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Materials for Drug & Gene Delivery

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Outline:

Figs 2.1-2.9 are from the author(s) reference: C.E. Mora-Huertas, H. Fessi, A. Elaissari, Polymer-based nanocapsules for drug delivery, **International Journal of Pharmaceutics**, Volume 385, Issues 1–2, 29 January 2010, Pages 113-142.

Introduction

International Union of Pure and Applied Chemistry (IUPAC) has defined nanomaterials as materials having sizes smaller than 100 nanometers (1 nm = 10^{-9} m) along at least one dimension (length, width, or height) [1]. Nanomaterials are a new step in the evolution of understanding and utilization of materials. They are investigated as promising tools for the advancement of diagnostic biosensors, drug/gene delivery and biomedical imaging for their unique physicochemical and biological properties. Many properties of nanomaterials, such as size, shape, chemical composition, surface structure, surface charge, aggregation, agglomeration, and solubility can greatly influence their interactions with biomolecules and cells [2]. The uniqueness of the structural characteristics, energetics, response, dynamics, and chemistry of nanostructures constitutes the basis of nanoscience [3]. Suitable control of these properties and responses of nanostructures can lead to new devices and technologies.
Although it is basically impossible to cover all the areas where nanoscale materials are involved, we have made a choice of topics for this book that will provide the reader not only with a broad overview of current hot topics in materials chemistry, but also with specific examples of the special properties of these materials and some particular applications of interest.

Nanoparticles

Nanoparticles may be defined as ultra dispersed solid supramolecular structures, generally (but not necessarily) made of polymers and displaying a sub-micrometer size, preferably smaller than 500 nm [4]. Polymers used in controlled drug delivery, including nanoparticles, may be classified as either (*i*) synthetic and natural, or (*ii*) biodegradable and nonbiodegradable. Synthetic biodegradable polymers used to prepare nanoparticles include: poly lactide-co-glycolide (PLGA), poly-ε-caprolactone, polylactic acid (PLA), Polyglycolic acid (PGA), polyanhydrides, and polyphosphazene. Synthetic nonbiodegradable polymers used in drug delivery include polymethyl methacrylate. Naturally occurring biodegradable and biocompatible polymers include: chitosan, gelatin, alginate, cellulose, pullulan, and gliadin [5].

Synthesis

Methods used in synthesis of nanoparticles can be divided into two groups (*i*) those based on polymerization (*ii*) those taking advantage of preformed polymers. The choice of the method for the preparation of nanoparticulate formulation depends upon various factors including (a) size of nanoparticles required (b) inherent properties of drug, e.g., aqueous solubility and stability (c) surface characteristics such as charge and permeability (d) degree of biodegradability, biocompatibility and toxicity (e) drug release profile desired (f) Antigenicity of the final product [6].

Solvent Evaporation

This method can be used for preparation of particles with sizes varying from a few nanometers to micrometers by controlling the stirring rates and conditions, showing high efficiency in incorporation of lipophillic drugs [6]. Polymer solution is prepared in volatile solvents and emulsion is formulated (either oil in water or water in oil in water). Earlier dichloromethane and chloroform preformed polymer were widely used, which is now replaced with ethyl acetate, having better toxicological profile. High speed homogenization or ultrasonication are utilized to reduce the particle size followed by evaporation of

the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent. Afterwards, the solidified nanoparticles can be collected by ultracentrifugation and washed with distilled water to remove additives such as surfactants. Finally, the product is lyophilized. The Schematic representation of solvent evaporation technique is shown in Figure 2.1 [7, 8].

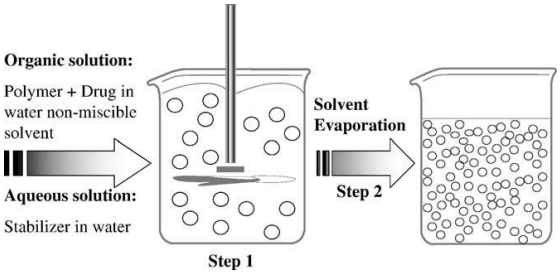

FIGURE 2.1
Solvent Evaporation technique [8].

Emulsification /solvent diffusion method

This is a modified version of solvent evaporation method. The polymer is dissolved in a partially water soluble solvent such as propylene carbonate and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. To produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent by diluting with excess of water or other organic solvent. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanoparticles. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point [8].
Emulsification /solvent diffusion method is efficient in encapsulating lipophilic drugs.
Several drug-loaded nanoparticles were produced by the ESD technique, including mesotetra(hydroxyphenyl)porphyrin-loaded PLGA (p-THPP) nanoparticles, doxorubicin-loaded PLGA nanoparticles, plasmid DNA-loaded PLA nanoparticles, coumarin-loaded PLA nanoparticles, indocyanine, cyclosporine (Cy-A)-loaded gelatin and cyclosporin (Cy-A)-loaded sodium glycolate nanoparticles [5, 9-15].

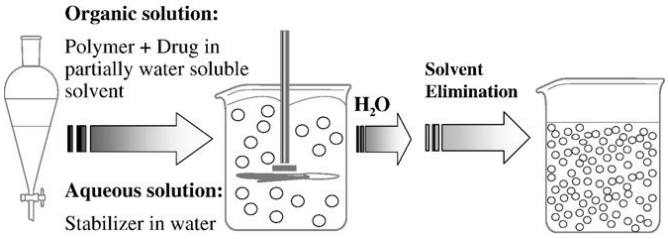

FIGURE 2.2
Emulsification /solvent diffusion method [8].

Salting Out

Salting out is based on the separation of a water miscible solvent from aqueous solution via a salting out effect. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes such as magnesium chloride, calcium chloride and magnesium acetate or non- electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of water to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. Both the solvent and the salting out agent are then eliminated by cross-flow filtration [8].

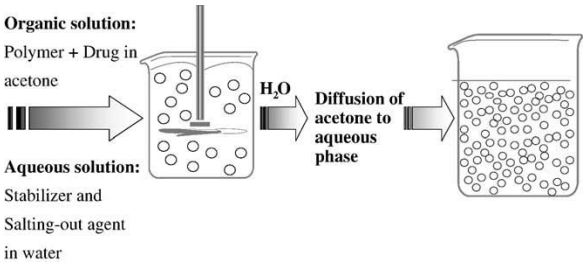

FIGURE 2.3
Salting out technique [8].

Solvent Displacement/ Nanoprecipitation

Nanoprecipitation is also called solvent displacement method. It involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant [16-19].
The polymer is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanoparticles. Acetone, dichloromethane are used to dissolve and increase the entrapment of drugs. The dichloromethane increases the mean particle size [20]. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. When both phases are in contact the solvent diffuses from the organic phase into the water and carries with it some polymer chains which are still in solution. As the solvent diffuses further into the water the associated polymer chains aggregate forming nanoparticles. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension [6, 18].
This method is basically applicable to lipophilic drugs because of the miscibility of the solvent with the aqueous phase, and it is not an efficient means to encapsulate water-soluble drugs. It has been applied to various polymeric materials such as PLGA, PLA, PCL, and poly (methyl vinyl ether-comaleic anhydride) (PVM/MA) [17, 21-24]. Nanoprecipitation is well adapted for the incorporation of cyclosporin A, because entrapment efficiencies as high as 98% were obtained [25].

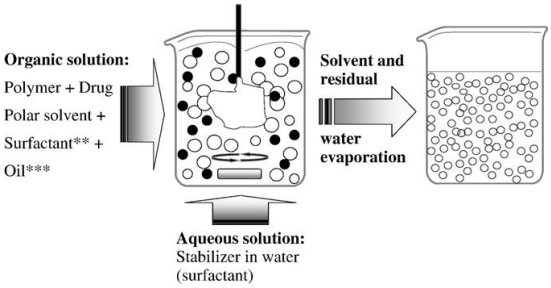

FIGURE 2.4
Solvent Displacement [8].

