



SOLID LIPID NANOPARTICLES AS DRUG DELIVERY SYSTEMS

Jaswinder Singh*

Asst. Professor, Department of Pharmaceutics, G.H.G. Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana-Punjab.

***Corresponding Author: Jaswinder Singh**

Asst. Professor, Department of Pharmaceutics, G.H.G. Khalsa College of Pharmacy, Gurusar Sadhar, Ludhiana-Punjab.

Article Received on 20/03/2018

Article Revised on 10/04/2018

Article Accepted on 01/05/2018

ABSTRACT

A solid lipid nanoparticle (SLN) is a typically colloidal spherical particle with an average diameter between 10 to 1000 nm. Solid lipid nanoparticles (SLNs) are being currently used as alternative drug delivery systems to colloidal drug delivery systems such as lipid emulsions, liposomes, and polymeric nanoparticles. Various drugs like proteins and antigens molecules for therapeutic purposes may be incorporated or adsorbed onto SLN, and further administered by parenteral routes or be alternative routes such as oral, nasal and pulmonary. The potential to incorporate drugs into nanocarriers offers a new archetype in drug delivery that could hold an immense promise in accomplishing the bioavailability enhancement accompanied by controlled and site specific drug delivery. This review presents a broad treatment of solid lipid nanoparticles discussing their advantages, limitations and their possible remedies, production procedures, factors affecting the formulation, characterization studies. The different types of nanocarriers which were based on solid lipid like solid lipid nanoparticles, nanostructured lipid carriers, lipid drug conjugates are also discussed.

KEYWORDS: Solid lipid nanoparticle, nanocarriers, nanostructured lipid carriers, lipid drug conjugates.

INTRODUCTION

Solid lipid nanoparticles (SLNs) introduced in 1991, signify an alternative and better carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles. SLNs combine the advantages of different colloidal carriers, for example like emulsions and liposomes, these are physiologically acceptable and like polymeric nanoparticles, controlled release of drug from lipid matrix can be predicted.^[1-3] SLN's are attracting major attention in the novel colloidal carrier for intravenous application. SLN's are a new generation of submicron-sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. SLN are sub-micron colloidal carriers ranging from 10 to 1000 nm, composed of physiological lipid dispersed in water or in aqueous surfactant solution. Due to their solid particle matrix, they can protect incorporated ingredients against chemical degradation and allow alteration of release of the active compounds. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at interfaces, and are attractive for their potential to improve performance of pharmaceuticals, nutraceuticals and other materials.^[4]

Advantages of Solid Lipid Nanoparticles (SLN's)^[5-8]

- Use of biodegradable physiological lipids which decreases the danger of acute and chronic toxicity

and avoidance of organic solvents in production methods

- Both lipophilic and hydrophilic compounds can be encapsulated and delivered by SLN with modification in the formulation
- Improve stability of pharmaceuticals.
- Protecting the labile and sensitive drugs from chemical, photochemical or oxidative degradation, due to immobilization of drug molecules by solid lipids
- Enhance the bioavailability of entrapped bioactive and chemical production of the labile incorporated compound.
- The possibility of scaling up.
- Site specific delivery of drugs, enhanced drug penetration into the skin via dermal application

Disadvantages of SLN^[9]

- Particle growth.
- Unpredictable gelation tendency.
- Unexpected dynamics of polymeric transitions
- Poor drug loading capacity.
- Drug expulsion after polymeric transition during storage.
- The low capacity to load water soluble drugs due to partitioning effects during the production process

Advanced Modifications of Solid Lipid Nanoparticles

To overcome the limitations of SLN, nanostructured lipid carriers (NLCs), Lipid drug conjugate (LDCs) and Polymer lipid hybrid nanoparticles (PLNs) were introduced.

