Review Article

Various Techniques for Preparation of Nanosuspension- A Review

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ABSTRACT

Many of the newly developed drugs are poorly soluble and they create major problems during formulation and shows poor bioavailability. The problem is even more complex for drugs which belong to BCS Class II category. To overcome these problems nanotechnology is used to improve the solubility as well as bioavailability of poorly soluble drugs. Nanotechnology is defined as the science and engineering carried out in the nanoscale that is 10^{-9} meters. Nanosuspensions are a part of Nanotechnology. Nanosuspensions are defined as the submicron colloidal dispersions of pharmaceutical active ingredient particles in a liquid phase, size below 1µm, without any matrix material which are stabilized by surfactants and polymers. Nanosuspensions differ from nanoparticles and solid lipid nanoparticles with respect to the fact that nanoparticles are polymeric colloidal carriers of drug while solid lipid nanoparticles are lipid carrier of drugs. Preparation of nanosuspension is simple and applicable to all drugs which are water insoluble. Nanosuspensions are prepared by using wet mill, high pressure homogenizer, emulsion solvent evaporation, melt emulsification and supercritical fluid techniques. Nanosuspensions can be delivered by oral, parenteral, pulmonary and ocular routes. Nanosuspensions can also be used for targeted drug delivery when incorporated in the ocular inserts and mucoadhesive hydrogels. This review article mainly focuses on preparation of nanosuspensions by various techniques with their advantages and disadvantages, formulation considerations, Characterization and their applications in drug delivery. Nanosupensions not only solves the problem of poor solubility and bioavailability but also alter the pharmacokinetics of the drug and thus improving safety and efficacy.

Keywords: Bioavailability, BCS Class II, solubility, nanotechnology, nanosuspensions

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INTRODUCTION

Many of the new chemical entities (approximately 40% or more) being developed through drug discovery programmers are poorly water soluble [1]. The formulation of poorly water soluble drugs has been always a challenging problem faced by pharmaceutical scientists [2]. The low saturated solubility and dissolution velocity leads to poor bioavailability. The problem is more severe for drugs belonging to BCS class II, such as Itraconazole and carbamazepine as they are poorly soluble in both aqueous and organic media [3]. The performance of these drugs is dissolution-rate-limited and is affected by fed/fasted state of the patient. Dissolution rates of sparingly soluble drugs are associated to the shape as well as the particle size. Hence decrease in particle size results in increase in dissolution rate.

There are number of formulation approaches that can be used to solve the problems associated with the low solubility and low bioavailability of class II drugs. Some of the approaches to increase solubility include micronization [4], solubilisation using cosolvents [5], use of permeation enhancers, surfactant dispersions [6], salt formation [7] and precipitation techniques [8,9]. Most of these techniques for solubility enhancement have advantages

as well as some limitations and hence have limited utility in solubility enhancement.

Other techniques used for solubility enhan cement like microspheres, emulsions, micro emulsions[10], Liposome's [11], supercriti cal processing, solid dispersions [12] and inclusion complexes using Cyclodextrins [13] show reasonable success but they lack in universal applicability to all drugs, which are not soluble in both aqueous and organic media. Nanosuspensions have revealed their potential to undertake the problems associated with delivery of poorly water-soluble and lipid soluble drugs and are unique because of their simplicity and the advantages they confer over other strategies.

NANOSUSPENSIONS

A pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for either oral and topical use or parenteral and pulmonary administration.

Nanosuspension is a sub-micron colloidal dispersion of drug particles which are stabilized by surfactants, polymers or a mixture of both. They can also define as the biphasic system consisting of pure drug particle dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1um in size. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 and 600 nm. Nanosuspensions differ from nanoparticles and solid lipid nanoparticles. Nanoparticles are commonly polymeric colloidal carriers of drugs whereas solid lipid nanoparticles are lipid carriers of drugs. In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particle size < 10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Nanosized particles can increase solution velocity and saturation solubility because of the vapor pressure effect. In addition, the diffusional distance on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. The increases in surface area and concentration gradient lead to a much more pronounced increase in the dissolution velocity as compared to a micronized product. Nano is a Greek word, which means 'dwarf'. Nano means it is the factor of 10^{-9}or one billionth. Some comparisons of nanoscale are given below,

0.1 nm = Diameter of one Hydrogen atom.

2.5 nm = Width of a DNA molecule

1 micron = 1000 nm.

1 nm = 10^{-9}m= 10^{-7} cm = 10^{-6} mm.

Micron = 10^{-6}m= 10^{-4} cm = 10^{-3}mm.

