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REVIEW

Drug Delivery Using Nanocarriers: Indian Perspective

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Abstract Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery technologies are patent protected formulation technologies that modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues or other diseased tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation. Nanocarrier delivery systems are extensively being investigated as a drug delivery strategy in the pharmaceutical research from the last 3 decades. Nanoparticulate technologies in general offer immense benefits such as solubilization of hydrophobic active pharmaceutical ingredient (API), improvement in bioavailability, improved (or altered) pharmacokinetics of API and protection of API from physical, chemical or biological degradation. As nanocarriers may also exert toxicological effects, nanotoxicology has emerged as a new branch of toxicology for studying undesirable effects of nanocarriers. Therefore, development of novel nanocarriers for therapeutics and diagnostics must proceed in tandem with assessment of any toxicological and environmental side effects of these carriers.

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Introduction

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery technologies are patent protected formulation technologies that modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Most common routes of administration include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes. Many medications such as peptide and protein, antibody, vaccine and gene based drugs, in general may not be delivered using these routes because they might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently due to molecular size and charge issues to be therapeutically effective. For this reason many protein and peptide drugs have to be delivered by injection. For example, many immunizations are based on the delivery of protein drugs and are often done by injection. Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues or other diseased tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation. Types of sustained release formulations include liposomes, drug loaded biodegradable nanoparticles, microspheres and drug polymer conjugates etc. [1].

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At present, there are 30 main drug delivery products on the market. The total annual income for all of these is approximately US$33 billion with an annual growth of 15 % (based on global product revenue). The reasons for this increasing interest in drug delivery are due to the increasing need of safe drugs, capable of reaching the target and with minimal side effects. In fact the main problems associated with systemic drug administration are essentially related to the bio-distribution of pharmaceuticals throughout the body. This indiscriminate distribution means that, to achieve a required therapeutic concentration the drug has to be administered in large quantities, the major part of which is just wasted in normal tissues. Ideally, a "perfect" drug should exert its pharmacological activity only at the target site, using the lowest concentration possible and without negative effects on non-target compartments [2]. The process for delivering a drug is as important as the actual activity of the drug in determining the therapeutic effect. For optimum therapeutic effect, the right amount of a drug needs to get to the right place at the right time. Consequently, advanced drug delivery formulations have been developed over the past 20 years that do not simply release a drug at a specific rate, but release the drug in a way that the pharmaceutical scientists and engineers have designed. Additionally, because drug delivery can improve safety, efficacy, convenience and patient compliance, improving delivery methods has become a major focus of pharmaceutical companies.

In traditional drug delivery, common delivery routes include oral, pulmonary, transdermal and injection, and they each have certain advantages and disadvantages associated with them. For instance, all of these routes, except for direct injection into a vein or muscle tissue, have cellular layers that are encountered, which function as a barrier to transport into the systemic circulation.

Controlled release in drug delivery can significantly enhance the therapeutic effect of a drug. Typically, controlled release is used to achieve sustained or pulsatile drug release. Sustained release is used to achieve a constant release of a drug over an extended period of time. For example, many drugs have an optimum range of concentrations that if the concentration of the drug is above or below this range, the drug is toxic or has no therapeutic effect, respectively. In this case, controlled release would be used to maintain the concentration of drug delivered within the optimum range for maximum therapeutic effect. In contrast, there are many situations where it is not optimal to have sustained release because it fails to mimic the body's natural response. For instance, a healthy individual produces insulin in a pulsatile manner, and therefore, a pulsatile delivery is typically utilized in the treatment of diabetes to mimic the insulin production of the body [3, 4].

Nanocarrier Delivery Systems

Nanocarrier delivery systems are extensively being investigated as a drug delivery strategy in the pharmaceutical research from the last 3 decades. Nanoparticulate technologies in general offer immense benefits such as solubilization of hydrophobic active pharmaceutical ingredient (API), improvement in bioavailability, improved (or altered) pharmacokinetics of API, protection of API from physical, chemical or biological degradation. Moreover, the nanosize of these systems allows efficient crossing of biological barriers, amelioration in tissue tolerance, improved cellular uptake and transport, thus enabling efficient delivery of the therapeutic agents to the target sites like liver, brain and solid tumor. Furthermore, by modulating the surface properties, composition and milieu, the desired release pattern of the therapeutic agent and biodistribution can be achieved. Apart from all the aforementioned advantages, one of the major advantages associated with nanoparticulate systems is their ability to withstand physiological stress or improved biological stability and possibility of oral delivery which makes them more attractive as a drug delivery strategy [5].

Nano-sized carriers (10–400 nm) are desirable as drug carriers because they possess the advantages of being capable of carrying large amount of drugs, having prolonged circulation time (especially when surface PEGylated), and facilitating selective tumour accumulation via the enhanced permeability and retention (EPR) effect. Nanocarriers are also helpful in addressing other limitations of conventional drugs, including poor aqueous solubility, low bioavailability and/or unfavourable pharmacokinetic properties. In addition, delivery via nanocarriers has been reported to overcome multidrug resistance (MDR) caused by drug efflux transporters such as the P-glycoprotein (P-gp), which are frequently overexpressed in cancer cells [6, 7]. To achieve higher specificity, nanocarriers can be surface modified with ligands that specifically recognize receptors on target cells as shown in Fig. 1. Combining passive and active targeting in a single platform may further improve the therapeutic index of nanocarrier delivered drugs.

Need of Nanocarriers

Development of nonocarriers is a novel area of science that provides, with a new hope, the tools and technology to work at atomic, molecular and supramolecular levels leading to creation of devices and delivery systems with fundamentally new properties and functions. These carriers offer a number of advantages making it an ideal drug delivery vehicle.

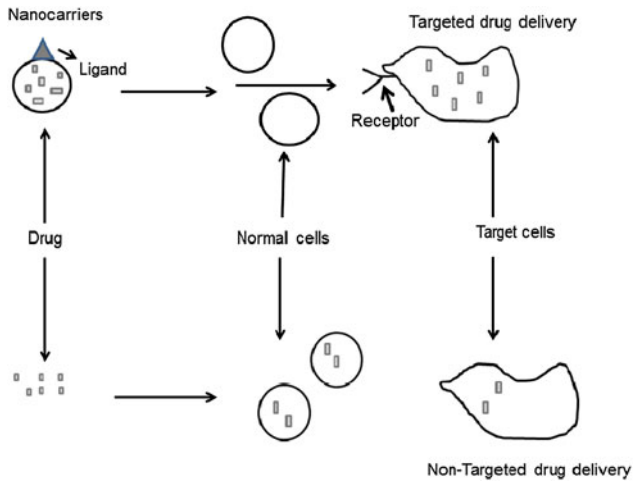

Fig. 1 Schematic presentation of site specific drug delivery using nanocarrier systems

1. Better drug delivery to certain stubborn or impermeable sites of body.
2. Owing to their small size, chemistry and distribution, these carriers better bridge the gaps between the structure and function of biomolecules.
3. Reaching the micron or nano range with these particles enables them to be highly potential carriers for many biological molecules as proteins, DNA, viruses and xenobiotics.
4. Better targeting to body tissues and sites where action is required, elimination of side effects and adverse effects.
5. Owing to size, nature and chemistry, these systems give better drug permeability from biological membranes and help in solubilization of some practically insoluble drugs and hence solve bioavailability problems of many drugs.
6. These carriers involve overlap of biotechnology, nanotechlogy and information technology, which might result in many important applications in life sciences including areas of gene therapy, drug delivery, imaging, biomarkers, biosensors and novel drug discovery techniques.
7. Nanocarriers offer an attractive solution for transformation of biosystems, and provide a broad platform in several areas of bioscience.
8. The surface properties of carriers 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.
9. Targeted drug carriers reduce drug toxicity and provide more efficient drug distribution.
10. Drug carriers hold promise to deliver biotech drugs over various anatomic extremities of body such as blood brain barrier (BBB), branching pathways of the pulmonary system, and the tight epithelial junctions of the skin etc.
11. Drug carriers better penetrate tumors due to their leaky constitution, containing pores ranging from 100 to 1,000 nm in diameter [8, 9].

Limitations of Nanocarriers

1. Drug carriers exhibit difficulty in handling, storage, and administration because of susceptibility to aggregation.
2. These are unsuitable for less potent drugs.
3. The key area of concern is related to its small size as nanocarriers can gain access to unintended environments with harmful consequences, e.g. it can cross the nuclear envelope of a cell and cause unintended genetic damage and mutations [10, 11].

Preparation Methods of Nanocarriers

Nanotechnology-based synthetic methods are most commonly developed on the basis of two rational designs; either top-down or bottom-up engineering of individual components. The top-down process involves starting with a larger object and breaking it up into nanostructures through etching, grinding, or ball milling. The process can be accelerated by addition of chemicals or using laser. Microscale or macroscale manufacturing, like silicon microfabrication and photolithography, is often accomplished as top-down process. However, the method is time consuming and often generates considerably broader particle size distribution [12]. The bottom-up technique refers to synthesis based on atom-by-atom or molecule-by-molecule arrangement in a controlled manner, which is regulated by thermodynamic means. The process takes place through controlled chemical reactions, either in gas or liquid phase, resulting in nucleation and growth of nanoparticles. Bottom-up techniques (like supercritical fluid (SCF) antisolvent techniques, precipitation methods, etc.) create heavily clustered masses of particles that do not break up on reconstitution [13, 14]. Various methods of preparation of nanocarriers are summarized in Fig. 2.

High-Pressure Homogenization Method

High-pressure homogenization (HPH) has been used as a reliable and powerful technique for the large-scale production of nanostructured lipid carriers (NLCs), lipid drug conjugate (LDC), solid lipid nanoparticles (SLNs), and parenteral emulsions. The lipid is pushed with high

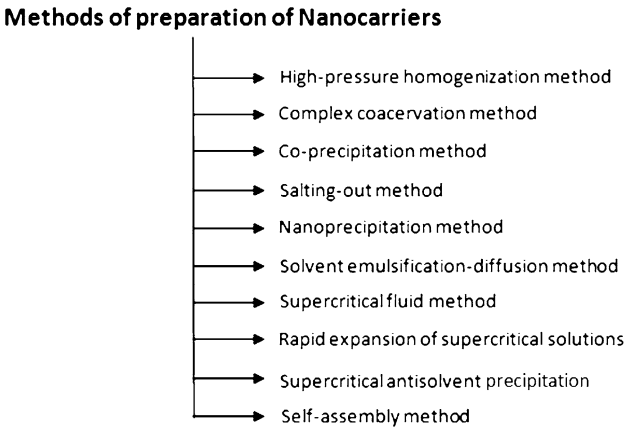

Fig. 2 Various methods of preparation of nanocarriers

pressure (100 to 2,000 bars) through a very high shear stress, which results in disruption of particles down to the sub micrometer or nanometer range. Homogenization may be performed either at elevated temperature (hot homogenization) or below room temperature (cold homogenization). In the hot homogenization process, the melted lipid containing solubilized drug is dispersed in a hot aqueous surfactant solution at identical temperature to form a pre-emulsion. It is subsequently homogenized at a high pressure (500 bars) and temperature above the melting point of lipid, resulting in formation of an oil-in-water (o/w) nanoemulsion. Upon being cooled down to room temperature, the lipid recrystallizes and gives rise to SLNs. The method gives lower particle size because of the decreased viscosity of the inner phase at higher temperatures; however, such a high temperature may result in an increased degradation rate of the active component and lipids. The cold homogenization method has been developed for thermolabile drugs in view of the limitations of the hot homogenization process. Typically, the method involves the solubilization or dispersion of drug in the lipid melt at a temperature 5 to 10 °C above the melting point of lipid. Solidification is performed by pouring the drug-loaded lipid in dry ice or liquid nitrogen. The solid, containing drug and lipid, is milled to obtain typical 50- to 100-μm particles, which are dispersed in a chilled emulsifier solution to form a presuspension. It is then homogenized at or below room temperature to obtain SLNs [15, 16].

Complex Coacervation Method

Complex coacervation is a spontaneous phase separation process of two liquid phases in colloidal systems, which results by the interaction of two oppositely charged polyelectrolytes upon mixing in an aqueous solution. The process leads to formation of micrometric or nanometric colloidal particles, depending on substrates or process variables, such as pH, temperature, molecular weight, ionic strength, polyelectrolyte concentration, and so forth. However, this method exhibits poor drug stability and less drug loading efficiency, which can be improved by cross-linking of the complex by chemical reagents, such as glutaraldehyde [17].

Co-Precipitation Method

Co-precipitation is a modified complex coacervation method for the preparation of nanoscale core–shell particles and provides good dispersion stability to poorly water-soluble drugs. Ibuprofen (Ib) nanoparticles were stabilized by diethyl amino ethyl cellulose (DEAE)-dextran (Ddex as water-soluble, positively charged resin). The latter was used as a coating layer to co precipitate with negatively charged drug. The method involved precipitation of Ib in a supersaturated solution, followed by deposition of Ddex onto the precipitated Ib particles through electrostatic interactions. Transmission electron microscopy (TEM), atomic force microscopy (AFM), and zeta potential studies showed typical core–shell nanoparticles, with high encapsulation efficiency and good stability with Ddex/Ib in a weight ratio of 5:1 [17]. Another method, evaporative precipitation into aqueous solution (EPAS), was suggested for preparing submicrometer particles of a poorly water-soluble drug, cyclosporin A. Incorporation of hydrophilic stabilizers within the aqueous phase resulted in minimized particle growth and thus restricted the crystallization of drug during storage. The resulting amorphous nanoparticle suspension could either be used in parenteral formulations or be dried to produce oral dosage forms with high dissolution rates [18].

Salting-Out Method

The salting-out method is widely used in the pharmaceutical industry owing to its high yield, purity, and speed and simplicity of the operation. The method does not demand thermal treatment at any stage of sample processing and, therefore, may be especially useful for the incorporation of thermolabile drugs [19]. It is based on the phenomenon in which solubility of a nonelectrolyte in water is decreased upon addition of an electrolyte. Though it involves an emulsification stage, it still avoids the use of surfactants and chlorinated solvents. A water-soluble stabilizing polymer is added to a saturated solution of electrolyte (e.g, sodium chloride, magnesium acetate, or magnesium chloride) to obtain a viscous gel. Subsequently, polymer and drug are dissolved separately in an organic solvent. Most often, acetone is used as solvent because of its solubilizing properties and well known separation from aqueous solution upon salting-out with electrolytes. Addition of viscous

gel into organic phase under continuous stirring causes salting out of the organic solvent, inducing formation of nanoparticles in organic-aqueous medium [20]. Finally, both solvent and electrolyte are eliminated by cross-flow filtration. Toth et al. [21] suggested the salting-out method for glycine as model substance, wherein sodium chloride was salted out from its aqueous solution using ethanol as an antisolvent. The process resulted in production of fine particles with minimum agglomeration.

Nanoprecipitation Method

Nanoprecipitation, also known as solvent displacement method, is based on interfacial deposition of a polymer after displacement of a semipolar solvent miscible with water from a lipophilic solution. Rapid diffusion of the solvent into aqueous phase results in a decrease in the interfacial tension between the two phases, which increases the surface area and leads to formation of small droplets of organic solvent even without any mechanical stirring. However, it provides poor entrapment efficiency for water-soluble drugs. Modified nanoprecipitation method has been developed by using poly (D, L-lactide-co-glycolide) (PLGA) nanoparticles, which includes various approaches like influence of aqueous phase pH and change in drug salt form to enhance the incorporation efficiency of highly water-soluble drugs like procaine hydrochloride [22].

Solvent Emulsification–Diffusion (SED) Method

SED is the most commonly used method for preparation of solid-lipid (SLNs) and polymeric nanoparticles (PNPs). An o/w emulsion is prepared with oil phase containing polymer and oil in an organic solvent. It is emulsified with the aqueous phase, containing stabilizer, in high shear mixer, which is followed by addition of water to induce the diffusion of organic solvent thus resulting in formation of nanoparticles. The selected organic solvent must be partially soluble in water (for diffusion step) and have the capacity to dissolve both the oil and polymer. Besides, it should be removed safely under reduced pressure. Ethyl acetate is a suitable solvent in the face of the above-mentioned qualities [23].

Supercritical Fluid (SCF) Methods

Submicrometer-sized and nano-sized particles can be designed by using various SCF methods. A SCF can either be a liquid or gas and used above its thermodynamic critical point of temperature and pressure. Most commonly used SCFs are carbon dioxide (CO_2) and water [24]. On the basis of formulatory approaches, two SCF methods are described below.

Rapid Expansion of Supercritical Solutions (RESS)

RESS is a useful technique for thermolabile drugs and it produces finely divided particles with a controlled size distribution. The process involves saturation of SCF with the substrate and depressurization of the obtained solution through a heated nozzle into a low-pressure chamber to cause rapid nucleation of the substrate. As the solution is allowed to expand across a calibrated orifice, the density decreases gradually and the solute is precipitated as finely divided solid fibers or crystals (Fig. 3). Expansion at high pressure keeps the density high and reduces the flow velocity of particles, thus providing the particles enough growth time for clustering and aggregation. Consequently, larger particles are produced at high expansion pressure. On the other hand, expansion into low pressure causes density to become low and velocity high, so that both the flow time and density are less favorable for the growth of larger clusters. Besides, maintenance of homogeneous experimental conditions gives rise to controlled and uniform particle size distribution. CO_2 has widely been used as SCF in most RESS techniques. For slightly soluble drugs, fluorinated hydrocarbons, like trifluoromethane (e.g., CHF_3), can be used owing to their higher polarity [25, 26].

Supercritical Antisolvent Precipitation (SAS)

SAS has recently been proposed as an alternative to the liquid antisolvent precipitation (LAS) for producing micronized particles of some antibiotics. In the case of liquid antisolvent processing, it is very difficult to remove solvent, whereas SAS allows removal of the solvent by pressure reduction of the gas phase and results in appearance of submicrometer-sized particles with narrow size distribution. SAS is based on the use of two completely

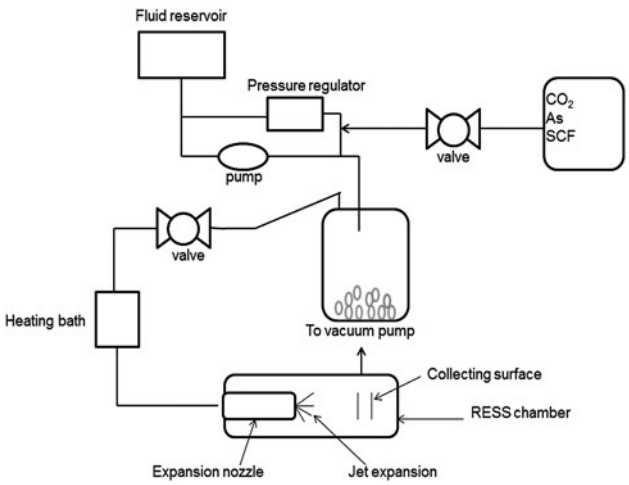

Fig. 3 Rapid expansion supercritical solution method

miscible liquid solvents; the drug or solute to be micronized should be soluble in the first solvent but not in the second one. Antisolvent addition can be carried out from the top or bottom of the precipitating chamber. A fast diffusion of the solvent results in supersaturation and therefore particles are precipitated in micronized form (Fig. 4). Supercritical CO_2 continues to flow through the chamber until the end of precipitation to wash the remaining liquid solvent. Otherwise, it may resolubilize the solutes during depressurization step, and thus product stability may be affected [27].

