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Nanosuspension for poorly water soluble drug & enhanced bioavailability- A Review

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ABSTRACT

Nanosuspensions are promising candidates that can be used for enhancing the dissolution of poorly water soluble drugs. Nanosuspension is emerging as a preferred approach to address challenges involved in the delivery of poorly soluble and high permeable (BCS Class-II) compounds. The development of nanoparticle formulations for BCS Class-II drugs would result in enhanced bioavailability, reduced systemic variability and more convenient dosing regimen. Nanosuspension consistof the pure poorly water soluble drug without any matrix material suspended in dispersion. The reduction of the drug particles into the sub-micron range leads to a significant increase in the dissolution rate and therefore enhances bioavailability. The review articles includes the methods of preparation with their advantages and disadvantages, characterization and evaluation parameters and pharmaceutical applications. A nanosuspensions not only solve the problems of poor solubility and bioavailability but also alters the pharmacokinetics of drug and thus improves the drug safety and efficacy to enhance the dissolution rate. This formulation is unproblematic, economical and applicable to all drugs which are less soluble in nature.

Keywords: Bioavailability, Solubility enhancement, Nanotechnology, Hydrophilic.

INTRODUCTION

A Pharmaceutical suspension is a coarse dispersion in which internal phase is dispersed uniformly throughout the external phase. The internal phase consisting of insoluble solid particles having a specific range of size which is maintained uniformly throughout the suspending vehicle with aid of single or combination of suspending agent. The external phase (suspending medium) is generally aqueous in some instance, may be an organic or oily liquid for non-oral use.

CLASSIFICATION OF SUSPENSION

1. Based on General Classes
   - Oral suspension
   - Externally applied suspension
   - Parenteral suspension
2. Based on Proportion of Solid Particles
   - Dilute suspension (2 to 10%w/v solid)
   - Concentrated suspension (50%w/v solid)
3. Based on Electrokinetic nature of SolidParticles
   - Flocculated Suspension
   - Deflocculated Suspension
4. Based on Size of Solid Particles
   - Colloidal Suspension (<1 micron)
   - Coarse Suspension (>1 micron)
   - Nano Suspension (10ng)

NANOSUSPENSION

Nanotechnology has emerged as an tremendous field in the medicine. The number of poorly soluble drugs increasing day by day requires innovative formulation approaches to reach a sufficiently high bioavailability after oral administration.

More than 40% of the new chemical entities being generated through drug discovery programmes are poorly water-soluble or lipophilic compunds. Formulating a poorly water soluble drug has always been a challenging problem confronted by the pharmaceutical scientist.

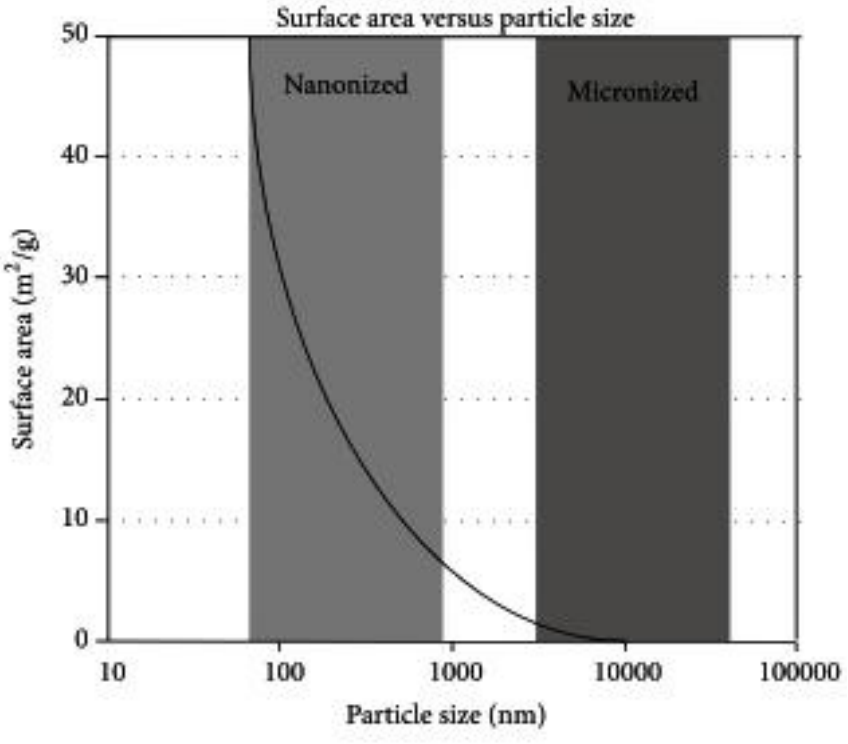
Biopharmaceutical Classification Scheme Class II (BCS class II) as classified by BCS System as they are poorly soluble in both aqueous and organic media, and for those drugs having a log P value of 2. The performance of these drugs is dissolution rate-limited (for class II and III drugs) and is affected by the fed/fasted state of the patient. 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.

Nanosuspension 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 1µm in size.

Nano is Greek word, which means “dwarf”. Nano means it is the factor of 10^{-9} or one billionth. Some comparisons of nanoscale are given below,

0.1nm = Diameter of one Hydrogen atom.
2.5nm = Width of DNA molecule.
1micron = 1000nm.

