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Review

NANOPARTICLES AS CARRIERS FOR DRUG TARGETING

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Abstract

In conventional dosage forms, only a small amount of administered dose reaches the target site. While majority of the drug distributes throughout the rest of the body in accordance with its physiochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task. One approach is the use of colloidal drug carriers that can provide site specific or targeted drug delivery with optimal drug release profiles. Among these carriers, nanoparticles have been the most extensively investigated. The present review outlines the advantages and disadvantages of nanoparticles with its preparation methods, drug release and therapeutic applications published over the past decade. From the literature survey, it is realized that research activities on nanoparticulate systems containing various drugs for different therapeutic applications have increased at the rapid rate. Hence, it may be used as a new alternative and cheaper carrier in therapy for reduction in dose and thereby dose related systemic toxicities.

Key Words: Nanoparticles, Colloidal drug carrier, Targeted drug delivery

Introduction

The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage form), only a small amount of administered dose reaches the target site. While majority of the drug distributes throughout the rest of the site in accordance with its physiochemical and biochemical properties. Therefore, developing a drug delivery system that optimizes the pharmaceutical action of a drug while reducing its toxic side effects *in vivo* is a challenging task.

One approach is the use of colloidal drug carriers that can provide site specific or targeted drug delivery combined with optimal drug release profiles. The idea of using submicron drug delivery

system for drug targeting was conceived and developed after Paul Ehrlich originally proposed the idea of tiny drug-loaded magic bullets over a hundred years ago. Among these carriers, liposome and micro/nanoparticles have been the most extensively investigated.¹⁻⁴

Nanoparticles can be defined as solid colloidal particles with diameter ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached.²

The advantages of using nanoparticles as a drug delivery system include the following:^{1,4-7}

- Particle size and surface characteristics of nanoparticles can be easily manipulated to

achieve both active and passive drug targeting after parenteral administration.

- Decreases toxicity and occurrence of adverse drug reactions.
- Better drug utilization.
- They control and sustain the release of the drug during the transportation and at the site of localization. They alter the 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.
- Drug loading is relatively high and drug can be incorporated into the systems without any chemical reaction this is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of the particles or use of magnetic guidance.
- The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular, etc.

In spite of these advantages, nanoparticles have some limitations. Their smaller size and large surface area can lead to particle-particle aggregation makes physical handling of nanoparticles difficult in liquid and dry form.⁴

Types of nanoparticles

There are two types of nanoparticles depending on the preparation process (Fig. 1):

- Nanospheres
- Nanocapsules

The term nanoparticles is a collective name for both nanospheres and nanocapsules.

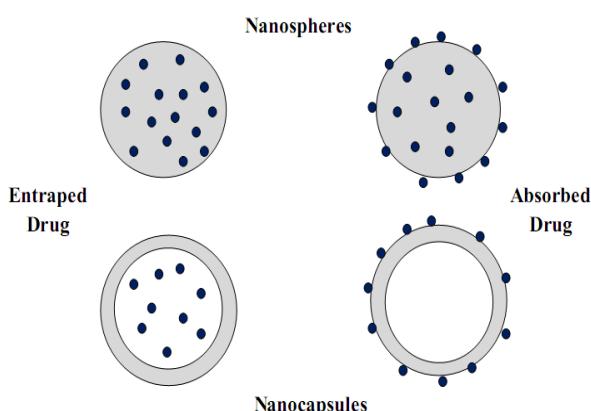


Fig. 1. Types of nanoparticles

Nanospheres: They have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed onto their surfaces or encapsulated within the particles.

Nanocapsules: They are the vesicular system in which the drug is confined to a cavity consisting of an inner liquid core surrounded by a polymeric membrane. In this case the active substance is usually dissolved in the inner core but may also be adsorbed to the capsule surface.^{1,8}

Carriers used in the preparation of nanoparticles

The criteria for selection of ideal polymeric carrier for nanoparticles include:¹

- Ease of synthesis and characterization
- Cost
- Biocompatibility
- Biodegradability
- Non-immunogenicity
- Water solubility

Nanoparticles are prepared using natural hydrophilic or synthetic hydrophobic polymer. The natural hydrophilic polymers include proteins (gelatin, albumin, lecithin, legumin, and vicilin) and polysaccharides (alginate, dextran, chitosan, agarose, and pullulan). These polymers have certain disadvantages such as poor batch to batch reproductivity, the specific conditions for their degradation and potential antigenicity.