Emulsion-Diffusion-Evaporation

This method incorporates both evaporation and diffusion process in nanoparticles formation. Polymer is dissolved in a volatile, slightly miscible organic solvent, like ethyl acetate, which is added to the aqueous phase under continuous stirring. The resulting emulsion is slowly diluted by sufficient water under continuous stirring resulting in nanoparticle formation. The basic methodology involves the dispersion of organic phase as globules in equilibrium with external aqueous phase due to continuous stirring. The emulsion is stabilized by adsorption of stabilizer at the interface. The globule size is further lowered by homogenization. Addition of water destabilizes the equilibrium and diffusion of organic solvent to aqueous phase causes local super-saturation near the interface resulting in nanoparticles formation. The organic phase is removed from the preparation by evaporation at 400˚C [26].

Spray Drying

In spray drying technique polymer solution is obtained by dissolving polymer and drug in dilute acetic acid at room temperature. The polymer solution is then added to the aqueous medium containing cross linking agent with magnetic stirring at room temperature. The resulting colloidal solution was stirred for 30 minutes before spray-dried at a feed rate of 6.0 ml/min. The spray-drying conditions were inlet temperature 128–132°C, outlet temperature 68–71°C, aspirator 90% and pump feed 20% [27]. The nature of solvent used, temperature of the solvent evaporation and feed rate affects the morphology of the microspheres. The main disadvantage of this process is the adhesion of the microparticles to the inner walls of the spray-dryer [28-30].

Materials

Poly (Lactide-Co-Glycolide) (PLGA)

PLGA, copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA), is widely used for DDS development because of its biodegradability, biocompatibility and ease of processing [31]. It is the best defined biomaterial available for drug delivery with respect to design and performance. Poly lactic acid contains an asymmetric carbon which is typically described as the D or L form in classical stereochemical terms and sometimes as R and S form, respectively. PLGA is generally an acronym for poly D,L-lactic-co-glycolic acid where D- and L- lactic acid forms are in equal ratio [32].

PLGA, which is hydrophobic in nature [32], can be processed into almost any shape/size, and can encapsulate molecules of virtually any size. It is soluble in wide range of common solvents including chlorinated solvents, tetrahydofuran, acetone and ethyl acetate [33, 34]. Crystalline PGA, when co-polymerized with PLA, reduces the degree of crystallinity of PLGA hence a higher content of PGA leads to quicker rates of degradation with an exception of 50:50 ratio of PLA/PGA, which exhibits the fastest degradation. Properties of PLGA like glass transition temperature (Tg), moisture content and molecular weight, changes during polymer biodegradation and has influences on the release and degradation rates of incorporated drug molecules. Properties like molecular weight and polydispersity index also affect the ability to be formulated as a drug delivery device and may control the device degradation rate and hydrolysis [32].
Sustained intracellular retention suggest that nanoparticles containing encapsulated plasmid DNA could serve as an efficient sustained release gene delivery system [35]. Therapeutic proteins and peptides can be encapsulated into nanoparticles using double emulsion solvent evaporation techniques. Adjuvant properties of PLGA nanoparticles containing encapsulated vaccines and drug have been extensively studied [36].

Polymethyl methacrylate (PMMA)

PMMA is a non-biodegradable synthetic homopolymer of methylmethacrylate monomer (MMA). It is classified as a hard, rigid but brittle material with a glass transition temperature of 105°C [37]. PMMA is rather hydrophobic but becomes slightly more hydrophilic after contact with water. The best organic solvents for PMMA are partly substituted hydrocarbons as trichloroethylene. At present, it is generally accepted that PMMA is a non-toxic polymer as it possesses a very good toxicological safety record in biomedical applications [38].
PMMA used as the carrier for daptomycin, non-steroidal anti-inflammatory drugs (NSAID) like indomethacin, tolmetin and mefenamic acid, antineoplastic and antiresorptive agents as methotrexate, doxorubicin and pamidronate and anti-fungal drugs as amphotericin B [39-43].

Poly-ϵ-Caprolactones (PCL)

Poly (ϵ-caprolactone) (PCL) is biodegradable industrial polyester with excellent mechanical strength, non-toxicity, and biocompatibility. It has been frequently used as implantable carriers for drug delivery systems or as surgical repair materials. It is hopeful to combine chitosan with the biodegradable polyester to create amphiphilic copolymer applicable to drug delivery systems.
Dextran-PCLn was prepared by coupling between carboxylic function present on preformed PCL monocarboxylic acid and the hydroxyl groups on dextran [44, 45]. The modification of the surface with dextran significantly reduced the cytotoxicity [46].
Poly-ϵ-caprolactone nanoparticles have been used as vehicles to deliver a wide range of drugs including tamoxifen, retinoic acid, and griseofulvin [47]. Bovine serum albumin and lectin were incorporated in the nanoparticles. Lectins could also be adsorbed onto the surface of the nanoparticles. Surface-bound lectin conserved its hemagglutinating activity, suggesting the possible application of this type of surface-modified nanoparticles for targeted oral administration [48].

Poly glycolic acid (PGA)

PGA is biocompatible and has been known since 1954 to be a potentially low-cost tough fibre forming polymer. PGA is the simplest aliphatic polyester. It has a glass transition temperature between 35–40˚*C*

and melting point ranging from 224– 227˚ C. Because of its simple chemical structure and stereoregularity, it occurs with different degree of crystallinity from completely amorphous to a maximum of 52% crystallinity. The crystallinity of PGA in Dexon Suture is typically in the range of 46–52% and it tend to lose mechanical strength rapidly, typically over a period of 2–4 weaks after implantation [49].

Poly Lactic Acid (PLA)

PLA is a synthetic, bioabsorbable, non-toxic and biodegradabile polymer [50]. PLA is chiral in nature, the chirality is seen in the carbon with four different substituents (hydrogen, oxygen, carbonyl, and methyl), and it is this that causes two different PLA polymers – PDLA and PLLA. PLLA has a crystallinity of 37%, a glass transition temperature between 50 and 80°C, and a melting temperature of 173-178° C. A polymerization of the racemic mixture produces PDLLA, which, due to the interference of stereochemistry in the chain alignment, is amorphous [51].

Chitosan

Chitason is a natural polymer obtained by deacetylation of chitin, a component of crab shells. It is a cationic polysaccharide composed of linear β (1,4)-linked d-glucosamine [52]. Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi [53-55]. Chitin is highly basic polysaccharides due to presence of primary amino group in its structure.
The main factors which may affect the chitason properties are its molecular weight and degree of deacetylation. The molecular weight of the chitason depends on viscosity, solubility, elasticity and tears strength. In alkaline or neutral medium, free amino group of chitosan is not protonated and therefore it is insoluble in water, while in acidic pH, it gets solubilized due to protonation of free amino groups and the resultant soluble polysaccharide is positively charged. Chitosan forms water-soluble salts with inorganic and organic acids includes glyoxylate, pyruvate, tartarate, malate, malonate, citrate, acetate, lactate, glycolate, ascorbate [56].
Chitosan used as carrier material for various drugs by numerous mechanisms including chemical cross-linking, ionic cross-linking, and ionic complexation [57]. Chitosan also used as a carrier for antibodies [58].

Alginates

Alginate is a water-soluble linear, polyanionic, polysaccharide extracted from brown seaweed and is composed of alternating blocks of 1–4 linked α-L-guluronic and β-D-mannuronic acid residues [59]. Alginate exhibits a pH-dependent anionic nature and has the ability to interact with cationic polyelectrolytes and proteoglycans [60]. In aqueous media, the sodium ions from salts of this anionic polymer exchange with divalent cations, such as calcium, to form water-insoluble gels [5]. Therefore, delivery systems for cationic drugs and molecules can be obtained through simple electrostatic interactions.
The molecular weight (MW) of alginate influences the degradation rate and mechanical properties of alginate-based biomaterials. Basically, higher MW decreases the number of reactive positions available for hydrolysis degradation, which further facilitates a slower degradation rate [60].
Alginates are ideal carriers for oligonucleotides, peptides, proteins, water-soluble drugs, or drugs that degrade in organic solvents [5].

Gelatin

Gelatin is a natural, biodegradable protein obtained by acid- or base-catalyzed hydrolysis of collagen. It is a heterogenous mixture of single- or multi-stranded polypeptides composed predominantly of glycine, proline, and hydroxyproline residues and is degraded in vivo to amino acids. Gelatin is a polyampholyte having both cationic and anionic groups along with hydrophobic group [61]. PEGylation of the particles significantly enhances their circulation time in the blood stream and increases their uptake into cells by endocytosis [62].
Gelatin nanoparticles have been used to deliver paclitaxel, methotrexate, doxorubicin, DNA, double-stranded oligonucleotides, and genes [62]. Antibody-modified gelatin nanoparticles have been used for targeted uptake by lymphocytes [63].