$1nm = 10^{-9} m = 10^{-7} cm = 10^{-6} mm.$
$Micron = 10^{-6} m = 10^{-4} cm = 10^{-3} mm.$



The plot demonstrates the increase in surface area obtained when solids are fractured from the micron-size range (microparticles) to the nanometer-size particles used in the various nanoparticle formulations to improve the performance of poorly water-soluble compounds.
The diagram demonstrates one of the primary issues associated with poorly water soluble molecules whose bioavailability is dissolution rate limited. On the left, large drug particles cannot adequate dissolve, which results in the inability to be absorbed. On the right, nanometer drug particles are rapidly dissolved during transit through the gut, thus maximizing absorption and improving bioavailability.

CRITERIA FOR NANOSUSPENSION

When to go for Nanosuspension Approach

Preparing nanosuspensions is preferred to 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.
In case of drugs that are insoluble in both water and in organic media instead of using lipidic systems. Nanosuspensions are used as a formulation approach.
Nanosuspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose.

Major issues associated with poorly water-soluble compounds

Poor bioavailability, Inability to optimized lead compound selection based on efficacy and safety, Fed/fasted variation in bioavailability. Lack of dose-response proportionality. Suboptimal dosing. Use of harsh excipients, i.e., excessive use of co-solvents and other excipients. Use of extreme basic or acidic conditions to enhance solubilisation.

Potential Benefits of Nanosuspension Technology for Poorly Soluble drugs

Reduced particle size, increased drug dissolution rate, increased rate and extent of absorption, increased bioavailability of drug, area under plasma versus time curve, onset time, peak drug level, reduced variability, reduced fed/fasted effects. Nanosuspensions can be used for compounds that are water insoluble but which are soluble in oil. On the other hand, Nanosuspensions can be used in contrast with lipidic systems, successfully formulate compounds that are insoluble in both water and oils. Nanoparticles can adhere to the gastrointestinal mucosa, prolonging the contact time of the drug and thereby enhancing its absorption. A pronounced advantage of Nanosuspension is that there are many administration routes for Nanosuspensions, such as oral, parenteral, pulmonary, dermal and ocular. Nanosuspension of nanoparticle (NPs) offers various advantages over conventional ocular dosage forms, including reduction in the amount of dose, maintenance of drug release over a prolonged period of time, reduction in systemic toxicity of drug, enhanced drug absorption due to longer residence time of nanoparticles on the corneal surface, higher drug concentrations in the infected tissue, suitability

for poorly water-soluble drugs and smaller particles are better tolerated by patients than larger particles, therefore nanoparticles may represent auspicious drug carries for ophthalmic applications. Nanosuspensions has low incidence of side effects by the excipients. Nanosuspensions overcome delivery issues for the compounds by obviating the need to dissolve them, and by maintaining the drug in a preferred crystalline state of size sufficiently small for pharmaceutical acceptability. Increased resistance to hydrolysis and oxidation, increased physical stability to settling. Reduced administration volumes; essential for intramuscular, subcutaneous, ophthalmic use. Finally, Nanosuspensions can provide the passive targeting.

Major Advantages of Nanosuspensions

Its general applicability to most drugs and its simplicity. Can be applied for the poorly water soluble drugs. Reduced tissue irritation in case of subcutaneous/intramuscular administration. Oral administration of nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability. The absorption from absorption window of the drugs can be increased, due to reduction in the particle size. Higher bioavailability and more consistent dosing in case of ocular administration and inhalation delivery. Drugs with log P value can be formulated as nanosuspensions to increase the bioavailability of such drugs. Improvement in biological performance due to high dissolution rate and saturation solubility of the drug. Ease of manufacture and little batch-to-batch variation. Long term physical stability (Due to absence of Ostwald ripening). Nanosuspensions can be incorporated in tablets, pellets, hydrogel and suppositories are suitable for various routes of administration. Increasing the amorphous fraction in the particles, leading to a potential change in the crystalline structure and higher solubility. Possibility of surface-modification of nanosuspension for site specific delivery.

FORMULATION CONSIDERATIONS

Stablizer

Stabilizer plays an important role in the formulations of nanosuspensions. In the absence of an appropriate stabilizer, the high surface energy of nanosized particles can induce agglomeration or aggregation of the drug crystals. The main function of a stabilizer is to wet the drug particles thoroughly, and to prevent Ostwlads ripening and agglomeration of nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behavior of nanosuspensions. In some cases, a mixture of stabilizers is required to obtain a stable nonosuspension. The drug-to-stabilizer ratio in the formulation may vary from 1:20 to 20:1 and should be investigated for a specific case. Example:Lecithine, PVPK30, PVA, SLS, Cellulose, Poloxamers, Polysorbates, Lecithine and Povidones.

Organic Solvents

Organic solvents may be required in the formulation of nanosuspensions if they are to be prepared using an emulsion or micro-emulsion as a template. As these techniques are still in their infancy, elaborate information on formulation considerations is not available. The acceptability of the organic solvents in the pharmaceutical area, their toxicity potential and the ease of their removal from the formulation need to be considered when formulating nanosuspensions using emulsions or micro-emulsions as templates. Partially water-miscible organic solvents like glycols can be used as the internal phase of the micro-emulsion when the nanosuspensions are to be produced using a micro-emulsion as a template. Example: The pharmaceutical acceptance & less suitable hazardous water miscible solvents, such as ethyl formate, butyl lactate, triacetin, propylene carbonate & benzyl alcohol are preferred in the formulation of nanosuspensions.

Surfactants

Surfactants are incorporated to improve the dispersion by reducing the interfacial tension. They also act as wetting or deflocculating agents. Example: Tweens and Spans-widely used surfactants.

Co-surfactants

The choice of co-surfactant is critical when using micro-emulsions to formulate nanosuspensions. Since co-surfactant can greatly influence phase behavior, the effect of co-surfactant on uptake of the internal phase for selected micro-emulsion composition and on drug loading should be investigated. Example:Transcutol, Glycufurol, Ethanol and Iso-propanol, Bile salts and Dipottasiumglycerrhizinate can be used as co-surfactants.

