

NEW APPROACHES IN NANOPARTICULATE DRUG DELIVERY SYSTEM - A REVIEW

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ABSTRACT

Nanoparticles are 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. 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. Present review reveals the methods of preparation, characterization and application of several nanoparticulate drug delivery systems.¹

Keywords: Nano particle drug delivery system, nanospheres, nanocapsules.

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. 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. In recent years, biodegradable polymeric nanoparticles, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as polyethylene 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 carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes. 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

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

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
2. 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.
3. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction.
4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.
6. Avoidance of coalescence leads to enhanced physical stability.
7. Reduced mobility of incorporated drug molecules leads to reduction of drug leakage.
8. Static interface solid/liquid facilitates surface modification.

Disadvantages

1. Potential toxicity

While the small size of nanoparticle is what makes them so useful in medicine, it is also the factor that might make them potentially dangerous to human health.

2. Environmental concerns

Artificially manufacture nanoparticles will be new to the environment in type and quantity and would constitute a new class of non biodegradable pollutants.

3. There are no convenient method by which exposure to nanoparticle in the workplace can be measured or assessed there is a need and for more research into the development of new improved methods, combinatic and strategies to improved assessment of exposure to nanoparticle and nanoparticle aerosol.

Theory^{1-7,10}

Ideal Properties

- 1 Natural or synthetic polymer
- 2 Inexpensive
- 3 Nontoxic
- 4 Biodegradable
- 5 Nonthrombogenic
- 6 Nonimmunogenic
- 7 Particle diameter <100nm
- 8 No platelet aggregation
- 9 Noninflammatory
- 10 Prolonged circulation time

Materials Used In the Preparation of Nanoparticles¹³

1. Poly(ethylene oxide)-poly(L-lactic acid)/poly(benzyl-L-aspartate)

Polymeric micelles often self-assemble when block copolymers are used for their preparation. Micelles, based on the biocompatible copolymers of poly (ethylene oxide) PEO with poly(L-Lactic acid) PLA or with poly(□benzyl-L-aspartate) PBLA, have been described in literature. Aldehyde groups on the surface of the PEO-PLA micelles may react with the lysine residues of cell's proteins. They may also be used for attachment of the amino-containing ligands. The hydroxyl groups on the surface of the PEO-PBLA micelles can be further derivatized and conjugated with molecules capable to pilot the modified micelles to specific sites of living organism. Such

nanospheres have been tested as vehicles for delivery of anti-inflammatory and anti-tumor drugs.

2. Poly(lactide-co-glycolide)-[poly(propylene oxide)-poly(ethylene oxide)]

Nanoparticles (80-150 nm) of the biocompatible and biodegradable polyester copolymer PLG [Poly(lactide-co-glycolide)] Figure 1 have been reported by the nanoprecipitation method (they have been precipitated with acetone from their oily colloidal nanodispersion in water). Thus formed particles of PLG were coated with 5-10 nm thick layer of the poly (propylene oxide) - poly (ethylene oxide) (PPO-PEO) block copolymer or with tetrafunctional (PEO-PPO)₂-N-CH₂-CH₂-N-(PPO-PEO)₂. Such coats are bound to the core of the nanosphere by the hydrophobic interactions of the PPO chains, while PEO chains protrude into the surrounding medium and form a steric barrier, which hinders the adsorption of certain plasma proteins onto the surface of such particles. On the other hand, the PEO coat enhances adsorption of certain other plasma compounds. In consequence, the PEO-coated nanospheres are not recognized by macrophages as foreign bodies and are not attacked by them.

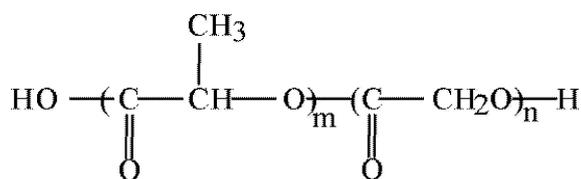


Fig. 1: Poly (lactide-co-glycolide) PLG

3. Polyphosphazene derivatives

Allock and coworkers developed derivatives of the phosphazene polymers suitable for biomedical applications. Long-circulating in the blood, 100-120 nm in diameter, PEO-coated nanoparticles of the poly (organophosphazenes) containing amino acid, have been prepared. PEO-polyphosphazene copolymer, or poloxamine 908 (a tetrafunctional PEO copolymer) has been deposited on their surface.

4. Poly (ethylene glycol) coated nanospheres

Poly (ethylene glycol) PEG-coated nanospheres from PLA, PLG, or other biodegradable polymers viz., poly (ϵ -caprolactone) (PCL), may be used for the intravenous drug delivery. PEG and PEO denote essentially identical polymers. The only difference between the respective notations is that methoxy groups in PEO may replace the terminal hydroxyls of PEG. It has been pointed out that PEG coating of nanospheres provides protection against interaction with the blood components, which induce removal of the foreign particles from the blood. It prolongs, therefore, their circulation in the blood stream. In consequence, thus coated nanospheres may function as circulation depots of the administered drugs. Slowly releasing drugs into plasma and thus altering their concentration profiles can achieve obvious therapeutical benefits. About 200 nm in diameter PEG-coated nanospheres, in which PEG is chemically bound to the core have been prepared, in the presence of monomethoxy PEG, by ring opening polymerization (with stannous octoate as a catalyst) of such monomers as ϵ -caprolactone, lactide, and/or glycolide. Ring opening polymerization of these monomers in the presence of such multifunctional hydroxy acids as citric or tartaric, to which several molecules of the monomethoxy monoamine of PEG (MPEG-NH₂) have been attached, yields multiblock (PEG)_n-(X)_m copolymers. PEG-PLA copolymer in which NH₂ terminated methoxy PEG molecules have been attached to tartaric acid.

