



## REVIEW : NANOSUSPENSIONS

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### ABSTRACT

Nanosuspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because of their versatile features and unique advantages. Techniques such as media milling and high pressure homogenization have been used commercially for producing nanosuspensions. Recently, the engineering of nanosuspensions employing emulsions and microemulsions as templates has been addressed in the literature. The unique features of nanosuspensions have enabled their use in various dosage forms, including specialized delivery systems such as mucoadhesive hydrogels. Rapid strides have been made in the delivery of nanosuspensions by parenteral, peroral, ocular and pulmonary routes. Currently, efforts are being directed to extending their applications in site-specific drug delivery.

**Keywords:** Nanosuspension, Lipid solubility, Particle size, Oral bioavailability.

### INTRODUCTION

Various formulation parameters that play a crucial role in the successful formulation of drugs are aqueous solubility, stability at ambient temperature and humidity, photostability, compatibility with solvent and excipient. Among this aqueous solubility became a hurdle for the formulation of new molecular entities. More than 40% of the new chemical entities being generated through drug discovery programmes are poorly water-soluble or lipophilic compounds. Formulating a poorly water soluble drug has always been a challenging problem confronted by the pharmaceutical scientist. The formulation of nano-sized particles can be implemented to all drug compounds belonging to biopharmaceutical classification system (BCS) classes II and IV to increase their solubility and hence partition into gastrointestinal barrier.

Micronization is used for class II drugs of (BCS), i.e. drugs having a good permeability and poor solubility. There are many conventional methods for increasing the solubility of poorly soluble drugs, which include micronization, solubilisation using co-solvents, salt form, surfactant dispersions, precipitation technique, and oily solution. Other techniques are like liposomes, emulsions, microemulsion, solid dispersion and inclusion complexation using cyclodextrins show sensible achiever, but they lack in universal applicability to all drugs. These techniques are not applicable for those drugs which are not soluble in aqueous and organic solvents. Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility and bioavailability enhancement. Nanosuspension is favoured for compounds that are insoluble in water (but are soluble in oil) with high log P value, high melting point and high doses. Nanosuspension technology can also be used for drugs which are insoluble in both water and organic solvents.

There are drug candidates that have poor solubility in water but can be dissolved by suitable conventional formulation strategies which include co-solvents, milling techniques, super critical processing and solid dispersions including complexation and precipitation techniques.<sup>1</sup>

Eg. Atorvastatin is currently used as calcium salt for the treatment of hypercholesterolemia. It is insoluble in aqueous solution of pH 4 and below; it is very slightly soluble in water and pH 7.4 phosphate buffer. The intestinal permeability of atorvastatin is high at the physiologically relevant intestinal pH. The drug is absorbed more in the upper duodenum and in the upper small intestine regions. However, it is reported that the absolute bioavailability (*F*) of atorvastatin is 12% after a 40 mg oral dose. In the present study, an attempt was made to enhance the solubility and dissolution characteristics of a poorly soluble model drug, atorvastatin calcium (AC) using nanosuspension technology.

### Definition

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 $\mu$ m in size. Reduction of drug particles to nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of saturation solubility.

The increase in the saturation solubility and solution velocity of nanoparticle is due to increase of vapour pressure of the particles. Nanosuspension have disclosed the problems associated with the delivery of poorly water-soluble and poorly water-and lipid soluble drugs and are unequalled because of their simplicity and rewards they confer over other strategies.<sup>2</sup>



**Table 1:** Lipid formulation classification system

Formulation Type	Materials	Characteristics	Advantages	Disadvantages
TYPE I	Oils without surfactant	Non-dispersing	Simple, excellent capsule compatibility	Formulation has poor solvent capacity
TYPE II	Oils & water insoluble surfactant	SEDDS formed without water soluble components	Lose solvent capacity on dispersion	Turbid o/w dispersion
TYPE III	Oils, surfactants, cosolvents	SEDDS formed with water soluble components	Clear dispersion, drug absorption without digestion	Less easily digested
TYPE IV	Water soluble surfactants & cosolvents	Formulation disperses typically to form a micellar solution	Formulation has good solvent capacity	May not be digestible

## METHODS

### Preparation of nanosuspension

There are two methods for preparation of nanosuspension. They are

1. 'Bottom up technology'
2. 'Top down technology'

#### 1. Bottom up technology

In Bottom up technology the drug is dissolved in a solvent, which is then added to non-solvent that causes precipitation of the fine drug particles. All-Trans retinoic acid nanosuspensions were prepared with a precipitation method.