Other additives

Nanosuspensions may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant, depending on either the route of administration or the properties of the drug moiety. Examples: Buffers (acetate, phosphate), cryptotectants (sucrose as sugar), osmogent (mannitol, sorbitol).

PROPERTIES OF NANOSUSPENSIONS

Physical Long-term stability

Ostwald ripening is responsible for crystal growth and subsequently formation of microparticles. Ostwald ripening is caused by the differences in dissolution pressure/saturation solubility between small and large particles. Molecules diffuse from the higher concentrated area around small particles (higher saturation solubility) to areas around larger particles possessing a lower drug concentration. This leads to the formation of a supersaturated solution around the large particles and consequently to drug crystallization and growth of the large particles. The diffusion process of the drug from the small particles to the large particles leaves an area around the small particles that is not saturated any more,

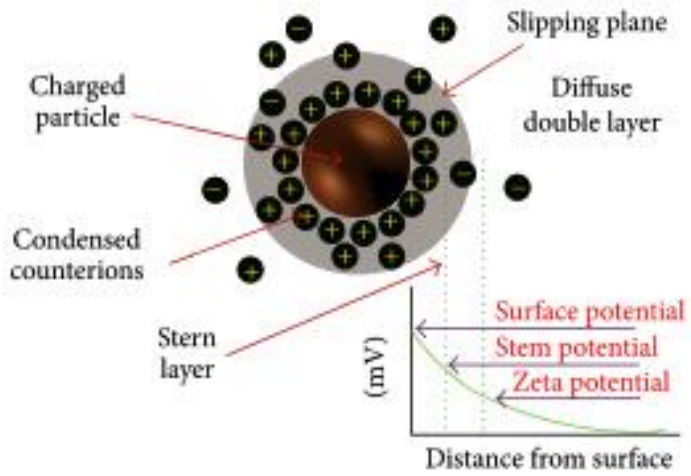
consequently leading to dissolution of the drug from the small particles and finally completes disappearance of the small particles.

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 homogenization, particles are transformed from crystalline state to amorphous state. The change in state depends upon the hardness of drug, number of homogenization cycles chemical nature of the drug and powder density applied by homogenizer.

Adhesiveness

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. A drastically remarkable report is that of the increase in bioavailability for danazol from 5% (as macrosuspension) to 82% (as nanosuspension).

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 pressure during the production of nanosuspensions was found to promote the amorphous state.

Increasing in Saturation Solubility and Dissolution Velocity of drug

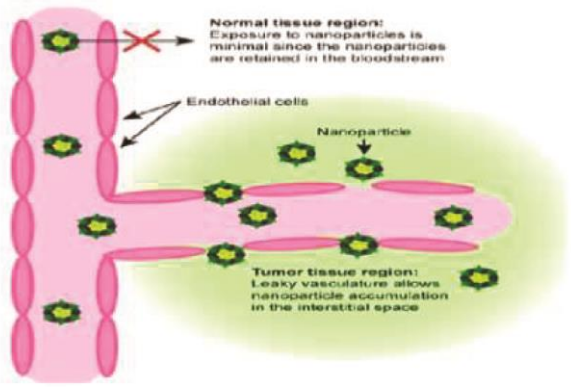
Dissolution of drug is increased due to increase in the surface area of the drug particles from micrometers to the nanometer size. According to Noyes-Whitney equation dissolution velocity increases due to increase in the surface area from micron size to particles of nanometer size.

$$dx/dt = [(D \times A)/h] [C_s - X/V]$$

Where,
D is diffusion coefficient, A is surface area of particle, dx/dt is the dissolution velocity, V is volume of dissolution medium, h-is the thickness of the diffusion layer and X is the concentration in surrounding liquid.

Nanosuspension provide Passive Targeting

Most of the drug have fail to achieve favorable outcomes because they do not have the ability to reach the site of action. A significant amount of the administrated drug is distributed over the normal tissues or organs that are not involved in the pathological process, often leading to serve side effects. An effective approach to overcome this critical issue to development of targeted drug delivery systems.



Nanosuspensions provide Versatility

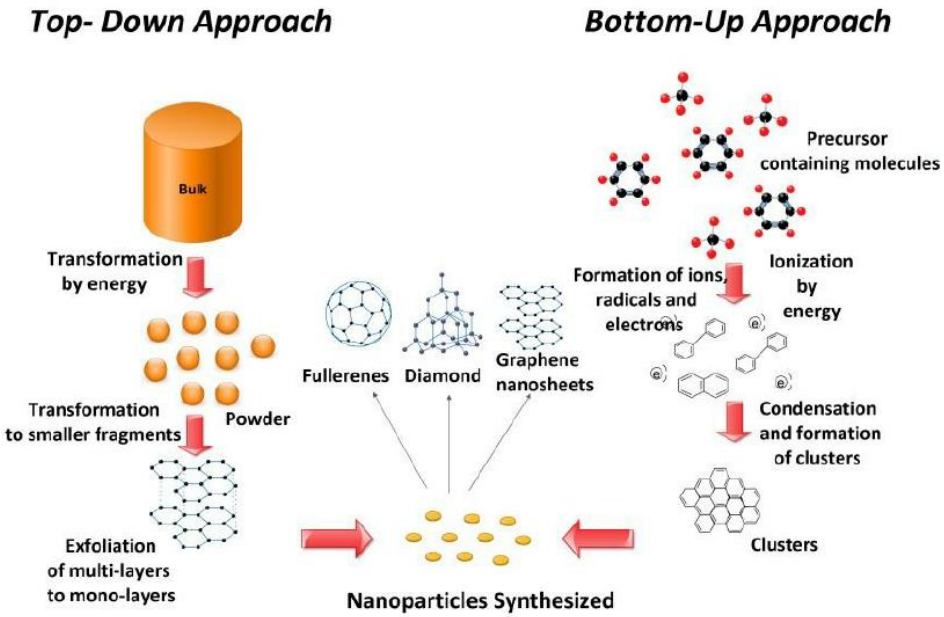
The flexibility offered in the modification of surface properties and particle size, and ease of post-production processing of nanosuspensions enables them to be incorporated in various dosage forms, such as tablets, pellets, suppositories and hydrogels, for various routes of administration, thus proving their versatility.