A modification of the conventional SAS process, termed SAS with enhanced mass transfer (SAS-EM), was proposed for the preparation of griseofulvin nanoparticles. Supercritical CO_2 was used as the antisolvent, but as a modification, the process used a deflecting surface, which vibrated at ultrasonic frequency to atomize the solution jet into micro-sized droplets, or to further break the particle jet into smaller particles. Use of ultrasound field and vibrating surface during the process greatly enhanced the turbulence and mixing within the supercritical phase. Consequently, mass transfer between the solution and antisolvent increased. It resulted in the reduction of solution droplet size and provided particles critically tenfold smaller (100 to 500 nm) than those obtained from the conventional SAS process [28].

One more method, known as SCF extraction of emulsions (SCFEE), has been suggested for biopharmaceutics classification system (BCS) class II drugs to enhance the dissolution rate of the resulting microparticles or nanopartices, which is brought about by the increased surface area. The process chiefly comprises an emulsification step that is followed by extraction of the prepared o/w emulsion using supercritical CO_2. Extraction temperature and pressure are maintained constant at 35 °C and 80 bars, respectively (if not specified), as they provide maximum mass-transfer efficiency [29].

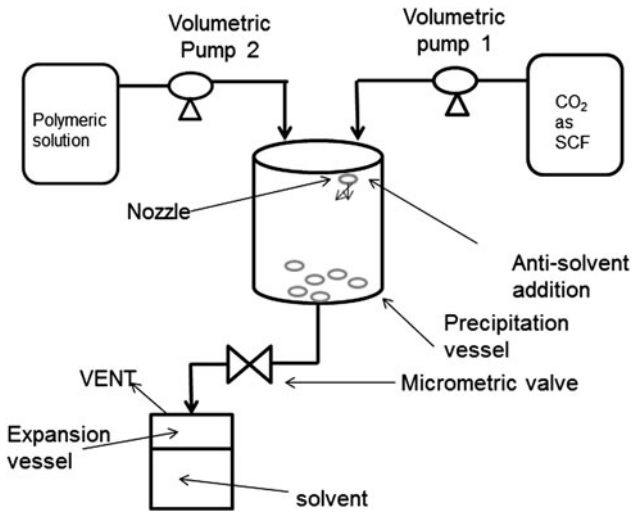

Fig. 4 Supercritical antisolvent precipitation method

Self-Assembly Methods

Self-assembly is the physical process wherein pre-existing disordered components, atoms or molecules, set themselves up into regulated nanoscale structures by physical or chemical reactions without assistance of any external source. For example, interaction and organization of hydrophilic and hydrophobic regions result in self-assembly of cell membranes. Self assembling may be intramolecular or intermolecular. Intramolecular self-assembling molecules are often complex polymers, with the ability to assemble from random coil conformations into well-defined secondary and tertiary structures. Protein folding is an example of intramolecular self-assembly. Intermolecular self-assembly is the ability of molecules to form supramolecular assemblies (quarternary structure); for example, formation of a polymeric micelle by surfactant molecules in solution [30, 31]. Thus, the technique provides a handy tool in the area of nanotechnology, in which desired structure could be encoded in the shape and properties of the molecules being used. However, it has not been used to its full potential as yet because experimental conditions under which a set of components self-assemble remain poorly understood.

Types of Nanocarriers

Various types of nanocarriers used in drug delivery are depicted in Fig. 5.

Polymeric Nanoparticles

These are solid colloidal particles, ranging in size from 1 to 1,000 nm, consisting of various biocompatible polymeric matrices in which therapeutic moiety can be adsorbed, entrapped, or covalently attached (Fig. 6). Due to their particulate nature, PNPs are rapidly cleared by cells of MPS (Mononuclear Phagocytic System) after i.v. injection. Moreover, in a manner similar to liposomes, size, surface properties, composition, concentration and hydrophilicity or hydrophobicity of nanoparticles play a major role in their in vivo performance [32]. Polymers approved by the US Food and Drug Administration (FDA) for administration in human beings are generally biodegradable and biocompatible synthetic polymers like polylactic acid (PLA), poly (glycolic acid) (PGA), PLGA, poly (ε-caprolactone), and poly (methyl methacrylate). For example, PLA and PLGA can easily be hydrolyzed into individual

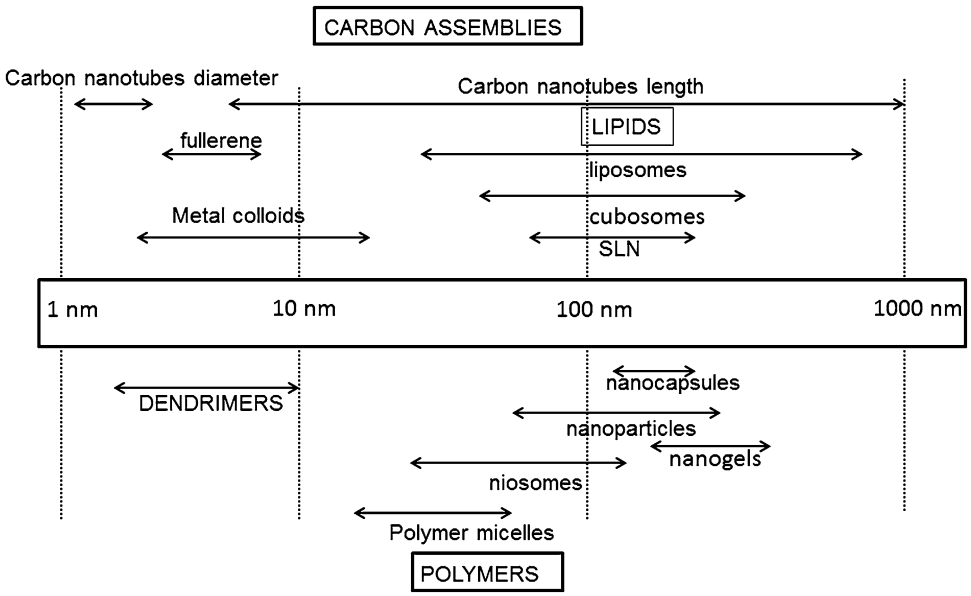

Fig. 5 Schematic diagram of various types of nanocarriers used for drug delivery

monomers (lactic acid or glycolic acid), which are removed from the body via normal metabolic pathways [12]. Reports show that higher entrapment efficiency in PNPs can be achieved by incorporation of drug during their preparation rather than adsorption on preformed nanoparticles [33]. Drug release takes place through their simultaneous biodegradation, followed by desorption, diffusion, or erosion [34].

In one study, Muthu et al. [35] developed extended-release risperidone nanoparticles for parenteral delivery (intravenous, i.v.) and to reduce the dose-dependent extrapyramidal side effects of risperidone. The in vivo efficacy of prepared formulations and the risperidone solution (RS) was studied by administering them intravenously to apomorphine-treated mice. During in vivo studies, prepared risperidone-containing formulations showed a significantly prolonged antipsychotic effect with reduced extrapyramidal side effects than that of RS. In another study; Agrahari et al. [36] developed PLGA nanoparticles of anticancer drug cisplatin with higher therapeutic efficacy and lesser side effects.

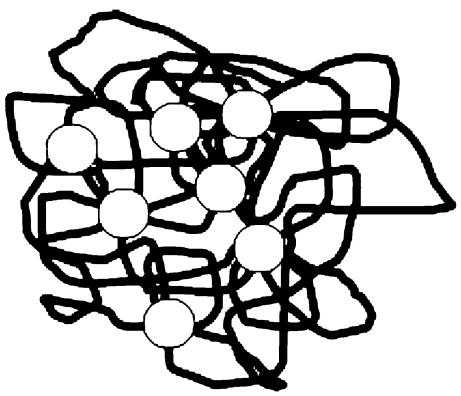

Fig. 6 Polymeric nanoparticle

Moreover, Bisht et al. [37] synthesized polymeric nanoparticle encapsulated formulation of curcumin -nanocurcumin—utilizing the micellar aggregates of cross-linked and random copolymers of *N*-isopropylacrylamide (NIPAAM), with *N*-vinyl-2-pyrrolidone (VP) and poly(ethyleneglycol) monoacrylate (PEG-A). Nanocurcumin provided an opportunity to expand the clinical repertoire of this efficacious agent by enabling ready aqueous dispersion. Future studies utilizing nanocurcumin are warranted in pre-clinical in vivo models of cancer and other diseases that might be benefited from the effects of curcumin.

Tripathi et al. [38] developed the PLGA nanoparticles loaded with rifampicin, intended to be administered intravenously which improved the therapeutic index of the drug. The release behaviour of rifampicin exhibited a biphasic pattern characterized by an initial burst (11.26 % in 1 day) release followed by a slower and continuous release (more than 30 days). Therefore, rifampicin loaded PLGA-nanoparticles might be considered as an effective antitubercular drug delivery system for therapy. In another study, Ahmad et al. [39] developed and evaluated econazole (ECZ)—and moxifloxacin (MOX)—loaded PLGA nanoparticles against murine tuberculosis (TB) (drug susceptible) in order to develop a more potent regimen for TB. PLGA nanoparticles were administered orally to mice. A single oral dose of PLGA nanoparticles resulted in therapeutic drug concentrations in plasma up to 5 days (ECZ) or 4 days (MOX), whilst in the organs (lungs, liver and spleen) it was up to 6 days. In comparison, free drugs were cleared from the

same organs within 12–24 h. In *M. tuberculosis*-infected mice, eight oral doses of the formulation administered weekly were found to be equipotent to 56 doses (MOX administered daily) or 112 doses (ECZ administered twice daily) of free drugs. Furthermore, the combination of MOX + ECZ was proved to be significantly efficacious compared with individual drugs. Addition of rifampicin to this combination resulted in total bacterial clearance from the organs of mice in 8 weeks. PLGA nanoparticles appeared to have the potential for intermittent therapy of TB, and combination of MOX, ECZ and RIF was the most potent.

Nanocapsules

Nanocapsules are submicroscopic colloidal drug delivery system and are composed of an oily or an aqueous core surrounded by a thin polymeric membrane (Fig. 7). Nanocapsules have recently generated lot of interest in the area of controlled release with availability of biocompatible and biodegradable polymers. Dispersed polymer nanocapsules can serve as nano-sized drug carriers to achieve controlled release as well as efficient drug targeting. The dispersion stability and the primary physiological response are mainly determined by the type of the surfactant and the nature of the outer coating. Their release and degradation properties largely depend on the composition and the structure of the capsule walls. Another important criterion is the capsule size, where an optimum is generally seen for radii ranging between 100 and 500 nm [40].

In one study, Kumar et al. [40] prepared nanocapsules of diltiazem with an objective of achieving controlled release of the drug in order to reduce the frequency of administration of drug, to obtain more uniform plasma concentration, and to improve patient compliance. In vitro release studies indicated prolonged release for all polymers for 48 h, with polycaprolactone as the best polymer releasing about 95–98 %. Thus, it could be concluded that nanocapsules are useful carriers for controlled release of diltiazem. Moreover, Bhowmik et al. [41] prepared testosterone-loaded nanocapsules using alginate, biodegradable hydropolymer, by in situ nanoemulsion-polymer cross-linking approach. Sustained diffusive drug release was observed in vitro, following zero order kinetics releasing the drug payload over a period of 48 h. Embedding testosterone in alginate provided sustained release. Therefore nanocapsulation technique can be a good choice for the development of different sustained steroid hormonal drug carriers. Furthermore, Nassar et al. [42] prepared double coated nanocapsules to improve the oral bioavailability of a P-gp substrate drug, tacrolimus, without modulating the physiological activity of the P-gp pump. The novel formulations that released mostly drug loaded nanocapsules in the intestine were shown to enhance markedly the oral absorption of tacrolimus. The relative oral bioavailability of tacrolimus was found to be 4.9 and 2.45 fold compared to the commercial product in rats and pigs respectively. Although there was no direct evidence that intact nanocapsules were internalized in the enterocytes, numerous small oil cores were detected within the enterocytes showing the potential of P-gp substrates incorporated in such nanocarriers to escape the efflux pump.

Nanospheres

Nanospheres are solid metrical structures with drug molecules within the matrices and/or adsorbed on the surfaces of the colloidal carriers (Fig. 8). Carboxylated polystyrene nanospheres (20 nm) were evaluated for CNS drug delivery [43]. After i.v. injection such nanospheres were remained in the vasculature under normal conditions. However, they extravasated into brain during cerebral ischemia-induced stress that partially opened the BBB [44, 45]. Such nanospheres might have potential for CNS delivery of drugs and imaging agents during ischemia, stroke and other conditions that disrupt the BBB.

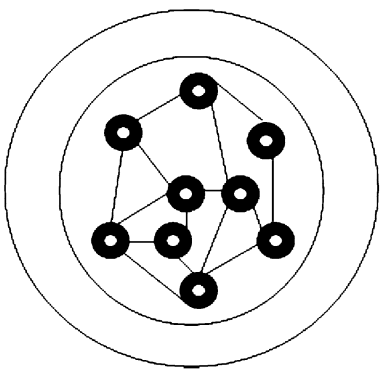

Fig. 7 Nanocapsule

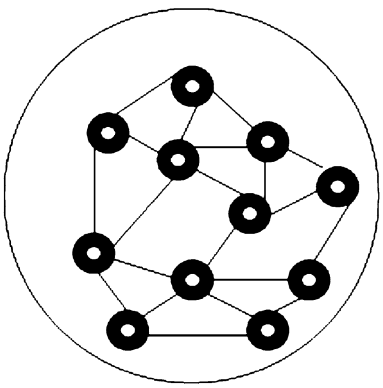

Fig. 8 Nanosphere

In one study, Mukherji et al. [46] used cellulose derivatives to prepare nanospheres entrapping the drug, 5-fluorouracil (5-FU). Investigation of the in vivo distribution showed a high concentration of the drug in lung tissue. Sangeetha et al. [47] developed sodium alginate nanospheres of ofloxacin by controlled gellification method and evaluated its in vitro release characteristics. The study revealed that the release of drug from the nanospheres followed fickian diffusion with acceptable release. The formulated ofloxacin nanospheres could be a possible approach to treat bacterial infections. In another study, Sangeetha et al. [48] developed sodium alginate nanospheres of amphotericin B by controlled gellification method. In vitro release kinetic study revealed that the release of drug from the nanospheres followed fickian diffusion. In vivo studies showed that the nanospheres bound drug produced a higher antifungal efficacy than the free drug. The formulated sodium alginate nanospheres containing amphotericin B was found to have better antifungal activity when compared to the free drug and also yielded sustained in vitro release. Furthermore, Verma et al. [49] developed hydrophilic nanospheres of copolymers NIPAAM and VP, encapsulating a bioactive derivative of 5-fluorouracil-hexyl-carbamoyl fluorouracil (HCFU). Slow drug release from nanospheres was observed in PBS and serum, with ~80 % released at 37 °C after 72 h. The HCFU loaded polymeric nanospheres were found to be stable in whole blood having negligible RBC toxicity. Cytotoxicity in Mia-Paca 3, pancreatic cancer cell line was done in a 24–72 h assay. Dose dependant cytotoxicity was observed when incubated with various concentrations of HCFU loaded polymeric nanospheres while HCFU (<1 mg) showed 90 % toxicity within 24 h.

Dubey et al. [50] investigated Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide anchored pegylated nanospheres (YIGSR-NS) loaded with 5-FU for selective and preferential presentation of carrier contents at angiogenic endothelial cells over-expressing laminin receptors on and around tumor tissue and thus for assessing their targetability. In vitro endothelial cell binding of nanospheres exhibited eightfold higher binding of YIGSR-NS to human umbilical vein endothelial cells (HUVEC) in comparison to the NS. Spontaneous lung metastasis and angiogenesis assays showed that YIGSR peptide anchored nanospheres were significantly ($P \leq 0.05$) effective in the prevention of lung metastasis and angiogenesis compared to free 5-FU and NS. In therapeutic experiments, 5-FU, NS, and YIGSR-NS were administered intravenously on day 4 at the dose of 10 mg 5-FU/kg body weight to B16F10 tumor bearing BALB/c mice resulting in effective regression of tumors in YIGSR-NS compared with free 5-FU and NS. Results indicated that YIGSR peptide anchored NS bearing 5-FU were significantly ($P \leq 0.05$) active against primary tumor and metastasis than the non-targeted NS and free drug. Thus, YIGSR-NS hold potential of targeted cancer chemotherapeutics.

Nanosuspensions

Nanosuspensions are colloidal dispersions of nanosized drug particles stabilized by surfactants. They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1 μm in size. Reduction of drug particles to nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of saturation solubility. The increase in the saturation solubility and solution velocity of nanoparticle is due to increase of vapour pressure of the particles. Nanosuspensions have disclosed the problems associated with the delivery of poorly water-soluble and poorly water-and lipid soluble drugs [51].

In one study, Kumar et al. [52] developed and characterized nanosuspension of a poorly soluble drug (atorvastatin calcium) in order to enhance its solubility and dissolution characteristics. The in vitro drug release studies showed a significant increase in the dissolution rate of nanosuspension as compared with pure drug. This study showed that initial crystalline state was reduced following particle size reduction and that the dissolution characteristics of atorvastatin nanosuspension were significantly increased in regards to the pure drug.

In another study, Muthu et al. [53] prepared poly (D, L lactide) nanoparticles suspensions containing risperidone by nanoprecipitation method using polymeric stabilizer (Pluronic® F-68 or Pluronic® F-127). The drug release from the risperidone nanosuspension was sustained in some batches for more than 24 h with 75 % drug release whereas risperidone solution showed release within 1.5 h. The release pattern of drug was analyzed and found to follow first order equation and fickian diffusion kinetics. The study suggested the feasibility of formulating risperidone loaded poly (D, L-lactide) nanoparticles suspension for the treatment of psychotic disorders.

Furthermore, Nakarani [54] prepared cyclosporine A-nanosuspension using zirconium oxide beads as a milling media, poloxamer 407 as a stabilizer and distilled water as an aqueous medium using the pearl milling technique. The formulation was found to be iso-osmolar with blood and stable up to 3 months at 2–8 °C. In vivo studies were carried out in albino rats and the pharmacokinetic parameters were compared with the marketed formulation (sandimmune i.v.), which indicated better results of the prepared formulation than the marketed formulation.

Also, Nakarani et al. [55] prepared itraconazole nanosuspension by pearl milling technique using zirconium

oxide beads as a milling media, poloxamer 407 as a stabilizer and glycerol as a wetting agent. The in vitro dissolution profile of the optimized formulation compared to the pure drug and marketed formulation (canditral capsule) by using 0.1 N hydrochloric acid as release medium showed higher drug release.