The nanoparticles, prepared using equimolar amounts of the PLLA-PEG and PDLA-PEG stereoisomers, are shaped as discs with PEG chains sticking out from their surface.

Their hydrophobic/hydrophilic content seems to be just right for applications in cancer and gene therapies. Such nanospheres are prepared by dispersing the methylene chloride solution of the copolymer in water and allowing the solvent to evaporate.

5. Poly (isobutylcyanoacrylate) nanocapsules

Intragastric administration of insulin-loaded poly (isobutylcyanoacrylate) nanocapsules induced a reduction of the glycemia to normal level in streptozotocin diabetic rats and is alloxan induced diabetic dogs. The hypoglycemic effect was characterized by surprising events including a lag time period of 2 days and a prolonged effect over 20 days. Insulin is a very hydrosoluble peptide and should be inactivated by the enzymes of the gastrointestinal tract. Thus, the reason why insulin could be encapsulated with high efficiency in nanocapsules containing an oily core and why these nanocapsules showed so unexpected biological effect remained unexplained. Nanocapsules can be prepared by interfacial polymerization of isobutylcyanoacrylate. Any nucleophilic group including those of some of the aminoacids of insulin could initiate the polymerization of such a monomer. In this case insulin could be found covalently attached to the polymer forming the nanocapsule wall as it was recently demonstrated with insulin-loaded nanospheres.

6. Poly(γ -benzyl-L-glutamate)/poly(ethylene oxide)

Nanoparticles have been widely investigated as the drug carriers. Biodegradable poly (D,L-lactide) polybutylcyanoacrylate and poly(ϵ -caprolactone) are widely being used to prepare nanoparticles. The advantages of the nanoparticles are the reduced drug toxicity, the improvement of biodistribution, and the increased therapeutic efficacy. Diblock copolymers have been studied in the sustained release system as an alternative drug carrier, since they are known to form a micelle structure. Hydrophilic-hydrophobic diblock copolymers exhibit amphiphilic behavior and form micelles with core-shell architecture. These polymeric carriers have been used to solubilize hydrophobic drugs, to increase blood circulation time, to obtain favorable biodistribution and to lower interactions with reticuloendothelial system. The nanoparticles are obtained from poly(γ -benzyl-L-glutamate)/poly(ethylene oxide) [PBLG/PEO] diblock copolymer, which form a hydrophobic inner core and a hydrophilic outer shell of micellar structure, by adopting dialysis procedure. Their results indicate that only 20% of the entrapped drug was released in 24 hr at 37 °C and the release were dependent on the molecular weight of hydrophobic polymer.

7. Chitosan-poly(ethylene oxide) nanoparticles

Hydrophilic nanoparticle carriers have important potential applications for the administration of therapeutic molecules. Most of the recently developed hydrophobic-hydrophilic carriers require the use of organic solvents for their preparation and have a limited protein-loading capacity. A new approach for the preparation of nanoparticles made solely of hydrophilic polymer, to address these limitations. The preparation technique, based on an ionic gelation process, is extremely mild and involves the mixture of two aqueous phases at room temperature. One phase contains the polysaccharide chitosan (CS) and a diblock copolymer of ethylene oxide and polyanion sodium tripolyphosphate (TPP). It was stated that, the size (200-1000 nm) and zeta potential (between + 20mv and +60mv) of nanoparticles can be conventionally modulated by varying the ratio CS/PEO-PPO. Furthermore, using bovine serum albumin (BSA) as a model protein, it was shown that these new nanoparticles have great protein loading capacity (entrapment efficiency up to 80% of the protein) and provide a continuous release of the entrapped protein for up to 1 week.

8. Methotrexate-o-carboxymethylate chitosan

Nanoparticles of methotrexate (MTX) were prepared using o-carboxymethylate chitosan (o-CMC) as wall forming materials, and an isoelectric-critical technique under ambient condition. Drug controlled releases were studied in several media including simulated gastric fluid, intestinal fluid and 1% fresh mice serum. It was found that acidic media provide a fast release rate than neutral media. The effect of MTX/o-CMC ratio and amount of crosslinking agents of drug release in different media were evaluated. The changes of size and effective diameter of o-CMC nanoparticles were detected by SEM and laser light scattering system before and after the drug release. The author claimed that, the o-CMC nanoparticles

constitute an attractive alternative to other anticancer drugs and enzyme carriers.

Methods of Preparation of Nanoparticles¹

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including:

- 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,
- e) Biocompatibility and toxicity;
- f) Drug release profile desired; and
- g) Antigenicity of the final product.

Nanoparticles have been prepared 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 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.