- *Nanostructured Lipid Carrier (NLC)* is a blend of mixture of solid lipids along with spatially incompatible liquid lipids. It remains solid at room temperature. It has various advantages similar to SLN such as use of bio-compatible lipids, controlled release of drug from the carrier, feasible to produce on large scale using the existing machinery at economical cost, avoids first pass metabolism by lymphatic transport and provides protection to drug moiety from biochemical degradation. It also overcomes the disadvantages of SLN such as NLC have better drug loading due to the use of liquid lipid, avoids drug expulsion during storage for long period, lipids can be used in higher ratio (up to 95%) when compared to SLN where above 30% it forms bicoherent systems.^[10]
- *Lipid drug conjugates (LDCs)*: A major problem of SLNs is the low capacity to load hydrophilic drugs 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. LDC enables the incorporation of both hydrophilic (e.g., doxorubicin and tobramycin) and lipophilic (e.g., progesterone and cyclosporine A) drugs.^[11]
- *Polymer lipid hybrid nanoparticles (PLNs)*: Polymer-lipid hybrid nanoparticles hold great promise as a drug delivery vehicle in the treatment of a myriad of diseases such as breast cancer. A PLN comprises three distinct functional components:
 - I. hydrophobic polymeric core to encapsulate poorly water-soluble drugs
 - II. hydrophilic polymeric shell to enhance PLN stability and circulation half-life
 - III. lipid monolayer at the core and shell interface to promote drug retention inside the polymeric core.

Interactions among these components play an important role for successful fabrication and performance of PLNs. These hybrid NPs combine the merits of both liposomes and polymeric nanoparticles, two of the most popular drug delivery vehicles approved for clinical use, thereby serving as a robust drug delivery platform. It has been shown in vitro that hybrid NPs possess the ability of carrying poorly water-soluble drugs with high encapsulation and loading yields, tunable and sustained drug release profiles, excellent serum stability, and differential targeting of cells.^[12, 13]

Factors To Be Considered In The Formulation of SLNs

Common ingredients used in the formulation of SLNs are lipids (matrix materials), emulsifiers, coemulsifiers,

and water. The lipids used may be triglycerides (tristearin), partial glycerides (Imwitor), fatty acids (stearic acid, palmitic acid), and steroids (cholesterol) and waxes (cetyl palmitate). Various emulsifiers and their combination (Pluronic F 68, F 127) have been used to stabilize the lipid dispersion. The combination of emulsifiers might prevent particle agglomeration more efficiently.^[14]

➤ Selection of lipids

Lipid matrices used for the production of SLNs for i.v. administration should have the following appropriate properties^[15]

- They should be capable of producing small size particles (in the nanometer size range) with low content of micro particles (>5 μ m).
- They should possess sufficient loading capacity for lipophilic and possible also hydrophilic drugs.
- They should be suitable for sterilization by autoclaving.
- They should be stable in aqueous dispersions, on long-term storage, or alternatively they can be lyophilized or spray dried.
- They should be toxicologically accepted and should not leave any toxic residues from the production process (e.g. solvents).
- They should be biodegradable

➤ Selection of emulsifier

Emulsifier should be nontoxic, compatible with other excipients, capable of producing desired size with minimum amount used, and also provide adequate stability to the SLNs, by covering their surface. Another aspect to be considered in the selection of emulsifier is its in vivo fate; for instance, the poloxamer series provide long-circulating properties to SLNs by preventing reticulo endothelial system (RES) uptake, which allows passive targeting whereas polysorbate 80 coated SLNs improved drug delivery to brain targeting.^[16, 17]

➤ Selection of co-emulsifier

Phospholipids used in the formulation of SLNs are neither soluble in continuous phase nor do they form highly dynamic micelles. The excess phospholipid molecules form small, predominantly unilamellar vesicles during homogenization process. Phospholipid molecules bound to vesicles, however, exhibit only a limited mobility. Therefore, they are not able to immediately cover the newly created interfaces, during recrystallization of solid lipids. Due to the low mobility of the phospholipid molecules, sudden lack of emulsifier on the surface of the particle leads to particle aggregation and increase in the particle size of SLNs. To avoid this, co-emulsifiers like glycocholate (ionic) as well as tyloxapol (nonionic polymer) are employed. These types of water-soluble emulsifiers are able to form micelles. Polymer molecules are able to diffuse to the particle surface in a much shorter time than do vesicles.

