CHAPTER 1

NANOPARTICLES

Introduction

In recent years a prompt attention has been given in development of Novel Drug Delivery System (NDDS). Nanoparticle has occupied the most integrated part in novel drug delivery system. They are specially designed to release the drug in vicinity of target tissue.

- Speiser and his co-workers who first developed the concept that nanoparticles are used as vehicle for drug delivery in late 1960s and early 1970s.
- The word 'nano' refers to size ranging from 10-1000 nm and 'particle' is defined as a small object that behaves as a whole unit in terms of its transport and properties.

Definition

Nanoparticles may be defined as, a submicron colloidal systems generally, made up of polymers (biodegradable or not) with particle size ranging from 1-1000 nm, in which, the active ingredient is dissolved, entrapped or encapsulated or adsorbed or attached. They do not have precise classification but are referred as nanospheres, rods, fibers, capsules and suspensions.

Advantages

- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
- They control and sustain the release of the drug during the transportation and at the site of localization, altering organ distribution of the drugs and subsequent clearance of the drugs, so as

to achieve increase in therapeutic efficacy of drugs and reduction in side effects.

- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents.
- Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction. This is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- The system can be used for various routes of administration including oral, nasal, parenteral and intra-ocular.
- Nanoparticles with diameter less than 200 nm are not screened out of circulation by liver and spleen.
- Nanoscale powders of anti-asthma and analgesic drugs are quickly absorbed in the human body in comparison to the traditional drug delivery systems.

Advantages of Nanoparticles Over other Drug Delivery System

An important area of application of nanotechnology includes novel drug delivery techniques, which are quicker and less risky, compared to the costs of developing new drugs.

- A large percent of ordinary drugs taken orally are destroyed by the stomach and liver and then distributed throughout the body, despite the fact that optimal effects would arise from focusing the drug to the target organ. Targeted drug delivery by nanoparticles has the potential to overcome some of these problems, and render treatment more effective, ensuring cost and safety benefits.
- The therapeutic potential of currently available drugs is also hampered by micro kinetics such as local instability issues and difficulties in crossing certain biological barriers such as blood brain barrier and placenta. Nanoparticles can help to address these problems, and several applications in drug delivery using this technology are being developed.

Limitations

In spite of these advantages, nanoparticles do have following limitations.

- For example, their small size and large surface area can lead to particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.
- In addition, small particles size and large surface area readily result in limited drug loading and burst release.
- These practical problems have to be over comed before nanoparticles can be used clinically or made commercially available.

Preparation Techniques of Nanoparticles

Based on the physicochemical characteristics of polymer and drug to be loaded appropriate method for preparation is selected. The drug can be either entrapped within the reservoir or the matrix or otherwise be adsorbed on the surface of the particulate system. The classifications of methodologies are:

1. Macromolecule cross linking:
   (a) Heat cross linking
   (b) Chemical cross linking
2. Polymerization based methods:
   (a) Polymerization of monomer
   (b) Emulsion (micellar) polymerization
   (c) Dispersion polymerization
   (d) Interfacial condensation polymerization
   (e) Interfacial complexation
3. Polymer precipitation methods:
   (a) Solvent extraction/evaporation
   (b) Solvent displacement (nano precipitation)
   (c) Salting out

1. Macromolecule Crosslinking

Nanoparticles are prepared from amphiphilic (affinity for aqueous and lipid solvents) macro molecules, proteins and polysaccharides.

- The technique involves aggregation of amphiphilic followed by stabilization by heat denaturation or chemical cross linking.
- These process occur in biphasic either o/w or w/o dispersed system.

Cross Linking in W/O Emulsion

- This method is widely used for nano encapsulation of drugs.
- This method involves emulsification of bovine serum albumin (BVA), human serum albumin (HSA) or protein aqueous solution in oil under high pressure homogenization.
- This w/o emulsion is poured into pre heated oil (100°C) and stirred for a specific time to denature and aggregate protein contents of aqueous phase.
- Water is evaporated, proteinaceous sub-nanoparticles are formed which are washed with organic solvents and collected by centrifugation.