The synthetic hydrophobic polymers are divided into two groups, first group include polyesters [poly (ε-caprolactone), poly (lactic acid), poly (lactide-co-glycolide), polystyrene] and the second group include poly (alkyl cyanoacrylates) like poly (isobutyl cyanoacrylates), poly (butyl cyanoacrylates), poly hexyl cyanoacrylates, polymethyl(methcyanoacrylates).^{8,9}

Methods of preparation of nanoparticles

Ionic Gelation

In this method, chitosan is dissolved in aqueous acidic solution to obtain cation of chitosan. This solution is then added droton and precipitates to form spherical particles. Three kinds of phenomena were observed: solution, aggregation and opalescent suspension. The resulting chitosan particle suspension were subsequently centrifuged and dried (Fig. 2).^{10,11}

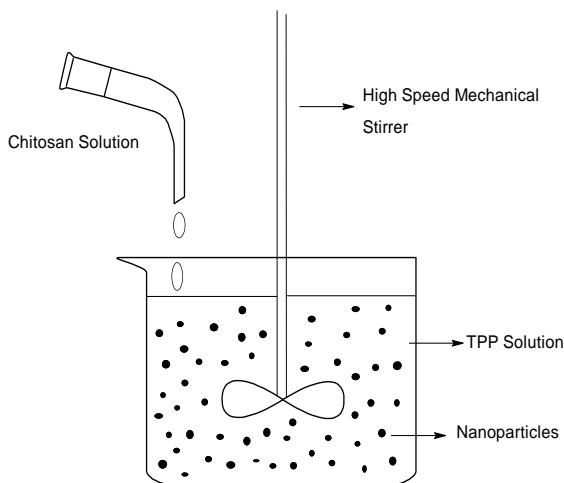


Fig. 2. Diagrammatic representation of preparation of nanoparticles by ionic gelatin method

Emulsion Cross-Linking Method

In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the chitosan solution in the oil phase (Fig. 3). Aqueous droplets are stabilized using a suitable surfactant, the stable emulsion is cross-linked by appropriate cross-linking agent such as glutaraldehyde to harden the droplets, and the particles are filtered and washed repeatedly. By this method particle size can be controlled by controlling the size of the aqueous droplets. However, the particle size of the final product depends upon the extent of the cross-linking agent used while hardening in addition to the speed of stirring during the formation of emulsion. The drawback of this method involves tedious procedure as well as use of harsh cross-linking agents, which might possibly induce chemical reaction with agents, however complete removal of the un-reacted cross-linking agent may be difficult in this process.¹²

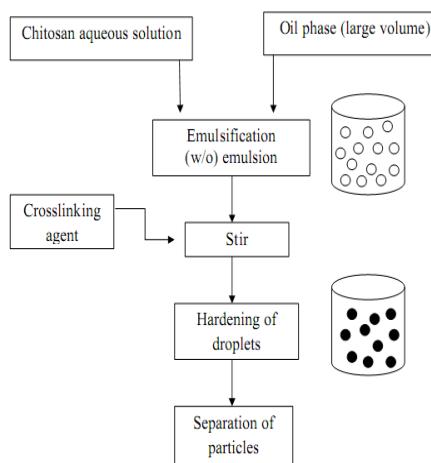


Fig. 3. Diagrammatic representation of preparation of nanoparticles by Emulsion-cross linking method

Nanoprecipitation

The nanoparticle formation is instantaneous and the entire procedure is carried out in only one step. Briefly, it requires two solvents that are miscible. Ideally, both the polymer and the drug must dissolve in the first one (the solvent), but not in the second system (the non-solvent). Nanoprecipitation occurs by a rapid desolvation of the polymer when the polymer solution is added to the nonsolvent.¹³

Coacervation / Precipitation

This method utilizes the physiochemical property of chitosan since it is insoluble in alkaline pH. When it comes in contact with alkaline solution, it precipitates or coacervates. Particles are produced by blowing the chitosan solution into the alkaline solution using a compressed air nozzle to form coacervate droplets. Separation and purification can be done by filtration/centrifugation followed by successive washing with hot and cold water (Fig. 4).¹⁴

