



Review Article

NANOPARTICLES – AN OVERVIEW

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ABSTRACT

Recently particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Drug delivery research is clearly moving from the micro- to the nanosize scale. Nanotechnology is therefore emerging as a field in medicine that is expected to elicit significant therapeutic benefits. The development of effective nanodelivery systems capable of carrying a drug specifically and safely to a desired site of action is one of the most challenging tasks of pharmaceutical formulation investigators. They are attempting to reformulate and add new indications to the existing blockbuster drugs to maintain positive scientific outcomes and therapeutic breakthroughs. The nanodelivery systems mainly include nanoemulsions, lipid or polymeric Nanoparticles and liposomes. For the past few years, there has been a considerable research on the basis of Novel drug delivery system, using particulate vesicle systems as such drug carriers for small and large molecules. Nanoparticles have been improving the therapeutic effect of drugs and minimize the side effects. Basically, Nanoparticles have been prepared by using various techniques as such dispersion of preformed polymers, polymerization of monomers and ionic gelation or co-accervation of hydrophilic polymer.

Keywords: Nanoparticles, Nanotechnology, Polymeric Nanoparticles, Particulate system, Nanodelivery.

INTRODUCTION

Nanotechnology employs knowledge from the fields of physics, chemistry, biology, materials science, health sciences, and engineering. It has immense applications in almost all the fields of science and human life. Nanoparticles can be defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.

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cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carrier of DNA in gene therapy, and their ability to deliver proteins, peptides and genes.

The input of today's nanotechnology is that it allows real progress to achieve temporal and spatial site-specific delivery. The market of nanotechnology and drug delivery systems based on this technology will be widely felt by the pharmaceutical industry. In recent years, the number of patents and products in this field is increasing significantly. Several terminologies have been used to describe

nanoparticulate drug delivery systems. In most cases, either polymers or lipids are used as carriers for the drug, and the delivery systems have particle size distribution from few nanometers to few hundred nanometers.

Nanomedicines is a large subject area and includes nanoparticles that act as biological mimetic (e.g. functionalized carbon nanotubes), "nanomachics" (e.g. those made from interchangeable DNA parts and DNA scaffolds such as octahedron and stick cube), nanofibers and polymeric nanoconstructs as biomaterials (e.g. molecular self-assembly and nano-fibers of peptides and peptide amphiphiles for tissue engineering), shape memory polymers as molecular switches, nanoporous membranes), and nanoscale micro-fabrication based devices (e.g. silicon microchips for drug release and micro machined hollow needles and two dimensional needles assay from single crystal silicon), sensors and laboratory diagnostics.

Recent developments in nanotechnology have shown that nanoparticles (structures smaller than 100 nm in at least one dimension) have a great potential as drug carriers. Due to their small sizes, the nanostructures exhibit unique physicochemical and biological properties (e.g., an enhanced reactive area as well as an ability to cross cell and tissue barriers) that make them a favorable material for biomedical applications. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties.

Nanoparticles Preparation [5]

Nanoparticles are aimed to be prepared from a variety of materials such as proteins, polysaccharides and synthetic

polymers. The selection criteria of matrix materials depends on many factors such as: (a) Size of nanoparticles required; (b) Inherent properties of the 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; and (f) Antigenicity of the final product.

Nanoparticles preparation is most frequently by three methods: (1) Dispersion of preformed polymers; (2) Polymerization of monomers; and (3) Ionic gelation or coacervation of hydrophilic polymers. However, other methods such as supercritical fluid technology [8] and particle replication in non-wetting templates have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in industry.

Dispersion of preformed polymers [6,7]

Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticle from poly (lactic acid) (PLA); poly (D,L-glycolide), PLG; poly (D,L-lactide-coglycolide) (PLGA) and poly(cyanoacrylate) (PCA). This technique can be used in various ways as described further:

Solvent evaporation method [8]

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

Spontaneous emulsification or solvent diffusion method [9]

This is a modified version of solvent evaporation method. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as

anol phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of watermiscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

Polymerization method [10-12]

In this method, monomers are polymerized to form nanoparticle in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles.

Coacervation or ionic gelation method [13]

The nanoparticles preparation is carried by using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Developing a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. In this method, positively charged amino-group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer.

Production of nanoparticles using supercritical fluid technology [14-15]

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe. A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO₂ (SC CO₂) is the most widely used supercritical fluid because of its mild critical conditions (T_c = 31.1 °C, P_c = 73.8 bars), nontoxicity, non-

flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid (SC CO₂), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles. RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure. Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates.

This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive.

Evaluation of Nanoparticles

Zeta potential^[16]

The Zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (±) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles.

Particle Shape^[17]

SEM characterizes the nanosuspension before going for evaluation; the nanosuspension is lyophilized to form solid particles. The solid particles are coated with platinum alloy using a sputter coater.

Particle size^[18]

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, biological fate, and toxicity and targeting ability of nanoparticle system. In addition, they can also influence the drug loading, drug release and stability of

nanoparticles. Currently, the faster and most routine method of determining particle size is by photon-correlation spectroscopy or dynamic light scattering. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM).

Drug Entrapment Efficiency^[19]

The nanoparticles were separated from the aqueous medium by ultracentrifugation at 10,000 rpm for 30 min at 50C. Then the resulting supernatant solution was decanted and dispersed into phosphate buffer saline pH 7.4. Thus the procedure was repeated twice to remove the untrapped drug molecules completely. The amount of drug entrapped in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium.