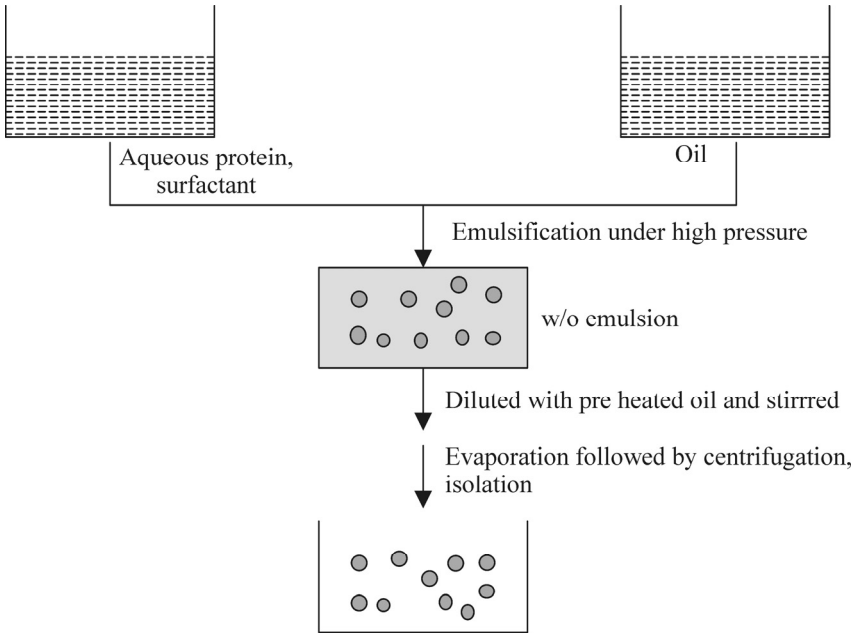

Fig. 1.1 Cross Linking in W/O Emulsion.

Phase Aeperation in Aqueous Medium (Desolvation)

- By changing PH or changing temperature or adding appropriate counter ions we can desolvate proteins or polysaccharides from aqueous phase.
- This method essentially proceeds by three steps
  - protein dissolution
  - protein aggregation
  - protein deaggregation
- All the three levels are maintained using appropriate levels of desolvation and resolvation and finally the aggregated particles are cross linked by using glutaraldehyde.

2. Polymerization based Methods

(a) Polymerization based Methods

- Two different approaches are adopted for preparation of nanospheres using polymerization technique.
- Emulsion polymerization: Non-solvent phase is used for the emulsification of the monomer to be polymerized.
- Monomer is dissolved in internal phase.
- Dispersion polymerization: Monomer is dissolved in a solvent in which polymer is immiscible.
- Monomer is taken as dispersed phase.

In both the cases polymer is insoluble in internal or dispersed phase resulting in a suspension of nanospheres.

(b) Emulsion Polymerization

- Depending on the nature of continuous phase the process of emulsion polymerization may be inverse or conventional.
- If continuous phase is aqueous (o/w emulsion) it is conventional. Whereas it is organic (w/o emulsion) it is inverse.

Two different mechanisms were proposed for emulsion polymerization.

(i) Micellar nucleation and polymerization

(ii) Homogenous nucleation and polymer

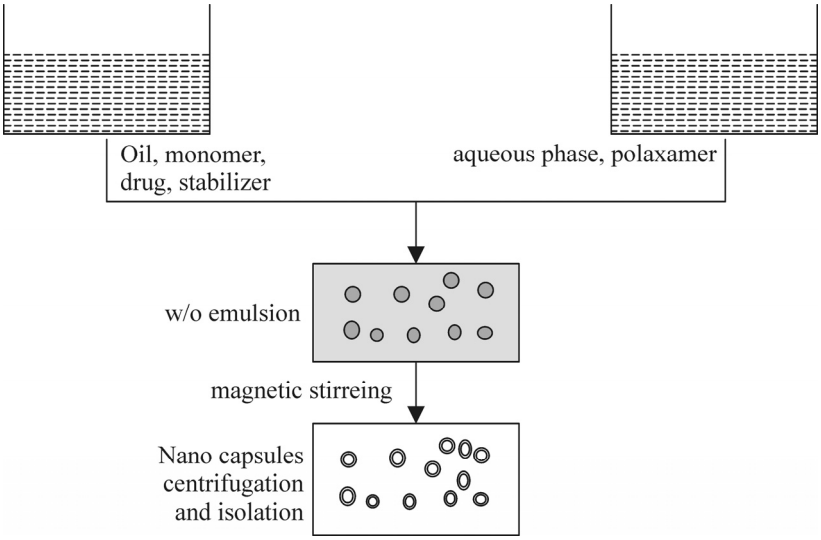

Fig. 1.2 Emulsion polymerization process.

