would play a crucial role in the treatment of human diseases and also in enhancement of normal human physiology.

One of the emerging branches among biomedical sciences is pharmaceutical technology. Pharmaceutical nanotechnology covers the applications of nanotechnology to pharmacy as nanomaterials, and as devices like drug delivery, diagnostic, imaging and biosensor. Pharmaceutical nanotechnology has provided more fine-tuned diagnosis and focused treatment of disease at a molecular level. Pharmaceutical nanotechnology offers various opportunities to fight against many diseases. It helps in detecting the antigen associated with diseases such as cancer, diabetes mellitus, neurodegenerative diseases, as well as detecting the microorganisms and viruses associated with infections. It is expected that in next 10 years market will be flooded with nanotechnology devised medicine. Applications of nanotechnology to pharmacy that provide intelligent and smart drug delivery systems is expected to emerge as most important and powerful tool as alternate to conventional dosage form. These nano-intelligent drug delivery systems need little investment while expected to be a high profit making deal due to new patent protection for current or soon-to-be off-patent drugs. A recent report claimed that 23 major pharmaceutical patents would expire by 2008 leading to revenue loss of US $ 46 billion and by 2011, US $ 70–80 billion loss is expected as various drugs go off-patent. Pharmaceutical Nanotechnology based systems presents two basic types of nano tools viz. nanomaterials and nanodevices, which play a key role in realm of pharmaceutical nanotechnology and related fields. Nanomaterials are biomaterials used, for example, in orthopedic or dental implants or as scaffolds for tissue-engineered products. Their surface modifications or coatings might greatly enhance the biocompatibility by favoring the interaction of living cells with the biomaterial. These materials can be sub classified into nano-crystalline and nanostructured materials. Nano crystalline materials are readily manufactured and can substitute the less performing bulk materials. Raw nanomaterials can be used in drug encapsulation, bone replacements, prostheses, and implants. Nanostructured materials are processed forms of raw nanomaterials that provide special shapes or functionality, for example quantum dots, dendrimers, fullerenes and carbon nanotubes. Nanodevices are miniature devices in the nanoscale and some of which include nano- and microelectromechanical systems, microfluidics, and microarrays. Examples include biosensors and detectors to detect trace quantities of bacteria, airborne pathogens, biological hazards, and disease signatures and some intelligent machines like respirocyte (Figs. 2.1 and 2.2). Various prominent features and applications of nanosystems are mentioned Table 2.1.
### Table 2.1 Various characteristics and brief applications of nanosystems [14]

<table>
<thead>
<tr>
<th>Types of Nanosystems</th>
<th>Size (nm)</th>
<th>Characteristics</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon nanotubes</td>
<td>0.5–3 diameter and 20–1000 length</td>
<td>Third allotropic crystalline form of carbon sheets either single layer (SWNT) or multiple layer (MWNT).</td>
<td>Functionalization enhanced solubility, penetration to cell cytoplasm and to nucleus, as carrier for gene delivery, peptide delivery</td>
</tr>
<tr>
<td>Dendrimer</td>
<td>&lt;10</td>
<td>Highly branched, nearly monodisperse polymer system produced by controlled polymerization; three main parts core, branch and surface</td>
<td>Long circulatory, controlled delivery of bioactives, targeted delivery of bioactives to macrophages, liver targeting</td>
</tr>
<tr>
<td>Liposome</td>
<td>50–100</td>
<td>Phospholipid vesicles, biocompatible, versatile, good entrapment efficiency, offer easy</td>
<td>Long circulatory, offer passive and active delivery of gene, protein, peptide and various other</td>
</tr>
<tr>
<td>Metallic nanoparticles</td>
<td>&lt;100</td>
<td>Gold and silver colloids, very small size resulting in high surface area available for functionalization, stable</td>
<td>Drug and gene delivery, highly sensitive diagnostic assays, thermal ablation and radiotherapy enhancement</td>
</tr>
<tr>
<td>Nanocrystals Quantum dots</td>
<td>2–9.5</td>
<td>Semi conducting material synthesized with II–VI and III–V column element; Size between 10 and 100 Å; Bright fluorescence, narrow emission, Broad UV excitation and high photo stability</td>
<td>Long term multiple color imaging of liver cell; DNA hybridization, immunoassay; receptor mediated endocytosis; labeling of breast cancer marker HeR2 surface of cancer cells</td>
</tr>
<tr>
<td>Polymeric micelles</td>
<td>10–100 nm</td>
<td>Block amphiphilic copolymer micelles, high drug entrapment, payload, biostability</td>
<td>Long circulatory, target specific active and passive drug delivery, diagnostic value</td>
</tr>
<tr>
<td>Polymeric nanoparticles</td>
<td>10–1000</td>
<td>Biodegradable, biocompatible, offer complete drug protection</td>
<td>Excellent carrier for controlled and sustained delivery of drugs. Stealth and surface modified nanoparticles can be used for active and passive delivery of bioactives</td>
</tr>
</tbody>
</table>
2.2 Classification of Nanoparticles

Nanoparticles are broadly classified into three classifications [15]

- **One dimension nanoparticles**
  One dimensional system (thin film or manufactured surfaces) has been used for decades. Thin films (sizes 1–100 nm) or monolayer is now common place in the field of solar cells offering, different technological applications, such as chemical and biological sensors, information storage systems, magneto-optic and optical device, fiber-optic systems.

- **Two dimension nanoparticles**
  Carbon nanotubes

- **Three dimension nanoparticles**
  Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (QDs)

2.3 Characterization of Nanoparticles

Characterization of nanoparticles is based on the size, morphology and surface charge, using such advanced microscopic techniques as atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Properties such as the size distribution, average particle diameter, charge affect the physical stability and the in vivo distribution of the nanoparticles. Properties like surface morphology, size and overall shape are determined by electron microscopy techniques. Features like physical stability and redispersibility of the polymer dispersion as well as their \textit{in vivo} performance are affected by the surface charge of the nanoparticles. Different characterization tools and methods for nanoparticles are mentioned in Table 2.2. Therefore it’s very important to evaluate the surface charge during characterization of nanoparticles.

2.3.1 Particle Size

Characterizations of nanoparticles are primarily evaluated by the particle size distribution and morphology. With the aid of electron microscopy it’s now possible to ascertain the morphology as well as the size of nanoparticles. Application of nanoparticles in drug release and drug targeting can be conveniently determined by various tools. It has already been reported that particle size of nanoparticles has profound effect on the drug release.
Table 2.2 Various characterization tools and methods for nanoparticles [16]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Characterization method</th>
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<tbody>
<tr>
<td>Carrier-drug interaction</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>Charge determination</td>
<td>Laser Doppler Anemometry</td>
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<tr>
<td></td>
<td>Zeta potentiometer</td>
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<tr>
<td>Chemical analysis of surface</td>
<td>Static secondary ion mass spectrometry</td>
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<tr>
<td></td>
<td>Sorptometer</td>
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<tr>
<td>Drug stability</td>
<td>Bioassay of drug extracted from Nanoparticles</td>
</tr>
<tr>
<td></td>
<td>Chemical analysis of drug</td>
</tr>
<tr>
<td>Nanoparticle dispersion stability</td>
<td>Critical flocculation temperature (CFT)</td>
</tr>
<tr>
<td>Particle size and distribution</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td></td>
<td>Laser defractometry</td>
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<tr>
<td></td>
<td>Photon correlation spectroscopy (PCS)</td>
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<tr>
<td></td>
<td>Scanning electron microscopy</td>
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<tr>
<td></td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>Release profile</td>
<td>In vitro release characteristics under physiologic and sink</td>
</tr>
<tr>
<td></td>
<td>conditions</td>
</tr>
<tr>
<td>Surface hydrophobicity</td>
<td>Rose Bengal(dye) binding</td>
</tr>
<tr>
<td></td>
<td>Water contact angle measurement</td>
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<tr>
<td></td>
<td>X-ray photoelectron spectroscopy</td>
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</tbody>
</table>

Smaller the size of nanoparticles larger surface area, which results in to fast drug release. Loaded drug when exposed to the particle surface area causes significant drug release. In contrast, inside the nanoparticles drugs slow diffusion of larger particles occurs. Consequently smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion. Therefore there is a mutual compromise between maximum stability and small size of nanoparticles [17]. In addition degradation of the polymer can also be affected by the particle size e.g. the extent of poly (lactic-co-glycolic acid) degradation was found to increase with increasing particle size in vitro [18]. With the advancement in analytical tools various techniques are now available for determining nanoparticle size as discussed below:

### 2.3.1.1 Photon-Correlation Spectroscopy (PCS) or Dynamic Light Scattering (DLS)

Current research demands the fastest and most popular method of determining particle size. The fastest and most popular techniques like photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS), widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. In this technique solution of spherical particles in Brownian motion causes a Doppler shift when they are exposed against shining monochromatic light (laser). Such monochromatic light exposure hits the moving particle which results in
changing the wavelength of the incoming light. Extent of this change in wavelength determines the size of the particle. This parameter assists in evaluation of the size distribution, particle's motion in the medium, which may further assist in measuring the diffusion coefficient of the particle and using the autocorrelation function. Dynamic light scattering (DLS) offer the most frequently used technique for accurate estimation of the particle size and size distribution [19].

2.3.1.2 Scanning Electron Microscopy (SEM)

This electron microscopy based technique determines the size, shape and surface morphology with direct visualization of the nanoparticles. Therefore scanning electron microscopy offer several advantages in morphological and sizing analysis. However they provide limited information about the size distribution and true population average. During the process of SEM characterization, solution of nanoparticles should be initially converted into a dry powder. This dry powder is then further mounted on a sample holder followed by coating with a conductive metal (e.g. gold) using a sputter coater. Whole sample is then analyzed by scanning with a focused fine beam of electrons [20]. Secondary electrons emitted from the sample surface determine the surface characteristics of the sample. This electron beam can often damage the polymer of the nanoparticles which must be able to withstand vacuum. Average mean size evaluated by SEM is comparable with results obtained by dynamic light scattering. In addition these techniques are time consuming, costly and frequently need complementary information about sizing distribution [21].

2.3.1.3 Transmission Electron Microscope

Experimental difficulties in studying nanostructures stem from their small size, which limits the use of traditional techniques for measuring their physical properties. Transmission electron microscopy techniques can provide imaging, diffraction and spectroscopic information, either simultaneously or in a serial manner, of the specimen with an atomic or a sub-nanometer spatial resolution. TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra thin for the electron transmittance. High-resolution TEM imaging, when combined with nanodiffraction, atomic resolution electron energy-loss spectroscopy and nanometer resolution X-ray energy dispersive spectroscopy techniques, is critical to the fundamental studies of importance to nanoscience and nanotechnology. During the TEM characterization nanoparticles dispersion is deposited onto support grids or films. After dispersion they are fixed using either a negative staining material (phosphotungstic acid or derivatives, uranyl acetate, etc., or by plastic embedding). This is done to make nanoparticles withstand against the instrument vacuum and facilitate handling. Alternatively nanonoparticles sample can also be exposing to liquid nitrogen temperatures after embedding in vitreous ice. When a
beam of electrons is transmitted through an ultra thin sample it interacts with the sample as it passes through The surface characteristics of the sample are obtained [21]. TEM imaging mode has certain benefits compared with the broad-beam illumination mode:

- Collection of the information about the specimen using a high angular annular dark field (HAADF) detector (in which the images registered have different levels of contrast related to the chemical composition of the sample)
- It can be utilized for the analysis of biological samples is its contrast for thick stained sections, since high angular annular dark field images (samples with thickness of 100–120 nm) have better contrast than those obtained by other techniques.
- Combining HAADF-TEM imaging leads to imaging the atomistic structure and composition of nanostructures at a sub-angstrom resolution.
- Availability of sub-nanometer or sub-angstrom electron probes in a TEM instrument, due to the use of a field emission gun and aberration correctors, ensures the greatest capabilities for studies of sizes, shapes, defects, crystal and surface structures, and compositions and electronic states of nanometer-size regions of thin films, nanoparticles and nanoparticle systems.

2.3.1.4 Atomic Force Microscopy

This technique is also known as scanning force microscopy (technique that forms images of surfaces using a prob that scans the specimen), very high resolution type of scanning probe microscopy, with reported resolution on the order of fractions of a nanometer, more than 100 times better than the optical diffraction limit. The atomic force microscopy is based on a physical scanning of samples at sub-micron level using a probe tip of atomic scale and offers ultra-high resolution in particle size measurement [22]. Depending upon properties, samples are usually scanned in contact or noncontact mode. During contact mode, the topographical map is generated by tapping the probe on to the surface across the sample and probe hovers over the conducting surface in non-contact mode. One of the prime advantage of AFM is its ability to image non-conducting samples without any specific treatment. This feature allows the imaging of delicate biological and polymeric nano and microstructures [23]. Moreover AFM (without any mathematical calculation) provides the most accurate description of size, size distribution and real picture which helps in understanding the effect of various biological conditions [24].

2.3.2 Surface Charge

Surface charge and intensity determines the interaction of nanoparticles with the biological environment as well as their electrostatic interaction with bioactive compounds. Stability of colloidal material is usually analyzed through zeta potential of
nanoparticles. Zeta potential is an indirect measure of the surface charge. It can be obtained by evaluating the potential difference between the outer Helmholtz plane and the surface of shear. Thus zeta potential of colloidal based dispersion assists in directly evaluating its storage stability. Zeta potential values (high zeta potential values, either positive or negative) are achieved in order to ensure stability and avoid aggregation of the particles. Zeta potential values can be utilized in evaluating surface hydrophobicity and the nature of material encapsulated within the nanocapsules or coated onto the surface [25].

