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REVIEW ARTICLE

Review on Solid Lipid Nanoparticles

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ABSTRACT

Solid lipid nanoparticles (SLN) have emerged as a next-generation drug delivery system with potential applications in pharmaceutical field, cosmetics, research, clinical medicine and other allied sciences. This paper gives an overview about the potential advantages and also the disadvantages of solid lipid nanoparticles, and all the different methods involved in their production. SLN Recently, increasing attention has been focused on these SLN as colloidal drug carriers for incorporating hydrophilic or lipophilic drugs. The present study focuses on the preparation of SLN for increasing permeability and enhancing bioavailability. In this study use of lipophilic lipid or hydrophilic drug and excipient co-surfactant, surfactant, solvent use. Nanoparticles where prepared by cold homogenization method and evaluate for it's particle size, entrapment efficiency, *in-vitro* drug release and permeability.

KEYWORDS

SLN (solid lipid nanoparticle), LDC (lipid drug conjugation)

INTRODUCTION

SLN are introduced in 1991 as an alternative carrier system for traditional colloidal carriers, such as liposomes, emulsions and polymeric micro and nanoparticles. Lipid nanoparticles are unique in size between 10 and 1000 nm are known as nanoparticles and their ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could be used for secondary and tertiary levels of drug targeting. Hence, solid lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and hence have attracted wide attention of researchers. SLNs are stabilized by surfactants and polymers. They are manufactured from synthetic/natural polymers and ideally suited to optimize drug delivery and reduce toxicity. Over the years, they have emerged as a variable substitute to liposomes as drug carriers. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size Solid matrix of nanoparticles are protecting incorporated active substances against chemical degradation and providing high flexibility to modify release profiles. Appropriate analytical techniques for the characterization of SLN like scanning electron microscopy, differential scanning calorimetry are highlighted. Nanoparticle formulations have many advantages over traditional dosage forms, such as enhanced dissolution properties and the potential for intracellular drug delivery. SLNs can efficiently incorporate lipophilic drugs because the latter

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can be incorporated easily within the lipid core. However, encapsulation of hydrophilic materials into the hydrophobic matrix of SLNs is a challenge, as these drugs tend to partition towards the aqueous phase during the production process. There are limited examples of hydrophilic drugs being encapsulated into SLNs. The potential of SLNs to incorporate hydrophilic drugs, can be efficiently harnessed by suitably selecting or modifying the constitution of the lipid matrix; a field hitherto under-explored.

Many drug having problem of permeability that is BCS class III and IV drug and it solve by suitable formulation. Solid lipid nanoparticle is capable to enhance the permeability. In this formulation lipid is use and nanoparticle formulation so it cross blood brain barrier (BBB). But complication in the formulation of solid lipid nanoparticle is drug hydrophic then it low entrapment in formulation it solve by using lipid drug conjugation (LDC).

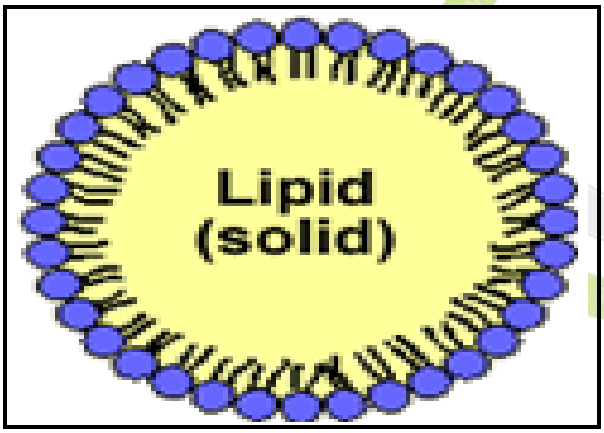

Figure 1: Structure of solid lipid Nanoparticle

Lipid Drug Conjugates (LDC)

SLNs have a problem of low capacity of hydrophilic drug loading due to partitioning effects during the production process. Only highly potent low dose hydrophilic drugs may be suitably incorporated in the solid lipid matrix. In order to overcome this limitation, the so called LDC nanoparticles with drug loading capacities of up to 33% have been developed. An insoluble drug-lipid conjugate bulk is first prepared either by salt formation (e.g. with a fatty acid) or by covalent linking (e.g. to ester or ethers). The obtained LDC is then processed with an aqueous surfactant solution (such as Tweens) to a nanoparticle formulation using high pressure homogenization (HPH). Such matrices may have potential application in brain targeting of hydrophilic drugs in serious protozoal infections.

