ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Nanoparticles: An Advance Technique for Drug Delivery

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ABSTRACT

Nanoparticles have attracted the interest of many research groups and had been utilized in an increasing number of fields during the last decades. Various methods can be used to produce nanoparticles such as solvent evaporation, salting-out, dialysis, supercritical fluid technology, micro-emulsion, emulsion cross linking and interfacial polymerization. This review covers the general description, preparation and the detailed description of the crucial parameters involved in techniques designed to obtain the desired properties of nanoparticles.

Keywords: Nanoparticles, Polymerization , Zeta Potential

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ISSN: 0975-8585

INTRODUCTION

Nanoparticles (NP) are a type of colloidal drug delivery system comprising particles with a size range from 10 to 1000nm in diameter and are used to deliver the drugs or biomolecules. The word 'nano' is derived from Latin word, which means dwarf. Nano size refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a metre (i.e. $1nm = 10^{-9}$ m)[30]. Generally, nanometric carriers also comprise sub-micron particles with size below 1000 nm. They are drug carriers of natural, semisynthetic, and synthetic polymeric nature in the nanometer size range. Nanoparticles may or may not be are a collective name for nanospheres and nanocapsules as illustrated in Fig. 1. Nanospheres possess a matrix type structure, where drugs or biomolecules are absorbed or dissolved or entrapped within the polymeric carrier. Nanocapsules are vesicular system in which the drug or bioactive molecule forms a core, which is surrounded by a polymeric membrane. In this case the active substances are usually dissolved in the inner core but may also be absorbed at their surface [59]. Conventional preparations like solution, suspension or emulsion suffer from certain limitations like higher dose requirements due to low bioavailability, often associated with first pass effect, intolerance and instability. Moreover, they are associated with fluctuations in plasma drug levels and do not provide sustained effect. Therefore, some novel carriers are need of the hour, which could meet ideal requirement of drug delivery system. Nanoparticles may or may not be associated with other nanosize-related properties that differ significantly from those observed in fine particles or bulk materials.

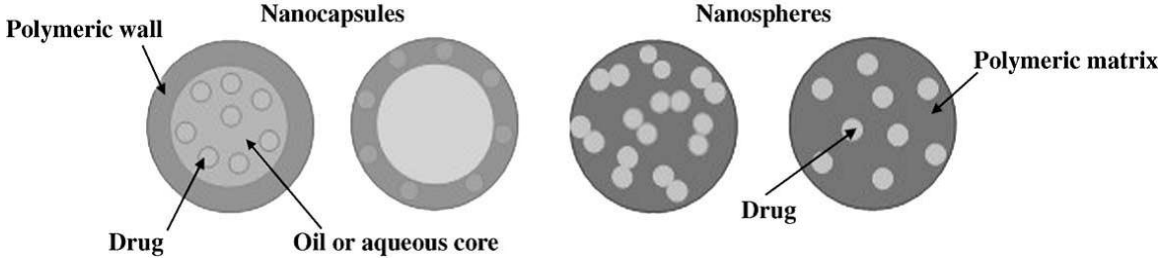

Fig1. Schematic representation of polymeric nanoparticles [59]

The ideal requirements for designing nanoparticulate delivery system are to effectively control particle size, surface properties, enhance solubility and release of pharmacologically active ingredients in order to achieve the site-specific delivery at predetermined rate. Although, liposomes have also been used as potential carriers with unique advantages including biocompatibility, targeting to site of action and reduction in toxicity or side effects, their applications are limited due to inherent problems like low encapsulation efficiency, rapid leakage of drug and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. Polymeric nanoparticles offer stability of drugs/proteins and possess useful controlled release properties.

Various methods had been utized for preparing nanoparticles depending on the

physicochemical properties of the polymer and the drug. Most of the methods involve the use of organic solvents, heat, sonication or vigorous agitation which may affect the stability of active pharmaceutical ingredients. Nanoparticles can also be formulated by utilizing electrostatic interaction between charged species. These polyelectrolyte complexes can be formulated by avoiding such stresses asoociated with other methods of preparation, therefore minimizing possible damage to drug during formulation. A variety of biodegradable polymers with diverse physicochemical properties are available to formulate nanoparticles. These polymers are either natural or synthetic. Natural polymeric carriers used for oraly delivered nanoparticles include chitosan, dextran, gelatine, alginate, agar among which chitosan is the most popular one.

Chitosan is biocompatible, non-toxic and mucoadhesive derivative obtained from natural source. It is a widely available modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin obtained from shells of crustaceans like crabs, prawns etc. The physicochemical properties of chitosan are significantly influenced by its molecular weight and degree of deacetylation. The presence of reactive functional groups in chitosan provides great opportunity for chemical modification, which allows formulation of a wide range of derivatives possessing unique properties. Chitosan is soluble at lower pH although it has limited solubility at pH above 6.5. Derivatives of chitosan, synthesized by introducing alkyl groups to amine groups, e.g. quaternized derivatives of chitosan, are permanently positively charged and posess better aqueous solubility. Chitosan is able to increase intestinal permeability by opening tight junctions. Chitosan can form polyelectrolyte complexes of approximately 200 to 400 nm. Overall, it is evident that Chitosan and its derivatives are useful carriers, owing to their biocompatible and biodegradable nature inaddition to other physicochemical properties. Currently, dietary supplements of chitosan are tested in clinical trials to lower blood cholesterol but no clinical trials with chitosan nanoparticles are ongoing. Other polysachharides like dextran, gelatine and alginate are also being investigated for medical applications due to their biocompatibility [53].

Advantages of Nanoparticles

- Improved stability i.e. long shelf life.
- Increased solubility of the drug.
- High carrier capacity or high drug entrapment efficiency.
- The drug can be incorporated without any chemical reaction, which is a prerequisite for preserving the drug activity.(Wu *et al.,*2011)
- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting.
- Targeted and controlled drug delivery is possible
- Increased therapeutic efficacy and reduction in side effects.(Gelperina *et al.,*2005)
- Drug and particle degradation characteristics can be easily modulated by proper selection of matrix constituents.
- Drug loading is relatively high
- Site-specific targeting can be achieved by attaching targeting ligands to surface of

particles or use of magnetic guidance.

