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Review Article

Appraisal on preparation and characterization of nanoparticles for parenteral and ophthalmic administration

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ABSTRACT

PAS71 document developed in the UK is: "A particle having one or more dimensions of the order of 100 nm or less". Various polymers have been used in the formulation of nanoparticles for drug delivery to increase the therapeutic benefits, while minimizing side effect. The polymeric nanoparticles can carry drugs, which are entrapped in the polymer matrix as particulates or may be bound to the particle surface by physical adsorption or chemically. This appraisal addresses how drug is dissolved, entrapped, encapsulated to a nanoparticles matrix and delivers a drug to specific targets by a physical approach to alter and improve the pharmacokinetic and pharmaco-dynamic properties of various types of drug molecules and to protect the drug entity in the systemic circulation and to study the biodistribution of nanoparticles through the ocular and parenteral rout. Nanoparticles not only deliver drug(s) to specific organs but also delivery rate in addition could be controlled as being by standers, burst, and controlled, pulsatile or modulated. However, special attention has been paid to its biopharmaceutical aspects, drug kinetics and its application in various areas. 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 regime. This review discussed about various methods of preparation, their characterization techniques, such as drug loading, release and the applications of Nanoparticles along with some marketed products and focusing on its properties and biological transport.

Keywords: Nanotechnology; Polymers; Particle size; Release kinetics; Bioavailability; Stability

INTRODUCTION

For the past few decades, there has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Nanoparticles are defined as per PAS71 document developed in the UK “particle having one or more dimensions of the order of 100 nm or less” (Vyas et al., 2002) 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. (Langer 2000; Bhadra et al., 2002; Kommareddy et al., 2005; Lee et al., 2005).

The major goals in designing nanoparticles as a delivery system are to control 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. (Vila et al., 2002; Mu et al., 2003).

Properties of nanoparticles

Particle size

Particle size and size distribution are the most important characteristics of nanoparticles systems. Smaller particles have larger surface area, whereas, larger particles have large cores, which allow more drugs to be encapsulated and slowly diffuse out. Two main techniques are being used to determine the particle size distribution of nanoparticles and include photon correlation spectroscopy (PCS) and electron microscopy (EM). The latter includes scanning electron microscopy

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(SEM), transmission electron microscopy (TEM) (Mohanraj et al., 2006).

Drug loading

Nonparticulate system should have a high drug-loading capacity. 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) (Mohanraj et al., 2006).

Drug release

Drug release rate depends on:

(1) Solubility of drug;

(2) Desorption of the surface bound or adsorbed drug;

(3) Drug diffusion through the nanoparticles matrix;

(4) Nanoparticles matrix erosion/degradation; and

(5) Combination of an erosion/diffusion process (Mohanraj et al., 2006).

SYNTHESIS OF NANOPARTICLES

Two basic strategies are used to produce nanoparticles: "top-down" and "bottom up".

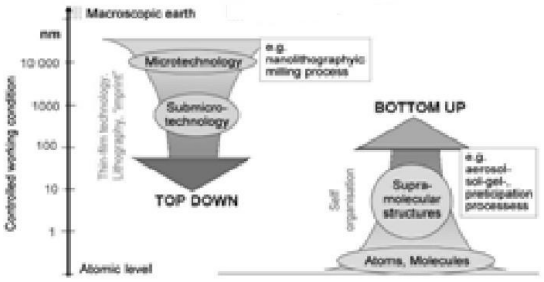

Figure 1: Methods of nanoparticles production: top-down and bottom-up

Top-Down/ mechanical-physical production processes

The mechanical production approach uses milling to crush microparticles. Milling involves thermal stress and is energy intensive. Here, a chemical or chemo-physical reaction accompanies the milling process by using mills to crush particles yields product powders with a relatively broad particle-size range. (Stefan et al., 2004).

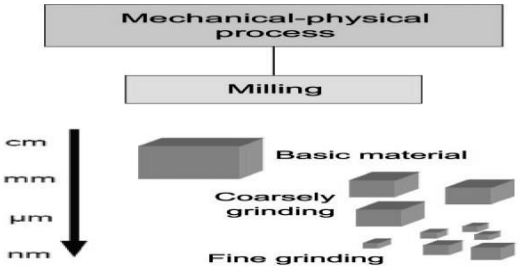

Figure 2: Overview of mechanical-physical nanoparticles production processes

Bottom-up/ Chemo-physical production processes

Bottom-up methods are used to produce selected, more complex structures from atoms or molecules, better controlling sizes, shapes and size ranges. It includes aerosol processes, precipitation reactions and sol gel process.

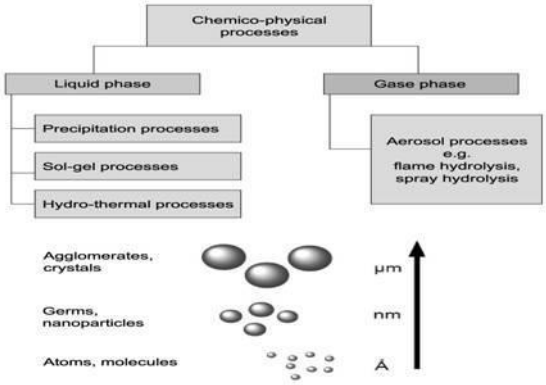

Figure 3: Chemo-physical processes in nanoparticles production

1. Gas phase processes (aerosol processes)

Nanoparticles are created from the gas phase by producing a vapor of the product material using chemical or physical means. The production of nanoparticles, which can be in a liquid or solid state, takes place via homogeneous nucleation and further particle growth involves condensation, chemical reaction(s) on the particle surface and/or coagulation processes (Rössler et al., 2001).

