ISSN 0975-6299 Vol.1/Issue-4/Oct-Dec.2010

International Journal of Pharma and Bio Sciences

NANOSUSPENSION A NOVEL DRUG DELIVERY SYSTEM

VISHVAJIT A. KAMBLE*[1], DEEPALI M. JAGDALE[1] AND VILASRAO J. KADAM[2]

[1]Department of Quality Assurance, Bharati Vidyapeeth's college of Pharmacy, Belapur, Navi mumbai. India. Pin-400614.

Corresponding Author vishvakamble@gmail.com.

ABSTRACT

Solubility is an essential factor for drug effectiveness, independent of the route of administration. Poorly soluble drugs are often a challenging task for formulators in the industry. Conventional approaches for enhancement of solubility have limited applicability, especially when the drugs are poorly soluble simultaneously in aqueous and in non-aqueous media. One of the critical problems associated with poorly soluble drugs is too low bioavailability. Nanosuspension technology can be used to improve the stability as well as the bioavailability of poorly soluble drugs. Nanosuspensions are biphasic systems consisting of pure drug particles dispersed in an aqueous vehicle, stabilized by surfactants. Nanotechnology can be used to resolve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} meters. The present article describes the details about nanosuspensions. Nanosuspensions consist of the pure poorly water-soluble drug without any matrix material suspended in dispersion. The review article includes the methods of preparation with their merits and demerits, characterization, application, and evaluation parameters.

KEY WORDS

Nanosuspension, Nanotechnology,Solubility enhancement,Bioavailability.

INTRODUCTION

More than 40 percent of the drugs coming from High-throughoutput screening are poorly soluble in water[1]. Obviously poorly water-soluble drugs show many problems in formulating them in conventional dosage forms. One of the main problems responsible for the low turnout in the development of new molecular entities as drug formulations is low solubility and low bioavailability of the lead compounds. The increasing frequency of poorly water soluble new chemical entities exhibiting therapeutic activity is of major concern to the pharmaceutical industry. There are number of formulation approaches to resolve the problems of low solubility and low bioavailability. The approaches include micronization[2], solublization using co-solvents, use of permeation enhancers, oily solutions, surfactant dispersions[2], salt formation[3] and

precipitation techniques[4, 5]. These techniques for solubility enhancement have some limitations and hence have limited utility in solubility enhancement.Other techniques like liposomes[6], emulsions, microemulsions[7], solid-dispersions[8] and inclusion complexes using Cyclodextrins[9] show reasonable success but they lack in universal applicability to all drugs. These techniques are not applicable to the drugs, which are not soluble in both aqueous and organic Medias. Hence there is need of some different and simple approach to tackle the formulation problems to improve their efficacy and to optimize the therapy with respect to pharmacoeconomics.

NANOSUSPENSION

Nanosuspensions are colloidal dispersions of nanosized drug particles stabilized by surfactants. They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1 µm in size. [10]Nanosuspensions can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result, the rate of flooding of the active compound increases and the maximum plasma level is reached faster. This is one of the unique advantages that it has over other approaches for enhancing solubility. It is useful for molecules with poor solubility, poor permeability or both, which poses a significant challenge for the formulators. Nanosuspensions differ from Nanoparticles[11], which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules), and from solid-lipid nanoparticles[12] (SLN), which are lipidic carriers of drug.

PREPARATION OF NANOSUSPENSIONS

Mainly there are two methods for preparation of nanosuspensions. The conventional methods of precipitation (Hydrosols[13]) are called 'Bottom Up technology'. In Bottom Up Technology the drug is dissolved in a solvent, which is then added to non-solvent to precipitate the crystals. Sucker and co-workers used a precipitation technique to produce nanoparticles by dissolving the drug in a solvent and adding the solvent to a non-solvent that cause precipitation of the fine drug particle. The basic advantage of precipitation technique is the use of simple and low cost equipments. The basic challenge of this technique is that during the precipitation procedure the growing of the drug crystals needs to be controlled by addition of surfactant to avoid formation of microparticles. The limitation of this precipitation technique is that the drug needs to be soluble in atleast one solvent and this solvent needs to be miscible with nonsolvent. Moreover precipitation technique is not applicable to drugs, which are simultaneously poorly soluble in aqueous and nonaqueous