#### Advantages

1. Use of simple and low cost equipment and
2. Higher saturation solubility is the advantage for precipitation technique compared to other methods of nanosuspension preparation.

#### Disadvantages

1. Precipitation technique is not applicable to drugs which are poorly soluble in aqueous and non aqueous media. In this technique, the drug needs to be soluble in at least one solvent which is miscible with nonsolvent.
2. Avoid crystal growth due to Ostwald ripening being caused by different saturation solubilities in the vicinity of differently sized particles.

#### 2. Top down technology

The top down technologies include

- (a) Media milling
- (b) High pressure homogenization
- (c) Emulsion diffusion method
- (d) Melt emulsification method

and these are preferred over the precipitation methods.

##### (a) Media milling (Nanocrystals or Nanosystems)

The method is first developed by liversidge *et. al.*, in this method 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 milling medium is framed of glass, zirconium oxide or highly cross-linked polystyrene resin.

#### Procedure

The milling chamber is charged with the milling media, water, drug and stabilizer, and the milling media or pearls are then rotated at a very high shear rate. The milling process is performed under controlled temperatures. The high energy and shear forces generated as a result of the impaction of the milling media with the drug provide the energy input to break the microparticulate drug into nano-sized particles. The unimodal distribution profile and mean diameter of <200, require a time profile of 30-60 min. The media milling procedure can successfully process micronized and non-micronized drug crystals. Once the formulation and the process are optimized, very short batch-to-batch variation is observed in the quality of the dispersion.

A nanosuspension of Naproxen with a mean particle size of 300-600 nm was prepared using pearl milling technique.

#### (b) homogenization

Homogenization involves the forcing of the suspension under pressure through a valve having a narrow aperture.

The most commonly used homogenizer in the preparation of nanosuspension is the APV micron LAB 40(APV Deutschland GmbH, Lubeck, Germany). However, other piston-gap homogenizers from Avestin (Avestin Inc., Ottawa, Canada) and Stansted (Stansted Fluid PowerLtd, Stansted, UK) can also be used.

The instrument can be operated at pressures varying from 100 to 1500 bars. In some instruments, a maximum pressure of 2000 bars can be reached. Most of the cases require multiple passes or cycles through the homogenizer, which depends on the hardness of the drug, the desired mean particle size, and required homogeneity.

High-pressure homogenizers are available with different capacities ranging from 40ml (for laboratory purposes) to a few thousand litres (for large-scale production). Before subjecting the drug to the homogenization process, it is essential to form a presuspension of the micronized drug in a surfactant solution using high-speed stirrers.



In the homogenization gap, according to Bernoulli's equation, the dynamic pressure of the fluid increases with the simultaneous decrease in static pressure below the boiling point of water at room temperature. In consequence, water starts boiling at room temperature, leading to the formation of gas bubbles, which go off when the suspension leaves the gap (called cavitation) and normal air pressure is reached again. The implosion forces are sufficiently high to break down the drug microparticles into nanoparticles. Additionally, the collision of the particles at high speed helps to achieve the nano-sizing of the drug.

The principle is employed in the APV gaulin micron LAB 40 homogenizer (APV homogenizer, Lubeck, Germany) and NS 100 1L-panda 2K high pressure homogenizer (NIROSUAVI. S.P.A., Parma, Italy).

Eg. Nimodipine nanosuspensions were prepared by using high pressure homogenizer. Nimodipine coarse powder was first disintegrated into microparticles by using fluid jet mill technology.