In another study, Dandagi et al. [56] formulated a novel ophthalmic nanosuspension (ONS), an alternative carrier system to traditional colloidal carriers for controlled release of acyclovir (ACV). In this study, ONS was employed to avoid some of the major disadvantages of colloidal carriers systems such as instability in cul de sac and short half life by increasing efficiency of drug encapsulation as well as by controlled release. In vivo studies showed ACV concentration of 82.83, 77.49 and 34.15 mg/ml in aqueous humor at 8 h from control, formulation 1 and formulation 2 respectively. Overall; the study revealed that ONS was capable of releasing the drug for a prolonged period of time and increased bioavailability.

Solid Lipid Nanoparticles

SLN are solid, submicronic particulate carriers with a size ranging from 1 to 1,000 nm, consist of physiological and biodegradable/biocompatible lipids, suitable for the incorporation of lipophilic and hydrophilic drugs within the lipid matrix in considerable amounts (Fig. 9). Generally, lipids that can be employed as a matrix for SLN are highly purified triglycerides, complex glyceride mixtures or even waxes. However, recently, SLN based on mixture of solid lipid and liquid lipids (so-called NLCs), high amounts of lecithins, amphiphilic cyclodextrins and para-acyl-calix-4-arenes have been investigated [57–60].

SLNs are comparatively stable colloidal carrier system in which melted lipid is dispersed in an aqueous surfactant by HPH or microemulsification [61]. They are generally made up of a solid hydrophobic core containing the drug dissolved or dispersed. SLNs exhibit certain potential advantages. They are safely taken up by brain and exhibit the least toxicity due to the biodegradable nature of the carrier lipid [62]. Smaller size (around 10–200 nm) and narrow size range (100–200 nm) allows them to cross tight endothelial cells of the blood–brain barrier (BBB), escape from the reticuloendothelial system (RES), and bypass liver. They have comparatively higher drug entrapment efficiency, render the drug more stable in their lipid matrix, and provide a controlled release lasting up to several weeks. Their production can be scaled up with excellent reproducibility. Surface coating of SLNs with hydrophilic polymers or surfactants, such as poly (ethylene glycol) (PEG), minimizes their uptake in liver cells and results in improved bioavailability. Stearic acid-PEG 2000 has been used for their stearic stabilization, whereas the use of complex lipids (mono-, di-, triglycerides of different chain lengths) results in an increased loading efficiency [63]. The incorporation of the therapeutic agent in SLN can be described by three models viz. homogenous matrix model (in which drug is either molecularly dispersed or present as amorphous clusters in the lipid matrix), drug-enriched shell model (outer lipid shell containing drug with lipid core) and drug-enriched core model (drug core surrounded by lipid layer or reservoir type system).

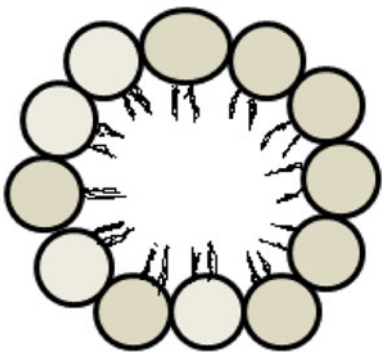

Fig. 9 Solid lipid nanoparticle

The characteristic features that make SLN an interesting carrier are as follows:

1. Improved body/tissue tolerance and less stringent regulatory requirements due to utilization of physiologically acceptable lipids.
2. Ability to entrap lipophilic as well as hydrophilic drugs by using various techniques of fabrication.
3. No biotoxicity of carrier.
4. Protection of labile compounds against chemical degradation has been shown, e.g. for retinal, coenzyme Q-10, tocopherol and TRE.
5. Depending on the produced SLN-type, modulation of drug release is possible depending upon the requirements. SLN with a drug-enriched shell show burst release characteristics whereas SLN with a drug-enriched core lead to sustained release.
6. Possibility of drug targeting and controlled delivery of active agent [16, 64].

In addition, due to their nano size range, SLNs can be an effective ocular drug delivery system by enhancing corneal absorption, improving ocular bioavailability, prolonging the ocular retention time, and providing a sustained drug release profile [65]. All types of drugs can be loaded into SLNs, the only disadvantage being the burst effect associated with hydrophilic drugs. The burst effect is often associated with drug adsorption on the surface of the nanoparticles, which results in the release of a major fraction of the dose in a short period of time. SLNs control and stop the degradation process of sensitive lipophilic

materials and drugs due to the fact that the mobility of the reactive agents is hindered in solid state when compared to liquid state [66].

Apart from their initial success, SLNs are associated with a few drawbacks. Limited drug loading capacity, possibility of drug expulsion during phase modifications, and high water content of SLNs aqueous dispersions (70–90 %) have led to the introduction of NLCs [67, 68].

In one study, Pandey et al. [69] developed the oral SLN and evaluated the chemotherapeutic potential of oral SLNs incorporating rifampicin, isoniazid and pyrazinamide against experimental TB. Following a single oral administration to mice, therapeutic drug concentrations were maintained in the plasma for 8 days and in the organs (lungs, liver and spleen) for 10 days whereas free drugs were cleared by 1–2 days. In *M. tuberculosis H37Rv* infected mice, no tubercle bacilli could be detected in the lungs/spleen after 5 oral doses of drug loaded SLNs administered at every 10th day whereas 46 daily doses of oral free drugs were required to obtain an equivalent therapeutic benefit. Thus, SLN based antitubercular drug therapy formed a sound basis for reducing dosing frequency and improving patient compliance for better management of TB. In another study, Nimje et al. [70] developed and evaluated the mannosylated SLNs of rifabutin for alveolar targeting. Ex vivo cellular uptake studies of SLNs formulations in alveolar macrophages depicted almost 6 times enhanced uptake due to mannose coating. Further the serum level and organ distribution studies demonstrated efficiency of the developed system for prolonged circulation and spatial delivery of rifabutin to alveolar tissues. It was concluded that mannose-conjugated SLNs can be exploited for effective and targeted delivery of rifabutin compared to its uncoated formulation and ultimately increasing the therapeutic margin of safety while reducing the side effects. Moreover, Doijad et al. [71] developed SLNs of cisplatin by microemulsification method using stearic acid, soy lecithin 95 % and sodium glycolate. The in vivo results of formulated SLNs revealed that the drug was preferentially targeted to liver followed by brain and lungs.

Furthermore, Reddy et al. [72] studied the tumoricidal effects of etoposide incorporated into SLNs after single-dose administration into Dalton's lymphoma ascites bearing mice. Etoposide and its nanoparticle formulations were administered intraperitoneally, and the cell cycle perturbation, cytogenetic damage, cell death (apoptosis), tumor regression, and animal survival were investigated as parameters of response with time. The tumor burden of mice treated with etoposide and its nanoparticle formulations decreased significantly ($P < 0.001$) compared with the initial up to 4–6 days, followed by an increase at later time intervals. Of the 3 different formulations, the survival time of mice was higher when treated with etoposide-loaded tripalmitin (ETP) nanoparticles, followed by etoposide-loaded glycerol monostearate (EGMS) (27.3 %) and etoposide-loaded glycerol distearate (EGDS) (27.3 %) nanoparticles compared with free etoposide. Cell cycle analysis revealed the hypodiploid peak (sub G_0/G_1cell population) as well as G_2 arrest in mice treated with etoposide and its nanoparticle formulations. The frequency of dead cells treated with the nanoparticle formulations remained high even after 8 days of treatment compared with free etoposide. The mice treated with nanoparticle formulations exhibited hypodiploid peaks and reduced S phase even 8 days after treatment, whereas the free etoposide-treated mice showed decrease in apoptosis after 3 days of treatment. The apoptotic frequency in cells 17 days after treatment was in the order of ETP > EGMS > EGDS > etoposide. The experimental results indicated that among the 3 nanoparticle formulations studied, the ETP nanoparticles showed greater and prolonged apoptotic induction properties, resulting in the higher increase in survival time of tumor bearing mice.

Kakkar et al. [73] prepared curcumin-loaded SLNs (C-SLNs) using a microemulsification technique. In vivo pharmacokinetics performed after oral administration of C-SLNs (50, 25, 12.5 and 1 mg/kg dose) and (free) solubilized curcumin (C–S; 50 mg/kg), using a validated LC–MS/MS method in rat plasma revealed significant improvement (at $P < 0.05$) in bioavailability (39 times at 50 mg/kg; 155 times at 1 mg/kg; and, 59 and 32 times at 12.5 and 25 mg/kg, respectively) after administration of C-SLNs at all the doses with respect to C–S. Enhanced and reliable bioavailability established its therapeutic usefulness especially for neurodegenerative and cancerous disorders in humans. In another study, Jain [74] prepared and evaluated nimesulide loaded SLNs. The cumulative percentage drug release of nimesulide was found approximately 60 % in 24 h and release behavior was in accordance with Higuchi-equation. The results indicated SLNs as a promising controlled-release system. Misra et al. [75] developed methotrexate-(MTx)-loaded SLN by hot microemulsion congealing technique. In vitro skin deposition studies showed significantly higher ($P < 0.05$) deposition of MTx from MTx-SLN gel. Clinical studies demonstrated improvement in therapeutic response ($P < 0.01$) at all evaluation time points and reduction in local side effects. Moreover, Pople et al. [76] investigated SLN for topical application of vitamin A palmitate and studied its beneficial effects on skin. In vitro penetration studies showed almost 2 times higher drug concentration in the skin with lipid nanoparticle-enriched gel as compared with conventional gel, thus indicating better localization of the drug in the skin. In vivo skin hydration studies in albino rats revealed increase in the thickness of the stratum

corneum with improved skin hydration. The developed formulation was non-irritant to the skin with no erythema or edema and had primary irritation index of 0.00. Shah et al. [77] developed SLNs of tretinoin (TRE) and evaluated the viability of SLN based gel in improving topical delivery of TRE. The skin irritation studies carried out on rabbits showed that SLN based TRE gel was significantly less irritating to skin as compared to marketed TRE cream and clearly indicated its potential in improving the skin tolerability of TRE. In vitro permeation studies through rat skin indicated that SLN based TRE gel had permeation profile comparable to that of the marketed TRE cream.

In another study, Jain et al. [78] formulated and evaluated miconazole nitrate (MN) loaded SLNs for topical application. In vivo studies were performed using candida infected rats. It was observed that MN-loaded SLN-bearing hydrogel was more efficient in the treatment of candidiasis. Results indicated that MN-loaded SLN-bearing hydrogel provided a sustained topical effect of MN as well as quicker relief from fungal infection. Furthermore, Thakkar et al. [79] prepared celecoxib-loaded SLNs. The biocompatibility of SLNs was evaluated by histopathology of the rat joints after intra-articular injection in normal rats. Celecoxib and celecoxib-loaded SLN were labelled with ^{99m}Tc and the labelling parameters were optimised to obtain maximum labelling efficiency. The labelled complexes were administered intra-articularly and the pharmacokinetics and biodistribution were determined. The nanoparticles showed no inflammatory infiltrates 3 and 7 days post-intra-articular injection, proving their biocompatibility and suitability for intra-articular use. Free celecoxib underwent rapid clearance from the inflamed articular joints into the systemic circulation, while the celecoxib-loaded SLN were associated with significantly lower blood levels compared with free celecoxib. Free celecoxib was found to have been extensively distributed to organs of the RES such as the liver, lungs and spleen. In contrast, celecoxib-loaded nanoparticles demonstrated significantly lower distribution to the reticuloendothelial organs. The articular concentrations of celecoxib-loaded nanoparticles in the inflamed joints were 16-fold higher at 4 h post-injection and 15-fold higher at 24 h post-injection than free celecoxib concentrations, indicating greater and prolonged retention in the inflamed articular joints. It was concluded that celecoxib-loaded SLN with its greater intra-articular retention and sustained-release properties could be a beneficial delivery system for the effective treatment of arthritis.

In another study, Varia et al. [80] prepared SLNs loaded with cyclosporine A using glyceryl monostearate (GMS) and glyceryl palmitostearate (GPS) as lipid matrices. In vitro release studies revealed that GMS based SLNs released the drug faster (41.12 % in 20 h) than GPS SLNs (7.958 % in 20 h). Release of cyclosporine A from GMS SLN followed Higuchi equation better than first order while release from GPS SLN followed first order better than Higuchi model. Bhalekar et al. [81] prepared MN loaded SLNs (MN-SLN) effective for topical delivery of MN. The penetration of MN from the gel formulated using selected MN-SLN dispersion as into cadaver skins was evaluated ex vivo using franz diffusion cell. The MN-SLN formulations could significantly increase the accumulative uptake of MN in skin over the marketed gel and showed a significantly enhanced skin targeting effect.

Magnetic Nanoparticles (MNPs)

Magnetically targeted nanoparticulate drug delivery systems (Fig. 10) involve binding of drug with MNPs, such as oxidized iron (Fe) or magnetite. By virtue of their controllable sizes (ranging from 10 to 100 nm) and capacity of delivering the drug or radionucleotide in the vicinity of a target site, they provide a good scope in drug delivery. For biomedical applications, magnetic carriers must be water based, biocompatible, nontoxic, and nonimmunogenic. Various magnetic carriers, which receive external magnetic field, include nickel, cobalt, iron, and magnetite. Iron oxide is most commonly used because of its biodegradable nature, biocompatibility, superparamagnetic effects, and capacity to serve as a contrast agent in magnetic resonance imaging (MRI). Iron oxide particles are phagocytosed or endocytosed by the kupffer cell in the RES of liver, spleen, lymph, and bone marrow. Once compartmentalized within the lysosomes of RES cells, they are broken down into ferritin and/or hemosiderin, which are antiferromagnetic forms of iron [82, 83]. The concentration of carriers at any specific location can be manipulated by calculation of capillary flow rate, vascular permeability, and hydrodynamic condition of the individual. For therapeutic effect, MNPs are injected into the bloodstream, and a high gradient magnetic field is generated outside the body so as to pull them out of suspension and deliver the drug to a

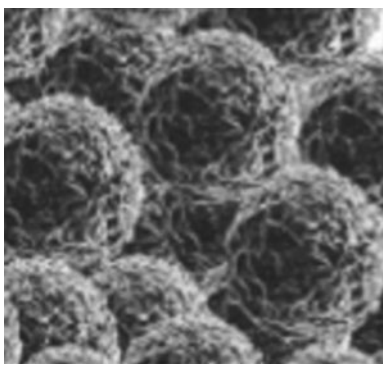

Fig. 10 Magnetic nanoparticles

localized disease site [84]. Coating with dextran or PEG improves its water dispersibility. Iron oxide MNPs, coated with oleic acid, were stabilized by pluronic F-127, to form a stable, water-dispersible system [85].

In one study, Gupta et al. [86] prepared super paramagnetic iron oxide nanoparticles with specific shape and size and coupled to insulin for their targeting to cell expressed surface receptors and thereby preventing the endocytosis. The influence of these nanoparticles on human fibroblasts is studied using various techniques to observe cell–nanoparticle interaction that includes light, scanning, and TEM studies. The derivatization of the nanoparticle surface with insulin-induced alterations in cell behavior that were distinct from the underivatized nanoparticles suggested that cell response could be directed via specifically engineered particle surfaces. The results from cell culture studies showed that the uncoated particles were internalized by the fibroblasts due to endocytosis, which resulted in disruption of the cell membrane. In contradiction, insulin-coated nanoparticles were attached to the cell membrane, most likely to the cell-expressed surface receptors, and were not endocytosed. The presence of insulin on the surface of the nanoparticles caused an apparent increase in cell proliferation and viability. One major problem with uncoated nanoparticles was the endocytosis of particles leading to irreversible entry. These results provided a route to prevent this problem. The derivatized nanoparticles showed high affinity for cell membrane and opened up new opportunities for magnetic cell separation and recovery that might be of crucial interest for the development of cellular therapies.

Metal and Inorganic Nanoparticles

Various metals, such as gold (Au), copper (Cu), and silver (Ag), and inorganic carriers, such as silica or alumina, have been used for the preparation of nanoparticles, among which Au nanoparticles are most promptly used due to their excellent optical and photoelectric properties. Au NPs consist of a core of Au atoms that can be functionalized by addition of a monolayer of moieties containing a thiol (SH) group [87]. Examples of these moieties include ligands for active targeting of the Au NP, such as masked phosphonioalkyl selenoates [88] peptides and glyconanoparticles. Au NPs can be synthesized using $NaBH_4$ to reduce $AuCl_4^-$ salts in the presence of SH containing moieties that subsequently form a monolayer around the core Au atom, depending on the stoichiometric Au/SH ratio. Synthesized NPs have a diameter of 1–150 nm. Further NP modification can be carried out using a place exchange reaction, in which SH-containing moieties are swapped. In this way, a single Au NP core can be functionalized with many different groups for targeting, stability, evasion of host defences and drug delivery [89]. Studies have confirmed that Au NPs are non-toxic at the cellular level in a number of human cell lines [90]. Studies in mice using Au NPs as an imaging agent revealed no evidence of toxicity over 30 days [91]. A pioneering study demonstrated that PEGylated Au NPs (10–30 nm) are unable to cross the human placenta within 6 h, which could be used to restrict drug delivery to just the mother while preventing teratogenic effects on the foetus [92].

Moreover, Au exhibits some specific advantages, like inertness and nontoxicity, higher stability, ease of preparation, and possibility of bioconjugation and biomodification with SH, disulfide, and amine functional groups [93]. Its dispersion stability can be enhanced by conjugation with thiolated PEG [94]. Au NPs are highly effective contrast agents in cancer diagnosis and photodermal cancer therapy [93]. Furthermore, they serve as a good vector for oligonucleotide [62], SH-conjugated small interfering RNA (Si-RNA) [95], insulin [96], and gene delivery [89]. Mesoporous silica has been used as a targeted nanocarrier due to its high encapsulation efficiency, controlled structural properties, and biocompatibility [97].

In one study, Shrivastava et al. [98] prepared silver nanoparticles (10–15 nm) with increased stability and enhanced antibacterial potency. The antibacterial effect of Ag nanoparticles was dose dependent and was more pronounced against gram-negative bacteria than gram-positive organisms. Although bacterial cell lysis could be one of the reasons for the observed antibacterial property, nanoparticles also modulated the phosphotyrosine profile of putative bacterial peptides, which could thus affect bacterial signal transduction and inhibit the growth of the organisms. In another study, Shrivastava et al. [99] studied that nanosilver had an innate antiplatelet property and effectively prevented integrin-mediated platelet responses, both in vivo and in vitro, in a concentration-dependent manner. Ultra structural studies showed that nanosilver was accumulated within platelet granules and reduced interplatelet proximity. The results suggested that these nanoparticles did not confer any lytic effect on platelets and thus hold potential to be promoted as antiplatelet/antithrombotic agents after careful evaluation of toxic effects. Moreover, Devasena [100] investigated antidandruff activity of ketoconazole coated silver nanoparticles (AgNp) of 4 ± 2 nm towards the dandruff scales collected from human volunteers by disc diffusion method. The minimal inhibitory concentration (MIC) of ketoconazole and ketoconazole coated AgNp during incubation with the dandruff causing fungi—*Malassezia furfur* was also studied. Antidandruff activity was highest with ketoconazole coated AgNp when compared to ketoconazole and AgNp individually. MIC was 0.06 mg/ml for ketoconazole, 0.026 for AgNp and 0.0135 mg/ml for ketoconazole coated AgNp. Results

revealed the synergistic antidandruff activity of ketoconazole and AgNp. The study concluded that AgNp enhanced the activity of ketoconazole. Furthermore, Mukesh et al. [101] formulated and characterized calcium phosphate nanoparticle containing anticancer drug, MTx. Confocal microscopy was performed using CHO cell lines, which showed intracellular localization of FITC-dextran loaded calcium phosphate nanoparticles. Results indicated that prepared nanoparticles could be served for intracellular drug delivery.

