Nanoparticles as Drug Delivery Systems

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Nanoparticles, one of the colloidal drug delivery systems, hold great promise for reaching the goal of controlled drug delivery as well as site specific delivery. This review presents various methods of preparation, characterization, stability, drug release and applications of nanoparticles. If appropriately investigated, nanoparticles may open new avenues in research and therapy.

Nanoparticles are a type of colloidal drug delivery systems where the particle size ranges from 10-1000 nm in diameter. These sub-particles are prepared from a variety of natural and synthetic polymers that include gelatin, dextran, polylactide, poly (alkyl 2-cyanoacrylate) and poly (methyl methacrylate). Drugs can be dissolved, entrapped or encapsulated into the nanoparticles, or simply absorbed on their surface. Nanospheres consist of a dense polymeric matrix, in which the drug can be dispersed, whereas, nanocapsules are constituted of a liquid core surrounded by a polymeric shell. Nanoparticles are formed by a single layered shell and are filled with oil which lend themselves ideally as carriers for lipophilic agents[2].

The choice of appropriate polymer, particle size, and manufacturing process will primarily depend on the bioacceptability of the polymer, followed by physicochemical properties of the drug and the therapeutic objective.

METHODS OF PREPARATION

1. Emulsion Polymerization[1,3]

The term Emulsion Polymerization was used because the monomer is emulsified in a nonsolvent by means of emulsifiers. The location of polymerization is in the emulsifier micelles. These micelles coexist with single emulsifier molecules those are present in solution and with the emulsifier molecules that are adsorbed at the emul-

sion/droplet interfaces, thus stabilizing the emulsion droplets. It is assumed that monomer molecules would diffuse from the emulsion droplets into the emulsifier micelles and that the solubilized monomer molecules in the micelles then polymerizes to form the polymer latex. After polymerization a small polymer particles suspension is obtained.

Emulsifier concentration does not affect the polymerization rate and the formation of particle is independent of the rate of polymerization. The location of the polymerization initiation is the solvent phase. Initiation takes place in this phase when dissolved monomer molecules are reacted with a starter molecule or exposed to high-energy radiation. The polymerization and chain growth is maintained by additional monomer molecules that diffuse to the growing polymer. Initially, during emulsion polymerization the growing polymer molecules remain dissolved in the continuous phase. After a certain molecular weight is reached, the polymer becomes insoluble, so that separation and particle formation occurs.

2. Polymerization in a continuous aqueous phase

In this method the monomers are dissolved in water and polymerization is induced chemically or by high energy radiations, as exemplified by the preparations of :

(i) Poly methyl methacrylate nanoparticles :

Monomeric methyl methacrylate is soluble in water

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in a concentration up to 1.5%. After the monomer completely dissolves, polymerization can be induced by either high energy radiation[4] (gamma rays) emitted by[60] Co source of chemical initiation by the addition of polymerization initiators[5] such as ammonium or potassium peroxodisulfate and heating to elevated temperature, generally above 65⁰.

Instead of pure water, a buffer solution or solution of a drug or any other material intended to be bound to the nanoparticles can be used as the polymerization medium. Macromolecules such as drugs may be present in the polymerization medium, or they may be added after polymerization has been achieved.

(ii) Poly (alkyl) cyanoacrylate nanoparticles :

The cyanoacrylate monomers are added to the aqueous polymerization medium in concentration between 0.05% and 7%[6]. The polymerization mechanism is initiated by bases present in the medium. These are mainly initiated by the OH ions resulting from the dissociation of water, but some basic drugs may also function as initiators.

(iii) Acrylic copolymer nanoparticles[7-14] :

The monomers that can be used are methyl methacrylate, 2-hydroxyethyl methacrylate, methacrylic acid, ethylene glycol dimethyl acrylate, acrylamide, N,N-bismethylene acrylamide, and 2-dimethylamine. Chemical initiation as well as γ irradiation can be employed for inducing polymerization.

(iv) Polystyrene nanoparticles :

Polystyrene nanoparticles can be produced by methods similar to those of polyacrylic nonoparticles[15]. As the water solubility of styrene is relatively lower than that of most acrylates surfactants are used to improve its solubility.

