INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Review Article

Nanosuspension: An Attempt to Enhance Bioavailability of Poorly Soluble Drugs

Sneha B Garasiya*

Dharmaj Degree Pharmacy college, Amrapalit township, Khambhat petlad road, Dharmai. Guiarat. India.

ABSTRACT

One of the critical problems associated with poorly soluble drugs is low bioavailability and or erratic absorption. The problem is even more complex for drugs such as itraconazole and Carbamazepine (belonging to BCS CLASS II) as they are poorly soluble in both aqueous and organic media.There are number of formulation approaches to resolve the problems of low solubility and low bioavailability. But all those have some limitations and hence have limited utility in solubility enhancement. Nanotechnology can be used to resolve these problems associated with conventional approaches. Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} meters. Nanosuspensions consist of the pure poorly water-soluble drug without any matrix material suspended in dispersion. A nanosuspension not only solves the problems of poor solubility and bioavailability but also alters the pharmacokinetics of drug and thus improves drug safety and efficacy.

Keywords: Nanotechnology; Nanosuspensions; drug delivery; bioavailability.

INTRODUCTION

More than 40 percent of the drugs coming from High-throughput screening are poorly soluble in water[1]. As per a recent report [2], 46% of the total New Drug Applications (NDA) filed between 1995 and 2002 were BCS class IV, while only 9% were BCS class I drugs, revealing that a majority of the approved new drugs were water insoluble Because of their poor solubility it will become more complicated to incorporate them into the conventional dosage forms and thus decreasing the bioavailability of the drugs [3].

The problem is even more complex for drugs such as itraconazole and Carbamazepine (belonging to BCS CLASS II) as classified by BCS System[4] as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. The performance of these drugs is dissolution rate-limited (for Class II and III drugs) and is affected by the fed/fasted state of the patient. Dissolution rates of sparingly soluble drugs are related to the shape as well as the particle size. Therefore decrease in particle size results in an increase in dissolution rate[5].

Of course, there are number of formulation approaches that can be used to resolve the problems associated with the low solubility and low bioavailability of these class II drugs. Some of the approaches to increase solubility include micronization [6], solubilisation using co-solvents, use of permeation enhancers, oily solutions, surfactant dispersions [6], salt formation [7] and precipitation techniques [8-9].

Most of these techniques for solubility enhancement have advantages as well as some limitations and hence have limited utility in solubility enhancement. Other techniques used for solubility enhancement include microspheres, emulsions, microemulsions [10], liposomes [11], super critical processing, solid-dispersions [12] and inclusion complexes using Cyclodextrins [13] 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 Media.

However, there still remains an unmet need to equip the pharmaceutical industry with particle engineering technologies capable of formulating the poorly soluble drugs to improve their efficacy and to optimize therapy with respect to pharmacoeconomics. One such novel technology is nanosuspension technology. Nanosuspensions are

sub-micron colloidal dispersions of nanosized drug particles stabilized by surfactants [14]. Nanosuspensions consist of the poorly water-soluble drug without any matrix material suspended in dispersion [15]. These can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result of increased solubility, 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. This approach is useful for molecules with poor solubility, poor permeability or both, which poses a significant challenge for the formulators. The reduced particle size renders the possibility of intravenous administration of poorly soluble drugs without any blockade of the blood capillaries. The suspensions can also be lyophilised and into a solid matrix. Apart from these advantages it is also having the advantages of liquid formulations over others. In the present review is mainly focussing on the different methods of preparation, critical parameters and evaluation of the nanosuspension as well as pharmaceutical applications of nanosuspensions.