1. Dispersion of preformed polymers

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

2. Solvent evaporation method

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 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.

3. Spontaneous emulsification or solvent diffusion method

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 an oil 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 water miscible 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.

4. Polymerization method

In this method, monomers are polymerized to form nanoparticles 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. Nanocapsule formation and their particle size depend on the concentration of the surfactants and stabilizers used.

5. Coacervation or ionic gelation method

Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. 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. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.

6. Production of nanoparticles using supercritical fluid technology

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. 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 in 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.

Characterisation of Nanoparticles⁴

Particles with a very small size (<100nm), low charge, and a hydrophilic surface are not recognised by the mononuclear phagocytic system (MPS) and, therefore, have a long half life in the blood circulation which is essential for targeting NPs to target brain.

Characterization is done by using a variety of different techniques, mainly drawn from materials science.

Common techniques ⁴

- (1) Electron microscopy [TEM,SEM]
- (2) Atomic force microscopy [AFM]
- (3) Dynamic light scattering [DLM]
- (4) X-ray photoelectron spectroscopy [XPS]
- (5) Powder x-ray diffractometry [XRD]

Effect of Characteristics of Nanoparticles on Drug Delivery¹

1. Particle size

Particle size and size distribution are the most important characteristics of nanoparticle systems. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. 100 nm nanoparticles had a 2.5 fold greater uptake than 1 μ m microparticles, and 6 fold greater uptake than 10 μ m microparticles in a Caco-2 cell line. In a subsequent study, the nanoparticles penetrated throughout the submucosal layers in a rat in situ intestinal loop model, while microparticles were predominantly localized in the epithelial lining. It was also reported that nanoparticles can cross the blood-brain barrier following the opening of tight junctions by hyper osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors. Tween 80 coated nanoparticles have been shown to cross the blood-brain barrier. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability.

Polymer degradation can also be affected by the particle size. For instance, the rate of PLGA polymer degradation was found to increase with increasing particle size *in vitro*. It was thought that in smaller particles, degradation products of PLGA formed can diffuse out of the particles easily while in large particles, degradation products are more likely remained within the polymer matrix for a longer period to cause autocatalytic degradation of the polymer material. Therefore, it was hypothesized that larger particles will contribute to faster polymer degradation as well as the drug release.

Currently, the fastest and most routine method of determining particle size is by photon-correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM).

2. Surface properties of nanoparticles

When nanoparticles are administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation. Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This in turn influences the *in vivo* fate of nanoparticles. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system (MPS) such as liver, spleen, lungs and bone marrow. Indeed, once in the blood stream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the macrophages of MPS rich organs. Generally, it is IgG, complement C3 components that are used for recognition of foreign substances, especially foreign macromolecules. Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles *in vivo*. This can be achieved by

1. Surface coating of nanoparticles with hydrophilic polymers/surfactants;
2. Formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG),

polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80).

Studies show that PEG conformation at the nanoparticle surface is of utmost importance for the opsonin repelling function of the PEG layer. PEG surfaces in brush-like and intermediate configurations reduced phagocytosis and complement activation whereas PEG surfaces in mushroom-like configuration were potent complement activators and favoured phagocytosis.

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. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the nanocapsule or adsorbed onto the surface.

3. Drug loading

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods:

- Incorporating at the time of nanoparticles production (incorporation method)
- Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption/absorption technique).

Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end functional groups (ester or carboxyl). The PEG moiety has no or little effect on drug loading. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption. For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading.

4. Drug release

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on:

- (1) Solubility of drug
- (2) Desorption of the surface bound/ adsorbed drug
- (3) Drug diffusion through the nanoparticle matrix
- (4) Nanoparticle matrix erosion/degradation
- (5) Combination of erosion/diffusion process

Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. In the case of nanospheres, where the drug is uniformly distributed, the release occurs by diffusion or erosion of the matrix under sink conditions. If the diffusion of the drug is faster than matrix erosion, the mechanism of release is largely controlled by a diffusion process. The rapid initial release or 'burst' is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles. It is evident that the method of incorporation has an effect on release profile. If the drug is loaded by incorporation method, the system has a relatively small burst effect and better sustained release characteristics. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. The membrane coating acts as a barrier to release, therefore, the solubility and diffusivity of drug in polymer membrane becomes determining factor in drug release. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxiliary

ingredients. When the drug is involved in interaction with auxiliary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect; whereas if the addition of auxiliary ingredients e.g., addition of ethylene oxide-propylene oxide block copolymer (PEO-PPO) to chitosan, reduces the interaction of the model drug bovine serum albumin (BSA) with the matrix material (chitosan) due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be observed.

Various methods which can be used to study the *in vitro* release of the drug are:

1. Side-by-side diffusion cells with artificial or biological membranes;
2. Dialysis bag diffusion technique;
3. Reverse dialysis bag technique;
4. Agitation followed by ultracentrifugation/centrifugation;
5. Ultra-filtration or centrifugal ultra-filtration techniques.

Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.

Types Of Nanoparticles^{2,4,11,12}.