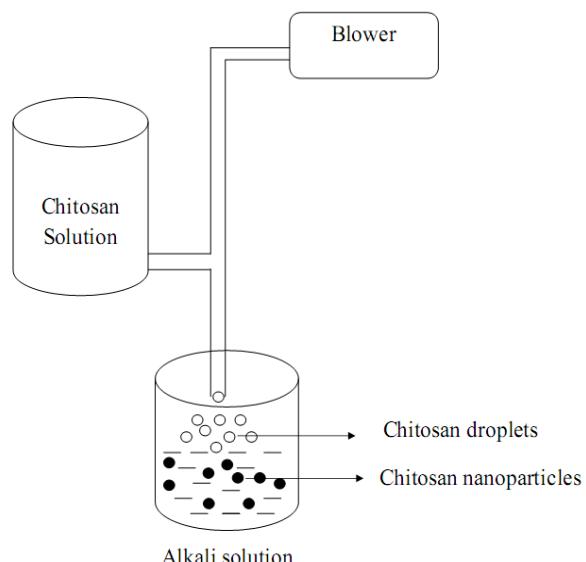


Fig. 4. Preparation of nanoparticles by coacervation precipitation method

Spray-Drying

In this method chitosan is first dissolved in acetic acid, drug is dissolved or dispersed in solution and then, suitable cross-linking agent is added, this solution or dispersion is then atomized in a stream of hot air. Atomization leads to the formation of small droplets, from which solvent evaporates leading to the formation of free flowing powders. Particle size depends upon size of the nozzle, spray flow rate, atomization pressure, and inlet air temperature and extent of cross-linking.¹⁵

Salting -out Method

In this method, acetone is chosen as the water-miscible organic solvent because of its pharmaceutical acceptance with regard to toxicity. The method consists of the addition of water soluble PVA in a highly concentrated salt solution in water (aqueous phase) to a polymer solution in acetone (organic phase). Although acetone is miscible with pure water in all ratios, the high salt concentration of the aqueous phase prevents mixing of the phase. After emulsification, the addition of pure water in a sufficient quantity causes acetone to diffuse into the aqueous phase, resulting in the formation of nanoparticles.^{16,17}

Emulsion-Droplet Coalescence

In this method, instead of cross-linking the stable droplets, precipitation is induced by allowing the coalescence of chitosan droplets with NaOH droplets. First, a stable emulsion containing aqueous solution of chitosan along with drug is produced paraffin oil and then, another stable emulsion containing chitosan aqueous solution of NaOH is produced in same manner. When both the emulsion is mixed under high speed stirring, droplets of each emulsion would collide at random and coalesce, thereby precipitating chitosan droplets to small size particles (Fig. 5).¹⁴

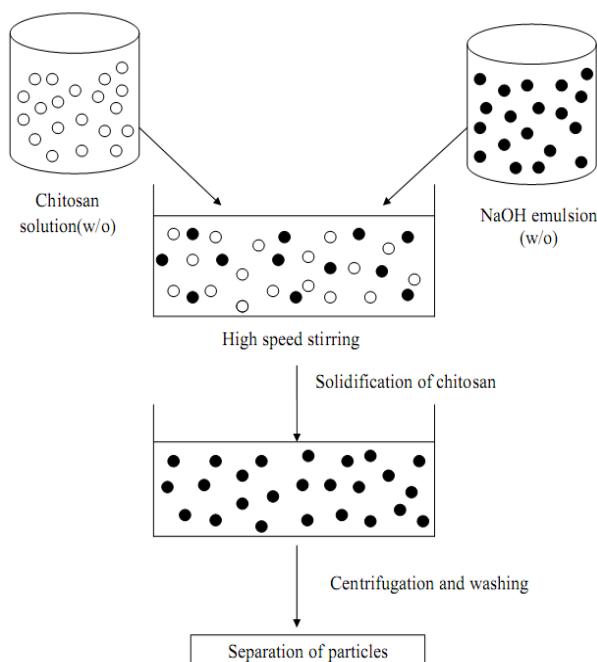


Fig. 5. Preparation of nanoparticles by emulsion-droplet coalescence method

Reverse Micellar Method

In this method, the surfactant is dissolved in an organic solvent to prepare reverse micelles. To this aqueous solution of chitosan and the drug is added with constant vortexing to avoid any turbidity. To this transparent solution, cross-linking agent is added with constant stirring, and cross-linking is achieved by stirring overnight. The organic solvent is then evaporated to obtain a dry mass. The material is dispersed in water and then addition of suitable salts precipitates the surfactant out. The mixture is centrifuged and the supernatant solution is immediately dialyzed and lyophilized to dry powder (Fig. 6).¹⁴