2. Liquid phase processes

The liquid phase processes are most important in nanomaterial production are precipitation, sol gel-processes.

a) Precipitation processes

In precipitation processes, particle size and size distribution, crystallinity and morphology (shape) are determined by reaction kinetics (reaction speed). The influencing factors include, beyond the concentration of the source material, the temperature, pH value of the solution, the sequence in which the source materials are added, and mixing processes. A good size control can be achieved by using self-assembled membranes, which in turn serve as nanoreactors for particle production. They are composed of a polar group and a non-polar hydrocarbon chain (Stefan et al., 2004).

b) Sol-gel processes

The sol-gel processes involve a three-dimensionalcross-linking of the nanoparticles in the solvent by add-ing organic substances. The pH value of the solution is adjusted with an acid or a base as a catalyst. The hydrolysis followed by condensation and polymerization reaction takes place, which depend on many factors: the composition of the initial solution, the type and

amount of catalyst, temperature as well as the reactor and mixing geometry. The different reaction and processing steps of the sol-gel process. (Sing N.H ., and Schubert U., 2003)

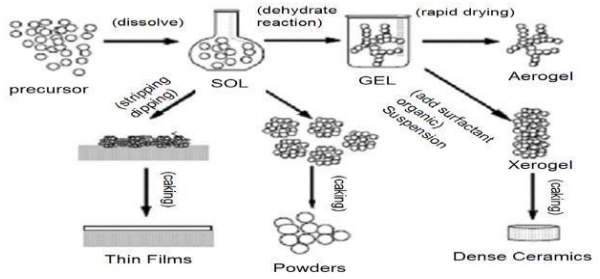

Figure 4: Sol-gel process

MATERIALS used in nanoparticles

Natural hydrophilic polymers

These are proteins such as albumin, gelatin, legumin or vicilin, as well as polysaccharides like alginates or agarose. They suffer disadvantages like batch to batch variation and conditional biodegradability (Shamkhani et al., 1991; Pangburn et al., 1984).

Table 1: Natural hydrophilic polymers

Proteins	Polysaccharides
Gelatin	Alginate
Albumin	Dextran
Lectins	Chitosan
Legumin	Agarose
Vicilin	Pullulan

Synthetic hydrophobic polymers

These are used for microsphere preparation. This contains polymers from the ester class poly (lactic acid) and poly (lactic-glycolic acid) copolymers (Lewis, 1990; Pitt, 1990).

Table 2: Synthetic hydrophobic polymers

Pre-polymerized	Polymerized in process
Poly(€-caprolactone) Poly(lactic acid) Poly(lactide-co-glycolide) Polystyrene	Poly(isobutylcyanoacrylates) Poly(butylcyanoacrylates) Polyhexylcyanoacrylates Poly methyl(methacrylate)

PREPARATION TECHNIQUES OF NANOPARTICLES

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; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity. (Kreuter, 1994). Two types of systems with different inner structures are apparently possible including; A matrix type system consisting of an entanglement of oligomers. (Nanoparticles/ nanospheres). A reservoir type of system comprised of an oily core surrounded by an embryonic polymeric shell (nanocapsules). The drug can either be entrapped within the reservoir or the matrix or otherwise be adsorbed on the surface of this particulate system (Vyas et al., 2002).

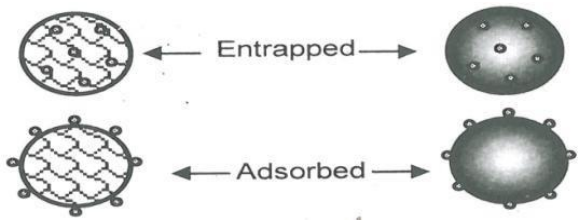

Figure 5: Nanoparticles / Nanosphere and Nanocapsule with the mode of Drug Entrapment

The preparation techniques are conveniently classified as follows;

1. Amphiphilic macromolecule cross-linking
   a. Heat cross-linking
   b. Chemical cross-linking
2. Polymerization based methods
   a. Polymerization of monomer in situ
   b. Emulsion (micellar) polymerization
   c. Dispersion polymerization
   d. Interfacial condensation polymerization
   e. Interfacial complexation
3. Polymer precipitation method
   a. Solvent extraction/evaporation
   b. Solvent displacement (nano precipitation)
   c. Salting out
4. Micellaneous

a) Amphiphilic macromolecule cross-linking

This technique involves firstly, the aggregation of amphiphile(s) followed by further stabilization either by heat denaturation or chemical cross-linking. These processes may occur in biphasic O/W or W/O type dispersed systems (Gupta et al., 1987a; Gupta et al., 1987b).

Cross-linking in W/O emulsion

The cross-linking method involves the emulsification of bovine serum albumin (BSE)/human serum albumin (HAS) in oil using high-pressure homogenization or high frequency sonication. The water in oil emulsion so formed is then poured into preheated oil and maintained temp. above 100^{o}C is held stirred for specified time in order to denature and aggregate the protein content completely and to evaporate the water. The particles are finally washed with an organic solvent to remove any adherent or adsorbed oil traces and collected variable size of nanoparticles by centrifugation (Kramer 1974; Sugibasayashi et al., 1979).

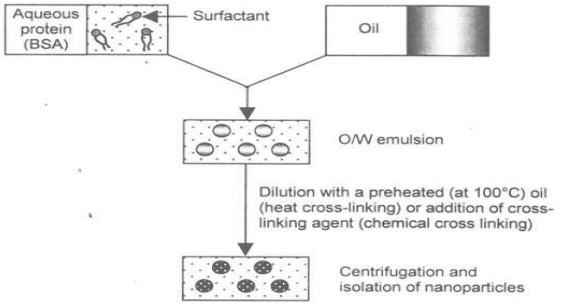

Figure 6: Schematic of Macromolecular Cross-linking in Water in Oil (W/O) Emulsion

b) Nanoparticles Preparation Using Polymerization Based Methods

Two different approaches are generally adopted for the preparation of nanospheres using in situ polymerization technique; Emulsion polymerization or Dispersion polymerization. (Kreuter, 1991).