Nanosuspension enhance Bioavailability

Drug with poor solubility or poor permeability in gastrointestinal tract will leads to poor oral bioavailability. Nanosuspension revolves the problem of poor bioavailability by solving the problem of poor solubility, and poor permeability across the membranes.

Nanosuspension provide Long-term Physical Stability

Dispersed systems show physical instability due to Ostwald ripening which is responsible for crystal growth to form micro-particles. Ostwald ripening is caused due to the difference in dissolution velocity, saturation solubility of small and large particles. In nanosuspensions all particle are of uniform size hence there is little difference between saturation solubility of the drug particles because of that Ostwald ripening is totally absent.



METHOD OF PREPARATION

A. Bottom up technology

The conventional methods of precipitation (hydrolysis) are called Bottom up technology. Using a precipitation technique, the drug is dissolved in an organic solvent and this solution is mixed with a miscible anti-solvent. In the water-solvent mixture the solubility is low and the drug precipitates. The limitation of this precipitation technique is that the drug needs to be soluble in at least one solvent and this solvent needs to be miscible with nanosolvent.

Nanoprecipitation method

This most common method of precipitation used in anti-solvent addition method in which drug is dissolved in suitable organic solvent and this solution mixed with a miscible anti-solvent. Nanoprecipitation has been coupled with high shear processing. The nanoedge process is reliable on precipitation of material for fragmentation under condition of high shear of

thermal energy. Rapid addition of a drug solution to a solvent leads to sudden saturation of the mixed solution and generation of fine crystalline solid.

Advantage: Simple process, Ease of scale up, Low cost equipment. Disadvantage: Drug is soluble in at least one solvent and this solvent needs to be miscible of non-solvent. Drug crystal needs to be limited by surfactant addition.

B. Top Down Technology

'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 (Disso cubes), 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, micro-emulsions as templates etc.

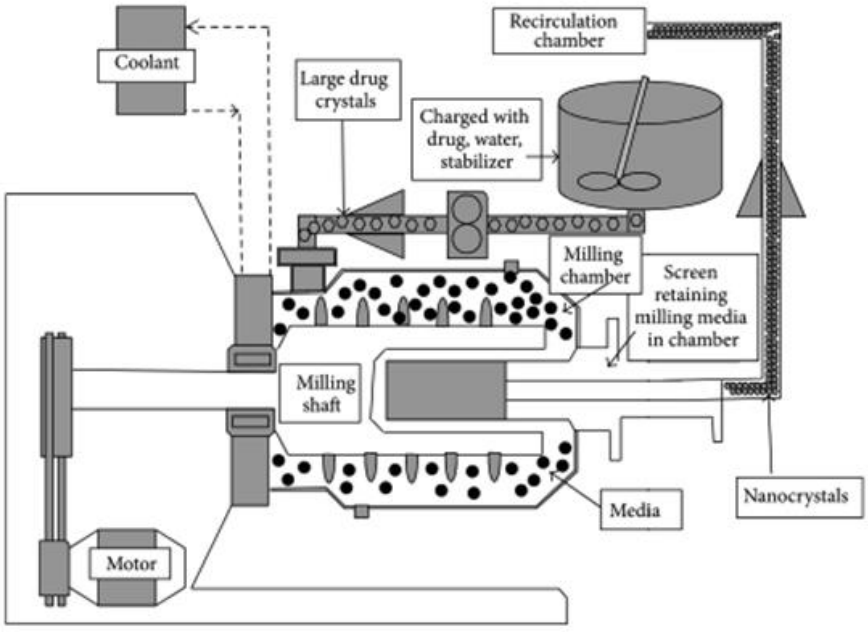
Media Milling

This patent-protected technology was developed by Liversidge et al. (1992). Formerly, the technology was owned by the company Nano systems but recently it has been acquired by an drug delivery. In this method, the nanosuspension 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 chamber charged with polymeric media is the active component of the mill. The mill can be operated in a batch or recirculation mode. Crude slurry consisting of drug, water and stabilizer is fed into the milling chamber and processed into nanocrystalline dispersion and the milling media or pearls are then rotated at a very high shear rate. The milling process is performed under controlled temperatures. The typical residence time generated for a nanometer-sized.

Principle: 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 in to nanosized particles. The milling medium is composed of glass, zirconium oxide or highly cross-linked polystyrene resin. The process can be performed in either batch or recirculation mode. In batch mode, the time required to obtain dispersions with unimodal distribution profiles and mean diameters <200nm is 30-60 mins.

Advantages: Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions. Ease of scale-up and little batch-to-batch variation. Narrow size distribution of the final nano-sized product. Flexibility in handling the drug quantity ranging from 1 to 400mg/ml, enabling formulation of very dilute as well as highly concentrated nanosuspensions.