(c) Dispersion Polymerization

- In this method the monomer is added to the aqueous phase which acts as precipitant for the polymer.
- So without catalyst only nucleation occurs in the aqueous monomer solution.
- The oligomers are formed and aggregated and above certain limit they precipitate as particles and transform into nanoparticles.
- This method is used to prepare biodegradable polyacrylamide and polymethyl-methacrylate nanoparticles.

(d) Interfacial Polymerization

- In this method polymer is preformed and finally transformed to an embryonic sheath.
- Polymer that forms core and drug to be loaded are dissolved in volatile solvents.
- Polymer and core phase are obtained by pouring the solution into non-solvent.

- At o/w interface the polymer phase is separated as a coacervation phase.
- Owing to the formation of nanocapsules the resultant mixture turns milky.
- E.g.: Interfacial polymeric condensation of 2, 2-bis-(4-hydroxy phenyl) propane and sebacoyl chloride.

(e) Interfacial Complexation

- In case of nanoparticle preparation with the help of surface active agents aqueous polyelectrolyte solution is carefully dissolved in reverse micelle in a polar bulk face.
- In soluble polyelectrolyte complex is formed by adding competing polyelectrolyte to coacervate at the interface.

3. Polymer Precipitation Methods

(a) Solvent Extraction

- Involves the formation of conventional o/w emulsion between partially water miscible solvent with polymer, drug and aqueous phase containing stabilizer.
- Solvent is removed by evaporation and addition of water.

Solvent Evaporation

- In this technique preformed polymer and drug are mixed with water immiscible organic solvent emulsified in a aqueous solution containing stabilizer.
- This crude emulsion is then exposed to ultra sonicator (a high energy source) or passed through homogenizers', colloid mills to reduce globule size.
- Fine dispersion of aqueous nanoparticles are formed by subsequent removal of organic solvent by heat and vacuum.
- Homogenization step is the key factor in obtaining submicron particles.

- E.g. PLGA (poly acetyl glycolic acid) to prepare PLGA nanospheres the polymer is solubilized in chloroform and dispersed in gelatin solution by sonication to form o/w emulsion.
- Solvent is removed by evaporation using sonicator, high-speed/pressure.
- Homogenizer which breaks the coarse emulsion to nano droplets yielding nanospheres.

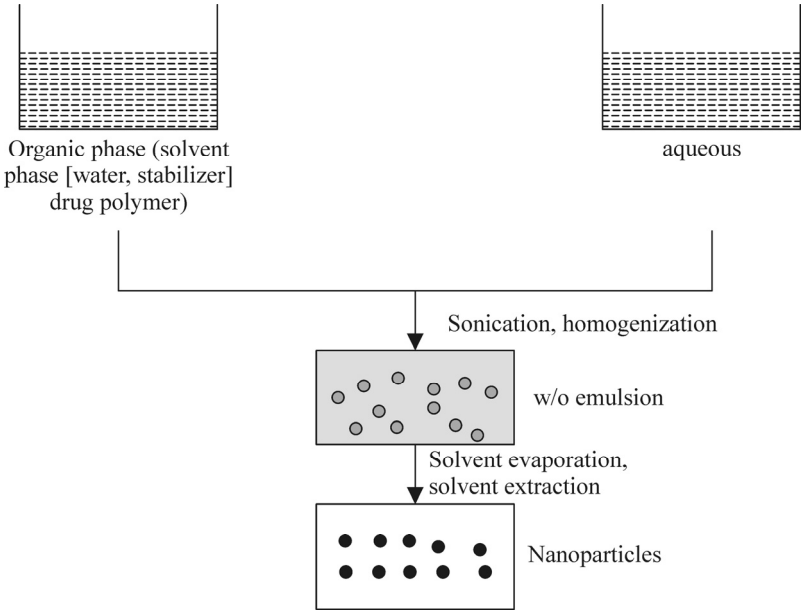

Fig. 1.3 Solvent evaporation method.

Multple Emulsion Solvent Evaporation Method

- It is modified form of solvent evaporation.
- In this method w/o/w is formed and later organic solvent is evaporated to form nanoparticles.
- This are washed with water and then lyophilized.