Emulsion polymerization

The process of emulsion polymerization, depending upon the nature of the continuous phase. Two different mechanisms were proposed for the emulsion polymerization process and they include: Micellar nucleation and polymerization and Homogenous nucleation and polymerization. (Durbin et al., 1979; Kreuter, 1983; Vanderhoff, 1985; Kreuter, 1994).

1. Micellar nucleation and polymerization

The polymerization reaction takes place in the presence of chemical or physical initiators. The energy provided by the initiator creates free reactive monomers in the continuous phase which then collide with surrounding unreactives monomer and initiate the polymerization chain reaction which slightly soluble in the surrounding phase, the monomer molecules reach the micelles by diffusion from the monomer droplets through the continuous phase, thus allowing the polymerization to progress within the micelles.

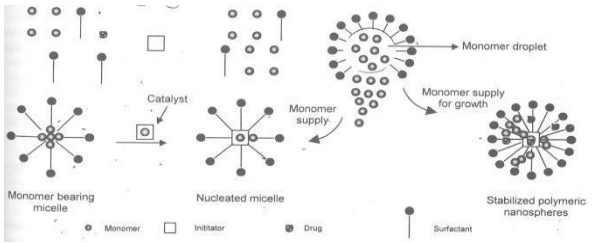

Figure 7: Emulsion polymerization (Micellar Polymerization Mechanism)

2. Homogenous nucleation and polymerization

The nucleation and polymerization stages in this both the micelles droplets play the role of monomer reservoirs throughout the polymer chain length. They precipitate and form primary particles, which are stabilized by the surfactant molecule provided by the micelles and droplets. Depending on the bulk condition and system stability, the end product nanospheres are formed by additional monomer input into the primary particles.

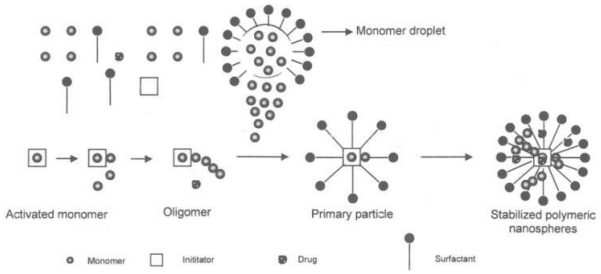

Figure 8: Emulsion polymerization (Homogenous Polymerization Mechanism)

Dispersion polymerization

The polymerization is initiated by adding catalyst and proceeds with nucleation phase followed by growth phase (propagation). In the case of dispersion polymerization, the nucleation is directly induced in the aqueous monomer solution and the presence of stabilizer or surfactant is not absolutely necessary for the formation of stable nanospheres (Kreuter and Speiser, 1979).

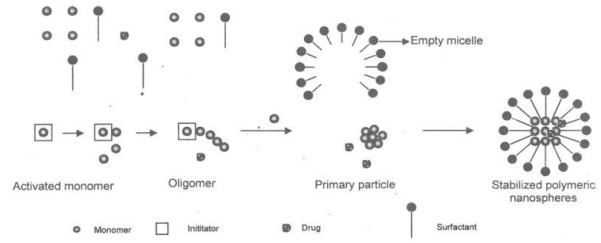

Figure 9: Dispersion Polymerization Mechanism

Interfacial Polymerization

In this method, the preformed polymer phase is finally transformed to an embryonic sheath. A polymer that eventually becomes a core of nanoparticles and drug molecules to be loaded are dissolved in a volatile solvent. The solution as then poured into a non-solvent for both polymer and core phase. The polymer phase is separated as a coacervate phase at O/W interface. The resultant mixture instantaneously turns milky owing to the formation of Nanocapsules. The solvent is removed under vacuum. The size of nanoparticles ranges from 30-300 nm. (Vyas et al., 2002).

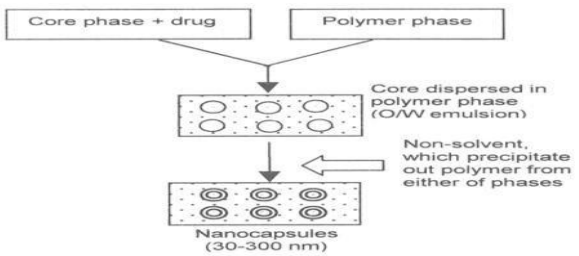

Figure 10: Preparation of Nanocapsules using Interfacial Polymer Condensation

Interfacial Complexation

In the case of nanoparticles preparation, aqueous polyelectrolyte solution is carefully dissolved in revers micelles in an apolar bulk phase with the help of an appropriate surface-active agent. Subsequently, competing polyelectrolyte is added to the bulk, which allow a layer of insoluble polyelectrolyte complex to coacervate at the interface (Vyas et al., 2002).

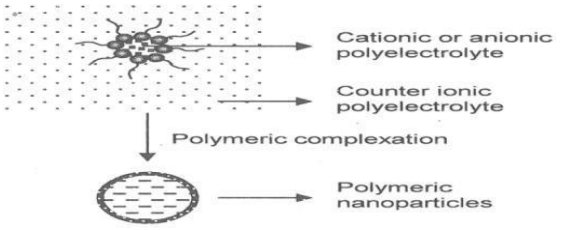

Figure 11: Interfacial Complexation Process Between Two competing Poly-electrolyte

3. Nanoparticles Preparation using Polymer Precipitation Method

In this method , the hydrophobic polymer and/ or hydrophobic drug is dissolved in a particular organic solvent followed by its dispersion in a continuous aqueous phase, In which the polymer is insoluble the external phase also contains the stabilizer. Depending upon solvent miscibility techniques they are designated as solvent extraction/evaporation method (Vyas et al., 2002).