- Nanoparticles can be used for various routes of administration including oral, nasal, parenterals, intra-ocular etc.
- They can be tailor-made to achieve both controlled drug release and disease specific localization by controlling the polymer characteristics and surface chemistry.
- Due to smaller size they are better suited for intravenous (i.v.) delivery without resulting in embolism.
- Nanoparticles are effective in sustaining the drugs thus allowing a more efficient interaction with the receptors which are cytoplasmic [22].
- Decrease the toxic side effects of the drug.
- Allow rapid formulation development.
- Scale up to large scale production is feasible.

Limitations of Nanoparticles

- Their small size and large surface area can lead to particle-particle aggregation.
- Making physical handling of nanoparticles difficult in liquid and dry forms.
- Small particles size and large surface area may result in limited drug loading and burst release.

Types of Nanoparticles

Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) are prepared from a synthetic or semisynthetic polymeric block to increase the circulation half-life and to reduce phagocytic uptake and inactivation of the therapeutic moiety and can be used to deliver and target therapeutic agents. They are formulated by incorporating biodegradable polymers inorder to maximize tissue compatibility and minimize cytotoxicity. A number of polymers are approved by the U.S. Food and Drug Administration (FDA) for administration in human beings viz. polylactic acid (PLA), poly(glycolic acid) (PGA), PLGA, poly-ecaprolactone, and poly(methyl methacrylate). PLA and PLGA can easily be hydrolyzed into individual monomers (lactic acid or glycolic acid), which are removed from the body via normal metabolic pathways. Methods of preparation of PNPs may be categorized two major classes: one deals with the polymerization of monomers (eg, emulsion and dispersion polymerization), whereas the other essentially involves dispersion of polymers (eg, salting out, emulsification- diffusion, and nanoprecipitation). It had been reported that higher entrapment efficiency in PNPs can be achieved by incorporation of drug during their preparation rather than adsorption on preformed nanoparticles.(Wong *et al.,*2010) Drug release takes place in polymeric nanoparticles through their simultaneous biodegradation, followed by desorption, diffusion, or erosion.

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are a comparatively stable colloidal carrier system in

which molten lipid is dispersed in an aqueous media containing surfactant by high-pressure homogenization or microemulsification [8]. They are generally made up of a solid hydrophobic core containing the drug dissolved or dispersed. SLNs exhibit certain potential advantages over PNPs. They can have better brain uptake and exhibit the least toxicity due to the biodegradable nature of the carrier lipid. Smaller size (around 10 to 200 nm) and narrow size range (100 to 200 nm) allows them to cross tight endothelial cells of the blood brain barrier (BBB), escape from the reticuloendothelial system (RES), and bypass the liver [47]. They have comparatively higher drug entrapment efficiency, render the drug more stable in their lipid matrix and provide sustained release of drug lasting up to several weeks. Their production can be scaled up with excellent reproducibility and therefore they can be easily transferred from labs to actual large scale production [49].

Magnetic nanoparticles

Magnetically targeted nanoparticulate drug delivery systems involve binding of drug with magnetic nanoparticles (MNPs), such as oxidized iron (Fe) or magnetite. By virtue of their controllable sizes (ranging from 10 to 100 nm) and capacity of delivering the drug or biomolecules in the vicinity of a target site, they hold a lot of potential for targetted drug delivery especially in treatment of cancer as well as in diagnostics. For biomedical applications, magnetic carriers must be water based, biocompatible, nontoxic, and nonimmunogenic. Various magnetic carriers, which receive external magnetic field, include nickel, cobalt, iron, and magnetite. Iron oxide is most commonly used due to its biodegradable nature, biocompatibility, super paramagnetic effects, and capacity to serve as a contrast agent in magnetic resonance imaging (MRI). Iron oxide particles are phagocytosed or endocytosed by the Kupffer cell in the RES of liver, spleen, lymph, and bone marrow. Once compartmentalized within the lysosomes of RES cells, they are broken down into ferritin and/or hemosiderin, which are antiferromagnetic forms of iron. The concentration of carriers at any specific location can be manipulated by calculation of capillary flow rate, vascular permeability, and hydrodynamic condition prevalent at the target site as well as pathophysiology of the individual. For therapeutic effect, MNPs are injected into the bloodstream and a high gradient magnetic field is generated outside the body so as to pull them out of biological stream and deliver the drug to a localized disease site[69].

Metal and Inorganic Nanoparticles

Nanoparticles have also been prepared using various metals, such as gold (Au), copper (Cu), and silver (Ag) and inorganic carriers, like silica or alumina, among which gold nanoparticles are widely being invesigated due to their excellent optical and photoelectric properties. Moreover, gold nanoparticles exhibits some specific advantages, like inertness and nontoxicity, higher stability, ease of preparation, and possibility of bioconjugation and biomodification with thiol, disulfide, and amine functional groups. Its dispersion stability can be enhanced by conjugation with thiolated PEG. Gold nanoparticles are highly effective contrast agents in cancer diagnosis and photodermal cancer therapy. Furthermore, they serve as a good vector for oligonucleotide, thiol-conjugated small interfering RNA (Si-RNA), insulin, and gene

delivery [60].

Quantum dots

Quantum dots (QDs) are colloidal semiconductor nanocrystals (up to 2 to 10 nm), composed of atoms from groups II–VI or III–V of the periodic table, having unique optical and fluorescent properties (Fig. 2). The most commonly used materials for preparation of quantum dots are cadmium selenide (CdSe), cadmium telluride (CdTe), and indium arsenide (InAs). Upon their interaction with photon, they get excited and emit energy in UV, visible, or near-infrared (IR) regions, which can be detected. Owing to their small size, they can be used for the tagging of biological macromolecules, such as nucleoside and proteins [48].