Advantages of SLN

- ✓ Use of biodegradable physiological lipids which decreases the danger of acute and chronic toxicity and avoidance of organic solvents in production methods (Rupenagunta et al., 2011)
- ✓ Improved bioavailability of poorly water soluble molecules (Fahr and Liu, 2007)
- ✓ Site specific delivery of drugs, enhanced drug penetration into the skin via dermal application
- ✓ Possibility of scaling up.
- ✓ Protection of chemically labile agents from degradation in the gut and sensitive molecules from outer environment
- ✓ SLNs have better stability compared to liposomes
- ✓ Enhance the bioavailability of entrapped bioactive and chemical production of labile incorporated com-pound.
- ✓ High concentration of functional compound achieved.
- ✓ Lyophilization possible

Disadvantages of SLN

- ✓ Poor drug loading capacity,
- ✓ Drug expulsion after polymeric transition during storage
- ✓ Relatively high water content of the dispersions (70-99.9%) (Schwarz et al., 1994).
- ✓ Particle growth.
- ✓ Unpredictable gelation tendency.
- ✓ Unexpected dynamics of polymeric transitions.
- ✓ Sometimes burst release.

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Types of Nanoparticles

Solid-Lipid Nanoparticles (SLNs)

SLNs is comparatively stable colloidal drug delivery system in which molten lipid is dispersed in water or an aqueous media containing surfactant for emulsification and generation of submicron-sized lipid emulsions. Diameters of SLNs range from 50-1000 nm. Generally SLNs are made up of a solid hydrophobic core having a monolayer of phospholipids coating in which the drug is dissolved or dispersed. The SLNs contain solid lipid (matrix material), emulsifiers, co emulsifiers and water. SLNs possess unique properties such as small size, large surface area, high drug loading, carrying lipophilic and hydrophilic drugs, and good biocompatibility. The interactions of phases at the interfaces are attractive for their potential to improve performance and stability of pharmaceuticals, neutraceuticals and other materials (Sagar R.M. et al 2011, Vivek R.S. et al 2010).

Nanostructured Lipid Carriers (NLC)

These are produced from blend of solid and liquid lipids, but particles are in solid state at body temperature. Lipids are versatile molecules that may form differently structured solid matrices, such as the NLC and the lipid drug conjugate nanoparticles that have been created to improve drug loading capacity. The production of NLC is based on solidified emulsion (dispersed phase) technologies. NLC can present an insufficient loading capacity due to drug expulsion after polymorphic transition during storage, particularly if the lipid matrix consists of similar molecules. Drug release from NLC occurs by diffusion and simultaneously by lipid particle degradation in the body. They have been utilized in the delivery of anti-inflammatory compounds, cosmetic preparation, topical cortico therapy (Sagar R.M. et al 2011).

Dendrimers

Dendrimers are unimolecular, monodisperse, micellar nanostructures with a well defined regularly branched symmetrical structure and a high density of functional end groups provides a high degree of surface functionality and versatility. Diameter of dendrimer is around 20 nm in size. The structure of dendrimer contains three regions that is core, branches and surface. It is a highly branched synthetic polymer and consists of a monomer unit attached core. Characteristics of dendrimer are: monodisperse, tree-like, star-shaped or generational structure with precise molecular weights, its unique architectural design and high degree of branching, multivalency and globular structure. Dendrimers are generally prepared by using either a divergent method or a convergent method or combined convergent-divergent synthesis method. Dendrimers composed of poly (amidoamine) (PAMAM), melamine, polyL-glutamic acid (PG), polyethyleneimine (PEI), polypropyleneimine (PPI), and polyethylene glycol (PEG), Chitin. The applications of dendrimers in the field of imaging, drug delivery, gene transfection and non-viral gene transfer (Varun T. et al 2012, Sagar R. M. et al 2011, Vidyavathi M. et al 2012).