The 'Top Down Technologies' are the disintegration methods and are preferred over the precipitation methods. The 'Top Down Technologies' include Media Milling (Nanocrystals), High Pressure Homogenization in water (Dissocubes), High Pressure Homogenization in nonaqueous media (Nanopure) and combination of Precipitation and High-Pressure Homogenization (Nanoedege) [14, 15]

PREPARATION METHOD OF NANOSUSPENSIONS

There are different methods of Nanosuspensions preparation like

1. Media milling (Nanocrystal or Nanosystems).
2. Homogenization in water (Dissocubes).
3. Homogenization in nonaqueous media (Nanopure).
4. Combined precipitation and homogenization (Nanoedege).
5. Nanojet technology

6. Emulsification-solvent evaporation technique.
7. Hydrosol method
8. Supercritical fluid method.

1. *Media milling (Nanocrystal or Nanosystems)*

The method is first developed and reported by Liversidge et.al. (1992).The nanosuspensions are prepared by using high-shear media mills. The milling chamber charged with milling media, water, drug and stabilizer is rotated at a very high shear rate under controlled temperatures for several days (at least 2-7 days). The milling medium is composed of glass, Zirconium oxide or highly cross-linked polystyrene resin. The high energy shear forces are generated as a result of the impaction of the milling media with the drug resulting into breaking of microparticulate drug to nanosized particles[16, 13].

Advantages:

- Media milling is applicable to the drugs that are poorly soluble in both aqueous and organic media.
- 2. Very dilute as well as highly concentrated nanosuspensions can be prepared by handling 1mg/ml to 400mg/ml drug quantity.
- 3. Nanosize distribution of final nanosize products.

Disadvantages:

- Nanosuspensions contaminated with materials eroded from balls may be problematic when it is used for long therapy.
- The media milling technique is time consuming.
- Some fractions of particles are in the micrometer range.
- Scale up is not easy due to mill size and weight.

2. *Homogenization in water (Dissocubes)*

Homogenization involves the forcing of the suspension under pressure through a valve having a narrow aperture. Dissocubes was developed by Muller *et al.* in 1999.[17] The instrument can be operated at pressure varying from 100 – 1500 bars (2800 –21300psi) and up to 2000 bars with volume capacity of 40ml (for laboratory scale).

Principle

In piston gap homogeniser particle size reduction is based on the cavitation principle. Particles are also reduced due to high shear forces and the collision of the particles against each other. The dispersion contained in 3cm diameter cylinder; suddenly passes through a very narrow gap of 25µm. According to Bernoulli's Law the flow volume of liquid in a closed system per cross section is constant. The reduction in diameter from 3cm to 25µm leads to increase in dynamic pressure and decrease of static pressure below the boiling point of water at room temperature. Due to this water starts boiling at room temperature and forms gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure, are reached. The size of the drug nanocrystals that can be achieved mainly depends on factors like temperature, number of homogenization cycles, and power density of homogeniser and homogenization pressure.

Advantages

- It does not cause the erosion of processed materials[18].
- Very dilute as well as highly concentrated nanosuspensions can be prepared by handling 1mg/ml to 400mg/ml drug quantity[19].
- It is applicable to the drugs that are poorly soluble in both aqueous and organic media.
- It allows aseptic production of nanosuspensions for parentral administration[20].

Disadvantages

- Preprocessing like micronization of drug is required.
- High cost instruments are required that increases the cost of dosage form.

3. *Homogenization in nonaqueous media (Nanopure)*

Nanopure is suspensions homogenized in water-free media or water mixtures.[21] In the Dissocubes technology, the cavitation is the determining factor of the process. But, in contrast to water, oils and oily fatty acids have very low vapour pressure and a high boiling point. Hence, the drop of static pressure will not be sufficient enough to initiate cavitation. Patents covering disintegration of polymeric material by high- pressure homogenization mention that higher temperatures of about 80^{o} C promoted disintegration, which cannot be used for thermolabile compounds. In nanopure technology, the drug suspensions in the non- aqueous media were homogenized at 0^{o} C or even below the freezing point and hence are called "deep-freeze" homogenization. The results obtained were comparable to Dissocubes and hence can be used effectively for thermolabile substances at milder conditions

4. *Combined precipitation and homogenization (Nanoedege).*

The basic principles of Nanoedge are the same as that of precipitation and homogenization. A combination of these techniques results in smaller particle size and better stability in a shorter time. The major drawback of the precipitation technique, such as crystal growth and long-term stability, can be resolved using the Nanoedge technology. In this technique, the precipitated suspension is further homogenized, leading to reduction in particle size and avoiding crystal growth.