1. Liposome
2. Gliadin Nanoparticles
3. Polymeric Nanoparticles
4. Solid Lipid Nanoparticles (SLN)
5. Others-gold, carbon, silver, etc.
6. Nanoparticles and nanospheres

1. Liposomes

A **liposome** is a spherical vesicle with a membrane composed of phospholipids bilayer used to deliver drugs or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg, phosphatidylethanolamine), or of pure components like DOPE (dioleoylphosphatidylethanolamine). The lipid bilayer can fuse with other bilayers (e.g., the cell membrane), thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs, (which would normally be unable to diffuse through the membrane), they can be (indiscriminately) delivered past the lipid bilayer.

2. Gliadin nanoparticles

In an effort to improve bioavailability anti-H.pylori effects of antibiotics, mucoadhesive gliadin nanoparticles (GNP) which have the ability to deliver the antibiotics at the sites of infection were prepared. GNP bearing clarithromycin (CGNP) and omeprazole (OGNP) were prepared by desolvation method. In vivo gastric mucoadhesive studies confirmed the strong mucoadhesive propensity and specificity and specificity of gliadin nanoparticles towards stomach. Gliadin nanoparticles show a higher tropism for the gastrointestinal regions and their presence in other intestinal regions is very low. This high capacity to interact with the mucosa may be explained by gliadin composition. In fact, this protein is rich in neutral and lipophilic residues. Neutral amino acid can promote hydrogen bonding interaction with the mucosa whereas the lipophilic components can interact within biological tissue by hydrophilic interaction. The related protein gliadin possessing an amino and disulphide groups on the side chain has a good probability of developing bonds with mucin gel.

3. Polymeric Nanoparticles

Polymeric nanoparticles have been invented by Speiser et al. They represent interesting alternative as drug delivery systems to liposomes. They usually exhibit a long shelf life and a good stability

on storage. These are superior to liposomes in targeting them to specific organs or tissues by adsorbing and coating their surface with different substances. Nanoparticles can be prepared either from preformed polymers, such as polyesters (i.e. polylactic acid), or from a monomer during its polymerization, as in the case of alkyl-cyanoacrylates. Most of the methods based on the polymerization of monomers consists in adding a monomer into the dispersed phase of an emulsion, an inverse microemulsion, or dissolved in a non-solvent of the polymer.

4. Solid Lipid Nanoparticles (SLN)

Solid lipid nanoparticles have been developed as alternative delivery system to conventional polymeric nanoparticles. SLNs are sub-micron colloidal carriers (50-1000nm) which are composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. SLNs combine advantages of polymeric nanoparticles, fat emulsions and liposomes, but avoid some of their disadvantages. They are biodegradable, biocompatible and non-toxic.

5. Gold Nanoparticles May Simplify Cancer Detection⁴

Binding gold nanoparticles to a specific antibody for cancer cells could make cancer detection much easier. A common synthesis involves the reduction of a gold salt in the presence of capping agent molecules such as thiols, citrates or phosphines. The functionalities of these capping agents can be altered to yield various chemical properties.

The synthesis of gold nanoparticles with a polymer-thiol monolayer involves the mechanism of particle formation in the presence of bulky ligands. TEM has been used extensively as a way of characterizing the particles. Figure shows an example of TEM imaged particle.

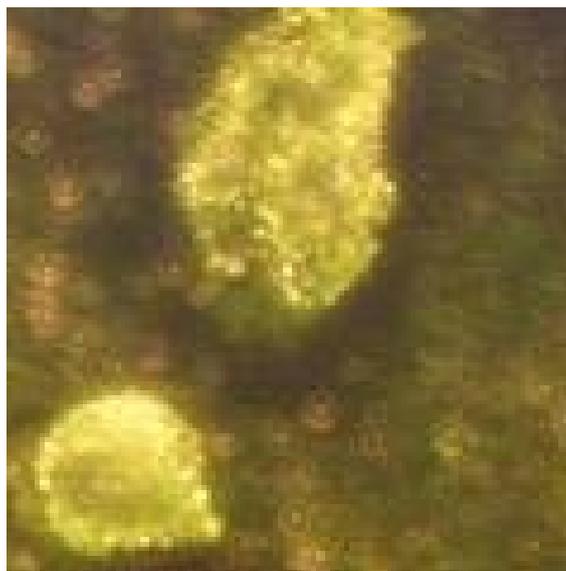


Fig. 2: Gold nanoparticles stick to cancer cells and make them shine

“Gold nanoparticles are very good at scattering and absorbing light. Many cancer cells have a protein, known as Epidermal Growth Factor Receptor (EGFR), all over their surface. By conjugating, or binding, the gold nanoparticles to an antibody for EGFR, suitably named anti-EGFR, researchers were able to get the nanoparticles to attach themselves to cancer cells. Gold nanoparticles don't stick as well to noncancerous cells. The results can be seen with a simple microscope. In the study, researchers found that the gold nanoparticles have 600 percent greater affinity for cancer cells than for noncancerous cells. The particles that worked the best were 35 nanometers in size. Researchers tested their technique using cell cultures of two different types of oral cancer and one nonmalignant cell line. The shapes of the strong absorption spectrum of the gold nanoparticles are also found to distinguish between cancer cells and noncancerous cells.