Moreover, micelles represent highly dynamic colloidal structures and may serve as reservoirs.^[18]

METHODS OF PREPARATION OF SOLID LIPID NANOPARTICLES (SLN's)

1. High-pressure homogenization
 - A. Hot homogenization
 - B. Cold homogenization
2. Ultrasonication/high-speed homogenization
3. Solvent evaporation method
4. Solvent emulsification-diffusion method
5. Supercritical fluid method
6. Microemulsion based method
7. Spray drying method
8. Double emulsion method
9. Precipitation technique
10. Film-ultrasound dispersion

1. High pressure homogenization (HPH)

It is a reliable and powerful technique, which is used for the production of SLN's. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap (in the range of a few microns). The fluid accelerates on a very short distance to very high velocity (over 1000 Km/h). Very high shear stress and cavitation forces disrupt the particles down to the sub-micron range. Generally, 5–10% lipid content is used but up to 40% lipid content has also been investigated. Two general approaches of HPH are hot homogenization and cold homogenization; work on the same concept of mixing the drug in bulk of lipid melt.

A. Hot homogenization

Hot homogenization is carried out at temperatures above the melting point of the lipid and is similar to the homogenization of an emulsion. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase (same temperature) is obtained by high-shear mixing device (like silversion-type homogenizer). The quality of the pre-emulsion affects the quality of the final product to a great extent and it is desirable to obtain droplets in the size range of a few micrometers. High pressure homogenization of the pre-emulsion is done above the lipid melting point. Usually, lower particle sizes are obtained at higher processing temperatures because of lowered viscosity of the lipid phase^[19], 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 bar).^[20]

B. Cold homogenization^[21–23]

Cold homogenization is carried out with the solid lipid containing drug and therefore called as milling of a suspension. Cold homogenization has been developed to prevent:

- Temperature induced drug degradation.
- Partitioning of hydrophilic drug from lipid phase to aqueous phase.

- Complexity of the crystallization step of the nanoemulsion leading to several modifications and/or super cooled melts.

2. Ultrasonication/high-speed homogenization

In this method, drug is added to hot lipid melt. Hot aqueous phase is added to the hot lipid melt, emulsified by probe sonicator or by using high speed stirrer. Pre-emulsion is formed, sonicate it using probe sonicator, o/w nanoemulsion is formed which is filtered to obtain SLNs (Mahajan and Kaur et al., 2014). The advantage of this method is that the equipment used is commonly available at lab scale and this method suffers from problems such as broader size distribution ranging into micrometer range, physical instability like particle growth upon storage are other drawbacks associated with this technique.^[24]

3. Solvent evaporation method^[25]

SLNs can be prepared by solvent evaporation method. The lipophilic material is dissolved in a waterimmiscible organic solvent (e.g. cyclohexane) that is emulsified in an aqueous phase. Upon evaporation of the solvent, nanoparticles dispersion is formed by precipitation of the lipid in the aqueous medium by giving the nanoparticles of 25 nm mean size. The solution was emulsified in an aqueous phase by high pressure homogenization. The organic solvent was removed from the emulsion by evaporation under reduced pressure (40–60 mbar).

Advantages

- Scalable.
- Mature technology.
- Continuous process.
- Commercially demonstrated.

Disadvantages

- Extremely energy intensive process.
- Polydisperse distributions.
- Biomolecule damage.

4. Solvent emulsification-diffusion method

The particles with average diameters of 30–100 nm can be obtained by this technique. Avoidance of heat during the preparation is the most important advantage of this technique.

Advantages

- Reduced shear stress.

Disadvantages

- Potential metal contamination.
- Physical instability like particle growth upon storage.^[26,27]

5. Supercritical fluid method^[28]

This is an alternative method of preparing SLN by particles from gas saturated solutions (PGSS). This is new technique and advantage of solvent less processing. SLN can be prepared by the rapid expansion of

supercritical carbon dioxide solutions this method is called as RESS method. Carbon dioxide with 99.99% is good solvent. This technique has several advantages such as

- Avoid the use of solvents.
- Particles are obtained as a dry powder, instead of suspensions.
- Mild pressure and temperature conditions.

6. Microemulsion based method^[29]

This method is based on the dilution of microemulsions. As micro-emulsions are two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions).