2.3.3 Surface Hydrophobicity

Techniques such as hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc. can be utilized for the determination of surface hydrophobicity. Recent advancement in research offers several sophisticated analytical tools for surface property analysis of nanoparticles. modern technique such as X-ray photon correlation spectroscopy not only determine surface hydrophobicity but also permits the identification of specific chemical groups on the surface of nanoparticles [26].

2.3.4 Drug Release

It’s very essential to determine extent of the drug release and in order to obtain such information most release methods require that the drug and its delivery vehicle be separated. drug loading capacity of the nanoparticles is defined as the amount of drug bound per mass of polymer or in another term it is the moles of drug per mg polymer or mg drug per mg polymer or it could also be given as percentage relative to the polymer. Various techniques such as UV spectroscopy or high performance liquid chromatography (HPLC) after ultracentrifugation, ultra filtration, gel filtration, or centrifugal ultrafiltration are used to determine this parameter. Methods that are employed for drug release analysis are also similar to drug loading assay which is more often assessed for a period of time to evaluate the drug release mechanism [27, 28].

2.4 Preparation of Nanoparticles

The selection of appropriate method for the preparation of nanoparticles depends on the physicochemical character of the polymer and the drug to be loaded. Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including [29]:

• Antigenicity of the final product.
• Biocompatibility and toxicity
2.4 Preparation of Nanoparticles

- Degree of biodegradability
- Drug release profile desired
- Inherent properties of the drug (aqueous solubility and stability)
- Size of nanoparticles required
- Surface characteristics (charge and permeability)

Nanoparticles have been usually prepared by three methods:

- Dispersion of preformed polymers
- Ionic gelation or coacervation of hydrophilic polymers
- Polymerization of monomers

However, other methods such as supercritical fluid technology [30] and particle replication in non-wetting templates [31] have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in industry.

Dispersion of preformed polymers: This technique is based on the preparation of biodregerable nanoparticles via dispersion of biodegradable polymers such as poly (D, L-glycolide), poly (lactic acid) (PLA); poly (cyanoacrylate) (PCA), and PLG; poly (D, L-lactide-co-glycolide) (PLGA) [32–34]. Dispersion of preformed polymers to prepare the nanoparticles can be used in various ways:

2.4.1 Solvent Evaporation Method

Solvent evaporation method is one of the most frequently used methods for the preparation of nanoparticles. This method involves two steps (first is emulsification of the polymer solution into an aqueous phase and second is evaporation of polymer solvent, inducing polymer precipitation as nanospheres). This method is based on the solubility of polymer and hydrophobic drug since both polymer and hydrophobic drug are dissolved in an organic solvent (dichloromethane, chloroform or ethyl acetate) which is also used as the solvent for dissolving the. Mixture obtained from polymer and drug solution is then emulsified in an aqueous solution. This aqueous solution contains surfactant or emulsifying agent to form oil in water (o/w) emulsion. Once the stable emulsion forms, the organic solvent is evaporated either by continuous stirring or by reducing the pressure. Size range of nanoparticles was found to be influenced by the concentrations and type of stabilizer, polymer concentration and homogenizer speed [35]. Ultrasonication or high-speed homogenization may be often employed in order to produce small particle size [36]. The nanoparticles are collected by ultracentrifugation and washed with distilled water to remove stabilizer residue or any free drug and lyophilized for storage [37]. Modification of this method is known as solvent evaporation method and high pressure emulsification [38]. This method involves preparation of a emulsion which is then subjected to homogenization under high pressure followed by overall stirring to remove organic solvent [39]. The size can be controlled by adjusting the stirring rate, type and amount of dispersing agent, viscosity of organic and aqueous phases and
temperature [40]. However this method can be applied to liposoluble drugs and limitation are imposed by the scale up issue. Polymers used in this method are PLGA [41], PLA [42], cellulose acetate phthalate [43], EC [44], Poly (β-hydroxy-butyrate) (PHB) [45], Poly (β-caprolactone) (PCL) [46].

### 2.4.2 Spontaneous Emulsification or Solvent Diffusion Method

This method is developed from solvent evaporation method [47], in which the water miscible solvent along with a small amount of the organic solvent (water immiscible) is used as an oil phase. During the spontaneous diffusion of solvents between the two phases an interfacial turbulence is generated which may ultimately leads to the formation of small particles. Smaller particle size can be achieved by increasing the concentration of water miscible solvent increases. This method can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

### 2.4.3 Double Emulsion and Evaporation Method

Most of the emulsion and evaporation based methods suffer from the limitation of poor entrapment of hydrophilic drugs. Therefore to encapsulate hydrophilic drug the double emulsion technique is employed, which involves the addition of aqueous drug solutions to organic polymer solution under vigorous stirring to form w/o emulsions. This w/o emulsion is added into second aqueous phase with continuous stirring to form the w/o/w emulsion. The emulsion then subjected to solvent removal by evaporation and nano particles can be isolated by centrifugation at high speed. The formed nanoparticles must be thoroughly washed before lyophilisation [48]. In this method the amount of hydrophilic drug to be incorporated, the concentration of stabilizer used, the polymer concentration, the volume of aqueous phase are the variables that affect the characterization of nanoparticles [48, 49].

### 2.4.4 Salting Out Method

Method involves the separation of a water-miscible solvent from aqueous solution via a salting-out effect [50]. It’s based on the on the separation of a water miscible solvent from aqueous solution via a salting-out effect. During the initial process polymer and drug are dissolved in a solvent which is subsequently emulsified into an aqueous gel containing the salting out agent and a colloidal stabilizer. Various types of salting out agents (electrolytes, such as magnesium chloride and calcium chloride, or non-electrolytes such as sucrose) and colloidal stabilizer (such as
polyvinylpyrrolidone or hydroxyethylcellulose) have been used. This lead to formation of oil/water emulsion which is further diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of solvent into the aqueous phase, ultimately induce the formation of nanospheres. Parameters such as stirring rate, internal/external phase ratio, concentration of polymers in the organic phase, type of electrolyte concentration and type of stabilizer in the aqueous phase can be varied in this process [43]. Salting out method is reported for preparation of ethyl cellulose and PLA, Poly(methacrylic) acids nanospheres. leads to high efficiency and is easily scaled up [51, 52].

2.4.4.1 Advantages

- Does not require an increase of temperature and therefore may be useful when heat sensitive substances have to be processed [53].

2.4.4.2 Disadvantages

- Limited application to lipophilic drug and the extensive nanoparticles washing steps

2.4.5 Emulsions-Diffusion Method

This is another widely used method to prepare nanoparticles. The encapsulating polymer is dissolved in a partially water-miscible solvent (such as propylene carbonate, benzyl alcohol), and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point. This technique presents several advantages, such as high encapsulation efficiencies (generally 70%), no need for homogenization, high batch-to-batch reproducibility, ease of scaleup, simplicity, and narrow size distribution.

2.4.5.1 Disadvantages

Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency [54]. Several drug-loaded nanoparticles were produced by the technique, including mesotetra...
(hydroxyphenyl) porphyrin-loaded PLGA (p-THPP) nanoparticles [55], doxorubicin-loaded PLGA nanoparticles, and cyclosporine (cy-A-); loaded sodium glycolate nanoparticles [56].

2.4.6 **Solvent Displacement/Precipitation Method**

In this method preformed polymer is precipitated in an organic solution and organic solvent is diffused in the aqueous medium. Diffusion of organic solvent can be achieved in the presence or absence of surfactant. Semi polar water miscible solvent such as acetone or ethanol can be used to dissolve the polymers, drug, and or lipophilic surfactant. After their complete dissolution, solution is then poured or injected into an aqueous solution containing stabilizer under magnetic stirring. Nano particles are formed immediately by the rapid solvent diffusion. This step is followed by the removal of solvent from the suspensions under reduced pressure. Particles size is dependent on the extent of addition of the organic phase into the aqueous phase. It was also found that decrease in both particles size and drug entrapment occurs as the mixing rate of the two phase increases [57]. This method is more suitable for poorly soluble drugs. Optimization of various parameters (preparation parameters) can effectively control size, drug release and yield of nanosphere.

Nanosphere size, drug release and yield were shown to be effectively controlled by adjusting preparation parameters. Regulation the concentration of polymer in the organic phase was reported to be useful in the production of smaller sized nanospheres. However size range is restricted to minimum range of the polymer to drug ratio.

2.4.7 **Coacervation or Ionic Gelation Method**

Recent exploration of biodegradable polymers such as gelatin and sodium alginate has been focused now to yield biodegradable nanoparticles having features like biocompatibility and low toxicity. Methods such as ionic gelation can be used for preparing hydrophilic polymer based nanoparticles. Calvo and co-workers developed method for preparing chitosan based nanoparticles by ionic gelation method [58, 59]. In this method two different aqueous phases are prepared for polymer (chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is for polyanion sodium tripolyphosphate. This method is based on the strong electrostatic interaction between positively charged amino group of chitosan and negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Existence of strong electrostatic interaction between two aqueous phases leads to the formation of coacervates. In contrast ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.
2.4 Preparation of Nanoparticles

2.4.8 Polymerization Method

This method involves polymerization of monomers to form nanoparticles in an aqueous solution. In polymerization drug is incorporated at two different stages (either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed) [60, 61]. Ultracentrifugation can be used to purify nanoparticle suspension by removing various stabilizers and surfactants employed for polymerization, followed by the re-suspension of particles in an isotonic surfactant-free medium. This technique is reported for making poly (alkyl-cyanoacrylate) or polybutylcyanoacrylate nanoparticles. Desirable size of nanocapsule can be achieved by optimization of concentration of the surfactants and stabilizers [62].

2.4.9 Production of Nanoparticles Using Supercritical Fluid Technology

Above mentioned conventional methods (such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods) obligatory use organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, there is an urgent requirement of suitable technology which avoid the usage of organic solvents or any other ingredient hazardous to health. Since supercritical fluids are environmentally safe, therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles [63]. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive. Supercritical fluids are those fluids which are at a temperature above its critical temperature remains in a single phase regardless of pressure [63]. CO₂ (SC CO₂) is the most widely used supercritical fluid because of its mild critical conditions, non-flammability, low price and nontoxicity. Among the various processing techniques involving supercritical fluids, supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS) are the most common one. In former process a liquid solvent (methanol) is selected on the basis of it’s completely miscibility with the supercritical fluid (SC CO₂). This is done to dissolve the solute to be micronized at the process conditions. Since the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, results in the formation of nanoparticles. This process is reported for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose. In later process called as RESS, solute is dissolved in a supercritical fluid such as supercritical methanol and then the solution is rapidly expanded through a small nozzle into a region lower pressure [63]. This dramatically affects the solvent power of supercritical fluids which is ultimately decreases and the solute eventually precipitates. RESS and its modified process have been used for the product of polymeric nanoparticles [64].
2.5 Most Favorable Requirements for Designing Therapeutic Nanoparticles

One of the most primary requirements before designing therapeutic nanoparticles is the rapid clearance during systemic delivery. After entering the bloodstream nanoparticles surface may experience nonspecific protein adsorption called as opsonization. This process makes them more visible to phagocytic cells. These opsonized nanoparticles could be easily cleared from the bloodstream through phagocytosis by the mononuclear phagocyte system (MPS) in the liver and by spleen filtration. Factors that govern the clearance and biodistribution of nanoparticles should be considered before designing therapeutic nanoparticles. Nanoparticle size plays an important role in controlling circulation and biodistribution of nanoparticles during its journey through physiological parameters such as hepatic filtration, tissue extravasation/diffusion, and kidney excretion. Nanoparticles size range <10 nm can be rapidly cleared by the kidneys or through extravasation, while larger particles size may have higher tendency to be cleared by cells of the mononuclear phagocyte system (MPS also referred to as reticuloendothelial system, RES). It was also found that PEGylated spherical nanoparticles (<100 nm, 100–200 nm, and >200 nm) showed different protein absorption rate since particle size influence its uptake by murine macrophages, and blood clearance kinetics. It was reported that nanoparticles <100 nm remain in the blood for long periods of time and experience reduced hepatic filtration. Additionally nanoparticle size also affects their accumulation rate in tumor or its surroundings. This accumulation is achieved through the EPR effect. Thus to take advantage of the EPR effect and to efficiently escape from the physiological barriers, many studies advocate the optimal nanoparticle size range of approximately 10–250 nm. Second factor that could affect nanoparticles uptake by the MPS cells is their surface charge. Positively charged nanoparticles generate a higher immune response compared to neutral or negatively charged nanoparticle formulations. Similarly neutrally charged particles have demonstrated much lower opsonization rates than charged particles. For reduced phagocytosis and minimized nonspecific, it has been demonstrated optimal range of nanoparticle is between −10 and +10 mV. Third factor, PEGylation, referred to the surface modification of nanoparticles with PEG, which has favorable intrinsic physicochemical properties which was found to reduce nanoparticle accumulation in off-target organs. A hydrophobic or charged particles PEG shell on the nanoparticle, leads to prolonged circulation half-life compared to non-PEGylated nanoparticles. Several factors such as length, shape, and density of PEG chains on the nanoparticle surface largely affect its surface hydrophilicity and phagocytosis. Brush configuration in PEGylated nanoparticles would create more effective blocking or repulsion of opsonins than the mushroom one. Fourth factor i.e. ligand functionalization is the conjugation of targeting ligands to the surface of PEGylated nanoparticles which has also been proven to affect their biodistribution. However targeting ligands could improve the cell- or tissue-specific delivery of nanoparticles. They may compromise the particle surface properties by masking the PEG layer and adversely affecting the nanoparticles’ antibiofouling properties in vivo.
2.6 Types of Pharmaceutical Nanosystems