Polymeric Nanoparticles (PNPs)

Polymeric nanoparticles are defined as colloidal particles ranging between 10-1000 nm in size and composed of natural or synthetic polymers. These are used to increase the circulation half-life, to reduce phagocytic uptake and inactivation of the therapeutic moiety and can be used to deliver and target therapeutic agents and also used to controlled drug release. To reduce immunological interactions (e.g. opsonization or presentation PNPs to CD8 T-lymphocytes) as well as intermolecular interactions between the surface chemical groups of PNPs. PNPs are usually coated with nonionic surfactants. Methods of preparation of PNPs may be categorized two major classes: one deal with the polymerization of monomers (eg. Emulsion and dispersion polymerization) and other essentially involves dispersion of polymers (eg. salting out, emulsification diffusion and nanoprecipitation). Drug release takes place in polymeric nanoparticles through their simultaneous biodegradation followed by desorption, diffusion or erosion (Kuldeep M. et al 2012, Archana S. et al).

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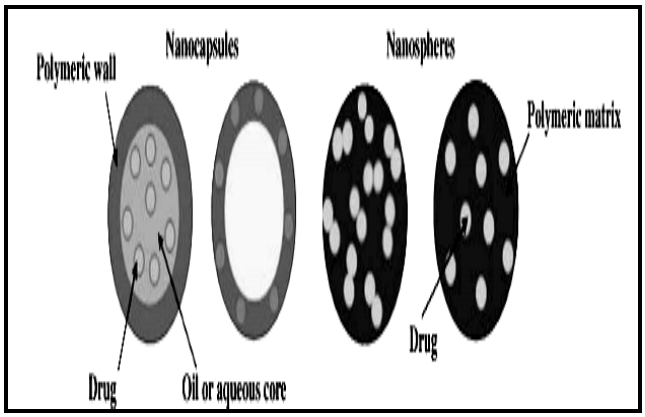

Figure 2: Schematic representation of polymeric Nanoparticles

Polymeric Carrier Used to Prepare Nanoparticles

Polymers have very large molecular weights made up of repeating units (or mers) throughout their chains. Polymers have unique cooperative properties that are not found with low-molecular-weight compounds. Many characteristics of polymers, including solubility, dissolution rate, rigidity, and tensile strength, are dependent on molecular weight (polymer science,). The ability of polymers to restrict the diffusion of low-molecular-weight compounds in matrix or nanomedicine arrangements. Polymers prolong the drug availability, alter biodistribution, enable hydrophobic drug administration and transport a drug to its specific site of action (Ijeoma F. Uchegbu).

Polymers used in controlled drug delivery may be classified as,

Natural and synthetic or biodegradable and nonbiodegradable

- *Natural Biodegradable Polymers Used to Prepare Nanoparticles*

E.g. Alginates, Albumin, Chitosan, Gelatin, Gliadin and Pollulan.

- *Synthetic Biodegradable Polymers Used to Prepare Nanoparticles*

E.g. Polylactide, Poly-(lactide-co glycolide), Polyanhydrides, Poly-Ɛ-caprolactones, Poly alkyl-cyanoacrylates, tristearin.

- *Nonbiodegradable Polymers Used to Prepare Nanoparticles*

E.g. polymethacrylate, Polymethyl methacrylate, polyurethane.