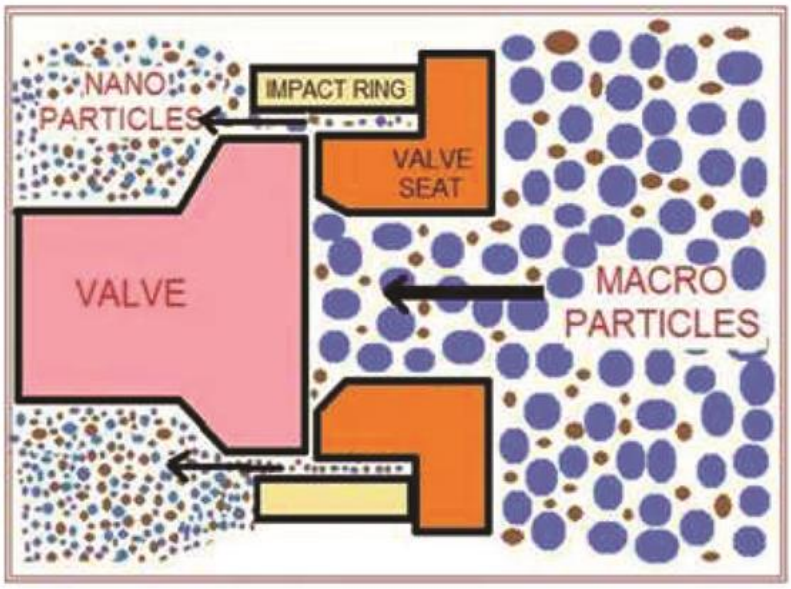
Limitations: The major concern is the generation of residues of milling media, which may be introduced in the final product as a result of erosion. This could be problematic when nanosuspensions are intended to be administered for a chronic therapy. The severity of this problem has been reduced to a great extent with the advent of polystyrene resin based milling medium. For this medium, residual monomers are typically 50ppb and the residuals generated during the milling processing are not more than 0.005% w/w of the final product or the resulting solid dosage form.



High pressure homogenization

R.H.Muller developed Dissocubes technology in 1999. The instrument can be operated at pressure varying from 1000-1500 bars (2800-21300psi) and up to 2000 bars with volume capacity of 40ml (for laboratory scale) High pressure homogenization has been used to prepare nanosuspension of many poorly water soluble drugs. 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. Different methods developed based on this principle for preparation of nano suspensions are Dissocubes, Nanopure, Nanoedge, Nanojet technology.

Principle: Based on cavitation in the aqueous phase. The particles cavitations forces are sufficiently high to convert the drug micro particles into nano particles. The concern with this method is the need for small sample particles before loading and the fact that many cycles of homogenization are required. Figure: Schematic representation of the high pressure homogenization process.
Advantages: Useful for formation of very dilute as well as highly concentratenanosuspensions aseptic production possible. Low risk of product contamination. Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions. Narrow size distribution of the nanoparticulate drug present in the final product. Allows aseptic production of nanosuspensions for parenteral administration. Flexibility in handling the drug quantity ranging from 1 to 400mg/ml, thus enabling formulation of very.
Disadvantages: Prerequisite of micronized drug particles. Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.



Homogenization in non-aqueous media

i.e. 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. The nanocrystals of the drug dispersed in liquid polyethylene glycol (PEG) or various oils can be directly filled as drug suspensions into HPMC capsules or gelatin.
Advantages: The dispersion medium need not be removed. Evaporation is faster and under milder conditions (when water and water miscible liquids are used). This is useful for temperature sensitive drugs. For i.v.injections, isotonic nanosuspensions are obtained by homogenizing in water glycerol mixtures.

Nanojet Technology

In this technique the precipitated suspensions is further homogenized to get smaller particle size and to avoid crystal growth is performed in water using water miscible solvent, as methanol, ethanol, and isopropanol. It is desired to remove the solvent completely by including evaporation step to provide a solvent free modified starting material followed by high pressure homogenization. Emulsions as templates apart from the use of emulsions as a drug delivery vehicle, they can also be used as templates to produce nanosuspensions. The use of emulsions as templates is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent.
Principle: An organic solvent or mixture of solvents loaded with the drug is dispersed in the aqueous phase containing suitable surfactants to form an emulsion. The organic phase is then evaporated under reduced pressure so that the drug particles precipitate instantaneously to form a nanosuspension stabilized by surfactants. 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.
Advantages: Use of specialized equipment is not necessary. Particle size can easily be controlled by controlling the size of the emulsion droplet. Ease of scale-up if formulation is optimized properly.
Disadvantages: Drugs that are poorly soluble in both aqueous and organic media cannot be formulated by this technique. Safety concerns because of the use of hazardous solvents in the process. Need for diultrafiltration for purification of the drug nanosuspensions, which may under the process costly. High amount of surfactant/stabilizers is required as compared to the production techniques described earlier. The production of drug nanosuspensions from emulsions templates has been successfully applied to the poorly.

Microemulsions as Templates

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 (Eccleston 1992).
Principle: The drug can be either loaded in the internal phase or pre-formed microemulsions can be saturated with the drug by intimate mixing. The suitable dilution of themicroemulsion yields the drug nanosuspension by the mechanism described earlier. If all the ingredients that are used for the production of the nanosuspension are present in

a concentration acceptable for the desired route of administration, then simple centrifugation or ultracentrifugation is sufficient to separate the nanosuspension.
Advantages: High drug solubilization, long shelf-life and ease of manufacture, make them an ideal drug delivery vehicle. The advantages and disadvantages are the same as for emulsion template. The only added advantage is the need for less energy input for the production of nanosuspensions by virtue of microemulsions.