Nanocapsules

Nanocapsules are defined as nano-vesicular systems that exhibit a typical core-shell structure in which the drug is confined to a reservoir or within a cavity surrounded by a polymer membrane or coating. The cavity can contain the active substance in liquid or solid form or as a molecular dispersion. Likewise, this reservoir can be lipophilic or hydrophobic according to the preparation method and raw materials used. Nanocapsules can also carry the active substance on their surfaces or imbibed in the polymeric membrane.

Synthesis

Generally, there are five classical methods for the preparation of nanocapsules: nanoprecipitation, emulsion–diffusion, double emulsification, emulsion-coacervation and layer by layer.

Nanoprecipitation method

Nanocapsule synthesis needs both solvent and non-solvent phases. The solvent phase (usually organic phase) essentially consisting of a solution in a solvent or in a mixture of solvents (i.e. ethanol, acetone, hexane, methylene chloride or dioxane) of a film-forming substance such as a polymer (synthetic, semi-synthetic or naturally occurring polymer), the active substance, oil, a lipophilic tensioactive and an active substance solvent. On the other hand, the non-solvent phase (usually aqueous phase) consisting of a non-solvent or a mixture of non-solvents for the film-forming substance, supplemented with one or more naturally occurring or synthetic surfactants.
In the nanoprecipitation method, the polymer is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. The process of particle formation in the nanoprecipitation method comprises three stages: nucleation, growth and aggregation. The rate of each step determines the particle size and the driving force of these phenomena is supersaturation. The separation between the nucleation and the growth stages is the key factor for uniform particle formation. The key variables of the procedure are those associated with the conditions of adding the organic phase to the aqueous phase, such as organic phase injection rate, aqueous phase agitation rate, the method of organic phase addition and the organic phase/aqueous phase ratio.

The polymers commonly used are biodegradable polyesters, especially poly-e-caprolactone (PCL), poly(lactide) (PLA) and poly(lactide-co-glicolide) (PLGA). Synthetic polymers have higher purity and better reproducibility than natural polymers.

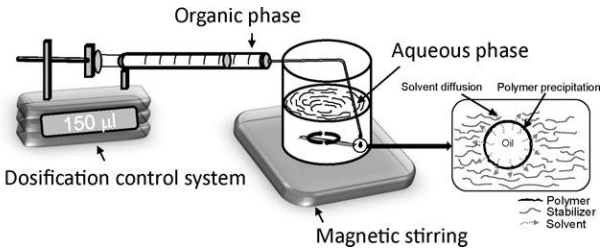

FIGURE 2.5
Nanoprecipitation.

Emulsion–diffusion method

Preparation of nanocapsules by the emulsion–diffusion method allows both lipophilic and hydrophilic active substance nanoencapsulation. The experimental procedure performed to achieve this requires three phases: organic, aqueous and dilution. The organic phase contains the polymer, the active substance, oil and an organic solvent (partially miscible with water). The aqueous phase comprises the aqueous dispersion of a stabilizing agent. Dilution phase is usually water.

For preparation of nanocapsules using the emulsion–diffusion method, the organic phase is emulsified under vigorous agitation in the aqueous phase. The subsequent addition of water to the system causes the diffusion of the solvent into the aqueous phase, resulting in nanocapsule formation. This can be eliminated by distillation or cross-flow filtration depending on the boiling point of the solvent.

The nanocapsule formation mechanism is based on the theory that each emulsion droplet produces several nanocapsules and that these are formed by the combination of polymer precipitation and interfacial phenomena during solvent diffusion. Consequently, solvent diffusion from the globules carries molecules into the aqueous phase forming local regions of supersaturation from which new globules or polymer aggregates are formed and stabilized by the stabilizing agent, which prevents their coalescence and the formation of agglomerates. If the stabilizer remains at the liquid–liquid interface during the diffusion process and if its protective effect is adequate, the nanocapsules will be formed after the complete diffusion of the solvent. The nanocapsule size is related to the shear rate used in the emulsification process, chemical composition of the organic phase, polymer concentration, oil-to-polymer ratio and the drop size of the primary emulsion.

The polymers commonly used are biodegradable polyesters, especially PCL, PLA and eudragit. Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBHV) may also be used. Ethyl acetate is the first option as a solvent though propylene carbonate, benzyl alcohol and dichloromethane can also be used. In regarding to the aqueous phase, the solvent used is water and poly(vinyl alcohol) (PVA) is preferred as the stabilizing agent. Other stabilizing agents such as poloxamer and ionic emulsifiers have been used. The dilution phase is often water.

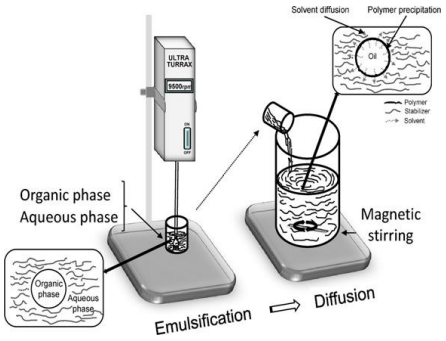

FIGURE 2.6
Emulsion–diffusion method.

Double emulsification method

Double emulsions are complex heterodisperse systems called "emulsions of emulsions", that can be classified into two major types: water-oil-water emulsion (w/o/w) and oil-water-oil emulsion (o/w/o). Double emulsions are usually prepared in a two step emulsification process using two surfactants: a hydrophobic one designed to stabilize the interface of the w/o internal emulsion and a hydrophilic one to stabilize the external interface of the oil globules for w/o/w emulsions.

In the primary w/o emulsion the oil is changed by an organic phase containing a solvent that is totally or partially miscible in water, film-forming polymer and a w/o surfactant. Then the water containing a stabilizing agent is added to the system to obtain the water in organic in water emulsion.

For the preparation of nanocapsules by double emulsification, the primary emulsion is formed by ultrasound and the w/o surfactant stabilizes the interface of the w/o internal emulsion. The second emulsion is also formed by ultrasound and nanocapsule dispersion is stabilized by the addition of the stabilizing agent. Finally, the solvents are removed by evaporation or extraction by vacuum, leaving hardened nanocapsules in an aqueous medium.

In the organic phase ethyl acetate, methylene chloride and dichloromethane have been used as solvents. Biodegradable polyesters such as PCL, PLA and PLGA have been frequently used. Sorbitan esters are preferred as o/w surfactants. PVA and polysorbates are used as stabilizing agents in external aqueous phase.

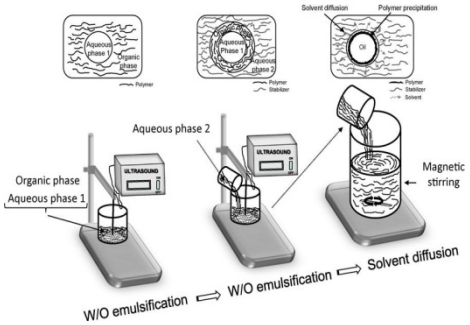

FIGURE 2.7
Double emulsification method.

Emulsion-coacervation method

The emulsion-coacervation process is mainly presented as a strategy for nanocapsules preparation from naturally occurring polymeric materials. Up to now, sodium alginate and gelatin have been used though synthetic polymeric materials could be used for this purpose.
The procedure involves the o/w emulsification of an organic phase (oil, active substance and active substance solvent if necessary) with an aqueous phase (water, polymer, stabilizing agent) by mechanical stirring or ultrasound. Then, a simple coacervation process is performed by using either electrolytes (sodium alginate–calcium chloride system) with the addition of a water miscible non-solvent or a dehydration agent with a gelatin–isopropanol–sodium sulfate system or by temperature modification with the application of triblock terpolymer in gold nanocapsule synthesis. Finally the coacervation process is complemented with additional crosslinked steps that make it possible to obtain a rigid nanocapsule shell structure.
Nanocapsule formation by the emulsion-coacervation method uses the emulsion as a template phase and the formation of a coacervate phase that causes polymer precipitation from the continuous emulsion-phase to form a film on the template forming the nanocapsule. Additionally, it can be stabilized by physical intermolecular or covalent cross-linking, which typically can be achieved by altering pH or temperature, or by adding a cross-linking agent.