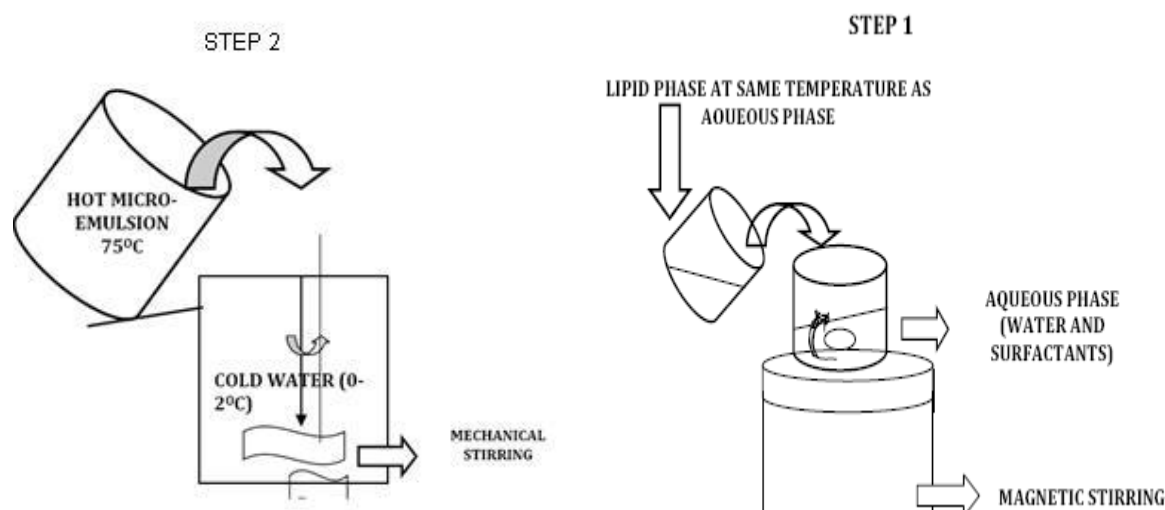


Fig. 1: Microemulsion method.

7. Spray drying method

It is an alternative technique to the lyophilization process. This recommends the use of lipid with the melting point more than 70°C. The best results were obtained with SLN concentration of 1% in a solution of trehalose in water or 20% trehalose in the ethanol-water mixture.

8. Double emulsion method

Warm w/o/w double microemulsion can be prepared in two steps. Firstly, w/o microemulsion is prepared by adding an aqueous solution containing drug to a mixture of melted lipid, surfactant and co-surfactant at a temperature slightly above the melting point of lipid to obtain a clear system. In the second step, formed w/o microemulsion is added to a mixture of water, surfactant and co-surfactant to obtain a clear w/o/w system. SLNs can be obtained by dispersing the warm micro double emulsions in cold then washed with dispersion medium by ultra-filtration system. Multiple emulsions have inherent instabilities due to coalescence of the internal aqueous droplets within the oil phase, coalescence of the oil droplets, and rupture of the layer on the surface of the internal droplets. In case of SLNs production, they have to be stable for few minutes, the time between the preparations of the clear double microemulsions and its

They are made by stirring an optically transparent mixture at 65-70°C, which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. SLN dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much of water needs to be removed. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation. Due to the dilution step; achievable lipid contents are considerably lower compared with the HPH based formulations.

quenching in cold aqueous medium, which is possible to achieve.^[30]

9. Precipitation technique

The glycerides are dissolved in an organic solvent (e.g. chloroform) and the solution will be emulsified in an aqueous phase. After evaporation of the organic solvent the lipid will be precipitated forming nanoparticles.^[29]

10. Film-ultrasound dispersion

The lipid and the drug were put into suitable organic solutions, after decompression, rotation and evaporation of the organic solutions, a lipid film is formed, then the aqueous solution which includes the emulsions was added. Using the ultrasound with the probe to diffuser at last, the SLN with the little and uniform particle size is formed.^[29]

Methods of Characterization of SLNs

Adequate and proper characterization of the SLNs is necessary for its quality control. However, characterization of SLN is a serious challenge due to the colloidal size of the particles and the complexity and dynamic nature of the delivery system. The important parameters which need to be evaluated for the SLNs are particle size, size distribution kinetics (zeta potential), degree of crystallinity and lipid modification

(polymorphism), coexistence of additional colloidal structures (micelles, liposome, super cooled, melts, drug nanoparticles), time scale of distribution processes, drug content, in vitro drug release and surface morphology.

