REVIEW ARTICLE

Nanosuspension technology and its applications in drug delivery

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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. 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. These are simple to prepare and are more advantageous than other approaches. Techniques such as wet milling, high-pressure homogenization, emulsification–solvent evaporation and super critical fluid have been used in the preparation of nanosuspensions. It has the advantage of delivery by various routes, including oral, parenteral, pulmonary and ocular routes. The present article reviews the current methods used to prepare nanosuspensions and their application in drug delivery.

Key words: *Dissolution, nanosuspension, solubility enhancement, saturation solubility*

INTRODUCTION

One of the main problems responsible for the low turnout in the development of new molecular entities as drug formulations is poor solubility and poor permeability 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. Various formulation parameters that play a crucial role for successful formulation are aqueous solubility, stability at ambient temperature and humidity, photostability, compatibility with solvents and excipients, etc. Of these, solubility is the most important property for developing formulations. A major hurdle that has prevented the commercialization of many promising poorly soluble drugs is dissolution rate-limited bioavailability.

Compounds exhibiting dissolution rate-limited bioavailability are considered class II according to the BCS classification.[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. There are drug candidates that have poor solubility in water but can be dissolved by suitable conventional formulation strategies, which include co-solvents,[3] milling techniques,[4] super critical processing,[5] solid dispersions,[6] including complexation,[7] and precipitation techniques.[8] However, there still remains an unmet need to equip the pharmaceutical industry with particle engineering technologies capable of enhancing the dissolution of poorly soluble compounds. One such novel technology is nanosuspension technology.

NANOSUSPENSIONS

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.[9] 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 (e.g., oral or intravenous [IV] administration of the nanosuspension). 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. The reduced particle size renders the possibility of

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intravenous administration of poorly soluble drugs without blockade of the blood capillaries. The nanosuspensions can also be lyophilized or spray dried and the nanoparticles of a nanosuspension can also be incorporated in a solid matrix. Apart from this, it has all other advantages of a liquid dosage form over the solid dosage forms. The present review is focused on various methods of preparing nanosuspensions, critical parameters to be characterized and the application of nanosuspension formulations.

PREPARATION OF NANOSUSPENSIONS

The most common approach that has been used for preparing nanosuspensions is micronization by colloid or jet milling.[10] This method increases the dissolution rate of the drug but does not have any impact on the saturation solubility and thus cannot improve the bioavailability of drugs.

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.[8] This has the advantage of using relatively simple and low-cost equipment. However, this created problems in stirring and mixing when taken up for large-scale production. The major challenge of this technique is to avoid crystal growth that occurs on storage due to Ostwald ripening.

The principle techniques used in recent years for preparing nanosuspensions can be classified into four basic methods: (a) wet milling, (b) homogenization, (c) emulsification–solvent evaporation and (d) supercritical fluid method.

Wet milling

Nanosuspensions are produced by using high-shear media mills or pearl mills. The mill consists of a milling chamber, milling shaft and a recirculation chamber. An aqueous suspension of the drug is then fed into the mill containing small grinding balls/pearls. As these balls rotate at a very high shear rate under controlled temperature, they fly through the grinding jar interior and impact against the sample on the opposite grinding jar wall. The combined forces of friction and impact produce a high degree of particle size reduction.

The milling media or balls are made of ceramic-sintered aluminium oxide or zirconium oxide or highly cross-linked polystyrene resin with high abrasion resistance. Planetary ball mills (PM100 and PM200; Retsch GmbH and Co., KG, Haan, Germany) is one example of an equipment that can be used to achieve a grind size below 0.1 μm. A nanosuspension of Zn-Insulin with a mean particle size of 150 nm was prepared using the wet milling technique. The major drawbacks of this technology include the erosion of balls/pearls that can leave residues as contaminants in the final product, degradation of the thermolabile drugs due to heat generated during the process and presence of relatively high proportions of particles $\geq 5\,\mu$m.

Homogenization

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.[11] In this case, the suspension of the drug is made to pass through a small orifice that results in a reduction of the static pressure below the boiling pressure of water, which leads to boiling of water and formation of gas bubbles. When the suspension leaves the gap and normal air pressure is reached again, the bubbles implode and the surrounding part containing the drug particles rushes to the center and in the process colloids, causing a reduction in the particle size. Most of the cases require multiple passes or cycles through the homogenizer, which depends on the hardness of drug, the desired mean particle size and the required homogeneity. This principle is employed in the APV Gaulin Micron LAB 40 Homogenizer (APV Homogenizer, Lübeck, Germany) and the NS 1001L-Panda 2K high-pressure homogenizer (Nirosuavi. S.P.A., Parma, Italy).

Scholer *et al*. prepared atovaquone naanosuspensions using this technique.[12] An aqueous suspension of atovaquone was dispersed using an Ultra turrax T25, IKA-Werke GmbH & Co. KG, Staufen, Germany and was further homogenized in a Gaulin Micron Lab 40 high-pressure homogenizer. After subjecting to pressures of 1.5×10^7 (two cycles), 5×10^7 (two cycles) and 1.5×10^8 (20 cycles) Pa, a nanosuspension of atovaquone with a mean diameter of 279 $\pm$ 7 nm and mean polydispersity index of 0.18 $\pm$ 0.001 was obtained. To produce a nanosuspension with a higher concentration of solids, it is preferred to start homogenization with very fine drug particles, which can be accomplished by pre-milling. The major advantage of high-pressure homogenization over media milling is that it can be used for both diluted as well as concentrated suspensions and also allows aseptic production.