Lipid Drug Conjugate (LDC) Nanoparticles

LDC has emerged as an approach to improve delivery of hydrophilic drugs. LDC nanoparticles can be described as a special form of nanoparticles consisting of 100 % LDC or a mixture of LDC with suitable lipids. LDC is obtained by converting hydrophilic drug to a lipophilic drug conjugate or prodrug mainly via esterification or amidation. Typically, LDC has a melting point in the range of 50–100 °C in order to facilitate their nanosizing with the help of HPH. LDC has been developed in order to improve biological transport and targeting of hydrophilic drugs. LDC technology has been applied to improve delivery of hydrophilic anti-trypanosomial drug diminazene diaceturate (DZA) for the treatment of chronic phase of human African trypanosomiasis where the targeting of the drug to brain was essential. Muller and coworkers in a series of experiments demonstrated that DZA containing LDC could be successfully targeted to the brain by virtue of selective adsorption of apolipoproteins E, A–I and A–IV which are key factors in mediating the delivery of drugs to brain. Furthermore, in vivo studies showed localization of LDC in the endothelial cells of the blood vessels in the brain as observed by confocal laser scanning microscopy. Moreover, they also observed that the in vivo performance and cytotoxicity potential of LDC were strongly governed by the type of lipid used for the conjugation and drug to lipid ratio. Much exploration is required in this technology to prove its success in parasitic diseases. Furthermore, the fact that LDC would be treated as new chemical entities (NCE) could be a limiting factor in their rapid commercialization. However, NCE status is acceptable, in case the LDC delivery systems prove to be highly efficient in therapy [102, 103].

Quantum Dots (QDs)

QDs are colloidal semiconductor nanocrystals (up to 2–10 nm), composed of atoms from groups II–VI or III–V of the periodic table, having unique optical and fluorescent properties (Fig. 11). The most commonly used are cadmium selenide (CdSe), cadmium telluride (CdTe), and

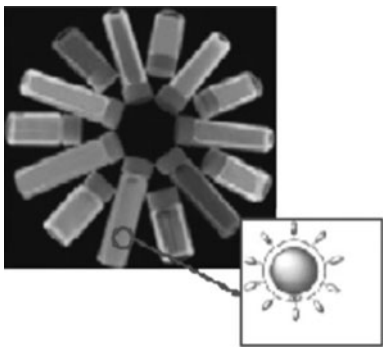

Fig. 11 Quantum dot

indium arsenide (InAs). Upon their interaction with photon, they get excited and emit energy in UV, visible, or near-infrared (IR) regions, which can be detected. Owing to their small size, they can be used for the tagging of biological macromolecules, such as nucleoside and proteins. Among different elements, dihydrolipoic acid (DHLA)-coated cadmium selenide-zinc sulfide (CdSe-ZnS) QDs have shown a more stable fluorescent intensity and higher photostability [104]. To enhance their water solubility and photostability, various surface modification techniques have been used; for example, surface coating with thiolated PEG, ligand grafting, use of dendrimers, and so forth [105]. Continuous use of QDs under certain conditions, like high radiation exposure or ultraviolet (UV) oxidation, may lead to the leakage of cytotoxic cadmium ions from CdSe QDs, thus generating oxidative free radicals, which could be lethal to liver and kidney cells. Cytotoxicity caused by QDs depends upon their dose, type of capping material, or cell surface chemistry [106, 107]. A recent report indicated that cadmium can induce cell death and hepatotoxicity due to reactive oxygen species (ROS), which causes apoptosis in the $HepG_2$ cells in a dose-dependent manner, as confirmed by DNA fragmentation analysis [108]. To minimize cytotoxicity, QDs are encapsulated with ZnS and bovine serum albumin (BSA), which provide surface protection of QDs [109].

QDs conjugated with antibodies or specific cellular markers allow the detection of multiple molecular targets in small tumor regions on the basis of which treatment guidelines can be prepared. Breast cancer cells overexpress certain protein biomarkers [e.g., estrogen receptor, progesterone receptor, endothelial growth factor receptors, such as ERB_1 and ERB_2 or herpes$_2$ (HER_2) receptor], which can be used for diagnosis and drug targeting [110]. Immunoglobulin-G (IgG) molecules have widely been used for bioconjugation with QDs in the diagnosis of breast cancer. They have been successfully labeled with IgG and streptavidin to target HER_2 on live breast cancer cells. The conjugate detected two targets in the same cell with

different colors, and provided good photostability and high drug entrapment efficiency [111]. Luminescent colloidal semiconductor nanocrystals containing CdSe-ZnS core–shell QDs or inorganic fluorophores are widely used for fluoroimmunoassay. Electrostatic interactions between negatively charged DHLA-capped CdSe-ZnS core–shell QDs and positively charged leucine provide a high quantum yield and resistance to photo degradation and therefore are widely used in pathogen detection and immunoassays [112]. Similarly, conjugated QDs have also been used for the detection of pathogen and food toxins. For example, conjugation of QDs with two surface-bound proteins, internalin A (InlA) and internalin B (InlB), results in a highly sensitive, reproducible, and rapid fluorescence-based immunoassay for detection of listeria monocytogenes, a pathogen responsible for food poisoning [113]. QDs have been used in simultaneous detection of two species, *Escherichia coli* (strain *O157:H7*) and *Salmonella typhimurium*, both being responsible for food-borne diseases. A novel immunofluorescent detection system for *Cryptosporidium parvum* and *Giardia lamblia* by QDs-conjugated biotinylated antibodies resulted in superior photostability and increased illumination efficiency [114]. Multiplexed toxin analysis (MTA) has been possible for different toxoids, like staphylococcal enterotoxin B, cholera toxin, shigalike toxin-1, and ricin toxin. It is performed by sandwich fluoroimmunoassay containing DHLA capped CdSe-ZnS core–shell QDs [115].

In one study, Koyakutty et al. [116] prepared a heavy-metal-free luminescent QDs based on doped ZnS, conjugated with a cancer-targeting ligand, folic acid (FA), as a promising bio-friendly system for targeted cancer imaging. The cytotoxicity of bare and FA conjugated QDs was tested in vitro using normal lung fibroblast cell line (L929), folate-receptor-positive (FR+) nasopharyngeal epidermoid carcinoma cell line (KB), and FR-negative (FR−) lung cancer cell line (A549). Both bare and FA-conjugated ZnS QDs elicited no apparent toxicity even at high concentrations of $\sim$100 μM and 48 h of incubation. In contrast, CdS QDs prepared under identical conditions showed relatively high toxicity even at low concentrations of $\sim$0.1 μM and 24 h of incubation. Interaction of FA-QDs with different cell lines showed highly specific attachment of QDs in the FR+ cancer cell line, leaving others unaffected. The bright and stable luminescence of the QDs could be used to image both single cancer cells and colonies of cancer cells without affecting their metabolic activity and morphology. Thus, this study presents, for the first time, the use of non-toxic, Cd-, Te-, Se-, Pb- and Hg-free luminescent QDs for targeted cancer imaging. In another study, Dwarakanath et al. [117] studied the effect of a 20-min exposure to antibody–quantum dot (Ab–QD) conjugates on colony counts of *Escherichia coli* and compared with exposure to unconjugated QDs having only amine or carboxyl groups on their surfaces. Under these conditions, Ab–QD conjugates generally exhibited >90 % reduction in colony-forming units as compared to untreated *E. coli* and *E. coli* treated with unconjugated QDs after incubation for as long as 41 h. The antibacterial effect of Ab–QD conjugates versus unconjugated QDs on *Salmonella enterica* subsp. *enterica serovar Typhimurium* was also assessed by means of a disk-diffusion technique which demonstrated greater growth inhibition ($\approx$3 mm greater) by Ab–QD conjugate-impregnated disks than by unconjugated-QD-only-impregnated disks at a 10-μg disk load. At a 25-μg disk load, both treatment groups exhibited nearly equal growth inhibition.

Polymeric Micelles (PMs)

PMs (‘‘micellar nanocontainers’’) are nanoscopic core–shell structures created by spontaneous self-assembly of individual amphiphilic di/tri-block co-polymers, with hydrophobic core and hydrophilic surface shells or vice versa (Fig. 12). They contain both hydrophilic and hydrophobic regions in their structure and serve as good candidates for poorly soluble drugs. Multifunctional PMs can be designed to facilitate simultaneous drug delivery and imaging. Their stability depends upon strong cohesive force between drug and core polymer segments as well as cross-linking of the shell or core, which is performed by radical polymerization. They form spontaneously in aqueous solutions of amphiphilic block copolymers and have a core–shell architecture with a core of hydrophobic polymer blocks (e.g., poly (propylene glycol) (PPG), poly (D, L-lactide), poly (caprolactone), etc.) and a shell of hydrophilic polymer blocks (often PEG).

The size of PMs usually varies from 10 to 100 nm. Their core can incorporate considerable amounts (up to 20–30 %wt) of water-insoluble drugs preventing premature drug release and degradation [118, 119]. The shell stabilizes micelles in dispersion and masks the drug from

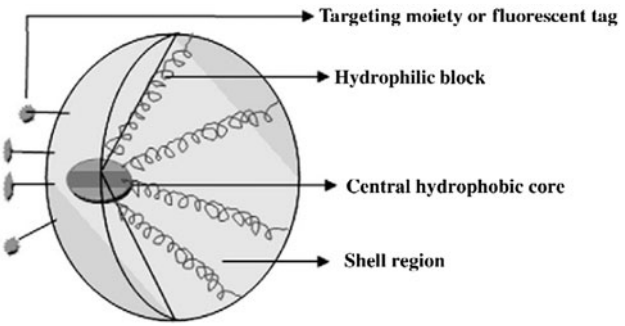

Fig. 12 Schematic presentation of a self-assembled block copolymeric micelle

interactions with serum proteins and untargeted cells. After reaching target cells drug is released from the micelle via diffusion. Several clinical trials are completed or underway to evaluate PMs for delivery of anti-cancer drugs [120].

Prolonged circulation and targeted delivery of PMs is possible by designing of environment-responsive PMs (pH, light, temperature, ultrasound, etc.). The pH at a tumor site is acidic (6.5–7.2) compared with that of healthy tissues. Therefore, PMs with hydrazone and acetal bonds, which are labile at lower pH (pH 5.0–7.0) and stable at physiologic pH (7.0–7.4), have been employed as pH-sensitive vehicles for drug delivery. Poly (L-histidine) (polyHis) has been used as a pH-sensitive carrier because of its imidazole ring, which is ionized at acidic pH [121]. Doxorubicin, attached to two block copolymers, 75 % polyHis/PEG-folate (poly-L-histidine–PEG-folate) and 25 % PLLA/PEG-folate (poly-L-lactic acid with PEG-folate) showed higher drug accumulation at the tumor site with low cytotoxicity [122]. Intracellular pH-dependent polymeric micellar carriers, based on poly (L-lactide)-poly (2-ethyl-2-oxazoline)-poly (L-lactide) triblock copolymer, have been suggested for the delivery of chemotherapeutic agents [123].

Micellar drug delivery using ultrasonic waves (20–40 kHz) has been explored because of its non invasiveness and capability of targeting the drug deeply into the tumor. Such waves increase the permeability and extravasation at the tumor site, thus enhancing the drug uptake. Drug release can be modulated by ultrasound frequency, insonation, power density, pulse length, and interpulse intervals [124]. Pluronics 105 are widely used vehicles for ultrasonic micellar delivery due to their low toxicity and ability to solubilize biologically active hydrophobic substances [125]. Myhr and Moan [126] have reported the effect of low frequency ultrasound waves on 5-FU micelles stabilized with Plurogel®, which showed a significant reduction in the tumor volume. An injectable micellar formulation of paclitaxel showed 20-fold increase in drug uptake under the influence of ultrasonic waves and has been used for drug resistant breast cancer [127].

Stimuli-responsive PMs are often designed for controlled release of drug into tumor tissue with external stimuli trigger, like temperature, pH, ultrasound, and special enzymes [128]. Among these stimuli, pH and temperature are of representativeness, because the external pH of cancerous tissue tends to be lower and the temperature is higher compared to the surrounding normal tissue, which are caused by abnormal metabolism of cancer tissues [129]. Lower critical solution temperature (LCST) polymers, such as poly (*N*-isopropylacrylamide) (PNIPAAm) with a cloud point around 32°C or some other poly (*N*-alkylacrylamide) compounds, were investigated as components of temperature-responsive copolymer micelles [130]. The micelles exhibited rapid and temperature responsive drug release in cancer cells, which was caused by the destruction of the hydrophobic-hydrophilic balance with the increase of temperature (Fig. 13). Nakayama et al. [131] developed temperature-sensitive PMs composed of biodegradable hydrophobic block poly (D, L-lactide) and thermoresponsive block [poly (*N*-isopropylacrylamide-co-*N*,*N*-dimethylacrylamide)] showing 90 % doxorubicin release over 48 h.

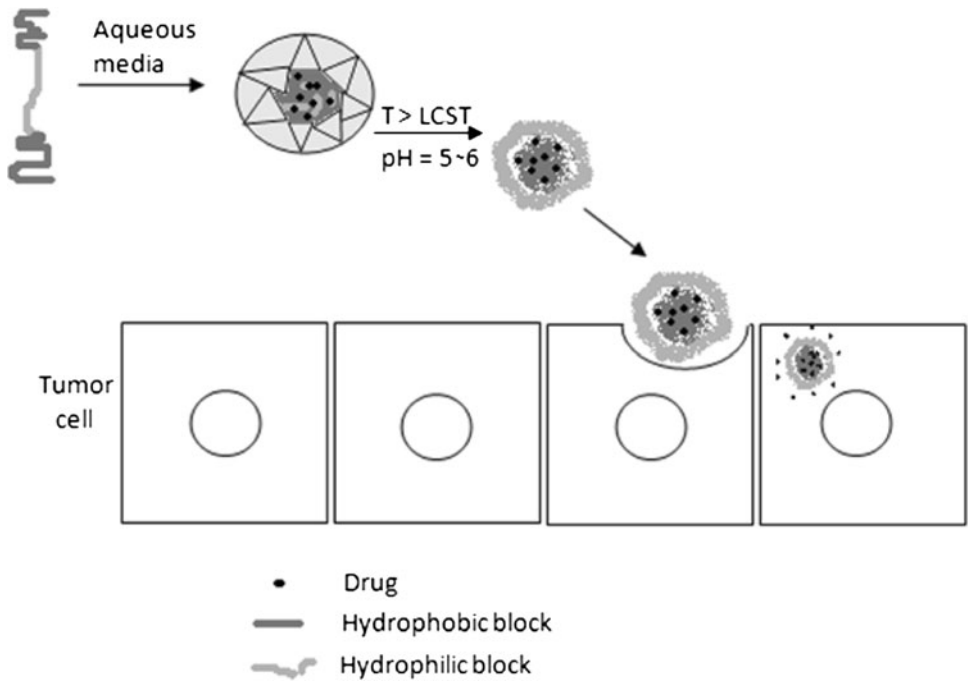

Fig. 13 Schematic of drug release from micelles with temperature or pH trigger after uptake by tumor cells

In one study, Gupta et al. [132] used PMs made of copolymer of NIPAAM, VP and acrylic acid (AA) having cross-linkage with N,N'-methylene bis-acrylamide (MBA) as host carrier in which up to 30 % w/w ketorolac (free acid) was entrapped to make the formulation. In vitro corneal permeation studies through excised rabbit cornea indicated twofold increase in ocular availability with no corneal damage compared to an aqueous suspension containing same amount of drug as in nanoparticles.

Polyion Complex Micelles (Also Termed ''Block Ionomer Complexes'')

Polyion complex micelles (also termed ''block ionomer complexes'') are novel nanosystems for incorporation of charged molecules. They are formed as a result of the reaction of double hydrophilic block copolymers containing ionic and non-ionic blocks with macromolecules of opposite charge including oligonucleotides, plasmid DNA and proteins or surfactants of opposite charge [133, 134]. For example, block ionomer complexes are prepared by reacting trypsin or lysozyme (that are positively charged under physiological conditions) with an anionic block copolymer, PEG-poly (α, β-aspartic acid). Such complexes spontaneously assemble into nanosized particles having core–shell architecture. The core contains polyion complexes of a biomacromolecule and ionic block of the copolymer. The shell is formed by the non-ionic block. In case of surfactant-based complexes the core is composed of mutually neutralized surfactant ions and polyion chains. It contains hydrophobic domains of surfactant tail groups and can additionally incorporate water-insoluble drugs [135, 136]. Depending on surfactant and block copolymer architectures the complexes assume different morphologies including vesicles and micelles of different shapes. These nanomaterials are versatile and can incorporate solutes of different structure with high loading capacity. Furthermore, they can release solutes upon change of environmental conditions such as pH (acidification), concentration and chemical structure of elementary salt [137]. These nanomaterials were shown to efficiently deliver DNA molecules in vitro and in vivo although no study on their delivery to CNS was reported so far [138, 139].

In one study, Jeong et al. [140] developed novel types of polyion complex micelles for drug delivery to brain tumor. Methoxy poly (ethylene glycol) (mPEG)-grafted chitosan (CP) was synthesized in order to make PMs encapsulating all-trans retinoic acid (ATRA) based on polyion complex formation. In the cell cytotoxicity study using U87MG cells in vitro, polyion complex micelles showed similar cytotoxicity to that of free ATRA. A migration test was performed to investigate the inhibition of tumor cell invasion in vitro. The results suggested that the polyion complex micelles were more effective at inhibiting tumor cell migration than free ATRA.

Phospholipid Micelles

Sterically stabilized micelles (SSMs), composed of polyethylene glycol (PEGylated) phospholipids, have been introduced as safe, biocompatible nanocarriers for the delivery of poorly water-soluble drugs, especially anticancer molecules. Koo and co-workers developed camptothecin-containing SSMs (CPT-SSMs) as a novel nanomedicine for parenteral administration, which showed higher solubilization potential, estimable stability, and less in vitro cytotoxicity. Furthermore, they were lyophilized without additional lyo-protectants and reconstituted without any significant change in properties [141]. PEG-phospholipid micelle-encapsulated QDs (QD-Ms) showed their maximum accumulation in the tumor area due to higher circulation time and EPR effect. Recently, Papagiannaros et al. [142] developed QD-Ms for visualization of both tumor and internal organs, which conglomerated maximally in the tumor area within 1 h of their administration compared with 4 h for that of the commercially available PEGylated QDs. Phospholipid-based nanomicelles of indisulam were prepared by co-precipitation and reconstitution of drug and lipids. It showed 40-fold increase in drug solubility and was successfully lyophilized without addition of any lyoprotectant or cryoprotectant. In vitro studies showed that indisulam in micellar system was more effective than free indisulam [143].