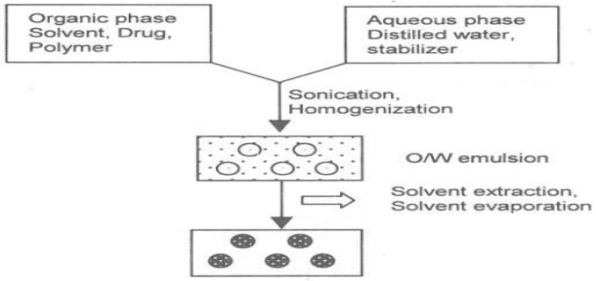

Figure 12: Nanoparticles Preparation using Emulsion Solvent Evaporation

Method

a) Solvent Extraction Method

This method involves the formation of conventional O/W emulsion between a partially water micssible solvent containing the polymer and the drug, and aqueous phase containing the stabilizer. The subsequent removal of solvent (Solvent evaporation method) or the addition of water to the system to affect diffusion of the solvent to the external phase (emulsification diffusion method) are two variance of the solvent extract on method (Vyas et al., 2002).

b) Double Emulsion Solvent Evaporation Method

Emulsion solvent evaporation techniques have been further modified and a double emulsion of water in oil in water type has been used. BSA and PGA are dissolved separately in aqueous and organic phases respectively and subjected to ultrasonication to yield water in oil emulsion. This W_1/O is further added to PVA aqueous solution to yield the water-in-oil-in-water ($W_1/O/W_2$) double emulsion. The organic solvent allowed to evaporate while being stirred first at atmosphere pressure for 16 h and then gradually at a reduced pressure (from 100 mm Hg to 30 mm Hg) to dried nanoparticles (Vyas et al., 2002).

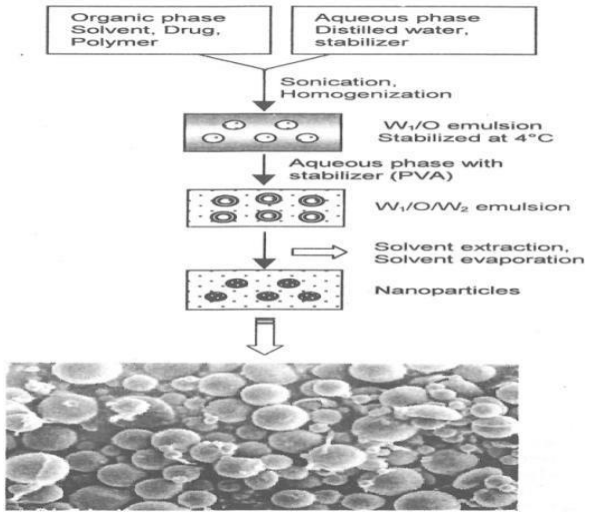

Figure 13: Preparation of nanoparticles using Double Emulsion Solvent Evaporation Method

c) Solvent Displacement or Nanoprecipitation

Solvent displacement method involves the use of an organic phase, which is completely soluble in the external aqueous phase. This method is particularly useful for drug that is slightly soluble in water. In this method, the drug is dissolved in the small volume of appropriate oil and then diluted in the polar organic solvent. When the organic solution is dispersed in the aqueous phase, the polymer precipitates around the nanodroplets, forming a reservoir system (Fessi et al., 1987).

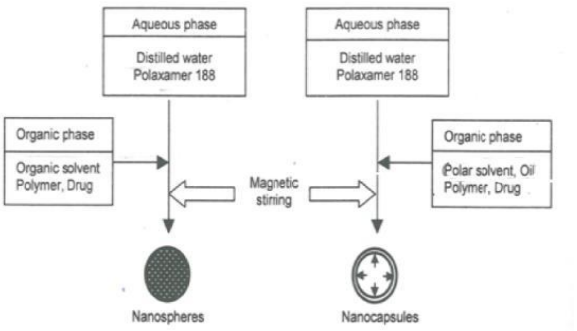

Figure 14: Solvent displacement method for prepare nanoparticles

c) Salting out

The method involves the incorporation of a saturated aqueous solution of polyvinyl alcohol (PVA) into an

acetone solution of the polymer under magnetic stirring to form an O/W emulsion. The precipitation of the polymer occurs when a sufficient amount of water is added to external phase to allow complete diffusion of the acetone from internal phase into the aqueous phase (Vyas et al., 2002).

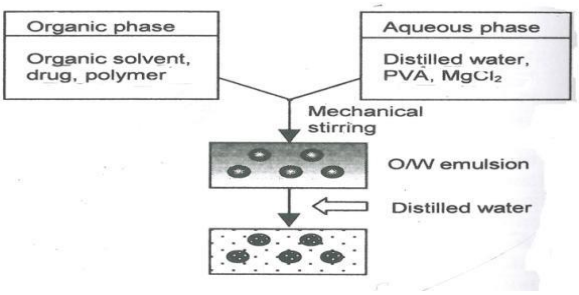

Figure 15: Nanoparticles preparation using Salting out of polymer

4. Miscellaneous

Nanoparticles have been prepared by using these methods: Ionic gelation or coacervation of hydrophilic polymers and supercritical fluid technology

a) Coacervation or ionic gelation method-

The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block copolymer 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 (Calvo et al., 1997).