Nanosuspensions differ from nanoparticles [17] which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules), and from solid-lipid nanoparticles [18] (SLN), which are lipidic carriers of drug. The potential benefits of nanosuspension used via various routes of administration are described in **Table 1** [19]**.**

Table 1: Potential benefits of nanosuspension

ROUTE OF ADMINISTRATION	POTENTIAL BENEFITS
Oral	➢ Rapid dissolution and ➢ High bioavailability ➢ Reduced fed/fasted ratio
Intravenous (I.V)	➢ Tissue targeting ➢ Rapid dissolution ➢ Longer duration of retention in systemic circulation
Ocular	➢ Higher bioavailability ➢ Less irritation ➢ More consistent dosing
Inhalation	➢ Higher bioavailability ➢ More consistent dosing
Subcutaneous/ intramuscular	➢ Higher bioavailability ➢ Rapid onset ➢ Reduced tissue irritation

When to go for Nanosuspensions Approach[20]?

Nanosuspension is preferred for the compounds that are insoluble in water (but are soluble in oil) with high log P value. Conventionally the drugs that are insoluble in water but soluble in oil phase system are formulated in liposome, emulsion systems but these lipidic formulation approaches are not applicable to all drugs. In these cases nanosuspensions are preferred. Nanosuspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose.Figure1 shows selection criteria for formulation approach to enhance solubility of poorly soluble drugs.

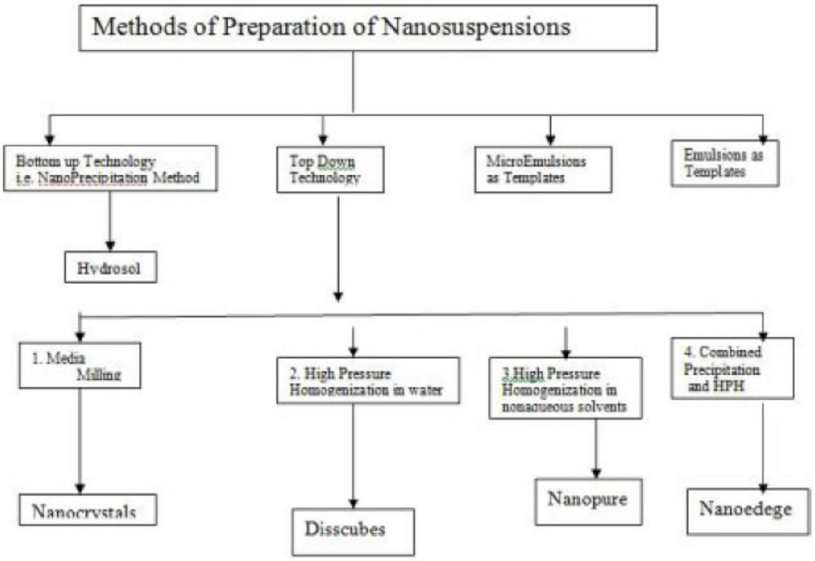

Fig. 1: The criteria for selection of various technologies to enhance solubility of poorly soluble drugs

Methods of Preparation of Nanosuspensions[19]

Preparation of nanosuspensions were reported to be a more cost effective and technically more simpler alternative than liposomes and other conventional colloidal drug carriers, particularly for poorly soluble drugs and yield a physically more stable product. The simplest method of preparation of nanosuspensions is micronization by colloid or jet milling 20, which improves the dissolution rate but is not having any effect on saturation solubility. Nanosuspension engineering processes currently used are preparation by precipitation, high pressure homogenization, emulsion and milling techniques. These techniques and the obtained compounds are summarized in **Table 2** and are briefly described in the following sections. Mainly there are two methods for preparation of nanosuspensions. The conventional methods of precipitation are called 'Bottom Up technology'. The 'Top Down Technologies' are the disintegration methods and are preferred over the precipitation methods. These 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). Few other techniques used for preparing nanosuspensions are emulsion as templates, microemulsion as templates etc.

Precipitation: The most common method of precipitation used is anti solvent addition method in which the drug is dissolved in an organic solvent and this solution is mixed with a miscible antisolvent. Mixing processes vary considerably. Precipitation has also been coupled with high shear processing. The NANOEDGE process (is a registered trademark of Baxter International Inc. and its subsidiaries) relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy[21].