6. Nanoparticles and Nanospheres^{4,8}

Nanoparticles were first developed around 1970. They were initially devised as carriers for vaccines and anticancer drugs. In order to enhance tumor uptake, the strategy of drug targeting was employed, and as a first important step, research focused on the development of methods to reduce the uptake of the nanoparticles by the cells of the reticuloendothelial system (RES). Simultaneously, the use of nanoparticles for ophthalmic and oral delivery was investigated.

7. Nano-capsules based drug delivery system^{4,10,12}

a) Introduction to nanocapsules

Nano-capsules have been made for many years following the example of nature, using molecules called phospholipids, which are hydrophobic on one end and hydrophilic on the other. When these molecules are placed in an aqueous environment, they can spontaneously form capsule in which hydrophobic portions are inside. Nano-capsules are vesicular system in which drug molecule is embedded in an aqueous or oily cavity surrounded by a single polymeric membrane. Nano- capsule may, thus be considered as a 'reservoir system'.

b) Overview of types of nanoparticles used as Nanocapsule in drug Delivery

Liposomes are micro- or nanoparticulate vesicles formed by self assembly of natural molecules such as phospholipids, cholesterol etc or synthetic amphiphiles in aqueous environment. In particular, liposome have been recognized as an effective nanoparticle drug delivery system and extensively used in research, analytical and therapeutic applications. Liposomes are extensively used as drug carrier.

Their amphiphilic properties make them versatile carrier of either water soluble or lipid soluble drug. Entrapped drug is protected from enzymes and metabolism, and can not be active until released. The ability of liposomes to alter drug pharmacokinetics makes it as an ideal drug carrier. The increased use of liposome as a drug carrier is due to its ability to increase drug concentration at targeted site and by decreasing drug concentration in sensitive normal tissues resulting in increased therapeutic index and reduces unwanted side effects.

Stealth liposomes: The major problem with liposomes are, they recognized by immune system as a foreign product and quickly removed from circulation before significant delivery of drug. Recently, lots of research has been done to develop so called stealth particles, which are invisible to macrophages. Stealth particles are composed of lipid particles that incorporate the polymers like polyethylene glycol (PEG) gangliosides coating. This coating evades the potential impact of immune system. The design of such carriers is based on the physico-chemical concept of steric repulsion. The PEG coating on liposomes reduces the adsorption of steric proteins due to steric hindrance. This approach allows stealth particles to remain in blood for long time and also offers protection from immune system. Stealth particles have ligands on their surface that target receptors expressed on diseased cells. For the disease of vasculature origin, stealth liposomes provide the best therapeutic effect over conventional drug delivery system.

Ceramic Nano-particles are made from silica and alumina. They make the entrapped drug invisible to immune system and protect from degradation. Although they are stable in a range of temperature and pH their slow dissolution raises questions.

Dendrimers are artificial polymers. The hollow space within it provides great potential for targeted delivery.

Hydrogels are natural polymer amphiphiles where cholesterol groups provide covalent cross linking. Hydrogels have good bioavailability but are quite unstable.

Micelles are an amphiphilic molecule that includes pluronics. They are thermo stable and can carry water insoluble drugs. It may protect the drug from enzyme and pH action. They can be complexed to ligands combining target ability with stimuli sensitivity.

Nanocrystals are aggregates of molecules with thin surfactant coating. The advantages of nanocrystals include, high dosages can be achieved and poorly soluble drugs can be formulated for improved bioavailability. Both oral and parenteral delivery can be achieved but poor stability is major limitation with the use of nanocrystals.

Nanotubes are self assembling sheets of atoms arranged in tubes. Researchers have discovered carbon nanotubes can enter the nuclei of cells and this ability of nanotubes may be used to deliver drugs and vaccines. Nanotubes have large

Internal space and external surface can be easily functionalized.

Solid lipid nanoparticles are lipid based submicron colloidal carriers. They require high amount of surfactants for stability. As compared to the polymer they are less toxic and can be used by various routes like oral, topical or pulmonary.

c) Drug release mechanisms from the Nano-capsules

Release of drug from the carrier is important step in nano-capsule based drug delivery system. PH controlled release of drug from carrier is one of the promising approaches to cancer therapy. In this approach acid-sensitive spacers are incorporated between drug and carrier enables release of an active drug from the carrier in a tumor tissue, either in slightly acidic extra cellular tissue fluids or after endocytosis, in endosomes or lysosomes of cancer cells.

In another innovative approach Disulfide bonds are used to assemble nanomolecular capsule around the drug of choice by linking capsule subunit together using disulfide bonds. Disulfide bonds are very stable in blood stream but reduction of the disulfide bond occurs in presence of glutathione. This may be basic principle behind drug release from disulfide nano-capsule. Tumour cells have large amount of glutathione and when nanocapsule reaches tumour site it release drug because of reduction in disulfide bonds.