Production of nanoparticles using supercritical fluid technology

A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. The process of SAS employs a liquid solvent, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles (Reverchon et al., 2006; Jung et al., 2001).

PHARMACEUTICAL ASPECTS OF NANOPARTICLES

Nano-particles in the point of view, three important process parameters are performed before releasing them for clinical-trails (Allemann et al., 1993a; Langer et al., 1994).

A. Purification of Nanoparticles (Allemann et al., 1993a)

Following methods are adopted for purification

a. **Gel filtration** – In this high molecular weight substances and impurities are difficult to remove.

b. **Dialysis –** High molecular weight impurities are difficult to remove. It is a time-consuming process.

c. **Ultra-centrifugation –** Aggregation of particles is observed. It is also a time-consuming process.

B. Cross-flow filtration method

Best method for purification of nanoparticles. In this method, the nanoparticle suspension is filtered through membranes, with the direction of fluid being tangential to the surface of the membrane due to which clogging of the filters is avoided, which is seen in perpendicular filtration modes. The suspension is subjected to several filtration cycles, which is followed by addition of purified water at the same filtration rate. It is simple to perform and filtration rate is fast (Allemann et al., 1993b).

C. Freeze drying of Nano-particles

This involves freezing of nanoparticles suspension and subsequent sublimation of its water content under reduced pressure to get a free flowing powdered material (Auvillain et al.,1989). It has been following advantages:

1. Prevention from degradation of polymer
2. Prevention from drug leakage, drug desorption and drug degradation.
3. Easy to handle and store and help in long term preservation.
4. Readily dispersible in water without modification in their physicochemical properties.

But there are some disadvantages like

1. Aggregation problems during the lyophilization process.
2. Full redispersion of the system may be difficult to achieve.

D. Sterilization of Nano-particles

Nanoparticles for parenteral use should be sterilized to make them pyrogen free. Sterilization of nanoparticles is best achieved by using an aseptic technique throughout their preparation and processing and formulation. Sterilization can also be achieved by autoclaving or γ-irradiation (Allemann et al., 1993b).

CHARACTERIZATION OF NANOPARTICLES

The nanoparticles are generally characterized for size, density, electrophoretic mobility, angle of contact, and specific surface area (Vyas et al., 2002).

1. Size and morphology

The particles size is one of the most important parameters of nanoparticles. Two main techniques are being used to determine the particle size distribution of nanoparticles and include photon correlation spectroscopy (PCS) and electron microscopy (EM) The latter includes scanning electron microscopy (SEM), transmission electron microscopy (TEM) (Douglas et al., 1987; Kreuter, 1883; Fusai et al., 1997).

2. Specific surface

The specific surface area of freeze dried nanoparticles is generally determined with the help of Sorptometer. The equation given below can be used in the calculation of specific surface area.

$$A=6/\delta.d$$

Where A is the specific surface area, δ is the density and d is the diameter of the particles. (Kreuter, 1883)

3. Surface charge and Electrophoretic mobility

Surface charge of nanoparticles can be determined by measuring the particle velocity in an electric field. Laser light scattering techniques, i.e. Laser Doppler Anemometry or Velocimetry, have become available as fast and high-resolution techniques for nanoparticles velocity. The surface charge can also be measured as electrophoretic mobility (Sestier et al., 1998).

4. Surface Hydrophobicity

Hydrophobicity regulates the extent and type of hydrophobic interactions of nanoparticulates with the blood components. Several methods, including hydrophobic interaction chromatography, two-phase partition, adsorption of hydrophobic fluorescent, etc has been adopted to evaluate surface hydrophobicity (Carstensen et al., 1991)

5. Density

The density of nanoparticles is determined with helium or air using a gas Pycnometer. The value obtained with air and with helium may differ noticeably from each other. The difference is much more pronounced due to specific surface area and porosity of the structure (Kreuter, 1883).

6. Molecular Weight Measurements of Nanoparticles

Molecular weight of polymer and its distribution in the matrix can be evaluated by gel permeation chromatography (GPC) using a refractive index detector (Van Snick et al., 1985)

7. Nanoparticles Recovery and Drug Incorporation Efficiency

The nanoparticles recovery; can be calculated using the following equation; (Govender et al., 1999).

$$Nanoparticles\ Recovery\ (\%) = \frac{Conc\ of\ drug\ in\ nanoparticles}{Conc\ of\ nanoparticles\ recovered} \times 100$$

8. Turbidimetry

For non absorbing particles, turbidity is the complement to light scattering because it represents the amount of incident radiations not reaching a detector, that is, light lost to scattering. This approach requires tiny amounts of sample and can be easily executed using a spectrophotometer (Mohanty et al., 2002).

9. Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by a chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanaoparticles (Mohanty et al., 2002).

10. Optical Microscope

Most nanoparticles are below the resolution limit (ca. 0.5 μm) of direct optical imaging, though microscopy is still useful to get an estimate of size and crystallinity of starting materials, as might be desirable in the instance of comminution or homogenization processing, or other larger particles (Mohanty et al., 2002).

11. Electron Microscopy

Scanning and transmission electron microscopy, SEM and TEM, respectively, provide a way to directly observe nanoparticles, with the former method being better for morphological examination (Mohanty et al., 2002).

12. Hydrophobic Interaction Chromatography

In this method, the analyte is first adsorbed onto a chromatographic stationary phase using a high concentration of an antichaotropic salt. Elution occurs using a gradient in which the salt concentration is decreased, so that those materials eluting first are the least hydrophobic because the salt concentration did not need to be decreased much before the analyte desorbed (Mohanty et al., 2002).