This is accomplished by a combination of rapid precipitation and high-pressure homogenization. Rapid addition of a drug solution to an antisolvent leads to sudden super saturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super saturation when the solubility of the amorphous state is exceeded. The success of drug nanosuspensions prepared by precipitation techniques has been reported in some journals [21-22].

Table 2: Summary of the Nanosuspension Formation Technologies

Technology	Advantage	Disadvantage	Drug
Precipitation	Simple process. Ease of scale up. Economical production.	Growing of crystals needs to be limit by surfactant addition. Drug must be soluble at least in one solvent.	Carbamazepine[23] Cyclosporine [24] Griseofulvin [25]
Emulsion /Microemulsion template	High drug solubilization. Long shelf life. Ease of manufacture.	Use of high amount of surfactant and stabilizers. Use of hazardous solvent in production.	Breviscapine [26] Griseofulvin [27]

High pressure Homogenization	Applicable to most of the drugs Very dilute as well as highly concentrate nanosuspension can be prepared. Aseptic production possible.	High number of homogenization cycles. Drug should be in micronized state. Possible contamination could occur from metal ions coming off from the walls.	Albendazole [28] Aphidicolin [29] Azithromycin [30] Fenofibrate [31]
Milling methods			
Media milling	Applicable to the drugs that are poorly soluble in both aqueous and organic media. Little batch to batch variation. High flexibility in handling large quantities of drugs.	Time consuming. Difficult to scale up. Prolonged milling may induce the formation of amorphous & instability.	Cilostazol [32]
Dry Co-grinding	Easy process and no organic solvent required. Require short grinding time.	Generation of residue of milling media.	Clarithromycin [33]

Lipid Emulsion/Microemulsion Template: Lipid emulsions as templates are applicable for drugs that are soluble in either volatile organic solvents or partially water miscible solvents. In this method the drug will be dissolved in the suitable organic solvent and then emulsified in aqueous phase using suitable surfactants. Then the organic solvent will be slowly evaporated under reduced pressure to form drug particles precipitating in the aqueous phase forming the aqueous suspension of the drug in the required particle size. Then the suspension formed can be diluted suitably to get nanosuspensions [34]. Moreover, microemulsions as templates can produce nanosuspensions. Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids such as oil and water stabilized by an interfacial film of surfactant and co-surfactant. The drug can be either loaded into the internal phase or the pre-formed microemulsion can be saturated with the drug by intimate mixing. Suitable dilution of the microemulsion yields the drug nanosuspension [34]. An example of this technique is the griseofulvin nanosuspension which is prepared by the microemulsion [34]. The advantages of lipid emulsions as templates for nanosuspension formation are that they easy to produce by controlling the emulsion droplet and easy for scale-up. However, the use of organic solvents affects the environment and large amounts of surfactant or stabilizer are required.

High Pressure Homogenization: It is the most widely used method for the preparation of the nanosuspensions of many poorly water soluble drugs [35-37]. Different methods developed based on this principle for preparation of nanosuspensions are *Dissocubes*, *Nanopure*, *Nanoedge*, *Nanojet technology*. In the high pressure homogenization method, the suspension of a drug and surfactant is forced under pressure through a nanosized aperture valve of a high pressure homogenizer.

The principle of this method is based on cavitation in the aqueous phase. The particles cavitations forces are sufficiently high to convert the drug microparticles into nanoparticles. The concern with this method is the need for small sample particles before loading and the fact that many cycles of homogenization are required [38-39].

Milling Techniques

Media milling: Media milling is a further technique used to prepare nanosuspensions [40]. This patent-protected technology was developed by Liversidge et al. [41]. Formerly, the technology was owned by the company NanoSystems but recently it has been acquired by Elan Drug Delivery. In this technique, the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. The drug nanoparticles are obtained by subjecting the drug to media milling. High energy and shear forces generated as a result of impaction of the milling media with the drug provide the necessary energy input to disintegrate the microparticulate drug into nanosized particles. The milling medium is usually composed of glass, zirconium oxide or highly cross-linked polystyrene resin. In batch mode, the time required to obtain dispersions with unimodal distribution profiles and mean diameters <200nm is 30–60 min. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer. Then milling media or pearls are rotated at a very high shear rate.