Magnetic field can be used for drug release. A focused magnetic field selectively activates the magnetic particle present in the nanocapsules. The magnetic field energy is converted to heat by magnetic particles causing a rapid temperature increase with resulting drug release. Incorporation of magnetic nanoparticle is not only useful for producing hyperthermia and magnetically guided drug release but also it gives enhanced and targeted Magnetic Resonance Imaging (MRI).

d) Characterizations of the nanocapsules

The sample preparation for examining the morphology, size range and structural information of the nanocapsules with selected anticancer drug in it involve dispersing them in hexane and deionised water respectively. A few drops of the liquid containing the dispersed nanocapsules are studied using transmission electron microscopy. Chemical absorption can be studied using Fourier transform infrared spectroscopy (FTIR). A simple experiment can be performed to study retention of magnetism of magnetic particle when encapsulated in hybrid nanocapsules. This experiment involves application of external magnetic field to the container holding the nanocapsules. The intensity with which nano capsules gets attracted towards magnetic field may determine the magnetism of nanocapsules

In vivo evaluation

- 1 Drug distribution
- 2 Drug delivery
- 3 Efficacy

Following are various methods that can be used for characterization of nanocapsules:

- 1 Transmission Electron Microscopy
- 2 Electron Scanning Environmental Microscopy
- 3 Raman Spectroscopy
- 4 Thermal analysis

Generally the drug release behaviour depends on a various factors including particle size, surface properties, degradation rate, and interaction force of the drug binding to the surface. Therefore it is necessary to study characterization of nanocapsules which gives evidence to move to the next step of the drug development which is *in vitro* testing.

In vitro evaluation

In vitro cytotoxicity assessment of nanocapsules with anticancer drug in it can be done on human carcinoma cells. Assay involves seeding of approximately 500 to 1000 human carcinoma cells per well in a 96 well plate in 200 μ L growth medium. After one day nanocapsule formulation have to be added at the indicated concentration and incubated for 4 days at 37°C. Sulforhodamine B assay can be used to measure tumour cell survival.

Result can be obtained by fitting the data to a sigmoidal dose response. Alternatively cytotoxicity can be measured on different hepatoma cell lines by calculating IC50 that is the 50% inhibiting concentration. In this experiment cells will be incubated for specific time with increasing concentration of nanocapsules drug against standard drug. Cells viability can be measured by neutral red assays. Results can be plotted with Excel software.

Intracellular accumulation of drugs

107 cells can be used to study the intracellular drug accumulation. The experiment involves incubation of nanocapsules containing drugs for 2 hours at concentration of 1, 10 and 50 μ mol/L. Immediately after incubation cells are washed twice with ice cold Phosphate Buffer Saline (PBS), harvested by scraping in ice cold PBS, centrifuged and resuspended in 1 ml milli-Q 800 μ L sample will have to dry by overnight centrifugation under vacuum and cell pellets formed will be digested in 65% v/v nitric acid at 75°C for 2 hour. After dilution in water drug content can be analyzed by non flame atomic absorption spectroscopy.

In vitro drug release

It involves five different sets of experiments. They include three different temperatures 40, 37 and 20°C and two different pH 5.3 and pH 7.4. Each experiment has similar procedure with 3.0 mg of the drug encapsulated in nanocapsules sealed in a dialysis membrane tube. The dialysis tube is submerged into 10 ml of Na2HPO4-KH2PO4 buffer solution which is placed in test tube with a closer. The test tube with closer is placed in a water bath maintained at 40°C, 37°C and 20°C. The release medium is withdrawn at predetermined time intervals. And the amount of free drugs in the buffer solution can be quantified using Lambert-Beer law.

In vivo evaluation

In vitro testing of product is only one phase of the clinical development. The next phase is testing its performance in the intended application environment that is, *In vivo* assessment.

There are several animal systems with which drug delivery, distribution and efficacy in pre clinical trial can be measured and explored.

Potential Applications

- 1 Biological toxin exposures
- 2 Radiological toxin exposure and radioprotection
- 3 Internal hemorrhage
- 4 Brain swelling
- 5 Stroke therapy
- 6 Cancer therapy
- 7 Acute trauma leading to kidney failure

Solid lipid nanoparticles^{2,11,12}

The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could be used for secondary and

tertiary levels of drug targeting. Hence, solid lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and hence have attracted wide attention of researchers. Different production methods which are suitable for large scale production and applications of solid lipid nanoparticles are described.

Appropriate analytical techniques for characterization of solid lipid nanoparticles like photon correlation spectroscopy, scanning electron microscopy, differential scanning calorimetry are highlighted. Aspects of solid lipid nanoparticles route of administration and their biodistribution are also incorporated. If appropriately investigated, solid lipid nanoparticles may open new vistas in therapy of complex diseases.

SLNs are attracting major attention as novel colloidal drug carrier for intravenous applications. The SLNs are sub-micron colloidal carrier which is composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. The Pubmed search till the date indicates the trends in SLN research, so if systematically investigated, SLNs may open new vista in research and therapy.

1. Nanostructure lipid carriers (NLC)

NLC were introduced to overcome the potential difficulties with SLNs. The goal was to increase the drug loading and prevent drug expulsion. This could be visualized in three ways. In the first model, spatially different lipids (like glycerides) composed of different fatty acids are mixed. The use of spatially different lipids leads to larger distances between the fatty acid chains of the glycerides and general imperfections in the crystal and thus provides more room for accommodation of guest molecules. The highest drug load could be achieved by mixing solid lipids with small amounts of liquid lipids (oils). This model is called imperfect type NLC. Drugs showing higher solubility in oils than in solid lipids can be dissolved in the oil and yet be protected from degradation by the surrounding solid lipids. These types of NLC are called multiple types NLC, and are analogous to w/o/w emulsions since it is an oil-in-solid lipid-in-water dispersion.