2.6.1 Carbon Based Structures

Carbon nano tubes are carbon based tubular structures that are discovered in 1991 [65]. These structures are arranged in fashion like a graphite sheet rolled up into a cylinder and capped at one or both ends by a buckyball. These are hexagonal networks of carbon atoms having diameter of one nanometer and length from 1 to 100 nm. These carbon networks are arranged layer of graphite rolled up into a cylinder. There are two carbon based configuration that have received much attention recently: single-walled nanotubes (SWNTs) and multiwalled nanotubes (MWNTs). In addition to these types C_{60} fullerenes is also a part of common configurations. These are hollow, carbon-based, cage-like architectures (nanotubes and fullerenes), also known as bucky balls, which are differ in the arrangement of their graphite cylinders. The size, geometry, and surface characteristics of these macromolecules make them appealing for drug carrier usage and have remarkable physical proper. SWNTs and C_{60} fullerenes have internal diameters range of 1–2 nm. This dimension is equivalent to about half the diameter of the average DNA helix, whereas MWNTs have diameters ranging from several nanometers to tens of nanometers with 0.36 nm distance between layers of MWCNT, depending on the number of walls in the structure. Size may vary in their length ranging from 1 μm to a few micrometers [66]. As far as their architecture is concerned fullerenes and carbon nanotubes are typically fabricated using laser ablation, chemical vapor deposition, electric arc discharge, or combustion processes. Characterization of these concentric forms is based on their strength and stability so that they can be used as stable drug carriers. Cellular entry of nanotubes may be mediated by endocytosis or by insertion through the cell membrane. Fullerenes have also shown drug targeting capability. Tissue-selective targeting and intracellular targeting of mitochondria have been shown with use of fullerene structures. Furthermore, experiments with fullerenes have also shown that they exhibit antioxidant and antimicrobial behavior. Carbon nanotubes and their applications are highlighted in Table 2.3. Some of well known examples of carbon nantubes and their respective applications are highlighted in Table 2.3.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type of disease</th>
<th>Type of CNTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Leishmania donovani (parasite)</td>
<td>MWCNTs</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>Bladder cancer</td>
<td>Not specified</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>Leukemia</td>
<td>SWCNTs</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Lymphoma</td>
<td>SWCNTs</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>Ovarian cancer</td>
<td>SWCNTs</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Breast cancer</td>
<td>MWCNs</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Breast cancer</td>
<td>SWCNTs</td>
</tr>
</tbody>
</table>
2.6.1.1 Applications

• **Cell specificity**
  Enhancement of cell specificity by conjugating antibodies to carbon nanotubes with fluorescent or radiolabelling [68]

• **Internalization**
  Internalization within mammalian cells can be achieved by surface-functionalized carbon nanotubes

• **Vaccine delivery**
  Conjugation with peptides may be used as vaccine delivery structures

• **Gene delivery**
  With the advancement in molecular dynamics simulations, the flow of water molecules through surface-functionalized carbon nanotubes has been modeled in such a way so that they can be conveniently utilized as small molecule transporters in transporting DNA, indicating potential use as a gene delivery tool. The ability of nanotubes to transport DNA across cell membrane is used in studies involving gene therapy. During this therapy DNA can be attached to the tips of nanotubes or can be incorporated within the tubes. It has been found that gene therapy with β galactosidase marker gene nanotubes showed greater expression compared to transfer of naked DNA. This assures the advantage of non immunogenicity in contrast to viral vectors used for gene transfer.

• **Transport of peptides, nucleic acids and other drug molecules**
  Incorporation of carboxylic or ammonium groups to carbon nanotubes enhances their solubility which makes them more suitable for the transport of peptides, nucleic acids and other drug molecules. Pristine carbon nanotubes is a common example of water insoluble forms having high in vitro toxicity compared to modified water dispersible forms of nanotubes. It has been also proven that the extent of toxicity decreases with functionalization. However functionalization also affects the elimination of the nanotube. Single-walled nanotubes without conjugation to monoclonal antibody have a high renal uptake. Whereas modest liver uptake as compared to single-walled nanotubes with conjugation to monoclonal antibody which is having higher liver uptake and lower renal uptake. In addition it was also reported that carbon nanotubes, except acetylated ones, when conjugated with peptide produce a higher immunological reactions when compared to free peptides. Such a property of inducing immunological responses can be utilized in vaccine production to enhance the efficacy of vaccines.

• **Reduced toxicity and increases the efficacy**
  Carbon nanotubes enhance drug delivery, efficacy and reduces the toxicity as found in the case of Amphotericin B nanotubes. It has been found that Amphotericin B nanotubes has shown enhanced drug delivery to the interior of cells, increased antifungal efficacy and reduced toxicity to mammalian cells when compared to amphotericin B administration without nanotubes [69]. The efficacy of amphotericin B nanotubes was also effective on strains of fungi which are usually resistant to amphotericin B alone [69].
• **Gene silencing**
  Highly selective therapy is required for cancer therapy where tumor cells will be selectively modulated. In this case gene silencing has been done with small interfering RNA. This can be achieved by targeting functionalized single walled carbon nanotubes with siRNA to silence targeted gene expression in the targeted cell [70].

• **In diagnostics**
  It was reported that compounds that are bound to nanotubes enhance the efficiency of diagnostic methods. This property of functionalization and high length to diameter aspect ratio (which provides a high surface to volume ratio), assists in designing the highly efficient biosensors [71].

Thus carbon nanotubes offer diverse advantages over other drug delivery and diagnostic systems due to very interesting physicochemical properties such as ordered structure with high aspect ratio, high electrical conductivity, high mechanical strength, ultra-light weight, high thermal conductivity, metallic or semi-metallic behavior and high surface area [72].

### 2.6.2 Fullerenes

They are also known as bucky balls, that are the carbon allotrope discovered in 1985 [72] having dimensions near around 7 Å in diameter and composed of 60 carbon atoms that are arranged in a shape known as truncated icosahedrons [73]. Its shape is quite similar to soccer ball with 20 hexagons and 12 pentagons and is highly symmetrical [74]. There are various types of fullerenes such as Alkali doped fullerenes, endohedral fullerenes, endohedral metallofullerenes, exohedral fullerenes and heterofullerenes. Alkali doped fullerenes are the carbon allotrope structures that contains alkali metal atoms in between fullerenes contributing valence electrons to neighboring fullerenes [75]. Similarly endohedral fullerenes have another atom enclosed inside the buckyball. If they are enclosed with metallic atom then they are called as metallofullerenes [76, 77]. Owing to the very small size of C-60 fullerene, it is difficult to synthesize endohedral $C_{60}$ fullerenes. Therefore larger fullerenes ($C_{82}$ or $C_{96}$) fullerenes are used for synthesizing endohedral fullerenes. Another type of fullerenes called as exohedral fullerenes or fullerene derivatives or functionalized fullerenes which are synthesized by chemical reaction between the fullerene and other chemical groups. Last class of fullerene compounds are heterofullerenes where one or more carbon atoms are replaced by other atoms like nitrogen or boron.

#### 2.6.2.1 Applications

• **Diagnostics**
  Endohedral metallofullerenes can be used for diagnostic purposes as radio contrast media in magnetic resonance imaging and other imaging procedures. Since
the radioactive metal is enclosed within the buckyball, these are less toxic and safer. This method can also be employed for imaging organs as radioactive tracers [77]. Animal studies with C_{60} fullerene conjugated with thyroglobulin have produced a C_{60} specific immunological response which can be detected by ELISA with IgG specific antibodies. This can be used to design methods of estimation of fullerene levels in the body when used for therapeutic or diagnostic purposes [78].

- **Drug transport**
  Fullerene are being investigated for drug transport of antiviral drugs, antibiotics and anticancer agents [79–82].

- **Free radical scavengers**
  Due to presence of high number of conjugated double bonds in the core structure fullerenes can also be used as free radical scavengers. They also provide protection to the mitochondria against injury induced by free radicals [83], owing to this property they can be used in cancer therapy [84].

- **Photosensitizers**
  Fullerenes especially exohedral fullerenes can be used as photosensitizers in photodynamic therapy against various types of malignancies. These fullerenes potentially generate reactive oxygen species when stimulated by light and kills the target cells. This method is now also being investigated for antimicrobial property as these cause cell membrane disruption especially in Gram positive bacteria and mycobacterium [79–82].

- **Stimulate host immune response and production of antibodies**
  Fullerenes are efficient in stimulating host immune response and production of fullerene specific antibodies.

### 2.6.2.2 Toxicity

After its parenteral administration through intravenous injection, these fullerenes get distributed to various parts of the body and finally get excreted unchanged through the kidney. In comparison to non soluble derivatives, soluble derivates of fullerenes are safer and biocompatible. They are also having low toxic potential even at higher doses [78]. Cost of fullerenes is dependent on its degree of purification. Purified fullerenes are very expensive, restricting its application in medical field.

### 2.6.3 Quantum Dots

Quantumdots (QDs) are nanocrystals of semiconducting materials measuring around 2–10 nm, consisting of a semiconductor inorganic core (CdSe), an aqueous organic coated shell (e.g., ZnS) to improve optical properties, and can be made to fluorescence when stimulated by light. Quantumdots bear a cap which enables them in improving their solubility in aqueous buffers. They are neither atomic nor bulk semiconductors. Core of the quantumdots determines the color emitted and outer
aqueous shell is available for conjugation with biomolecules. Biomolecular conjugation of the quantum dots can be modified according to target various biomarkers [85]. Their properties originate from their physical size, which ranges from 2 to 10 nm in radius. Owing to the to their narrow emission, bright fluorescence, high photostability and broad UV excitation QDs have been adopted for tracking of intracellular process for longer time, for in vitro bioimaging and for real time monitoring (Fig. 2.1). As far as the its applications are concerned QDs covers medical areas as a diagnostic as well as therapeutic tool for in vitro and in vivo detection and analysis of biomolecules, immunoassays, DNA hybridization, diagnostic tools (magnetic resonance imaging, MRI), time graded fluorescence imaging of tissue, development of non-viral vectors for gene therapy, labeling of cells, as therapeutic tools for cancer treatment and transport vehicles for DNA, protein, drugs or cells [86]. In addition they can be also tagged with biomolecules and used as highly sensitive probes. Quantum dots and their therapeutic applications are highlighted in Table 2.4.

**2.6 Types of Pharmaceutical Nanosystems**

<table>
<thead>
<tr>
<th>Therapeutics</th>
<th>Target cells/ diseases</th>
<th>Type of QDs</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Fluorouracil</td>
<td>Breast cancer</td>
<td>ZnS QDs</td>
<td>Targeting and controlled drug delivery to cancer cells.</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>Leukaemia</td>
<td>CdTe QDs</td>
<td>Enhanced drug uptake</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>Leukemia K562 cells</td>
<td>CdS QDs</td>
<td>Inhibit multidrug resistance</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Ovarian cancer</td>
<td>Mucin1- aptamer QD</td>
<td>Higher accumulation on target</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>HIV-1</td>
<td>Carboxyl-terminated QDs</td>
<td>High site-specificity and can cross BBB</td>
</tr>
</tbody>
</table>

**2.6.3.1 Applications**

- **Cancer therapy**
  In one report it was proven that quantum dots are accumulated in prostate cancer developed in nude mice by enhanced permeability and retention [87]

- **Bioconjugation with polymer**
  It was reported that its conjugation with polyethylene glycol (PEG) and antibody and targeting to prostate specific membrane antigen enhances its accumulation and retention [87] in the tumor tissue

- **Imaging**
  They can also be utilized for imaging of sentinel node in cancer patients for tumor staging and planning of therapy. This application assists in detecting suitable therapy and stage for various malignancies like melanoma, breast, lung and gastrointestinal tumors [87]. In addition quantum dot probes provide real time imaging of the sentinel node with Near Infra Red (NIR) fluorescence system which is having the potential to produce reduced [88] background noise and deeper penetration of rays into the biological sample.
2.6.3.2 Toxicity

QDs utilization in clinical practice is limited since it has serious elimination problems which cause extreme toxicity. After functionalization of the QDs size increases. This size range is greater than the pore size of endothelium and renal capillaries, thus reducing its elimination and resulting in toxicity. Additionally there are very less reports available on the metabolism and excretion of quantum dots making their utilization more difficult clinically \[86\].

2.6.4 Nanoshells

Nanoshells are the new modified forms of targeted therapy, having core of silica and a metallic outer layer \[89\]. These thin coated core particles of different material have gained considerable attention now days. The properties of nanoshells can be altered by simply tuning the core to shell ratio. With the recent advancement in new techniques it is now possible to synthesize these nanostructures in desired shape, size and morphology. Nanoshells are synthesized to create novel structures with different morphologies, since not possible to synthesize all the materials in desired morphologies. For obtaining desirable morphology core particles of different morphologies such as rods, wires, tubes, rings, cubes, etc. can be coated with thin shell in core shell structures. These shells are inexpensive as precious materials can be deposited on inexpensive cores. Therefore while synthesizing nanoshells expensive material is required in lesser amount than usual. Targeting of nanoshells can be achieved by using immunological methods. Nanoshells occupies variety of applications in diverse areas such as providing chemical stability to colloids, enhancing luminescence properties, engineering band structures, biosensors, drug delivery, etc. Synthesis of nanoshells can be useful for creating.

