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NANOSUSPENSION: BIOAVAILABILITY ENHANCING NOVEL APPROACH

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ABSTRACT

A Nanosuspension is a submicron colloidal dispersion of drug particles. The objective of this study to review on novel aspects of a novel dosage form Nanosuspension. A pharmaceutical nanosuspension is very finely colloid, Biphasic, dispersed, solid drug particles in an aqueous vehicle, size below 1 µm, without any matrix material, stabilized by surfactants and polymers, prepared by suitable methods for Drug Delivery applications, through various routes of administration like oral, topical, parenteral, ocular and pulmonary routes. An increase in saturation solubility is postulated by particle size reduction due to an increased dissolution pressure. Depending on the production technique applied changes in crystalline structure of drug particles may also occur. An increasing amount of amorphous drug fraction could induce higher saturation solubility. It was hypothesized that nanosuspensions will enhance drug flux resulting from higher trans-membranous concentration gradients. 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. The nanosuspension were prepared by Homogenization in water (DissoCubes), Media milling (Nanocrystal or Nano Systems), Homogenization in non-aqueous media (Nanopure), Combined precipitation and homogenization (Nanoedge), Nanojet technology, Emulsification-solvent evaporation technique, Hydrosol method, Supercritical fluid method, Dry co-grinding, Emulsion as template and Microemulsion as template. The nanosuspensions were characterized for droplet size, viscosity, refractive index, pH, zeta potential, drug entrapment study, drug release study with kinetics and stability studies. The study concluded that the nanosuspension is an emerging novel dosage form and more study to be done to optimize the formulation and manufacturing difficulties.

KEY WORDS

Solubility, Bioavailability, nanosuspension, Colloids, Biopharmaceuticals.

INTRODUCTIONS:

A Nanosuspension is a submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very finely colloid, biphasic, dispersed, solid drug particles in an aqueous vehicle, size below 1µm, without any matrix material, stabilized by surfactants and polymers, prepared by suitable methods for drug delivery applications, through various routes of administration like oral, topical, parenteral, ocular and pulmonary routes [1-3]. Nanosuspensions differ from nanoparticles, which are polymeric colloidal carriers of drugs (Nanospheres and nanocapsules), and from solid lipid nanoparticles (SLN), which are lipidic carriers of drug [4, 5]. 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 [6, 7]. Nanosuspension has been reported to enhance absorption and bioavailability it may help to reduce the dose of the conventional oral dosage forms. Drug particle size reduction leads to an increase in surface area and consequently in the rate of dissolution as described by the Nernst–Brunner and Levich modification of the Noyes–Whitney equation [8]. In addition, an increase in saturation solubility is postulated by particle size reduction due to an increased dissolution pressure explained by the Ostwald–Freundlich equation [9]. Depending on the production technique applied changes in crystalline structure of drug particles may also occur [10.11]. An increasing amount of amorphous drug fraction could induce higher saturation solubility. It is effective for those molecules insoluble in oils; secondly, the high drug loading can be achieved as a drug exists in the form of pure solids and can significantly reduce the administration volume of high dose; thirdly, nanosuspensions can increase the physical and chemical stability of drugs as they are actually in the solid state; finally, nanosuspensions can provide the passive targeting [12-15].

IMPORTANCE OF SOLUBILITY:

Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost effectiveness, least sterility constraints and flexibility in the design of dosage form [16]. The oral bioavailability depends on several factors including aqueous solubility, drug permeability, dissolution rate, first-pass metabolism, pre-systemic metabolism and susceptibility to efflux mechanisms. The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability.

Need of solubility enhancement:

Solubility also plays a major role for other dosage forms like parenteral formulations as well [17]. Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for achieving required pharmacological response [18]. Poorly water-soluble drugs often require high doses in order to reach therapeutic plasma concentrations after oral administration. More than 40% NCEs (New chemical entities) developed in pharmaceutical industry are practically insoluble in water. These poorly water-soluble drugs having slow drug absorption leads to inadequate and variable bioavailability and gastrointestinal mucosal toxicity. For orally administered drugs, solubility is the most important one rate limiting parameter to achieve their desired concentration in systemic circulation for pharmacological response [19, 20].