Supercritical fluid method

The organic solvents used in the preparation of conventional methods as solvent extraction evaporation, solvent diffusion and organic phase separation methods are hazardous to environment and physiological systems. To rectify the problem occurred through the conventional method supercritical fluid technology has been investigated for the preparation of biodegradable micro and nanoparticles, because supercritical fluids are environmentally safe. The most common techniques using supercritical fluids are supercritical anti-solvents(SAS), precipitation with compressed anti-solvent process(PCS) and rapid expansion of supercritical solution(RESS). The process of SAS employs a liquid solvent, e.g. methanol, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized, at the process condition, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting in the formation of nanoparticles.
Dexamethasone 49 phosphate drug nanoparticles(for microencapsulation) and griseofulvin 50 nanoparticles were prepared by using SAS method. RESS differs from the SAS process in that its solute is dissolved in supercritical fluid and then the solution is rapidly expanded through a small nozzle into a region lower pressure, thus the solvent power of supercritical fluid dramatically decreases and solute eventually.

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. 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. Many soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrin derivatives have been used.
Physicochemical properties and dissolution of poorlywater soluble drugs were improved by co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug precipitates.

POST-PRODUCTION PROCESSION

Post production procession of nanosuspensions becomes essential when the drug is highly susceptible to hydrolytic cleavage or chemical degradation. Procession may also be required when the best possible stabilizer is not able to stabilize the nanosuspension for a longer period of time or there are acceptability restrictions with respect to the desired route. Considering these aspects, techniques such as lyophilization or spray drying may be employed to produce a dry powder of nano-sized drug particles. Rational selection has to be made in these unit operations considering the drug properties and economic aspects. Generally, spray drying is more economical and convenient than lyophilization. The effect of post-production processing on the particle size of the nanosuspension and moisture content of dried nanosized drug should be given due consideration.

Solidification Techniques

In this case, solid dosage forms are considered more attractive, due to their patient convenience (marketing aspects) and good stability. Therefore, transformation of nanosuspensions into the solid dosage form is desirable. Solidification methods of the nanosuspensions include some unit-operations such as pellatization, granulation, spray drying or lyophilization. As the primary objective of the nanoparticulate system is rapid dissolution, disintegration of the solid form and redispersionof the individual nanoparticles should be rather rapid, so that it does not impose a barrier on the integrated dissolution process. Drying of nanoparticles can create stress on the particles that can cause aggregation. For example, drying may lead to crystallization of the polymers such as polyoxamers, thereby compromising their ability to prevent aggregation. Drying can also create additional thermal stresses that may destabilize the particles. Due to the above considerations, adding matrix-formers to the suspension prior to solidification is necessary. Microcrystalline cellulose has been successfully used to displace sucrose as a matrix former during freeze-drying of itraconazolenanosuspensions in addition, the effect of surface hydrophobicity.

Surface Modification Techniques

Nanosuspensions have the particular characteristics to increase the saturation solubility and dissolution rate for the poorly soluble drugs. But in some cases, the rapid or burst release of nanosuspensions may result in the side effect and toxicity. As a colloid nanoparticle system, nanosuspensions usually can target the Monocyte Phagocytic System (MPS), which can aid in the treatment of Lymphatic-mediated diseases, like *Mycobacterium tuberculosis,Listeria monogyna, Leishmania sp.* The action is called as ‘passive targeting’. However, the passive targeting process could pose an obstacle when either macrophages are not the desired targets or accumulated drug is toxic to MPS cells. Hence, in order to bypass the phagocytic uptake of the drug, its surface properties need to be tuned, just like stealth liposomes and nanoparticles. Faced with the above problems, the surface modification of nanosuspensions will be very necessary. In the case of burst release and passive targeting, the controlled release and long residence at site of action may be effective.

For example, *Tanet al*. had prepared layer-by-layer selfassembly coated procaine hydrochloride.

EVALUATION OF NANOSUSPENSION

In vitro Evaluations

1. Mean particle size and size distribution

The mean particle size and the width of particle size distribution called polydispersity index are determined by Photon Correlation Spectroscopy (PCS). Particle size and polydispersity index (PI) governs the saturation solubility, dissolution velocity and biological performance. It is proved that change in particle size changes saturation solubility and dissolution velocity. PCS measures the particle size in the range of 3nm-3µm only. PI governs the physical stability of nanosuspension and should be as low as possible for long-term stability. PCS is a versatile technique but has low measuring range. In addition to PCS analysis nanosuspensions are analyzed by Laser Diffractometry(LD). LD measures volume size distribution and measures particles ranging from 0.05-80µm up to 2000µm. Atomic Force microscope is used for visualization of particle shape.

2. Particle Charge (Zeta potential)

Particle charge determines the stability of nanosuspensions. For electrostatically stabilized nanosuspension a minimum zeta potential of ± 30mv and for combined steric and electrostatic stabilization it should be a minimum of ± 20mv.

3. Crystalline state and particle morphology

Differential Scanning Colorimeter (DCS) determines the crystalline structure. When nanosuspensions are prepared drug particles get converted to amorphous form hence it is essential to measure extent of amorphous drug generated during the production of nanosuspensions. The X-ray Diffraction (XRD) is also used for determining change in physical state and extent of amorphous drug. It is important a know the crystal morphology of the drug in the nanosuspensions. Polymorphic or morphological changes in drug that occur during nano-sizing can be determined by X-ray diffraction analysis. It gives information about the changes in the physical state of the drug particles as well as the extent of the amorphous fraction. Differential scanning calorimetry can be used additionally. Scanning electron microscopy is also used to get exact information about particle morphology. Effect of high pressure homogenization on the crystalline structure of the drug is estimated by X-ray diffraction analysis.