5. *Nanojet technology*

This technique, called opposite stream or nanojet technology, uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure. The high shear force produced during the process results in particle size reduction. Equipment using this principle includes the M110L and M110S microfluidizers (Microfluidics). Dearn prepared nanosuspensions of atovaquone using the microfluidization process.[22] The major disadvantage of this technique is the high number of passes through the microfluidizer and that the product obtained contains a relatively larger fraction of microparticles

6. *Emulsification-solvent evaporation technique*

This technique involves preparing a solution of drug followed by its emulsification in another liquid that is a non-solvent for the drug. Evaporation of the solvent leads to precipitation of the drug. Crystal growth and particle aggregation can be controlled by creating high shear forces using a high-speed stirrer.

7. *Hydrosol method*

This is similar to the emulsification- solvent evaporation method. The only difference between the two methods is that the drug solvent is miscible with the drug anti-solvent.[23]Higher shear force prevents crystal growth and Ostwald ripening and ensures that the precipitates remain smaller in size.

8. *Supercritical fluid method*

Supercritical fluid technology can be used to produce nanoparticles from drug solutions. The various methods attempted are rapid expansion of supercritical solution process (RESS), supercritical anti-solvent process and precipitation with compressed anti-solvent process (PCA).The RESS involves expansion of the drug solution in supercritical fluid through a nozzle, which leads to loss of solvent power of the supercritical fluid resulting in precipitation of the drug as fine particles. Young *et al.* prepared cyclosporine Nanoparticles in the size range of 400-700 nm using this process.[24] In the PCA method, the drug solution is atomized into a chamber containing compressed CO_2. As the solvent is removed, the solution gets supersaturated and thus precipitates as fine crystals. The supercritical anti- solvent process uses a supercritical fluid in which a drug is poorly

soluble and a solvent for the drug that is also miscible with the supercritical fluid. The drug solution is injected into the supercritical fluid and the solvent gets extracted by the supercritical fluid and the drug solution gets supersaturated. The drug is then precipitated as fine crystals. Nanoparticles of griseofulvin, a drug with poor solubility, were prepared by Chattopadhyay *et al*. using this method. The disadvantages of the above methods are use of hazardous solvents and use of high proportions of surfactants and stabilizers as compared with other techniques, particle nucleation overgrowth due to transient high super saturation, which may also result in the development of an amorphous form or another undesired polymorph.

PROPERTIES OF NANOSUSPENSIONS

1. Physical Long-term stability.

The high surface energy of nanosized particles induces agglomeration of the drug crystals. The main function of the stabilizer is to wet the drug particles thoroughly to prevent Ostwald ripening and agglomeration of the nanosuspension and form a physically stable formulation by providing a steric or an ionic barrier. Typical examples of stabilizers used in nanosuspensions are cellulosics, poloxamer, polysorbates, lecithin, polyoleate and povidones. Lecithin may be preferred in developing parenteral nanosuspensions. [25]

2. Internal structure of Nanosuspensions.

The high-energy input during disintegration process causes structural changes inside the drug particles. When the drug particles are exposed to high-pressure homogenisation particles are transformed from crystalline state to amorphous state. The change in state depends upon the hardness of drug, number of homogenisation cycles chemical nature of drug and power density applied by homogeniser[13, 26].

3. Adhesiveness.

There is a distinct increase in adhesiveness of ultra fine powders compared to coarse powders. This adhesiveness of small drug nanoparticles can be exploited for improved oral delivery of poorly soluble drugs. Improved bioavailability, improved dose proportionality, reduced fed / fasted variability, reduced inter-subject variability and enhanced absorbtion rate (both human and animal data)[27] are some of the important benchmarking effects of a drug formulated as nanoparticles in oral administration. These data have been acquired in vivo in animals but also in humans as reported by the company NanoSystems. A drastically remarkable report is that of the increase in bioavailability for danazole from 5 % (as macrosuspension) to 82% (as nanosuspension).

4. Crystalline state and morphology

A potential change in the crystalline structure of nanosuspensions saying increasing the amorphous fraction in the particle or even creating completely amorphous particles is a characteristic of consideration. The application of high pressures during the production of nanosuspensions was found to promote the amorphous state.[2]

EVALUATION OF NANOSUSPENSIONS[13, 29]

Nanosuspensions evaluation is done in similar ways as those used for conventional suspensions such as appearance, color, odor, assay, related impurities, etc. Apart from the aforementioned parameters, the nanosuspensions should be evaluated for their particle size, zeta potential, crystalline status, dissolution studies and *in vivo* studies.