Nano-Liposomes

Liposomes are small artificial vesicles of globular shape composed of aquatic pores encapsulated with amphiphilic phospholipids and cholesterol bilayer (Fig. 14). They are

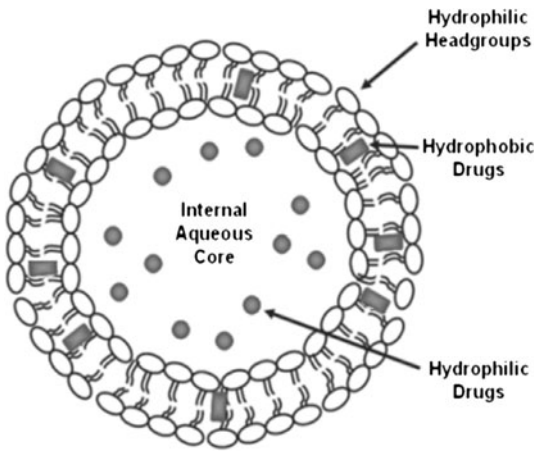

Fig. 14 Schematic depicting the structure of liposomes which can accommodate both hydrophilic as well as hydrophobic drugs either in the internal aqueous core or phospholipid bilayer respectively

widely used because of their size, both hydrophilic as well as hydrophobic character, and tissue biocompatibility. Depending on size and number of phospholipid bilayers, liposomes can be classified into small unilamellar vesicles (SUVs; single lipid layer 25–50 nm in diameter), large unilamellar vesicles (LUVs; heterogeneous group of vesicles), and multilamellar vesicles (MLVs; several lipid layers separated from one another by a layer of aqueous solution) [144].

Liposomes have been investigated for the delivery of vaccine, toxoids, gene, anticancer, and anti-HIV drugs. Their blood circulation time can be increased through surface modification (e.g., by attaching PEG [145], dextran [146], or poly-N vinylpyrrolidones [147] to the lipid bilayer). Furthermore, conjugation with targeting ligands, like monoclonal antibodies or aptamers, can enhance their tissue specificity. Liposome technology has existed for the past four decades, but they do not have enough market shares due to some of their potential drawbacks, like batch-to-batch variation in manufacturing, low drug loading efficiency, and poor stability. Relatively large amounts of drug molecules can be incorporated into liposome aqueous compartments (water soluble compounds) or within lipid bilayers (lipophilic compounds). Conventional liposomes usually are rapidly cleared from circulation by RES. Extended circulation time can be accomplished with small sized liposomes (<10 nm) composed of neutral, saturated phospholipids and cholesterol. Furthermore, many modern studies use liposomes with a surface modified with polyethylene glycol (PEG) [148]. Such modification (''PEGylation'') reduces opsonisation of liposomes in plasma and decreases its recognition and removal by the MP system in liver and spleen. PEGylated (or ''stealth'') liposomes have circulation half-life as long as 50 h in humans [149]. One example, a doxorubicin encapsulated in PEGylated liposome, Doxils, was approved for treatment of ovarian cancer, AIDS related Kaposi's sarcoma (ARKS) [150] and metastatic breast cancer [151]. Overall, encapsulation of a drug into liposomes may prolong drug circulation time in blood stream, reduce drug side effects, and enhance drug therapeutic effects in CNS.

Rudra et al. [152] developed, characterized, and investigated phosphatidylethanolamine (PE)-conjugated nanoliposomes for their accumulation in liver, kidneys, and lungs in rats. Fluorescence microscopic study showed that liposomes were well distributed in liver, lungs, and kidneys. Data suggested that PE-conjugated nanoliposomes released the drug in a sustained manner and were capable of distributing them in various organs. This strategy might be used for cell/tissue targeting; attaching specific antibodies to PE. Patel et al. [153] developed and proved the superiority of liposomal dry powder formulations (DPFs) over conventional DPFs due to favorably improved pharmacokinetics and pharmacodynamics of entrapped drugs, and thus, reduced local and systemic toxicities. Nanoliposomal DPFs (NLDPFs) provided stable, high aerosolization efficiency to deep lung, prolonged drug release, slow systemic dilution, and avoided macrophage uptake of encapsulated drug by carrier-based delivery of nano-range liposomes.

Nanogels

Nanogels are nanosized networks of cross-linked polymers that often combine ionic and non-ionic chains, such as polyethyleneimine (PEI) and PEG or poly (acrylic acid) and pluronic's. Such networks swell in water and can incorporate oligonucleotides, siRNA, DNA, proteins and low molecular mass drugs. Their loading proceeds with very high capacity (up to 40–60 % wt) which is not achieved with conventional Nanoparticles [154, 155]. Because of solubility of PEG chains, individual collapsed nanogel particles do not phase separate and form stable dispersions.

Vinogradov et al. [156] prepared polyplex nanogel formulations for delivery of cytotoxic nucleoside analog, 5′-triphosphate of cytotoxic 5-fluoroadenosine arabinoside (fludarabine) (FATP). The drug-nanogel formulation compared to the drug demonstrated a significantly enhanced cytotoxicity in cultured cancer cells. Cancer cell-targeting molecules, such as folate, could be easily attached to nanogels and this modification resulted in a strong ten-fold increase of the carrier's internalization in human breast carcinoma MCF-7 cells. Moreover, transcellular transport of the folate-nanogel polyplexes was found to be 4 times more effective compared to the drug alone using Caco-2 cell monolayers as an in vitro intestinal model. The data demonstrated that this carrier-based approach for delivery of cytotoxic drugs might enhance tumor specificity and significantly reduce side effects related to systemic toxicity usually observed during cancer chemotherapy.

Nanofibers and Nanotubes

Nanofibers and nanotubes (Fig. 15) are carbon vapor grown, self-assembled from peptide amphiphiles or

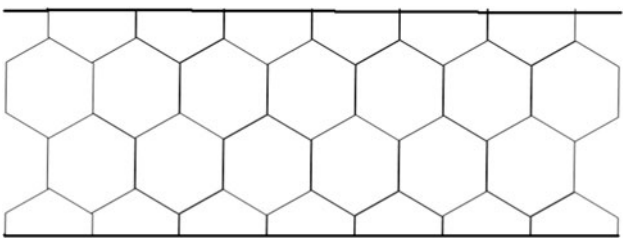

Fig. 15 Carbon nanotube

electrospun from most polymer materials. Carbon nanotubes (CNTs) have attracted attention in nanomedicine although there are also serious concerns regarding their safety. Electrospun continuous nanofibers are unique since they represent nanostructures in two dimensions and macroscopic structures in another dimension [157, 158]. They are safer to manufacture than CNTs and pose less risk of air pollution. Electrospun nanofibers of a degradable polymer, PLGA loaded with dexamethasone have been used for neural prosthetic applications [159]. A conducting polymer, poly (3,4-ethylenedioxythiophene), was deposited to the nanofiber surface and the coated nanofibers were then mounted on the microfabricated neural microelectrodes, which were implanted into brain. The drug was released by electrical stimulation that induced a local dilation of the coat and increased permeability. In future, nanotubes and nanofibers can be administered systemically, if the toxicity issues are addressed, for example, by appropriate polymer coating. Continuous nanofibers are more likely to be used in implants and tissue engineering applications.

Naveen et al. [160] synthesized polyhydroxybutyrate (PHB) nanofibers by electrospinning of a PHB solution prepared using hexafluoroisopropanol as the solvent. The nanofibrous scaffold supported rapid cell growth with normal morphology and attained a viability of 87 % after 48 h. Kanamycin sulphate-loaded PHB nanofiber mats were synthesized, with the antibiotic on the surface and sandwiched within the nanofiber mats: their antimicrobial property was proved by the good zone of inhibition tested against *Staphylococcus aureus*. The drug showed more than 95 % release within 8 h. These results indicated that nanofibers loaded with the antibiotic had potential applications as a template for tissue engineering and as a drug carrier.

Dendrimers

Dendrimer is a Greek word consisting of 'dendron' meaning tree and 'meros' means part; structurally it is hyperbranched, monodisperse, three-dimensional macromolecules resembling the architecture of a tree (Fig. 16). Dendrimers are one of the newest carrier systems used for delivery of drug(s) with better prospects.

Typically, a dendrimer consists of three main structural components: a multifunctional central core, branched units and surface groups. The partical size of dendrimers, ranging from 1 to 100 nm, makes them less susceptible to uptake by the RES. Dendrimers today are known for their three dimensional, monodispersed, highly branched, macromolecular nano-scopic architecture with number of reactive end groups. Dendrimers have been reported to act as solubilizing agents to host both hydrophilic and hydrophobic drugs [161, 162].

Khairnar et al. [163] investigated the potential of poly (amido amine) (PAMAM) and poly (propylene imine) (PPI) dendrimers of different generations as tool for enhancement of antifungal activity of selected two antifungal drugs. Microbilogical study showed that PAMAM and PPI dendrimers could enhance the antifungal activities of nystatin and terbinafine against *Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevisiae*. Results showed increase in the antifungal activity of nystatin and terbinafine in dendrimer solution compared to pure nystatin and terbinafine dissolved in DMSO (dimethylsulfoxide). The antifungal activity studies indicated PAMAM and PPI dendrimers as potential tool for enhancement of antifungal activity of nystatin and terbinafine. Agrawal et al. [164] synthesized poly-L-lysine dendrimers having polyethyleneglycol (PEG-1000) as core, up to fourth generation. Chloroquine phosphate (CP)-loaded uncoated and galactose coated dendrimers were evaluated for in vitro drug release rate, hemolytic toxicity and stability studies. Ex vivo cellular uptake studies of uncoated and coated drug dendrimer formulations in macrophages revealed almost 5 times reduced phagocytosis due to galactose coating ($P < 0.0001$). In vitro-in vivo release behavior indicated possibilities of galactose-coated drug dendrimers formulation in controlled drug delivery of CP. Galactose coated formulations drastically reduced hemolytic toxicity compared to uncoated poly-L-lysine formulation as well as plain drug. Hematological data suggested that galactose-coated formulations were less immunogenic compared to uncoated formulations. Finally, it was concluded that galactose-coated polylysine dendrimers could be utilized for controlled delivery of CP more safely compared to its uncoated formulation both in vitro and in vivo.

Niosomes

Niosomes are non-ionic surfactant-based unilamellar and multilamellar vesicles used as a carrier system for drug delivery having a size range of 20–200 nm. These are able to encapsulate both hydrophilic and lipophilic molecules. Moreoften, niosomes are preferred as a drug carrier because of their advantages related to stability, low cost and availability of materials [165].

Sathali et al. [166] developed niosomal formulations of terbinafine hydrochloride for targeted delivery to the fungal affected cells. The formulations were tested for in vitro antifungal activity using the strain *A. niger* and compared with pure drug solution (as standard). All the niosomal formulations showed gradual increase in zone of inhibition due to the controlled release of medicament. The best formulation with maximum zone of inhibition and

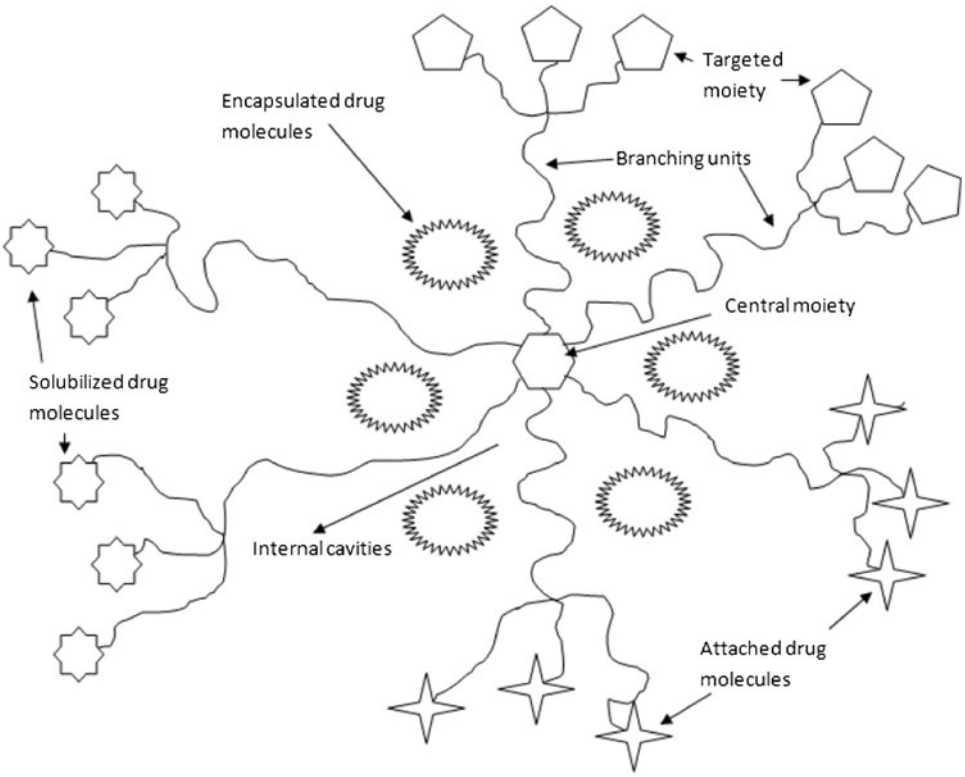

Fig. 16 Dendrimer

sustained release of drug (tween 40 niosomes) was incorporated into gel bases and evaluated. The studies revealed that gel containing total niosomes possessed maximum zone of inhibition values (12 mm) initially followed by sustained release (12–16 mm) comparing to gel containing drug entrapped niosomes, gel containing pure drug and marketed preparation. Jain et al. [167] prepared niosomes containing rifampicin using various nonionic surfactants of sorbitan ester class and cholesterol in 50:50 % mol fraction ratios. The study revealed that effective compartmentalisation of the drug took place in the lymphatic system following intraperitoneal administration of niosome-encapsulated rifampicin. Thus rifampicin encapsulated in niosomes could successfully be used for treatment of TB along lymphatic system.

Nanoemulsions

Nanoemulsions are thermodynamically stable isotropic system in which two immiscible liquid (water and oil) are mixed to form a single phase by means of an appropriate surfactants. Nanoemulsion droplet sizes fall typically in the range of 20–200 nm and show narrow size distributions. Unlike microemulsions, they are non-equilibrium structures and rely on energetic input to form, often from an emulsion. This makes a nanoemulsion metastable, at best. A nanoemulsion can nonetheless "survive" long before changing, e.g. into a coalesced form. Shearing, especially in the high concentration range, accelerates physical deterioration of any nanoemulsion. This may be problematic if a concentrated nanoemulsion is squeezed through a nanoporous membrane, such as skin. First advocated for dermal usage in the early 90 s, nanoemulsions have since been explored extensively for the purpose [168, 169]. Nanoemulsions are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. They are by far the most advanced nanoparticle systems for the systemic delivery of biologically active agents for controlled drug delivery and targeting [170].

In one study, Bhanushali et al. [171] developed intranasal (i.n.) nanoemulsion and gel formulations of rizatriptan benzoate for prolonged action. Comparative evaluation of i.n. nanoemulsions and i.n. mucoadhesive gels indicated that greater brain-targeting could be achieved with nanoemulsions. Moreover; Shakeel et al. [172] investigated the potential of a nanoemulsion formulation for transdermal delivery of aceclofenac. Transdermal permeation of aceclofenac through rat abdominal skin was determined by Franz diffusion cell. The in vitro skin permeation profile of optimized formulations was compared with that of aceclofenac conventional gel and nanoemulsion gel. A significant increase in permeability parameters such as steady-state flux (Jss), permeability coefficient (Kp), and enhancement ratio (Er) was observed in optimized nanoemulsion formulation F1, which consisted of 2 % wt/wt of aceclofenac, 10 % wt/wt of Labrafil®, 5 % wt/wt of

Triacetin®, 35.33 % wt/wt of Tween 80®, 17.66 % wt/wt of Transcutol P®, and 32 % wt/wt of distilled water. The anti-inflammatory effects of formulation F1 showed a significant increase ($P < 0.05$) in percent inhibition value after 24 h when compared with aceclofenac conventional gel and nanoemulsion gel on carrageenan induced paw edema in rats. These results suggested that nanoemulsions were potential vehicles for improved transdermal delivery of aceclofenac. Venkateshwarlu et al. [173] developed stable lipid nanoemulsions (LNEs) for delivery of docetaxel for treatment of cancer. During in vitro studies on cancer cell lines, optimized formulations showed similar values of IC_{50} (half maximal inhibitory concentration) in comparison to docetaxel solution. Based on this, it was concluded that the optimized LNEs were efficacious for the delivery of docetaxel and could act as alternative delivery systems to overcome the poor solubility, hydrolytic instability, and drug-induced and vehicle-related side effects of docetaxel.

Furthermore, Kumar et al. [174] prepared nanoemulsion containing risperidone to accomplish the delivery of drug to the brain via nose. Biodistribution of risperidone nanoemulsion (RNE), risperidone mucoadhesive nanoemulsion (RMNE), and RS in the brain and blood of Swiss albino rats following i.n. and i.v. administration was examined using optimized technetium labeled (^{99m}Tc-labeled) risperidone formulations. Gamma scintigraphy imaging of rat brain following i.v. and i.n. administrations were performed to ascertain the localization of drug in brain. The brain/blood uptake ratio of 0.617, 0.754, 0.948, and 0.054 for RS (i.n.), RNE (i.n.), RMNE (i.n.), and RNE (i.v.), respectively, at 0.5 h were indicative of direct nose to brain transport bypassing the BBB. Higher drug transport efficiency (DTE %) and direct nose to brain drug transport (direct transport percentage, DTP %) for mucoadhesive nanoemulsions indicated more effective and best brain targeting of resperidone amongst the prepared nanoemulsions. Studies conclusively demonstrated rapid and larger extent of transport of resperidone by RMNE (i.n.) when compared to RS (i.n.), RNE (i.n.) and RNE (i.v.) into the rat brain. In another study, Kumar et al. [175] formulated an olanzapine nanoemulsion that could potentially deliver the drug directly to the brain following i.n. administration. The optimized olanzapine nanoemulsion exhibited a high diffusion coefficient and no nasal cilio-toxicity.

Emulsomes

Emulsomes are pharmaceutical compositions comprising nanoemulsions of particles comprising a lipid core composed of lipid which is in a solid or liquid crystalline phase at at least 25 °C, stabilized by at least one phospholipid envelope. Phospholipid envelope surrounds the lipid core at the aqueous interface thereby stabilizing the emulsion. A key feature of these particles is that the core is composed of lipid which in bulk form is in a solid or liquid crystalline phase, rather than oil in a fluid phase. Emulsomes, having the characteristics of both liposomes and emulsions, provide the advantages of high hydrophobic drug loading in the internal solid lipid core and the ability to encapsulate water-soluble medicaments in the aqueous compartments of surrounding phospholipid layers. Vyas et al. [176] prepared plain emulsomes and stearylamine containing cationic emulsomes loaded with an antiviral drug (zidovudine). In vivo organ distribution studies in rats demonstrated better uptake of emulsomal formulations by the liver cells. Further, a significantly higher ($P < 0.05$) liver concentration of drug was estimated over a period of 24 h for cationic emulsomes than for plain emulsomes.