TECHNIQUES FOR PREPARATION OF NANOSUSPENSIONS

Technically preparations of nanosuspensions are simpler alternative than liposome's and other conventional colloidal drug carriers but reported to be more cost effective. it is particularly for poorly soluble drugs and to yield a physically more stable product. For manufacturing nanosuspensions there are two converse methods, "Top-down process technology" and "Bottom-up process technology".

The top - down process follows disintegration approa ch from large particles, microparticles to Nanosized particles [14].

Examples are

- High pressure homogenization
- Nanoedge
- Nanopure
- Media milling (Nanocrystals).

Bottom-up process is an assembly method forms nanoparticles from molecules [15]. Examples includes

- Solvent-Antisolvent method
- Super critical fluid process
- Emulsification Solvent evaporation techni - que
- Lipid emulsion/Micro-emulsion template.

The principle techniques used in recent years for preparing nanosuspensions are:

A.HIGH PRESSURE HOMOGENIZATION:

It is most widely used method for preparing nanosuspensions of many poorly aqueous soluble drugs [16]. It involves three steps. First drug powders are dispersed in stabilizer solution to form presuspension, and then the presuspension

is homogenized in high pressure homogenizer at a low pressure for premilling, and finally homogenized at high pressure for 10 to 25 cycles until the nanosuspensions of desired size are formed. Different methods are developed based on this principle for preparations of nanosuspensions are Disso cubes, Nanopure, Nanoedge and Nanojet [17].

Homogenization in aqueous media (Disso cubes):

This technology was developed by R.H.Muller using a piston-gap type high pressure homogenizer in 1999 [18]. In this method, the suspension containing a drug and surfactant is forced under pressure through a Nanosized aperture valve of a high pressure homogenizer.

Principle:

This method is based on cavitation principle. The dispersion present in 3cm diameter cylinder is suddenly passed through a very narrow gap of 25µm.According to Bernoulli's law the flow volume of liquid in a closed system per cross section is constant. It leads to increase in dynamic pressure and decrease of static pressure below the boiling point of water at room temperature due to reduction in diameter from 3cm to 25µm.Then water starts boiling at room temperature and forms gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached. The particles cavitation forces are sufficiently high to convert the drug micro particles into nanoparticles.

Advantages

1. It does not cause the erosion of processed materials.
2. It is applicable to the drugs that are poorly soluble in both aqueous and organic media.

Disadvantages

1. Pre-processing like micronization of drug is required.
2. High cost instruments are required that increases the cost of dosage form.

Homogenization in nonaqueous media (Nanopure):

Nanopure is suspensions homogenized in water-free media or water mixtures like PEG 400, PEG 1000 etc. The homogenization can be done at room temperature, 0^0C and below freezing point (-20^0C), hence it is known as "deep freeze" homogenization [19].

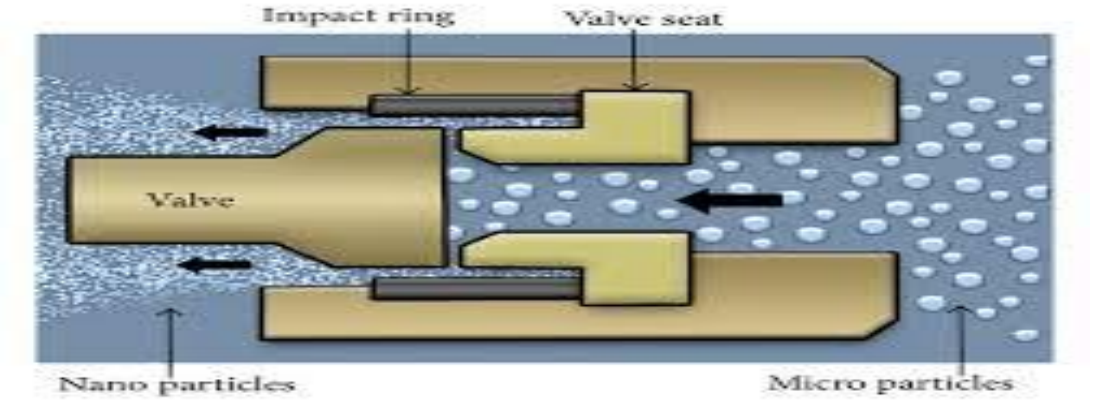

Figure 1: Schematic Cartoon of the High-Pressure Homogenization Process

Nanoedge:

Nanoedge technology is the combination of both precipitation and homogenization. The basic principle is same as that of precipitation and homogenization [20]. The major disadvantage of precipitation technique such as crystal growth and long term stability can be overcome by using the Nanoedge technology. Particles of smaller size and better stability in short time can be achieved.

Nanojet:

It is also called as opposite stream technology, uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure, due to the high shear forces

produced during the process particle size is reduced [21].