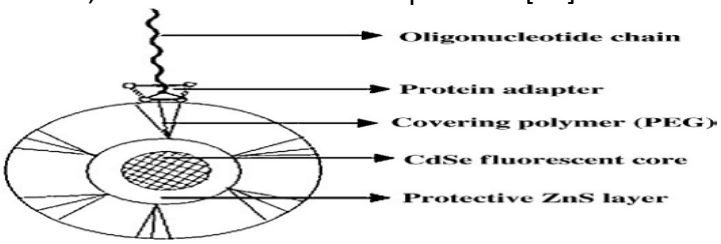

Fig.2 Fluorescent quantum dots

Polymeric Micelles

Polymeric micelles (PMs) are nanosized core-shell structures formed by spontaneous self-assembly of individual amphiphilic di/tri-block co-polymers with hydrophobic core and hydrophilic surface shells or vice versa (Fig.3). They contain both hydrophilic and hydrophobic regions in their structure and serve as good carrier systems for poorly soluble drugs. Multifunctional polymeric micelles can be also being designed to facilitate simultaneous drug delivery and imaging. Their stability depends on strong cohesive force between drug and core polymer segments as well as cross-linking of the shell or core, which is performed by radical polymerization. Prolonged circulation and targeted delivery of PMs is possible by designing of environment-responsive polymeric micelles (pH, light, temperature, ultrasound, etc.).

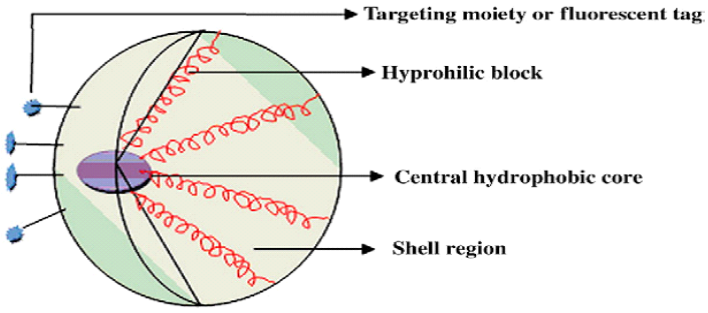

Fig.3 Schematic representation of a self-assembled block copolymeric micelle [48]

Sterically Stabilized Micelles

Sterically stabilized micelles (SSMs), containing polyethylene glycol (PEGylated) and phospholipids (Phospholipid Micelles), have also been prepared as a safe, biocompatible

nanocarriers for the delivery of poorly water-soluble as well as cytotoxic drugs like anticancer drugs. Camptothecin-containing SSMs (CPT-SSMs) has been prepared as a novel nanomedicine for parenteral administration, which showed higher solubilization potential, estimable stability, and less *in vitro* cytotoxicity.

Colloidal Nano-liposomes

Liposomes are small artificial vesicles of globular shape composed of aqueous pores encapsulated with amphiphilic phospholipids and cholesterol bilayer (Fig. 4) [62]. They are widely used owing to their ability to act as carrier for both hydrophilic as well as hydrophobic drugs, and better tissue biocompatibility along with the fact that their size can be suitably monitored [37]. Depending on size and number of phospholipid bilayers, liposomes had been classified into small unilamellar vesicles (SUVs; single lipid layer 25 to 50 nm in diameter), large unilamellar vesicles (LUVs; heterogeneous group of vesicles), and multilamellar vesicles (MLVs; several lipid layers separated from one another by a layer of aqueous solution). Liposomes have been widely investigated for the delivery of vaccine, toxoids, gene, anticancer, and anti-HIV drugs.

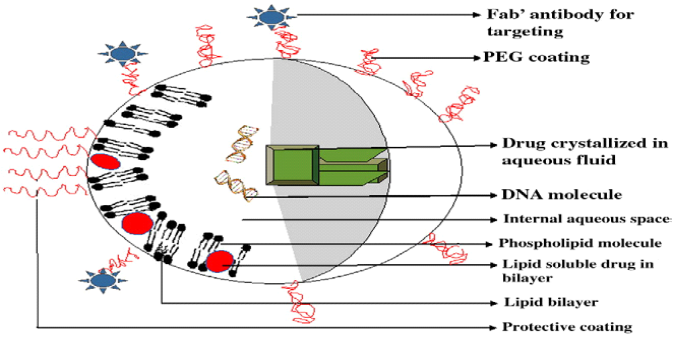

Fig. 4 Schematic representation of sterically stabilized liposomes. [48]

Hydrogel nanoparticles (Nanogels)

Hydrogel nanoparticles (NPs) (recently referred to as nanogels) as a family of nanoscale particulate materials, have recently attracted a lot of attention of scientists dealing with development of newer drug delivery systems. Interestingly, hydrogel nanoparticulate materials would demonstrate the features and characteristics of both hydrogels and NPs simultaneously. Therefore, it seems that the pharmacy world will benefit from both the hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility of these particles and all the advantages of the NPs, mainly long life span in circulation and the possibility of being actively or passively targeted to the desired biophase, e.g. tumor sites. Different methods have been adopted to prepare NPs of hydrogel consistency. Besides the commonly used synthetic polymers, active research is now focused on the preparation of NPs using naturally occurring hydrophilic polymers and hydrocolloids.

ISSN: 0975-8585

Methods of Preparation of Nanoparticles

Nanoparticles can be prepared using a wide variety of materials such as proteins, polysaccharides and synthetic and semisynthetic polymers as well as natural gums and hydrocolloids. The selection of ideal matrix forming material/ polymeric carrier is made after careful consideration of many factors such as:

- Size of nanoparticles required
- Inherent properties of the drug, e.g., pKa, aqueous solubility and stability
- Surface characteristics such as charge and permeability
- Degree of biodegradability, biocompatibility and toxicity
- Drug release profile desired
- Antigenicity of the final product.
- Method of preparation being used, etc

Emulsion cross-linking

This method utilizes the reactive functional amine group of CS to cross-link with aldehyde groups of the cross-linking agent. In this method, water-in-oil (w/o) emulsion is prepared by emulsifying the CS aqueous solution in the oil phase. Aqueous droplets are stabilized with the help of suitable surfactant (Low HLB value). The stable emulsion is cross-linked by using an appropriate cross-linking agent such as glutaraldehyde, which results into hardening of the droplets. The resulting nanoparticles are seperated by centrifugation, filtered and washed repeatedly with n-hexane followed by alcohol and then dried. By this method, size of the particles can be controlled by controlling the size of aqueous droplets. However, the particle size of final product depends upon the extent of cross-linking agent used while hardening, in addition to nature and concentration of surfactant along with speed of stirring during the formation of emulsion. This method is schematically represented in Fig.5. The emulsion cross-linking method has few drawbacks since it involves tedious procedures as well as use of harsh cross-linking agents, which might possibly induce chemical reactions with the active agent. Moreover, complete removal of the un-reacted crosslinking agent may be difficult in this process.