Gupta et al. [177] developed and characterized amphotericin B (AmB) bearing emulsomes for passive and active macrophage targeting. Emulsomes were modified by coating them with macrophage-specific ligand (O-palmitoyl mannan, OPM). In vivo organ distribution studies in albino rats demonstrated that extent of accumulation of emulsome entrapped AmB in macrophage rich organs, particularly liver, spleen and lungs was significantly high when compared against the free drug (AmB-deoxycholate). The rate and extent of accumulation were found to increase further on ligand anchoring. Further, a significantly higher ($P < 0.05$) drug concentration in the liver was estimated over a period of 24 h for OPM coated emulsomes than for plain emulsomes. In another study Gupta et al. [178] demonstrated antileishmanial efficacy of AmB bearing emulsomes against experimental visceral leishmaniasis. AmB was formulated in uncoated and OPM coated trilaurin emulsomes. In terms of reduction in splenic parasite burden in *L. donovani* infected hamsters, OPM coated AmB emulsomes were found to be more efficient (showed 73.7 ± 6.7 % parasite inhibition, PI) as compared to uncoated AmB emulsomes (showed 51.7 ± 5.4 % PI) and AmB deoxycholate (showed 30.4 ± 4.8 % PI), when administered twice at a dose of 0.5 mg/kg i.c on alternate days.

Virosomes

Virosomes are spherical unilamellar vesicle with a mean diameter of 150 nm possessing short surface projections of 10–15 nm. Virosomes hold potential applications in the field of vaccine development, drug and gene delivery. By and large 'virosomes' are liposomes spiked with viral proteins extracted from envelop of viral virion, which acquires viral functions, such as cell surface adherence and fusogenicity to cellular and organelle membranes however they free from virulence and infectinity. Virosomes can be

considered as hydrids of viral and liposomal carrier systems, combining the characteristics of cellular interactions of viral vectors while biosafety of liposomes. Virosomes have attracted attention as delivery vesicles especially for cytosolic drug delivery as they are capable of transporting therapeutic molecules into the cytoplasm, owing to efficient drug encapsulation efficiency with fusogenicity which provide for them a virtual sink in the cellular cytoplasm. Possibly they also avert the effect of p-glycoprotein efflux pumps by trans-membrane diffusion and cytosolic sustained and controlled release of drug. In one study, virosomes have been exploited as a drug delivery system for cytotoxic drug doxorubicin and found to be capable of binding and penetrating deep into tumor cells mass, ensuring effective delivery of cytotoxic drugs. Further, virosomes have been conjugated with fab' fragments of an anti-rat Neu monoclonal antibody to achieve efficient cell specific targeting to the rat Neu-over-expressing cells of breast tumors [179].

Applications of Nanocarriers in Drug Delivery

Ocular Drug Delivery

Nanocarriers are changing the perception of drug administration using conventional dosage forms. Nanocarriers have the potential to revolutionize the way that we develop new therapies, as well as optimize existing ones. In the pharmaceutical sciences, the term nanocarrier refers to a particulate drug delivery system where particle size is in the nanometre range (1–1,000 nm). Nanocarriers are being investigated extensively in order to develop drug delivery systems capable of allowing penetration through physiological barriers. Nanocarriers are either in the form of matrix-dispersion (nanospheres) or a membrane-reservoir type (nanocapsules), where drugs can be dissolved, entrapped, encapsulated, and dispersed within the particles or adsorbed on the surface of these particles [180].

A wide range of chemical and physiological materials have been used to prepare SLNs including polymers, lipids, phospholipids, and metals. These multifunctional drug carriers are expected to accommodate large drug loads, help target them to the site of action, and promote sustained/controlled drug delivery while maintaining a minimum size of 30–300 nm [181]. Submicron sized particles have a very high surface-to-volume ratio. Therefore, according to the Noyes Whitney and Kelvin equations, they have increased dissolution rates enabling them to enhance the absorption of poorly soluble drugs such as cyclosporine, paclitaxel, or amphotericin B. On the contrary, there are a few disadvantages associated with nanoparticles, such as difficulty of production, stability during their storage, aggregation, and complexity of administration [63]. Another drawback is the faster release rates associated with nanoparticles when compared to microspheres. Physicochemical properties such as particle size, surface net charge, shape, solubility, degree of ionization, and lipophilicity influence drug ocular absorption and determine the route of administration [182]. These factors can be tailored using novel nanoparticulate drug delivery systems that enhance the ocular bioavailability of drugs.

In recent years, scientists have been interested in incorporating drugs and other therapeutics into nanoparticulate carriers, administered as modified eye drops which are cost effective and therapeutically efficient. The modified eye drops provide better penetration, extended ocular surface residence time, minimized drainage owing to mucoadhesive properties, simple administration, and patient compatibility [183]. Moreover, colloidal and particulate drug delivery systems can also be utilized for sub-conjunctival, periocular, and intraocular injections [184]. Many nano-structured systems such as SLNs, niosomes, nanocapsules, nanospheres, dendrimers, nanosuspensions, liposomes, and nanoemulsions have been employed in ocular drug delivery. These have alleviated problems associated with poorly soluble drugs, increasing their bioavailability while decreasing their administered dose and toxicity [63]. For many serious eye conditions (e.g. chronic cytomegalovirus rhinitis (CMV)), a constant and prolonged controlled drug release is necessary to achieve therapeutic goals.

Nanocarriers-based delivery systems including SLNs can ensure sustained and controlled release of drugs and medicaments avoiding frequent administrations associated with conventional delivery systems. Further, nanomedicines have several advantages, such as the possibility of formulation as modified eye drops which are easily self-administered by the patient, elimination of the need for repeated administration, and offering protection against metabolic enzymes present on the ocular surfaces by constructing a protective barrier. Due to their submicron size range they have the added advantage of not impairing vision [185, 186]. Nanocarriers such as positively charged liposomes and SLNs are expected to increase corneal absorption of drugs by increasing drug residence time by ionic interactions. The common problems associated with liposomes are that they are in liquid form, which limits their pharmaceutical formulation feasibility. Moreover, most methods of sterilization are considered unsuitable for liposomes. The heating involved in autoclaving can irreversibly damage their vesicular structure and filtration reduces the liposomes to an average of 200 nm which limits their application [187].

Targeted Drug Delivery to Brain

Human brain is restricted and separated from circulatory network by a highly efficient BBB. This is constituted by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems of the MDR pathways which control and limit the access of molecules to the brain, either by paracellular or transcellular pathways (Fig. 17). Physiologically, BBB is designed in such a manner that it can only permit the transport of molecules essential for functional activity of brain. It efficiently prevents flow of water-soluble molecules from blood circulation into central nervous system (CNS), and can also decrease concentration of lipid-soluble molecules by the action of enzymes or efflux pumps.

Presently, drug delivery to CNS is a major menace as multiple cerebral diseases like alzheimer's, brain tumors and prion diseases are cropping up. The BBB represents an insurmountable obstacle for a large number of drugs including antibiotics, antineoplastic agents and a variety of CNS-active drugs like neuropeptides [45, 188]. This creates a considerable threat for the therapy of cerebral diseases. Currently, nanocarriers are utilized as a drug delivery vehicle. The use of nanocarriers to deliver drugs to the brain by infiltrating BBB may provide a significant strategy to break this impasse. The primary advantage of nanocarriers is that they can cross BBB entrapping the original characteristics of the therapeutic drug molecule. Furthermore, this system may reduce drug leaching in the brain and decrease peripheral toxicity [32]. Drugs that have successfully been transported into the brain using nanocarrier include the hexapeptide dalargin, the dipeptide kytorphin, loperamide, tubocurarine, the *N*-methyl D-aspartate (NMDA) receptor antagonist MRZ 2/576, doxorubicin, etc. The nanocarriers may be special vehicle for the treatment of the brain tumors [45] especially for primary and metastatic brain tumors [189]. Currently, nanoparticle iron chelators have been used to treat alzheimer disease and other neurologic disorders associated with trace metal imbalance [190]. It has been reported that PEGylated PNPs are efficient drug carrier for the delivery of active therapeutic molecules in prion diseases [191]. Strategies for nanocarriers targeting to the brain rely on the presence of its interaction with specific receptor-mediated transport systems in the BBB. For example, polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melano transferrin have been shown its efficacy of delivering a self non-transportable drug into the brain via chimeric construct that can undergo receptor-mediated transcytosis [192]. It has been reported that poly (butylcyanoacrylate) (PBCA) nanoparticles are able to deliver hexapeptide dalargin, doxorubicin and other agents into the brain which are significantly obstructed by BBB [193]. Polysorbate 80 [194] and OX26 Mabs (anti-transferrin receptor MAbs) are the most studied BBB targeting molecule, which have been used to amplify the BBB penetration of liposomes [195]. Studies have shown that delivery to the brain of compounds normally excluded by the BBB could be significantly enhanced by prior binding of a drug or centrally active agent to PBCA nanoparticles, that are then overcoated with surfactant such as polysorbate 80 (Tween 80) [196]. Adsorption of drugs to polysorbate 80-coated nanoparticles has been shown to increase the transport of a number of substances across the BBB including the polar hexapeptide dalargin [197], tubocurarine [198] and the lipid-soluble P-gp substrates loperamide [199] and doxorubicin [45]. However, toxicity induced by the nanoparticles has also been repeatedly reported [200]. For example, TiO_2 nanoparticles could damage brain-cells [201]. On the other hand, if a nanoparticle is biodegradable (e.g. lipidic nanoparticle), it will become less toxic when compared to PNPs [202].

Figure 18 presents a proposed scheme depicting how nanocarriers can be used to improve drug transport across the BBB. Overall, nanocarriers can enhance brain delivery by three major pathways, which include: (1) increasing the local drug gradient at the BBB by passive targeting, (2) Allowing drug-trafficking by non-specific or receptor-mediated endocytosis and (3) blocking drug efflux transporters at the BBB.

Drug Delivery for Cancer

Cancer treatment involving chemotherapy is typically accompanied by toxic side effects, thereby limiting the amount of the drug that can be given to a patient. As a result, all of the tumor tissue may not be exposed to a lethal dose of the drug. The use of nanocarriers such as liposomes and micelles can improve the pharmacological properties of traditional chemotherapeutics. Their small size ($\sim$100 nm or less) allows them to readily extravasate from circulation through vascular.

Defects typically present at tumor sites due to ongoing angiogenesis [203], where they can then deliver encapsulated cytotoxic agents to tumor tissue. This, coupled with the fact that there is generally poor lymphatic drainage at tumor sites, results in a phenomenon known as the EPR effect [204]. The principle pathway for the movement of liposomes into the tumour interstitium is via extravastion through the discontinuous endothelium of the tumour microvasculature. Even the size of the liposomes in passive targeting approach determines the degree or extent of extravasation from the normal vasculature (Fig. 19) which in part explains their clinical success. For example, both DaunoXome and Doxil are examples of clinically-approved liposomal-based drugs

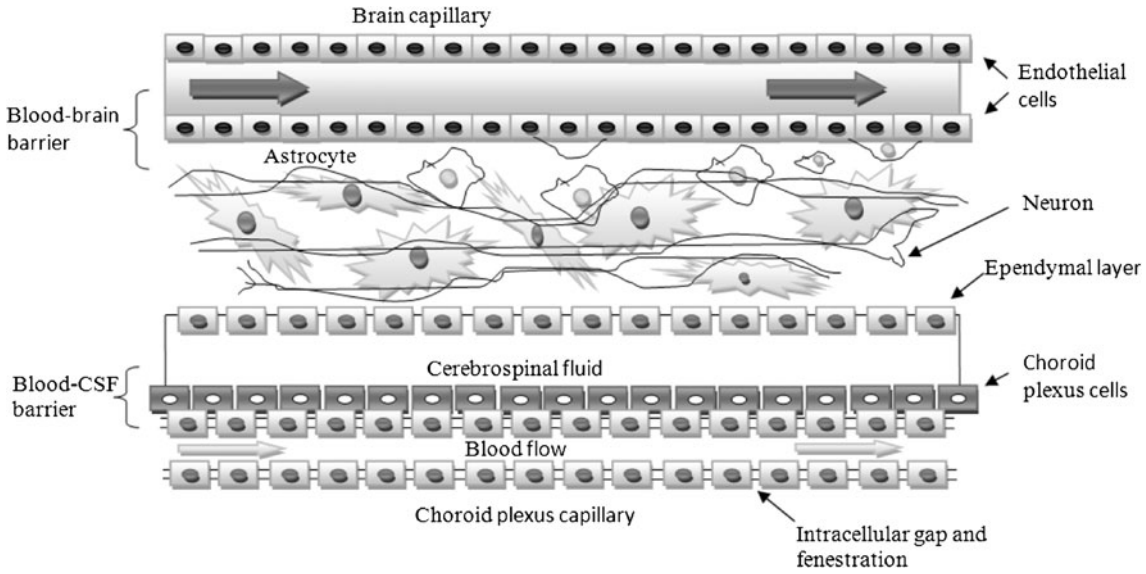

Fig. 17 Schematic diagram depicting various barriers in the targeted drug delivery to CNS

that are currently used to treat either Kaposi's sarcoma [205, 206] or both ovarian and recurrent breast cancer [207]. Alternatively, micellar based drugs containing doxorubicin, paclitaxel, or cisplatin are in various stages of clinical trials [208–210]. While the use of both liposomes and micelles in cancer therapy seems promising, obstacles associated with drug transfer from these nanocarriers to tumor cells within the tumor site remain particularly challenging. The fact that these unmodified drug delivery systems are susceptible to opsonization while in circulation results in low tumor site accumulation. However, surface coating of these nanocarriers with PEG allows for improved circulation times in vivo, and thereby the preferential accumulation of the drug within tumors [204, 211]. As a result, various clinically approved nanocarrier-based formulations such as Doxil are PEGylated. In addition, PEG-lipids used as hydrophilic corona-forming blocks in many micellar-based drugs also allow for longer circulation times [212]. However, while the presence of the PEG moiety improves tumor site accumulation of the drug, it also presents a steric barrier between the nanocarrier and tumor cells, which results in a dramatic reduction in tumor cellular uptake [213, 214]. Therefore, delivery of the encapsulated chemotherapeutic is based on leakage in the tumor microenvironment, followed by the subsequent cellular uptake of the free drug. Further limiting the overall effectiveness of the drug is due to the fact that some cytotoxic agents commonly used in these formulations, such as doxorubicin; have limited tumor tissue penetrability following escape from its nanocarrier due to a high affinity between this drug and various components of the extracellular environment [215]. Therefore, uniform distribution of the drug within the tumor microenvironment is not achieved, and all of the tumor tissue is not necessarily exposed to a lethal dose of the drug. As a result, many research groups are currently working on replacing this form of passive drug delivery with an active one in order to further improve the co-localization between the drug and cancer cells. This type of targeted drug delivery usually involves surface modifications made to these nanocarriers in order to accommodate surface ligands, which recognize and bind certain over expressed receptors present on the cells of interest. Although there are numerous nanocarriers available for such delivery, the use of liposomes and micelles is particularly ideal as surface modifications made to them eliminate the need for direct chemical conjugation between the drug and targeting moiety which is typically required with this form of delivery. This is a particularly important aspect associated with their use as conjugation of drugs directly to the targeting ligand can negatively affect the targeting molecule in a manner that disrupts receptor/ligand recognition [216] and may alter the cytotoxicity of the drug [217].

Targeted Drug Delivery to Cardiovascular System

Cardiovascular diseases constitute and represent foremost vulnerable target diseases particularly in the developed countries. These diseases are typically localized to discrete vascular regions, affording great opportunity for targeted pharmacological treatment. Indeed, vascular thrombosis accounts for about half of all deaths in these countries as a result of myocardial infarction, stroke, pulmonary emboli etc. The best way to improve patient survival and decrease morbidity is prompt detection and treatment of thrombosis using thrombolytic therapy. Therefore, a variety of thrombolytic agents including tissue plasminogen activator, urokinase and streptokinase have been developed.

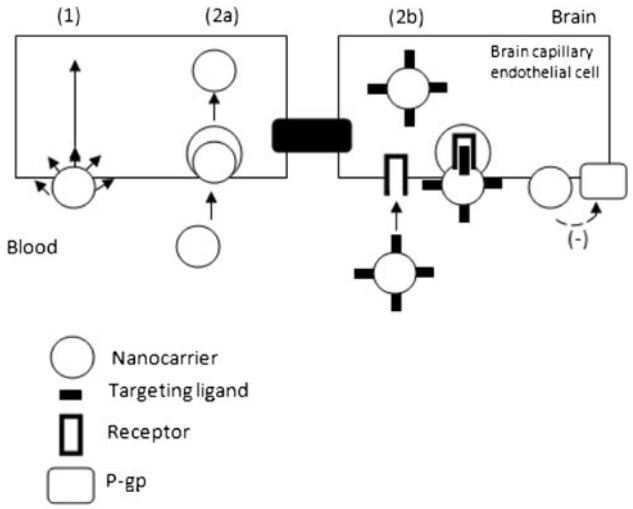

Fig. 18 Major pathways used by nanocarrier systems to improve antiretroviral penetration across the BBB. (*1*) Increasing the local drug gradient at the BBB by passive targeting, (*2a*) allowing drug-trafficking by endocytosis (non-specific or receptor-mediated), (*2b*) blocking drug efflux transporters (*solid line*) inhibitory effect

These agents act by activating the protein plasminogen into plasmin. When this occurs, activated plasmin circulates throughout the vascular system, triggering the fibrinolytic cascade of events that dissolves the thrombi. The short half-life of thrombolytic drugs necessitates the administration of high dosages of these agents in systemically active forms over an extended period of time, which in turn, increases the risk for side effects (uncontrolled hemorrhage).

For optimal drug delivery, delivery systems should be localized to the site of thrombus as well as should maintain a reservoir which avoids uptake into unwanted tissue. Nanocarriers are appropriate options to fulfill such requirements. Small size of nanocarriers allows rapid incorporation of drug into the thrombus interior. Encapsulation of streptokinase (immunogenic thrombolytic) in the liposomes may also decrease the immunogenicity of the streptokinase. Thus by using nanocarriers based formulations it could be possible to treat cardiovascular diseases more effectively and safely. Erdogen et al. [218] prepared three types of vesicular drug delivery systems, namely niosomes, liposomes and sphingosomes containing streptokinase, for achieving the slow release of entrapped proteins in the circulation to mask immunogenic properties, increase half-life and protect against loss of enzymatic activity. Biodistribution of these vesicular dispersions was monitored using radiolabelled streptokinase and compared with i.v. injected free streptokinase. The results demonstrated the highest level of concentration of liposomes and sphingosomes in the spleen and kidneys. Uptake of Tc-^{99m}-labelled streptokinase by the thrombus can be explained by the mechanism of thrombolysis produced by streptokinase. This mechanism involves a series of reactions where streptokinase absorbs to and penetrates in and around the thrombus; it activates plasminogen located within the thrombus, and yields sufficient plasmin for fibrin dissolution and thrombolysis [219].