A) In-Vitro Evaluations.

1. Particle size and size distribution.
2. Particle charge (Zeta Potential).
3. Dissolution velocity and saturation solubility.

4. Crystalline state and morphology.

B) In-Vivo Evaluation.

C) Evaluation for surface-modified Nanosuspensions.[29]

1. Surface hydrophilicity.
2. Adhesion properties.
3. Interaction with body proteins.

1. Particle Size and Size Distribution.

Particle Size and Size distribution determines the physiochemical behavior of the formulation, such as saturation solubility, dissolution velocity, physical stability, etc. The particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) and coulter counter multisizer. The PCS method can measure particles in the size range of 3 nm to 3 µm and the LD method has a measuring range of 0.05-80 µm. The coulter counter multisizer gives the absolute number of particles, in contrast to the LD method, which gives only a relative size distribution. For IV use, particles should be less than 5 µm, considering that the smallest size of the capillaries is 5-6 µm and hence a higher particle size can lead to capillary blockade and embolism.

2. Particle charge (Zeta potential).

Zeta potential is an indication of the stability of the suspension. For a stable suspension stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is required whereas in case of a combined electrostatic and steric stabilizer, a zeta potential of ±20 mV would be sufficient.

3. Dissolution velocity and saturation solubility.

Nanosuspensions have an important advantage over other techniques, that it can increase the dissolution velocity as well as the saturation solubility. These two parameters should be determined in various physiological solutions. The assessment of saturation solubility and dissolution velocity helps in determining the *in vitro* behavior of the formulation. Böhm *et al.* reported an increase in the dissolution pressure as well as dissolution velocity with a reduction in the particle size to the nanometer range.[30] Size reduction leads to an increase in the dissolution pressure. An increase in solubility that occurs with relatively low particle size reduction may be mainly due to a change in the surface tension leading to increased saturation solubility. Muller explained that the energy introduced during the particle size reduction process leads to an increase in the surface tension and an associated increase in the dissolution pressure.

4. Crystalline state and morphology.

To characterize the polymorphic changes due to the impact of high-pressure homogenization in the crystalline structure of the drug, techniques like X-ray diffraction analysis in combination with differential scanning calorimetry or differential thermal analysis can be utilized. Nanosuspensions can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms because of high-pressure homogenization.

APPLICATION

1. Intravenous administration.

The parenteral route of administration provides a quick onset of action, rapid targeting and reduced dosage of the drug. It is the preferred route for drugs undergoing first-pass metabolism and those that are not absorbed in the GIT or degraded in the GIT. One of the important applications of nanosuspension technology is the formulation of intravenously administered products. IV administration results in several advantages, such as administration of poorly soluble drugs without using a higher concentration of toxic co-solvents, improving the therapeutic effect of the drug available as conventional oral formulations and targeting the drug to

macrophages and the pathogenic microorganisms residing in the macrophages.[31]

2. Bioavailability enhancement.

The poor oral bioavailability of the drug may be due to poor solubility, poor permeability or poor stability in the gastrointestinal tract (GIT). Nanosuspensions resolve the problem of poor bioavailability by solving the twin problems of poor solubility and poor permeability across the membrane. Bioavailability of poorly soluble oleanolic acid, a hepatoprotective agent, was improved using a nanosuspension formulation. The therapeutic effect was significantly enhanced, which indicated higher bioavailability. This was due to the faster dissolution (90% in 20 min) of the lyophilized nanosuspension powder when compared with the dissolution from a coarse powder (15% in 20 min). [32]

3. Pulmonary administration.

Aqueous nanosuspensions can be nebulized using mechanical or ultrasonic nebulizers for lung delivery. Because of their small size, it is likely that in each aerosol droplet at least one drug particle is contained, leading to a more uniform distribution of the drug in lungs. They also increase adhesiveness and thus cause a prolonged residence time. Budenoside drug nanoparticles were successfully nebulized using an ultrasonic nebulizer. [33]

4. Ocular administration.

Ocular delivery of the drugs as nanosuspensions to provide a sustained release of drug. Pignatello *et al.* prepared Eudragit retard nanosuspensions of cloricromene for ocular delivery. [34] They observed that the drug showed a higher availability in rabbit aqueous humor and the formulation appeared to offer a promising means of improving the shelf-life and the bioavailability of this drug after ophthalmic application.