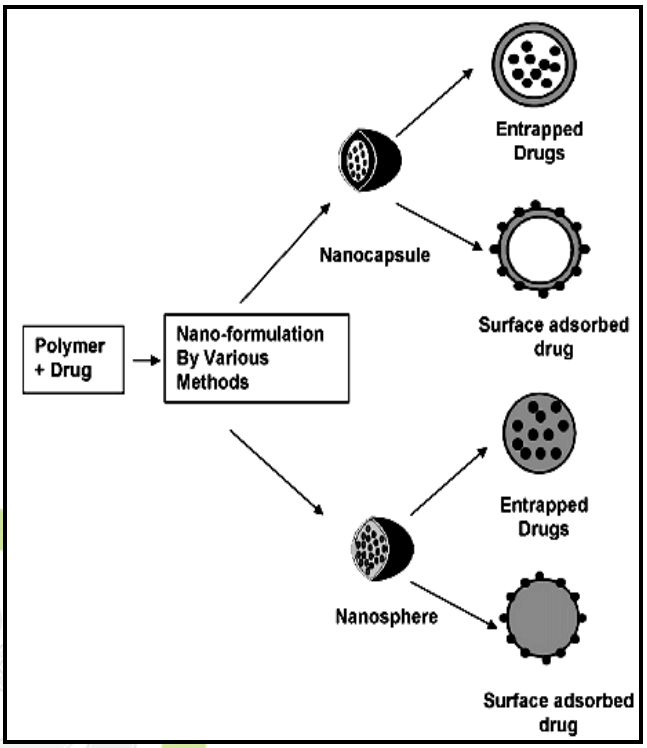

Figure 3: Types of biodegradable nanoparticles

Magnetic Nanoparticles

It involves binding of drug with magnetic nanoparticles (MNPs), such as oxidized iron (Fe) or magnetite. Due to controllable sizes ranging from 10-100 nm and capacity of delivering the drug or biomolecules to the target site, they hold a lot of potential for targeted drug delivery as well as in diagnostics. For biomedical applications, magnetic carriers must be water based, biocompatible, nontoxic and non-immunogenic MNPs.

Method of Preparation

High Shear Homogenization

High shear homogenization technique was initially used for the solid lipid nanodispersions (Domb, 1993). HSH method is used to produce SLN by melt emulsification. Homogenization is a fluid mechanical process that involves the subdivision of droplets or particles into micro- or nanosize to create a stable emulsion or

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dispersion. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the range of few microns) lipids used in this study include trimyristin, tripalmitin, a mixture of mono, di and triglycerides (Witepsol W35, Witepsol H35) with glyceryl behenate and polaxomer 188 used as stearic stabilizers (0.5% w/w). HPH method involves 2 processing procedures (Mukherjee). They are

a. Hot homogenization, b. Cold homogenization

a. Hot Homogenization

This is applied to lipophilic and insoluble drugs. This technique does not suit for hydrophilic drugs into SLN because of higher partition of drug in water. Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. Usually, lower particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase (Lander, 2000), although this might also accelerate the drug and carrier degradation. Better products are obtained after several passes through the high-pressure homogenizer (HPH), typically 3-5 passes.

High pressure processing always increases the temperature of the sample (approximately 10° at 500 bars) (Jahnke, 1998). In most cases, 3-5homogenization cycles at 500-1500 bar are sufficient. Increasing the homogenization leads to an increase of the particle size due to particle coalescence, this occurs because of the high kinetic energy of the particles.

b. Cold Homogenization

Cold homogenization technique is used for hydrophilic drugs. If the drugs have low aqueous solubility in the melted lipid, then surfactants can be used for solubilization of the drug. The solid particles are dispersed in an aqueous surfactant solution at a temperature below the lipid melting point, forming a 'pre-suspension'. The pre suspension is then subjected to HPH below the lipid melting temperature to reduce the solid particle size. The advantage of this method is avoidance of or minimizes the melting process of lipid and hence it is suitable for thermo sensitive and thermo labile drugs relative to hot HPH, Cold HPH generally produces larger mean particle sizes and broader particle size distributions (Mehnert and Mader, 2001).

