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Review Article

NANOSUSPENSION: A PROMISING DRUG DELIVERY SYSTEM

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ABSTRACT

Solubility proves to be a major hurdle for the successful development and commercialization of new drug products. Since 40% of the active substances being identified through the new paradigm in high – throughput screening are lipophilic. So, its viability as a potential new drug candidate reduces manifold. Because of this limitation, many pharmacologically active molecules have failed to reach the market. Therefore, Nanosuspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because of their versatile features and unique advantages. Techniques such as media milling and high pressure homogenization have been used commercially for producing nanosuspensions. Recently, the engineering of nanosuspensions employing emulsions and microemulsions as templates has been addressed in the literature. The unique features of nanosuspensions have enabled their use in various dosage forms, including specialized delivery systems such as mucoadhesive hydrogels. Rapid strides have been made in the delivery of nanosuspensions by parenteral, per-oral, ocular and pulmonary routes. Currently, efforts are being directed to extending their applications in site-specific drug delivery. The following work deals with the special features of nanosuspensions, the preparation methods, advantages of such methods, characterization of nanosuspensions, patents, marketed products and their applications for hoping to make easy, the future research in this area.

Keywords: Nanosuspensions, High pressure homogenization, Media milling, Ostwald ripening, Saturation solubility, Dissolution velocity, Noyes-Whitney equation.

INTRODUCTION

Pharmaceutical industries are constantly seeking new approaches in order to obtain an adequate oral bioavailability, as most of biological properties exhibiting NCEs are poorly water - soluble. The increasing frequency of poorly water soluble NCEs exhibiting therapeutic activity is of major concern to the development of new formulations in pharmaceutical industry which leads to low turnout in the development of new molecular entities as drug formulations is poor solubility and poor permeability of the lead compounds. Recently, the formulation of such drugs as nanoscale systems (which have a size below 1μm) has rapidly evolved as a new and novel drug delivery system. The major characteristic of these systems is the rapid dissolution rate, which enhance bioavailability after oral administration.[1] The aim of the present article is to review the nanosuspensions as an emerging and promising tool for the formulation of poorly soluble drugs.

Definition

A pharmaceutical nanosuspension is defined as "very finely dispersed solid drug particles in an

aqueous vehicle, stabilized by surfactants, for either oral and topical use or parentral and pulmonary administration, with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability". The diameter of the suspended particle is less than 1 µm in size (i.e. 0.1nm-1000 nm).[2,5] 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.[3] 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. Nano size particles can increase dissolution velocity and saturation solubility because of the vapor pressure effect.[1]

Need of Nanosuspension

More than 40% of drugs are poorly soluble in water, so they show problems in formulating them in conventional dosage forms. Also, for class II drugs which are poorly soluble in aqueous and organic media, the problem is more complex.[6] Preparing nanosuspension is preferred for such compounds that are insoluble in water (but are soluble in oil) with high log P value. Various approaches to resolve problems of low solubility and low bioavailability micronization, co-solvency, oily solution, salt formation- some other techniques are liposomes, emulsions, microemulsion, solid dispersion, ß- cyclodextrin inclusion complex etc. But, many of these techniques are not universally applicable to all drugs.[6] In these cases nanosuspensions are preferred. In case of drugs that are insoluble in both water and in inorganic media instead of using lipidic systems, nanosuspensions are used as a formulation approach. It is most suitable for the compounds with high log P value, high melting point, and high dose. Nanosuspensions can be used to enhance the solubility of drugs that are poorly soluble in aqueous as well as lipid media. As a result, the rate of flooding of the active compound increases and the maximum plasma level is reached faster (e.g., oral or intravenous (IV) administration of the nanosuspension). This is one of the unique advantages that it has over other approaches for enhancing solubility. It is useful for molecules with poor solubility, poor permeability or both, which poses a significant challenge for the formulators. Major issues associated with poorly water-soluble compounds[4]**:**

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

Although, all marketed products, currently are produced by so-called 'top-down techniques', in which the nanoparticles are obtained through size reduction into the submicron-range, bottom-up techniques and especially controlled precipitation method, are methods of interest for nanozation of poorly soluble drugs. In this method, without any harsh conditions and only with simple equipments one could reduce the particle size to few hundred nanometers range. Therefore, whatever method which is used for the production of nanosuspension, a careful evaluation of the type and concentration of the stabilizer is a critical stage for the successful production of nanosuspension. Both polymeric and surfactant stabilizers can be used for this purpose.[11] 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. The key difference from conventional formulations of suspensions is that the particle size distribution of the solid particles in nanosuspensions is usually less than 1 µm (i.e. 0.1nm-1000nm), with an average particle size range between 200–600 nm. On the other hand, the particle diameter required in most good pharmaceutical suspensions, is 1 to 50 µm. In nanosuspensions, the overall bioavailability is improved by an increase in surface area and

saturation solubility via particle size reduction. This system cannot be achieved by the conventional milling techniques (Figure 1).[2]