4.Saturation solubility and dissolution velocity

The nanosuspension increase the saturation solubility as well as dissolution velocity. Saturation solubility is compound specific constant depending upon temperature and the properties of dissolution medium. Kelvin equation and the Ostwsald-Freundlich equations can explain increase in saturation solubility.

5.Stability

Nanosuspension stability depends on the particle size of the suspended particles. Decrease in the particle size to the nano range increase the surface energy of the particles, and the tendency of the particles to agglomerate increases. Therefore the stabilizers are used to decrease the chance of Ostwald ripening and to improve the stability of the suspension by providing a steric or ionic barrier. Stabilizers like cellulosic, poloxamers, polysarbates, lecithin, polyoleate and povidones are generally used in the nanosuspensions. Nanosuspensions can be stored at different stress-conditions like different temperature (15, 25, 35, 45°c), thermal cycling and mechanical shaking and change in their mean particle size can be followed for three months. Different constructions of small molecule surfactants (like hydroxyl propyl methyl cellulose) HPMC can be evaluated to determine the effect of stabilizer type and solubilized drug on Ostwald ripening.

6.pH

Prepared nanosuspensions was taken in 10ml beaker and pH was measured using pH meter.

7.Osmolarity

Osmolarity of nanosupension can be measured by using Osmometer.

8.Drug content

Drug content of nanosuspension formulation was carried out by taking lyophilized powder (weigh equivalent to 5mg of drug) in Methanol:THF (1:1) mixture shaken well, mannitol is slightly soluble in methanol:THF (1:1) mixture so it was then centrifuged at 8000rpm for 10min. The supernatants were taken and diluted with methanol:THF (1:1) mixture and the absorbance was measured at 210nm. The drug content was calculated using the calibration curve.

Total volume of nanosuspension = Total volume of nanosuspension × Amount of drug in a liquid/volume of liquid

In Vivo Evaluation

Particular drug and route of administration requires the specific in vivo evaluation of the nanosuspensions. Generally the formulations are administered by required route and the plasma drug concentrations are determined by HPLC-UV visible spectrophotometry.

Surface hydrophilicity/hydrophobicity (which determines interaction with cells prior to phagocytosis), adhesion properties and the interaction with body proteins are generally evaluated by in vivo parameters. The monitoring of the in vivo performance of the nanosuspensions and the establishment of relationship between in vitro release and in-vivo absorption are required in order to prepare a successful preparation irrespective of the administration and the delivery systems. Rate of dissolution influences the in-vivo biological performance of oral nanosuspensions. Size of nanoparticle

and surface properties of the particles determine the organ distribution for intravenously injected nanosuspensions.

Evaluation of the surface modified of particles

Surface Hydrophilicity

For intravenously injected nanosuspensions, additional parameters need to be determined which affect the in vivo fate of the drug nanoparticles. Surface hydrophilicity / hydrophobicity is considered as one of the important parameters affecting the in vivo organ distribution after i.v. injection. The surface hydrophobicity determines the interaction with cells prior to phagocytosis and in addition, it is a relevant parameter for the adsorption of plasma proteins the key factor for organ distribution. To avoid artifacts, the surface hydrophobicity needs to be determined in the original environment of the drug nanoparticles, which means is aqueous dispersion medium. A suitable technique is hydrophobic interaction chromatography (HIC), previously employed to determine the surface hydrophobicity of bacteria, and then transferred to the characterization of nanoparticulate drug carriers.

Adhesion Properties

In vivo bio-adhesive study is performed where Male Wister rats can be used. In general, each animalreceives a single oral dose of 1ml aqueous suspension containing 10mg of the nanoparticles loaded with the drug (approximately 45mg particles/kg body weight). The animal is sacrificed by cervical dislocation 1 and 3 post administration. The abdominal cavity is opened and the stomach, small intestine and cecum is removed, opened lengthwise along the mesentery and rinsed with phosphate saline buffer (pH 7.4). Further, the stomach, small intestine and cecum is cut into segments of 2cm length and digested in suitable alkali for 24 hrs. Drug extracted from the digested samples by addition of 2ml methanol for 1min centrifuged. A liquid (1ml) of the supernatant into be assayed for the drug by spectrofluorimeter estimate the fraction of adhered nanoparticles to the mucosa. For calculations, standard curve of the drug can also be prepared.

Interaction with body proteins

In vivo interaction between nanoparticles can be studied by incubation of nanoparticles (1:4 weight ratio) either in acidic or in neutral medium. The incubation is carried out under stirring at temperature of 37°C. The dispersions is then be centrifuged and 150µl of each supernatant is placed in a test plate. Micro BCA Protein Assay Reagent Kit (150µl) then added to the supernatants and the plate is incubated for 2hr at 37°C. According to this procedure, the absorbance measured by colorimetry at ʎ-max of the drug. The amount of the drug adsorbed to the nanoparticles can be determined as a difference between its initial concentration and the concentration found in the dispersion after incubation and centrifugation. The calculations can be made on the basis of drug standard curves.

PHARMACEUTICAL APPLICATIONS

1. Oral drug delivery

The oral route is the preferred route of drug delivery because of its numerous well known advantages. The efficacy of performance of the orally administered drug generally depends on its solubility and absorption through the GIT. Nano-sizing of such drugs can lead to dramatic increase in their oral absorption and subsequently bioavailability and increased saturation solubility, leading to an increased concentration gradient between the GIT lumen and blood, and increased dissolution velocity. This enhancement in bioavailability will lead to a subsequent reduction in drug dose, rendering the therapy cost-effective and obliterating any undue drug dumping in the body.