Dry Co-Grinding: Recently, nanosuspensions can be obtained by dry milling techniques. Successful work in preparing stable nanosuspensions using dry-grinding of poorly soluble drugs with soluble polymers and copolymers after dispersing in a liquid media has been reported [42].

Many soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrin derivatives have been used [43]. Physicochemical properties and dissolution of poorly water soluble drugs were improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug [44]. Dry co-grinding can be carried out easily

and economically and can be conducted without organic solvents. The co-grinding technique can reduce particles to the submicron level and a stable.

Physical, Chemical and Biological Properties of Nanosuspensions: Nanosuspension formulation increases the saturation solubility as well as dissolution rate. Basically the saturation solubility is a compound specific constant which is temperature dependent. The saturation solubility also depends on the polymorphism of the drug as different polymorphs have different solubilities. It is also dependent on the particle size. This size-depend encycomes only into effect for particles having a size below approximately 1 µm. Another marked property is the adhesiveness generally described for nanoparticles [45].

As the particle size decreases the adhesive properties of the particles will be improved and thus improved oral delivery of poorly soluble drugs. Improved bioavailability, improved dose proportionality, reduced fed/fasted variability, reduced inter-subject variability and enhanced absorption rate (both human and animal data) [46] are some of the main effects observed on oral administration. These data have been acquired *in vivo* in animals but also in humans as reported by the company Nano Systems. A drastically remarkable report is that of the increase in bioavailability for danazole from 5 % (as macrosuspension) to 82% (as nanosuspension) [46]. The application of high pressures during the production of nanosuspensions was found to promote the amorphous state [47]. The degree of particle fineness and the fraction of amorphous particles in the nanosuspensions were found to be dependent on production pressure number of cycles of homogenisation and hardness of drug. The increase in the amorphous fraction leads to a further increase of the saturation solubility. The homogenization process (giving uniform particle size) was able to overcome Ostwald ripening [48] which means physical long-term stability as an aqueous suspension [49]. In oral drug administration, the bioavailability mainly depends upon the solubility of the drug, highly active compounds have failed in the past because their poor solubility has limited *in vivo* absorption and did not lead to effective therapeutic concentrations. As an example, Atovaquone is given orally three times 750 mg daily, because of the low absorption of only 10–15%. Oral administration of nanosuspensions can overcome this problem because of the high adhesiveness of drug particles sticking on biological surfaces and prolonging the absorption time.

Evaluation of Nanosuspensions [50-51]: The characterisation of the nanosuspensions is also similar to that of the suspensions such as colour, odour, presence of impurities and other important characteristics as mentioned below.

***In-Vitro* Evaluations**

- **Particle size and size distribution**
- **Particle charge (Zeta Potential)**
- **Crystalline state and morphology**
- **Saturation solubility and dissolution velocity**
- **Stability**

***In-vivo* evaluation**

***In-Vitro* Evaluations**

Particle size and size distribution: It is the most important parameter in the evaluation of the suspensions as it is having the direct effect on the solubility and dissolution rate and the physical stability of the formulation. The mean particle size and the width of particle size can be determined by Photon Correlation Spectroscopy (PCS) [52], laser diffraction and coulter current multisizer. Particle size and polydispersity index (PI) governs the saturation solubility, dissolution velocity and biological performance. PCS measures the particle size in the range of 3nm-3 µm only. PI governs the physical stability of nanosuspension and should be as low as possible for long-term stability (Should be close to zero). LD measures volume size distribution and measures particles ranging from 0.05- 80µm upto 2000µm. Atomic Force Microscopy is used for visualization of particle shape [53]. 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.