Biopharmaceutical classification system (BCS):

The BCS classification system is based on the scientific rationale that, if the highest dose of a drug candidate is readily soluble in the fluid volume on average present in the stomach (250 ml) and the drug is more than >90% absorbed, then the *in vitro* drug product dissolution profiles should allow assessment of the equivalence of different drug formulations. Solubility and dissolution can be easily measured *in vitro*. The effective intestinal permeability of therapeutic agents correlates well with total fraction absorbed in both humans as well as rats, and to a lesser extent *in vitro* tissue culture system. All drugs have been divided into four classes: class I-highly soluble and highly permeable, class II-low soluble and high permeable, class III—low soluble and high permeable and class IV—low soluble and low permeable [21-22].

BIOAVAILABILITY:

Factors affecting bioavailability:

Low bioavailability is most common with oral dosage forms of poorly water-soluble, slowly absorbed drugs. Age, sex, physical activity, genetic phenotype, stress, disorders (e.g. achlorhydria, malabsorption syndromes), or previous GI surgery (e.g. bariatric surgery) can also affect drug bioavailability [23].

Improvement of bioavailability:

The drugs for which solubility has presented a challenge to the development of a suitable formulation for oral administration. Examples are griseofulvin, digoxin, phenytoin, sulphathiazole etc. Consideration of the modified Noyes-Whitney equation provides some hints as to how the dissolution rate of even very poorly soluble compounds might be improved to minimize the limitations to oral availability [24]. The main possibilities for improving dissolution according to this analysis are: to increase the surface area available for dissolution by decreasing the particle size of the solid compound, by optimizing the wetting characteristics of the compound surface, to decrease the diffusion layer thickness, to ensure sink conditions for dissolution and, to improve

the apparent solubility of the drug under physiologically relevant conditions [25]. A fundamental step in the solubilization of drug compound is the selection of an appropriate salt form, or for liquid drugs, adjustment of pH of the solution. Traditional approaches to drug solubilization include either chemical or mechanical modification of the drug molecule, or physically altering the macromolecular characteristics of aggregated drug particles. Improvement of bioavailability can be obtained by following measures: addition of solubilizing excipients, inclusion complexes, polymorphism, lipid-based emulsion systems, salt form, solid dispersions and particle size reduction, eutechtic mixture etc [26].

Need of nanosuspension for bioavailability enhancement:

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. Increase in surface area as well as concentration gradient lead to a much more pronounced increase in the dissolution velocity as compared to a micronized product. 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. 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. Ostwald ripening is responsible for crystal growth and subsequently formation of microparticles [27, 28].

ADVANTAGES OF NANOSUSPENSIONS:

The major advantages [29, 30] of nanosuspension technology are provides ease of manufacture and scale-up for large scale production, long-term physical stability due to the presence of stabilizers, oral administration of nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability, rapid dissolution and tissue targeting can be achieved by IV route of administration, reduction in tissue irritation in case of subcutaneous/intramuscular administration, higher bioavailability in case of ocular administration and inhalation delivery, drugs with high 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, nanosuspensions can be incorporated in tablets, pellets, hydrogels and suppositories are suitable for various routes of administration, the flexibility offered in the modification of surface properties and particle size and ease of postproduction processing of nanosuspensions enables them to be incorporated in various dosage forms for various routes of administration, thus proving their versatility.

SELECTION CRITERIA OF DRUG FOR NANO SUSPENSIONS:

Nanosuspension can be prepared for the API that is having either of the following characteristics: water insoluble but which are soluble in oil (high log P) or API are insoluble in both water and oils, drugs with reduced tendency of the crystal to dissolve, regardless of the solvent, API with very large dose [31].

FORMULATION OF NANOSUSPENSION [32-36].

Stabilizer: The main function of a stabilizer is to wet the drug particles thoroughly, and to prevent ostwald's ripening and agglomeration of Nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barrier. The type and amount of stabilizer has a pronounced effect on the physical stability and *in vivo* behavior of nanosuspension. Stabilizers that have been used are poloxomers, polysorbate, cellulosics, povidones, and lecithins. Lecithin is the stabilizer of choice if one intends to develop a parentally acceptable and autoclavable nanosuspension.

Organic Solvent: Organic solvents are used in the formulation of Nanosuspension if emulsions or micro emulsions are used as a template. The pharmaceutically acceptable less hazardous water miscible solvent, such as methanol, ethanol, chloroform, ispropanol, and

partially water miscible solvents ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate, benzyl alcohol, are preferment in the formulation over the conventional hazardous solvents, such as dichloromethane.

Co-Surfactants: The choice of co-surfactant is critical when using micro emulsions to formulate Nanosuspensions. Since co-surfactants 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. Although the literature describes the use of bile salts and dipotassium glycerrhizinate as cosurfactants, various solubilizers, such as Transcutol, glycofurol, ethanol and isopropanol, can be safely used as co-surfactants in the formulation of nanosuspensions.