5. Drug targeting.

Nanosuspensions can also be used for targeting as their surface properties and changing of the stabilizer can easily alter the *in vivo* behavior. The drug will be up taken by the mononuclear phagocytic system to allow regional-specific delivery. This can be used for targeting anti-mycobacterial, fungal or leishmanial drugs to the macrophages if the infectious pathogen is persisting intracellularly. [35]

6. Mucoadhesion of the nanoparticles.

Nanoparticles orally administered in the form of a suspension diffuse into the liquid media and rapidly encounter the mucosal surface. The particles are immobilized at the intestinal surface by an adhesion mechanism referred to as "bioadhesion." From this moment on, the concentrated suspension acts as a reservoir of particles and an adsorption process takes place very rapidly. The direct contact of the particles with the intestinal cells through a bioadhesive phase is the first step before particle absorption. [36] The adhesiveness of the nanosuspensions not only helps to improve bioavailability but also improves targeting of the parasites persisting in the GIT.

REFERENCES

1. Lipinski C. Poor aqueous solubility-an industry wide problem in drug discovery. Am.pharm.Rev.2002; 5: 82-85.
2. S.M.Wong,I.W.Kellaway, S.Murdan. Enhancement of the dissolution rate and oral absorption of a poorly water soluble drug by formation of surfactant-containing microparticles. Int.J.Pharm.2006; 317:61-68.
3. Parikh R.K.,Manusun SN.S.,Gohel M.C. and Soniwala M.M. Dissolution enhancement of Nimesulide using complexation and salt formation

techniques.Indian drugs. 2005; 42(3):149-154.
4. Marazban Sarkari, Judith Brown, Xiaoxia Chen, Steve Swinnea, Robert O. Williams III, Keith P. Johnston.Enhanced drug dissolution using evaporative precipitation into aqueous solution. Int.J.Pharm.2002; 243:17-31.
5. True L.Rogers, Ian B. Gillespie, James E. Hitt, Kevin L.Fransen,Clindy A. Crowl,Chritoper J.Tucker, et.al. Development and characterization of a scalable controlled precipitation process to enhance the dissolution of poorly soluble drugs. Pharm.Res.2004; 21(11).
6. Riaz M., Stability and uses of liposomes. Pak.Pharm.Sci.1995; 8(2): 69-79.
7. Jadhav KR,Shaikh IM,Ambade KW, Kadam VJ. Applications of microemulsion based drug delivery system. Cur. Dr. Del.2006; 3(3): 267-273.
8. Leuner C.Dressman J.Improving drug solubility for oral delivery using solid dispersions. Eur.J.Pharm.Biopharm.2000; 50(1): 47-60.
9. Challa R.,Ahuja A., Ali J., Khar RK. Cyclodextrins in drug delivery: an updated Review. AAPS Pharm.Sci.Tech. 2005; 6(2): E329-357.
10. Dubey R. Pure drug nanosuspensions-Impact of nanosuspension technology on drug discovery and development. Drug Del Tech 2006; 6:65-71.
11. Shobha Rani,R.Hiremath and Hota A. Nanoparticles as drug delivery systems. Ind.J.Pharm.Sci.1999; 61(2): 69-75.
12. Mehnertw, Mader K. Solid lipid nanoparticles: Production, characterization and applications. Adv.Drug Deliv. Rev. 2000; 47(2-3): 165-96.
13. R.H.Muller, B.H.L.Bohm and .J.Grau. Nanosuspensions : a formulation approach for poorly soluble and poorly bioavailable drugs. In D.Wise (Ed) Handbook of pharmaceutical controlled release technology.2000; 345-357.
14. Chowdary K.P.R. and Madhavi B.L.R.,Novel drug delivery technologies for insoluble drugs. Ind.Drugs.2005; 42(9): 557-563.
15. Cornelia M. Keck,, Rainer H. Muller.Drug nanocrystals of poorly soluble drugs produced by high-pressure homogenisation. Eur. J. Pharm.Biopharm.2006; 62: 3–16.
16. V.B.Patravale, Abhijit A.Date and R.M.Kulkarni. Nanosuspensions: a promising drug delivery strategy. J.Pharm.Pharcol.2004; 56: 827-840.
17. Móller RH, Grau MJ, Hildebrand GE. Increase of solubility of poorly soluble drugs by transfer to Dissocubes using high pressure homogenization. Proc Int Symp Control Rel Bioact Mater 1999; 26: 112-3.
18. K.P. Krause, O. Kayser, K. Mader, R. Gust, R.H. Muller. Heavy metal intamination of nanosuspensions produced by high-pressure homogenisation. Int J. Pharm.2000; 196: 169–172.
19. K.P.Krause, R.H.Muller.Production and characterization of highly concentrated nanosuspensions by high pressure homogenisation. Int.J.Pharm.2001; 214:21-24.
20. Jan Moschwitzer,Georgr Achleitner, Herberk Pomper, Rainer H.Muller. Development of an intraveneously injectable chemically stable aqueous omeprazole formulation using nanosuspension. Eur. J. Pharm. Biopharm.2004; 58:615-619.
21. Radtke M. Nanopure: Poure drug nanoparticles for the formulation of poorly soluble drugs. New Drugs 2001; 3: 62-8.
22. Dearns R. Atovaquone pharmaceutical compositions. US Patent US 6018080, 2000.
23. Sucker H. Hydrosole, eine Alternative fór die parenterale Anwendung von schwer wasserloslichen Wirkstoffen. In: Muller RH, Hildebrand GE, editors. Pharmazeutische Technologie; Modern Arzneiformen. 2nd ed. Stuttgart: WVG; 1998.
24. Young TJ, Mawson S, Johnston KP, Henriska IB, Pace GW, Mishra AK.Rapid expansion from supercritical to aqueous solution to produce submicron suspension of water insoluble drugs. Biotechnol Prog 2000;16: 402-7.