2.6.4.1 Applications

- **Cancer therapy**
  This technology is being evaluated for cancer therapy. Nanoshells are tuned to absorb infra red rays when exposed from a source outside the body and get heated and cause destruction of the tissue. This has been studied in both \textit{in vitro} and \textit{in vivo} experiments on various cell lines \[89\]

- **Diagnostic purposes**
  They are useful for diagnostic purposes in whole blood immunoassays e.g. coupling of gold nanoshells to antibodies to detect immunoglobulins in plasma and whole blood

- **Hydrogel mediated delivery**
  Nanoshells can be easily embedded in hydrogel polymer containing the drug. Such type of delivery system can be used for targeting tumor cells. Mechanism
of action is based on the targeting of gel to tumor tissue by immunological methods and exposed under infrared laser beam to heat the polymer which facilitates the release of the drug at the desirable site.

- **Micro metastasis and diabetes**
  Nanoshells are currently studied for micro metastasis of tumors and also for treatment of diabetes [90]

### 2.6.5 Nanobubbles

Nanobubbles (NBs) are nanoscaled bubble like structures that are generated in the interface of hydrophobic surfaces in liquids. These nanobubbles remain stable at room temperature and when heated to physiological temperature within the body coalesce to form microbubbles. The mechanism of NB formation is based on the nucleation of gas at the hydrophobic surface from a supersaturated solution, leading to trap atmospheric gases. However the formation of NBs is thermodynamically forbidden, but the life time of NBs is reached event to the orders of hours. There are four types of nanobubbles: bulk, interfacial, plasmonic and oscillating nanobubbles. Cancer therapeutic drugs can be incorporated into these nanoscaled bubbles like structures. Nanobubbles potentially exhibit advantages in targeting the tumor tissue and delivering the drug selectively under the influence of ultrasound exposure. This may enhance the intracellular uptake of the drug by the tumor cells. Additionally these nanobubbles can be easily visualized in tumor by means of various ultrasound methods [91, 92].

#### 2.6.5.1 Applications

- **Delivery of drugs**
  NBs can be potentially utilized in delivery of drugs like doxorubicin *in vitro* and *in vivo*. These NBs reach the tumor and get accumulated which is followed by formation of microbubbles by coalescing of nanobubbles. Disruption of the microbubbles occurs when the site is focused with high intensity focused ultrasound, which ultimately causes release of the drug. This may results in accumulation of higher levels of drug in the target cells and reduced toxicity and increased efficacy. This method needs further exploration for its utility in treatment of various malignancies.

- **Gene therapy**
  Liposomal nanobubbles and microbubbles are also being studied for effective non viral vectors for gene therapy. Nanobubbles combined with ultrasound exposure have shown improved transfer of gene in both *in vitro* and *in vivo* studies [93, 94].

- **Thrombolysis**
  Nanobubbles are also being investigated for removal of clot in vascular system in combination with ultrasound. This process is called as sonothrombolysis. This method is non invasive and causing less damage to endothelium [95].
- Toxicity
  NBs are not that much toxic since the disruption of the microbubbles occurs only at the targeted site when it’s being exposed to ultra sound waves. Therefore drug is released at a particular site.

### 2.6.6 Paramagnetic Nanoparticles

Magnetic drug targeting is conceptualized with an objective to target magnetic drug carrier particles at a specific site in the body using an externally applied magnetic field. Magnetic nanoparticles are a class of particulate materials of less than 100 nm size that can be manipulated under the magnetic field. These particles are composed magnetic elements such as cobalt, nickel, iron and their respective oxides such as magnetite, maghemite, cobalt ferrite and chromium dioxide. The classification of these particles is based on their magnetic susceptibility which is defined as ratio of induced magnetization to the applied field. Paramagnetic nanoparticles have a greater magnetic susceptibility than conventional contrast agents. They are investigated for both diagnostic and therapeutic purposes. For diagnostic purpose paramagnetic iron oxide nanoparticles are used as contrast agents in magnetic resonance imaging. Targeting with paramagnetic nanoparticles enables identification of specific organs and tissues [96].

### 2.6.6.1 Applications

- **Cancer therapy**
  Conjugation of paramagnetic nanoparticles with antibodies and their expression in breast cancer cells have been used with MRI to detect breast cancer cells *in vitro* [97]. Moreover conjugation of paramagnetic nanoparticles with luteinizing hormone (releasing hormone as breast cancer cells express LHRH receptors) studied for the detection of breast cancer cells *in vivo*. Magnetic nanoprobes such as iron nanoparticles coated with monoclonal antibodies are used for cancer therapy. These nanoparticles are targeted towards tumor cells to generate high levels of heat after their accumulation at the target site by means of an alternating magnetic field applied externally. Such procedure kills the cancer cells selectively [98].

- **Eliminate plasma opsonins and increase in circulation time**
  On intravenous administration of decoy of nanoparticle plasma opsonins can be eliminated and reduces uptake of the nanoparticles. Moreover alteration in surface charge of the nanoparticle to neutral by covalent coupling to chemicals leads to an increase in circulation time

- **For internalization**
  Paramagnetic nanoparticles internalization by macrophages can be overcome by treatment with drugs like lovastatin which reduce macrophage receptor expression for the nanoparticle.
2.6 Types of Pharmaceutical Nanosystems

- **Identification of proteins**
  Magnetic microparticle probes with nanoparticle probes have been used for identification of proteins like prostate specific antigen. Here magnetic microparticles coated with antibodies together with nanoprobes with similar coating and a unique hybridized DNA barcode are used [99].

- **Imaging**
  Utilization of iron oxide in MRI imaging faces limitations like specificity and internalization by macrophages. Monocrystalline iron oxide nanoparticles have been studied for magnetic resonance imaging of brain. These are rapidly taken up by the tumor cells [100]. Hence give long lasting contrast enhancement of tumor. The remaining nanoparticles are removed from the circulation by reticuloendothelial system [100].

- **Targeted action**
  Conjugation of antibodies with paramagnetic nanoparticles to direct the nanoparticle to the target site helped to overcome problems with specificity of action.

2.6.7 Nanosomes

Nanosomes are currently being used for medical applications such as targeting, diagnosis and therapy.

- **Brain targeting**
  These nanosomes are being developed for treatment of various tumors (CNS tumors) e.g. silica coated iron oxide nanoparticles coated with polyethylene glycol used to access specific areas of brain involved with tumor [101].

- **Tumor targeting**
  Nanosomal delivery with magnetic resonance imaging and laser assist in targeting the nanoparticle specifically to the tumor cells and destroy the cells loaded with these nanoparticles by the heat generated by iron oxide particles by absorbing the infra red light.

- **ROS production**
  Their stable integration with photocatalyst produces reactive oxygen species when stimulated by light and destroy the target tissue. These nanoform exhibit advantages over conventional drugs in being much safer without the adverse effects of cancer chemotherapy drugs and also the absence of development of drug resistance.

2.6.8 Pharmacyte

Pharmacyte is an ideal nanotechnology-based drug delivery system, which is a self-powered, computer controlled medical nanorobot system capable of digitally precise transport, timing, and targeted delivery of pharmaceutical agents to specific cellular and intracellular destinations within the human body. This nanotechnology
may be constructed using future molecular manufacturing technologies such as diamond mechanosynthesis. Pharmacytes will have many applications in nanomedicine such as initiation of apoptosis in cancer cells and direct control of cell signaling processes [102].

2.6.8.1 Niosome

Niosome is a class of molecular cluster formed by self-association of non-ionic surfactants in an aqueous phase. The unique structure of niosome presents an effective novel drug delivery system (NDDS) with ability of loading both hydrophilic and lipophilic drugs. Niosomes are vesicles composed of non-ionic surfactants, which are biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes. Niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. As with liposomes, the properties of niosomes depend on the composition of the bilayer as well as method of their production. It is reported that the intercalation of cholesterol in the bilayers decreases the entrapment volume during formulation, and thus entrapment efficiency. However, differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol, whereas liposomes are prepared from double-chain phospholipids (neutral or charged). The concentration of cholesterol in liposomes is much more than that in niosomes. As a result, drug entrapment efficiency of liposomes becomes lesser than niosomes. Besides, liposomes are expensive, and its ingredients, such as phospholipids, are chemically unstable because of their predisposition to oxidative degradation; moreover, these require special storage and handling and purity of natural phospholipids is variable. Current opinions for the utilization of niosomes in the delivery of biomolecules can be unsubstantiated with a wide scope in encapsulating toxic drugs such as anti-AIDS drugs, anticancer drugs, and anti-viral drugs. Niosomes offers a promising carrier system in comparison with ionic drug carriers, which are relatively toxic and unsuitable. However, the technology utilized in niosomes is still in pipeline. Therefore researches are going on to develop a suitable technology for large production because it is a promising targeted drug delivery system.

2.6.9 Dendrimers

Dendrimers are a unique class of polymers, are hyperbranched, tree-like structures, whose size and shape can be precisely controlled and have compartmentalized chemical polymer. Dendrimers are fabricated from monomers using either convergent or divergent step growth polymerization. Size of these regular branching polymeric nanostructures is dependent on the number of branching which can be controlled. These nanostructures arise several branches from the core in shape of a spherical structure by means of polymerisation, resulting in formation of cavities
within the dendrimer molecule which can be used for drug transport. Free ends of dendrimer can be utilized for conjugation or attachment to other molecule. These end groups that can be tailored according to requirements. Such interconnecting networks transport the attached molecules at desirable site and give dendrimers various functional applications \[103\]. These well defined nanostructures are equipped with surface functionalization capability, monodispersity of size, and stability properties that make them attractive drug carrier candidates. Incorporation of drug molecule can be easily achieved via either complexation or encapsulation. As far as the construction is concerned it contains three different basic regions: core, branches, and surface (Fig. 2.1). Branches or end groups can be tailored or modified into biocompatible compounds with low cytotoxicity and high biopermeability. Such branches or networks assist in delivery of bioactive ranging from vaccines, drugs, genes and metal to desired sites. Hollow networks present in dendrimers presents space to incorporate drugs and other bioactive physically or by various interactions to act as drug delivery vehicles. Dendrimers covers various distinct applications mainly are solubilization, gene therapy, dendrimer based drug delivery, immunoassay and MRI contrast agent. List of the various drugs that can be delivered through dendrimers are highlighted in Table 2.5. This hollow branched nanostructure is an ideal carrier for drug delivery due to several advantages like:

- Can be modulated for target-specific drug delivery
- Feasibility to develop with defined molecular weight
- Good entrapment efficiency
- Offering surface for functionalization
- Very low polydispersity index
- Very low size (1–5 nm)

Variety of polymers (single or in combinations) are used for designing drug delivery system in form of dendrimers:
- Andpoly(ethylene glycol)
- Chitin
- Melamine, poly(l-glutamic acid)
- Poly(propyleneimine),
- Polyamidoamine
- Polyethyleneimine

2.6.9.1 Applications

- **Drug and gene delivery**
  Dendrimers are being investigated for both drug and gene delivery, as carriers for penicillin, and for use in anticancer therapy.

- **Imaging, targeting & diagnosis of disease**
  Complexes of dendrimers such as tectodendrimers with each dendrimer module of the complex performing different functions such as targeting, diagnosis of disease state, delivery of drug and imaging.
**Chemotherapy**
Tectodendrimers are the nano device that acquires potential applications in cancer chemotherapy as a mode of targeted drug therapy [104]

**Gene therapy**
Dendrimers can be used for gene therapy where these can replace conventional viral vectors. They enter the cells by endocytosis and the DNA gets transported into nucleus for transcription of the applied gene.

**Stimulation of immune reaction**
The advantage of dendrimer based therapy is absence of stimulation of immune reaction.