Wang et al. [220] prepared thrombus-targeted RGD peptide conjugated urokinase liposomes and observed its thrombolytic efficacy on thrombus model rats. In this study the ligand H-Arg-Gly-Asp-Ser-OH (RGDS) conjugated DSPE-$PEG_{3,500}$-COOH was incorporated in liposomal lipid bilayers, to produce thrombus-targeted long-circulatory liposomes. The thrombolytic activity was measured in terms of dry thrombi weights. It was observed that the

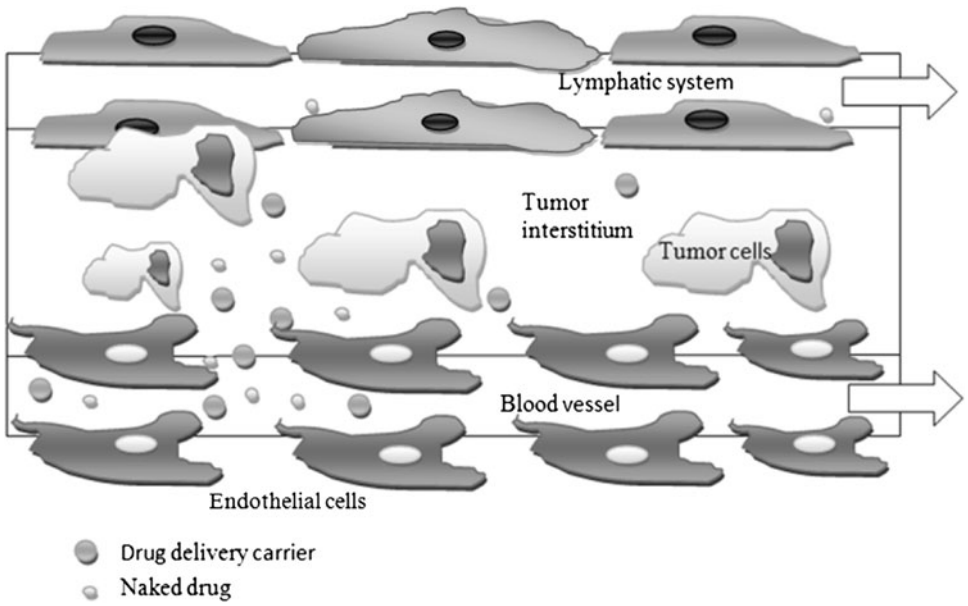

Fig. 19 Schematic showing enhanced permeability and retention effects

targeted urokinase liposomes showed significantly improved thrombolytic efficacy as compared to conventional urokinase liposomes. Hypothetical mechanism for the improved thrombolytic activity of these types of carrier systems is shown in Fig. 20. It was demonstrated that urokinase was released selectively at the site of thrombus, acted on plasminogen and converting it into plasmin near the clot thus reduce the bleeding complications.

Intracellular Organelle Targeting

Many molecular drug targets are associated with specific subcellular compartments [221–223]. Therefore, specifically directing therapeutic agents to an individual organelle is an attractive strategy for drug delivery. Nanocarriers can be designed as efficient delivery vehicles to intracellular organelles, including early-late endosomes, lysosomes, cytoplasm, mitochondria and nucleus.

The sequestration of drug nanocarriers within endosomes/lysosomes following endocytosis is one of the most critical bottlenecks for cytoplasmic drug delivery, especially for high molecular weight drugs such as plasmid DNA and siRNAs. Strategies have been designed to increase endosome/lysosome escape through controlled membrane destabilization (e.g., triggered by acidic pH or reducing environment, or proteases). This can be accomplished, e.g., by incorporation of a fusogenic peptide [224, 225]. Alternately, nanocarriers can be designed to bypass the endosomal route. This strategy can be achieved by conjugating cell-penetrating peptides to nanocarriers [226, 227]. In order to direct drug to mitochondria or the nucleus, specific trafficking signals may be attached on the nanocarriers, e.g., a nuclear localization signal (NLS) [228, 229] and a mitochondrial targeting signal peptide (MTS).

Mitochondrion is a promising therapeutic drug target due to its important role in energy supply and cell death regulation. A variety of methods have been developed to enhance mitochondrial accumulation of drugs. Harashima and Yamada [230] described a lipid-based carrier multifunctional envelope-type nano-device (MEND) and MITO-Porter. MITO-Porter consists of liposomes conjugated to octa-arginine (R8) peptides, which facilitate uptake of the entire assembly into cells by macropinocytosis. Following endosomal escape, MITO-Porter further fuses with the mitochondrial outer membrane due to fusogenic lipids in the liposome. The dequalinium (DQA)-based liposomes constitute another class of mitochondrial delivery nanocarriers. DQA is a symmetrical delocalized lipophilic divalent cation, which promotes efficient mitochondrial localization [231, 232]. Furthermore, surface modification of liposomes with mitochondriotropic triphenylphosphonium (TPP) cations has been reported to promote the efficient subcellular delivery of a model drug to mitochondria both in vitro and in vivo [233].

Cheng et al. [234] studied on DQAsome encapsulation of paclitaxel, a drug which induces apoptosis by direct action on mitochondrial membrane, and found that paclitaxel-loaded DQAsomes inhibited the gowth of human colon cancer in nude mice by 50 % compared to controls. The hypothetical mechanism of action of paclitaxel-loaded DQAsomes is summarized in Fig. 21. When DQAsomes reach the endosome by the process of endocytosis they disrupt the endosomal membrane and attract towards the mitochondrial membrane. The DQAsomes are then destabilized following interaction with the mitochondrial membrane releasing the paclitaxel. The released paclitaxel acts on the mitochondrial membrane resulting in the release of cytochrome C which as a result induces apoptosis and ultimately cell death.

Gene Delivery

Rapid escape of nanocarriers from the degradative endo-lysosomal compartment to the cytoplasmic compartment and their sustained intracellular retention suggest that nanocarriers containing encapsulated plasmid DNA could serve as an efficient sustained release gene delivery system [235, 236]. The therapeutic efficacy of the nanocarriers could be due to their ability to protect the therapeutic agent from degradation due to lysosomal enzymes.

One limiting factor in gene therapy is the toxicity of expression vectors [237], which most often limits the dose of DNA that can be delivered. Nanocarriers are neither toxic in vitro to cultured cells nor in vivo as shown in various studies [238, 239]. Nanocarriers administered intravascularly in arterial tissue did not show untoward effects in chronic pig and rat models of restenosis, demonstrating the long-term biocompatibility of Nanoparticles [240]. Therefore, even if the amount of DNA associated with nanoparticles (1:50 w/w) is relatively lower than that in cationic polymer (1:0.4 to 1:6) or lipid based systems (1:2 to 1:6), the dose of nanoparticles could be increased to deliver the required amounts of DNA without the concerns over nanoparticle-associated toxicity. Recently, new efforts are being made to enhance DNA entrapment in nanoparticles by condensing them prior to encapsulation, or synthesizing novel polymers with cationic groups that would condense DNA into the nanoparticle matrix. It is quite possible that some transfecting agents, who show high transfection efficiency in vitro, are not as effective in vivo because the vector itself causes tissue toxicity, thus reducing the transfection efficiency [241]. It is now an accepted fact that the safety of the expression vector in vivo is equally important as the efficiency of gene expression to the success of gene therapy [242]. It is hypothesized that slow release of DNA from PLGA nanoparticles intracellularly would be effective in

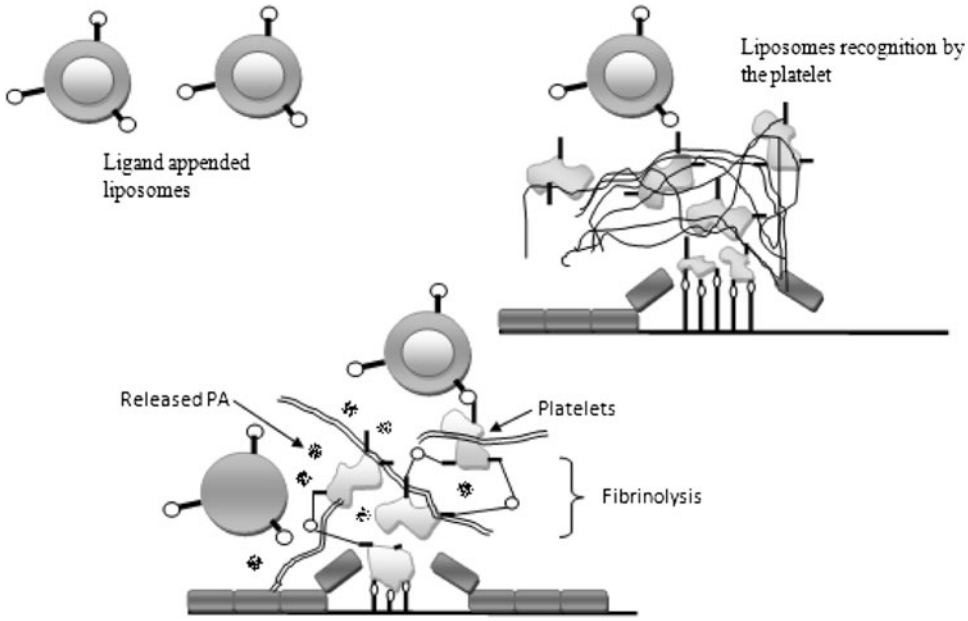

Fig. 20 Schematic presentation of mechanism of action of PA loaded surface modified liposomes

achieving sustained gene expression in the target tissue. Sustained and regulated gene expression is probably more important in treating certain localized disease conditions such as cardiac and limb ischemia by inducing neovascularization in the damaged tissue using genes encoding pro-angiogenic growth factors [243]. It has been shown that unregulated over-expression of growth factors for angiogenesis therapy could lead to tumor formation instead of therapeutic angiogenesis [244]. Similarly, sustained gene expression has been shown to be effective in bone regeneration using a collagen matrix for DNA delivery [245]. Restenosis, a vasculo proliferative condition that occurs following coronary balloon angioplasty procedure, is another example of a pathological condition where sustained expression of a gene in the target artery having anti-proliferative effect could be more effective [246]. Thus, just as in drug therapy where the optimal dose and the duration of therapy is important, in gene therapy the optimized level of gene expression for a sufficient period of time could be more effective in certain disease conditions. Therefore, optimal gene delivery would depend on the requirements of different disease conditions. Certain disease conditions are localized whereas others require systemic therapy. For systemic gene therapy, however, the knowledge of optimal plasma levels of expressed proteins is necessary. Felgner et al. [247] proposed the use of cationic liposomes as efficient carriers for the intracellular delivery of DNA. DOPE can fuse with endosomal membrane. DNA on mixing with cationic liposomes produces a condensed DNA along with tubular structure and liposome aggregate. The mechanism by which DNA-cationic liposome complex delivers the DNA is understood to be as follows. The complex first interacts with the cell membrane, followed by endocytosis and finally disruption of endosomes (Fig. 22).

Protein Delivery

Therapeutic proteins and peptides can be encapsulated into nanocarriers using double emulsion solvent evaporation technique. One primary concern with protein encapsulation in PLGA nano- and microparticles is the loss of therapeutic efficacy of the protein due to the degradation/denaturation of the protein. For example, about 30 % of the tetanus toxoid (TT) activity was lost following its encapsulation in Nanoparticles [248]. Inactivation of protein could occur through two different mechanisms. First, protein is exposed to organic solvents during the formulation procedure, leading to protein adsorption at the oil–water interface and consequent denaturation and aggregation of the protein [249, 250]. Second, the acidic environment generated during the degradation of PLGA matrix due to the formation of acidic monomers and oligomers could produce similar inactivation. Protein aggregation at the interface can be inhibited by addition of either human or BSA to the aqueous phase before emulsification [251]. BSA protects the therapeutic protein by preferentially adsorbing to the interface. Similarly, increased protein release and protection of antigenicity of the tetanus toxoid was achieved by using 0.2 % gelatin in the nanospheres formulation [252]. Therapeutic protein can be protected from degradation due to acidic microenvironment by including a buffering base such as magnesium hydroxide into the formulation [253]. Addition of magnesium hydroxide to the PLGA nanosphere formulation was shown to protect the encapsulated BSA from aggregation and increase its in vitro release from the nanospheres.

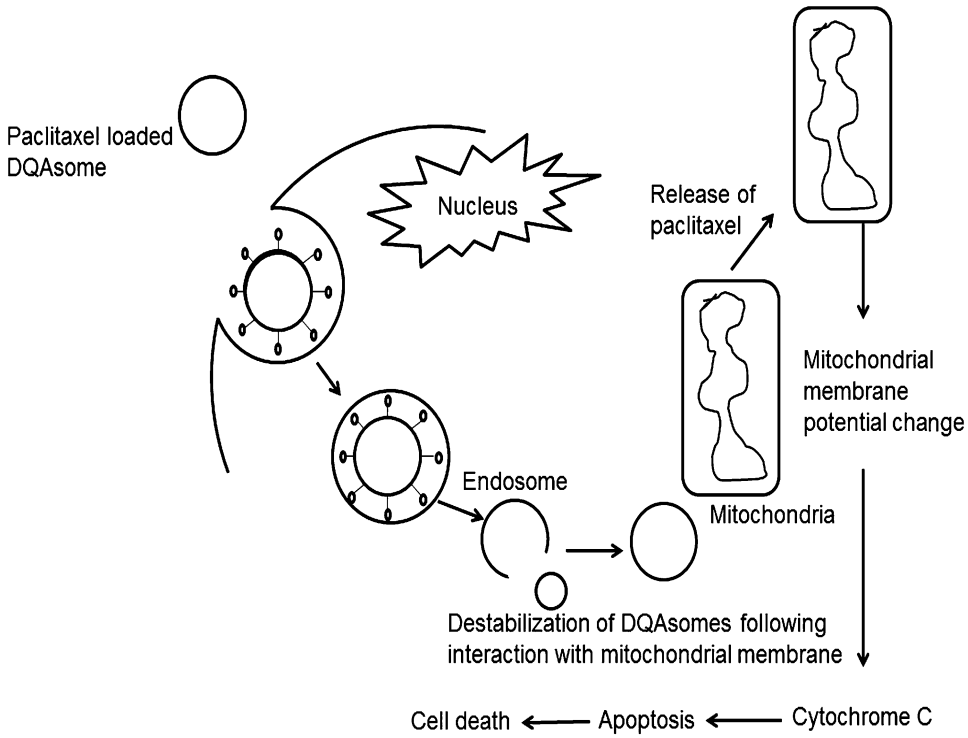

Fig. 21 Schematic presentation of possible role of paclitaxel loaded DQAsomes in colon cancer

Vaccine Adjuvant

Nanocarriers containing entrapped or adsorbed antigens are being investigated as vaccine adjuvant alternatives to the currently used alum with an objective to develop better vaccine adjuvants and minimize the frequency of immunization [248, 254]. Nanocarriers containing encapsulated antigen can be effective as an adjuvant because they could provide sustained release of the antigen. Kreuter and Speiser originally demonstrated the adjuvant properties of poly (methyl methacrylate) nanocarriers in 1976 [255]. Nanocarriers have also been investigated for oral immunization to induce systemic and mucosal immunity [256]. Desai et al. [248] demonstrated adjuvant properties of PLGA nanoparticles containing encapsulated *Staphylococcal enterotoxin* B toxoid. The systemic immune response (IgG, IgA and IgM titers) of animals injected with nanoparticles was comparable to that obtained following injection of alum. The immune response was reached a maximum at 7 weeks post-immunization, which was then gradually declined with time. A booster dose of toxoid at 19 weeks induced a similar secondary immune response in both groups, which was higher than the primary immune response. While these studies suggested that PLGA nanoparticles could be used as vaccine adjuvants, another study with TT, demonstrated that co-injecting TT-alum along with TT-loaded nanoparticles induced a synergistic immune response [254]. The combination induced a four-fold greater mean serum anti-TT IgG response than a single injection of TT-nanoparticles alone. The mean immune response obtained with the combination was comparable with that obtained from two injections of alum alone. The additional benefit of the combination was that it resulted in a much stronger immune response as early as at 3 weeks. Thus the system could be useful in inducing an early immune response in case of an epidemic outbreak.

Toxicity of Nanocarrier Systems

Natural nanosized particles serve as models for the description of a possible toxicological profile for nanocarrier systems. It is worth noting that toxicological research has been mainly conducted using occupational and environmental data that involves natural nanomaterials. This can be applied to the study of man-made nanoparticles since the same toxicological principles apply to both types of nanosized particles [257]. Indeed, certain workplace conditions can generate nanosized particles that reach higher concentrations than typically found in the environment. There has not been extensive research conducted to analyze the toxicity of nanocarriers per se [258]. However, Curtis et al. [259] hypothesized possible mechanisms of toxicity for nanoparticles: (1) Toxicology of bulk materials is relatively well defined as in the case of heavy metals as this toxicity is quite facile to quantify, (2) The electrical properties of nanoparticles differ from bulk material. Nanomaterials can create and/or scavenge ROS

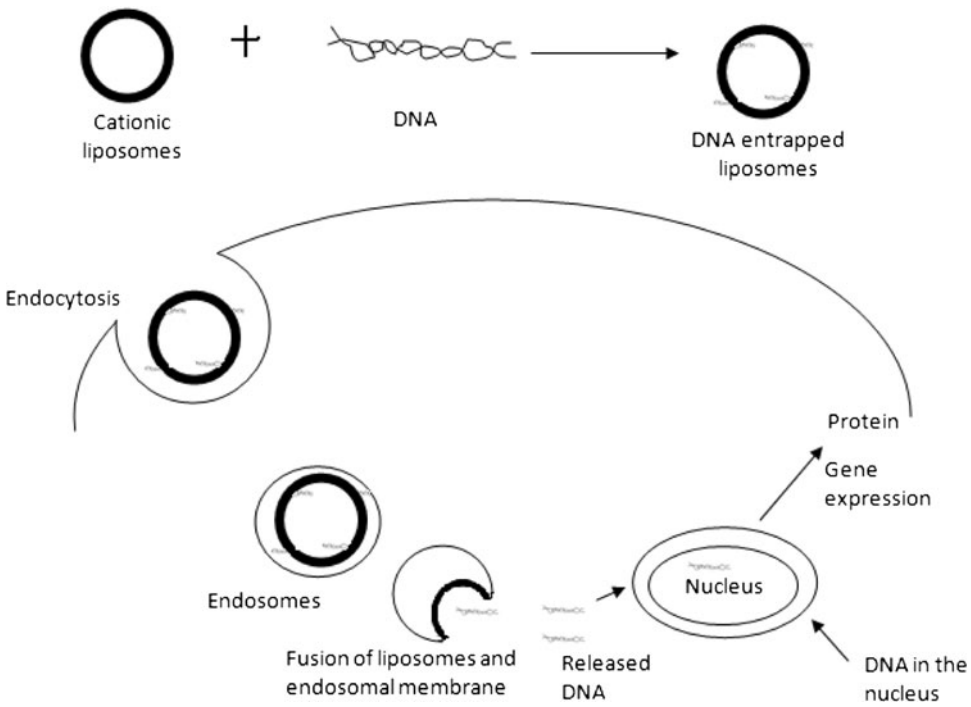

Fig. 22 Schematic mechanism showing DNA delivery through cationic liposomes

and free radicals [260]; (3) Toxicity of nanoparticles may be linked to their size as suggested by studies of ultrafine particles in the respiratory tract. The toxicological effects of ultrafine particles are determined by their size and their propensity to agglomerate. They are also known to pass biological barriers, like skin, vascular endothelium and the BBB; therefore, affecting absorption, distribution, and excretion of these particles [261]; (4) Shape may also be a factor that determines toxicity, as is the case of CNTs; (5) It has not been clearly delineated how nanoparticles can trigger immune responses; however, there is a growing concern about their role in possible allergic reactions [262, 263].

