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NANOSUSPENSION: FORMULATION, CHARCTERIZATION AND EVALUATION

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ABSTRACT

Disperse system of solid-in-liquid or solid-in-semisolid, the dispersed phase comprising pure active compound or an active compound mixture. The average diameter of the dispersed phase is between 10 nm and 1,000 nm (determined by photon correlation spectroscopy), the distribution of the population being quite narrow, that is to say the proportion of microparticles in the particle population is very low. The nanosuspension can be surfactant-free, but can also comprise surfactants or stabilizers or both. The nanosuspension can also be lyophilized or spray dried, and the nanoparticles of a nanosuspension can also be incorporated into a solid carrier matrix. They form a depot where injected and slowely release the drug to the systemic circulation control manner. By using various polymers it can work as sustained release of numbers of drugs.

KEYWORDS

Micro particles, lyophilized, nanosuspension, sustained release Nanosuspension, solid-in-semisolid, microparticles, sustained release, Disperse system

INTRODUCTION

A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants. 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. 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 [1,2].Nanosuspensions differ from nanoparticles. Nanoparticles are commonly polymeric colloidal carriers of drugs whereas solid

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lipid nanoparticles are lipidic 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 [1]. 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. Furthermore, the saturation solubility is increased as well. Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles. Dissolution experiments can be performed to quantify the increase in the saturation solubility of a drug when formulated into a nanosuspension [3].The stability of the particles obtained in the nanosuspension is attributed to their uniform particle size which is created by various manufacturing processes. The absence of particles with large differences in their size in nanosuspensions prevents the existence of different saturation solubilities and concentration gradients, consequently preventing the Oswald ripening effect [4].

Formulation considerations

Stabilizer: Stabilizer plays an important role in the formulation of nanosuspensions. In the absence of an appropriate stabilizer, the high surface energy of nano-sized particles can induce agglomeration or aggregation of the drug crystals. The main functions of a stabilizer are to wet the drug particles thoroughly, and to prevent Ostwald's ripening [5,6] 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 behaviour of nanosuspensions. In some cases, a mixture of stabilizers is required to obtain a stable nanosuspension. The drug-to-stabilizer ratio in the formulation may vary from 1:20 to 20:1 and should be investigated for a specific case. Stabilizers that have been explored so far include cellulosics, poloxamers, polysorbates, lecithins and povidones [7]. Lecithin is the stabilizer of choice if one intends to develop a parenterally acceptable and autoclavable nanosuspension.

Organic solvents:

Organic solvents may be required in the formulation of nanosuspensions if they are to be prepared using an emulsion or microemulsion 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 arena, their toxicity potential and the ease of their removal from the formulation need to be considered when formulating nanosuspensions using emulsions or microemulsions as templates. The pharmaceutically acceptable and less hazardous water-miscible solvents, such as ethanol and isopropanol, and partially water-miscible solvents, such as ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate and benzyl alcohol, are preferred in the formulation over the conventional hazardous solvents, such as

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dichloromethane. Additionally, partially watermiscible organic solvents can be used as the internal phase of the microemulsion when the nanosuspensions are to be produced using a microemulsion as a template.

Co-surfactants

The choice of co-surfactant is critical when using microemulsions to formulate nanosuspensions. Since co-surfactants can greatly influence phase behaviour, the effect of co-surfactant on uptake of the internal phase for selected microemulsion composition and on drug loading should be investigated. Although the literature describes the use of bile salts and dipotassium glycerrhizinate as co-surfactants, various solubilizers, such as Transcutol, glycofurol, ethanol and isopropanol, can be safely used as co-surfactants in the formulation of microemulsions.

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.

Methods of production:

Media milling (NanoCrystals) 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 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 unimodel distribution profiles and mean diameters<200nm is 30–60 min. The media milling process can successfully process micronized and non-micronized drug crystals. Once the formulation and the process are optimized, very little batch-to-batch variation is observed in the quality of the dispersion.

Dry co-grinding: Nanosuspensions prepared by high pressure homogenization and media milling using pearl-ball mill are wet–grinding processes. 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[8, 9, 10]. Itoh et al[9] reported the colloidal particles formation of many poorly water soluble drugs griseofulvin, glibenclamide and nifedipine obtained by grinding with polyvinylpyrrolidone (PVP) and sodium dodecylsulfate (SDS). Many soluble polymers and co-polymers such as PVP, polyethylene glycol (PEG), hydroxypropyl methylcellulose (HPMC) and cyclodextrin derivatives have been used [8, 11]. 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 [8, 12]. Dry co-grinding can be carried out easily and economically and can be conducted without organic solvents. The co-grinding technique can reduce particles to the submicron level and a stable

High-pressure homogenizers (Disso Cubes) During homogenization, the fracture of drug particles is brought about by capitation, high-shear forces and the collision of the particles against each other. The drug suspension, contained in a cylinder of diameter about 3 mm, passes suddenly through a

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very narrow homogenization gap of 25 mm, which leads to a high streaming velocity. 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 implode 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. To improve the efficiency of nano-sizing, the addition of viscosity enhancers is advantageous in certain cases as increasing the viscosity increases the powder density within the dispersion zone (homogenization gap). In order to obtain an optimized formulation, the effect of the following process variables should be investigated.