SLN Preparation: SLNs are made up of solid lipid, emulsifier and water/solvent. The lipids used may be triglycerides (tri-stearin), partial glycerides (Imwitor), fatty acids (stearic acid, palmitic acid), and steroids (cholesterol) and waxes (cetyl palmitate). Various emulsifiers and their combination (Pluronic F 68, F 127) have been used to stabilize the lipid dispersion. The combination of emulsifiers might prevent particle agglomeration more efficiently. A clear advantage of SLN is the fact that the lipid matrix is made from physiological lipids which decreases the danger of acute and chronic toxicity. The choice of the emulsifier depends on the administration route with a suitable number of emulsifier suitable for parenteral administration.

Method of SLN Preparation⁴

1. High shear homogenization

High shear homogenization technique was initially used for the production of solid lipid nanodispersions. Both methods are widespread and easy to handle. However, dispersion quality is often compromised by the presence of micro particles. High-speed homogenization method is used to produce SLN by melt emulsification. Olbrich *et al.* investigated the influence of different process parameters, including emulsification time, stirring rate and cooling condition on the particle size and zeta potential. Lipids used in this study included trimyristin, tripalmitin, a mixture of mono, di and triglycerides (Witepsol W35, Witepsol H35) with glycerol behenate and poloxamer 188 used as steric stabilizers (0.5% w/w). For Witepsol W35 dispersions the best SLN quality was obtained after stirring for 8 min at 20,000 rpm followed by cooling 10 min and stirring at 5000 rpm at a room temp. In contrast, the best conditions for Dynasan 116 dispersions were a 10-min emulsification at 25,000 rpm and 5 min of cooling at 5,000 rpm in cool water ($\approx 16^\circ$). Higher stirring rates did not significantly change the particle size, but slightly improved the polydispersity index.

2. Hot homogenization

Hot homogenization is carried out at temperatures above the melting point of the lipid and is similar to the homogenization of an emulsion. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device (like silverson-type homogenizer). The quality of the pre-emulsion affects the quality of the final product to a great extent and it is desirable to obtain droplets in the size range of a few micrometers. High pressure homogenization of the pre-emulsion is done above the lipid melting point. Usually, lower particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase, although this might also accelerate the drug and carrier degradation. Better products are obtained after several passes through the high-pressure homogenizer (HPH), typically 3-5 passes. High pressure processing always increases the temperature of the sample (approximately 10° at 500 bar). In most cases, 3-5 homogenization cycles at 500-1500 bar are sufficient. Increasing the homogenization leads to an increase of the particle size due to particle coalescence, this occurs because of the high kinetic energy of the particles.

3. Cold homogenization

The cold homogenization process is carried out with the solid lipid and therefore is similar to milling of a suspension at elevated pressure. To ensure the solid state of the lipid during homogenization, effective temperature regulation is needed. Cold homogenization has been developed to overcome the following problems of the hot homogenization technique such as: Temperature mediated accelerated degradation of the drug payload, Partitioning and hence loss of drug into the aqueous phase during homogenization, Uncertain polymorphic transitions of the lipid due to complexity of the crystallization step of the nanoemulsion leading to several modifications and/or super cooled melts. The first preparatory step is the same as in the hot homogenization procedure and includes the solubilization or dispersion of the drug in the lipid melt. However, the subsequent steps differ. The drug containing melt is cooled rapidly (using dry ice or liquid nitrogen) to favor homogenous drug distribution in the lipid matrix. In effect, the drug containing solid lipid is pulverized to microparticles by ball/mortar milling. Typical particle sizes attained are in the range 50-100 microns. Chilled processing further facilitated particle milling by increasing the lipid fragility. The SLNs are dispersed in a chilled emulsifier solution. The dispersion is subjected to high pressure homogenization at or below room temperature with appropriate temperature control keeping in view the usual rise in temperature during high pressure processing. However, compared to hot homogenization, larger particle sizes and a broader size distribution are typical of cold homogenized samples. The method of cold homogenization minimizes the thermal exposure of the sample, but it does not avoid it due to the melting of the lipid/drug mixture in the initial step.

4. Ultrasonication or high speed homogenization

SLN were also developed by high speed stirring or sonication. A most advantages are that, equipments that are used here are very common in every lab. The problem of this method is broader particle size distribution ranging into micrometer range. This lead physical instability likes particle growth upon storage. Potential metal contamination due to ultrasonication is also a big problem in this method. So for making a stable formulation, studies have been performed by various research groups that high speed stirring and ultrasonication are used combined and performed at high temperature.

5. Spray drying method

It's an alternative procedure to lyophilization in order to transform an aqueous SLN dispersion into a drug product. It's a cheaper method than lyophilization. This method cause particle aggregation due to high temperature, shear forces and partial melting of the particle. The use of lipid with melting point >70 ° for spray drying. The best result was obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in ethanol-water mixtures (10/90 v/v).