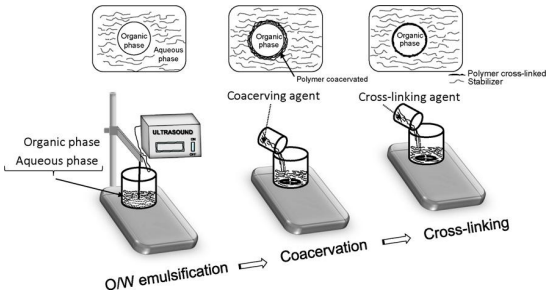

FIGURE 2.8
Emulsion-coacervation method.

Layer-by-layer method

The layer-by-layer assembly process developed for colloidal particle preparation makes it possible to obtain vesicular particles, called polyelectrolyte capsules, with well-defined chemical and structural properties. The layer by layer technique is based on alternate adsorption of oppositely charged materials, mostly linear polyelectrolytes, via electrostatic interactions. Multilayer ultrathin films can be developed with "molecular architecture" design with precise control of thickness and molecular composition

The mechanism of nanocapsule formation is based on irreversible electrostatic attraction that leads to polyelectrolyte adsorption at supersaturating bulk polyelectrolyte concentrations. This method requires a colloidal template onto which is adsorbed a polymer layer either by incubation in the polymer solution, subsequently washed, or by decreasing polymer solubility by drop-wise addition of a miscible solvent. This procedure is then repeated with a second polymer and multiple polymer layers are deposited sequentially. The solid form of the active substances, biological cells, compact forms of DNA, protein aggregates and gel beads can be used as a template.

The polycations used in layer-by-layer method are polylysine, chitosan, gelatin B, poly(allylamine) (PAA), poly(ethyleneimine) (PEI), aminidextran and protamine sulfate. The polyanions are poly(styrene sulfonate) (PSS), sodium alginate, poly(acrylic acid), dextran sulfate, carboxymethyl cellulose, hyaluronic acid, gelatin A, chondroitin and heparin [64, 3].

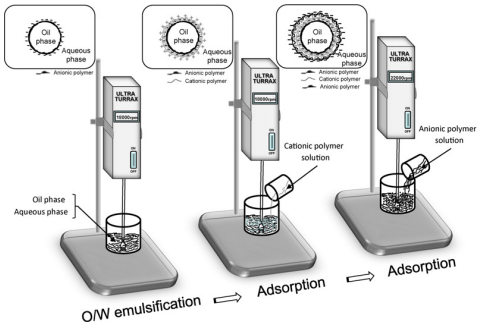

FIGURE 2.9
Layer-by-layer method.

Materials

The polymers commonly used are poly-e-caprolactone (PCL), poly(lactide) (PLA), poly (lactide-co-glicolide) (PLGA), poly(alkyl cyanoacrylate) (PACA) and Eudragit [64]. PCL, PLA and PLGA are discussed earlier in this chapter.

Poly alkyl cyanoacrylate (PACA)

Alkyl cyanoacrylate monomers are highly reactive and polymerized via anionic, zwitterionic or radical mechanism in suitable polymerization medium to form various types of nanocarriers - nanospheres, core-shell nanoparticles (with covalently attached hydrophilic polymers on the surface), nanocapsules (with oily or aqueous core), hybrid nanoparticles with magnetic core etc [65].

Nanoparticles of PACA homopolymers have relatively hydrophobic surfaces and adsorb larger amounts of proteins [65]. The PEGylation concept, either via a simple adsorption of PEG chains onto the

nanoparticles or by a covalent linkage of PEG chains with PACA polymers, allows different types of hydrophilic molecules to anchor on to the surface of PACA nanoparticles [66].
Different types of PACA-based nanocarriers incorporate a great variety of drugs, such as cytostatics, antibiotics, antiviral agents, anti-fungal drugs, non-steroidal anti-inflammatory drugs etc [65].

Eudragit

Eudragit is a trade name of Poly(meth)acrylates prepared by the polymerization of acrylic and methacrylic acids or their esters, e.g., butyl ester or dimethylaminoethyl ester [67]. Eudragit polymers are available in a wide range of different physical forms (aqueous dispersion, organic solution granules and powders). The flexibility to combine the different polymers enables to achieve the desired drug release profile by releasing the drug at the right place and at the right time and, if necessary, over a desired period of time [68].
Eudragit has a glass transition temperature 48˚C. It is soluble in gastric fluid to pH=5 [67]. Eudragit L and S polymers are preferred choice of coating polymers. They enable targeting specific areas of the intestine [68].
Eudragit used in delivery of drugs like Ibuprofen, Acetaminophen, Morphine HCl, Roxithromycin, Nizatidine, Cetraxate HCl, Ciprofloxacin, Ibuprofen, Bifemelane HCl etc [67].

Fullerenes

Fullerenes are closedcage carbon molecules with three-coordinate carbon atoms tiling the spherical or nearly-spherical surfaces, the best known example being C60, with a truncated icosahedral structure formed by twelve pentagonal rings and twenty hexagonal rings. Subsequent studies have shown that fullerenes actually represent a family of related structures containing 20, 40, 60, 70, or 84 carbons.

A key attribute of the fullerene molecules is their numerous points of attachment, allowing for precise grafting of active chemical groups in 3D orientations. This attribute, the hallmark of rational drug design, allows for positional control in matching fullerene compounds to biological targets [69].

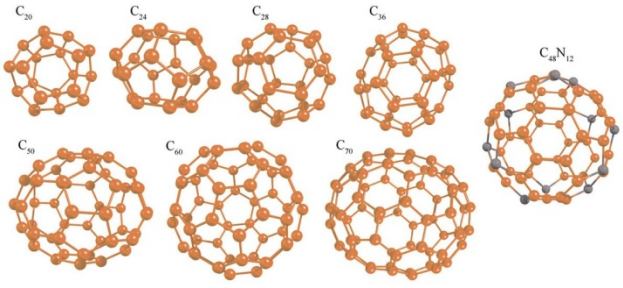

FIGURE 2.10
Structure of fullerenes [70].

Synthesis

Two high purity graphite rods are clamped to the high current feedthrougs. The chamber is then pumped down to ≤ 10^{-3} torr and refilled with He gas to a pressure of 150-250 torr. Because both oxygen and water significantly inhibit the formation of fullerenes, it is important to evacuate the chamber carefully and refill it using purified helium. The electrides are positioned so that the carbon rods are just touching, and then the vaporization is initiated by passing a high current through the rods. For 6.25mm diameter rods, current between 100-200A leads to efficient fullerene formation. Under these conditions, the 6.25mm rods are consumed at a rate of about 5-10 mm/min. The crude carbon product or soot produced by this vaporization collect on the water cooled inner surface of the fullerene apparatus and is readily removed from the walls and collected using a stiff brush. This soot contains a variety of carbon products including C_{60} and larger fullerenes [71].

Properties

The diameter of a C60 molecule is about 7 Å. The C60 molecule, also termed as 'buckminsterfullerene' and 'buckyball' has two bond lengths. The 6:6 ring bonds (between two hexagons) can be considered 'double bonds' and are shorter than the 6:5 bonds (between a hexagon and a pentagon). The carbon atoms in fullerene are in sp2 and sp3 hybridized state. The free electrons on the cage build a strong localized p-electron system. This electron system influences the chemical reactions of the fullerenes. In chemical reactions, these molecules do not exhibit aromatic behavior. Instead, they show aliphatic behavior.
Fullerenes are insoluble in water. However, they are soluble in other solvents like carbon disulphide, toluene and o-dichlorobenzene. Solutions of pure C60 have a deep purple color [72]. The solubility in a solvent generally increases with increasing molecular weight of the solvent [73].

Applications

Fullerenes have potential applications in the treatment of diseases where oxidative stress plays a role in the pathogenesis. These include Degenerative diseases of the CNS including PD, AD, and amyotrophic lateral sclerosis, Multiple sclerosis, Ischemic cardiovascular diseases, Atherosclerosis, Major long-term complications of diabetes, Sun-induced skin damage and physical manifestations of aging etc [69].
Fullerenes are used in treatment of cancer cells, the surface of fullerenes can be 'decorated' with chemotherapeutic agents. An antibody is attached, which serves as a guidance system. metallo fullerenes are being investigated for loading radioactive atoms and then firing them like guided missiles at diseased cells [72].