- **Particle charge (Zeta Potential):** The particle charge is of importance in the study of the stability of the suspensions. Usually the zeta potential of more than ±40mV will be considered to be required for the stabilisation of the dispersions. For electrostatically stabilized nanosuspension a minimum zeta potential of ±30mV is required and in case of combined steric and electrostatic stabilization it should be a minimum of ±20mV of zeta potential is required.
- **Crystalline Sate and Particle Morphology:** It is of importance as there are chances of the polymorphism during the storage of the nanosuspensions. Hence it is necessary to study the crystal morphology of the drug in suspension. Differential Scanning Calorimetry (DSC) is most commonly used for such studies [54]. When nanosuspensions are prepared drug particles may get converted to amorphous form hence it is essential to measure the

extent of amorphous drug generated during the production of nanosuspensions. The X-Ray Diffraction (XRD) is commonly used for determining change in crystallinity and the extent of the amorphous form of drug [55].

- **Saturation solubility and Dissolution Velocity:** The main advantage associated with the nanosuspensions is improved saturation solubility as well as dissolution velocity. These are studied in different physiological solutions at different pH. Kelvin equation and the Ostwald-Freundlich equations can explain increase in saturation solubility. Determination of these parameters is useful to assess *in vivo* performance of the formulation.
- **Stability of Nanosuspensions:** Stability of the suspensions is dependent on the particle size. As the particle size reduces to the nanosize the surface energy of the particles will be increased and they tend to agglomerate. So stabilizers are used which will decrease the chances of Ostwald ripening and improving the stability of the suspension by providing a steric or 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 40.
- ***In vivo* evaluation:** The *in vivo* evaluation of the nanosuspensions is specific to drug and route of administration. Most commonly the formulation was given by required route of administration and the plasma drug levels were estimated using HPLC-UV visible Spectrophotometry. Other parameters which are generally evaluated *in vivo are*

- Surface hydrophilicity/hydrophobicity (determines interaction with cells prior to phagocytosis)
- Adhesion properties
- Interaction with body proteins

APPLICATIONS OF NANOSUSPENSIONS IN DRUG DELIVERY

Oral drug delivery

The oral route is the preferred route for drug delivery because of its numerous well-known advantages. The efficacy or performance of the orally administered drug generally depends on its solubility and absorption through the gastrointestinal tract. Hence, a drug candidate that exhibit s poor aqueous solubility and / or dissolution rate limited absorption is believed to possess slow and/or highly variable oral bioavailability.

Danazol is poorly bioavailable gonadotropin inhibitor, showed a drastic improvement in bioavailability when administered as a nanosuspension as compared to the commercial danazol macrosuspension Danocrine. Danazol nanosuspension led to an absolute bioavailability of 82.3%, where as the marketed danazol suspension Danocrine was 5.2% bioavailable[52].

Parentral drug delivery

From the formulation perspective, nanosuspensions meet almost all the requirements of an ideal drug delivery system for the parentral route. Since the drug particles are directly nanosized, it becomes easy to process almost all drugs for parentral administration.Hence, nanosuspensions enable significant improvement in the parentrally tolerable dose of the drug, leading to a reduction in the cost of the therapy and also improved therapeutic performance.

The maximum tolerable dose of paclitaxel nanosuspension was found to be three times higher than the currently marketed Taxol, which uses Cremophore EL and ethanol to solubilize the drug[53].

Ocular drug delivery

Nanosuspension can also be used for the drugs that exhibit poor solubility in lachrymal secretions. To achieve sustained release of the drug for a stipulated time period, nanosuspension can be incorporated in a suitable hydrogel base or mucoadhesive base or even in ocular inserts. The designed polymeric nanosuspensions revealed superior in-vivo activity over the existing marketed formulations and could sustain drug release for twenty four hours[54].