---

**Table 2.5** List of drugs that can be delivered through Dendrimers [67]

<table>
<thead>
<tr>
<th>Drugs/therapeutics</th>
<th>Type of dendrimers/conjugates</th>
<th>Target cells/indications/functions</th>
<th>Advantages/features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boron</td>
<td>EGF-carrying PAMAM dendrimers</td>
<td>Neuron capture technology</td>
<td>Intratumoral injection or CED</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>2,2 bis(hydroxymethyl) propanoic acid-based dendrimers</td>
<td>Colon carcinoma cells of rat</td>
<td>In vitro and in vivo, dendrimer product was ten times less toxic</td>
</tr>
<tr>
<td>Efavirenz</td>
<td>Tuftsin-conjugated PPE dendrimers</td>
<td>HIV</td>
<td>Targeted delivery to macrophages</td>
</tr>
<tr>
<td>EGFR siRNA</td>
<td>Dendriworms</td>
<td>Knockdown EGFR expression</td>
<td>IV or CED</td>
</tr>
<tr>
<td>Galactosyleramide analogues</td>
<td>Multivalent phosphorus-containing catanionic dendrimers</td>
<td>HIV-1</td>
<td>Higher stability and anti-viral property, lower toxicity</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>Mannose-capped PPE dendrimers</td>
<td>HIV</td>
<td>Increased cellular uptake, reduced cytotoxicity</td>
</tr>
<tr>
<td>Plasmid pEGFP-N2</td>
<td>Angiopep-carrying PEGylated PAMAM dendrimer G5.0</td>
<td>Encode green fluorescence protein</td>
<td>IV</td>
</tr>
<tr>
<td>siRNA</td>
<td>Amino-terminated carbosilane dendrimers</td>
<td>Lymphocytes</td>
<td>Reduced HIV infection, in-vitro</td>
</tr>
<tr>
<td>SN38</td>
<td>G3.5 PAMAM dendrimers</td>
<td>Hepatic colorectal cancer cells</td>
<td>Increase oral bioavailability and decrease gastrointestinal toxicity</td>
</tr>
<tr>
<td>Sulphated oligosaccharides</td>
<td>Polylysine dendrimers</td>
<td>HIV</td>
<td>Higher activity due to dendrimer product</td>
</tr>
</tbody>
</table>

_PAMAM_ Poly(amido amine), _PPE_ poly(propyleneimine)
• **Gene transfer**
  It was studied that the potential use of transferring conjugated gene transfer for tumors of various tumors PEG modified polyamidoamine dendrimers and magnetic nanoparticle modified dendrimers for targeted gene delivery to the brain and in transfer of antisense surviving oligonucleotides in tumor cell lines. These methods provide an effective alternative to viral vectors of gene transfer for treatment of various tumors [105]

• **Transfection**
  Various dendrimer based DNA transfection kits (Nanojuice™ Transfection Kit produced by EMD Chemicals Inc. and Superfect® Transfection Reagent of Qiagen) are used for delivering DNA into the cell. These are claimed to have improved transfection efficacy and low toxicity to cells [106]

• **Antiretroviral therapy**
  Dendrimer based drugs are being tried for antiretroviral therapy. Some of the dendrimer based drugs was found to successfully prevent simian HIV infection

• **Treatment of cancer**
  Treatment of cancer by conjugating with anti-cancer drugs like cisplatin, adriamycin or methotrexate [107]. PAMAM dendrimers can also be used in treatment of cancer.

• **Reduces the cytotoxicity**
  While antibacterial investigation it was observed that PEG coating of the dendrimer reduces the cytotoxicity of unmodified PAMAM dendrimers. Hover reduces the efficacy against Gram positive bacteria without change in efficacy against Gram negative bacteria like *Pseudomonas*

• **Contrast agents for imaging**
  Dendrimers are also used as contrast agents for imaging. The 1, 4-diaminobutane core dendrimer and the PAMAM dendrimer are well studied commercially available dendrimers for imaging studies. Renal excretion is the main route of clearance and is dependent on the size of the particle and more than 60% of injected DAB or PAMAM dendrimer is cleared from circulation within 15 min

• **Rapid clearance**
  Smaller sized dendrimers undergo rapid renal clearance whereas dendrimers with charged surface or hydrophobic surfaces are rapidly cleared by the liver. Those dendrimers with a hydrophilic surface escape renal clearance and have a greater circulation time

### 2.6.9.2 Toxicity

Toxicity profile of dendrimers renders them not very popular system for use as delivery means. Cationic dendrimers have a greater potential to cause cytotoxicity compared to anionic dendrimer or PAMAM dendrimers. It is proposed to cause cell membrane instability and cell lysis. The toxicity of dendrimer is dependent on the size of the particle and increase with size. It can be reduced by means of surface modification of the dendrimers with incorporation of PEG or fatty acids
2.6.10 Nanopores

Nanopores were designed in 1977, consist of wafers with highly dense pore of size 20 nm (diameter). Main advantage of these nanopores that they doesn’t allow the entry of oxygen glucose and other products. They can be potentially utilized to protect transplanted tissues from the host immune system.

2.6.10.1 Application

- **DNA sequencing**
  Currently several researchers are working on modified nanopores that have the ability to differentiate DNA strands based on differences in base pair sequences. Nanopores are also being developed with ability to differentiate purines from pyrimidines.

- **Pharmacogenomics in drug development process**
  DNA sequencing via nanopores could possibly read a thousand bases per second per pore. These can be used for low cost high throughput genome sequencing which would be of great benefit for application of pharmacogenomics in drug development process.

- **Treatment for insulin dependent diabetes mellitus**
  β cells of pancreas can be enclosed within the nanopore device and implanted in the recipient’s body.

2.6.11 Microbivores

Function of these nano based hypothetical forms is to trap circulating microbes just like the function of white blood cells in the blood stream. They are designed in such a way so that they acquire greater efficacy than cellular blood cells in phagocytosis. Their surface is arranged in such a fashion which can extend in length and secure the microbe which gets in contact with it. Entrapped microbe will be gradually transferred to the ingestion port and undergoes the process of morcellization and enzymatic degradation. Degraded products are ultimately released as amino acids, fatty acids, nucleotides and sugars.

2.6.11.1 Application

- **Clear the blood circulation**
  Microbivores could theoretically clear the blood stream in septicaemia at a much greater rate than the natural defense mechanism with antibiotics [108]
2.6.12 Nanocrystals and Nanosuspension

These are aggregated structures that are formed by the combination of various particles of drug in crystalline form coated with surfactant or combination of surfactants. To achieve static and electrostatic surface stabilization a minimum quantity of surfactants needs to be added in nanocrystals. These aggregated forms reduce limitations of several drugs that are suffering from bioavailability and absorption problems. In addition problems of preparing the parenteral dosage form may be resolved by formulation as nanocrystals. Loading capacity especially in carrier-based nanoparticles is quite low however administration of high drug levels with depot release can be achieved if dissolution is sufficiently slow. Nanocrystal technology can be utilized for many dosage forms.

2.6.12.1 Applications

- **Drugs in pipeline**
  Nanocrystals such as Rapamune®, containing sirolimus which is an immunosuppressant drug and Emend®, which contains aprepitant, MK 869, are in pipeline
- **Favorable drug delivery system**
  Serve as a favorable delivery system for drugs like amphotericin B, tacrolimus, etc.
- **Safe and effective passage**
  The size of nanocrystals allows for safe and effective passage through capillaries.
- **Targeting**
  Nanoparticles offer the potential for targeting the mucosa of the gastrointestinal tract after oral administration, and targeting the cells of the mononuclear phagocytic system (MPS) to treat infections of the MPS such as fungal mycobacterial infections and leishmaniasis.

2.6.12.2 Toxicity

Since pure drug is used and no carrier is needed, eliminating potential toxicity issues associated with the carrier molecule.

2.6.13 Solid Lipid Nanoparticles

Solid lipid nanoparticles were developed as an alternative carrier system to liposomes, polymeric nanoparticles and emulsions as a colloidal carrier system for controlled drug delivery. Solid lipid nanoparticles carry distinct advantages that make them unique carriers systems than others like liposomes and polmeric nanoparticles. This type of nanoparticles constitute solid lipid matrix with an average diameter below 1 μm. Drug is normally incorporated in this matrix. These nanoparticles can
also be produced by high pressure homogenization. Different surfactants are used to avoid aggregation and to stabilize the dispersion. These surfactants have an accepted GRAS (Generally Recognized as Safe) status.

2.6.13.1 Applications

- **Can be used for diverse route system**
  SLN have been developed and investigated for parenteral, pulmonal and dermal application routes.
- **Non-viral transfection**
  SLN have been considered as new transfection agents using cationic lipids for the matrix lipid composition. Cationic solid lipid nanoparticles for gene transfer can be formulated using the same cationic lipids as for liposomal transfection agents. Cationic lipid composition seems to be more dominant for *in vitro* transfection performance than the kind of colloidal structure it is arranged in. Hence, cationic SLN extend the range of highly potent non-viral transfection agents by one with favorable and distinct technological properties.

2.6.14 Silicon-Based Structures

These silicon-based structures can be fabricated by techniques such as etching, photolithography, and deposition commonly used in the manufacture of and microelectromechanical systems and semiconductors. Among various silicon-based materials, porous silicon and silica, or silicon dioxide are the most materials that are architecture in form of calcified nanopores, platinum materials containing nanopores, porous nanoparticles, and nanoneedles. Nanopores size (diameter) and density can be accurately controlled to achieve a constant drug delivery rate through the pores. There are various forms (porous hollow silica nanoparticles) that are fabricated in a suspension containing sacrificial nanoscale templates. This followed by the addition of silica precursors, such as sodium silicate, into the suspension, which is then dried and calcined. Template material is then dissolved further leaving behind the porous silica shell. These nanoparticles mixed with the drug molecule and subsequently drying the mixture to coalesce the drug molecules to the surface of the silica nanoparticles.

2.6.14.1 Applications

Various examples of therapies being studied for use with silicon-based delivery systems include

- **For delivery of antitumor agent**
  Porous silicon embedded with platinum is reported
2.6.15 **Metallic Nanoparticles**

Currently these nanoparticles are emerging as good delivery carrier for drug and biosensor. For the synthesis of metallic nanoparticles diverse metals have been explored though silver and gold nanoparticles are of prime importance for biomedical use (Fig. 2.1). Surface functionalization on these nanonarticles can easily been done and various ligands have been decorated onto the surface. Variety of ligands such as sugars, peptide, protein and DNA has been linked to nanoparticles.

2.6.15.1 **Applications**

Metallic nanoparticles have been used for active delivery of bioactive, drug discovery, bioassays, detection, imaging and many other applications due to surface functionalization ability, as an alternative to quantum dots.

2.6.16 **Liposomes**

Liposomes are lipid based vesicles that are extensively explored and most developed nanocarriers for novel and targeted drug delivery. Drugs that can deliver through liposomal delivery system are highlighted in Table 2.6. These vesicles are synthesized by hydration of dry phospholipids. Depending upon on their size and number of bilayers they are classified into three basic types:

- **Multilamellar vesicles**  
  These vesicles consist of several lipid bilayers separated from one another by aqueous spaces. These entities are heterogeneous in size, often ranging from a few hundreds to thousands of nanometers in diameter.

- **Small unilamellar vesicles**  
  Small unilamellar vesicles consist of a single bilayer surrounding the entrapped aqueous space having size range less than 100 nm.

- **Large unilamellar vesicles**  
  These vesicles consist of a single bilayer surrounding the entrapped aqueous space having diameters larger than 100 nm.
Based on the physicochemical characteristics drug molecules can be entrapped in the aqueous space or intercalated into the lipid bilayer of liposomes. Liposomes are prepared with distinct structure, composition, size, flexibility with variety of surface modification. Such availability of liposomes with enormous diverse properties makes them most intelligent carrier system for both active and passive delivery of bioactive.

### 2.6.16.1 Applications

They have been successfully exploited in cancer therapy, carrier for antigens, pulmonary delivery, leishmaniasis, ophthalmic drug delivery etc. Some of liposome-based formulations are already in market (Table 2.5).

### 2.6.17 Polymeric Micelles

Polymeric micelles contains amphiphilic block copolymers assemble to form nanoscopic supramolecular core-shell structures called as ‘polymeric micelles’. These micelles are formed in solution as aggregates in which the component molecules are generally arranged in a spheroidal structure with hydrophobic cores shielded from water by a mantle of hydrophilic groups (Fig. 2.1). There are several examples of component molecule such as Amphiphilic AB-type or ABA-type block copolymers, where A and B are hydrophobic and Hydrophilic components, respectively. These polymeric micelles are usually <100 nm and are used for the systemic delivery of water-insoluble drugs. Their hydrophilic surface of these dynamic systems protects their nonspecific uptake by reticuloendothelial system. Polymeric micelle

| Table 2.6 Liposomes and their respective model drugs [67] |
|----------------|----------------|----------------|
| Therapeutics   | Type of liposome | Indications    |
| Amilorida hydrochloride | Small molecular liposome | Cystic fibrosis |
| Budesonide     | Small molecular liposome | Asthma        |
| Doxorubicin + Verapamil | Transferrin- (Tf-) conjugated PEG-Liposome | MDR-leukemia |
| Insulin        | Protein liposome  | Diabetes       |
| Interleukin-2  | Protein liposome  | Lung cancers   |
| Irinotecan + Cisplatin | Mixture of two liposomes | Small-cell lung cancer |
| Ketotifen      | Small molecular liposome | Asthma       |
| siRNA + Doxorubicin | PEG-Liposome | MDR-breast cancer |
| Tobramycin     | Small molecular liposome | Pulmonary infections |
| Topotecan + Vincristine | PEG-Liposome | Brain cancer   |
| VEGF gene      | Gene liposome    | Pulmonary hypertension |

*VEGF vascular endothelial growth factor*
carries advantage in trapping drugs or contrast agents physically within the hydrophobic cores or can be linked covalently to component molecules of the micelle. Additionally they are proved as an excellent novel drug delivery system due to their high stability in physiological conditions, high and versatile loading capacity, high accumulation of drug at target site, possibility of functionalization of end group for conjugation of targeting ligands and slower rate of dissolution.

2.6.18 Polymer Drug Conjugate

Polymer drug conjugate formed by the conjugation of low molecular weight drugs with polymer. This interaction/conjugation causes drastic change in pharmacokinetic disposition of drug in whole body and at cellular level. They are designed to increase the overall molecular weight, which facilitates their retention in cancer cells through enhanced permeation and retention effect using passive delivery approach.