Nanotubes

The Major Colloidal drug delivery system includes liposome and polymeric nanoparticles [74]. The increase in therapeutic range of the targeted delivery with the help of nanoparticles helped in decreasing the toxicity and side effects [75]. Carbon nanotubes have become the most popular candidates in the field of biomedical engineering, biotechnology, defense research and pharmaceutical

industry after their discovery in 1991 [76]. The introduction of DNA, proteins or drug molecules into the living cells is important to therapeutics. Nanotubes have advanced physical and chemical properties which make them highly promising for biological applications [77].

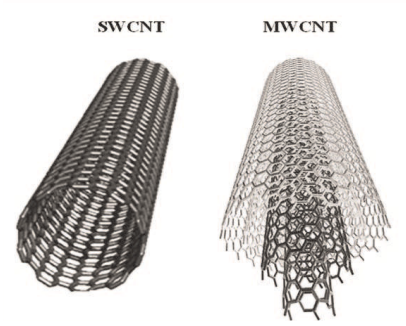

FIGURE 2.11
Single-wall and Multi-wall Carbon Nanotubes [78].

Synthesis

Among the various nanomaterials being currently developed carbon nanotubes (CNTs) have attracted considerable interest due to their great properties and potential benefits in many industrial applications (from materials engineering and electronics to medical devices and drug delivery systems). [79].

Arc-vaporization

The arc vaporization technique generally involves the use of two high-purity graphite electrodes. The anode is either pure graphite or contains metals and cathode is made of metals, mixed with the graphite powder. Cathod is introduced in a hole made in the anode center. The electrodes are momentarily brought into contact and an arc is struck. The synthesis is carried out at low pressure (30-130 torr or 500 torr) in controlled atmosphere composed of inert and/or reactant gas. The distance between the electrodes is reduced until the flowing of a current (50–150 A). The temperature in the inter-electrode zone is so high (>3000°C) that carbon sublimes from the positive electrode (anode) that is consumed. A constant gap (1mm) between the anode and cathode is maintained by adjusting the position of the anode. Plasma is formed between the electrodes which can be stabilized for a long reaction time by controlling the distance between the electrodes by means of the voltage (25–40 V) control. The reaction time varies from 30–60 seconds to 2–10 minutes [80].

Laser ablation

In the laser ablation technique, a high power laser was used to vaporize carbon from a graphite target at high temperature. In order to generate nanotubes, metal particles as catalysts must be added to the graphite targets similar to the arc discharge technique. The quantity and quality of produced carbon nanotubes depend on several factors such as the amount and type of catalysts, laser power and wavelength, temperature, pressure, type of inert gas, and the fluid dynamics near the carbon target. The laser beam (532 nm) is focused onto a carbon targets containing 1.2 % of cobalt/nickel with 98.8 % of graphite composite, placed in a 1200°C quartz tube furnace under the argon atmosphere (~500

Torr). The laser beam scans across the target surface under computer control to maintain a smooth, uniform face for vaporization. The soot produced by the laser vaporization was swept by the flowing Ar gas from the high-temperature zone, and deposited onto a water-cooled copper collector positioned downstream just outside the furnace. The nanotubes will self-assemble from carbon vapors and condense on the walls of the flow tube. The diameter distribution of SWNTs from this method varies about 1.0 - 1.6 nm. Carbon nanotubes produced by laser ablation were purer (up to 90 % purity)**.**
The targets were uniformly mixed composite rods made by the following three-step procedure: (i) a paste produced from mixing high-purity metal or metal-oxide with graphite powder and carbon cement at room temperature was placed in a mold; (ii) the mold was placed in a hydraulic press equipped with heating plates and baked at 130°C for 4–5 h under constant pressure (iii) the baked rod was then cured at 810°C for 8 h under Ar flow. Fresh targets were heated at 1200°C under flowing Ar for 12 h. Subsequent runs with the same target proceeded after two additional hours heating at 1200 °C. The following metals were used: Co, Cu, Nb, Ni, Pt, Co/Ni, Co/Pt, Co/Cu, Ni/Pt [80-82].

Chemical Vapor Deposition

The process involves passing a hydrocarbon vapor (typically for 15-60 minutes) through a tube furnace in which a catalyst material is present at sufficiently high temperature (600-1200°C) to decompose the hydrocarbon. CNTs grow over the catalyst and are collected upon cooling the system to room temperature. For liquid hydrocarbon (benzene, alcohol, *etc*.), the liquid is heated in a flask and an inert gas purged through it to carry the vapor into the reaction furnace. The vaporization of a solid hydrocarbon (camphor, naphthalene, *etc*.) can be conveniently achieved in another furnace at low temperature before the main, high temperature reaction furnace. The catalyst material may also be solid, liquid, or gas and can be placed inside the furnace or fed in from outside. Pyrolysis of the catalyst vapor at a suitable temperature liberates metal nanoparticles [83].

Applications

- Single-walled carbon nanotubes are molecular transporters or carriers with very high optical absorbance in the where biological systems are transparent. This intrinsic property stems from the electronic band structures of nanotubes and is unique among transporters.
- Carbon nanotube (CNT) membranes present the opportunity to create a transdermal patch that can vary its rate of delivery throughout its application to the skin to attain therapeutic plasma levels and plasma profiles of a specific drug [84].
- Cisplatin is a platinum based anticancer drug which is used to treat a wide range of tumors, despite its adverse side effects. It is expected that this form of targeted nanoscale drug delivery will significantly reduce these adverse side effects. The most ideal delivery capsule in terms of minimizing the amount of material required for encapsulation, thus providing the least toxicity. This technique, used to represent the encapsulation of cisplatin entering carbon, boron nitride, boron carbide and silicon nanotubes, can be extended to any number of drug molecules or alternative nanotube materials [85].

Lipid Based Carriers

Encapsulating drugs began in 1970`s with different level of success with vesicles such as liposome that a entrap a solvent core and separate it from the surrounding. Depending on the method of

preparation, lipid vesicles can be multi-, oligo- or unilamellar, containing many, a few, or one bilayer shell(s) respectively. The diameter of the lipid vesicles may vary between about 20 nm and a few hundred micrometers. Small unilamellar vesicles (SUVs) are surrounded by single lipid layer (25–50 nm), whereas several lipid layers separated by intermittent aqueous layer surround large unilamellar vesicles (LUV) (100–200 μm). Giant unilamellar vesicles (GUV) have a mean diameter of 1–2 μm, multilamellar vesicles (MLV) have a mean diameter between 1 μm and 2 μm (10 layers). Multivesicular vesicles are liposomes with lots of vesicles inside [86]. Vesicles are composed of various lipids such as phospatidylcholines, phosphotidylglycerols, and cholesterols. These lipids aggregates, fuses and releases their contents [87]. Lipid based drug delivery system has the advantage of being modified according to the requirement by adjusting the content of different lipid excipients and additives. Increase interest in the lipid base system is due to the versatility of the lipidic excipients, formulation versatility, enhanced permeation capacity, better characterization [88]. The important parameters that defines the oral drug delivery is solubilization of the drug and absorption, drugs which have poor solubility can be overcome using lipid based systems and due to the lipidic nature the absorption can be increased [89].

Type of Lipid Carrier Materials

There are many type of lipid carrier materials few of them includes:

a) **Lipophilic liquid:** Drugs like steroids have good solubility in triacylgylcerols, therefore such drugs can be encapsulated in for delivery. The drawback of this type system is that it limits the use of complex formulation.

b) **Micro-emulsifying systems:** Micro-emulsion systems are essentially surfactant micelles with oil and drug.

c) **Liposomes:** They are spherical bilayered structures consisting of a fatty acid component. the hydrophilic components are entrapped in the internal spaces of the system. They have to ability to penetrate through and delivery the drug, that is the reason they are popular among the lipid based system.

d) **Modified Lipoproteins:** The use of apoprotiens and recognition markers can be helpful in modifying the lipoprotein by using LDL and HDL [90].

e) **Solid Liquid Nanoparticles:** Developed in 1990s produced by replacing the liquid from the emulsion with a solid lipid or blended solid lipid. These are the stable nano-lipid carriers in which the drug is either dissolved or displaced.

f) **Nano-structured lipids:** They are produced by blending solid and liquid lipids. The use of this system is to improve the poor loading of the drug while preserving controlled release features [91].

g) **Lipospheres:** First reported by Domb as water dispersible solid micro-particles composing of solid hydrophobic fat core stabilized by a monolayer of phospholipid molecule embedded in a microparticle surface. The core contains the active ingredient [92].