Other additives: According to the requirement of the route of administration or the properties of the drug moiety, nanosuspensions may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant.

PROPERTIES OF NANOSUSPENSIONS [37-40]:

Physical Long-term stability: Another special feature of nanosuspensions is the absence of Ostwald ripening, which is suggestive of their long-term physical 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.

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 drug and power density applied by homogenizer.

Adhesiveness: There is a distinct 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.

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

Increase 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.

$dx/dt = [(D \times A)/ h] [Cs-X/V]$ ..... [1]

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

PREPARATION METHODS OF NANO- SUSPENSION:

Bottom up techniques:

It is the technique in which the Nano size is obtained by increasing the size of particles from molecular range to Nano range [41]. The conventional methods of precipitation ('Hydrosol') 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. Advantage of this method is the use of simple and low-cost equipment's, whereas limitations are as follows: the drug needs to be soluble in at least one solvent and the solvent needs to be miscible with non-solvent and moreover, it is not applicable to the drugs, which are poorly soluble in both aqueous and non-aqueous media.

Top down techniques:

High pressure homogenization (disso cubes):

Disso Cubes are engineered using piston-gap-type high-pressure homogenizers [42]. High pressure homogenization has been used to prepare nanosuspension of many poorly water-soluble drugs. Homogenization involves the forcing of the suspension under pressure through a valve having a narrow aperture. The instrument can be operated at pressure varying from 100-1500 bars with volume capacity of 40 ml. The concern with this method is the need for small sample particles before loading and the fact that many cycles of homogenization are required. Before subjecting the drug to the homogenization process, it is

essential to form a pre-suspension 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.

Media Milling (Nano-Crystals):

In this method, the nanosuspensions are produced using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber. The milling 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 nano-crystalline 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 dispersion with a mean diameter of <200 nm is 30–60 min [43]. 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 uni-modal distribution profiles and mean diameters <200 nm 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 [44].

Combined Precipitation and Homogenization (Nanoedge):

The drug is dissolved in an organic solvent and this solution is mixed with a miscible anti-solvent for precipitation. In the water-solvent mixture, the solubility is low and the drug precipitates. Precipitation has also been coupled with high shear processing. The basic principles of Nanoedge are the same as that of precipitation and homogenization. This is accomplished by a combination of rapid precipitation and high-pressure homogenization. The Nanoedge patented technology by Baxter depends on the precipitation of friable materials for fragmentation under conditions of high shear and/or thermal energy. Rapid addition of a drug solution to an anti-solvent leads to sudden super saturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high super saturation when the solubility of the amorphous state is exceeded [45]. The basic principles of Nanoedge are the same as that of precipitation and homogenization. A combination of these techniques results in smaller particle size and better stability in a shorter time. The major drawback of the precipitation technique, such as crystal growth and long-term stability, can be resolved using the Nanoedge technology [45].

Nanojet Technology:

This technique, called 'opposite stream or Nanojet technology', uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure up to 4000 bar at the high velocity of 1000 m/s.7 The high shear force produced during the process results in particle size reduction. Equipment using this principle includes the M110L and M110S microfluidizers (Microfluidics). The major limitation of this technique is the high number of passes through the micro-fluidizer (up to 75 passes) and that the product obtained contains a relatively larger fraction of microparticles. A limitation of this process is the large production time [46].

Emulsification-Solvent Evaporation Technique:

This technique involves preparing a solution of drug followed by its emulsification in another liquid that is a non-solvent for the drug. Evaporation of the solvent leads to precipitation of the drug. Crystal growth and particle aggregation can be controlled by creating high shear forces using a high-speed stirrer [47].

Supercritical Fluid Method:

Supercritical fluid technology can be used to produce nanoparticles from drug solutions. The various methods attempted are rapid expansion of supercritical solution process (RESS), supercritical anti-solvent process and precipitation with compressed anti-solvent process (PCA). The RESS involves expansion of the drug solution in supercritical fluid through a nozzle, which leads to loss of solvent power of the supercritical fluid resulting in precipitation of the drug as fine particles. In the PCA method, the drug solution is atomized into a chamber containing compressed CO_2. As the solvent is removed, the solution gets supersaturated and thus precipitates as fine crystals. The supercritical anti- solvent process uses a supercritical fluid in which a drug is poorly soluble

and a solvent for the drug that is also miscible with the supercritical fluid. The drug solution is injected into the supercritical fluid and the solvent gets extracted by the supercritical fluid and the drug solution gets supersaturated. The drug is then precipitated as fine crystals [48].