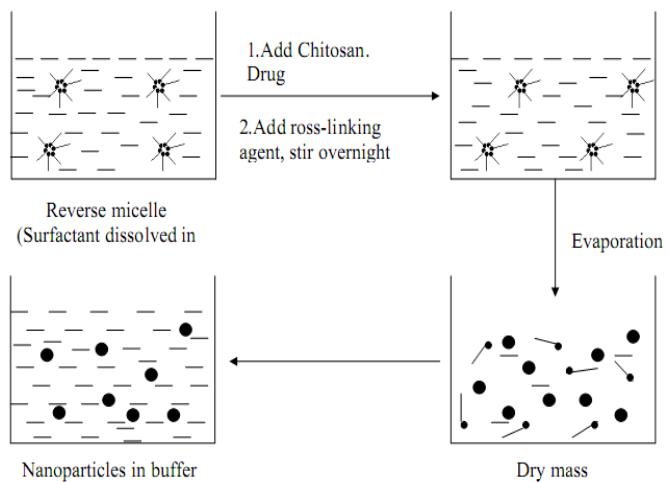


Fig. 6. Reverse micellar method of preparation of nanoparticles

Sieving Method

In this method, nanoparticles are obtained by cross-linking chitosan to obtain a non sticky glassy hydrogel followed by passing through a sieve with suitable mesh size. The nanoparticles are washed with NaOH solution and dried overnight in a hot air oven at 40°C.

Emulsification Diffusion Method

In this method, PLGA is dissolved in measured amount of solvent (ethyl acetate, benzyl alcohol, propylene carbonate, methyl ethyl ketone). This organic phase is added into required amount of aqueous phase containing the stabilizer. After mutual saturation of organic and continuous phase, the mixture is emulsified with a high speed homogenizer. For full diffusion into the water phase, excess amount of water is added to the oil in water emulsion under magnetic stirring, leading to the nanoprecipitation of the polymer.¹⁵

w/o/w Emulsification Solvent Evaporation

In this method measured amount of distilled water (w_1) is emulsified with a solution of PLGA in dichloromethane using an ultrasonic probe. This primary w/o emulsion is poured into a stabilizer solution (w_2) and sonicated. Finally, the w₁/o/w₂ emulsion is poured into a large volume of a stabilizer solution (w_3) in order to increase the distance between emulsion droplets and to minimize coalescence and aggregation of the particles being formed. The preparation is stirred with a propeller and the dichloromethane is allowed to evaporate and the PLGA to precipitate as nanoparticles (Fig. 7).¹⁶

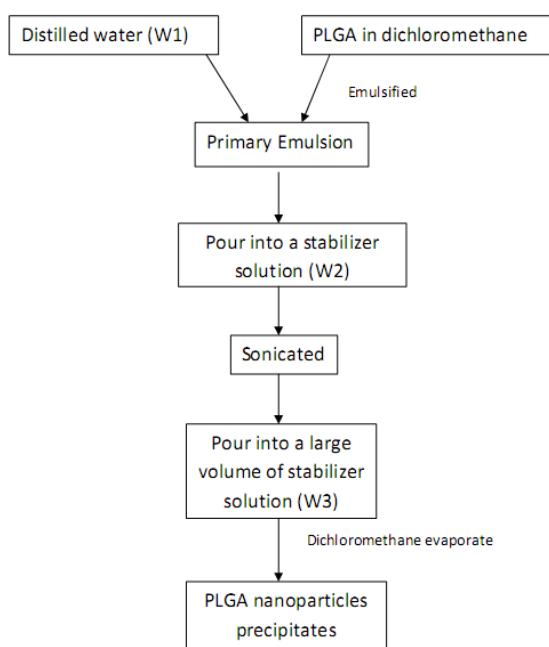


Fig. 7. W/o/w Emulsification solvent evaporation method for preparation of nanoparticles

Polyelectrolyte Complex

Polyelectrolyte complex or self assemble polyelectrolyte is a term describe complexes formed by self-assembly of cationic charged polymer and plasmid DNA. Mechanism of formation involves charge neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self-assemble. This technique offers simple and mild preparation method without harsh conditions involved.¹