Pulmonary drug delivery

Nanosuspension can be used for delivering drugs that exhibit poor solubility in pulmonary secretions. Currently such drugs are delivered as suspension aerosol or as dry powder inhalers. The drugs used in suspension aerosols and dry powder inhalers have micron size particle. Budesonide is poorly water -soluble corticosteroid, has been success fully formulated as a nanosuspension for pulmonary delivery[55].

Targeted drug delivery

Nanosuspension can be used for targeted deliver as their surface properties & changing of the stabilizer can easily alter in vivo behavior. Their versatility and ease of scale up and commercial production enables the development of commercially viable

nanosuspensions for targeted drug delivery. Kayser developed the formulation of aphidicolin as a nanosuspension to improve the drug targeting effect against Leishmania-infected macrophages, and stated that aphidicolin was highly active at a concentration in the microgram range[56].

CONCLUSION

Poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientists working on drug delivery of various drug compounds and leads to employment of novel formulation technologies.The use of drug nanosuspension is a universal formulation approach to increase the therapeutic performance of these drugs in any route of administration. Almost any drug can be reduced in size to the nanometer range. Production techniques such as media milling and high pressure homogenization have been successfully employed for large-scale production of nanosuspensions. The advances in production methodologies using emulsions or micro emulsions as templates have provided still simpler approaches for production but with limitations. Further investigation in this regard is still essential.

REFERENCES

1. Lipinski C. Poor aqueous solubility- an industry wide problem in drug discovery. Am.pharm.Rev.2002;5: 82-85.
2. Clewlow PJ: Survival of the smartest. Scrip′s Target world drug delivery news, 2004; 35:316-323
3. Elaine ML, Gary G L, and Eugene RC: Nanosizing: A formulation approach for poorly water-soluble compounds. Eur J Pharm Sci, 2003; 18:113-120.
4. The BCS Guidance from FDA, "Waiver of In-vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System." Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070246 [last accessed on 2010 April 10].
5. Mitra Mosharraf, Christer Nystrom. The effect of particle size and shape on the surface specific dissolution rate of microsized practically insoluble drugs. Int.J. Pharm.1995; 122:35-47.
6. Wong SM, Kellaway IW, Murdan S: 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.
7. Parikh RK, Manusun SN, Gohel MC. And Soniwala MM: Dissolution enhancement of Nimesulide using complexation and salt formation techniques. Indian drugs. 2005; 42(3):149-154..
8. Marazban S, Judith B, Xiaoxia C, Steve S, Robert OW, and Keith PJ: Enhanced drug dissolution using evaporative precipitation into aqueous solution. Int J Pharm, 2002; 243, 17-31.
9. True LR, Ian BG, James EH, Kevin LF, Clindy AC, Chritoper JT et.al: Development and characterization of a scalable controlled precipitation process to enhance the dissolution of poorly soluble drugs. Pharm Res, 2004; 21(11): 2048-2057.
10. Jadhav KR, Shaikh IM, Ambade KW, Kadam VJ: Applications of microemulsion based drug delivery system. Cur Dr Delivery, 2006; 3(3): 267-273.
11. Riaz M: Stability and uses of liposomes. Pak Pharm Sci, 1995; 8(2): 69-79.
12. Christian L and Jennifer D: Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm, 2000; 50(1): 47-60.
13. Challa R, Ahuja A, Ali J, Khar R: Cyclodextrins in Drug Delivery: An Updated Review. AAPS PharmSciTech, 2005; 06(02): E329-E357.
14. Barret ER: Nanosuspensions in drug delivery. Nat rev, 2004 ;(3): 785-796.
15. R H Muller, Gohla S, Dingler A, Schneppe T: Large-scale production of solid-lipid nanoparticles (SLN) and nanosuspension (Dissocubes). In D.Wise (Ed.) Handbook of pharmaceutical controlled release technology, 2000; 359-375.
16. Vyas SP, Khar RK. Targeted and Controlled Drug Delivery, Novel Carrier Systems. 1st ed. New Delhi: CBS Publishers; 2002; 173–247.
17. Shobha R, Hiremath R and Hota A: Nanoparticles as drug delivery systems. Ind J Pharm Sci, 1999; 61(2): 69-75.
18. Cornelia MK, Muller RH: Drug nanocrystals of poorly soluble drugs produced by high-pressure homogenisation. Eur J Pharm Biopharm, 2006; 62: 3–16.
19. Banavath H, Sivarama R K, Md. Tahir Ansari, Md. Sajid Ali and Pattnaik G, Nanosuspension: an attempt to enhance bioavailability of poorly soluble drugs.Int J Pharm Sci & Res, 2010;1(9):1-11.