The nimodipine suspension was then subjected to three types of homogenizers, such as microfluidizer processor M-110EH (MFIC, USA), niro-soavi NS1001L (ATS Co.ltd.,Italy), and emulsiflex C3 (Avestin Inc., Canada). At first 200 bar with 2 cycles 500 bar with five cycles and then 15-20 cycles at 1500bar were run. Drugs such as carbazepine, bupravaquone, aphidocolin, cyclosporine, paclitaxel, prednisolone and atorvastatin are also prepared by using high pressure homogenizer.

### Factors affecting

#### (A) Effect of homogenization pressure

As the pressure increases particle size decreases.

7-bis(cis-2, 6-dimethylmorpholin-4-yl)-6-phenylpteridine, revealed that an inverse relationship exists between the homogenization pressure and the particle size.

#### (B) Number of homogenization cycles

It is anticipated that as the number of homogenization cycles increases the particle size decreases.

It is not possible to achieve the desired particle size in single homogenization cycle. Typically multiple cycles are required. The number of cycles depends on the hardness of the drug, required homogeneity, and the desired mean particle size required.

Eg. An omeprazole nanosuspension was prepared by using this technique.

#### (c) Emulsion diffusion method

Apart from the use of emulsion as drug delivering vehicle they can also be used as templates to produce nanosuspension. The use of emulsions as templates is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent. Such solvents can be used as the dispersed phase of the emulsion. An organic solvent or mixture of solvents

loaded with the drug is dispersed in the aqueous phase containing suitable surfactants with stirring to form an emulsion. The obtained emulsion was further homogenized by high pressure homogenization. After homogenization cycles the emulsion was diluted with water, homogenized by homogenizer to diffuse the organic solvent and convert the droplets into solid particles. Since one particle is formed in each emulsion droplet, it is possible to control the particle size of the nanosuspension by controlling the size of the emulsion. Optimizing the surfactant composition increases the intake of organic phase and ultimately the drug loading in the emulsion. Originally methanol, ethanol, ethyl acetate, chloroform are used as organic solvents. However, environmental hazards and human safety concerns about residual solvents have limited their use in routine manufacturing processes. Nanosuspension of ibuprofen<sup>4</sup>, diclofenac, acyclovir were prepared by this method.<sup>3</sup>

#### (d) Melt emulsification method

In this method drug is dispersed in the aqueous solution of stabilizer and heated above the melting point of the drug and homogenized to give an emulsion.

During this process, the sample holder was enwrapped with a heating tape fitted with temperature controller and the temperature of emulsion was maintained above the melting point of the drug. The emulsion was then cooled down either slowly to room temperature or on an ice-bath.

### Advantages

Melt emulsification technique relative to the solvent diffusion method is total avoidance of organic solvents during the production process.

Eg. Nanosuspension of ibuprofen was prepared by this method. Formulating ibuprofen nanosuspension by melt emulsification method show greater dissolution rate than formulating by solvent diffusion method.<sup>2</sup>

## EVALUATION

#### (A) Colour, odour, taste

These characteristics are especially important in orally administered formulation. Variations in taste, especially of active constituents, can offered be attributed to changes in particle size, crystal habit and subsequent particle dissolution. Changes in colour, odour and taste can also indicate chemical instability.

#### (B) Particle size

The most important charecterization parameter for the nanosuspension are the mean particle size and width of particle size distribution (called polydispersity index) which governs the physicochemical properties like saturation solubility, dissolution velocity, physical stability and even biological performance. It is proved that change in particle size changes saturated solubility and dissolution velocity.



Different methods for determining particle size distribution are

1. photon correlation spectroscopy (PCS),
2. laser diffraction (LD), and
3. coulter counter multisizer

**PCS** can even be used for determining the width of the particle size distribution (polydispersity index, PI). The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. A PI value of 0.1–0.25 indicates a fairly narrow size distribution where as a PI value greater than 0.5 indicates a very broad distribution. PCS determines the particle size in the range of (3nm to 3  $\mu$ m) it becomes difficult to determine the possibility of contamination of the nanosuspension by microparticulate drugs (having particle size greater than 3 $\mu$ m).