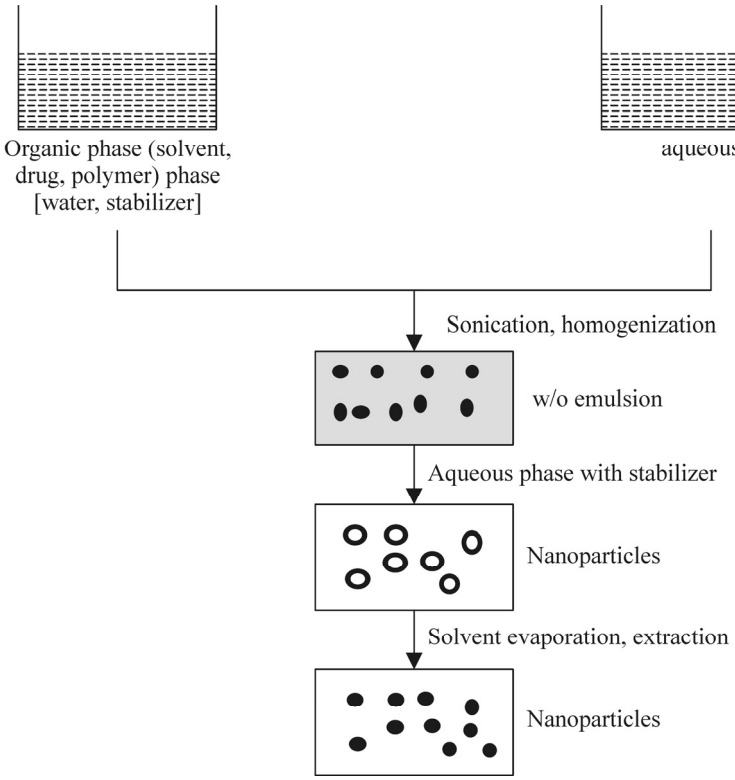

Fig. 1.4 Double emulsion solvent evaporation.

(b) Solvent Displacement (Nano Precipitation)

- This method involves interfacial deposition of polymer following displacement of semi polar solvent (miscible with water) from lipophilic solution.
- It involves use of organic phase and external phase which are completely miscible.
- As they are completely miscible the organic solvent diffuse to the external aqueous phase inducing polymer precipitation.
- For slightly soluble drugs after nano particle preparation solvent is eliminated and free flowing particles are obtained under reduced pressure.

- For hydrophilic drugs –drug diffuse out in to external phase.
- For highly hydrophobic drugs –drug precipitates in aqueous phase as crystals which grow during storage.

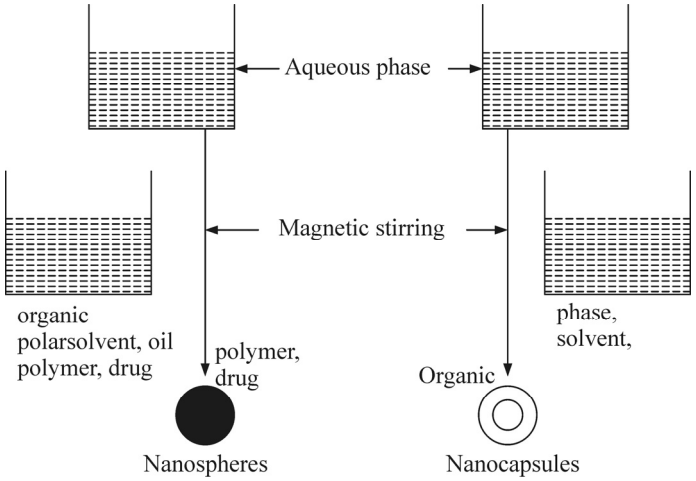

Fig. 1.5 Solvent displacement method.

(c) Salting Out

- Most common method used for preparation. It involves incorporation of saturated aqueous solution of poly vinyl alcohol (PVA) solution into organic phase.
- They are stirred under magnetic stirrer to form o/w emulsion.
- In this method miscibility of phases is retarded by adding PVA in external aqueous phase.
- When sufficient amount of water is added to the external phase it allows complete diffusion of acetone from internal phase to aqueous phase.
- This method is used for drugs and polymers which are soluble in polar solvents.