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 [13]. Their advantages, such as high drug solubilization, long shelf-life and ease of manufacture, make them an ideal drug delivery vehicle. There are several research papers available that describe the use of microemulsions as drug delivery vehicles [14,15, 16, 17, 18]. Recently, the use of microemulsions as templates for the production of solid lipid nanoparticles [19] and polymeric nanoparticles [20] has been described.

Precipitation

Using a precipitation technique, the drug is dissolved in an organic solvent and this solution is mixed with a miscible antisolvent. In the water-solvent mixture the solubility is low and the drug precipitates. Mixing processes vary considerably. Precipitation has also been coupled with high shear processing. The NANOEDGE process (is a registered trademark of Baxter International Inc. and its subsidiaries) relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy [21]. This is accomplished by a combination of rapid precipitation and high-pressure homogenization. Rapid addition of a drug solution to an antisolvent leads to sudden supersaturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high supersaturation when the solubility of the amorphous state is exceeded. The success of drug nanosuspensions prepared by precipitation techniques has been reported [18, 22, 23, 24].

Another method makes use of partially water-miscible

Solvents such as butyl lactate, benzyl alcohol and triacetin as the dispersed phase instead of hazardous solvents [22].The emulsion is formed by the conventional method and the drug nanosuspension is obtained by just diluting the emulsion. Dilution of the emulsion with water causes complete diffusion of the internal phase into the external phase, leading to instantaneous formation of a nanosuspension. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of diultrafiltration in order to make it suitable for

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administration. However, 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.

Disso Cubes technology was developed by [23] .The patent rights of Disso Cubes were initially owned by DDS (Drug Delivery Services) GmbH but currently they are owned by SkyePharma plc. Disso Cubes are engineered using piston-gap-type high-pressure homogenizers. A commonly used homogenizer 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 Power Ltd, Stansted, UK) can also be used. A high-pressure homogenizer consists of a high-pressure plunger pump with a subsequent relief valve (homogenizing valve). The task of the plunger pump is to provide the energy level required for the relief. The relief valve consists of a fixed valve seat and an adjustable valve. These parts form an adjustable radial precision gap. The gap conditions, the resistance and thus the homogenizing pressure vary as a function of the force acting on the valve. An external impact ring forms a defined outlet cross-section and prevents the valve casing from being damaged due to the flow [25]. The instrument is available in discontinuous and continuous versions. The continuous version is suitable for optimizing the various parameters of the homogenization process. Use of the discontinuous version is sensible if the drug is very costly or of limited availability. 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. High-pressure homogenizers are available with different capacities ranging from 40mL (for laboratory purposes) to a few thousand litres (for large-scale production). It is advisable to start with the micronized drug (particle size<25 _m) for production of nanosuspensions in order to prevent blocking of the homogenization gap. Hence, generally a jet-milled drug is employed as the starting material for producing Disso Cubes. 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. During the homogenization process, the drug suspension is pressed through the homogenization gap in order to achieve nano-sizing of the drug.

Characterization of nanosuspensions

The essential characterization parameters for nanosuspensions are as follows.

Mean particle size and particle size distribution.

The mean particle size and the width of particle size distribution are important characterization parameters as they govern the saturation solubility, dissolution velocity, physical stability and even biological performance of nanosuspensions. It has been indicated by [3] that saturation solubility and dissolution velocity show considerable variation with the changing particle size of the drug. Photon correlation spectroscopy (PCS)[26] can be used for rapid and accurate determination of the mean particle diameter of nanosuspensions. Moreover, 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

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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 whereas a PI value greater than 0.5 indicates a very broad distribution. No logarithmic normal distribution can definitely be attributed to such a high PI value. Although PCS is a versatile technique, because of its low measuring range (3nm to 3 nm) it becomes difficult to determine the possibility of contamination of the nanosuspension by microparticulate drugs (having particle size greater than 3 µm). Hence, in addition to PCS analysis, 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. Laser diffractometry yields a volume size distribution and can be used to measure particles ranging from 0.05–80 _m and in certain instruments particle sizes up to 2000 _m can be measured. The typical LD characterization includes determination of diameter 50% LD [18] and diameter 99% LD (99) values, which indicate that either 50 or 99% of the particles are below the indicated size.

Crystalline state and particle morphology.