13. Electrophoresis

This process will determine the clearance and biodistribution of the colloid, so evaluating the exact nature of the surface coverage is required to achieve a useful understanding. The small size of nanoparticles allows their electrophoretic behavior to be observed using bioanalytical tools such as isoelectric focusing and 2-D polyacrylamide gel electrophoresis (Mohanty et al., 2002).

14. Zeta Potential

Zeta potential is used as a surrogate for surface change, and is often measured by observing the oscillations in signal that result from light scattered by particles located in an electric field, though there are other approaches (Mohanty et al., 2002).

Table 3: Different Parameters and characterization Methods for Nanoparticles

S.No	Parameter	Characterization method (s)
1	Particle size and size distribution	Photon correlation spectroscopy (PCS) Laser defractometry Transmission electron microscopy Scanning electron microscopy Atomic force microscopy Mercury porositometry
2	Charge determination	Laser Doppler Anemometry Zeta potentiometer
3	Surface hydrophobicity	Water contact angle measurement Rose bengal (dye) binding Hydrophobic interaction chromatography X-ray photoelectron spectroscopy
4	Chemical analysis of surface	Static secondary ion mass Spectrometry Sorptometer
5	Carrier-drug interaction	Differential scanning calorimatry
6	Nanoparticle dispersion stability	Critical flocculation temperature (CFT)
7	Release profile	*In vitro* release characteristics under physiologic and sink conditions
8	Drug stability	Bioassay of drug extracted from nanoparticles Chemical analysis of drug

15. Differential Scanning Calorimetry (DSC)

DSC can be used to determine the nature and speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperatures and their associated enthalpies (Mohanty et al., 2002).

NANOPARTICLE CLASSIFICATION

Table 4: Classification of nanaoparticles (Mohanty et al., 2002)

Category	Examples
Nanotube	Carbon (fullerences)
Nanowire	Metal, Semiconductors, Oxides, Sulphides, Nitrides
Nanocrystals	Quantum dot insulators, Semiconductors, Metal, Magnetic matrrials
Other Nanoparticles	Ceramic oxides, Metal
Nanobotes	Biochip, Nubotes

IN VIVO FATE OF NANOPARTICLES (PARENTERALS)

Intravenous administration, the colloids carriers first come into contact with plasma/serum protein before they reach target cells. Most notably, the interaction of the colloidal carriers with the phagocytes often requires some serum components and then subsequently interaction with complement receptors, Fc receptor and sugar/lectin receptors on the macrophage, lymphocytes or other cells. Soluble carriers can be pinocytosed via Fc or lectin receptors are expressed in different cell types, which negotiate the transportation of various endogenous ligands or colloidal carriers appended with their synthetic mimics (Vyas and Sihorkar, 2000; Vyas et al., 2001). Normally intravenous injections of colloidal carriers follow their interactions with at least two distinct group of plasma protein. It is now recognized that phagocytosis of particulate by elements of RES (liver, spleen, bone marrow) and specially concerning liver (Kupffer cells) is regulated by the presence and balance between two groups of serum components :Opsonin that promote the phagocytosis, and dysopsonins that suppress the process. The so-called opsonin adsorbs on to the surface of the colloidal carriers and renders particles recognizable and more 'palatable' to the RES

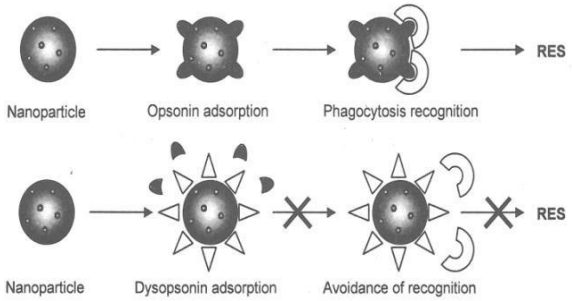

Figure 16: In Vivo Fate of Nanoparticles after Adsorption of Serum Components

Thus, they mediate their endocytosis by the fixed macrophages of the RES and circulating monocytes (Absolom, 1986; Moghimi et al., 1989; Moghimi et al.,1998).

BIOLOGICAL TRANSPORT OF NANOPARTICLES

Regarding the human body, the major passages are the blood vessels through which materials are transported in the body. For any moiety to remain in the vasculature, it needs to have its one dimension narrower than the cross-sectional diameter of the narrowest capillaries, which is about 2000 nm. Actually, for efficient

transport the nanoparticle should be smaller than 300 nm. But, just moving in the vessels does not serve the drug delivery purpose.

The delivery system must reach the site at the destination level. This requires crossing of the blood capillary wall to reach the extracellular fluid of the tissue and then again, crossing of other cells, if they are in the way, and entering the target cell. These are the major barriers in the transit. A nanoparticle has to do a lot during this so journey of the carrier through the vessels (capillaries) and across the barriers. There are two routes for crossing the blood capillaries and other cell layers, i.e., Ttranscellular and paracellular. In the transcellular route, the particulate system has to enter the cell from one side and exit the cell from the other side to reach the tissue. Paracellular movement of moieties including ions, larger molecules, and leukocytes is controlled by the cytoskeleton association of tight junctions and the adherence junctions called apical junction complex. While tight junctions act as a regulated barrier, the adherence junctions are responsible for the development and stabilization of the tight junctions. The tight junctions control the paracellular transport (Mohanty et al., 2002).