B.MILLING TECHNIQUES

i) Media Milling:

This method was first developed and reported by Liversidge (1992) [22]. The nanosuspensions by this method are prepared by high shear media mill. The milling chamber was charged with the milling media, water, drug and stabilizer and rotated at a very high shear rate under controlled temperature at least 2-7 days [23]. The milling medium is composed of glass, Zirconium oxide or highly cross linked polystyrene resin. The high energy shear forces are formed as a result of impaction of milling media with the drug which results in breaking of drug micro particles to nanosized particles.

Advantages

1. Very dilute as well as highly concentrated nanosuspensions can be prepared by handling 1 mg/ml to 400 mg/ml drug quantity.
2. Nanosized distribution of final nanosized product.

Disadvantages

1. The media milling technique is time consuming.
2. Some fractions of particles are in the micrometer range.
3. Scale up is not easy due to mill size and weight.

ii) Dry-Co-grinding:

Recently many nanosuspensions are prepared by dry milling technique. Dry- co-grinding can be carried out easily and economically and can be conducted without organic solvents. Physicochemical properties and dissolution of poorly water soluble drugs are improved by Co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug.

Advantages

1. Easy process and no organic solvent required.
2. Require short grinding time.

Disadvantages

Generation of residue of milling media.

C. EMULSIFICATION-SOLVENT EVAPORATION TECHNIQUE

This technique involves preparing a solution of drug followed by its emulsification in \another liquid that is a nonsolvent 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.

D. PRECIPITATION

Within the last decade, precipitation has been applied to prepare submicron particles, especially for the poorly soluble drugs [24]. The drug is first dissolved in a solvent, then this solution is mixed with a miscible antisolvent in the presence of surfactants. Rapid addition of a drug solution to the antisolvent leads to sudden super saturation of drug and formation of ultrafine crystalline or amorphous drug solids [25].

Advantages

Simple process, Ease of scale up and Economical production.

Disadvantages

Growing of crystals needs to be limit by surfactant addition. Drug must be soluble at least in one solvent.

E. SUPERCRITICAL FLUID PROCESS

The particle size reduction was achieved more by the solubilization and nanosizing technologies through the super critical fluid process. Super critical fluids (SCF) are noncondensable dense fluids whose temperature and pressure are greater than its critical temperature (T_c) and critical pressure (T_p).This process allows the micronization of drug particles to submicron level. Recent advances in SCF process are to create nanoparticulate suspension of particle size of 5 to 2000nm in diameter [26]. The low solubility of poorly water-soluble drugs and surfactants in supercritical CO_2 and the high pressure required for these processes restrict the utility of this technology in the pharmaceutical industry.

F. 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 evaporation method is total avoidance of organic solvents during the production process.

Disadvantages;

Formation of larger particles and few compliant objects than solvent evaporation.

G.LIPID EMULSION/MICROEMULSION TEMPLATE:

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

Advantages

-High drug solubilization

-Long shelf life

-easy to manufacture

Disadvantages

-Use of hazardous solvent

-Use of high amount of surfactant and stabilizers

H. SOLVENT EVAPORATION:

In the solvent evaporation method, the solutions of polymer are prepared in volatile solvents and emulsions. But from the past years dichloromethane and chloroform were used which was now replaced by ethyl acetate which has a better profile of toxicology. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. In the conventional methods, two main strategies are being used for the formation of emulsions, the preparation of single-emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These methods require high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. By ultracentrifugation the solidified nanoparticles are collected which was washed with distilled water to remove the additives like surfactants, and then it was lyophilized. The particle size was influenced by the concentration of polymer, stabilizer and the speed of homogenizer.

FORMULATION OF NANOSUSPENSIONS

Nanosuspension formulation requires basically stabilizer or surfactant, proper solvent system and others ingredients for its preparation.

a) Stabilizers:

Stabilizer is used to wet the surface of solute or drug particle and retard the Ostwald ripening and agglomeration in order to provide high physical stability which further reflects to its performance. Commonly used stabilizers are polysorbate (Tween/Span series), povidone, cellulosics, poloxomers and lecithin.

b) Organic solvent:

Organic solvents are generally used in preparation of nanosuspension if emulsion or microemulsions technologies are used as template for this. These solvents are very hazardous in physiologic and environmental means but still some less hazardous water miscible solvents like methanol, ethanol,

chloroform, isopropanol, and partially water miscible solvents ethyl acetate, ethyl formate, butyl lactate, triacetine, propylene carbonate, benzyl alcohol are used over the dichloromethane (reported as a conventional hazardous solvent).

c) Other additives:

Uses of other ingredients depends on either the route of administration or physicochemical properties of candidate drug but some additives such as buffers, salts, polyols, osmogent and cryoprotectant are normally used.