Synthesis

Melt dispersion technique

In this method, drug is dissolved or dispersed in the molten lipidic phase. Aqueous phase is composed of water or suitable buffer which is heated to the same temperature as lipid phase. The aqueous phase is kept under stirring during which emulsifier is added. To the aqueous phase containing emulsifier, lipid phase containing drug is added drop wise while maintaining the temperature and stirring speed. The temperature of the mixture is rapidly brought down to room temperature or below room temperature by adding ice cold water or ice under continuous stirring. This cold resolidification results in the formation of discrete lipospheres which can be filtered.
Several drugs like bupivacaine, glipizide, aceclofenac, retinyl acetate, progesterone, sodium cromglycate, diclofenac, carbamazepine, C14-diazepam, proteins like somatostatin, thymocartin, casein, bovine serum albumin, R32NS1 malaria antigen, tripalmitin based lipospheres for labon-chip applications have been prepared by melt dispersion methods.
Lipids carrying antigens exert their adjuvant effect to immunogenicity of antigens and the effect was found to decrease in the following order for the lipids studied: ethyl stearate> olive oil> tristearin> tricaprin> corn oil> stearic acid. Also inclusion of negatively charged lipids like dimyristoyl phosphotidyglycerol in the lipid core was found to improve the antibody response to encapsulated malaria antigen [93].

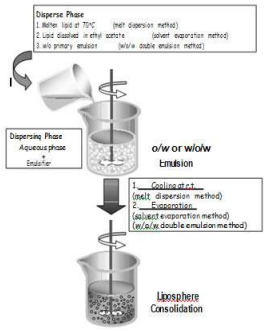

FIGURE 2.12
Melt dispersion technique.

Detergent removal method

Detergents can be defined as a subgroup of surfactants that are able to solubilize lipid membranes. Sufficient amount of detergents lead to the reorganization of lipid bilayers to form smaller, soluble detergent–lipid aggregates of various shapes.
The lipids and lipophilic substances, to be incorporated into the liposomal membrane, are dissolved together with the detergent in an organic solvent or solvent mixture to obtain a clear solution. In most cases methanol, ethanol, or mixtures with chloroform are used as solvent. The solvent is then removed in a rotary evaporator by reduced pressure at a moderate temperature. Residual solvent should be removed by high vacuum for at least 1hr. The dry film is normally clear when bile salts are used as detergent but with nonionic detergents the film will be turbid. A suitable buffer, optionally together with hydrophilic substances to be encapsulated, is added to yield the desired lipid concentration and

the temperature is adjusted. With octylglucoside, after adding the buffer, the dispersion may be opalescent for some seconds before clearing. A preformed liposome dispersion may be dissolved successively with detergent at the desired preparation temperature until a clear solution is achieved [94].

Reserves Phase Evaporation Method

In this method first water in oil emulsion is formed by brief sonication of a two phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. High encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength (0.01 M NaCl). This method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands [95].

Materials

Solid lipid Nanoparticles

Solid lipid Nanoparticles (SLN) are composed of a core solid lipid with bioactive material constituting a part of the lipid matrix. Such particles are stabilized by the surfactant layer. The term lipid indicates the use of trigylcerides, partial glycerides and fatty acids. An advantage of SLN is that they can reduce the risk of acute and chronic poisoning [96].

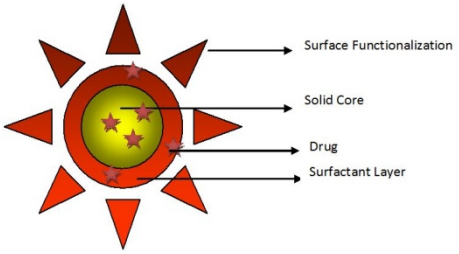

FIGURE 2.13
Solid lipid nanoparticles [97].

SLN permeation across blood brain barrier: Blood brain barrier penetration is one of the most difficult and crucial challenges in pharmaceutical research. Two anti cancer drugs namely camptothecin and doxorubicin when loaded with SLN resulted in drug accumulation into the brain after oral and IV route [98].

Transdermal application: The smallest particle size is observed with SLN, incorporation of SLN in gel like systems which is acceptable for direct application on the skin. SLN have also been to modulate the release of drug into the skin and to improve drug delivery to the particular skin layer [99].

Nanostructure Lipids

Solid nanostructure lipids had attracted attention as drug delivery system as an alternative carrier system to liposome, emulsion and polymeric nanoparticles due to exceptional stability, scaling up potential and biocompatible components. The application of NLC is enhanced by eliminating the use of organic solvents in the preparation stage and using high pressure homogenization technique [100].
Encapsulation of water soluble anticancer compounds: An approach to allow water soluble anticancer pharmaceuticals NLC have been very effective. These drugs can be encapsulated in the hydrophilic cavity of the NLC and outer coating of lipid gives good permeability for absorption.
NLC able to avoid reticuloendothelial scheme(RES): Number off cytotoxic drug which own undertaking to overcome the multi drug resistance phenotypes in units resistant to cytotoxic drug have been emerged. The use of NLC helps to overcome water solubility, drug release and clearing RES system [101].

Liposome

Physico-chemical properties: It readily disperse in aqueous media to reform the original colloidal dispersion. The typical range for lipid based formulation includes a particle size less than 1micro-meter. The solubility differs according to the type of the surfactant used and type of acidic or basic drug.

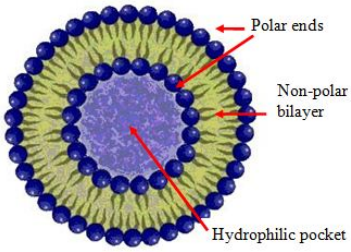

FIGURE 2.14
Liposomes [102].

Applications

- Delivery of nucleic acid and DNA: Liposome could be effective delivery system of Nucleic acid and DNA. Liposomal system with low surface area and small size using detergent dialysis procedure could exhibit the long term circulation of active ingredient. A recent advancement for manufacture of siRNA systems has been the application of microfluidic mixing and encapsulating siRNA with the control over the size has been achieved [103].
- Liposomal delivery as a mechanism to enhance synergism between anticancer drugs: Liposome can serve as a controlled release carrier these include clearance from reticuloendothelial system, longer systemic circulation, hepatic and spleen distribution. Drugs entrapped under liposome are not biologically active and must be released to gain access to the intracellular target [104].
- Breast cancer involving the chest wall: In a multimodality strategy, hyperthermia has been used to modulate delivery of liposomal drugs. Long circulation of liposomal accumulation with tumor tissue to be heated induces vascular permeability and microcirculatory dynamics which further facilitates liposome extraversion from tumor vessels [105].

- Application in ophthalmic drug delivery: Liposome can deliver ophthalmic drugs due to biodegradable and biocompatible in nature. Verteporfin is being clinically used in photodynamic therapy for treatment of subfoveal chorodial neovascularization, ocular histoplamosis or pathological myopia [106].
- Liposomes can be used to entrap anti-asthma drugs (salbutamol, beclometason) and in gastric ulceration [107, 108]

Nanogels

Nanogels are nanoscalar polymer network, with the tendency to imbibe water when placed in an aqueous environment. The advantage of nanogels over nanoparticles is the high degree of encapsulation [109]. Nanogels uses burst release system. They distinguish themselves from the bulk delivery system in that, they can be enter cells to delivers the drug. This property can be very helpful in cancer therapy, where the size of the delivery system is the key to target cancer through enhanced permeability and retention [110]. Nanogels can also be used for encapsulating cytoxic drug, due to the presence of polymeric network. The flexibility in the polymeric network has application in oligonucleotide binding [111].