2.6.19 Polyplexes/Lipopolyplexes

Polyplexes/Lipopolyplexes are the assemblies which are used in transfection protocols. These assemblies are formed by spontaneous interaction between nucleic acids and polycations or cationic liposomes (or polycations conjugated to targeting ligands or hydrophilic polymers). Usually composition and charge ratio of nucleic acid to that of cationic lipid/polymer determines the shape, size distribution, and transfection potential of these complexes. Current research offers various types of polycations that have been used in gene transfer/therapy protocols:

- Cationic cyclodextrin
- Linear- and branched-poly (ethyleneimine)
- Poly (amidoamine)
- Poly-amino esters
- Poly-L-lysine

2.6.20 Respirocytes

These are hypothetical nanodevices or called as artificial red blood cells and function as red blood cells but with greater efficacy. Respirocytes are having higher capacity to deliver oxygen to tissues. Their oxygen supplying capacity is 236 times more oxygen per unit volume than natural red blood cells. Respirocytes equipped with sensors on their surface which can detect changes in the environment. There is
also a provision to regulate the intake and output of the oxygen and carbon dioxide molecules. According to past investigation an infusion of 1 L dose of 50% respirocytes saline suspension in a human can theoretically keep the patient oxygenated up to four hours following cardiac arrest \[109\]. According to FDA these devices are regulated under the provisions of the Medical Device Amendments of 1976, Safe Medical Devices Act of 1990, and the Medical Device Amendments of 1992 \[110\].

### 2.6.21 Polymeric Nanoparticles

The main objective of our book is to explore the recent nano application of wide array of natural polymers obtained from different sources. Natural polymers based nano-conjugates and their advance applications are discussed in this chapter. In addition various drug delivery and targeting based considerations are also discussed. Natural polymer based nanoparticles are usually biocompatible and non toxic, although often suffer from stability problems when delivered across the various biological membranes. Such delivery exposed nanoparticles against various pH. This variation in pH and certain other problems limit their use sometime.

Polymeric nanoparticles consist of a biodegradable polymer which is biocompatible and non toxic. Feature such as biocompatibility is required for potential application in tissue engineering, drug and gene delivery and new vaccination strategies.

Recently research explore some advance modification of natural polymers which consists of synthetic polyesters like poly(D, L-lactide) or polycyanoacrylate and related polymers like poly(lactide-co-glycolide) PLA or poly(lactid acid). Among natural polymers the most widely used polymer which is used now days is chitosan. In addition to chitosan many other such as gelatin, and sodium alginate overcome some toxicological problems with the synthetic polymers. Natural polymer based nanoparticles offers a signifi cant improvement over traditional oral and intravenous methods of drug delivery system in terms of efficiency and effectiveness. The various natural polymers like gelatin, albumin and alginate are used to prepare the nanoparticles. However they have some inherent disadvantages like poor batch-to-batch reproducibility, prone to degradation and potential antigenicity. Various polymeric nanoparticles and their respective model drugs are highlighted in Table 2.7.

- Synthetic polymers used for nanoparticles preparation may be in the form of pre-formed polymer e.g. polyesters like polycaprolactone (PCL), poly lactic acid (PLA) or monomers that can be polymerized in situ e.g. polyalkyl cyanocrylate. There are many advantages of using polymeric nanoparticles in drug delivery:
  - Biocompatible and biodregerable
  - Increase the stability of any volatile pharmaceutical agents
  - Less toxic
  - They are easily cheaply fabricated in large quantities by a multitude of methods
  - Have engineered specificity, allowing them to deliver a higher concentration of pharmaceutical agent to a desired location
  - Nonimmunogenicity and nontoxicity
Various polymers that have been used recently for the preparation of nanoparticles are mentioned in Table 2.8.

Polymeric nanoparticles (Fig. 2.1) provide an alternative to abovementioned nanosystems due to some inherent properties like biocompatibility, nonimmunogenicity, nontoxicity and biodegradability. These are colloidal carrier, 10 nm−1 μm in size, consisting of synthetic or natural polymers. Polymeric nanoparticles are a broad class comprised of both vesicular systems (nanocapsules) and matrix systems (nanospheres). Nanocapsules are systems in which the drug is confined to a cavity surrounded by unique polymeric membrane whereas nanospheres are systems in which the drug is dispersed throughout the polymer matrix. Polymeric nanoparticles are considered as a matrix system in which the matrix in uniformly dispersed. It should be mentioned, that besides of these spherical vesicular systems nanocapsules are also known, where a polymeric membrane surrounds the drug in a matrix core. The candidate drug is dissolved, entrapped, attached or encapsulated throughout or within the polymeric shell/matrix. The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics. Moreover, polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering, and into drug delivery for species other than humans. Depending on the method of preparation, the release characteristic of the incorporated drug can be controlled. Polymeric nanoparticulate systems are attractive modules for intracellular and site specific delivery.

**Table 2.7** Different types of Polymer used for the preparation of nanoparticle [16]

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Model drug</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophilic Albumin, Gelatin</td>
<td>Hydrophilic and protein affinity</td>
<td>Desolvation and cross linking in water</td>
</tr>
<tr>
<td>Hydrophilic Albumin,Gelatin</td>
<td>Hydrophilic</td>
<td>Heat denaturation and cross linking in w/o emulsion</td>
</tr>
<tr>
<td>Hydrophilic Alginites and chitosan</td>
<td>Hydrophilic and protein affinity</td>
<td>Cross-linking in water</td>
</tr>
<tr>
<td>Hydrophilic Dextran</td>
<td>Hydrophilic</td>
<td>Polymer precipitation in an organic solvent</td>
</tr>
<tr>
<td>Hydrophobic Poly(alkylcyanoacrylates)</td>
<td>Hydrophilic</td>
<td>Emulsion polymerization</td>
</tr>
<tr>
<td>Hydrophobic Poly(alkylcyanoacrylates)</td>
<td>Hydrophobic</td>
<td>Interfacial O/W polymerization</td>
</tr>
<tr>
<td>Polysters Poly (lactic acid), poly(caprolactone)</td>
<td>Hydrophilic and Hydrophobic Soluble in polar solvent</td>
<td>Solvent extraction evaporation</td>
</tr>
<tr>
<td>Polysters Poly (lactic acid), Poly (lactide-co-glycolide),</td>
<td>Hydrophilic and Hydrophobic Soluble in polar solvent</td>
<td>Solvent displacement</td>
</tr>
<tr>
<td>Polysters Poly (lactic acid), Poly (lactide-co-glycolide)</td>
<td>Soluble in polar solvent</td>
<td>Salting out</td>
</tr>
</tbody>
</table>
Nanoparticles can be made to reach a target site by virtue of their size and surface modification with a specific recognition ligand. Their surface can be easily modified and functionalized. From the polymer chemistry viewpoint, there will be in the future a challenging field to create new polymers matching hydrophilic and lipophilic properties of upcoming drugs for smart formulation.

### 2.6.22 Applications of Nanoparticulate Delivery Systems

Targeting of the drug to cells or tissue of choice is the potential area in drug delivery. With the assistance of proficient drug targeting systems it’s now possible to decide the fate of a drug entering in the body. Modern drug delivery systems and technologies are far away from the model of magic bullet (proposed by Paul Ehrlich) in which the drug is precisely targeted to the exact side of action. Nanotechnology present challenge to achieve this goal (to deliver the drug in the right place at the right time) a bit closer [111]. Branch of nanotechnology is anticipated to bring a fundamental transformation in manufacturing and have an enormous impact on Life Sciences especially in delivery, diagnostics, nutraceuticals and the production of biomaterials. In delivery systems targeting is the ability to direct the drug-loaded system to desirable site. There are two major mechanisms (addressing the desired sites for drug release) involved in this process (Table 2.9):

#### Table 2.8 Various examples of drugs those can be delivered through polymeric nanoparticles [67]

<table>
<thead>
<tr>
<th>Therapeutics</th>
<th>Type of polymer/functionalization</th>
<th>Indication/activity</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>PLA-b-PEG</td>
<td>Neurodegenerative diseases</td>
<td>Improved transport across the BBB</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Aptamer-PEG-PLGA</td>
<td>Prostate cancer</td>
<td>Higher efficiency</td>
</tr>
<tr>
<td>Doxorubicin+Cyclosporine A</td>
<td>PACA</td>
<td>Various cancers</td>
<td>Synergistic effect.</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>Methylmethacrylate-sulfopropylmethacrylate</td>
<td>HIV/AIDS</td>
<td>100% increased BBB permeability</td>
</tr>
<tr>
<td>Nerve growth factor (NGF)</td>
<td>Polysorbate 80 coated PBCA</td>
<td>Parkinsonism</td>
<td>Improved transport across the BBB</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Aptamer-PEG-PLGA</td>
<td>Gliomas</td>
<td>Enhanced delivery</td>
</tr>
<tr>
<td>Stavudine</td>
<td>Polybutylcyanoacrylate (PBCA)</td>
<td>HIV/AIDS</td>
<td>8–20 times higher Permeability</td>
</tr>
<tr>
<td>Vincristine + Verapamil</td>
<td>PLGA</td>
<td>Hepatocellular carcinoma</td>
<td>Reduced multidrug resistance</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>Poly (isohexyl cyanate)</td>
<td>Targeting lymphoid tissue</td>
<td>Drug levels is four times higher</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>Polyhexylcyanoacrylate</td>
<td>Targeting lymphoid tissue</td>
<td>Higher Zidovudine levels in the body</td>
</tr>
</tbody>
</table>

PACA Polyalkylcyanoacrylate, PLA-b-PEG Polysorbate 80 coated poly(lactic acid)-b-poly(ethylene glycol), PLGA poly (lactide-co-glycolide)
### 2.6.23 Passive Targeting

Most popular example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors. This results in the enhanced vascular permeability of tumor tissues compared with healthy tissue. In passive targeting, ligand–receptor interactions can be highly selective; hence precise targeting at the site of interest \[112\] is possible. In this process, targeting with nanoparticles encounters multiple obstacles on the way to their target. These include mucosal barriers, nonspecific uptake of the particle and non-specific delivery of the drug (as a result of uncontrolled release).

### 2.6.24 Active Targeting

Active targeting allows the surface functionalization of drug carriers with ligands that are selectively recognized by receptors on the surface of the cells of interest. Therefore, two most important aspects of nanoparticle drug delivery must be:

- **Specific targeting of the diseased tissue with nanoparticles**
  Appropriate size and functionalization with antibodies or other means of selective binding provides means of enhanced delivery of drugs and reduced nonspecific toxicity. This issue can be resolved by functionalization of the nanoparticles with recognition elements on their surfaces towards receptors present on the particular diseased tissue. Conjugation with short chain variable fragments (scFvs) or antibodies will provide selective binding to the specific cell’s surface, and their endocytosis will be enhanced with suitably adjusted binding affinities.

- **Timed release of the drug**
  To prevent nonspecific toxicity the drug must not diffuse out of the particle while it is still in the circulatory system, and must remain encapsulated until the particle

<table>
<thead>
<tr>
<th>Applied field</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agriculture</td>
<td>Atomic force, microscopic and scanning tunnelling microscope</td>
</tr>
<tr>
<td>Chemical and Cosmetics</td>
<td>Nanoscale chemicals and compounds, paints, coatings etc.</td>
</tr>
<tr>
<td>Electronics</td>
<td>Semiconductors chips, memory storage, photonica, optoelectronics</td>
</tr>
<tr>
<td>Environment and Energy</td>
<td>Water and air purification filters, fuel cells, Photovoltaic</td>
</tr>
<tr>
<td>Food Sciences</td>
<td>Processing, nutraceutical food, nanocapsules</td>
</tr>
<tr>
<td>Materials</td>
<td>Nanoparticles, carbon nanotubes, biopolymers, points, coatings</td>
</tr>
<tr>
<td>Military and Energy</td>
<td>Biosensors, weapons, sensory enhancement</td>
</tr>
<tr>
<td>Nanomedicines</td>
<td>Nano drugs, Medical devices, Tissue Engineering</td>
</tr>
<tr>
<td>Scientific Tools</td>
<td>Atomic force, microscopic and scanning tunnelling microscope</td>
</tr>
</tbody>
</table>
binds to the target. For addressing this issue, nanoparticles with multilayeres can be engineered, where each layer will contain one drug from the cocktail, and their release will be sequenced in accordance with the appropriate timing of the delivery of each drug for combination therapy.

Nanoparticles can be significantly used in targeted drug delivery at the site of disease

• Improve the drug bioavailability
• Targeting of drugs to a specific site
• To improve the uptake of poorly soluble drugs

Chemotherapeutic agents such as dexamethasone, doxorubicin 5-fluorouracil and paclitaxel have been successfully formulated using nanomaterials. To encapsulate dexamethasone (a glucocorticoid with an intracellular site of action) polyactic/glycolic acid (PLGA) and polyactic acid (PLA) based nanoparticles have been used. Dexamethasone potentially binds to the cytoplasmic receptors and the subsequent drug-receptor complex is transported to the nucleus resulting in the expression of certain genes that control cell proliferation [113]. Site-specific-targeted drug delivery is important for such class of drugs in the therapeutic modulation of effective drug dose and disease control. NPs have been reported for their potential use in targeting to improve the bioavailability, reducing side effects, decreasing toxicity to other organs. This less costly NP-based drug delivery is feasible in hydrophilic and hydrophobic states through variable routes of administration such as oral, vascular, and inhalation. Advantages of using nanoparticles in drug development and discovery

• Nanoparticles can better deliver drugs to tiny areas within the body.
• Engineering on this scale enables researchers to exercise exquisite and previously unthinkable control over the physical attributes of polymers and other biomaterials.
• Nanocarriers holds promise to deliver biotech drugs over various anatomic extremities of body such as blood brain barrier [114]
• Nanoparticles aid in efficient drug delivery to improve aqueous solubility of poorly soluble drugs that enhance Bioavailability for timed release of drug molecules, and precise drug targeting.
• Nanoparticles overcome the resistance offered by the physiological barriers in the body
• Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
• Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
• Targeted nano drug carriers reduce drug toxicity and provide more efficient drug distribution.
• The surface properties of nanoparticles can be modified for targeted drug delivery for e.g. small molecules, proteins, peptides, and nucleic acids loaded nanoparticles are not recognized by immune system and efficiently targeted to particular tissue types.
• The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc. [115]
• They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects because efficient delivery of drug to various parts of the body is directly affected by particle size.