25. Shah T, Patel D, Hirani J, Amin AF. Nanosuspensions as a drug delivery systems-A comprehensice review. Drug Del Tech 2007;7: 42-53.
26. V.B.Patravale, Abhijit A.Date and R.M.Kulkarni. Nanosuspensions: a promising drug delivery strategy. J.Pharm.Pharcol.2004; 56: 827-840.
27. G.C. Liversidge, Drug nanocrystals for improved drug delivery, in: Int. Symp.Control. Release Bioact. Mater, Workshop on Particulate Drug Delivery Systems,Vol. 23, 1996.
28. M.J. Grau, R.H. M üller, Increase of dissolution velocity and solubility of poorly soluble drugs by formulation as nanosuspension, in: Proceedings 2nd World Meeting APGI/APV, Paris, 1998, pp. 623–624.
29. R.H.Muller, C.Jacobs, O. Kayser. Nanosuspensions as particulate drug formulations in therapy Rationale for development and what we can expect for the future. Ad.Drug Del.Rev.2001; 47:3-19.
30. Bohm BH, Muller RH. Lab-scale production unit design for nanosuspensions of sparingly soluble cytotoxic drugs.PSTT 1999; 2: 336-9.
31. Peters K, Leitzke S, Diederichs JE, Borner K, Hahn H, Móller RH, *et al*. Preparation of a clofazimine nanosuspensions for intravenous use and evaluation of its therapeutic efficacy in murine mycobacterium avium infection. J Antimicrob Chemother 2000; 45:77-83.
32. Chen Y, Liu J, Yang X, Zhao X, Xu H. Oleanolic acid nanosuspensions: Preparation, *in vitro*characterization and enhanced hepato protective effect. J Pharm Pharmacol 2005; 57:259-64.
33. Muller RH, Jacobs C. Production and Characterization of Budenoside nanosuspension for pulmonary administration. Pharm Res 2002; 19:189-94.
34. Pignatello R, Ricupero N, Bucolo C, Maugeri F, Maltese A, Puglisi G. Preparation and characterization of Eudragit retard nanosuspensions for the ocular delivery of cloricromene. AAPS Pharmscitech 2006; 7:E27.
35. Kayser O, Lemke A, Hernandz.Trejo N. The impact of Nanobiotechnology on the development of new drug delivery systems. Current Pharm Biotech 2005; 6:3-5.
36. Ponchel G, Montisci MJ, Dembri A, Durrer C, Duchкne. D. Mucoadhesion of colloidal particulate systems in the gastrointestinal tract. Eur J Pharm Biopharm1997; 44: 25-31.