Nanopure

Nanopure is suspensions homogenized in water-free media or water mixtures.[13] 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°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°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.

Nanoedge™

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.

Precipitation is performed in water using water-miscible solvents such as methanol, ethanol and isopropanol. It is desirable to remove those solvents completely, although they can be tolerated to a certain extent in the formulation. For an effective production of nanosuspensions using the Nanoedge technology, an evaporation step can be included to provide a solvent-free modified starting material followed by high-pressure homogenization.

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.[14] 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.

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.

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.[8] Higher shear force prevents crystal growth and Ostwald ripening and ensures that the precipitates remain smaller in size.

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.[15] 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.[16] 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 supersaturation, which may also result in the development of an amorphous form or an other undesired polymorph.

CHARACTERIZATION OF NANOSUSPENSIONS

Nanosuspensions are characterized 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.

Particle size distribution

Particle 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.

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 $\pm$30 mV is required whereas in case of a combined electrostatic and steric stabilizer, a zeta potential of $\pm$20 mV would be sufficient.

Crystal 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.

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.[17] 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 an 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.[18]

STABILITY OF NANOSUSPENSIONS

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.[31]

APPLICATIONS OF NANOSUSPENSIONS

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. The oral administration of naproxen nanoparticles lead to an area under the curve (AUC) (0–24 h) of 97.5 mg-h/l compared with just 44.7 mg-h/l for naprosyn suspensions and 32.7 mg-h/l for anaprox tablets.[19] Oral administration of the gonadotrophin inhibitor Danazol as a nanosuspension leads to an absolute bioavailability of 82.3 and the conventional dispersion (Danocrine) only to 5.2%.[20] A nanosuspension of Amphotericin B developed by Kayser *et al.* showed a significant improvement in its oral absorption in comparison with the conventional commercial formulation.[21]

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).[22] Kocbek *et al.* showed a significant improvement in the dissolution rate (65% in 10 min) of Ibuprofen made as a lyophilized nanosuspension powder as compared with (<15% in 10 min) that of the micronized drug.[23] The ocular anti-inflammatory activity of Ibuprofen-Eudragit RS100 nanosuspensions was greatly improved when compared with an aqueous solution of Ibuprofen lysinate. Further, the aqueous humor drug concentration was significantly higher in groups treated with Ibuprofen-Eudragit RS when compared with the Ibuprofen-treated group. Langutth *et al.* showed a nearly 5.7-fold increase in the AUC for spiranolactone, a low solubility drug made as a solid lipid nanoparticle. Dissocubes type showed about 3.3-fold increase in the AUC.[24] They observed that the improvement in drug solubility in the intestine as well as in the dissolution rate of spiranolactone is the most likely mechanism for the increase in the AUC.

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.

Peters *et al.* prepared clofazimine nanosuspensions for IV use and showed that the drug concentrations in the liver, spleen and lungs reached a comparably higher level, well in excess of the minimum inhibitory concentration for most *Mycobacterium avium* strains.[25] Further, the study also indicates that the nanoparticle formulation accumulated more in the liver than the liposomal formulation, indicating a better targeting potential of the nanoparticle formulation. Injectable nanosuspensions of poorly soluble drug tarazepide have been prepared to overcome the limited success achieved using conventional solubilization techniques, such as use of surfactants, cyclodextrins, etc., to improve bioavailability.[26] A stable intravenously injectable formulation of omeprazole has been prepared to prevent the degradation of orally administered omeprazole.[27]

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.[28] The pharmacokinetics of the nebulized nanocrystal budenoside suspension showed comparable AUC, higher Cmax and lower Tmax as that of the pulmicort respules.

Other applications include 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.[29] 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.

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.[30] Kayser formulated a nanosuspension of Aphidicolin to improve drug targeting against leishmania-infected macrophages. He stated that the drug in the conventional form had an effective concentration (EC 50) of 0.16 mcg/ml whereas the nanosuspension formulation had an enhanced activity with an EC (50) of 0.003 mcg/ml.[32] Scholer *et al.* showed an improved drug targeting to the brain in the treatment of toxoplasmic encephalitis in a new murine model infected with *Toxoplasma gondii* using a nanosuspension formulation of Atovaquone.[12]

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.[33] The adhesiveness of the nanosuspensions not only helps to improve bioavailability but also improves targeting of the parasites persisting in the GIT, e.g., *Cryptosporidium parvum*. Bupravaquone nanosuspensions have been reported to demonstrate an advantage in TRC-alpha-deficient mice infected with *Cryptosporidium parvum* oocytes. The bioadhesion can also be improved by including a mucoadhesive polymer in the formulation.[34]

CONCLUSION

Nanosuspensions of pure drug offer a method to formulate poorly soluble drug and enhance the bioavailability of several drugs. It has many formulations and therapeutic advantages, such as simple method of preparation, less requirement of excipients, increased dissolution velocity and saturation solubility, improved adhesion, increases the bioavailability leading to a decrease in the dose and fast-fed variability and ease of large-scale manufacturing. Nanosuspensions can be formulated for various routes of administration, such as oral, parenteral, ocular, topical and pulmonary routes. This technology is gaining significance as the number of molecules with solubility and bioavailability related problems are increasing day by day. Thus, nanotechnology can play a vital role in drug discovery programs to increase aqueous solubility as well as bioavailability of poorly soluble drugs.

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