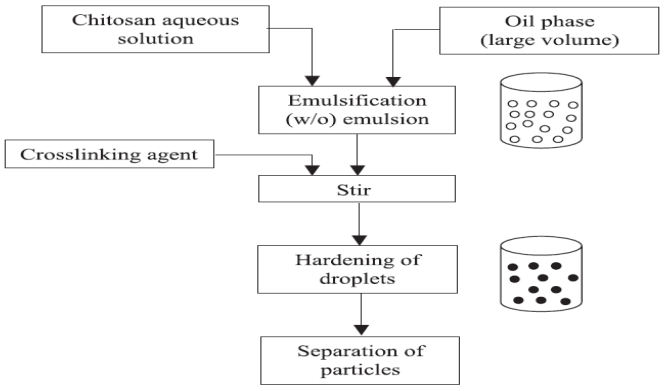

Fig. 5: Schematic representation of preparation of chitosan nanoparticulate systems by emulsion cross-linking method. [68]

ISSN: 0975-8585

Solvent evaporation method

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion.(Fig.6) After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication along with suitable surfactant and stabilizer may be employed [78].

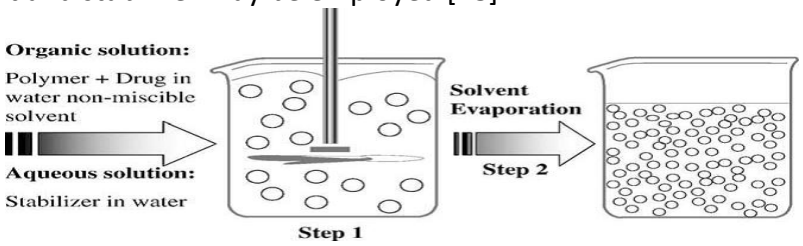

Fig.6 Schematic representation of the emulsification-evaporation technique.

Spontaneous Emulsification or Solvent Diffusion Method

Spontaneous emulsification or Solvent diffusion method is a modified version of solvent evaporation method. In this method, the oil phase consists of a water miscible solvent along with a small amount of the water immiscible organic solvent and due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both hydrophobic and hydrophilic drugs can be formulated into nanoparticles by solvent evaporation and solvent diffusion methods. In the case of hydrophilic drug, usually a multiple w/o/w emulsion is formulated with the drug dissolved in the internal aqueous phase [23].

Emulsification/solvent diffusion (ESD) is based on the use of organic solvents, and then it was suitably modified in accordance with the salting-out technique. The polymeric carrier is dissolved in a water miscible solvent such as propylene carbonate and then is saturated with water to ensure the initial thermodynamic equilibrium between both the liquids. In order to induce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to facilitate the diffusion of the solvent of the dispersed phase by dilution with an excess of water, when the organic solvent is partly miscible with water or with another organic solvent in the case where organic solvent is immiscible with water. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, depending on the oil-to-polymer ratio. Finally, the solvent is removed by evaporation or filtration, depending on its boiling point. This technique offers several advantages, such as high

encapsulation efficiencies (generally more than 70%), no requirement of homogenizers, high batch-to-batch reproducibility, easier scale-up, simplicity, and capability to yield narrow size distribution. Although there are certain limitations/ drawbacks as higher volume of water is to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, which may lower the encapsulation efficiency. As compared with some of the other techniques, this method is efficient in encapsulating lipophilic drugs.

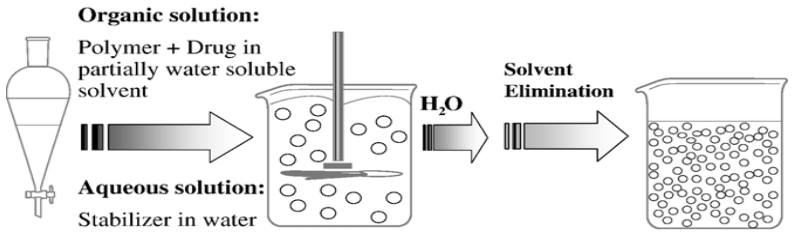

Fig. 7 Schematic illustration of the ESD technique [59]

Polymerization Method

In polymerization methods, monomers are polymerized with sugseqent entrapment of drug particles to form nanoparticles or adsorbed on their surface. Drug is incorporated either by dissolving in the polymerization medium or by adsorption onto the nanoparticles after completion of polymerization [61]. The nanoparticle suspension is then purified to remove traces of free stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium.(Fig.8) This technique has widely been utilized for formulating polybutylcyanoacrylate or poly(alkylcyanoacrylate) nanoparticles.

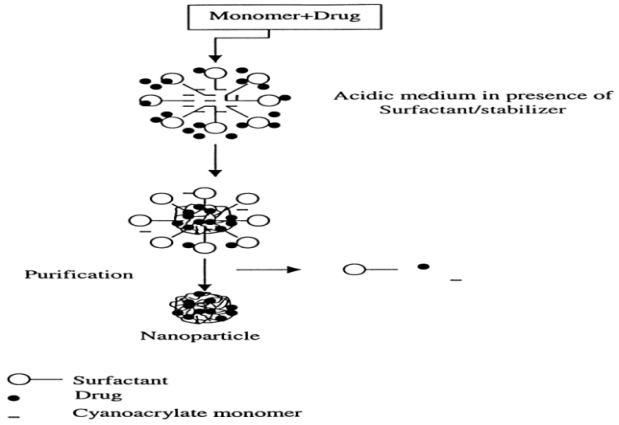

Fig.8 Schematic representation of polymerization method for the production of nanoparticles

Coacervation or Ionic Gelation Method

The use of ionic interaction between oppositely charged macromolecules to prepare CS nanoparticles has attracted much attention because the simplicity of process avoiding other stress conditions. In addition, reversible physical cross-linking by electrostatic interaction, instead of chemical cross-linking, has been applied to avoid the possible toxicity of reagents and

other undesirable effects. Tripolyphosphate (TPP) is a polyanion, which can interact with the cationic CS by electrostatic forces. The preparations of TPP–CS complex by dropping CS droplets into a TPP solution have been explored for its potential pharmaceutical usage. In the ionic gelation method, CS is dissolved in aqueous acetic acid solution to obtain the cation of CS (Fig.9), which is then added dropwise under constant stirring to polyanionic TPP solution. Due to the complexation between oppositely charged species, CS undergoes ionic gelation and precipitates to form spherical particles. However, TPP/CS microparticles formed have poor mechanical strength thus, limiting their usage in drug delivery [51].