NANOPARTICLES FOR OCULAR DRUG DELIVERY

The human eye can be divided into the anterior and posterior anatomical segments. Drug delivery to the anterior segment is primarily achieved through topical application and the delivery of drugs to the posterior segment of the eye poses a great challenge. The posterior segment disease treatment focuses on four approaches to deliver drugs - topical, systemic, intraocular, and periocular (Geroski et al., 2000). In all of these modes, the drug is interfaced with the sclera. There is substantial evidence indicating that drugs administered subconjunctivally can reach the vitreous effectively (Graham et al., 1989; Ahmed et al., 1985). Intravitreal injections are an effective way of delivering drugs to the vitreoretinal region. (Herrero-Vanrell et al., 2001). In recent times, nano- and microparticulate systems have generated considerable interest for sustaining drug delivery. Nanoparticle suspensions have been shown to improve the residence time of topically applied ophthalmic formulations. (Joshi, 1994).

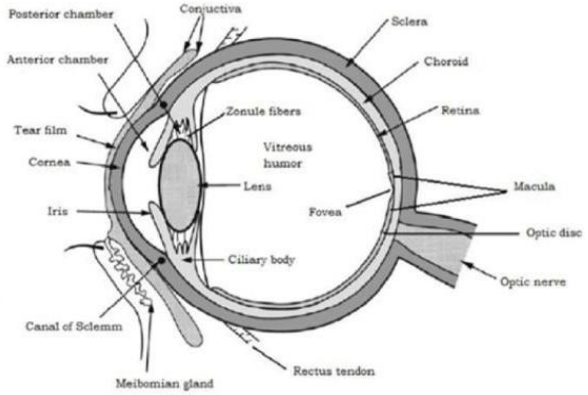

Figure 17: Anatomy of eye

The use of colloidal drug delivery systems, such as liposomes, biodegradable nanoparticles and nanocapsules in the ocular mucosa presents a major challenge for the therapy of extraocular diseases, such as keratoconjunctivitis sicca or dry eye disease. The major goal has design the topical ocular delivery systems which promote the concentration of the drug on the eye surface, and facilitate the drug transfer from the extraocular tissues to the internal structures of the eye and the design of injectable controlled release systems, which deliver the drug directly to the sclera (subconjunctival injection) or to the internal structures of the eye (intravitreal injection), for extended periods of time (Ameller et al., 2003; Machado et al., 2005; Allen et al., 2000; Hede et al., 2006; Damge et al., 1990).

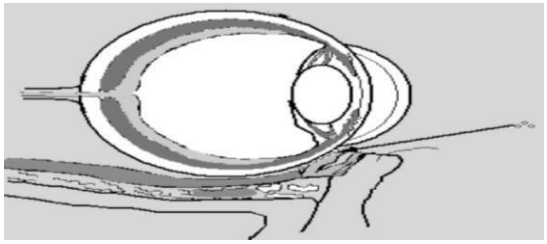

Figure 18: Schematic representation of the mammalian eye with particular drug delivery System (Kompella, 2003)

Whenever an ophthalmic drug is applied topically to the anterior segment of the eye, only a small amount (5%) actually penetrates the cornea and reaches the internal anterior tissue of the eyes. Rapid and efficient drainage by the nasolacrimal apparatus, non corneal absorption, and the relative impermeability of the cornea to both hydrophilic and hydrophobic molecules, all account for such a poor ocular bioavailability. The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems, which provide the controlled and continuous delivery of ophthalmic drugs, such as implants, inserts, and colloids. The second involves maximizing corneal drug absorption and minimizing precorneal drug loss through viscosity and penetration enhancers, prodrugs, and colloids.

Two types of nanoparticulates carries have been described for ocular drug delivery: Matrice-type nanoparticles- In which the biologically active molecule is entrapped or simply adsorbed onto their surface; and Reservoir-type nanocapsules- Which consist of a polymeric wall surrounding a liquid drug-containing core. These nanocarriers are classified into three categories: first generation of basic nanoparticles and nanocapsules, second generation of nanoparticles and nanocapsules with a hydrophilic polymer coating, and the third generation of functionalized nanoparticles/nanocapsules. (Ameller et al., 2003; Machado et al., 2005; Allen et al., 2000; Hede et al., 2006; Damge et al., 1990).

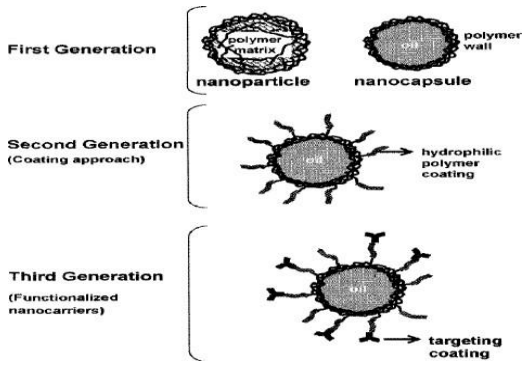

Figure 19: Schematic representation of different nano systems intended for ocular drug delivery

BIOPHARMACEUTICAL BARRIERS IN OCULAR DRUG DELIVERY

Major problem encountered with the conventional topical delivery of ophthalmic drugs is the rapid and extensive pre-corneal loss caused by the drainage and high tear fluid turnover. Most efforts in ophthalmic drug delivery have been focused on increasing the corneal penetration of drugs for treatments of different ocular diseases. Liquid formulations, solutions and suspensions, are the most commonly applied for topical ocular administration, since they are easy to use and do not interfere with vision and these formulations are often quite ineffective. Most of the drug applied topically onto the eye is immediately diluted in the precorneal tear film. The excess fluid spills over the lid margin and the remainder is rapidly drained into the nasolachrymal duct and most of the applied drug solution is cleared within 2-4 min. The very important additional barrier represented by the cornea, which is the main entrance to the inner eye. This small fraction of drug in contact with the cornea is then confronted with the very restrictive sub-barriers such as the epithelium, the stroma and the endothelium. In some instances, the required posologic regimen is unviable and hence the intravitreal injection becomes necessary to achieve significant drug levels in the intraocular structures. These biopharmaceutical constraints clearly evidence the necessity to conceive new ocular drug delivery strategies aimed at overcoming the above indicated barriers (Allen et al., 2000; Catarina et al., 2006; Couvreur et al., 1995; Couvreur et al., 2002).