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 co-polymers after dispersing in a liquid. Literature reported the colloidal particles formation of many poorly water-soluble drugs; Griseofulvin, Glibenclamide and Nifedipine obtained by grinding with polyvinyl pyrrolidone (PVP) and Sodium dodecyl sulfate (SDS). Many soluble polymers and co-polymers such as PVP, Polyethylene glycol (PEG), Hydrox ypropyl methyl cellulose (HPMC) and cyclodextrin derivatives have been used. 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. Recently, nanosuspensions can be obtained by dry milling techniques. 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 sub-micron level and a stable amorphous solid can be obtained [49].

Emulsion as Template:

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. Such solvents can be used as the dispersed phase of the emulsion. There are two ways of fabricating drug nanosuspensions by the emulsification method. In the first method, 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. Optimizing the surfactant composition increases the intake of organic phase and ultimately the drug loading in the emulsion. Originally, organic solvents such as methylene chloride and chloroform were used. However, environmental hazards and human safety concerns about residual solvents have limited their use in routine manufacturing processes. Relatively safer solvents such as ethyl acetate and ethyl formate can still be considered for use [49].

Microemulsion as Template/Lipid Emulsion:

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. Their advantages, such as high drug solubilization, long shelf-life and ease of manufacture, make them an ideal drug delivery vehicle. Taking advantage of the microemulsion structure, one can use microemulsions even for the production of nanosuspensions. Oil-in-water microemulsions are preferred for this purpose. The internal phase of these microemulsions could be either a partially miscible liquid or a suitable organic solvent, as described earlier [49]. 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 the microemulsion yields the drug nanosuspension by the mechanism described earlier. The influence of the amount and ratio of surfactant to co-surfactant on the uptake of internal phase and on the globule size of the microemulsion should be investigated and optimized in order to achieve the desired drug loading. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of di-ultrafiltration in order to make it suitable for administration.

Post-Production Procession:

Post-production processing of nanosuspensions becomes essential when the drug candidate is highly susceptible to hydrolytic cleavage or chemical degradation. Processing 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 [50].

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 palletization, granulation, spray drying or lyophilization. As the primary objective of the nanoparticulate system is rapid dissolution, disintegration of the solid form and redispersion of 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 Poloxamers, 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 [50].

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 [48, 49]. 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 [50].

CHARACTERIZATION OF NANOSUSPENSION [48-52]:

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

Particle size distribution: Particle size distribution determines the physiochemical behavior of the formulation, such as saturation solubility, dissolution velocity, physical stability, etc. The particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) and coulter counter multi-sizer. The PCS method can measure particles in the size range of 3 nm to 3µm and the LD method has a measuring range of 0.05-80 µm. The coulter counter multi-sizer gives the absolute number of particles, in contrast to the LD method, which gives only a relative size distribution. For IV use, particles should be less than 5µm, considering that the smallest size of the capillaries is 5-6 µm and hence a higher particle size can lead to capillary blockade and embolism.

Zeta potential: Zeta potential is an indication of the stability of the suspension. For a stable suspension stabilized only by electrostatic repulsion, a minimum zeta potential of ±30 mV is required whereas in case of a combined electrostatic and steric stabilizer, a zeta potential of ±20 mV would be sufficient.

Crystal morphology: To characterize the polymorphic changes due to the impact of high-pressure homogenization in the crystalline structure of the drug, techniques like X-ray diffraction analysis in combination with differential scanning calorimetry or differential thermal analysis can be utilized. Nanosuspensions can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms because of high-pressure homogenization.

Dissolution Velocity and Saturation Solubility: Nanosuspensions have an important advantage over other techniques, that it can increase the dissolution velocity as well as the saturation solubility. These two parameters should be determined in various physiological solutions. The assessment of saturation

solubility and dissolution velocity helps in determining the *in vitro* behavior of the formulation.

Density: Specific gravity or density of the formulation is an important parameter. A decrease in density often indicates the presence of entrapped air within the structure of the formulation. Density measurements at a given temperature should be made using well mixed, uniform formulation; precision hydrometer facilitate such measurements.

pH: The pH value of aqueous formulation should be taken at a given temperature and only after settling equilibrium has been reached, to minimize "pH drift" and electrolyte should not be added to the external phase of the formulation to stabilized the pH.

Droplet size: The droplet size distribution of micro emulsion vesicles can be determined by either light scattering technique or electron microscopy. Dynamic light scattering spectrophotometer which uses a neon laser of wavelength 632 nm.