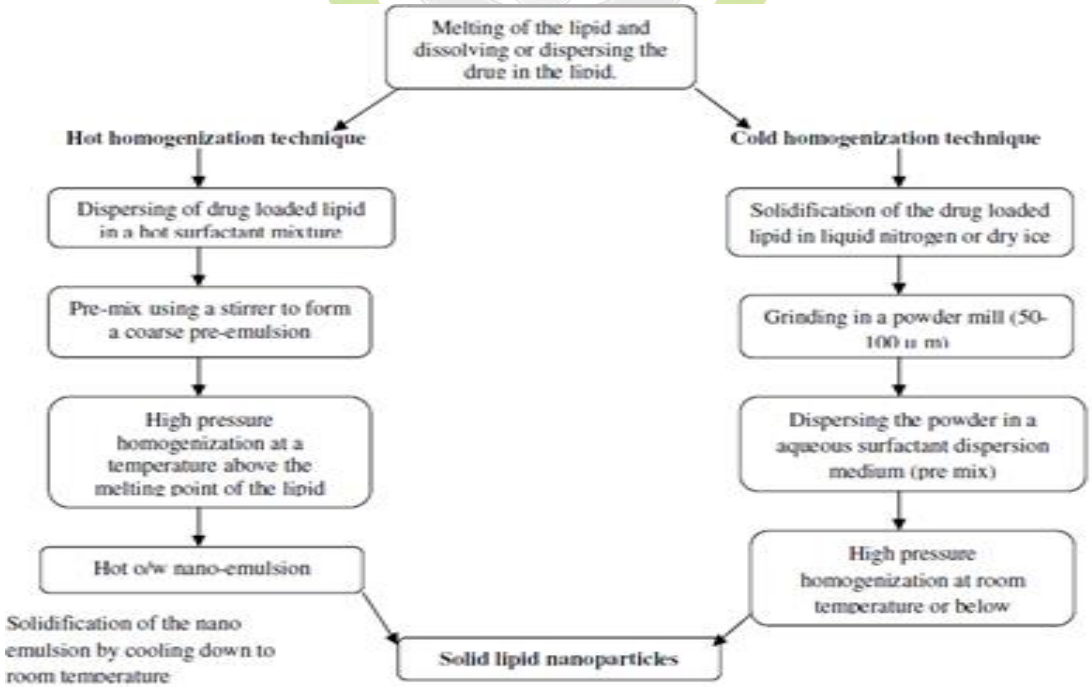

Figure 4: Flow Chart diagram showing different step in cold homoginizatiion and hot homogenization techniques in the manufacturing of solid lipid nano particles

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Microemulsion Based Method

SLN's can be produced by micro emulsification method of molten lipids as the internal phase, and the subsequent dispersion of the microemulsion in an aqueous medium under mechanical stirring.

They are made by stirring an optically transparent mixture at 65-70oc which is typically composed of a low melting fatty acid (stearic acid), an emulsifier (polysorbate 20, polysorbate 60, soy phosphatidylcholine, and sodium tauro deoxycholate), co-emulsifiers (Sodium mono octyl phosphate) and water. The hot microemulsion is dispersed in cold water under stirring.

Typical volume ratios of the hot microemulsion to cold water are in the range of 1:25 to 1:50. Nanoparticles were produced only with solvents which distribute very rapidly into the aqueous phase (acetone), while larger particle sizes were obtained with more lipophilic solvents. The dilution process is critically determined by the composition of the microemulsion.

According to the literature (Gasco, 1997; Boltri, 1993) the droplet structure is already contained in the microemulsion and therefore, no energy is required to achieve submicron particle size. The hydrophilic co-solvents of the microemulsion might play similar role in the formation of lipid nanoparticles as the acetone for the formation of polymer nanoparticles.

Multiple Microemulsification

Multiple microemulsification is also used for production of SLN's.

For the preparation of hydrophilic loaded SLN, a novel method based on solvent emulsification-evaporation has been used (Cortesi, 2002). Here the drug is encapsulated with a stabilizer to prevent drug partitioning to external water phase of w/o/w double emulsion.

But it has inherent instabilities due to coalescence of the internal aqueous droplets with in the oil phase, coalescence of droplets and rupture of the oil layer on the surface of the internal droplets (Florence and Whitehill, 1982).

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 or dispersing the drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent like gelatin, poly(vinyl alcohol), polysorbate-80, poloxamer-188,sodium dodecyl sulfate etc. to form either oil in water i.e. o/w emulsion (for encapsulation of hydrophobic drugs) or water in- oil i.e. w/o nanoemulsion (for encapsulation of hydrophilic drugs).

After formation of a stable emulsion, the organic solvent is evaporated either by increasing the temperature or under reduced pressure or by continuous stirring formed nanoparticles can be concentrated by filtration, centrifugation or lyophilization. Emulsification is done by high-speed homogenization or sonication to produce small particles. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. Most frequently used polymers are PLA, PLGA, ethyl cellulose, cellulose acetate phthalate, poly-ε-caprolactone and poly (h-hydroxybutyrate).

Drugs encapsulated were Albumin, Texanus toxoid, Loperamide, Testosterone, Prazinquante, Cyclosporin A, Nucleic acid and Indomethacin.

SLN Preparation by Using Supercritical Fluid

This is a relatively new technique for SLN production and has the advantage of solvent-less processing (Chen, 2006; Kaiser, 2001). There are several variations in this platform technology for powder and nanoparticle preparation. SLN can be prepared by the rapid expansion of supercritical carbon dioxide solutions method. Carbon dioxide (99.99%) was the good choice as a solvent for this method (Gosselin, 2003).