1. Particle Size and Shape

SLNs are submicron sized, particle size and shape is determined by:

a) Photon Correlation Spectroscopy (PCS)

It is an established method which is based on dynamic scattering of laser light due to Brownian motion of particles in solution/suspension. This method is suitable for the measurement of particles in the range of 3 nm to 3 μ m. The PCS device consists of laser source, a sample cell (temperature controlled) and a detector. Photomultiplier is used as detector to detect the scattered light. The PCS diameter is based on the intensity of the light scattering from the particles.

b) Electron Microscopy

Electron Microscopy methods such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to measure the physical characterization like overall shape and morphology of lipid nanoparticles. It permits the determination of particle size and distributions. SEM uses electrons transmitted from the surface of the sample while TEM uses electrons transmitted through the sample. TEM has a smaller size limit of detection.^[31]

2. Measurement of zeta potential

Zeta potential is used to measure the charge on the particles. It allows prediction about the storage stability of colloidal dispersion because of repulsion between particles. Malvern Zetasizer is most widely used instrument for measurement of Zeta potential. A zeta potential measurement can also be helpful in designing particles with reduced RES uptake. Zeta potential below -25 mV and above + 25mV are required for full electrostatic stabilization of the formulation.

3. X-ray diffraction (powder X-ray diffraction) and differential scanning calorimetry (DSC)

The geometric scattering of radiation from crystal planes within a solid allow the presence or absence of the former to be determined thus permitting the degree of crystallinity to be assessed. Another method that is a little different from its implementation with bulk materials, DSC can be used to determine the nature and speciation of crystallinity within nanoparticles through the measurement of glass and melting point temperatures and their associated enthalpies.^[32]

4. Determination of Incorporated Drug

Amount of drug incorporated in SLNs influences the release characteristics; hence it is very important to measure the amount of incorporated drug. The amount of drug encapsulated per unit weight of nanoparticles is determined after separation of the free drug and solid lipids from the aqueous medium by ultracentrifugation, centrifugation filtration or gel permeation

chromatography. The drug can be assayed by standard analytical technique such as spectroscopy and HPLC methods.

CONCLUSION

SLN as colloidal drug carrier combines the advantage of polymeric nanoparticles, fat emulsions and liposome; due to various advantages, including feasibility of incorporation of lipophilic and hydrophilic drugs, improved physical stability, low cost, ease of scale-up, and manufacturing. SLNs are prepared by various advanced techniques. The site specific and sustained release effect of drug can better be achieved by using SLNs. To exploit the broad applications of lipid based nanoparticulate formulations, it is essential that the pharmaceutical industries specialized in the development of new drug delivery systems should engage in novel formulation technology to promote their scale up and bring them onto the pharmacist's shelves. We can expect many patented dosage forms in the form of SLNs in the future.

REFERENCES

1. Muller RH, Karsten M, Sven G. Solid lipid nanoparticles (SLN) for controlled drug delivery—A review of the state of the art. *Eur J Pharm Biopharm*, 2000; 50: 161-77.
2. Stevens PJ, Sekido M, Lee RJ. Synthesis and evaluation of hematoporphyrin derivative in a folate receptor-targeted solid lipid nanoparticle formulation. *Anticancer Res*, 2004; 24(1): 161-165.
3. Mehenert W, Mader K. Solid lipid nanoparticles production, characterization and applications. *Adv Drug Deliv Rev*, 2001; 47: 165-96.
4. Mukherjee S, Ray S, Thakur, RS. Solid Lipid Nanoparticles: A Modern Formulation Approach In Drug Delivery System. Department Of Pharmaceutics, Krupanidhi College Of Pharmacy, Bangalore-560 034, India.1-30.
5. Rupenagunta A, Somasundaram I, Ravichandiram V, Kausalya J, Senthilnathan B. Solid lipid nanoparticles- A versatile carrier system. *J Pharm Res*, 2011; 4(7): 2069-2075.
6. Westesen K, Siekmann B, Koch MHJ. Investigations on the physical state of lipid nanoparticles by synchrotron radiation X-ray diffraction. *Int J Pharm*, 1993; 93: 189-99.
7. Hu FQ, Yuan H, Zhang HH, Fang M. Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. *Int J Pharm*, 2002; 239: 121-28.
8. Fuentes MG, Torres D, Alonso MJ. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. *Coll Surf B Bioint*, 2002; 27: 159-68.
9. Melike U, Gulgun Y. Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives *Int J Nanomedicine*, 2007; 2(3): 289-300.