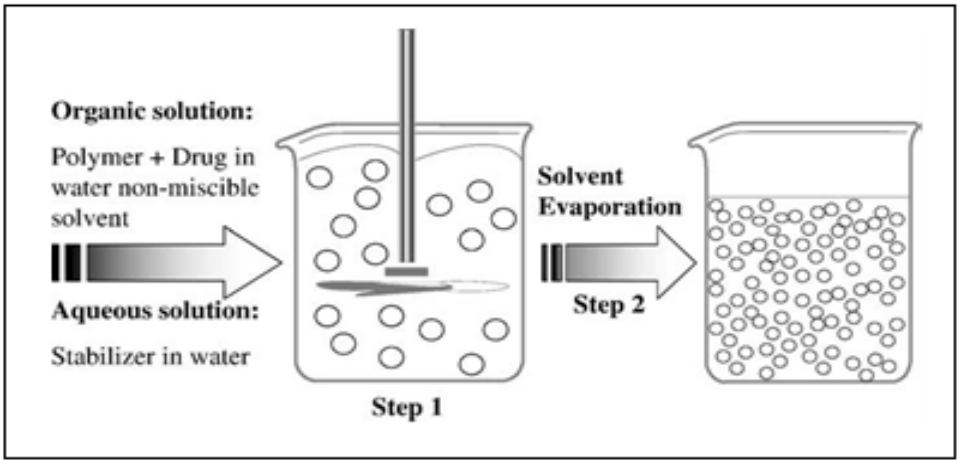

Figure 2: Schematic representation of the solvent-evaporation technique

CHARACTERIZATION OF NANOSUSPENSIONS:

Nanosuspensions are characterized for app earance, colour, odor, assay, related impurit ies, particle size, zeta potential, crystalline morphology status, dissolution studies and in vivo studies. Among these the essential characterization techniques were discussed.

1. Mean particle size and particle size distribution:

The mean particle size and particle size distribution affect the saturation solubility, dissolution rate, physical stability, even in-vivo behavior of nanosuspensions. The particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) and Coulter counter multisizer [27]. PCS can also be used for identifying the width of particle size distribution (polydisperity index, PI).A PI value of 0.1-0.25 indicates a fairly narrow size distribution, if PI value greater than 0.5 indicates a very broad distribution [28]. The coulter-counter gives the absolute no of particles per volume unit for the different size classes and it is more efficient and appropriate technique than LD for quantifying the contamination of nanosuspensions by micro particulate drugs.

2. Surface charge (Zeta potential):

Zeta potential gives information about the surface charge properties and the long-term physical stability of the nanosuspensions. For a stable suspension stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is essential, where as in case of a combined electrostatic and steric stabilizer, a zeta potential of ±20 mV would be sufficient.

3. Crystalline state and particle morphology:

The evaluation of the crystalline state and particle morphology helps in understanding the polymorphic or morphological changes that a drug may undergo when subjects to nanosizing. Because of High pressure homogenization nanosuspensions can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms. The changes in the solid state of the drug particles and extent of the amorphous fraction can be determined by X-ray diffraction analysis and supplemented by DSC. To get an actual idea of particle morphology, scanning electron microscopy is preferred.

4. Saturation solubility and Dissolution velocity:

The saturation solubility of the drug in different physiological buffers as well as at different temperatures should be assessed using methods described in the literature. The investigation of the dissolution velocity of nanosuspensions reflect the advantages that can be achieved over conventional formulations, especially when designing the sustained release dosage forms based on nanoparticulate drugs. The evaluation of saturation solubility and dissolution velocity helps in determining the invitro behavior of the formulation.

APPLICATION OF NANOSUSPENSIONS:

Nanosuspensions have various pharmaceutical and biopharmaceutical application a few of them highlighted here are:

1. Formulating the drug as nanosuspensions increases the saturable concentration, dissolution rate as well as bioavailability of the drug.
2. Nanosuspensions can prove to be a boon f or drugs that exhibit poor solubility in lachrymal fluids. For delivery of such drugs, approaches such as suspensions and ointments have been recommended.
3. These nanosuspensions are having application in different routes of administrations like oral, parenteral, topical, ophthalmic, mucoadhesive, pulmonary and targeted drug delivery.

CONCLUSION

The main goal of this review was to describe the various preparation techniques for production of nanosuspensions. it was observed that preparing nanosuspensions is a state -of-art technology that requires a suitable technique among the various possible methods. Production techniques such as media milling and high-pressure homogenization have been successfully employed for large scale production. The advances in production methodologies using emulsions or microemulsions as templates have provided still simpler approaches for production but with some limitations.

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