Solvent Displacement and Interfacial Deposition

Methods based on spontaneous emulsification of the organic internal phase containing the dissolved polymer into the aqueous external

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phase. Solvent displacement forms nanospheres or nanocapsules, whereas interfacial deposition forms only nanocapsules. Solvent displacement involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of a surfactant. The polymer is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension. Solvent and the nonsolvent of the polymer must be mutually miscible. The progressive addition of the polymer solution to the non-solvent generally leads to the formation of nanospheres close to 200 nm in size (Reis et al. 2006).

Nanoprecipitation

This method is also called as solvent displacement method. Nanoprecipitation method is based on interfacial deposition of a polymer after displacement of a semi polar solvent miscible with water from a lipophilic solution. It involves addition of drug and polymer in water-miscible organic solvent (acetone) into large amount of nonsolvent, usually water containing surfactant. Nanoprecipitation occurs by a rapid desolvation of the polymer when the polymer solution is added to the non-solvent. As soon as the polymer-containing solvent has diffused into the dispersing medium, the polymer precipitates, involving immediate drug entrapment. 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, extended shearing/stirring rates, sonication or very high temperatures. A problem associated with this technology is that the formed nanoparticles need to be stabilized to avoid growth in micrometer crystals and it provides poor entrapment efficiency for water-soluble drugs. Most commonly used method for the preparation of PLGA nanoparticles (Vidyavathi M. et al 2012, Kuldeep M. et al 2012).

Desolvation Technique

In desolvation process, nanoparticles are obtained by an intermittent or continuous drop-wise addition of ethanol/acetone to an aqueous solution of albumin (pH5.5) under continuous stirring until the solution became turbid. During the addition of ethanol/acetone into the solution, albumin is phase separated due to its diminished water-solubility. The morphologically formed albumin particles being not sufficiently stabilized could consequently redissolve again after dispersion with water. Therefore, co-acervates were hardened by cross-linking with glutaraldehyde where the amino moieties in lysine residues and arginine moieties in guanidino side chains of albumin are solidified by a condensation reaction with the aldehyde group of glutaraldehyde. Fig. 4 illustrates the steps of albumin nanoparticles preparation by desolvation method (Elzoghby A. O. et al 2012).

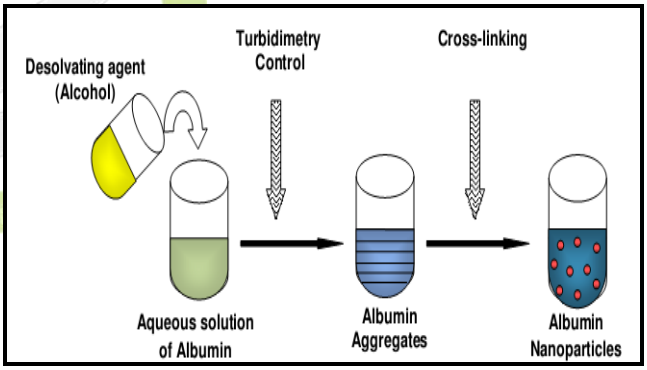

Figure 5: Preparation of albumin NPs by desolvation method

Salting Out

Salting-out method is based on the separation of a water miscible solvent from aqueous solution via a salting-out effect. This method involves an emulsification step by avoiding the use of surfactants and chlorinated solvents. It is based on the phenomenon in which solubility of a non-electrolyte in water is decreased upon addition of an electrolyte. The preparation method consists of an electrolyte-saturated aqueous solution (usually magnesium chloride hexahydrate,

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sodium chloride, magnesium acetate) containing PVA as a viscosity increasing and stabilizing agent to obtain viscous gel. The organic phase composed of the polymer and the drug dissolved in acetone under continuous mechanical stirring at room temperature. Most commonly acetone is used as solvent because of its solubilizing properties and easily removed from aqueous solution upon salting-out with electrolytes.

After addition of viscous gel into organic phase under continuous stirring causes salting out of the organic solvent, inducing formation of nanoparticles. Finally both solvent and electrolyte are eliminated by cross-flow filtration. This method is widely used in the pharmaceutical industry because its purity, high yield, speed and simplicity of the operation. The thermal treatment does not require at any stage of sample processing and therefore it may be especially useful for the incorporation of thermolabile drugs (Vidyavathi M. et al 2012, Kuldeep M. et al 2012).