Viscosity: The viscosity of lipid-based formulations of several compositions can be measured at different shear rates at different temperatures using Brookfield type rotary viscometer. The sample room of the instrument must be maintained at 37°C by a thermo bath and the samples, for the measurement are to be immersed in it.

Stability of Nanosuspension: The high surface energy of nanosized particles induces agglomeration of the drug crystals. The main function of the stabilizer is to wet the drug particles thoroughly to prevent Ostwald ripening and agglomeration of the Nanosuspension and form a physically stable formulation by providing a steric or an ionic barrier. Typical examples of stabilizers used in Nanosuspensions are cellulosics, poloxamer, polysorbates, lecithin, polyoleate and povidones. Lecithin may be preferred in developing parenteral Nanosuspensions.

Osmolarity: Practically, Osmolarity of nanosuspension can be measured by using Osmometer.

Drug content: Drug content of nanosuspension formulation can be carried out by extracting the nanosuspension in suitable solvent mixture, like Methanol: THF (1:1) mixture, shaken well, and then centrifuged. The supernatants can be separated and diluted with same solvent mixture and the absorbance can be measured at suitable λ_{max}.

***In vitro* dissolution study:** Dissolution rate may be defined as amount of drug substance that goes in the solution per unit time under standard conditions of liquid/solid interface, temperature and solvent composition. It can be considered as a specific type of certain heterogeneous reaction in which a mass transfer results as a net effect between escape and deposition of solute molecules at a solid surface. *In vitro* dissolution screening should be the first line of biopharmaceutical evaluation of nano-formulations. Since oral nano-formulations are designed to disperse in the stomach contents, dissolution in Simulated Gastric Fluid (SGF) should provide an initial estimate of the dissolution rate enhancement. For insoluble compounds, where dissolution is expected to mainly occur in the intestinal region, further *in vitro* testing in simulated intestinal media will provide additional insight on expected bioperformance. Several reports in the literature report an increased *in vitro* dissolution rate for nanosized APIs. However, one should keep in mind that the small particle size for nano-formulations may pose additional needs in terms of analytical sample handling and processing to ensure that no un-dissolved API is assayed during the dissolution test. Filtering through smaller pore size filters or (ultra)centrifugation to separate un-dissolved API has been implemented.

Interaction with body proteins: *In vitro* interaction between nanoparticles and mucin can be studied by incubation of mucin and nanoparticles (1:4 weight ratio) either in acidic or in neutral medium. The incubation is carried out under stirring at temperature of 37ºC. The dispersions is then be centrifuged and 150 µl 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 2 h at 37º C. According to this procedure, the absorbance of mucin can be measured by colorimetry at λ_{max} of the drug. The amount of the mucin 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 mucin standard curves.

Surface Hydrophilicity: For intravenously injected nanosuspensions, additional parameters need to be determined which affect the in vivo fate of the drug nanoparticles. Surface hydrophilicity / hydro- phobicity 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 in aqueous dispersion medium.

Adhesion properties: *In vivo* bioadhesive study is performed where Male Wistar rats can be used. In general, each animal receives a single oral dose of 1ml aqueous suspension containing 10 mg of the nanoparticles loaded with the drug (approximately 45 mg particles/kg body Weight). The animal is sacrificed by cervical dislocation at 1 and 3 h 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 2 cm length and digested in suitable alkali for 24 h. Drug is extracted from the digested samples by addition of 2 ml methanol, vortexed for 1 min and centrifuged. Aliquot (1 ml) of the supernatants is to be assayed for the drug by spectrofluorimetry to estimate the fraction of adhered nanoparticles to the mucosa. For calculations, standard curves of the drug can also be prepared.

***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 *in-vivo* behavior 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 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. Hence, suitable techniques have to be used in order to evaluate the surface properties and protein interactions to get an idea of *in vivo* behavior. Techniques such as hydrophobic interaction chromatography can be used to determine surface hydrophobicity, whereas 2-D PAGE can be employed for the quantitative and qualitative measurement of protein adsorption after intravenous injection of drug nanosuspensions in animals.

APPLICATIONS OF NANOSUSPENSIONS [52-55]:

Parenteral administration.