20. Nagaraju. P, Krishnachaithanya. K, Srinivas. V.D.N and Padma. S.V.N, Nanosuspensions: A Promising Drug Delivery Systems, Int J Pharm.Sci. Nano.2010;2(4):679-684
21. Kipp JE, Wong JC, Doty MJ and Rebbeck CL: Microprecipitation method for preparing submicron suspensions. US Patent no. 6,607,784 2003.
22. Zhang X, Xia Q and Gu N: Preparation of all-trans retinoic acid nanosuspensions using a modified precipitation method. Drug Dev Ind Pharm, 2006; 32, 857-63.
23. Marazban S, Judith B, Xiaoxia C, Steve S, Robert OW, and Keith PJ: Enhanced drug dissolution using evaporative precipitation into aqueous solution. Int J Pharm, 2002; 243, 17-31.
24. Xiaoxia C, Timothy JY, Marazban S, Robert OW and Keith PJ: Preparation of cyclosporine a nanoparticles by evaporative precipitation into aqueous solution. Int J Pharm, 2002; 242, 3-14.
25. Zohra Z, Souad S, Hatem F: Preparation and characterization of poly-ε-carprolactone nanoparticles containing griseofulvin. Int J Pharm, 2005; 294,261-7.
26. She ZY, Ke X, Ping QN, Xu BH and Chen LL: Preparation of breviscapine nanosuspension and its pharmacokinetic behavior in rats. Chin J Nat Med, 2007; 5, 50-5.
27. Michele T, Marina G, Maria EC and Silvia M: Preparation of griseofulvin nanoparticles from water-dilutable microemulsions. Int J Pharm, 2003; 254, 235-42.
28. Pavan KM, Madhusudan RY, Shashank A: Improved bioavailability of albendazole following oral administration of nanosuspension in rats. Curr Nano sci, 2007; 3, 191-4.
29. Kayser O: Nanosuspensions for the formulation of aphidicolin to improve drug targeting effects against Leishmania infected macrophages. Int J Pharm, 2000; 196, 253-6
30. Zhang D, Tan T, Gao L, Zhao W and Wang P: Preparation of azithromycin nanosuspensions by high pressure homogenization and its physicochemical characteristics studies. Drug Dev Ind Pharm, 2007; 33, 569-75.
31. Hanafy A, Spahn LH, Vergnault G, Grenier P, Grozdanis MT, Lenhardt T: Pharmacokinetic evaluation of oral fenofibrate nanosuspension and SLN in comparison to conventional suspensions of micronized drug. Adv Drug Del Rev, 2007; 59, 419-26
32. Jun-ichi J, Naoki K, Masateru M, Keigo Y, Tadashi M, Masaaki O et.al: Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J Control Release 2006; 111, 56-64.
33. Etsuo Y, Shinichi K, Shuei M, Shigeo Y, Toshio O, Keiji Y: Physicochemical properties of amorphous clarithromycin obtained by grinding andspray drying. Eur J Pharm Sci, 1999; 7, 331-8.
34. Patravale VB, Date AA and Kulkarni RM: Nanosuspension: a promising drug delivery strategy. J Pharm Pharmacology, 2004; 56, 827-40.
35. Jacobs C, Kayser O and Müller RH: Production and characterization of mucoadhesive nanosuspensions for the formulation of bupravaquone. Int J Pharm, 2001; 214, 3-7
36. Müller RH and Jacobs C: Buparvaquone mucoadhesive nanosuspension: preparation, optimization and long-term stability. Int J Pharm, 2002; 237, 151- 61
37. Moschwitzer J, Achleitner G, Promper H, Muller RH: Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur J Pharm Biopharm, 2004; 58, 615-19.
38. Norbert R and Bernd WM: Dissolution rate enhancement by in situ micronization of poorly water soluble drugs. Pharm Res, 2002; 19, 1894-900.
39. Hartwig S, Norbert R, Bernd WM: In situ micronization of disodium cromoglycate for pulmonary delivery. Eur J Pharm Biopharm, 2003; 55, 173-80.
40. Tejal S, Dharmesh P, Jayshukh H and Avani FA: Nanosuspensions as a drug delivery system: A comprehensive review. Drug Deliv Tech, 2007; 7, 42-53
41. Gary GL, Phil C: Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats. Int J Pharm, 1995; 125, 309-13
42. Wongmekiat A, Tozuka Y, Oguchi T and Yamamoto K: Formation of fine drug particles by co-grinding with cyclodextrin. I. the use of β-cyclodextrin anhydrate and hydrate. Pharm Res, 2002; 19,
43. Sugimoto M, Okagaki T, Narisawa S, Koida Y and Nakajima K: Improvement of dissolution characteristics and bioavailability of poorly water-soluble drugs by novel co-grinding method using