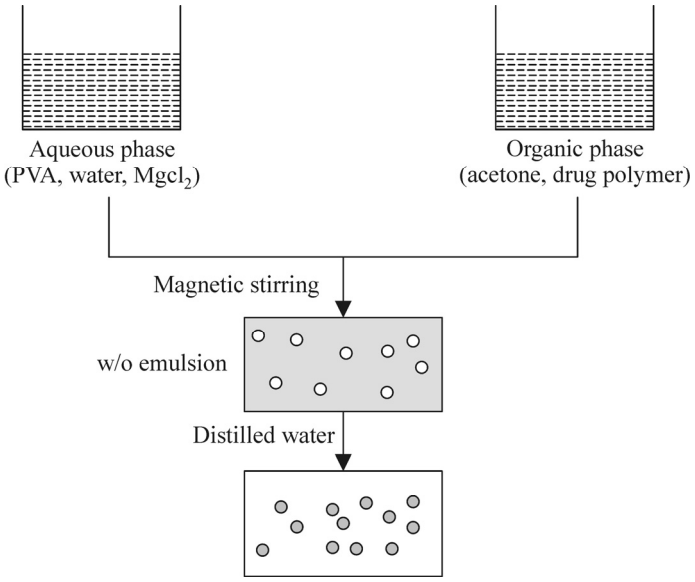

Fig. 1.6

Characterization/Evaluation of Nano Particles

1. Particle size and morphology
2. Molecular weight
3. Density
4. Surface properties
   (a) Surface charge
   (b) Surface hydrophobicity
5. Drug release and Drug loading

1. Particle Size and Morphology

One of the most important parameters of nanoparticles is particle size. It is determined by using following techniques,

(a) Photon correlation spectroscopy (PCS)

(b) Electron microscopy
   (i) Scanning electron microscopy (SEM)
   (ii) Transmission electron microscopy (TEM)

2. Molecular Weight

Molecular weights of nanoparticles are usually determined by Gel-permeation chromatography. It is determined by dissolving the particles in appropriate solvent.

Gel chromatography requires total dissolution of the polymer so molecular weight determination of cross linked polymers is not possible.

3. Density

Gas pycnometer with helium or air is used to measure the density. The value obtained with air and helium differs from each other due to specific surface area and porosity of structure.

4. Surface Properties

(a) Surface Charge

As surface charge determines the interaction of nanoparticles with biological environment and bioactive compounds, determining and studying the surface charge plays a important role.

The surface charge can be determined by

- Electrophoresis
- Laser Doppler Anemometry (LDA) (velocimeter)

(b) Surface Hydrophobicity

- Interaction of colloidal particles with the biological environment (e.g., protein adsorption, cell adhesion) are influenced by surface hydrophobicity.
- Biofate of nanoparticles and its contents are determined by hydrophobicity and hydrophilicity.
- Hydrophobicity and hydrophilicity of nanoparticles are determined by measuring angle of contact.

5. Drug Release and Drug Loading

Drug Loading

- High loading capacity is the property of the successful nanoparticulate system.

- Drug loading is done by two methods,
  (i) In corporation at the time of production
  (ii) Adsorption of drug after the formation of nanoparticles
- Drug loading and entrapment depend on the
  - Solid dissolution or solid dispersion
  - Molecular weight
  - Drug polymer interactions
  - Presence of end functional groups
  - Macro molecule or protein show good loading efficiency

Drug Release

- Drug release and polymer biodegradation are important consideration factors for a successful nanoparticulate system.

  Drug release rate depends on:

  (i) Solubility of drug
  (ii) Desorption of the surface bound/adsorbed drug
  (iii) Drug diffusion through the nanoparticle matrix
  (iv) Nanoparticle matrix erosion/degradation
  (v) Combination of erosion/diffusion process
- Various methods which can be used to study the *in vitro* release of the drug are,
  (i) Side-by-side diffusion cells with artificial or biological membranes
  (ii) Dialysis bag diffusion technique
  (iii) Reverse dialysis bag technique
  (iv) Agitation followed by ultracentrifugation/centrifugation
  (v) Ultra-filtration or centrifugal ultra-filtration techniques

Application of Nanoparticles

Nanoparticles are widely applied for different therapeutic application they are

- These are used in intracellular targeting of anti-infective to reduce the difficulty to treat intracellular infections in human

body. E.g., ampicillin to nanoparticles for intra-bacterial infections.

- To reduce the toxicity and increase the therapeutic activity of cytostatic drugs.
- For specific targeting of anti-inflammatory drugs to minimize the side effects.
- Normal aqueous eye drops have half-life 1-3 minutes but nanoparticles has half-life of 15-20 minutes.
- In nuclear medicine they are used as carriers for radio nucleotides for diagnostic purpose.
- To improve the solubility and bioavailability of poorly soluble drugs.
- They are used as solid lipid nanoparticles in skin and hair care.
- To deliver drug across blood brain barrier.
- For targeted delivery of peptides and proteins.
- Used as adjuvant for vaccines. E.g., polymethyl methacrylate.