Pharmaceutical considerations

Isolation

Nanoparticles are normally isolated by freeze drying using cryoprotective agents (sugars such as

glucose and trehalose) to assess the redispersibility of the colloidal system and to prevent the aggregation of nanoparticles during freeze drying process.⁸

Purification

Nanoparticles should be free from impurities and the degree of purification depends upon the final purpose of the formulation developed. The most commonly used procedures are gel filtration, ultracentrifugation, centrifugal filtration, dialysis and cross-flow filtration.⁷

Stability

Generally a colloidal dispersion is stable and does not tend to separate as a result of slow deposition of particles or due to the mixing tendencies of diffusion and convection. However, some agglomeration can occur. To prevent complete precipitation, it is necessary to incorporate some additives. Chemical integrity of the drug is also a fundamental aspect of the overall stability of nanoparticles. Some other parameters are also crucial for the stability, such as the duration of contact with the aqueous environment when the drug is water soluble, the surrounding pH when the drug degradation is pH dependent and light exposure when the drug is light sensitive. It is observed that the presence of anionic surfactants in the dispersion causes rapid degradation of nanoparticles as they are made up of hydrolytic degradable polymer. The degradation pathway varies from polymer to polymer. However, the common pathways are by erosion of polymer backbone and cleavage of ester. Hence stability studies are important and can be performed according to the drug and polymer properties.^{7,8}

Physiochemical characterization

The physicochemical methods for the characterization of nanoparticles are listed in Table 1.^{2,20-25}

Drug loading

The drug loading in the nanoparticulate system can be done by two methods, namely incorporation method and incubation method. In the incorporation method, the drug is incorporated at the time of nanoparticle production. Whereas in incubation method, the drug is absorbed/ adsorbed after formation of nanoparticles by incubating the carrier with the concentrated drug solution²⁵. Both the methods result in a solid solution of drug in the

polymer, or a solid dispersion of drug in the polymer, or surface adsorption of drug, or chemical bonding of drug in the polymer.²

Table 1. Physiochemical methods for the characterization of nanoparticles

PARAMETER	METHOD
Particle size	Photon-correlation spectroscopy Transmission electron microscopy Scanning electron microscopy Scanned probe microscope Fraunhofer diffraction Freeze-fracture electron microscopy
Molecular weight	Gel chromatography
Density	Helium compression pycnometry
Crystallinity	X-ray diffraction Differential scanning calorimetry
Surface charge	Zeta potential measurement Electrophoresis Laser droplet anemometry Amplitude-weighted phase structure determination
Hydrophobicity	Hydrophobic interaction chromatography Contact angle measurement Rose Bengal binding
Surface properties	Static secondary-ion mass spectroscopy
Surface element analysis	X-ray photon spectroscopy Molecular magnetic resonance Fourier transform infrared spectroscopy

In these systems, drug is physically embedded into the matrix or adsorbed on to the surface. Various methods of loading have been developed to improve the efficiency of loading which largely depend upon the method of preparation as well as physicochemical properties of the drug and the polymer. Maximum loading can be achieved by incorporating the drug during the time of formation of particles but it may get affected by the process parameters such as method of preparation, presence of additives, etc.

Both water soluble and insoluble drugs can be loaded into chitosan based particulate system. Water soluble drugs are mixed with chitosan solution to

form a homogenous mixture. Water insoluble drugs and the drug that precipitate at acidic pH solution can be loaded after formation of particles by soaking the particles with the saturated solutions of drug.¹⁴

The precise determination of drug content in the nanoparticles can be a problem because of colloidal nature of the carrier. The best method is separation of particles by ultracentrifugation followed by quantitative analysis of the drug after dissolution of the pelleted polymer. Other useful methods are ultrafiltration or gel filtration. Alternatively, the drug content can be determined in the supernatant or the filtrate. The amount of the drug bound to the particles can then be calculated by subtracting this amount from the total amount of the drug present in the suspension.²

Drug release and release kinetics

Drug release from the carrier based particulate system depends upon the cross-linking, morphology, size and density of the particulate system, physicochemical properties of drug as well as presence of adjuvant. *In vitro* drug release also depends upon pH, polarity and presence of enzymes in the dissolution medium.