From the formulation perspective, nanosuspensions meet almost all the requirements of an ideal drug delivery system for the parenteral route. Since the drug particles are directly nanosized, it becomes easy to process almost all drugs for parenteral administration. Hence, nanosuspensions enable significant improvement in the parenterally tolerable dose of the drug, leading to a reduction in the cost of the therapy and also improved therapeutic performance. The maximum tolerable dose of paclitaxel nanosuspension was found to be three times higher than the currently marketed Taxol, which uses Cremophore EL and ethanol to solubilize the drug. Nanosuspensions can be administered via different parenteral administration routes ranging from intra-articular via intra peritonal to intravenous injection. For administration by the parenteral route, the drug either has to be solubilized or has particle/globule size below 5 µm to avoid capillary blockage. In this regard, liposomes are much more tolerable and versatile in terms of parenteral delivery. However, they often suffer from problems such as physical instability, high manufacturing cost and difficulties in scale-up. Nanosuspensions would be able to solve the problems mentioned above. In addition, nanosuspensions have been found to increase the efficacy of parenterally administered drugs.

Oral administration.

The oral route is the preferred route for drug delivery because of its numerous well-known advantages. The efficacy or performance of the orally administered drug generally depends on its solubility and absorption through the gastrointestinal tract. Hence, a drug candidate that exhibits poor aqueous solubility and / or dissolution rate limited absorption is believed to possess slow and/or highly variable oral bioavailability. Danazol is poorly bioavailable gonadotropin inhibitor, showed a drastic improvement in bioavailability when administered as a nanosuspension as compared to the commercial danazol macro suspension Danocrine. Danazol nanosuspension led to an absolute bioavailability of 82.3%, whereas the marketed danazol suspension Danocrine was 5.2% bioavailable.11 Nanosizing of drugs can lead to a dramatic increase in their oral absorption and subsequent bioavailability. Improved bioavailability can be explained by the adhesiveness of drug nanoparticles to the mucosa, the

increased saturation solubility leading to an increased concentration gradient between gastrointestinal tract lumen and blood as well as the increased dissolution velocity of the drug. Aqueous nanosuspensions can be used directly in a liquid dosage form and a dry dosage form such as tablet or hard gelatin capsule with pellets. The aqueous nanosuspension can be used directly in the granulation process or as a wetting agent for preparing the extrusion mass pellets. A similar process has been reported for incorporating solid lipid nanoparticles into pellets. Granulates can also be produced by spray drying of nanosuspensions.

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 culde-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. Thus the intrinsic dissolution rate of the drug in lachrymal fluids controls 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. One example of a nanosuspension intended for ophthalmic controlled delivery was developed as a polymeric nanosuspension of ibuprofen. This nanosuspension is successfully prepared using Eudragit RS100 by a quasi-emulsion and solvent diffusion method. Nanosuspensions of glucocorticoid drugs; hydrocortisone, prednisolone and dexamethasone enhance rate, drug absorption and increase the duration of drug action. To achieve sustained release of the drug for a stipulated time period, nanosuspensions can be incorporated in a suitable hydrogel base or mucoadhesive base or even in ocular inserts. The bio-erodible as well as water soluble/permeable polymers possessing ocular tolerability61 could be used to sustain the release of the medication. The polymeric nanosuspension of flurbiprofen has been successfully formulated using acrylate polymers such as Eudragit RS 100 and Eudragit RL 100.62-64 The polymeric nanosuspensions have been characterized for drug loading, particle size, zeta potential, *in-vitro* drug release, ocular tolerability and *in-vivo* biological performance. The designed polymeric nanosuspensions revealed superior *in vivo* performance over the existing marketed formulations and could sustain drug release for 24 h. The scope of this strategy could be extended by using various polymers with ocular tolerability.

Pulmonary drug delivery.

Nanosuspensions may prove to be an ideal approach for delivering drugs that exhibit poor solubility in pulmonary secretions. Currently such drugs are delivered as suspension aerosols or as dry powders by means of dry powder inhalers. The drugs used in suspension aerosols and dry powder inhalers are often jet milled and have particle sizes of microns. Because of the microparticulate nature and wide particle size distribution of the drug moiety present in suspension aerosols and dry powder inhalers, some disadvantages are encountered: like limited diffusion and dissolution of the drug at the site of action, rapid clearance of the drug from the lungs, less residence time for the drugs, unwanted deposition of the drug particles in pharynx and mouth.