Drug Entrapment efficiency (%) = $\frac{\text{Amount of released from the lysed nanoparticle}}{\text{Amount of drug Initially taken to prepare the Nanoparticles}} \times 100$

Advantages of Nanoparticles^[20]

The advantages of using nanoparticles as a drug delivery system include the following:

- a) Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
- b) They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
- c) Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- d) Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
- e) The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

Limitations of Nanoparticles^[20-21]

a) Small size and large surface area can lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.

b) In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

Applications of Nanoparticles:

Tumor targeting using Nanoparticulate delivery system^[22]

The rationale of using nanoparticles for tumor targeting is based on (1) Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active nanoparticles. (2) Nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ. An experiment demonstrated in mice treated with doxorubicin incorporated into poly (isohexylcyanoacrylate) nanospheres that higher concentration of doxorubicin manifested in the liver, spleen and lungs than in mice treated with free doxorubicin.

Nanoparticles for oral delivery of peptides and proteins^[22]

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration. The surface area of human mucosa extends to 200 times that of skin. The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g., (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin; (b) proteolytic enzymes at the brush border membrane (endopeptidases); (c) bacterial gut flora; and (d) mucus layer and epithelial cell lining itself. The histological architecture of the mucosa is designed to

efficiently prevent uptake of particulate matter from the environment. One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles, which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract. .

Nanoparticles for Gene delivery ^[23]

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system.

Targeting of nanoparticles to epithelial cells in the GI tract using ligands

Targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Peyer's patches in the GI tract can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. The surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption. Vitamin B-12 absorption from the gut under physiological conditions occurs via receptor-mediated endocytosis. The ability to increase oral bioavailability of various peptides (e.g., granulocyte colony stimulating factor, erythropoietin) and particles by covalent coupling to vitamin B-12 has been studied. For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. The mucoprotein completely reaches the ileum where resorption is mediated by specific receptors.

Nanotechnology in Medicine Application:

Anti-Microbial Techniques^[24]

One of the earliest nanomedicine applications was the use of nanocrystalline silver, which is as an antimicrobial agent for the treatment of wounds, A nanoparticle cream has been

shown to fight staph infections. The nanoparticles contain nitric oxide gas, which is known to kill bacteria. Studies on mice have shown that using the nanoparticle cream to release nitric oxide gas at the site of staph abscesses significantly reduced the infection. Burn dressing that is coated with nanocapsules containing antibiotics. If a infection starts the harmful bacteria in the wound causes the nanocapsules to break open, releasing the antibiotics. This allows much quicker treatment of an infection and reduces the number of times a dressing has to be changed. A welcome idea in the early study stages is the elimination of bacterial infections in a patient within minutes, instead of delivering treatment with antibiotics over a period of weeks.

Absorption enhancement using non-specific interactions

In general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytotic pathway. The paracellular route of absorption of nanoparticles utilizes less than 1% of mucosal surface area. Using polymers such as chitosan, starch or poly (acrylate) can increase the paracellular permeability of macromolecules. Endocytotic pathway for absorption of nanoparticles is either by receptor-mediated endocytosis, that is, active targeting, or adsorptive endocytosis which does not need any ligands. This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions. Adsorptive endocytosis depends primarily on the size and surface properties of the material. If the surface charge of the nanoparticles is positive or uncharged, it will provide an affinity to adsorptive enterocytes though hydrophobic, whereas if it is negatively charged and hydrophilic, it shows greater affinity to adsorptive enterocytes and M cells. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated.

Nanotechnology in Medicine Application: Cell Repair ^[25]

Nanorobots could actually be programmed to repair specific diseased cells, functioning in a similar way to antibodies in our natural healing processes. Read about design analysis for one such cell repair nanorobot in this article:

The Ideal Gene Delivery Vector: Chromalloytes, Cell Repair Nanorobots for Chromosome Repair Therapy.

Nanoparticles for drug delivery into the brain^[26]

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. Relatively impermeable endothelial cells characterize the BBB with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function. Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor-mediated transport systems in the BBB. For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cellpenetrating peptides and melanotransferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis. It has been reported poly(butylcyanoacrylate) nanoparticles was able to deliver hexapeptidedalargin, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB. Despite some reported success with polysorbate 80 coated NPs, this system does have many shortcomings including desorption of polysorbate coating, rapid NP degradation and toxicity caused by presence of high concentration of polysorbate 80. OX26MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of liposomes. However, recently, Jiet al. demonstrated that brain uptake of lactoferrin, an iron-binding glycoprotein belonging to the transferrin (Tf) family, is twice that of OX26 and transferrin *in vivo*. It is possible soon we will see these BBB specific molecules used for targeting nanoparticles to the brain.

CONCLUSION:

A real therapeutic breakthrough can be achieved solely by carrying out painstaking studies in the field of nano-therapy. Using nanosystems in therapies of diseases may contribute to achieving an effective cancer treatment. The key applications of nanoparticles in medicine are diagnosis and target

therapy, however, their wider use is still the future. Nanoparticle have relatively higher intracellular uptake compared to microparticles and available to a wide range of biological targets due to their small size and relative mobility. The core of this system can enclose a variety of drugs, enzymes, genes and is characterized by a long circulation time due to the hydrophilic shell which prevents recognition by the reticular-endothelial system. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering is still required. Further advances are needed in order to turn the concept of nanoparticle technology into a realistic practical application as the next generation of drug delivery system.

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