The release of drug from the nanoparticulate system depends upon three different mechanisms, namely, release from the surface of particles, diffusion through the swollen rubbery matrix, and release due to erosion. In majority of cases, the drug release follows more than one type of mechanisms. When nanoparticle comes in contact with the release medium the drug instantaneously dissolves thus affecting its release from the surface. Drug entrapped in the surface layers of the particles also follows this mechanism. This type of drug release leads to burst effect.

Drug release through diffusion involves three steps (Fig. 8):

- Penetration into the particulate system which causes swelling of the matrix
- Conversion of glassy polymer into rubbery matrix
- Diffusion of drug from the rubbery matrix

Various methods which can be used to study the *in vitro* release of drug include side by side diffusion cells with artificial or biological membrane, dialysis bag method, ultracentrifugation, centrifugal ultra filtration. Analysis of nanoparticles release profiles frequently shows a biphasic release pattern that can

be described by a bi exponential function given by the equation².

$$C_t = Ae^{\alpha t} + Be^{\beta t}$$

Where, C_t = concentration of compound remaining in the nanoparticles at the time t ; α and β = System characteristic constants; A and B = Rate constants.

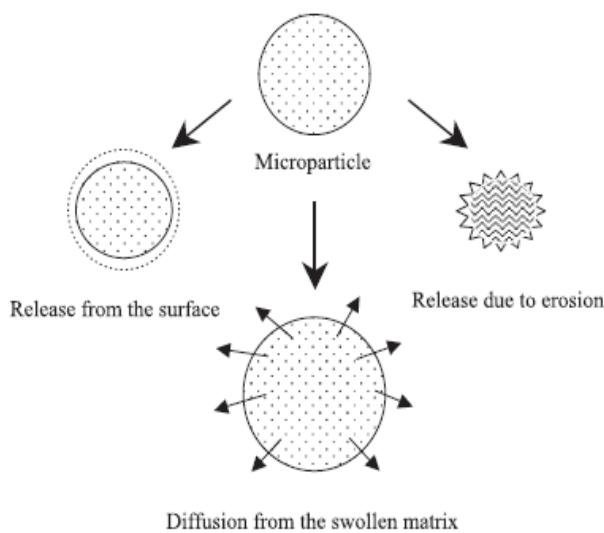


Fig. 8. Mechanisms of drug diffusion from nanoparticles

Uptake and distribution of nanoparticles

The main administration routes for nanoparticles are intravenous injection, subcutaneous or intramuscular injection, peroral administration and ophthalmic application.

Intravenous Administration

Nanoparticles, like other colloidal carriers such as liposome, micro emulsions and erythrocyte ghosts are taken up by the reticuloendothelial systems (RES) after intravenous injection and distributed mainly to liver, spleen and to a lesser degree bone marrow; varying amounts can be found in the lungs. All these organs contain sessile actively phagocytosing cells. Other phagocytosing cells are found in the blood and lymphatic system and in the endothelial lining of the blood vessels. These cells can take up nanoparticles by varying degree. The sequence of events during the distribution of nanoparticles and the phagocytosis is not currently known. It is believed that immediately after injection the particles are coated by the serum components, the opsonins, which act as labels to passively target the nanoparticles to certain phagocytic cells.

Passive Targeting: Passive targeting refers to the natural distribution pattern of the drug carrier in vivo. The nanoparticles distribution by the above method is passive targeting. The mechanical entrapment of large microspheres and large nanoparticles agglomerates ($>4 \mu\text{m}$) by capillary blockage also is referred to as passive targeting. This process can be exploited to target passively to lungs via the venous supply or to other organs via the appropriate arterial supply.

Active Targeting: Active targeting refers to a change in the natural distribution pattern of a carrier particle by deliberate modification of its properties, thereby directing it to specific cells, tissues, or organs. The following methods are mainly used;

- Alteration of the surface properties by coating the nanoparticles with the surfactants or macromolecules.
- Incorporation of magnetite particles into the particles and application of magnetical field
- Alteration of the surface charge and
- Attachment of specific antibiotics to the nanoparticles surface

The first two methods seem to be promising. Coating of the nanoparticles with the surfactants, for instance, can drastically reduce the liver uptake from 80% to 30% and the increase in blood concentration can be from 0.14 up to about 40%. Poloxamine 1508 is the principle agent for reducing liver uptake and increasing the blood concentration, whereas polysorbate 80 increase the concentration in non-RES-organs. Other surfactants such as poloxamer 184 and 407 have a high targeting capacity to the bone marrow. The possibility of bone marrow targeting is size dependent.