Synthesis

Different Nanogels uses different methods for synthesis. Carboxymethyl chitosan nanogel was prepared by carboxymethylation in which the hydroxyl groups are substituted with alkyacid groups, the acid and amino groups help in chelation [112]. Similarly nanogels of pullulan was synthesized in which hydroxyethyl methyacrylate and vinyl methylacrylate is grafted on the glucose residue [113].

Noval pullulan chemistry modification

Synthesis of cholesterol based pullulan nanogel was done by reacting mixture of cholesterol isocynate in dimethyl sulfoxide and pyridine. Pullulan was substituted with 1.4 cholesterol moieties per 100 anhydrous glucoside units. The preparation was freeze dried and in aqueous phase it formed nanogel. It further modified with acrylate group and their thiol group was modified with polyethylene glycol by adopting Michael addition reaction, this allowed reduction in mesh size to 40 nm encapsulating 96% interleukin-12. These nanosystems have also been investigated by modifying cholesterol units by 1.1 units of cholesteryl group per 100 glucose units of parent pullulan, shown significant interaction with Aβ oligomer and monomer for alzhiemer's disease treatment by enhancing microglia and cortical cell viability.

Novel photochemical approach

Photochemical approach have been developed to produce ferric oxide nanoparticles nanogel for MRI application by coating oxide with N-(2-aminoethyl)methyiacrylamide and N,N'-methylene bis acrylamide treared with UV radiation at 388 nm for 10 minutes recovering the product after washing with water. Likewise, diacrylated pluronic and glycidyl methyacrylated chitooligosacchride were loaded with plasmid DNA at different ratios and were photo irradiated with long wave length UV light at 365 nm, the photo initiator was igracure added to the mixture for cross linking offering advantage to gene delivery.

Emulsion photopolymerisation process

Emulsion photopolymerisation using UV was utilized for preparing cationic dextran nanogel in which the dextran hydroxyethyl methacrylate was emulsified with ABIL EM 90 as emulgent in mineral oil, the product was obtained in acetone:hexane(1:1), the precipitate was centrifuged, lyophilized and dessicated. The photosensitizer meso-tetraphenylporphine disulfonate was introduced in the preparation to cause breakage of endosomal membranes in cell and release of genes in cytoplasm and nuclease [114].

Applications

Nanogels have been shown to promising result for drug delivery of cancer drugs, other drugs that can be delivered includes delivery of anti-inflammatory drug for the treatment of rheumatoid arthritis. Chitosan based nanogels can be used to target macrophages [110].
Pullulan nanogels crosslinked with poly ethylene glycol were used to prepare biodegradable hydrogel. Galation with pullulan nanogels were used for preparing homogenized hydrogel. This hydrogel can be efficiently used for the delivery of anabolic agents in bone and cytokines [115].

Dendrimers

Dendrimers are synthetic nanostructures ranging from 10 to 200 Angstroms in diameter. They are hyper branched and monodisperse three-dimensional molecules with defined molecular weights, large numbers of functional groups on the surface and well-established host-guest entrapment properties. The surface of a dendrimer is characterized by the presence of functional groups that together can be utilized as a backbone for the attachment of several types of biological materials [116,117].

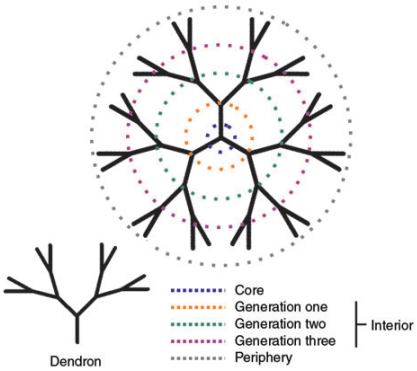

FIGURE 2.15
Anatomy of a dendrimers [118].

Synthesis

Divergent dendrimer synthesis

In the divergent approach, the construction of the dendrimer takes place in a stepwise manner starting from the core and building up the molecule towards the periphery using two basic operations (1) coupling of the monomer and (2) deprotection or transformation of the monomer end-group to create a new reactive surface functionality and then coupling of a new monomer *etc*., in a manner, somewhat similar to that known from solid-phase synthesis of peptides or oligonucleotides
For the poly (propyleneimine) dendrimers, which are based on a skeleton of poly alkylamines, where each nitrogen atom serves as a branching point, the synthetic basic operations consist of repeated double alkylation of the amines with acrylonitrile by "Michael addition" results in a branched alkyl chain structure. Subsequent reduction yields a new set of primary amines, which may then be double alkylated to provide further branching *etc* [119].
Polyamidoamine (PAMAM) dendrimers being based on a dendritic mixed structure of tertiary alkylamines as branching points and secondary amides as chain extension points was synthesised by Michael alkylation of the amine with acrylic acid methyl ester to yield a tertiary amine as the branching point followed by aminolysis of the resulting methyl ester by ethylene diamine.
The divergent synthesis was initially applied extensively in the synthesis of PPI and PAMAM dendrimers, but has also found wide use in the synthesis of dendrimers having other structural designs, *e.g*. dendrimers containing third period heteroatoms such as silicium and phosphorous [120].

Convergent Method

The second method developed by Hawker and Fréchet follows a "convergent growth process" In which several dendrons are reacted with a multifunctional core to obtain a product [121]. The convergent approach was developed as a response to the weakness of divergent synthesis. Convergent growth begins at what will end up being the surface of the dendrimer, and works inwards by gradually linking surface units together with more [122]. The advantage of convergent growth over divergent growth stem is that, only two simultaneous reactions are required for any generation adding step. Recently a breakthrough in the practice of dendrimer synthesis has come with the concept of double exponential growth. This approach allows the preparation of monomers of both convergent and divergent growth from a single starting material [123, 124].

Materials

Polyamidoamine (PAMAM)

PAMAM dendrimers are biocompatible, non-immunogenic, water soluble and possess terminal modifiable amine functional groups for binding various targeting or guest molecules [125]. The high density of amino groups and internal cavities in PAMAM dendrimers is expected to have potential applications in enhancing the aqueous solubility of low solubility drugs [126, 127] Caminade et al investigated that the water solubility of phosphorus-containing dendrimers was mainly due to the presence of hydrophilic end groups, which bear either positive or negative charges. These dendrimers can be used as in vitro DNA transfecting agents or in vivo anti-prion agents [128].
Dendrimers provide unique solutions to minimize delivery problems for ocular drug delivery. Recent research efforts for improving residence time of pilocarpine in the eye was increased by using PAMAM

dendrimers with carboxylic or hydroxyl surface groups. These surface-modified dendrimers were predicted to enhance pilocarpine bioavailability [129-130]. Many surface modified PAMAM dendrimers are non-immunogenic, water-soluble and possess terminal-modifiable amine functional groups for binding various targeting or guest molecules. PAMAM dendrimers are hydrolytically degradable only under harsh conditions because of their amide backbones, and hydrolysis proceeds slowly at physiological temperatures.

Polypropylenimine (PPI)

Cationic dendrimers (Polypropylenimine (PPI) dendrimers) deliver genetic materials into cells by forming complexes with negatively charged genetic materials through electrostatic interaction. Cationic dendrimers lend themselves as non-viral vectors for gene delivery because of their ability to form compact complexes with DNA

Applications

- Dendrimers have narrow polydispersity; nanometer size range of dendrimers can allow easier passage across biological barriers. All these properties make dendrimers suitable as host either binding guest molecules in the interior of dendrimers or on the periphery of the dendrimers.
- The family of dendrimers most investigated in drug delivery is the poly (amido amine) dendrimers (PAMAM). PAMAM dendrimers are biocompatible, non-immunogenic, water soluble and possess terminal modifiable amine functional groups for binding various targeting or guest molecules [125].
- For the in vivo pharmacokinetic and pharmacodynamic studies, indomethacin and dendrimer formulations were applied to the abdominal skin of the Wistar rats and blood collected from the tail vein at the scheduled time. The indomethacin concentration was significantly higher with PAMAM dendrimers when compared to the pure drug suspension. The results showed that effective concentration could be maintained for 24 h in the blood with the G4 dendrimer–indomethacin formulation. Therefore, data suggested that the dendrimer–indomethacin based transdermal delivery system was effective and might be a safe and efficacious method for treating various diseases [131].
- The anticancer drug paclitaxel (PTX) is a mitotic inhibitor used in chemotherapy to treat patients with lung, ovarian, breast, and head and neck cancers as well as advanced forms of Kaposis sarcoma. The drug works by interfering with normal microtubule growth during cell division, which especially affects fast growing cancer cells. In order to enhance its poor water solubility, paclitaxel has been encapsulated mainly into micelle-based formulations [132-135].
- The encapsulation of silver salts within PAMAM dendrimers produced conjugates exhibiting slow silver release rates and antimicrobial activity against various Gram-positive bacteria [136].
- Dendrimers can act as carriers, called vectors, in gene therapy. PAMAM dendrimers have also been tested as genetic material carriers. Cationic dendrimers (Polypropylenimine (PPI) dendrimer) deliver genetic materials into cells by forming complexes with negatively charged genetic materials through electrostatic interaction. Cationic dendrimers lend themselves as non-viral vectors for gene delivery because of their ability to form compact complexes with DNA. PAMAM dendrimers functionalized with α-cyclodextrin showed luciferase gene

expression about 100 times higher than for unfunctionalized PAMAM or for non-covalent mixtures of PAMAM and α-cyclodextrin [137].