10. Natarajan J, Karri V, Anindita D. Nanostructured Lipid Carrier (NLC): A Promising Drug Delivery System. *Glob J Nano*, 2017; 1(5): 555575.
11. Jaiswal, S. Gupta GD Recent advances in solid lipid nanoparticles and challenges Indo American Journal of Pharmaceutical Research, 2013; 3(12): 1601-1611
12. Pardeshi CS, Rajput P, Belgamwar V, Tekade A, Patil G, Chaudhary K, Sonje A. Solid lipid based nanocarriers: An overview. *Acta Pharm*, 2012; 62:433–472.
13. Ocheke NA, Olorunfemi PO, Ngwuluka NC. Nanotechnology and Drug Delivery Part 2: Nanostructures for Drug Delivery. *Tropical Journal of Pharmaceutical Research*, 2009; 8(3): 275-287.
14. Cavalli R, Caputo O, Gasco MR. Solid lipospheres of doxorubicin and idarubicin. *Int J Pharm*, 1993; 89: R9-R12.
15. Muller RH, Karsten M, Sven G. Solid lipid nanoparticles (SLN) for controlled drug delivery—A review of the state of the art. *Eur J Pharm Biopharm*, 2000; 50: 161-77.
16. Muller RH., Wallis KH Surface modification of i.v. injectable biodegradable nanoparticles with poloxamer polymers and poloxamine 908. *Int J Pharm* 1993; 89: 25-31.
17. Olbrich C, Gessner A, Kayser O, Muller RH Lipid drug conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazenediaceturate. *J Drug Target*, 2002; 10(5): 387-96.
18. Westesen K, Siekmann B. Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles. *Int J Pharm.*, 1997; 151: 35-45.
19. Lander R, Manger W, Scouloudis M, Ku A, Davis C, Lee A. Gaulin homogenization: a mechanistic study. *Biotechnol Prog*, 2000; 16: 80-5.
20. Jahnke S. The theory of high pressure homogenization. In: Muller RH, Benita S, Bohm B. editors. *Emulsions and nanosuspensions for the formulation of poorly soluble drugs*. Stuttgart: Medpharm Scientific Publishers, 1998; 177-200.
21. Rabinarayan P, Padilama S. Production of solid lipid nanoparticles-drug loading and release mechanism. *J Chem Pharm Res.*, 2010; 2(1): 211-27.
22. Nilesh J, Ruchi J, Navneet T, Brham P G, Deepak K J, Jeetendra B, et al., Nanotechnology: A safe and effective drug delivery system. *Asian Journal of Pharmaceutical and Clinical Research*, 2010; 3(3): 1974-2441.
23. Muller RH, Runge SA. Solid lipid nanoparticles (SLN) for controlled drug delivery. In: Benita S, editor. *Submicron emulsions in drug targeting and delivery*. Amsterdam: Harwood Academic Publishers, 1998; 219-34.
24. Elldem T, Speiser P, Hineal. A; Optimization of spray-dried and congealed lipid microparticles and characterization of their surface morphology by scanning electron microscopy. *Pharm. Res.*, 1991; 8: 47-54.
25. Krishna SA, Amareshwar P, Chakravarty P. Formulation of solid lipid nanoparticles and their applications. *CPR*, 2011; 1(2): 197-203.
26. Siekmann B, Westesen K. Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions. *Eur J Pharm Biopharm*, 1996; 43: 104-109.
27. Muller RH, Mäder K, Gohla SH. Solid Lipid Nanoparticles For Controlled Drug Delivery- A Review of the State of the art. *Eur J Pharm Bio Pharm*, 2000; 50(1): 161-177.
28. Nilesh J, Ruchi J, Navneet T, Brham P G, Deepak K J, Jeetendra B, et al., Nanotechnology: A safe and effective drug delivery system, *Asian Journal of Pharmaceutical and Clinical Research*, 2010; 3(3): 1974-2441.
29. Ekambaram P, Sathali AH, Priyanka K. Solid lipid nanoparticles: A Review *Sci. Revs. Chem. Commun*, 2012; 2(1): 80-102.
30. Lv Q, Yu A, Xi Y, Li H, Song Z, Cui J, Cao F, Zhai G. Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery. *International Journal of Pharmaceutics*, 2009; 372: 191–198.
31. Tiwari A, Rashi S. Anand S. *WJPPS*, 2015; 4(8): 337-355.
32. Drake B, Prater CB, Weisenhorn AL, Gould SAC, Albrecht TR, Quate CF, et al. Imaging crystals polymers and process in water with the AFM. *Science*, 1989; 243: 1586-9.