**Laser diffractometry (LD)** analysis of nanosuspensions should be carried out in order to detect as well as quantify the drug microparticles that might have been generated during the production process. LD determines the particle size in the range of 0.05–80 $\mu$ m up to 2000 $\mu$ m. The typical LD characterization includes determination of diameter 50% LD (50) and diameter 99% LD (99) values, which indicate that either 50 or 99% of the particles are below the indicated size. For parental use the particle size should be less than 5 $\mu$ m, considering that the smaller size of the capillaries is 5–6 $\mu$ m and hence a higher particle size can lead to capillary blockade and embolism. For nanosuspensions that are intended for intravenous administration, particle size analysis by the

**Coulter counter technique** is essential in addition to PCS and LD analysis. Since the Coulter counter gives the absolute number of particles per volume unit for the different size classes.

#### (C) Density

Specific gravity or density of the formulation is an important parameter. A decrease in density often indicates the presence of entrapped air within the structure of the formulation.

Density measurements at a given temperature should be made using well mixed, uniform formulation; precision hydrometer facilitate such measurements.

#### (D) pH VALUE

The pH value of aqueous formulation should be taken at a given temperature and only after settling equilibrium has been reached, to minimize "pH drift" and electrode surface coating with suspended particles. Electrolyte should not be added to the external phase of the formulation to stabilized the pH.

#### (E) Droplet size

The droplet size distribution of microemulsion vesicles can be determined by either light scattering technique or

electron microscopy. Dynamic light scattering spectrophotometer which uses a neon laser of wavelength 632 nm.

#### (F) Zeta potential

The surface charge is determined using a zeta potential of the preparation. ZP characterizes the surface charge of particles and thus it gives information about repulsive forces between particles and droplets.

To obtain stable nanoemulsion by preventing flocculation and coalescence of nano droplets. Zeta potential determines the physical stability of nanosuspension.

Zeta potential is an indirect measurement of the thickness of the diffusion layer, i.e. can be used to predict long term stability.

In order to obtain a nanosuspension exhibiting good stability, for an electrostatically stabilized nanosuspension a minimum zeta potential of  $\pm$  30mv is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of  $\pm$  20mV is desirable.

#### (G) Viscosity measurement

The viscosity of lipid based formulations of several compositions can be measured at different shear rates at different temperatures using Brookfield type rotary viscometer.

The sample room of the instrument must be maintained at 37°C by a thermo bath and the samples, for the measurement are to be immersed in it.

#### (H) Powder x-ray diffraction

Powder X-ray diffraction (PXRD) diffractograms of each of the excipients, and all of the un-milled and milled atorvastatin formulations were recorded using a Siemens Diffractometer D5000 (Siemens, Germany) with Ni-filtered Cu K $\alpha$  radiation. The 2 $\theta$  scan range was 5–60° with a step size of 0.02° and the scan speed was 3° per min. X-ray diffraction analysis in combination with differential scanning calorimetry, scanning electron microscopy is used to determine the polymorphic changes due to impact of high pressure homogenization in the crystalline structure of the drug. Nanosuspension 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.<sup>10</sup>

### APPLICATION OF NANOSUSPENSIONS

#### 1. Bioavailability enhancement

Drug with poor solubility, poor permeability or poor solubility in gastrointestinal tract will leads to poor oral bioavailability.

Nanosuspension resolves the problem of poor bioavailability by solving the problem of poor solubility, and poor permeability across the membranes.





Dissolution rate was increased in diclofenac when formulated in nanosuspension form. The dissolution rate of diclofenac 1 nanosuspension after 60 min in SGF and H<sub>2</sub>O is 25% and 10% in SIF compared to relative coarse suspension and the dissolution rate of diclofenac 2 after 60 min in SGF and H<sub>2</sub>O is 50% and 35% in SIF compared to coarse suspension.