2.6.25 Tumor Targeting Using Nanoparticulate Delivery Systems

The rationale of using nanoparticles for tumor targeting is based on one of the most efficiency of nanoparticles is delivering drug in the area of the tumor targets via the enhanced permeability and retention effect. This can also be achieved by active targeting by ligands on the surface of nanoparticles.

Nanoparticles limits the drug distribution to target organ, hence reduces the drug exposure against healthy tissues. It was reported that mice treated with doxorubicin based poly (isohexylcyanoacrylate) nanopsheres showed higher concentrations of doxorubicin in the liver, spleen and lungs than in mice treated with free doxorubicin [116]. It was also demonstrated that polymeric composition of nanoparticles such as biodegradation profile of the polymer along with the associated drug’s molecular weight, polymer type, its localization in the nanospheres and mode of incorporation technique, adsorption or incorporation and hydrophobicity have a great influence on the drug distribution pattern in vivo. In addition nanaoparticles are advantageous in their rapid nanoparticles rate, within 1/2 h to 3 h, and it likely involves MPS and endocytosis/phagocytosis process [117].

Earlier report suggested the biodistribution and pharmacokinetics (PK) pattern of a cyclic RGD doxorubicin-nanoparticle formulation in tumor bearing mice [118]. During this biodistribution study it was revealed that drug concentrations over time in the heart, lung, kidney and plasma was decreases and drug accumulation has been found in the liver, spleen and tumor. Maximum of the injected dose was observed in the liver (56%) and only 1.6% in the tumor at 48 h post injection. This study ensures that nanoparticles have a great tendency to be captured by liver. This and several other studies indicates the greatest challenge of using nanoparticles for tumor targeting is to avoid particle uptake by mononuclear phagocytic system (MPS) in liver and spleen. Such tendency of mononuclear phagocytic system for endocytosis/phagocytosis of nanoparticles provides an opportunity to effectively deliver therapeutic agents to these cells. This biodistribution can be of benefit for the chemotherapeutic treatment of mononuclear phagocytic system rich organs/tissues localized tumors

• Brochopulmonary tumors
• Gynaecological cancers
• Hepatic metastasis arising from digestive tract
• Hepatocarcinoma,
• Mall cell tumors
• Myeloma and leukemia
• Primitive tumors and metastasis

According to earlier report it has been proven that the utilization of doxorubicin loaded conventional nanoparticles was effective against hepatic metastasis model in mice. Moreover it was also discovered that greater reduction in the degree of metastasis than when free drug was used. This is possible due to the allocation of drug reservoir to the malignant tissues and transfer of doxorubicin from healthy tissue, resulting in increased therapeutic efficacy of the formulation [119]. Several other parameters such as histological examination of tissue ensures considerable accumulation of nanoparticles in the lysosomal vesicles of Kupffer cells, whereas nanoparticles could not be clearly identified in tumoral cells [119].

Following a massive uptake of nanoparticles by phagocytosis, Kupffer cells, potentially induce the release of doxorubicin, resulting in to drug concentration gradient, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells [119].

When conventional nanoparticles are used during chemotherapy, toxicity up to certain extent against the Kupffer cells can be expected would result in deficiency of Kupffer cells. This may finally resulted in to the reduced liver uptake and decreased therapeutic effect [120]. In addition, conventional nanoparticles can also be a excellent target for bone marrow. Bone marrow site is an important but unfavorable site of action for most anticancer drugs since treatment with chemotherapeutic agents at this site increase myelosuppresive effect. Thus potential of conventional nanoparticles to improve anticancer drugs efficacy is limited to targeting tumors at the level of mononuclear phagocytic system-rich organs. Furthermore, targeting anticancer drug-loaded nanoparticles to other tumoral sites is not feasible if a rapid clearance of nanoparticles occurs shortly after intravenous administration.

2.6.26 Long-Circulating and Target-Specific Nanoparticles

Nanoparticles are known to be the most successful way of delivering drug at desirable site. They can be used to target tumors which are localized outside mononuclear phagocytic system rich organs. Instantaneous identification of colloidal carriers (liposomes and polymeric nanospheres) from the blood by Kupffer cells, has begin a surge of development for “Kupffer cell-evading” or long-circulating particles. These carriers have major applications in vascular drug delivery and release, site-specific targeting (passive as well as active targeting), as well as transfusion medicine. So much effort has been done to develop so-called “stealth” particles (PEGylated nanoparticles), which are invisible to macrophages or phagocytes [121].

Utilization of hydrophilic polymers (poloxamines, poloxamers, polyethylene glycol, and polysaccharides) has begun a major breakthrough in the nanotechnol-
ogy field to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the mononuclear phagocytic system \[122\]. coating around these nanoparticles provide a active “cloud” of hydrophilic and neutral chains at the particle surface which repel plasma proteins \[123\], resulting in to the invisibility of coated nanoparticles against mononuclear phagocytic system, therefore, remained in the circulation for a longer period of time. Introduction of hydrophilic polymers can be achieved in two ways, either by adsorption of surfactants or by use of block or branched copolymers for production of nanoparticles.

Several studies also proved that PEG coated nanoparticles have a prolonged half-life in the blood compartment. However they can be selectively extravasate in pathological sites such as tumors or inflamed regions with a leaky vasculature, resulting in increase in potential of long-circulating nanoparticles directly towards targeted tumors located outside MPS-rich regions. Several characteristics such as size of the colloidal carriers and surface characteristics are critical to the biological fate of nanoparticles.

Nanoparticle size (less than 100 nm) and surface characteristics (hydrophilic surface) obligations are required in achieving the reduction of opsonisation reactions and subsequent clearance by macrophages. Surface coating of conventional nanoparticles with PEG or surfactants to obtain a long-circulating carrier has now been used as a standard strategy for drug targeting \textit{in vivo}. Various researches have been contributed to achieve “active targeting” of nanoparticles in order to deliver drugs to the right targets. This is particularly based on molecular recognition processes such as ligand-receptor or antigen-antibody interaction. Based on earlier reports related with overexpression of folate receptors on the surface of some human malignant cells and the cell adhesion molecules (selectins and integrins), it was concluded that nanoparticles bearing specific ligands such as folate may be used to target ovarian carcinoma while specific peptides or carbohydrates may be used to target integrins and selectins \[124\]. In one more report it was suggested that the benefits of folate ligand coating were to facilitate tumor cell internalization and retention of Gd-nanoparticles in the tumor tissue \[125\]. Small ligands based targeting emerges more likely to be successful since they are easier to handle and manufacture.

In addition they could be more significant when the active targeting ligands are used in combination with the long-circulating nanoparticles. Such combination can be used to maximize the likelihood of the success in active targeting of nanoparticles. Since cancer cells are able to develop mechanisms of resistance, deterioration of multidrug resistance anticancer drugs in tumor cells or in the tumor interstitium occurs, resulting in to their limited efficacy against numerous solid tumors. Such mechanism facilitates tumors to evade chemotherapy. One of the most serious problems in chemotherapy is multidrug resistance, mainly due to the over expression of the plasma membrane pglycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells. Several strategies including the use of colloidal carriers have been applied in order to restore the tumoral cells’ sensitivity to anticancer drugs by circumventing Pgp-mediated MDR. The underlying principle behind the drugs association with colloidal carriers (such as nanoparticles), against drug resistance derives from the fact that
Pgp probably recognizes the drug to be effluxed out of the tumoral cells. This can be achieved only when this drug is present in the plasma membrane, and not when it is located in the cytoplasm or lysosomes after endocytosis [126].

2.6.27 Nanoparticles for Oral Delivery of Peptides and Proteins

Exploration of more antigenic substances in biotechnology and biochemistry field persist the production of vaccines. Owing to the recent advancement biotechnology various bio macromolecules and vaccines are explored. Thus there is an urgent requirement of their suitable carriers system which still remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymer based nanoparticles facilitates the encapsulation of bio-active molecules and protect them against enzymatic and hydrolytic degradation e.g. insulin-loaded polymeric nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration. It’s universally known that the surface area of human mucosa extends to 200 times that of skin [127]. Protein or peptide based drug delivery encountered a variety of physiological and morphological barriers:

- Bacterial gut flora
- Mucus layer and epithelial cell lining itself
- Proteolytic enzymes at the brush border membrane (endopeptidases)
- Proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin

Histological feature of intestinal mucosa is designed in such a way to efficiently prevent uptake of particulate matter from the environment. To overcome the gastrointestinal barrier, drug can be delivered in a colloidal carrier system (nanoparticles), which can potentially enhance the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract. Nanoparticles based targeting to epithelial cells using ligands is an potential strategy to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer’s patches in the GI tract. Such type of targeting can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. M cells display cell-specific carbohydrates and enterocytes surface may serve as binding sites to colloidal drug carriers containing appropriate ligands. Several glycoproteins and lectins efficiently bind to this type of surface structure by specific receptor-mediated mechanism. Lectins such as tomato lectin and bean lectin have been studied to enhance oral peptide adsorption [128]. For an instance vitamin B-12 absorption from the gut under physiological conditions (occurs via receptor-mediated endocytosis) can be enhanced by covalent coupling with peptides such as granulocyte colony stimulating factor, erythropoietin, resulting in enhancement of oral bioavailability [129]. For making process more efficient and selective mucoprotein is required,
which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. After reaching to the ileum mucoprotein resorption is mediated by selective binding to specific receptors which can further enhance the absorption. Absorption can also be enhanced by using non-specific interactions. Generally, absorption of macromolecules and particulate materials in the gastrointestinal tract is achieved by either paracellular route or endocytotic pathway. Less than 1% of mucosal surface area is utilized for the paracellular route of absorption of nanoparticles having polymeric material such as starch or chitosan, poly(acrylate) [130–132]. Such polymers enhance the paracellular permeability of macromolecules. Absorption of nanoparticles mediated by endocytotic pathway can be accomplish by receptor-mediated endocytosis (active targeting) or adsorptive endocytosis which does not need any ligands. Existence of electrostatic forces (hydrogen bonding or hydrophobic interactions) between the cell surface and absorbed material encourages this whole process. Adsorptive endocytosis primarily dependent on the size and surface properties of the material e.g. positive surface charged nanoparticles provide an affinity to adsorptive enterocytes though hydrophobic, whereas negative surface charged nanoparticles and hydrophilic shows greater affinity to adsorptive enterocytes and M cells. So it has been concluded that of size, surface charge and hydrophilicity play a major role in absorption of material.

### 2.6.28 Nanoparticles for Gene Delivery

Several vaccines based nanomedicines functions to deliver genes to host cells and show their expression by production of antigenic protein to initiate immune response. One of the recent example of polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells (where they are expressed) producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such types of vaccines are responsible for the both humoral and cell-mediated immunity since intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system. Polynucleotide vaccines are composed of a key ingredient called as DNA, can be produced economically and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines. Based on the potential immunotherapy these polynucleotide vaccines are set to supersede many conventional vaccines. Nevertheless these polynucleotides based vaccine suffers from several issues which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site are still in consideration. Owing to the rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment, plasmid DNA based nanoparticulate system serve as a potential sustained release gene delivery system. Previous finding suggested the intracellular uptake and endolysosomal escape of these nanoparticles offer sustained release of DNA.
resulting in sustained gene expression. Such strategy could be utilized to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein [133, 134].