Important Routes of Exposure

Mammalian systems have been used as the main in vivo model to test toxicity of nanocarrier systems; in particular, several studies described the exposure of the respiratory system to airborne ultrafine particles in order to test the hypothesis that they cause significant health effects. Other exposure routes, such as skin and GI tract, have not been considered as extensively as the respiratory tract as portals of entry for nanocarrier systems [264].

Skin

It has been hypothesized that dermal exposure might be the most significant route of exposure; however, few literature reports are available that refer to the absorption and effects of nanoparticles in the skin. Contact with nanoparticles through the skin can occur due to occupational exposure during the manufacturing of solvents, pesticides, or pharmaceuticals. Skin exposure to nanoparticles can also occur during non-occupational situations from the use of cosmetics and in the intentional application of topical creams and other drug treatments. Initial studies of nanoparticle absorption through the skin are inconclusive; some demonstrate little penetration into the epidermis while others using more complex flexing protocols show deep absorption [265]. Due to their unique physicochemical properties, nanoparticles are rendered more biologically active than structures of the same chemical make-up, which is apparent by the inflammatory, oxidant, and anti-oxidant capacities described for nanocarrier systems. Evidence of mitochondrial distribution and oxidative stress also exists for nanocarrier systems [257].

Broken skin represents a readily available portal of entry even for large (0.5–7.0 μm) micron size particles. Even intact skin, when flexed, makes epidermis permeable to nanoparticles. A recent study demonstrated that fluorospheres (0.5–1 μm) can penetrate the epidermis and reach the epidermis employing a skin flexing protocol that is likely to be representative of physiological conditions [266, 267]. Once in the epidermis, nanoparticles reach the lymphatic system and regional lymph, and from there they can translocate to the systemic vasculature. Nanoparticles can also reach sensory skin nerves; as reported after the injection of nanoparticles in the tongue and facial muscles of mice. Cationized nanoparticles can reach cell bodies of

facial neurons, high lighting the importance of electric charge on nanoparticle incorporation and disposition into axons. To better understand dermal absorption of nanoparticles more research on regular skin, dry skin, and damaged skin is necessary [268, 269].

Respiratory Tract

Exposure to nanosized materials has increased since new anthropogenic sources developed around three decades ago. Inhalation nanoparticles are deposited in all regions of the respiratory tract, however, larger particles may be filtered out in the upper airways, whereas smaller particles reach distal airways [259]. The respiratory tract can be divided into three regions: nasopharyngeal, tracheobronchial, and alveolar regions. Significant amounts of certain particle size ranges can deposit in each region, for example, 90 % of nanoparticles of 1 nm in diameter deposit in the nasopharyngeal region, whereas only 10 % of these nanoparticles deposit in the tracheobronchial region and almost none reach the alveolar region. In comparison, 15 % of nanoparticles of 20 nm in diameter deposit in the nasopharyngeal region, 15 % in the tracheobronchial region, and approximately 50 % in the alveolar region [263]. After absorption across the lung epithelium nanoparticles can enter the blood and lymph to reach cells in the bone marrow, lymph nodes, spleen, and heart [262, 268]. Nanoparticles can even reach the CNS and ganglia following translocation [270].

Epidemiologic analysis and controlled clinical trial studies in humans have been used to describe the toxicology of airborne natural nanosized materials. These nanosized materials often have cardiovascular and respiratory effects that result in significant morbidity and mortality in susceptible segments of the population. Subjects with asthma or chronic obstructive pulmonary disease show greater deposition of natural nanosized materials in the respiratory tract than healthy individuals. The presence of natural nanosized particles is associated with the formation of blood markers of coagulation, has effects on the systemic inflammation and pulmonary diffusion capacity, and increases the risk of ventricular disrhythmias [271]. Ultrafine particles are described to be more toxic than larger particles with the same chemical make-up due to their large surface area, causing cytotoxicity, allergic response or inflammation [262]. Further studies are needed to investigate the toxic effects and fate of nanoparticles after their deposition in the respiratory tract.

Translocation, the transport of dissolved materials within the body, has been proposed as a mechanism for nanosized particles to reach extrapulmonary sites and then other target tissues. Nanoparticles can access the systemic vasculature directly or via lymphatic transfer by transcytosis, crossing the epithelia of the respiratory tract into the interstitium, phagocytosis, endocytosis or some other transmembrane process. A second target after translocation is suggested to be the sensory nerve endings embedded in the airway epithelia, followed by translocation to ganglia and the CNS via axons [262, 269].

In addition to epidemiological and controlled clinical studies, the effects of nanoparticles in the respiratory tract have been studied through inhalation and instillation studies in rodents and in vitro cell culture systems. In rodents, ultrafine particles cause mild pulmonary inflammatory responses and have effects on extrapulmonary organs. Dosing with both natural and anthropogenic nanosized particles, in vitro studies showed pro-inflammatory and oxidative stress related cellular response [259]. Interpretation of in vitro studies needs to be prudently evaluated to consider differential chemical disposition, different cell types and targets, and the use of high doses and consequent levels. In other words, cautious interpretation needs to be undertaken with concentrations that are orders of magnitude higher than those predicted from relevant ambient exposures [262, 272]. Shape and structure of nanoparticles may also predispose them to inhalational toxicity. For example, CNTs have distinct pulmonary effects as compared to carbon black and graphite, which are larger structures of similar chemical make-up. CNTs are arranged helically into cylindrical structures, and they form single-walled (SWCNTs), or multi-walled (MWCNTs) carbon nanotubes. The usual shape is elongated from 0.4 nm in diameter to hundreds of nm in length [273, 274]. When toxicity of CNTs was compared to that of carbon black after intratracheal instillation in mice, CNTs proved to be significantly more harmful [262]. Carbon black was ingested by macrophages in the alveolar region and resided predominantly at this site. In comparison, macrophages that ingested CNTs migrated to centrilobular locations and caused interstitial granulomas [259]. Pharyngeal aspiration of SWCNTs caused increased inflammation and cell damage. Two patterns of lung remodeling were present depending on whether SWCNTs aggregate (granuloma) or distribute (interstitial fibrosis) through the lung space [257]. Either pro-inflammatory (TNF-α, IL-1β) or anti-inflammatory profibrogenic cytokines (TGF-β, IL-10) are expressed in tissue affected by Nanoparticles [275]. When SWCNTs and MWCNTs were compared to C60 fullerenes they showed greater cytotoxicity to alveolar macrophages [258]. C60 fullerenes are allotropic forms of carbon that are arranged in clusters and are also used as nanocarrier systems [261].

Gastrointestinal Tract

Nanoparticles can reach the GI tract after mucociliary clearance from the respiratory tract through the nasal

region, or can be ingested directly in food, water, cosmetics, drugs, and drug delivery devices [262, 275]. The utility of biodegradable nanoparticles in the delivery of oral vaccines has been proposed for antigens known to be susceptible to proteolysis [276]. Few studies have looked at toxicity of nanoparticles following oral ingestion. Acute toxicity of Cu particles and nanocopper was measured in mice; LD_{50} for nanocopper is 413 mg/kg compared to >5,000 mg/kg for Cu [259]. Nanocopper was also reported to cause pathological damage to the liver, the kidney, and the spleen. Muller and Keck [277] noted that recrystallisation is possible in a supersaturated nanosuspension which can occur during dissolution in the GI tract. New studies that can overcome recrystallisation issues will be helpful to accurately assess toxicity of nanoparticles in the GI tract. Further studies on gastrointestinal lymphatic uptake and transport, and direct toxicological effects on the GI tract are required.

Toxicity in Brain, Circulatory and Central Nervous System

Regarding the passage of nanoparticles to CNS, the BBB permeability is mainly dependent on the charge of nanoparticles. It allows a large number of cationic nanoparticles to pass as compared to neutral or anionic particles, due to the disruption of its integrity. In the case of specific circulatory diseases like hypertension, brain inflammation and respiratory tract inflammation, increased levels of cytokines that cross the BBB and induce inflammation may have increased BBB permeability, which will also allow nanoparticles access to the nervous system. Experimental evidence suggested that the initiation and promotion of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and Pick's disease, are associated with oxidative stress and accumulation of high concentrations of metals such as copper, aluminium, zinc and especially iron in brain regions associated with the function loss and cell damage.

Interestingly, nanoparticles could avoid normal phagocytic defences in the respiratory system and gain access to the systematic circulation or even to the CNS. Once inhaled and deposited, nanoparticles can translocate to extrapulmonary sites and reach other target organs by different mechanisms. The first mechanism involves passing of nanoparticles across epithelia of the respiratory tract into the interstitium followed by an access to the blood stream directly or via lymphatic pathways, resulting in systemic distribution of nanoparticles. Nanoparticles can be rapidly observed in rat platelets after intratracheal instillation of particles of colloidal gold (30 nm). It is reported that inhaled (99 m) Tc-labelled carbon particles (100 nm) pass to the blood circulation 1 min after exposure. However, they did not find an accumulation of the same radiolabel in the liver after exposure. Once nanoparticles are translocated into the blood stream they could induce adverse biological effects. Mixed carbon nanoparticles and nanotubes, both MWCNT and SWCNT, are able to induce platelet aggregation in vitro and in addition accelerate the rate of vascular thrombosis in rat carotid artery. Furthermore, it has been found that nanoparticles can directly induce cytotoxic morphological changes in HUVEC, induction of proinflammatory responses, inhibition of cell growth and reduction of endothelial nitric oxide synthase.

The translocation of nanoparticles to CNS may not only take place as a result of systemic distribution. The other mechanism involves the uptake of nanoparticles by sensory nerve endings embedded in airway epithelia, followed by axonal translocation to ganglionic and CNS structures. In addition, nanoparticles, can be taken up by the nerve endings of the olfactory bulb and translocated to the CNS. It has been found that C60 fullerenes can induce oxidative stress in the brain of largemouth bass via the olfactory bulb.

Nanoparticle uptake by red blood cells that do not have phagocytic abilities, due to the lack of phagocytic receptors is entirely dictated by size, while the nanoparticle charge or material type plays little importance. In contrast, nanoparticle charge plays an essential role in their uptake by platelets and their influence on blood clot formation. Uncharged polystyrene particles do not have a effect on blood clot formation. Negatively charged nanoparticles significantly inhibit thrombi formation, while positively charged nanoparticles enhance platelet aggregation and thrombosis. The interaction between platelets and positively charged particles seems to be due to the net negative charge that platelets have. The positively charged nanoparticles interact with the negatively charged platelets and reduce their surface charge, making them more prone to aggregation. Until now, it was thought that blood clots can be formed due to three main causes: when the blood flow is obstructed or slowed down, when the vascular endothelial cells are damaged, or due to the blood chemistry. However, nanoparticles may act as nucleating centers for blood clots. It is important to note that pulmonary instillation of large nanoparticles 400 nm caused pulmonary inflammation of similar intensity as it is caused by 60 nm particles, but did not lead to peripheral thrombosis. The fact that the larger particles failed to produce a thrombotic effect suggests that pulmonary inflammation itself is insufficient to cause peripheral thrombosis and that thrombi formation occurs via direct activation of platelets.

Toxicity in Lymphatic System

Translocation of nanoparticles to lymph nodes is a topic of deep investigation today for drug delivery and tumor

imaging. Progression of many cancers; lung, esophageal, mesothelioma, etc. is seen in the spread of tumor cells to local lymph nodes. The detection and targeted drug delivery to these sites are the steps involved in the therapeutic treatment of cancer. Several studies showed that interstitially injected particles pass preferentially through the lymphatic system and not through the circulatory system, probably due to permeability differences. After entering the lymphatic system, they locate in the lymph nodes. The free nanoparticles reaching the lymph nodes are ingested by resident macrophages. Nanoparticles that are able to enter the circulatory system can also gain access to the interstitium and from there they are drained through the lymphatic system to the lymph nodes as free nanoparticles and/or inside macrophages. The adverse health effects of nanoparticle uptake by the lymphatic system are not sufficiently explored. However, one can hypothesize that oxidative stress created by certain types of nanoparticles could lead to damage of lymphocytes type of white blood cell, lymph nodes, and/or spleen [286].

Toxicological Effects of Nanocarrier Systems

Physicochemical Determinants

Due to their size, nanoparticles have a large specific surface area. This may translate into increased biological activity, due to different contact interactions with cells and its components, and variable biokinetics. Physicochemical properties of nanoparticles vary widely from the properties of bulk materials [257, 261]. The stability of nanoparticles requires further detailed investigation; however, the possibility of Ostwald ripening and agglomeration exists. Few studies have looked specifically at the stability of nanoparticles, for example, amphiphilic β-cyclodextrin nanosphere suspensions with and without poloxamer, a stabilizing agent, demonstrated good physical stability even after 3 years of storage at room temperature due to small size and structural organization of the Nanoparticles [278]. Additionally, Liu et al. [279] reported for the first time the effect of Ostwald ripening in nanoparticle formulations. The addition of antisolvent results in the reduction of the ripening rate for β-carotene nanoparticle dispersions by dramatically decreasing bulk solubility. It has also been reported that dispersant layers can increase the stability of nanoparticles in aqueous solutions and avoid agglomeration. Using pyrogallol-PEG architecture to adsorb on the surface of alumina nanoparticles can also counterbalance vanderwall forces that are responsible for agglomeration [280].

Molecular Determinants

Nanoparticles favor the formation of pro-oxidants, especially under exposure to light, UV light, or transition metals; thereby, destabilizing the balance between the production of ROS and the biological system's ability to detoxify or repair the system. Nanoparticles can modify mitochondrial function, as well as cellular redox signaling. ROS can also be produced by the NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) in phagocytic cells or as a product of P_{450} cytochrome metabolism. Oxidative stress induced by nanoparticles is reported to enhance inflammation through up regulation of redox-sensitive transcription factors including nuclear factor kappa B (NFκB), activating protein 1 (AP-1), extracellular signal regulated kinases (ERK), c-Jun *N*-terminal kinases (JNK) and p38 mitogen-activated protein kinases pathways [259, 281].

Genotoxicity and Antigenicity

Gene therapy investigations have recently discovered and incorporated the advantages of using nanocarrier systems to deliver genes more efficiently. Gene transfer is based on the destabilization of internalized vesicles via the surface charge effect [282]. For example, DNA, antisense oligonucleotides, and Si-RNAs are condensed into nanostructures that facilitate internalization into the cell via endocytosis. Poly (ethylenimine) induces membrane rupture by attracting protons, and facilitates the release of polycationnucleic complexes into the cytoplasm [283, 284]. Differential gene expression after delivery of micelles-carrying cisplatin has been reported in certain cells which resulted in induced cell death via apoptosis or necrosis. Cationic formulations have been described to affect cell proliferation, differentiation, and pro-apoptotic genes in human epithelial cells [285]. For example; synthetic polycationic non-viral gene transfer systems are proposed to improve the poor success of human gene therapy that utilizes viruses to transport the therapeutic genes. The polycationic nature of these delivery systems induces cytotoxicity by necrosis and apoptosis. Necrosis occurs when cationic components of the carrier and cell surface proteoglycans or proteins in the cytoskeleton of the target cell interact, disestablishing the membrane and causing the formation of pores. In comparison, apoptosis occurs in Jurkat cells via cytochrome c release from the mitochondria of target cells due to Bcl-2-sensitivity [263]. It is known that the use of different cationic materials initiates apoptosis at variable chronology through a variety of different pathways [284]. It is suggested that carriers may exacerbate, soothe, or mask the effects of delivered nucleic acids. In spite of the current application of nanomaterials in gene therapy and gene delivery in pre-clinical research, few studies have

focused on the toxigenomic responses [281]. Therefore, assessment of the toxicity of nanomaterials used in these areas of research is fundamental to maximize future clinical outcomes. As stated before, ultrafine particles are described to be more toxic than larger particles with the same chemical make-up, causing cytotoxicity, an allergic responses or inflammation. Further studies of the antigenicity of nanoparticles need to be extended to determine when nanoparticles are recognized by the immune system, and whether or not they cause specific immune responses with antigen formation [257]. PEG-grafted liposome infusion was described to trigger non-IgE-mediated signs of hypersensitivity. In comparison, peptide-functionalized CNTs form immunogenic complexes, enhancing the antibody response. Based on this research and the growing body of scientific evidence of nanoparticles, possible vaccine creation has been proposed [259, 263].

Adverse Health Effects and Treatment of Nanoparticle Toxicity

Nanoparticles, due to their small size, can influence basic cellular processes, such as proliferation, metabolism, and death. Many diseases can be associated with dysfunction of these basic processes. For example, cancer results from uncontrolled cell proliferation, while neurodegenerative diseases are caused in part by premature cell death. Oxidative stress has been implicated in many diseases, including cardiovascular and neurological diseases, pancreatitis and cancer. Severe inflammation is assumed to be the initiating step in the appearance of autoimmune diseases systemic lupus erythematosus, scleroderma and rheumatoid arthritis that can sometimes be associated with exposure to some nanoparticles, such as silica and asbestos.

Regarding the treatment of adverse health effects caused by nanoparticle cytotoxicity, antioxidants, anti-inflammatory drugs and metal chelators show promising effects. It has been reported that rats that underwent instillation of nanoparticles into the lungs together with an antioxidant nacystelin showed inflammation reduced by up to 60 % in comparison to those exposed to nanoparticles alone. Antioxidant therapy has been found to protect against the development of hypertension, arteriosclerosis, cardiomyopathies and coronary heart disease, providing further evidence of the link between the oxidative stress response and cardiovascular effects. The adverse health effects of transition metals can be minimized by metal chelators [286].

Conclusions

Nanocarriers in drug delivery applications have a bright future with the emergence of several promising approaches like dendrimers, SLNs, PNPs, PMs, liposomes, nanosuspensions and nanocrystals, ceramic nanoparticles, CNTs, QDs, Au nanoparticles, polymersomes etc. Nanocarriers are likely to be cornerstones of innovative nanomedical devices to be used for drug discovery and delivery, discovery of biomarkers and molecular diagnostics. As nanocarriers may also exert toxicological effects, nanotoxicology has emerged as a new branch of toxicology for studying undesirable effects of nanocarriers. Therefore, development of novel nanocarriers for pharmacology, therapeutics and diagnostics must proceed in tandem with assessment of any toxicological and environmental side effects of these carriers. For the pharmaceutical industry, novel nano drug delivery technologies represent a strategic tool for expanding drug markets as far as Indian health science is concerned. The technology can address issues associated with current pharmaceuticals such as extending product life (line extension), or can add to their performance and acceptability, either by increasing efficacy or improving safety and patient compliance. It is expected that novel nano drug delivery systems can make a significant contribution to global pharmaceutical sales. However, the cost of these 'nano-deliveries' should be in acceptable low range to be successful in the Indian clinics. In future, various multifunctional novel nanocarrier-based drug deliveries may be designed and developed. We are optimistic that an increasing number of novel 'nano-deliveries' for the treatment of various deadly diseases will emerge. We expect that with continued research and support, drug delivery applications will be an important beneficiary of nanotechnology in India for years to come.

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