water soluble polymer. Int J Pharm, 1998; 160, 11-9.
44. Tomoyuki W, Ikumasa O, Naoki W, Akira K, Mamoru S: Stabilization of amorphous indomethacin by co-grinding in a ternary mixture. Int J Pharm, 2002; 241, 103-11.
45. Dominique D, Gilles P: Bioadhesion of solid oral dosage forms, why and how? , Eur J Pharm Biopharm, 44 (1997) 15–23.
46. Gary GL: Drug nanocrystals for improved drug delivery. Int Symp Control Release Bioact Mater, Workshop on Particulate Drug Delivery Systems, Vol. 23, 1996.
47. Grau MJ, Müller RH: Increase of dissolution velocity and solubility of poorly soluble drugs by formulation as nanosuspension. Proceedings 2nd World Meeting APGI/APV, Paris, 1998, pp. 623–624.
48. EA Rawlin: Bentley's Textbook of Pharmaceutics, 8th Edition, Bailliere Tindall, London, 198
49. Peters K, Müller RH: Nanosuspensions for the oral application of poorly soluble drugs. Proceeding European Symposium on Formulation of Poorly-available Drugs for Oral Administration, APGI, Paris, 1996, pp. 330–333.
50. Muller RH, Bohm BH and Grau J: Nanosuspensions: a formulation approach for poorly soluble and poorly bioavailable drugs. In D.Wise (Ed.) Handbook of pharmaceutical controlled release technology, 2000; 345-357.
51. Muller RH, Jacobs C, Kayser O: 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
52. Liversidge G G, Cundy CK, Particle size reduction for improvement of oral bioavailability of hydrophobic drugs. I Absolute oral bioavailability of nanocrystalline danazol in beagle dogs, Int. J. Pharm, 127, 1995, 91–97.
53. Merisko L, Liversidge GG, et al. Formulation and anti-tumor activity evaluation of nanocrystalline suspensions of poorly soluble anti-cancer drugs, Pharm. Res, 13, 1996, 272–278.
54. Pignatello R, Bucolo C, Ferrara P, Maltese A, Puleo A, Puglisi G , Eudragit RS100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen, Eur. J. Pharm. Sci, 16,2002, 53–61.
55. Muller RH, Jacobs C, Production and characterization of a Budesonide nanosuspension for pulmonary administration, Pharm. Res, 19,2002,189–194.
56. Kayser O, Nanosuspensions for the formulation of aphidicolin to improve drug targeting effects against Leishmania infected macrophages,Int. J.Pharm. 2000, 196, 253-256.