6. Applications of Nanoparticulate Drug Delivery Systems ^{1,2,9-11}

1. Medicine

The biological and medical research communities have exploited the unique properties of nanomaterials for various applications (e.g., contrast agents for cell imaging and therapeutics for treating cancer). Terms such as biomedical nanotechnology, bionanotechnology, and nanomedicine are used to describe this hybrid field. Functionalities can be added to nanomaterials by interfacing them with biological molecules or structures. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. Thus far, the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug-delivery vehicles.

2. Diagnostics

Nanotechnology-on-a-chip is one more dimension of lab-on-a-chip technology. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive and more flexible when certain nanoscale particles are put to work as tags or labels. Magnetic nanoparticles, bound to a suitable antibody, are used to label specific molecules, structures or microorganisms. Gold nanoparticles, tagged with short segments of DNA can be used for detection of genetic sequence in a sample. Multicolor optical coding for biological assays has been achieved by embedding different-sized quantum dots, into polymeric micro beads. Nanopore technology for analysis of nucleic acids converts strings of nucleotides directly into electronic signatures.

3. Drug delivery

The overall drug consumption and side-effects can be lowered significantly by depositing the active agent in the morbid region only and in no higher dose than needed. This highly selective approach reduces costs and human suffering. An example can be found in dendrimers and nanoporous materials. They could hold small drug molecules transporting them to the desired location. Another vision is based on small electromechanical systems: NEMS are being investigated for the active release of drugs. Some potentially important applications include cancer treatment with iron nanoparticles or gold shells. A targeted or personalized medicine reduces the drug consumption and treatment expenses resulting in an overall societal benefit by reducing the costs to the public health system.

4. Tissue engineering

Nanotechnology can help to reproduce or to repair damaged tissue. This so called "tissue engineering" makes use of artificially stimulated cell proliferation by using suitable nanomaterial-based scaffolds and growth factors. Tissue engineering might replace today's conventional treatments, e.g. transplantation of organs or artificial implants. On the other hand, tissue engineering is closely related to the ethical debate on human stem cells and its ethical implications

5. Tumor targeting using nanoparticulate delivery systems⁵

The rationale of using nanoparticles for tumor targeting is based on 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 targeting by ligands on the surface of nanoparticles;

6. Long circulating nanoparticles

To be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. In the past decade, a great deal of work has been devoted to developing so-called "stealth" particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes. A major breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamines, poloxamers,

and polysaccharides) to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the MPS. These coatings provide a dynamic "cloud" of hydrophilic and neutral chains at the particle surface which repel plasma proteins. As a result, those coated nanoparticles become invisible to MPS, therefore, remained in the circulation for a longer period of time. Hydrophilic polymers can be introduced at the surface in two ways, either by adsorption of surfactants or by use of block or branched copolymers for production of nanoparticles.

7. Reversion of multidrug resistance in tumour cells

Anticancer drugs, even if they are located in the tumour interstitium, can turn out to be of limited efficacy against numerous solid tumour types, because cancer cells are able to develop mechanisms of resistance. These mechanisms allow tumors to evade chemotherapy. Multidrug resistance (MDR) is one of the most serious problems in chemotherapy. MDR occurs mainly due to the over expression of the plasma membrane glycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells. In order to restore the tumoral cells' sensitivity to anticancer drugs by circumventing Pgp-mediated MDR, several strategies including the use of colloidal carriers have been applied.

8. Nanoparticles for oral delivery of peptides and proteins

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.

9. 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 surfaces 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.

10. Nanoparticles for drug delivery into the brain

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also

reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps.

11. SLNs as gene vector carrier

SLN can be used in the gene vector formulation. In one work, the gene transfer was optimized by incorporation of a diametric HIV-1 HAT peptide (TAT 2) into SLN gene vector. There are several recent reports of SLN carrying genetic/peptide materials such as DNA, plasmid DNA and other nucleic acids. The lipid nucleic acid nanoparticles were prepared from a liquid nanophase containing water and a water miscible organic solvent where both lipid and DNA are separately dissolved by removing the organic solvent, stable and homogeneously sized lipid-nucleic acid nanoparticle (70-100 nm) were formed. It's called genospheres. It is targeted specific by insertion of an antibody-lipo polymer conjugated in the particle.

12. SLNs as cosmeceuticals

The SLNs have been applied in the preparation of sunscreens and as an active carrier agent for molecular sunscreens and UV blockers. The *in vivo* study showed that skin hydration will be increased by 31% after 4 weeks by addition of 4% SLN to a conventional cream. SLN and NLCs have proved to be controlled release innovative occlusive topical. Better localization has been achieved for vitamin A in upper layers of skin with glyceryl behenate SLNs compared to conventional formulations.

13. Nanosphere Blood Cleansing^{9,13}

Intravenously injected into victims of radiological, chemical or biological attack, biodegradable nanospheres circulate through the bloodstream, where surface proteins bind to the targeted toxins. They are removed from the bloodstream by a small dual-channel shunt, inserted into an arm or leg artery that circulates the blood through an external magnetic separator. Strong magnets in the shunt immobilize the iron-based particles, and clean blood flows back into the bloodstream.

CONCLUSION

The foregoing show that nanoparticulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs. 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.¹

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