2.6.29 Nanoparticles for Drug Delivery into the Brain

Nervous system is one of the most delicate microenvironments of the body which is protected by the blood–brain barrier (BBB) regulating its homeostasis. The blood–brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. BBB is a highly complex structure that tightly regulates the movement of ions of a limited number of small molecules and of an even more restricted number of macromolecules from the blood to the brain, protecting it from injuries and diseases. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. BBB only permits selective transport of molecules that are essential for brain function, consequently, the BBB also significantly precludes the delivery of drugs to the brain, thus, preventing the therapy of a number of neurological disorders. As a consequence, several strategies are currently being sought after to enhance the delivery of drugs across the BBB. For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melano transferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis [135–139]. It has been discovered that poly(butylcyanoacrylate) based nanoparticles was able to deliver dalargin, hexapeptide, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB. In addition to several reports which are based on the success of polysorbate coated NPs, this system does have many shortcomings including rapid NP degradation, toxicity caused by presence of high concentration of polysorbate and desorption of polysorbate coating. In addition to some reports OX26 MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of liposomes. Presently, there are no effective therapies for many diseases include neurodegeneration (e.g., amyotrophic lateral sclerosis, Alzheimer’s, Parkinson’s, Huntington disease, and Prion disease), genetic deficiencies (e.g. lysosomal storage diseases, leukodystrophy), and several types of brain cancer. Even if candidate drugs for therapy of such diseases may be already available in line of principle, they cannot be currently utilized because of their insignificant access to the central nervous system (CNS), due to the presence of the blood–brain barrier (BBB) preventing the passage from blood to the brain. It is possible soon we will see these BBB specific molecules used for targeting nanoparticles to the brain.
2.6.30 Anthrax Vaccine Uses Nanoparticles to Produce Immunity

Anthrax is caused by the spore-forming, Gram-positive bacterium *Bacillus anthracis*. The toxic effects of *B. anthracis* are predominantly due to an AB-type toxin made up of the receptor-binding subunit protective antigen (PA) and two enzymatic subunits called lethal factor and edema factor. Protective immunity to *B. anthracis* infection is conferred by antibodies against PA, which is the primary component of the current anthrax vaccine. A vaccine against anthrax that is more effective and easier to administer than the present vaccine has proved highly effective in tests in mice and guinea pigs, report University of Michigan Medical School scientists in the August issue of Infection and Immunity. Although the vaccine is safe and effective, it requires multiple injections followed by annual boosters. The scientists were able to trigger a strong immune response by treating the inside of the animals’ noses with a “nanoemulsion” a suspension of water, soybean oil, alcohol and surfactant emulsified to create droplets of only 200–300 nm in size. The oil particles are small enough to ferry a key anthrax protein inside the nasal membranes, allowing immune-system cells to react to the protein and initiate a protective immune response. That primes the immune system to promptly fight off infection when it encounters the whole microbe. It would take about 265 of the droplets lined up side by side to equal the width of a human hair. The oil particles are small enough to ferry a key anthrax protein inside the nasal membranes, allowing immune-system cells to react to the protein and initiate a protective immune response. That primes the immune system to promptly fight off infection when it encounters the whole microbe. Besides eliminating the need for needles, the nanoemulsion anthrax vaccine has another advantage, the researchers say: It is easy to store and use in places where refrigeration is not available. An effective and easy-to-administer vaccine would be a valuable tool for health authorities dealing with any future attack in which a terrorist might spread anthrax microbes. The researchers say a nasal nanoemulsion-based anthrax vaccine, if it proves effective in humans, could be given easily to people even after they are exposed in an anthrax attack, along with antibiotics. With some diseases, vaccines given after exposure are used to boost the speed of the immune response.

2.6.31 Stem Cell Therapy

Nanotechnology presents efficient tools for improving stem cell therapy. The synergy between size, structure and physical properties of NPs makes them key players in revealing the fate and performance of stem cell therapy. Clearly NPs have much to offer in stem cell research and therapy. Stem cell therapies offer great potentials in the treatment for a wide range of diseases and conditions. With so many stem cell replacement therapies going through clinical trials currently, there is a great need to understand the mechanisms behind a successful therapy, and one of the critical
points of discovering them is to track stem cell migration, proliferation and differentiation in vivo. To be of most use tracking methods should ideally be noninvasive, high resolution and allow tracking in three dimensions. Magnetic resonance imaging is one of the ideal methods, but requires a suitable contrast agent to be loaded to the cells to be tracked, and one of the most wide-spread in stem cell tracking is a group of agents known as magnetic nanoparticles. Researchers have successfully used nanoparticles to improve stem cells potential in stimulating the regeneration of damaged vascular tissue and reduce muscle degeneration in mice (published online on October 5 in Proceedings of the National Academy of Sciences). In addition researchers investigating stem cells role in stimulating new blood vessel formation. This was investigated after their implantation into a living organism. Cells may not continue to renew tissue effectively enough to keep the tissue alive long-term. Hence cells can benefit from help with performance-enhancing genes, which promote growth in the target tissue. Researchers usually rely on viral vectors to deliver these therapeutic genes to stem cells. It has been now investigated that (Chemistry researchers at the University of Warwick) tiny nanoparticles can be utilized for delivering this therapeutic gene since tiny nanoparticles could be twice as likely to stick to the interface of two non mixing liquids than previously believed. This research open gateways for the utilization of nanoparticles in living cells, polymer composites, and high-tech foams, gels, and paints.

### 2.6.32 Gold Nanoparticles Detect Cancer

Metallic nanostructures are more flexible particles compared to other nanomaterials owed to the possibility of controlling the size, shape, structure, composition, assembly, encapsulation and tunable optical properties. Between the metallic nanostructures possible applied, AuNPs appears of great interest in the medical field, showing great efficiency towards cancer therapy [140–143]. The continuous interest in AuNPs is based in their tunable optical properties that can be controlled and modulated for the treatment and diagnosis of diseases. Various researchers have utilized gold nanoparticles as ultrasensitive fluorescent probes to detect cancer biomarkers in human blood. High sensitivity of this approach surpass the current methods by several orders of magnitude and make the process more suitable for direct detection of viral or bacterial DNA. These nanoparticles are promising probes for biomedical applications since they can be easily manufactured and, unlike other fluorescent probes (quantum dots or organic dyes), they don’t get heated and burn out after long exposure to light. In one report, china based researchers apply the particles to detect carcinoembryonic antigen (CEA) and alpha foetal protein (AFP) - two important biomarkers in the diagnosis of various cancers, including liver, breast and lung cancer. In this work they have conjugated nanoparticles with antibodies to measure the amount of biomarker levels present in the sample.
2.6.32.1 AuNPs in Cancer Therapy

- **Photothermal Therapy**
  AuNPs presents tunable optical properties that allow the absorption of light at near UV to near infrared, being the last one a characteristic that allows nanoparticles to enter cells, constituting a major breakthrough for its application in photothermal therapy or hyperthermia [144].

- **Radiotherapy**
  AuNPs have been reviewed in radiotherapy experiments in order to overcome the problems associated to the healthy tissue damage imposed by radiotherapy [144].

- **Angiogenesis inhibition**
  The inhibition of angiogenesis, i.e. the formation process of new blood vessels, is also a potent mechanism by which AuNPs can operate for cancer therapy [144].

2.6.32.1.1 AuNPs as Delivery Systems

The well-known application of AuNPs in cancer therapy described above, lead to further investigation of new potential therapeutic strategies and was verified that AuNPs can be used in the design of delivery systems [145]. AuNPs as a potential nanocarrier have the possibility to carry different payloads, such as small drug molecules for drug delivery or biomolecules like DNA, proteins and RNA (siRNAs), being recognized as an attractive gene delivery system.

- **Specific targeting**
  The potency of such systems is achieved by the enhancement of cellular accumulation of AuNPs by an active targeting to cancer cells compared to a free drug that passively enters the cells, which simultaneously avoid the biological response and biophysical barriers *in vivo* [146].

- **AuNPs for drug/cargo delivery**
  The construction of DDSs depends on size, charge and surface functionalities of the AuNPs, once they dictate the uptake capacity of such nanovectorization systems as well as its intracellular fate [147].

- **AuNPs for gene therapy**
  Gene therapy is though as a hopeful strategy in cancer therapy being considered as a powerful treatment like chemotherapy and radiotherapy, however the implementation of such systems is based in viral vectors that raise cytotoxic and immune response problems [148]. When conjugated to AuNPs, siRNAs have been shown to exhibit increased stability, cellular uptake and efficacy in physiological conditions, retaining the ability to act through the RNAi pathway.
2.6.32.2 Toxicity of AuNPs

One major concern regarding AuNPs application in medical field relies in its toxicity in the biological systems, i.e. the production of a general toxicity response not only in cancer cells but also reaching healthy cells at the vicinity. Taken into account the size, surface modifications and solubility in promoting biocompatibility of the nanovectorization systems, they can be safer to apply in the medical field to the treatment of cancer. In fact, nanoparticles size is an important feature because it turns possible to circumvent the immune response and renal clearance, which maintains the therapeutic capacity of such systems [149].

2.7 Hazards and Toxicity Profile of Nanoparticles

Various reports are available on the toxicity of nanoparticles (Table 2.10) that are originated from the inhalation toxicology including particulate matter with a size below 10 nm.

2.7.1 Health Implication of Nanoparticles

It’s very essential to recognize and differentiate ‘free’ and ‘fixed’ nanoparticles. Free nanoparticles exhibit serious health threat since they are more difficult to contain due to airborne and can be inhaled. Nanoparticles can be entered in human body via

- Absorptions by the intestinal tract
- Absorptions by the skin [151]
- Lungs where a rapid translocation through the blood stream to vital organ is possible, including crossing the BBB and

Nanoparticles affect the following organs in several ways:

- Lungs
  It has been already observed that titanium dioxide (TiO₂) carbon black and the diesel particles exhibit various adverse effects. Based on previous findings it has been observed that the administration of ultrafine nanoparticles to the lung produce more potent adverse effect in the form of inflammation and subsequent tumors compared with larger sized particles, of identical chemical composition at equivalent mass concentration. Toxicity of these particles is dependent on their surface characteristics such as surface chemistry [152]

- Intestinal tract
  To facilitate the absorption of the food particles, the epithelium of the small and large intestinal is in close contact with ingested material. These food particles are converted in to a mixture of disaccharides, peptides, fatty acids and monoglycerides generated
<table>
<thead>
<tr>
<th>Type of nanoparticles</th>
<th>Toxicity</th>
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| Carbon nanotubes      | • MWCNT: Elicit proinflammatory responses in keratinocytes; Platelet aggregation  
• On a dose per mass basis carbon nanotubes are more toxic than quartz particles which are well known for their lung toxicity  
• SWCNT: *In vitro* incubation of high dose of SWCNT with keratinocytes and bronchial epithelial cells results in ROS generation, oxidative stress, lipid peroxidation, mitochondrial dysfunction and changes in cell morphology; Platelet aggregation; Intratracheal instillation of high doses of nanotubes causes chronic lung inflammation, foreign body granuloma formation, interstitial fibrosis; *in vivo* studies SWCNT induce lung granuloma |
| Dendrimers           | • Albertazzi et al. [150] demonstrated the *in vivo* distribution and toxicity of PAMAM dendrimers in the central nervous system depend on their surface chemistry  
• Limited clinical experience using dendrimers make it impossible to designate any particular chemistry intrinsically “safe” or “toxic” |
| Fullerenes            | • Sonicated c-60 fullerenes LC50 was relatively found to be very high, causes lipid peroxidation and related toxicity in brain |
| Gold nanoparticles    | • For gold nanorods the cytotoxicity is attributed to the presence of stabilizer CTAB  
• Gold nanoparticlese can cause cell death in the presence of activated laser light |
| Quantum dots          | • Cadmium-containing QDs can kill cells in culture  
• QDs undergo design-dependent intracellular localization and they can cause cytotoxicity by releasing free cadmium into solution and by generating free radical species. In animal experiments, QDs preferentially enter the liver and spleens following intravascular injection, undergo minimal excretion if larger than 6 nm, and appear to be safe to the animal  
• In vitro and *in vivo* studies show an apparent discrepancy with regard to toxicity. Dosing provides one explanation for these findings. Under culture conditions, a cell experiences a constant QD dose, but the *in vivo* QD concentration can vary, and the organ-specific dose may not be high enough to induce detectable toxicity  
• Surface coating of quantum dots during in vitro studies might be toxic. In contrast many studies also report that the surface modification decrease the toxicity induced by naked quantum dots |
| Silica nanoparticles  | • For silica nanoparticles both *in vitro* toxic and non toxic responses have been found  
• Silica exposure results in an increased ROS levels indicating increase in oxidative stress  
• Cell with long doubling time is more toxic against silica nanoparticles then with short doubling time  
• Alveolar macrophage cell line is more susceptible against cytotoxicity then lung epithelial cell line |
however digestion in small intestine are further transformed and taken in the villi. Particles having charge (e.g. like carboxylated polystyrene nano particles or those composed of positively charged polymer) exhibit poor oral bioavailability through electrostatic repulsions and means entrapment \([153]\). Smaller the size of particles facilitate the faster penetration (within 2 mints.); 415 nm particles took 30 mints whereas 1000 nm particles were not capable to translocate through this barrier \([154]\).

- **Skin**
  Particles having size range 500–1000 nm, theoretically afar from the area of nanotechnology can infiltrate and reach the lower level of human skin. Size range smaller than 128 are more likely penetrate deeper in to the skin \([155]\).

- **Blood and cardiovascular system**
  Cationic nanoparticles including gold and polystyrene have been shown to cause hemolysis and blood clotting while anionic nanoparticle are non toxic. Combustion and model nanoparticles can gain access to blood following inhalation and can enhance the experimental thrombosis. High exposure to DEP by inhalation cause altered heart rate in hypertensive rats. Inhalation of PM causes atheromatous plaque and destabilization in rabbits. Recent data showed that Carbon derived nanomaterials induce platelet aggregation

- **Brain**
  High concentrations of anionic and cationic nanoparticles are toxic to brain. Nanoparticles have been shown to produce reactive oxygen species and oxidative stress. This has been confirmed in the brain after inhalation of MNO\(_2\) nanoparticles. Oxidative stress induced by nanoparticles causes various neurodegenerative disease such as Parkinson and Alzheimer. In addition inhalation of nanoparticles in balb/c mice to particulate matter showed the activation of pro-inflammatory cytokines in the brain

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