Gold Nanoparticles

Gold has been one of the most coveted and prized metals since the very ancient times. In 1857, Faraday first reported that gold was pink when its size was extremely small [138]. Gold nanostructures have attracted considerable scientific interest in recent years for their potential to enhance both the diagnosis and treatment of cancer through their advantageous chemical and physical properties. The key feature of Au nanostructures for enabling this diverse array of biomedical applications is their attractive optical properties [139].

Types of Gold Nanoparticles

Gold nanoshells
Gold nanocages
Gold nanorods
Gold nanosphere
SERS nanoparticles [140].

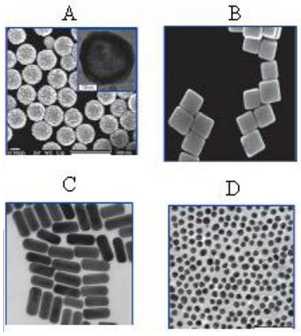

FIGURE 2.16
a) Gold nanoshells b) Gold nanocages c) Gold nanorods d) Gold nanosphere [141].

Gold Nanoshells

Gold nanoshells are spherical particles with diameters typically ranging in size from 10 to 200 nm composed of a dielectric core covered by a thin gold shell. They possess a remarkable set of optical, chemical and physical properties, which make them ideal candidates for enhancing cancer detection, cancer treatment, cellular imaging and medical biosensing [142].

Synthesis

Gold nanoshells with SPR peaks in the NIR region can be prepared by coating silica or polymer beads with gold shells of variable thickness. Silica cores are grown using the Stöber process, the basic

reduction of tetraethyl orthosilicate in ethanol. To coat the silica nanoparticles with gold in an aqueous environment, a seeded growth technique is typically used. Small gold nanospheres (2–4 nm in diameter) can be attached to the silica core using an amine-terminated silane as a liner molecule, allowing additional gold to be reduced until the seed particles coalesced into a complete shell. The diameter of the gold nanoshell is largely determined by the diameter of the silica core, and the shell thickness can be controlled through the amount of gold deposited on the surface of the core.
Gold nanoshells have also been synthesized via in situ gold nanoparticle formation using thermosensitive core-shell particles as the template. The use of microgel as the core offers signifi cantly reduced particle aggregation, as well as thickness control of the gold nanoshells using electroless gold plating. In one study, a virus scaffold has been used to assemble gold nanoshells. This approach may potentially provide cores with a narrower size distribution and smaller diameters (80 nm) than those of silica [143].

Properties

The properties of metallic nanoshells include optical, magnetic, photothermal, and catalytic. Gold nanoshells have been used for biomedical imaging and therapeutic applications because they offer highly favourable optical and chemical properties.
Gold nanoshells particles conjugated with enzymes and antibodies can be embedded in a matrix of the polymer. These polymers, such as Nisopropylacrylamide (NIPAAm), and acrylamide (AAm), have a melting temperature which is slightly above body temperature. When such a nanoshell and polymer matrix is illuminated with resonant wavelength, nanoshells absorb heat and transfer to the local environment.
Nanoshells function as useful and versatile imaging agents because of their large extinction cross - sections, immunity to photobleaching, spectral tunability, absorption/scattering ratio tunability, electromagnetic near – field enhancement, and enhanced luminescence. These optical phenomena are in large part due to a resonance phenomenon, known as surface plasmon resonance [144-146]

Applications

Gold Shells are used for drug delivery of Tumor necrosis factor-alpha (TNF-α), Methotrexate, methylene blue, insulin, and lysozyme [143].

Gold Nanocages

Noble-metal nanocages represent a novel class of nanostructures with hollow interiors and porous walls. They are prepared using the remarkably simple galvanic replacement reaction between solutions containing metal precursor salts and Ag nanostructures prepared by polyol reduction [147].

Synthesis

Gold nanocages with controllable pores on the surface have been synthesized via galvanic replacement reaction between truncated silver nanocubes and aqueous $HAuCl_4$. Silver nanostructures with controlled morphologies can be generated through polyol reduction, where $AgNO_3$ is reduced by ethylene glycol to generate silver atoms and then nanocrystals or seeds. Subsequent addition of silver atoms to the seeds produces the desired nanostructures through controlling the silver seed crystalline

structures in the presence of poly(vinylpyrrolidone), a polymer that is capable of selectively binding to the surface. The silver nanostructures, used as a sacrificial template, can then be transformed into gold nanostructures with hollow interiors via the galvanic replacement. The dimension and wall thickness of the resultant gold nanocages could be readily controlled, to very high precision, by adjusting the molar ratio of silver to HAuCl [143].

Properties

AuNCs have a range of hidden qualities that make them unique for theranostic applications.

- They are single crystals with good mechanical flexibility and stability, as well as atomically flat surfaces.
- They can be routinely produced in large quantities with wall thicknesses tunable in the range of 2–10 nm with an accuracy of 0.5 nm.
- LSPR peaks can be easily and precisely tuned to any wavelength of interest in the range of 600–1200 nm by simply controlling the amount of HAuCl4 added to the reaction.
- The hollow interiors can be used for encapsulation.
- Their porous walls can be used for drug delivery, with the release being controlled by various stimuli.
- Their sizes can be readily varied from 20 to 500 nm to optimize biodistribution, facilitate particle permeation through epithelial tissues, or increasing drug loading.
- Their LSPR peaks can be dominated by absorption or scattering to adapt to different imaging modalities.
- Other noble metals such as Pd and Pt can be incorporated into the walls during a synthesis to maneuver their optical properties [139].

Applications

The using of Ag nanocubes as a template for galvanic replacement with HAuCl4 offers an elegant way to make complementary hollow gold nanocages with controllable void size, wall thickness, and wall porosity [148-150]. Nanocages are used in cancer targeting, photothermal cancer treatment, controlled release of a drug such as doxorubicin [139].

Future Perspective

A desirable situation in drug delivery is to have smart drug delivery systems that can be integrated into the human body. This area of nanotechnology will play an extremely important role. Time-release tablets, which have a relatively simple coating that dissolves in specific locations, also involve the use of nanoparticles. Pharmaceutical companies are using nanotechnology to create intelligent drug-release devices. For example, the control of the interface between the drug/particle and the human body can be programmed so that when the drug reaches its target, it can then become active. The use of nanotechnology for drug-release devices requires autonomous device operation. For example, in contrast to converting a biochemical signal into a mechanical signal and being able to control and communicate with the device, autonomous device operation would require biochemical recognition to

generate forces to stimulate various valves and channels in the drug delivery systems, so that it does not require any external control.
It is now appears that we are on the verge of bioengineering molecular motors for specialized applications on nanoscale. These systems might be the key to yet unsolved biomedical applications that include nonviral gene therapy and interneuron drug delivery [151]

Conclusions

Numerous fields of science are converging to study science at a very fundamental level or building block level, namely nanoscience. The majority of studies have focused on materials sciences with some applications emerging in the biomedical field. Very few fundamental studies have been performed in the pharmaceutical field discussing the fundamentally different properties of materials at the nanolevel. The application is clear and promising; however, the basics of nanoscience in drug delivery are poorly understood. With sound investigation of these basic properties, the scope of pharmaceutical sciences within the invisible nanoworld seems poised to result in a revolution in medical world.

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