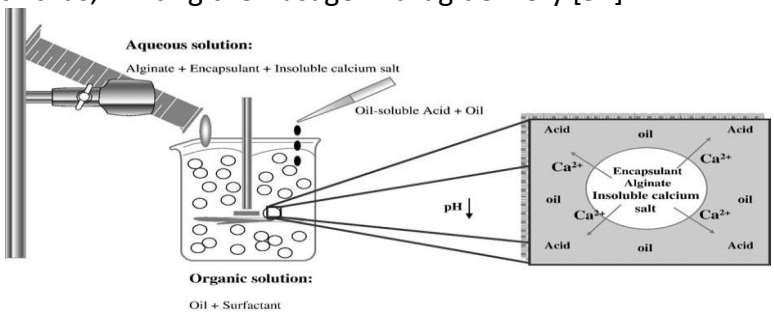

Fig.9. Schematic representation of the emulsification–internal gelation technique using alginate. [59]

Complex Coacervation Methods

Complex coacervation is a spontaneous phase separation process involving two liquid phases in colloidal systems, which results by the interaction of two oppositely charged polyelectrolytes upon mixing in an aqueous solution. The process leads to formation of micrometric or nanometric colloidal particles, depending on substrates or process variables, such as pH, temperature, molecular weight, ionic strength, polyelectrolyte concentration, and so forth The major drawbacks of this method is poor drug stability and lower drug loading efficiency, which, however, can be overcome by cross-linking of the complex by chemical reagents, such as glutaraldehyde.

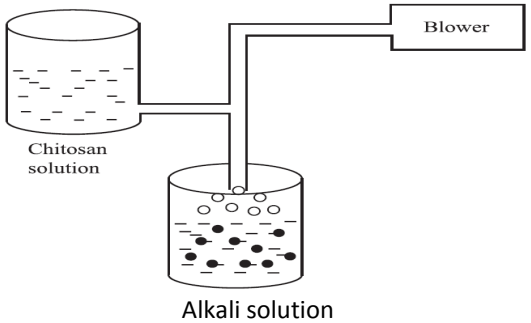

Fig.10 Schematic representation of preparation of chitosan nanoparticles by coacervation/precipitation method

Co-precipitation Method

Co-precipitation is a modified complex coacervation method for the preparation of nanosized core-shell particles having good dispersion stability of hydrophobic (poorly water-soluble) drugs. Drug loaded nanoparticles are stabilized by diethyl amino ethylcellulose (DEAE)-

dextran (Ddex as water-soluble, positively charged resin). Ddex is used as a coating layer to coprecipitate with negatively charged drug. The method involved precipitation of drug in a supersaturated solution, followed by deposition of Ddex onto the precipitated drug particles through electrostatic interactions. Transmission electron microscopy (TEM), atomic force microscopy (AFM), and zeta potential studies showed typical core-shell nanoparticles, with high encapsulation efficiency and good stability [23].

Spray-drying

Spray-drying is a well-known technique to produce powders, granules or agglomerates from the mixture of drug and excipient solutions as well as suspensions. The method is based on drying of atomized droplets in a stream of hot air and can be applied for formulation of nanoparticles [54]. In this method, CS is first dissolved in aqueous acetic acid solution, drug is then dissolved or dispersed in the solution along with a suitable cross-linking agent. This solution or dispersion is then atomized in a stream of hot air [78]. Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles as depicted in Fig.11.Various process parameters are required to be carefully controlled in order to get the desired size of particles. Particle size depends upon the size of nozzle, spray flow rate, atomization pressure, inlet air temperature and extent of cross linking [19].

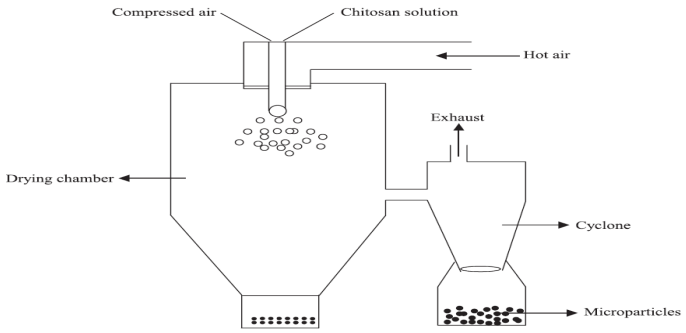

Fig.11 Schematic representation of preparation of chitosan particulate systems by spray drying method

Salting-out Method

The salting-out method is widely used in the pharmaceutical industry owing to its high yield, purity, speed and simplicity of the operation. The method does not demand thermal treatment at any stage of sample processing and therefore, may be especially useful for the incorporation of thermolabile drugs. It is based on the phenomenon in which solubility of a non-electrolyte in water is decreased upon addition of an electrolyte. This method involves an emulsification step avoiding the use of surfactants and chlorinated solvents, where a water-soluble stabilizing polymer is added to a saturated solution of electrolyte (e.g, sodium chloride, magnesium acetate, or magnesium chloride) to obtain a viscous gel. Subsequently, polymer and drug are dissolved separately in an organic solvent. Most often, acetone is used as solvent because of its solubilizing properties and easier separation from aqueous solution upon salting-out with electrolytes. Addition of viscous gel into organic phase under continuous stirring

causes salting out of the organic solvent, inducing formation of nanoparticles in organic-aqueous medium (Fig. 12). Finally, both solvent and electrolyte are eliminated by cross-flow filtration [50].