(v) Poly (vinyl pyridine) nanoparticles[8,9] :

The polymerization of vinyl pyridine was carried out in aqueous methanol or acetone solutions containing N,N-bis-methylene acrylamide as a cross linking agent. Poly (ethyleneoxide) was also present in amounts of 0.1 to 4% (W/V) for the stabilization of the formed particles.One percent higher concentration of polymer in water yielded crosslinked gels when exposed to ionizing radiation.

(vi) Polyacrolein nanoparticles[16,17] :

Polyacrolein nanoparticles can be produced by aqueous polymerization of acrolein using γ irradiation or by polymerization under alkaline conditions with sodium hydroxide solution (2h, pH 10.5). The particle size may be controlled by addition of surfactants.

(vii) Poly glutaraldehyde nanoparticles[18-21] :

This reaction is carried out by aldol condensation of monomeric glutaraldehyde at alkaline pH[18]. In presence of surfactants and in basic aqueous solution, poly glutaraldehyde precipitates out in the form of spherical colloidal particles, the diameter of the particles increases with increasing amounts of monomer or with decreasing surfactant concentration.

Increasing pH also causes reduction of particle size presumably due to the occurance of cannizzaro reaction leading to the formation of acid and alcohol. 5-flurouracil containing polyglutaraldehyde nonoparticles of a size of about 270 nm were produced by polymerization at a pH of 8.2 for 24h. Five percent tween-80 and 0.1% sodium carboxymethyl cellulose were used as surfactant and stabilizer, respectively.

(viii) Poly (alkyl methylidenemalonate) nanoparticles[22]:

Dialkyl methylidenemalonic acid esters can also be polymerized by OH induced cationic polymerization similar to the poly (alkyl cyanoacrylates) at neutral pH.

3. Emulsion polymerization in a continuous organic phase[23-27]

The initiation of this polymerization reaction can be carried out using N, N, N¹, N¹- tetramethyl ethylendiamine and potassium peroxodisulfate as initiators or by γ, UV, or visible light-irradiation. In the case of light initiation, riboflavin-5-sodium phosphate and potassium peroxodisulfate have to be added to the reaction mixture, as catalysts.

4. Interfacial polymerization

This involves polymerization of alkyl cyanoacrylates

in an organic solvent containing water swollen micelles leading to the formation of a polymer wall at the solvent micellar water interface as evident in case of Poly (N^{α}, N^{β}-L-Lysine diylterephthaloyl) nanoparticles[28] and Poly (alkyl cyanoacrylate) nanoparticles.[29,30]

5. Solvent deposition

In this process, the polymer (Poly (D,L) lactide) and phospholipids are dissolved in acetone[31]. The solution of the drug is then added to the organic phase and this mixure is subsequently, poured into water containing 0.5% poloxamer 188 under moderate stirring nanocapsules with the poly(lactic acid) wall surrounding an oily core are formed instantaneously.[32]

6. Solvent evaporation[33]

The preformed polymer is dissolved together with the drug in an organic solvent, which is then emulsified in water and subsequently evaporated by heating and/or under reduced pressure. Poly(L-lactide) has been cast into nanoparticles by a modified solvent evaporation process. The poly(L-lactide) and active agent (Triamcinolone-acetonide) were codissolved in chloroform. The chloroform solution (2ml) was homogenized ultrasonically at 15^{0} in a 0.5% aqueous gelatin solution (40 ml). Sonication was continued while the system was heated to 45^{0} and chloroform was allowed to evaporate. The residue consisting of the nanoparticles was collected.

7. Desolvation[34,35]

The solution of the polymer and of the drug to be entrapped are poured into water, resulting in the spontaneous formation of nanoparticles of size between 90 and 205nm. Polyacrylic nanoparticles can be prepared by dissolving relatively hydrophilic copolymer such as Eudragit R (RS) or Eudragit R (RL) in water-miscible solvents such as acetone and ethanol.

8. Production of albumin nanoparticles in an oil emulsion[36]

Nanoparticles consisting of albumin or other macromolecules may be produced by emulsification of aqueous solution of these macromolecules and of the drug to be incorporated into the particles in an oil. The resulting droplets can then be hardened by crosslinking with alde-

hydes or other crosslinking agents or by denaturation of the molecules at high temperature.

The use of high efficiency homogenization or ultrasonication as in the case of solvent evaporation enables the production of nanometer-sized emulsion droplets and after hardening lead to formation of nanoparticles.