Subcutaneous and Intramuscular Injection

After subcutaneous and intramuscular injection of ^{14}C -labeled polymethacrylate nanoparticles in rat, over 99% of the injected radioactivity remains at the injection site. The elimination rate of administered dose per day in the form of oligomeric components of the nanoparticulate polymer is found to be lesser in the urine and feces initially and later the elimination is found to be more in feces.

Peroral Administration

Nanoparticles are retained in the guts of rats and mice up to 6 days. In addition, they are taken up in the intestine and appear in lymph nodes, blood, liver, spleen, and site of inflammation in the body. Three different mechanisms are possible;

- Intracellular uptake
- Intracellular-paracellular uptake
- Uptake via the M-cells and Peyer's patches in the gut

Ophthalmic Administration

After ophthalmic application to rabbits, polyhexyl cyanoacrylate nanoparticles are eliminated from the tears with a half life of about 15 to 20 min. Aqueous eye drops, on the other hand, have a half life of 1-3 min. A small amount of polycyanoacrylate nanoparticles adheres mainly to the conjunctiva, also to the cornea and to the nictitating membrane of rabbit and penetrate into the first two layers of the cell layers.²

Therapeutic applications

One of the major challenges in drug delivery is to get the drug at the place it is needed in the body thereby avoiding potential side effects to non diseased organs. This is especially challenging in cancer treatment where the tumor may be localized as distinct metastases in various organs. The non restricted (cyto) toxicity of chemotherapeutics thus limits the full use of their therapeutic potential.

Local drug delivery or drug targeting results in increased local drug concentrations and provides strategies for more specific therapy. Nanoparticles have specific particles as tools to enable these strategies. These include benefits such as their small size which allows penetration of cell membranes, binding and stabilization of proteins, and lysosomal escape after endocytosis. The aims for nanoparticle entrapment of drugs are enhanced delivery to, or uptake by, target cells and/or a reduction in the toxicity of the free drug to non-target organs. For these aims, creation of long-lived and target-specific nanoparticles is needed. Various therapeutic applications of nanoparticles are listed in Table 2.^{8,25-31}

Conclusion

Stable nanoparticles can be easily formulated. They give good payload, *in vitro* release profile and better targeting to RES organs. Hence, nanoparticles may form new alternative and cheaper carriers in therapy for reduction in dose, and thereby, dose related systemic toxicities. In turn, they can reduce the cost of the therapy.

Table 2. Therapeutic applications of nanoparticles

Application	Material	Purpose
Cancer therapy	Poly (alkyl cyanoacrylate) nanoparticles with anticancer agents, oligonucleotides	Targeting, reducing toxicity, enhanced uptake of antitumour agents, improved in <i>in vitro</i> and in vivo stability
Intracellular targeting	Poly (alkyl cyanoacrylate) polyesters nanoparticles with anti-parasitic or antiviral agents	Target reticuloendothelial intercellular infections
Prolonged systemic circulation	Polyesters with adsorbed poly ethylene glycols or pluronic	Prolonged systemic effect, avoid by the uptake of reticuloendothelial system
Vaccine adjuvant	Poly (methyl methacrylate) nanoparticles with vaccines (oral and IM immunization)	Enhanced immune response, alternate acceptable adjuvant
Peroral absorption	Poly (methyl methacrylate) nanoparticles with proteins and therapeutic agents	Enhanced bioavailability, protection from GIT enzymes
Occular delivery	Poly (alkyl cyanoacrylate) nanoparticles with steroids, anti-inflammatory agents, anti-bacterial agents for Glaucoma	Improved retention of drug/ reduced wash out
DNA delivery	DNA-gelatin nanoparticles, DNA-chitosan nanoparticles, PDNA-poly (D,L lactide-co-glycolide) nanoparticles	Enhanced delivery and significantly higher expression levels
Oligonucleotide delivery	Alginate nanoparticles, poly (D,L lactic acid) nanoparticles	Enhanced delivery of oligonucleotides

Declaration of Interest

It is hereby declared that this paper does not have any conflict of interest.

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