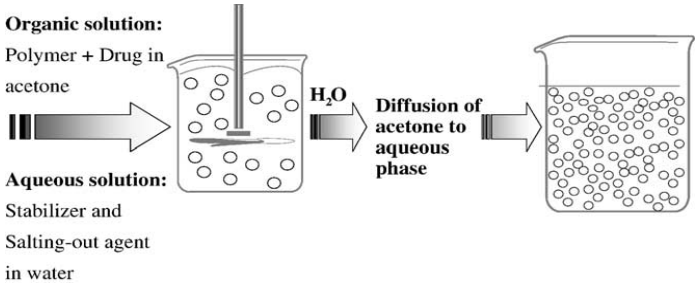

Fig 12 Schematic of the salting-out technique [59]

Nanoprecipitation Method

Nanoprecipitation, also known as solvent displacement method, is based on interfacial deposition of a polymer after displacement of a semi polar solvent miscible with water from a lipophilic solution. Rapid diffusion of the solvent into aqueous phase results in a decrease in the interfacial tension between the two phases, which increases the surface area and leads to formation of small droplets of organic solvent even without any mechanical stirring. However, it provides poor entrapment efficiency for water-soluble drugs [23].

Supercritical Fluid Methods

Submicrometer-sized and nano-sized particles can also be prepared by using supercritical fluid (SCF) technology. A supercritical fluid can either be a liquid or gas and used above its thermodynamic critical point of temperature and pressure. Most commonly used SCFs are carbon dioxide (CO_2) and water [13].

Rapid Expansion of Supercritical Solutions

Rapid expansion of supercritical solutions (RESS) is a useful technique for thermolabile drugs and is able to produce finely divided particles of nanometer size range with a precisely controlled size distribution (Fig13). The process involves saturation of SCF with the drug and depressurization of the obtained solution through a heated nozzle into a low-pressure chamber to cause rapid nucleation of the drug yielding more uniform particle size. As the solution is allowed to expand across a calibrated orifice, the density decreases gradually and the solute is precipitated as finely divided solid fibers or crystals. Expansion at high pressure keeps the density high and reduces the flow velocity of particles, thus providing the particles enough growth time for clustering and aggregation. Consequently, larger particles are produced at high expansion pressure, whereas, expansion into low pressure causes density to become low with higher velocity, so that both the flow time and density are less favorable for the growth of larger clusters.

ISSN: 0975-8585

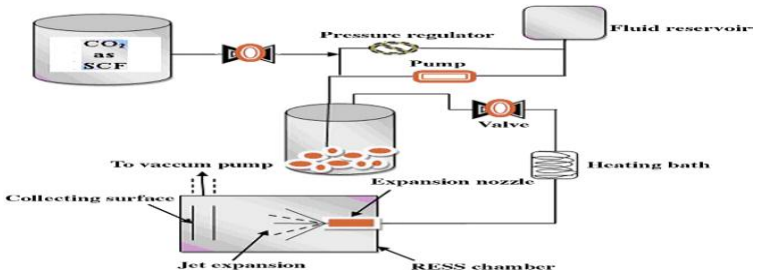

Fig.13 Rapid expansion supercritical solution method [48]

Supercritical Antisolvent Precipitation

Supercritical antisolvent precipitation (SAS) is now widely being investigated as an alternative to the liquid antisolvent precipitation (LAS) for producing micronized particles of some antibiotics.(Huerates *et al.,*2010) In the case of liquid antisolvent processing, removal of solvent is a cumbersome process, whereas SAS allows removal of the solvent under reduced pressure and results in appearance of sub micrometer-sized particles with narrow size distribution.(Amidi *et al.,*2010) Selection of proper solvent is a prerequisite as SAS technique is based on the use of two completely miscible liquid solvents; the drug or solute to be micronized should be soluble in the first solvent but not in the second one.(Nagarwal *et al.,*2009) Antisolvent addition can be carried out from the top or bottom of the precipitating chamber.(Rao *et al.,*2004) A faster diffusion of the solvent results in super saturation resulting in precipitation of particles in microsized form. Supercritical CO_2 is allowed to flow through the chamber until the end of precipitation in order to remove the remaining liquid solvent (Fig14). Otherwise, the remaining liquid solvent may resolubilize the solutes during depressurization step affecting the product quality and stability [16].

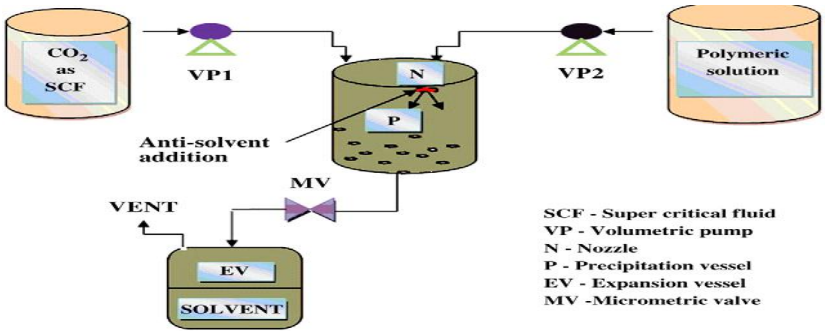

Fig.14 Supercritical antisolvent precipitation method

Osmosis Based Method

A novel osmosis based method had recently been proposed (Fig. 15) for the preparation of various natural- and synthetic Polymeric Nanoparticles. This method is based on the use of a physical barrier, particularly dialysis membrane or common semi-permeable membrane (SPM) that allow the passive transport of solvents in order to reduce the mixing of the polymer solution with a non solvent.

ISSN: 0975-8585

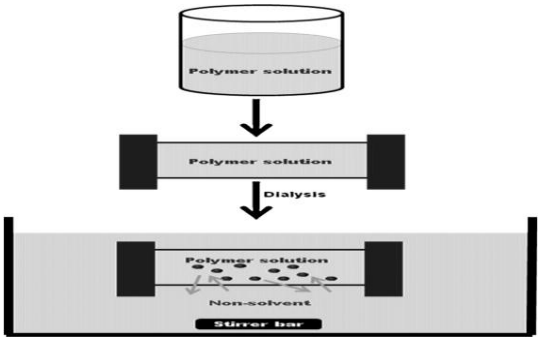

Fig.15 Schematic representation of osmosis based method for preparation of polymer nanoparticles [58]

Polymers used in Preparation of Nanoparticles

The polymers used in the preparation of polymeric nanoparticles can be divided into two broad categories.