9. Gelatin nanoparticles

In this method an emulsion consisting of equal volumes of oil and aqueous gelatin solution with a surfactant and the drug is prepared. Nanoparticles are generated at 60^{0} with vigorous stirring. The emulsion is then cooled in ice bath and added to acetone with stirring. The product is then filtered and dried[37].

10. Nanoparticles produced by desolvation of macromolecules

In order to prepare nanoparticles, a solution of the natural macromolecule and an active ingredient associated with it (e.g. protein binding) is prepared. This system is then desolvated by adding a solvent competing solute such as sodium sulfate or an alcohol. By controlling the desolvation process such that it can be terminated just outside the conservation region, colloidal-size particles rather than large aggregates may be obtained. These can be carefully resolvated until the desired state is reached.[37]

11. Carbohydrate nanoparticles

Carbohydrate nanoparticles consisting of acryloylated dextran, maltodextran, mannan or other starch derivatives were produced by polymerization of the acryloyl side chain after emulsification of the aqueous starch derivative solution in a toluene:chloroform (4:1) solution.[38-53]

CHARACTERIZATION

The parameters used to characterize nanoparticles are optical appearance, particle size distribution, lamellarity, tapped volume and stability (Leakage). Preparations of particles with diameters above 150 nm are white in colour even in diluted dispersions. Preparations containing particles of 100 nm become opaque. Emulsions containing particles below 60 nm result in clear transparent dispersions. The particle size, as well as the particle

size distribution can be determined by photon correlation spectroscopy[54], transmission electron microscopy (TEM), scanning electron microscopy (SEM)[55], SEM combined with energy-dispersive X-ray spectroscopy, scanned probe microscopes and frauhofer diffraction. Molecular weight is determined by gel chromatography, density by helium compression pycnometry[55], surface charge by electrophoresis[55] laser doppler anemometry, amplitude weighted phase structuration and surface properties by static secondary ion mass spectrometry (SSIMS). Freeze fracture electron microscope (FFEM) has been used to exactly characterize the size and shape of the lipid particles. Information about nanoparticle structure may also be obtained by X-ray diffraction and thermoanalytical techniques such as differential scanning calorimetry (DSC) defferential thermal analysis (DTA), thermogravimetric analysis (TGA), thermal mechanical analysis (TMA), and thermal optical analysis (TOA)[56,57].

DETERMINATION OF DRUG CONTENT

The precise drug content determination can be a problem due to the colloidal nature of the drug carried. However, the method of choice is separation of the particles by ultracentrifugation followed by quantitative analysis of the drug after dissolving the pelleted polymer. Ultrafiltration and gel filtration are the other useful separation techniques. The drug content can also be determined in the supernatant or the filtrate. The amount of drug bound to the particles can then be calculated by difference of this amount from the total amount of drug present in the suspension.[66]

STABILITY OF NANOPARTICLES[58-61]

Nanoparticles, due to their small size degrade faster than larger microspheres. The degradation pathways vary from polymer to polymer. However, the common pathways are by erosion of polymer backbone, and cleavage of the ester to name a few.

DRUG RELEASE[66]

The following methods have been used for the determination of in vitro release.

1. Using diffusion cells with artificial or biological membrane

2. Using dialysis bag

3. Reverse dialysis sac technique

4. Ultra centrifugation

5. Centrifugal ultrafiltration technique

The dissolution media will be a buffer solution of the required pH.

The drug release is found to occur by any one of the following mechanisms

1. Desorption of surface bound drug

2. Diffusion through the matrix or the polymer wall

3. Erosion

4. Combined erosion and diffusion.

APPLICATIONS

Nanoparticles possess a better stability as compared to liposomes. This property may be very important for many modes of targeting. Nanoparticles form the basis of colloidal drug delivery system which is biodegradable and capable of being stored for periods of at least one year. They can deliver drugs to the liver in vivo and in vitro to cells which are actively phagocytic.

Among the mononuclear phagocyte system cells (MPS), the macrophages of the liver, called Kupffer cells[62], are normally the most efficient cells for the phagocytosis of injected particles. Polymethacrylic nanoparticles have thus been designed for passively targeting the anticancer drug doxorubicin (DXR), to the liver to reduce toxicity and increase the therapeutic activity.