Bioavailability of poorly soluble, a COX2 inhibitor, celecoxib was improved using a nanosuspension formulation. The crystalline nanosized celecoxib alone or in tablet showed a dramatic increase of dissolution rate and extent compared to micronized tablet. Spironolactone and budesonide are poorly soluble drugs.

The nanosuspension prepared with different surfactant concentration form shows an increase in flux across the coca-2 cell monolayer compared to saturated solution. The higher flux contributes to the higher bioavailability of nanosuspension formulation.<sup>7</sup>

The bioavailability of poorly soluble fenofibrate following oral administration was increased compared to the suspensions of micronized fenofibrate.

Significant difference ( $p < 0.05$ ) was observed between the fluxes from saturated solution vs. nanosuspension at all concentrations of surfactant.

Oral administration of micronized Amphotericin B does not show any significant effect. However administration in nanosuspension form, showed a significant reduction ( $P < 0.5\%$ ) of the liver parasite load by 28.6%, it indicates that the nanosuspension of amphotericin B has high systemic effect and superior oral uptake in nanosuspension form.

The dissolution rate (65% in 10 min) of ibuprofen made as lyophilized nanosuspension power is greater than the micronized drug (<15% in 10min). Oral administration of gonadotrophin inhibitor danazol as a nanosuspension leads to an absolute bioavailability of 82.3 and the conventional dispersion (Danocrine) only to 5.2%.

The bioavailability of poorly soluble oleanolic acid was enhanced in the nanosuspension formulation. This is due to faster dissolution rate (90% in 10 min) in the lyophilized nanosuspension power form compared to the coarse powder (15% in 10 min).

Antibiotics like atovaquone and bupraquone has poor aqueous solubility. Nanosizing of the drugs leads to increase in oral absorption and subsequent bioavailability.

## 2. Ocular administration

For delivery of poorly soluble drug in cul-de-sac suspensions and ointments are recommended. Suspensions have advantages of prolonged residual time in cul-de-sac and avoidance of higher tonicity produced by water soluble drugs.

The ocular bioavailability of suspensions depends on the dissolution rate of the drug in lachrymal fluid. However

the inflow and outflow of lacrimal fluid causes variation in the dissolution rate of the drug.

Nanosuspension attains saturation solubility in the lachrymal fluid, representing an ideal approach for the ocular delivery of the hydrophobic drugs.

The nanosized drug particles had shown a prolonged residual time in cul-de-sac, giving sustained release of drug. The sustained release of drug for specified time can be achieved by incorporating nanosuspension in hydrogel base, mucoadhesive base, or in ocular inserts.

The sustained release in the cul-de-sac can also be achieved by loading the nanosuspension in the polymers.<sup>2</sup>

Diclofenac loaded bipolymeric nanosuspension for ophthalmic application showed higher bioavailability in rabbit aqueous humor and improved shelf life. The ocular delivery of hydrocortisone nanosuspension has been shown to enhance drug absorption rate and increase the duration of drug action.

Acyclovir loaded nanoparticles have achieved the objectives of increased contact time, prolonged release and decreased frequency of administration<sup>48</sup>. Cloricromene hydrochloride showed a higher drug availability in the aqueous humor after drug administration in eudragit nanosuspension, cloricromene loaded eudragit retard nanoparticle suspension appear to offer a promising means of improving the shelf life and bioavailability of this drug after ophthalmic application.<sup>[10]</sup>

## 3. Intravenous administration

The parenteral route is an invading route. Despite all these limitations, the parenteral route still retains its value because of its special advantages, such as quick onset of action in case of emergency, reduction in dose of the drug and the ability to target the drug quickly to the desired site of action, especially in the case of severe infections. The parenteral route is often employed as a substitute when the drug is either not absorbed through the gastrointestinal tract or undergoes extensive first-pass metabolism.