Biodegradable Polymers

- Albumin
- Gelatin
- Alginic acid/alginates
- Chitosan and chitin derivatives
- Polylactic acid (PLA)
- Polyglycolic acid (PGA)
- Polylactide-co-glycolide (PLGA)
- Poly-e-caprolactone (PCL)
- Polylactide-co-caprolactone (PLC)
- Polyalkyl cyanoacrylates [31]

Non-biodegradable Polymers

- Polymethyl vinyl ether/maleic anhydride
- Gantrez
- Polymethyl methacrylates (Eudragit)
- Polyamidoamines. (PAMAM) [9, 17, 38]

EVALUATION OF NANOPARTICLES

It is very important to characterize the freeze-dried product and to investigate the conservation of the nanoparticle properties.

Structural characterization

Structural characterization plays an important role in determining various attributes of a

nanoparticulate system like shape, size, surface morphology, spatial distribution, density, geometric feature etc. Scanning electron microscopy (SEM) produces the image down to length scales of 10 nm and provides valuable information regarding surface topology, structural arrangement, spatial distribution as well as surface morphology of nanoparticles. Transmission electron microscopy (TEM) and high resolution TEM are more powerful imaging tools than SEM and give more detailed geometrical features and information like crystal structure, quality, and orientation of nanoparticles along with varying density of the phases involved. Moreover, scanning tunneling probe such as scanning tunneling microscope (STM), electrical field gradient microscopy (EFM), and scanning thermal microscopy, combined with atomic force microscopy(AFM) are alsonow being employed to illustrate structural, electronic, magnetic and thermal properties besides topographical properties of nanosystems [72].

Particle Size Distribution

Particle size distribution and polydispersity index are one of the most important aspect of formulation of nanosystems, efforts are always made to achieve a system with narrow particle size distribution with lowest polydispersity index. Some techniques to determine the particle size distribution are dynamic light scattering techniques, microscopic techniques etc. The laser diffraction technique is used to detect microparticles or possible aggregates of drug nanoparticles.

Particle Charge / Zeta Potential

Zeta potential is the charge at particles mobile surface and is used to determine the degree of flocculation or deflocculation in nanosystems. Zeta potential measurement is carried out to optimize formulation parameters and to make predictions regarding the storage stability of the colloidal dispersion. Its value may be positive or negative depending on nature of, drug, polymer adsorbed ions. A sufficiently high zeta potential (positive or negative) indicates that the system shall be deflocculated as for aggregation particles have to overcome the electrostatic energy barrier.

Crystalline Status

Differential scanning calorimetric, X ray diffraction and other analytical methods are used to assess any possible changes brought about in the physical form, amorphous or crystalline structure and other polymorphic changes in the drug during formulation. Presence of different polymorphs can also be assessed by X ray diffractometer.

Toxicity Evaluation

Nanoparticles are also associated with some acute and long term toxicities determined in various animal models. Some important acute toxicities associated with nanosystem are enhanced endocytosis resulting in inflammation and granuloma formation; oxidative stress causing cell death due to free radical generation and altered and/or modified protein/gene

structure resulting in immune responses. The long-term toxicities associated with nanosystems are bioaccumulation, poor biodistribution and ultimate fate of nanosystem in body [30].

Macroscopic aspect of freeze-dried product

A freeze-dried product is observed to assess of the final volume and the appearance of the cake. One of the desired characteristics of a freeze-dried pharmaceutical form is to yield an intact cake occupying the same volume as the original mass. An attentive microscopic examination of the freeze dried cake must be carried out in order to detect any shrinkage or collapse of the formulation.

Reconstitution time

To rehydrate the freeze-dried nanoparticles one must add the same volume of water lost after lyophilization. The time of reconstitution may be recorded. In general, freeze-dried product rehydrates immediately after the addition of water, but in some cases, a long reconstitution time could be obtained as in the case of collapsed formulations. Many methods could be used to achieve the re-suspension of freeze dried nanoparticles after the addition of water, as manual shaking, vortexing or sonication to ensure full re-suspension. Measurement of nanoparticles size and zeta potential after freeze-drying after reconstitution, nanoparticles size must be measured by photon correlation spectroscopy or another technique. The conservation of a nanoparticle diameter size after freeze-drying is considered as a good indication of a successful freeze-drying cycle. In general, the ratio of nanoparticles size after and before freeze-drying may be calculated. A value near from one indicates the conservation of nanoparticles size, whereas an important value of this ratio indicates the aggregation of nanoparticles. Furthermore, the index of polydispersity may be recorded after lyophilization. This index gives also an idea about the distribution of nanoparticles size and its value must be compared to the value before freezedrying, to evaluate the conservation of nanoparticles distribution. The measurement of zeta potential is a good method to evaluate the state of nanoparticles surface and to detect any eventual modification after freeze-drying. Furthermore, it can be used to study the interaction between the cryoprotectant molecules and the nanoparticles surface. It has been found that the addition of 10% of sucrose to itraconazole loaded poly(ε-caprolactone) nanospheres suspension before freeze-drying decreased the negative surface charge from −40.9 mV to −20.4 mV. The authors explain this by the fact that nanosphere surface being masked as a result of hydrogen bonding between OH groups of the cryoprotectant agent and the surface of the nanospheres. After freeze-drying, the decrease in the negative surface charge is accentuated, showing a rearrangement of the surfactants (poloxamer) at the surface of the nanospheres, leading to a possible desorption of itraconazole molecules [1].