The ability of nanosuspensions to offer quick onset of action initially and then controlled release of the active moiety is highly beneficial and is required by most pulmonary diseases. Moreover, as nanosuspensions generally contain a very low fraction of microparticulate drug, they prevent unwanted deposition of particles in the mouth and pharynx, leading to decreased local and systemic side-effects of the drug. Additionally, because of the nanoparticulate nature and uniform size distribution of nanosuspensions, it is very likely that in each aerosol droplet at least one drug nanoparticle is contained, leading to even distribution of the drug in the lungs as compared to the microparticulate form of the drug. In conventional suspension aerosols many droplets are drug free and others are highly loaded with the drug, leading to uneven delivery and distribution of the drug in the lungs. Nanosuspensions could be used in all available types of nebulizer. However, the extent of influence exerted by the nebulizer type as well as the nebulization process on the particle size of nanosuspensions should be ascertained.

Bioavailability enhancement.

Drug with poor solubility or permeability in gastrointestinal tract leads to poor oral bioavailability. Nanosuspension resolves the problem of poor bioavailability by solving the problem of poor solubility, and poor permeability across the membranes. Dissolution rate was increased in diclofenac when formulated in nanosuspension form from 25 to 50 % in

SGF and H_2O while in case of SIF it was increased from 10 to 35 % as compared to coarse suspension. Bioavailability of poorly soluble, a COX-2 inhibitor, celecoxib was improved using a nanosuspension formulation. The crystalline nanosized celecoxib alone or in tablet showed a dramatic increase of dissolution rate and extent compared to micronized tablet. Spironolactone and budesonide are poorly soluble drugs. The higher flux contributes to the higher bioavailability of nanosuspension formulation. The bioavailability of poorly soluble fenofibrate following oral administration was increased compared to the suspensions of micronized fenofibrate.

Target drug delivery.

Nanosuspensions can also be used for targeted delivery as their surface properties and *in vivo* behavior can easily be altered by changing either the stabilizer or the milieu. Their versatility, ease of scale up and commercial product enable the development of commercial viable nanosuspensions for 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 mucoadhesive nanosuspensions of bupravaquone. Similarly, conditions such as pulmonary aspergillosis can easily be targeted by using suitable drug candidates, such as amphotericin B, in the form of pulmonary nanosuspensions instead of using stealth liposomes. Nanosuspensions can also be used for targeting as their surface properties and changing of the stabilizer can easily alter the *in vivo* behavior. The drug will be up taken by the mononuclear phagocytic system to allow regional-specific delivery. This can be used for targeting anti-mycobacterial, fungal or leishmanial drugs to the macrophages if the infectious pathogen is persisting intracellularly.

Topical formulations.

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

Mucoadhesion of the nanoparticles.

Nanosuspension containing drug nanoparticles orally diffuse into the liquid media and rapidly encounter the mucosal surface. The particles are immobilized at the intestinal surface by an adhesion mechanism referred to as "bioadhesion." From this moment on, the concentrated suspension acts as a reservoir of particles and an adsorption process takes place very rapidly. The direct contact of the particles with the intestinal cells through a bioadhesive phase is the first step before particle absorption [66]. The adhesiveness of the nanosuspensions not only helps to improve bioavailability but also improves targeting of the parasites persisting in the GIT. The oral route, since it avoids the pain and discomfort associated with injections and is more attractive from a marketing and patient compliance perspective. Finally, the major advantage of nanocrystals for oral delivery is generally regarded as being on the increased specific surface area of the particles. However, EMEND® and TriglideTM are formulated as nanosuspension to reduce fed/fasted variability.

PATENTS ON NANOSUSPENSIONS:

Because of such a versatile technology of nano-sizing, there are many patents on this technology (Table 2) [56].

Table 1. Current Marketed Pharmaceutical Products Utilizing Nano-crystalline Formulation.

Product	Drug compound	Indication	Company	Nanoparticle technology
RAPAMUNE®	Sirolimus	Immuno-suppressant	Wyeth	Élan Drug Delivery Nanocrystals®
EMEND®	Aprepitant	Anti-emetic	Merck	Élan Drug Delivery Nanocrystals®
TriCor®	Fenofibrate	Hypo-cholesteremic	Abbott	Élan Drug Delivery Nanocrystals®
MEGACE ES®	Megestrol Acetate	Appetite stimulant	PAR Pharmaceutical	Élan Drug Delivery Nanocrystals®
TRIGLIDE™	Fenofibrate	Hypo-cholesteremic	First Horizon Pharmaceutical	SkyePharma IDD®-P technology
EMEND®	Aprepitant	Anti-emetic	Merck	Élan Drug Delivery Nanocrystals®

Table 2. The New Drug Application Based on Nanosuspensions Technique Reported and Marketed by Now.