Nanoparticles are also used for ophthalmic drug delivery. The most commonly used polymers for preparation of ophthalmic nanoparticles are polyalkylcyanoacrylates. They are prepared by emulsion polymerization. The pH of the polymerization medium has to be kept below 3. After polymerization, pH may be adjusted. The polymers used are rapidly biodegradable. Nanoparticles of pilocarpine have shown enhanced miotic response by about 22-23%.[63] Eudragit self assembling nanoparticles of diclofenac diethylammonium showed improved efficacy against turpentine liniment-induced inflammation[63], as compared to the pure drug.

The attachment of certain hydrophilic polymers on the surface of the carriers reduces the uptake by the immune system (RES) therefore prolonging the systemic half-life of the nanospheres and thus they can be administered intravenously.

Poly(methyl methacrylate) nanoparticles are biodegraded at a very slow rate, for this reason, they are suitable as adjuvants for vaccines when the achievement of a very prolonged immune response is desired. Polystyrene nanoparticles are non biodegradable. For this reason, they are mainly used as immunosorbents for basic biodistribution and vaccination studies. Poly (vinyl pyridine) nanoparticles have the advantage of being able to corporate or bind metals through complexing their aromatic nitrogen. The ring itself can undergo chemical modification to introduce other functional groups[64], which would help in the easy uptake of drugs.

Ligands such as protein drugs, enzymes, and antibiotics can be bound covalently through their amino group via formation of Schiff's bases to the polyacrolein nanoparticles[16,17]. The preparation of albumin nanoparticles in an emulsion was first reported by Zolle et al[36]. They used nanoparticles as carriers for radionucleotides for diagnostic purposes in nuclear medicine. Similarly ^{99m}TC nanoparticles are used for the study of morphology, blood flow and functions of liver.

Nanoparticles formulated as amorphous spheres offer higher solubility than standard crystalline formulations, thus improving the poor aqueous solubility of the drug and hence its bioavailability. This improved solubility arises for any given dose, as particle diameter decreases, surface area increases, which results in an increase in disolution rate.

Nanoparticles can be formulated as injections consisting of spherical amorphous particles which do not aggregate, hence they can be safely administered by the intravenous route. Since no cosolvent is used to solubilize the drug, the overall toxicity of the formulation is reduced.

Mibelle AG cosmetics, Switzerland have prepared nanoparticles for skin and hair care. Lipid nanoparticles formed by lecithin encithin encapsulating an oil core are ideal carriers for lipophilic substances. They enhance the bioavailability of the encapsulated material to the skin. High pressure homogenization results in a 100% encapsulation of the oily phase with positively charged nanoparticles. Lipophilic UV filters and other active agents can efficiently be targeted to hair. The core of particles can contain a wide variety of different cosmetic oil (triglycerides, jojoba oil, borage oil, wheat germ oil, macadamia nut oil) and lipophilic agents (vitamin A palmitate, vitamin E acetate, retinol, tretinoin, UV filters, fragrances)[54]. Nanoparticles are also reported to be a suitable tool for delivery of drugs, such as, the dipeptide kyotorphim across the blood brain barrier.

Solid lipid nanoparticles, an alternative particulate carrier system to polymeric nanoparticles of prednisolone were prepared using high pressure homogenization. Sufficient long term stability, controlled release, the possibility of sterilization, low *in vivo* toxicity are some of the advantages achieved for parenteral drug delivery. Due to the small size and ability to disperse in water to form clear colloidal solution, nanoparticles have potential use as sustained release formulations.

Disinfectants or an algaecides can be delivered in a controlled manner into large reservoirs of water. It has been found that the larvae of some aquatic species and insect pests feed on colloidal particles. Thus it may be possible to deliver nutrients, medicinal agents or poisons to these creatures by feeding them drug impregnated nanoparticles[65].

In conclusion, nanoparticles are one of the promising drug delivery systems, which can be of potential use in controlling and targeting drug delivery. The methods of preparation are simple. Different polymers such as gelatin, albumin, polystyrene, polyacrylic acid can be used in the preparation. They possess better stability when compared to liposomes. The methods reported for the *in vitro* release are simple. They have varied applications such as opthalmic drug delivery, intravenous delivery, as carries for radionucleotides in nuclear medicine, as cosmetics for skin and hair care, sustained release formulations and many more.

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