Co-Acervation or Ionic Gelation Method

This method is commonly used for the preparation of chitosan, gelatin and sodium alginate nanoparticles. Formation of nanoparticles is based on ionic interaction between oppositely charged macromolecules. The method involves a mixture of two aqueous phases, in which one is the polymer and the other is a polyanion sodium tripolyphosphate. In this method, cationic group of polymer interacts with polyanion 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 (VJ Mohanraj et al 2006, Kuldeep M. et al 2012).

Spray Drying Technique

This is one of the preparation method of nanoparticles and usually used for drying of solutions and suspensions. The method is based on drying of atomized droplets in a stream of hot air and can be applied for formulation of nanoparticles. In this method, polymer is first dissolved in aqueous solvent; 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. Atomization leads to the formation of small droplets from which solvent evaporates instantaneously leading to the formation of free flowing particles.

Various process parameters like size of nozzle, spray flow rate, atomization pressure, inlet air temperature, compressed spray air flow and extent of cross linking are required to be carefully controlled in order to get the desired size of particles. Higher encapsulation efficiency for hydrophilic drugs can be achieved with the spray-drying method using aqueous solutions. When spray drying method compared with other methods, it provides a relatively rapid and convenient production technique that is easy to scale up and involves mild processing conditions (Pathak et al. 2009, Kuldeep M. et al 2012).

There is use of excipients like lactose, mannitol, sucrose, dextrose in spray drying which facilitates redispersion of the spray dried powder. Sugars with low glass transition temperatures resulted in a sticky powders (e.g. dextrose and sucrose), where lactose and mannitol provide easily flowable powders (Chaubal et al. 2008).

Polymerization Method

In polymerization methods, monomers are polymerized with subsequent entrapment of drug particles to form nanoparticles or adsorbed on their surface in an aqueous solution. Drug is incorporated either by dissolving in the polymerization medium or by adsorption onto the nanoparticles after completion of polymerization. The nanoparticles suspension is then purified to remove traces of various free stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. Nanocapsules formation and their particle size depend on the concentration of the surfactants and stabilizers used (Amit S.M. et al 2009, Kuldeep M. et al 2012).

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Evaluation of SLN

Particle Size and Zeta Potential

The physical stability of SLNs depends on their particlesize. Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for determination of particle size. PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light, which is caused by particle movement. The particle size determination by photon correlation spectroscopy (PCS) detects size range of 3nm to 3µm and by laser diffraction in size range of 100 nm to 180 µm. Although PCS is a good tool to characterize nano-particles, but is capable for the detection of larger microparticles. The LD method is based on the dependence of the diffraction angle on the particle size (Fraunhofer spectra). Smaller particles cause more intense scattering at high angles compared to the larger ones. Zeta potential measurement can be carried out using zeta potential analyzer or zetameter. Before measurement, SLN dispersions are diluted 50-fold with the original dispersion preparation medium for size determination and zeta potential measurement. Higher value of zeta potential may lead to deaggregation of particles in the absence of other complicating factors such as steric stabilizers or hydrophilic surface appendages. Zeta potential measurements allow predictions about the storage stability of colloidal dispersions.

Electron Microscopy

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provide way to directly observe nanoparticles. SEM is however better for morphological examination. TEM has a small size limit of detection.

Atomic Force Microscopy (AFM)

In this technique, a probe tip with atomic scale sharpness is raftered across a sample to produce a topological map based on the forces at play between the tip and the surface. The probe can be dragged across the sample (contact mode), or allowed to hover just above (non contact mode), with the exact nature of the particular force employed serving to distinguish among the sub techniques.

That ultra-high resolution is obtainable with this approach, which along with the ability to map a sample according to properties in addition to size, e.g., colloidal attraction or resistance to deformation, makes AFM a valuable tool.

Dynamic Light Scattering (DLS)

DLS, also known as PCS or quasi-elastic light scattering (QELS) records the variation in the intensity of scattered light on the microsecond time scale.

This variation results from interference of light scattered by individual particles under the influence of Brownian motion, and is quantified by compilation of an autocorrelation function. The advantages of the method are the speed of analysis, lack of required calibration and sensitivity to submicrometer particles.