The assessment of the crystalline state and particle morphology together helps in understanding the polymorphic or morphological changes that a drug might undergo when subjected to nanosizing. Additionally, when nanosuspensions are prepared drug particles in an amorphous state are likely to be generated. Hence, it is essential to investigate the extent of amorphous drug nanoparticles generated during the production of nanosuspensions. The changes in the physical state of the drug particles as well as the extent of the amorphous fraction can be determined by X-ray diffraction analysis [27, 28] and can be supplemented by differential scanning calorimetry [29]

Particle charge (zeta potential).

The determination of the zeta potential of a nanosuspension is essential as it gives an idea about the physical stability of the nanosuspension. The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself. In order to obtain a nanosuspension exhibiting good stability, for an electrostatically stabilized nanosuspension a minimum zeta potential of _30mV is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of 20mV is desirable [30].

Saturation solubility and dissolution velocity.

The determination of the saturation solubility and dissolution velocity is very important as these two parameters together help to anticipate any change in the in-vivo performance (blood profiles, plasma peaks and bioavailability) of the drug. As nanosuspensions are known to improve the saturation solubility of the drug, the determination of the saturation solubility rather than an increase in saturation solubility remains an important investigational parameter. 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 reflects the advantages that can be achieved over conventional formulations, especially when designing the sustained-release dosage forms based on nanoparticulate drugs. The dissolution velocity of drug nanosuspensions in various

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physiological buffers should be determined according to methods reported in the pharmacopoeia.

In-vivo biological performance.

The establishment of an in-vitro/in-vivo correlation and the monitoring of the in-vivo performance of the drug is an essential part of the study, irrespective of the route and the delivery system employed. It is of the utmost importance in the case of intravenously injected nanosuspensions since the Nanosuspensions: a promising drug delivery strategy 833 in-vivo behaviour of the drug depends on the organ distribution, which in turn depends on its surface properties, such as surface hydrophobicity and interactions with plasma proteins [5,6,7]. In fact, the qualitative and quantitative composition of the protein absorption pattern observed after the intravenous injection of nanoparticles is recognized as the essential factor for organ distribution [5,6,7,8]. Hence, suitable techniques have to be used in order to evaluate the surface properties and protein interactions to get an idea of in-vivo behaviour. Techniques such as hydrophobic interaction chromatography can be used to determine surface hydrophobicity , whereas 2-D PAGE [5] can be employed for the quantitative and qualitative measurement of protein adsorption after intravenous injection of drug nanosuspensions in animals.

Evaluation of nanosuspensions [61, 62]:–

In-Vitro Evaluations

Mean particle size and size distribution

The mean particle size and the width of particle size distribution (called Polydidpersity 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-3 µm only. PI governs the physical stability of nanosuspension and should be as low as possible for long-term stability. (Should be close to zero). 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 upto 2000µm. Atomic Force Microscopy is used for visualization of particle shape.

Particle charge (Zeta Potential)

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

Crystalline state and particle morphology

Differential Scanning Calorimetry (DSC) determines the crystalline structure. When nanosuspensions are prepared drug particles get converted to amorphous form hence it is essential to measure the extent of amorphous drug generated during the production of nanosuspensions. The X-Ray Diffraction (XRD) is also used for determining change in physical state and extent of amorphous drug.

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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 Ostwald-Freundlich equations can explain increase in saturation solubility

In-Vivo Evaluation

Mice were orally infected with 10 cysts of the ME49 strain of *T. gondii*. Mice were treated with sulfadiazine (Sigma-Aldrich) in drinking water (400 mg/liter) for 3 weeks beginning 2 days after infection [60] Two days after discontinuation of sulfadiazine, mice were treated with different concentrations of ANS (0.1, 1.0, and 10.0 mg/kg of body weight) administered as a single i.v. dose every other day. Control mice were treated with diluent only (a solution of Tween 80 at a concentration equivalent to that in a 10.0-mg/kg ANS solution) in the same manner. As a separate treatment control, ICSBP$^{-/-}$ mice were treated with a 100-mg/kg atovaquone suspension orally every other day. At day 8 after discontinuation of sulfadiazine—the time point at which control mice showed symptoms of disease and/or began to succumb—their brains, livers, lungs, and sera were obtained and fixed for histological examination or stored at −40°C for high-performance liquid chromatography (HPLC) analysis.

Evaluation for surface-modified Nanosuspensions[29]

1. Surface hydrophilic

2. Adhesion properties

3. Interaction with body proteins

CONCLUSION

The dissolution problems of poorly water soluble drugs have been largely solved to improve drug absorption and bioavailability. Nanosuspension formulations are promising candidates for enhancing the solubility of poorly water soluble drugs. Nanosuspension technology can be combined with traditional dosage forms: tablets, capsules, pellets, and can be used for parenteral products. To take advantage of nanosuspension drug delivery, simple formation technologies and variety applications, nanosuspensions will continue to be of interest as oral formulations and non-oral administration.

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