ISSN: 0975-8585

Table 1: Nanoparticles available in National and International Market

Sr No.	Brand Name	Active Ingredients	Indications	Name of Company
1	Adagen	Adinosine deaminase	Adenosine deaminase (ADA) enzyme deficiency	Ezon Pharmaceuticals inc., Bridgewater, NJ, USA
2	Onscaspar	L-asparginase	Acute Lymphoblastic leukaemia	Enzon Pharmaceuticals inc., NJ, USA
3	Copaxone	Glatiramer Acetate	Relapsing-remitting multiple sclerosis	Teva pharmaceuticals, Tikva, Isreal
4	Mucogen	Pegaptanib Sodium	All types of neovascular agerelated macular degeneration	Nektar Therapeutics, San Carlos, CA, USA; OSI Pharmaceuticals, Melville, NY, USA
5	Pegasys	Pegylated interferon alfa-2a	Hepatitis C	Nektar Therapeutics, CA, USA
6	Neulasta	Pegfilgrastim	Neutopenia	Nektar Therapeutics, CA, USA; Amgen inc, Thousand Oaks, CA, USA
7	PEG-INTRON	Peginterferon alfa-2b	Hepatitis C	Nektar therapeutics, CA, USA
8	Somavert	Pegvisomant	Acromegaly	Nektar Therapeutics, CA, USA
9	Protein (Albumin) Nanoparticles	Abraxane	Paclitaxel	Abraxix bioscience, Losangles, CA, USA, Astra Zeneca, Landon, UK
10	Rapamune	Sirolimus	Immunosupressant	Wyeth Pharmaceuticals
11	Megace ES	Megestrol acetate	Treatment of anorexia, cachexia	Par Pharmaceuticals
12	Emend	Aprepitant	Antiemetic	Merck
13	Tricor	Fenofibrate	Antihyperlipidemic agent	Abbott Laboratories
14	Triglide	Fenofibrate	Antihyperlipidemic agent	Skye Pharma
15	Panzem NCD	2-Methoxy estradiol	Estrozen metabolite	Entre Med Inc.
16	Sandummine Neoral	Cyclosporine	Immunosupressant	Novartis
17	Gengraf	Cyclosporine	Immunosupressant	Abbott Laboratories
18	Norvir	Ritonavir	Anti-retrovial	Abbott Laboratories
19	Fortovase	Saquinavir	Anti-retrovial	Hoffman-La Roche
20	Feridex/Endorm	Iron Nanoparticles	Liver Tumor Imaging	Advance Magnetics(USA)

Microscopic observation of freeze-dried product

The microscopic visualization of freeze-dried product is a direct way on the one hand to observe the microstructure of the freeze-dried matrix, on the other hand to prove the conservation of nanoparticles integrity and to observe whether any modification has occurred on their morphology. Many high resolution microscopic techniques are being used to observe the nanoparticle formulation after freeze-drying: transmission electron microscopy (TEM),

cryogenic transmittance electron microscopy (cryo-TEM), atomic force microscopy (AFM), scanning electronic microscopy (SEM), environmental scanning electronic microscopy (ESEM). TEM was also used to observe freeze-dried itraconazole- loaded nanospheres and poly (ε-caprolactone) nanocapsules after reconstitution. It is clear from TEM image that nanocapsules were well conserved after freeze-drying using PVP as cryoprotectant. The polymeric membrane was intact around the oily cavity of nanocapsules. An amorphous matrix of PVP could be observed at the outer surface of nanocapsules. Furthermore, freeze-dried core shell nanoparticles have been imaged by cryogenic transmittance electron microscopy to verify the formation of core/shell nanoparticles. Freeze-dried cationically modified silica nanoparticles using 5% of trehalose as cryoprotectant could be observed by AFM. It could be found from AFM images, that trehalose formed a matrix into which the nanoparticles were inter dispersed. ESEM imaging showed spherical mono disperse nanocapsules being well conserved after freeze-drying. ESEM offers the possibility to control the dehydration of sample by gradual reduction of pressure and temperature in the sample chamber. Such samples can be observed in a hydrated state without a complete drying which prevents the observation of individual nanocapsules. Furthermore, this technique has the ability to image wet systems without prior sample preparation. Finally, ESEM is the best technique for observing of lyophilized nanocapsules in a hydrated state. The advantages of ESEM over SEM for observing colloidal particles with minimal perturbation are the possibility to observe hydrated samples in their native state, without need of conductive coating of the samples and no need of the preparation of the samples. ESEM is the most adequate technique to observe nanoparticles in a hydrated state [75].

Thermal analysis by differential scanning calorimetry (DSC)

During storage, freeze-dried nanoparticles included within a vitrified matrix of lyoprotectant must be stored at a temperature below the temperature of glass transition (Tg) of the dried formulation to prevent any shrinkage of the freeze-dried cake or any destabilization of included nanoparticles as a result of lyoprotectant crystallization. The temperature of glass transition may be determined by differential scanning calorimetry. Furthermore, this technique is very useful to study the interaction between the lyoprotectant and the nanoparticles. For example, in the case of solid lipid nanocapsules freeze-dried with trehalose, DSC study points out a complexation between lecithin (forming the shell of nanocapsules) and trehalose, reinforcing the stabilizing properties of lecithin [7].

Drug content determination

The drug content in nanoparticles must be determined by an adequate analytical method measuring both free drug concentration as well as entrapped drug concentration using High and its value must be compared to that before freeze-drying to detect any leakage of drug from nanoparticles during freeze-drying.

Powder surface analysis

The elemental composition of the powder surface of freeze-dried nanocapsules can be analyzed by electron spectroscopy for chemical analysis (ESCA). This technique is based on the emission of electrons, in response to irradiation by photons of sufficient energy. These electrons are emitted at energies characteristics of the atoms from which they are emitted. ESCA has been repoted to be used for studying the surface modification of nanoparticles, and the adsorption of proteins at the air/liquid interface during spray-drying and adsorption on the ice crystals surface in the freeze-dried product. Such studies have a significant importance especially in the case of freeze-drying of immuno-nanoparticles which have antibodies adsorbed at their surface. The adsorption of protein at the interface ice/liquid during the freezing can disrpt their native fold and results in surface induced denaturation of proteins. Surfactants may drop surface tension of protein solutions and reduce the driving force of protein adsorption at the interface ice/liquid. Low concentrations of nonionic surfactants such as tween 80 are often sufficient to prevent surface adsorption.

Study of water sorption and determination of residual moisture

The thermal and the structure properties of freeze dried nanoparticles are influenced by residual moisture present in the product. Residual moisture is determined by the water desorption process during secondary drying. Sorption isotherm of water study is realized in order to determine on the one hand the degree of hygroscopicity of the product and to assess the ease in secondary drying. In general, the easier the water adsorption, the easier water desorption. The content of residual moisture in freeze-dried nanoparticles can be determined by Karl Fischer titration or by other methods as the gravimetric method or the thermal gravimetric analysis (TGA).

CONCLUSION

Nanoparticle technology is a fast growing field of activity that will allow development of materials with brand new properties. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties, enhance solubility 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. A successful NPs system may be one which has a high loading capacity to reduce the quantity of the carrier required for administration.

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