Static Light Scattering (SLS)/Fraunhofer Diffraction

This method studies the pattern of light scattered from a solution of particles is collected and fit to fundamental electromagnetic equations in which size is the primary variable. It is fast and rugged method, but requires more cleanliness than DLS, and advance knowledge of the particles' optical qualities.

Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction[35]

DSC and powder X-ray diffractometry (PXRD) is performed for the determination of the degree of crystallinity of the particle dispersion. The rate of crystallinity using DSC is estimated by comparison of the melting enthalpy/g of the bulk material with the melting enthalpy/g of the dispersion (Siekmann and Westesen, 1994). Thermodynamic stability, lipid packing density and quantification are a serious challenge due to the increase, while drug incorporation rates decrease in the following order:

Super cooled melt < α-modification < β9-modification < β-modification

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Due to the small size of the particles and the presence of emulsifiers, lipid crystallization modification changes might be highly retarded. Differential scanning calorimetry (DSC) and X-ray scattering are widely used to investigate the status of the lipid. Infrared and Raman spectroscopy are useful tools for investigating structural properties of lipids. Their potential to characterize SLN dispersions has yet to be explored.

Acoustic Methods

Another resemble approach, acoustic spectroscopy, measures the attenuation of sound waves as a means of determining size through the fitting of physically relevant equations. In addition, the oscillating electric field generated by the movement of charged particles under the influence of acoustic energy can be detected to provide information on surface charge.

Co – Existence of Additional Structures

The magnetic resonance techniques, nuclear magnetic resonance (NMR) and electron spin resonance (ESR) are powerful tools to investigate dynamic phenomena and the nano-compartments in the colloidal lipid dispersions. Dilution of the original SLN dispersion with water might cause the removal of the surfactant molecules from the particle surface and induce further changes such as crystallization changes of the lipid modification.

Parameter Method of Analysis

Molecular weight gel chromatography, X-ray photoelectron spectroscopy, Surface element analysis Electrophoresis, Laser Doppler anemometry.

Statistical Analysis

Size and entrapment efficiency of SLNs are compared using the Student's t-test. Statistical analyses are also performed.

Stability Studies

Drug loaded SLNs are stored at 25 °C for 6 months and average size and entrapment efficiency are determined.

Effect of Sterilization

To see the effect of sterilization on particle size, zeta potential and entrapment efficiency, blank and drug dispersions are autoclave at 121 °C for 20 min.

Everted Gut Sac Experiment Using Rat Intestine

Intestinal permeability studies using everted gut sac were performed using established methods adopted from literature [13,14] (Ruan *et al.*, 2006, Mariappan and Singh, 2006). Male Wistar rats (body wt. 250-300 g, n = 4) were used for the study. Prior to the surgical procedure, the rats were fasted overnight (16–20 h) with water *ad libitum*. The rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). The intestine of the rats was exposed by a midline abdominal incision and a 20-25 cm segment of the proximal rat jejunum was excised and placed in oxygenated TC 199 medium. The intestine was gently everted over a glass rod, divided into segments of length of approximately 4 cm each, filled with oxygenated TC 199 medium and tied using surgical suture (Braided silk wax, Pearsalls Ltd, USA) to prepare sacs. The sacs were placed in flasks containing 20 ml of caffeine, paracetamol and sulfasalazine (prepared in TC 199 at a concentration of 100 µM each) either separately or in a combination of all three drugs. Lucifer yellow (10µg/ml) was added to all the solutions as an internal standard. The flasks containing sacs were incubated for the period of 60 min, at 37°C in an oscillating water bath (80 cycles per min). After the incubation period, the sacs were cut open and the contents obtained were centrifuged at 3000 g for 5 min at 4 °C. The supernatants were analyzed for marker compounds using the validated method described earlier. Lucifer yellow was quantified by spectrofluorimetry at excitation and emission wavelengths of 485 nm and 530 nm, respectively, using POLARstar OPTIMA (BMG LABTECH, Germany), controlled by FLUOstar OPTIMA (version 1.30 R3). The apparent permeability coefficient (Papp) of the marker drugs was calculated by using the following equation: Papp = [V/ (A*T)]* (C60/C0) Where V is volume of

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serosal content, A is the area of the intestinal segment, T is the time of incubation, C0 is the initial concentration on mucosal side, while C60 is the concentration of the compound on serosal side after 60 minutes.

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