Drugs	Indication	Author or company	Route	Status
Danazol	Hormone	Rogers T.L.	Oral	Reported
Paclitaxel	Anticancer	American Bioscience	I.V.	Marketed
Naproxen	Anti-inflammatory	Anchalee Ain-Ai	Oral/ parenteral	Reported
Probucol	Lipid lowering	Jyutaro Shudo	Oral	Reported
Megestrol acetate	Steroid hormone	Par pharmaceutical	Oral	Marketed
Paliperidone palmitate	Anti-schizophrenia	Johnson and Johnson	Oral	Phase III
Busulfan	Anticancer	SkyePharma	Intrathecal	Undisclosed
Clofazimine	Anti-mycobacterial	K. Peters	I.V.	Reported
Buparvaquone	Antibiotic	Muller R. H.	Oral	Reported
Oridonin	Anticancer	Lei Gao	I.V.	Reported
Ascorbyl palmitate	Ascorbyl palmitate	Veerawat T.	I.V.	Reported
Prednisolone	Glucocorticoid	M.A. Kassem	Ophthalmic	Reported
Dihydro-artemisinin	Antimalarial	Jiraporn C.	I.V.	Reported
Cilostazol	Anti-platelet agent	Jun-ichi Jinno	Oral	Reported
Carbamazepine	Psychotolytic	D. Douroumis	Oral	Reported
Omeprazole	Proton pump inhibitor	Jan Moschwitzer	I.V.	Reported
Mitotane	Adrenal cortex hormone	M. trotta	Oral	Reported
Griseofulvin	Antifungal	Boris Y. Shekunov	Oral	Reported

MARKETED PRODUCTS:

Preferred dosage forms of nanosuspensions:

Aqueous or non-aqueous drug nanosuspensions exhibiting a physical long-term stability should be sufficient to place them on the market as liquid products. In the case of drug nanosuspensions in pure water or in water containing mixtures, they can be used as granulation fluid in the granulation process for the production of tablets or alternatively as wetting agents for the extrusion mass to produce pellets. Spray-drying of the nanosuspension is also possible. The produced powders can then be used again for tablet or pellet production or alternatively be filled in hard gelatin or HPMC capsules. The drug nanocrystals produced in non-aqueous media such as oils or liquid/solid PEG can be used directly for filling in capsules. Production of drug nanosuspensions in melted PEG which is solid at room temperature opens further perspectives [57].

Direct filling of capsules with the hot nanosuspension is possible. Alternatively, after solidification of the PEG, the drug nanocrystals containing mass can be ground and filled as a powder into the capsules. To summarize, there are different ways to transfer the drug nanocrystals to a final dry oral dosage form for the patient. With regard to parentral products, the drug nanosuspensions can be used as they are; a shelf life of up to three years was shown for selected nanosuspensions. Alternatively, lyophilized products can be offered to be reconstituted prior to administration. Table 2 summarizes current marketed nanosuspension formulations while Table 1 And 2 summarizes the New Drug Application Based on Nanosuspensions Technique Reported and Marketed by now [58-61].

Table 3. The New Drug Application Based on Nanosuspensions Technique Reported and Marketed by Now.

Drug	Indications	Route	Status
Paclitaxel	Anticancer	I.V.	Phase IV
Rapamune	Immunosuppressant	Oral	Marketed
Emend	Antiemetic	Oral	Marketed
Budesonide	Anti-asthamatic	Pulmonary	Phase I
Busulfan	Anticancer	Intrathecal	Phase I
Fenofibrate	Hypo-lipidemic	Oral	Phase I
Thymectacin	Anticancer	I.V.	Phase I/II
Insulin	Anti-diabetic	Oral	Phase I
Calcium Phosphate	Mucosal vaccine adjuvant for Herpes	Oral	____
Silver	Eczema, atopic dermatitis	Topical	Phase I
Cytokine Inhibitor	Crohn's disease	Oral	Phase II

CONCLUSION:

Present review demonstrates the current progress in therapeutic nanosuspensions produced by various techniques. Nanosuspensions are distinctive and commercially feasible approach to solve the problems of hydrophobic drug such as poor solubility and poor bioavailability. For large-scale production of nanosuspensions, media milling and high-pressure homogenization technology have been successfully used. The characteristics, like improvement of dissolution velocity, increased saturation solubility, improved bioadhesivity, versatility in surface modification, and ease of postproduction processing, have widened the applications of nanosuspensions for various routes of administration. The applications of nanosuspensions in oral and parental routes have been very well established. Further study has to be done to evaluate nanosuspension applications in pulmonary and ocular delivery.

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