*In vivo* studies in mouse model of sarcoma-180 solid tumour demonstrated significantly greater inhibition of tumour growth following a treatment with oridonin nanosuspension than oridonin solution at the same dosage. The mice injected with oridonin nanosuspension showed a highest reduction in tumour volume and tumour weight at the dose of 20mg/kg compared to the oridonin solution, with the tumour inhibition rate increased from 42.49% for oridonin solution to 60.23% for oridonin nanosuspension. Atovaquone when taken orally shows poor therapeutic activity against TE because of its poor absorption.

The bioavailability of nimodipine is low when given orally due to first pass metabolism in liver. But the nanosuspension of nimodipine given through i.v., the saturation solubility is increased.



The concentration of clofazimine nanosuspension after i.v concentration in livers, lungs and spleens reached comparably high, well in excess of the minimum inhibitory concentration for most mycobacterium avium strains. Further study indicates that the nanocrystalline clofazimine was as effective as liposomal clofazimine in reducing bacterial loads in the liver, spleen and lungs.<sup>11</sup>

IV administration of omeprazole nanosuspension is suitable in order to protect it from chemical degradation of orally administered omeprazole. The bioavailability of poorly soluble drug tarazepide is increased in the nanosuspension form than the conventional solubilization techniques such as surfactants, cyclodextrins.

#### 4. Pulmonary administration

Aqueous nanosuspension can be nebulized using mechanical or ultrasonic nebulizer for lung delivery. The nanoparticulate nature of the drug allows the rapid diffusion and dissolution of the drug at the site of action. At the same time, the increased adhesiveness of the drug to mucosal surfaces offers a prolonged residence time for the drug at the absorption site.

This ability of nanosuspensions to offer quick onset of action initially and then controlled release of the active moiety is highly beneficial and is required by most pulmonary diseases. Moreover, as nanosuspensions generally contain a very low fraction of microparticulate drug, they prevent unwanted deposition of particles in the mouth and pharynx, leading to decreased local and systemic side-effects of the drug.

The pharmacokinetic studies of fluticasone after the intra tracheal administration of nanosuspensions showed deep lung deposition and fast lung absorption, with solubility playing an important role in lung retention and duration of action.

#### 5. Targeted drug delivery

Nanosuspensions can also be used as targeted drug delivery. The targeted drug delivery can be designed by incorporating the drug into the mononuclear phagocytic system. Targeted drug delivery can be used for the anti-mycobacterial, fungal or leishmanial drugs to macrophages if the infectious pathogen is persisting intracellular.

The further plan of action for targeted drug delivery system is by using various surface coatings for active or passive targeting. Peter formulated a nanosuspension of clofazimine, permitting passive targeting to the reticuloendothelial system. Nanocrystalline drug concentration of clofazimine in liver, spleen, and lungs reached comparably high concentrations than liposomal formulation for most mycobacterium avium strains. Similarly, conditions such as pulmonary aspergillosis can easily be targeted by using suitable drug candidates, such as amphotericin B, in the form of pulmonary nanosuspensions instead of using stealth liposomes.<sup>12</sup>

#### 6. Mucoadhesion of the nanoparticles

A nanoparticle has an ability to adhere to the mucosa surface due to small particles. The adhesion of the particles is the first step before particle absorption. To further increase the adhesive time nanosuspensions are formulated with hydrogels made from mucoadhesive polymers, e.g. different types of carbopol and chitosan. The adhesiveness of the nanosuspension not only helps to improve the bioavailability but also improves targeting of the parasites persisting in the GIT. eg *cryptosporidium parvum*.

Bupravaquone mucoadhesive nanosuspensions have been reported to demonstrate an advantage in TRC alpha-deficient mice infected with *cryptosporidium parvum* oocytes.<sup>8</sup>

Table 2: Marketed Products

S.No	Product	Drug	Indication	Company
1	Rapamune	Sirolimus	Immunosuppressant	WYETH
2	Emend	Aprepitant	Antiemetic	MERCK
3	Tricor	Fenofibrate	Tretment of hypercholesterolemia	ABBOTT
4	Megace	Megestrol acetate	Appetite stimulant	PAR Pharmaceutical
5	Triglide	Fenofibrate	Tretment of hypercholesterolemia	First horizon pharmaceutical

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