Nanosuspensions are also advantages in achieving quick onset of action for drugs that are completely but slowly absorbed, i.e. those high tmax values. Apart from improving oral absorption, nanosuspensions offer the following advantages.

- Improved dose proportionality
- Reduced inter-subject variability
- Reduced fed/fasted variability

2. Parenteral Administration

Nanosuspensions can be administered via different parenteral administration routes ranging from intra-articular via intraperitonal to intravenous injections. For administration by the parenteral route, the drug either has to be solubilized or has particle/globule size below 5µm to avoid capillary blockage. The current approaches for parenteral delivery include salt formation, solubilization using co-solvent, micellar solutions, complexation with cyclodextrin and recently liposomes. However, there are limitations on the use of these approaches because of the limitations on their solubilization capacity and parenteral acceptability. Nanosuspensions would be able to solve the problems mentioned above. In addition, nanosuspensions have been found to increase the efficacy of parenterally administered drugs. Paclitaxel nanosuspensions revealed their superiority over taxol in reducing the median tumor burden. Similarly, aphidicolin a poor water soluble new anti-parasitic lead molecule, in an improvement in EC50 in comparison to DMSO dissolved drug.

3. Ophthalmic drug delivery

Nanosuspensions could prove to be vital for drugs that exhibit poor solubility in lachrymal fluids. Suspensions offer advantages such as prolonged residence time in a cul-de-sac, which is desirable for most ocular diseases for effective treatment and avoidance of high tonicity created by water soluble drugs. Their actual performance depends on the intrinsic solubility of the drug in lachrymal fluids as it governs its release and ocular bioavailability. However, the intrinsic dissolution rate of the drug will vary because of the constant inflow and outflow of lachrymal fluids.

Nanosuspensions of glucocorticoid drugs, hydrocortisone, prednisolone and dexamethasone enhance rate, drug absorption and increase the duration of drug action.

Nanosupensions provide the following benefits for ocular drug delivery.

- Prolonged residence time of drug in the cul-de-sac (desired for most ocular diseases for effective treatment).
- Avoidance of high tonicity created by water soluble drugs.
- Sustained release of the drug can be obtained by incorporation of nanosuspension in a suitable hydrogel base or mucoadhesive base.

4. Pulmonary drug delivery

Aqueous nanosuspensions can be nebulized using mechanical or ultrasonic nebulizers for lung delivery. Basically the nanosuspensions can be used in all nebulizers. The dispersions can be relatively high concentrated. Due to the presence of many small particles instead of a few large microparticles, all aerosol droplets are likely to contain drug nanoparticle. Budesonide, a poorly water soluble corticosteroid, has been successfully prepared as a nanosuspension for pulmonary delivery. Nanosuspensions provide following advantages over the conventional pulmonary formulations.

- Rapid diffusion and dissolution of the drug at the site of action (which increases the bioavailability of the drug).
- Increased adhesiveness of the drug to mucosal surfaces.
- Prolonged residence time of the drugs at absorption site which prolongs the effect of the drug.
- Initial quick onset of action and then controlled release of the active moiety (which is required by most pulmonary diseases).
- Decreased local and systemic side-effects of the drug due to prevention of unwanted deposition of particles in the mouth and pharynx.
- Even distribution of the drug in the lungs as compared to the microparticulate form of the drug as all droplets of aerosols contains drug nanoparticles.

5. Target drug delivery

Nanosuspensions can also be used for targeted drug delivery as their surface properties and in vivo behavior can easily be altered by changing the stabilizer. Their versatility, ease of scale up and commercial product enable the development of commercial viable nanosuspensions of targeted delivery. The engineering of stealth nanosuspensions by using various surface coatings for active or passive targeting of the desired site is the future of targeted drug delivery systems. Targeting of cryptosporidium parvum, the organism responsible for cryptosporidiosis was achieved by using surface modified mucoadhesivenanosuspensions.

6. Topical Formulations

Drug nanoparticles can be incorporated into creams and water-free ointments. The nanocrystalline form leads to an increased saturation solubility of the topical dosage form, thus enhancing the diffusion of the drug into the skin.

7. Mucoadhesion of the Nanoparticles

When nanoparticles are administered orally in the form of a suspension, they diffuse into the liquid media and rapidly encounter the mucosal surface. They adhere to the intestinal surface (bioadhesion) and get immobilized. After adhesion, the concentrated suspensions an act as a reservoir of particles and enables the rapid adsorption. The first step before particle absorption is the direct contact of the particles with the nanosupensions improves bioavailability as well as the targeting of the parasites persisting in the GIT.

CONCLUSION

Nanosuspensions appear to be a unique and yet commercially viable approach to combating such as poor bioavailability that are associated with the delivery of hydrophobic drugs, including those that are poorly soluble in aqueous as well as organic media. Production techniques such as media milling and high pressure homogenization have been successfully for large-scale production of nanosuspensions. The advances in production methodologies using emulsions or microemulsions as templates have provided still simpler approaches for production but with limitations. Further investigation in this regard is still essential. Attractive features, such as increased dissolution velocity, increased saturation solubility, improved bioavailability, improved bio-adhesivity, versatility in surface modification and ease of post-production processing, have winded the applications of nanosuspensions for various routes. The applications of nanosuspensions in parenteral and oral routes have been very well investigated and applications in pulmonary and ocular delivery have been realized. However, their applications in buccal, nasal and topical delivery are still awaiting exploration. Poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientists working on oral delivery off drug compounds and leads to employment of novel formulation technologies. The use of drug nanocrystals is a formulation approach to increase the therapeutic performance of these drugs in any route of administration.

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