TOXICOLOGICAL STUDY

The same properties (small size, chemical composition, structure, large surface area and shape), which make nanoparticles so attractive in medicine, may contribute to the toxicological profile of nanoparticles in biological systems. In fact, smaller the particles more the surface area per unit mass and this property makes nanoparticles very reactive in the cellular environment. Nanoparticles present possible dangers, both medically and environmentally. Most of these are due to the high surface to a volume ratio, which can make the particles very reactive or catalytic. They are also able to pass through cell membranes in organisms, and their interactions with biological systems are relatively unknown. However, free nanoparticles in the environment quickly tend to agglomerate and thus leave the nano-regime, and nature itself presents many nanoparticles to which organisms on earth may have evolved immunity (such as salt particulates from ocean aerosols, terpenes from plants, or dust from volcanic eruption A fuller analysis is provided in the article on nanotechnology. Therefore, any intrinsic toxicity of the particle surface will be enhanced. The respiratory system, blood, central nervous system (CNS), gastrointestinal (GI) tract, and skin have been shown to be targeted by nanoparticles (Ahmed et al., 2004; Yoo et al., 2001; Alexandridis et al., 1994; Kataoka et al., 2000; Zhang et al.,1999).

CONCLUSION

Nanoparticals improves the biopharmaceutical aspects like controlled or sustained release and better drug targeting. Nanoparticulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering, is still required. Growth in the applications of nanosuspension technology has occurred in response to the voluminous number of water-insoluble drug candidates who have emerged from discovery programs. Parenteral applications for subcutaneous, intramuscular, intradermal, intravenous, epidural, and intrathecal delivery have been studied in animals, with enhanced efficacy. At the same time, the safety profile has been observed to be improved in many cases when compared to conventional solution forms of the drugs. This therapeutic based on the successful clinical applications, it is anticipated that growth of this formulation tool will accelerate. Further advances are needed in order to turn the concept of nanoparticles technology into a realistic practical application as the next generation of the drug system. Ocular approach to the nanoparticulates drug delivery system for subconjunctivally administered micro- and nano-particles failed to reach the intraocular tissues under the conditions of this study. If subconjunctivally administered particulate systems remain in the subconjunctival space, such systems can be used to sustain drug delivery to the intraocular tissues. With the wide application to the nanoparticulates drug delivery system one can modify the solubility as well as the bioavailability of poorly soluble drug with respect to the permeability which having the prominent need in the pharmaceutical area.

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Table 5: Applications of Nanoparticle (Vyas et al., 2002)

Application	Material	Purpose
Cancer therapy	Poly(alkylcyanoacrylate) nanoparticles with anticancer agents, oligonucleotides	Targeting, reduced toxicity, enhanced uptake of antitumour agents, improved in vitro and in vivo stability.
Intracellular targeting	Poly(alkylcyanoacrylate) Polyester nanoparticles with antiparasitic or antiviral agents	Target reticuloendothelial systems for intracellular infections.
Prolonged systemic circulation	Polyesters with adsorbed polyethylene glycols or pluronics or derivatized Polyeseters Poly (methylmethacrylate) nanoparticles with vaccines (oral and intramuscular immunization).	Prolonged systemic drug effect, avoid uptake by the reticuloendothelial system
Vaccine adjuvant	Poly (methylmethacrylate) nanoparticles with proteins and therapeutic agents	Enhances Immune response, alternate acceptable adjuvants
Peroral absorption of proteins and peptides	Poly (alkylcyanoacrylate) nanoparticles with steroids, anti-inflammatory agents, antibacterial agents for glaucoma	Enhanced bioavailability, protection from git enzymes
Ocular delivery	DNA-gelatin nanoparticles, DNA-chitosan nanoparticles, PDNA-poly(DL-lactide-co-glycolide) nanoparticles	Improved retention of drug/reduced wash out
DNA delivery	Alginate nanoparticles, poly (D,L) lactic acid nanoparticles	Enhanced delivery and significantly higher expression level
Oligonucleotide delivery	Poly(alkylcyanoacrylate)nanoparticles with peptides	Enhanced delinery of oligonucleotide
Other applications	Poly(alkylcyanoacrylate) nanoparticles for transdermal application	Cross blood-brain barrier Improved absorption and penetration.
	Nanoparticles with adsorbed enzymes	Enzyme immunoassays
	Nanoparticle with radioactive or contrast agents	Radio-imaging
	Copolymerized peptide nanoparticles of butyl cyanoacrylates and activated peptides	Oral delivery of peptides

Table 6: List of some marketed products (Zhang et al., 2007)

Company	Trade name	Composition	Indication	Administration
Enzon	Abelect	Liposomal amphotericin B	Fungal infection	Intravenous
Berna Biotech	Epaxal	Liposomal IRIV Vaccine	Hepatitis A	Intramuscular
Novavax	Estrasorb	Micellular estradiol	Menopausal therapy	Topical
Nektal, Hoffmann-La Roche	Pegasys	PEG-a-interferon 2a	Hepatitis B, Hepatitis C	Subcutaneous
Genzyme	Renagel	Poly(allylamine hydrochloride)	End-stage renal disease	Oral
Elan-Merck	Emend	Nanocrystalline aprepitant	Antiemetic	Oral
Elan , Abbott	Tricor	Nanocrystalline finofibrate	Antihyperlipidemic	Oral
Elan, Wyeth	Rapamune	Nanocrystalline sirolimus	Pharmaceutical Immunosuppressant	Oral

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