Major Advantages of Nanosuspensions[6] (Table 1)

- Its general applicability to most drugs and its simplicity.
- Can be applied for the poorly water soluble drugs.
- Can be given by any route.
- Reduced tissue irritation in case of subcutaneous/intramuscular administration.
- Rapid dissolution and tissue targeting can be achieved by IV route of administration.
- Oral administration of nanosuspensions provide rapid onset, reduced fed/fasted ratio and improved bioavailability.
- The absorption from absorption window of the drugs can be increased, due to reduction in the particle size (Figure 2).
- Higher bioavailability and more consistent dosing in case of ocular administration and inhalation delivery (Figure 3 & 4).
- 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.
- Ease of manufacture and little batch-to-batch variation.
- Long term physical stability (Due to absence of Ostwald ripening).
- Nanosuspensions can be incorporated in tablets, pellets, hydrogel and suppositories are suitable for various routes of administration.
- Increasing the amorphous fraction in the particles, leading to a potential change in the crystalline structure and higher solubility.
- Possibility of surface-modification of nanosuspension for site specific delivery.
- Possibility of large-scale production, the pre-requisite for the introduction of a delivery system to the market.

Formulation Considerations

Stabilizer[6]

Stabilizer plays an important role in the formulation of nanosuspensions. In the absence of an appropriate stabilizer, the high surface energy of nanosized particles can induce agglomeration or aggregation of the drug crystals. The main function of a stabilizer is to wet the drug particles thoroughly, and to prevent Ostwald's ripening and agglomeration of nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behavior of nanosuspensions. In some cases, a mixture of stabilizers is required to obtain a stable 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 e.g. Cellulosics, Poloxamers, Polysorbates, Lecithin and Povidones. Lecithin is the stabilizer of choice if one intends to develop a parenterally acceptable and autoclavable nanosuspension.

Organic solvents[6]

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 (Table 2). Partially water-miscible organic solvents like glycols can be used as the internal phase of the microemulsion when the nanosuspensions are to be produced using a microemulsion as a template.

Surfactants[10]

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

Co-surfactants[6]

The choice of co-surfactant is critical when using microemulsions to formulate nanosuspensions. Since co-surfactants can greatly influence phase behavior, the effect of co-surfactant on uptake of

the internal phase for selected microemulsion composition and on drug loading should be investigated e.g. Transcutol, glycofurol, ethanol and iso-propanol - safely used as co-surfactants. Also, bile salts and Dipotassium glycerrhizinate can be used as co-surfactants.

Other additives[6]

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.

Properties of Nanosuspensions

Physical Long-term stability[6]

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. Molecules diffuse from the higher concentrated area around small particles (higher saturation solubility) to areas around larger particles possessing a lower drug concentration. This leads to the formation of a supersaturated solution around the large particles and consequently to drug crystallization and growth of the large particles. The diffusion process of the drug from the small particles to the large particles leaves an area around the small particles that is not saturated any more, consequently leading to dissolution of the drug from the small particles and finally completes disappearance of the small particles.

Internal structure of Nanosuspensions[2]

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 (Figure 5).

Adhesiveness[2]

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

Crystalline state and morphology[2]

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[5]

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 (Equation no.1), dissolution velocity increases due to increase in the surface area from micron size to particles of nanometer size.

$$dx/dt = [(D \times A)/ h] [Cs-X/V] \text{ -----Equation (1)}$$

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

There are different methods of Nanosuspensions preparation (Figure 6) [2]

- Homogenization in water (DissoCubes).
- Media milling (Nanocrystal or NanoSystems).
- 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
- Microemulsion as template

Current techniques used to obtain drug nanoparticles can be divided into two categories:

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.[1] 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. Basic challenge is that during the precipitation procedure growing of the crystals need to be controlled by addition of surfactant to avoid formation of microparticles.

Advantage

- The use of simple and low cost equipments.

Limitations

- The drug needs to be soluble in at least one solvent and the solvent needs to be miscible with non-solvent.
- Moreover, it is not applicable to the drugs, which are poorly soluble in both aqueous and non-aqueous media.

Top down techniques

The techniques in which nano size range of particles is obtained by reduction in size of larger particles.[1]

High pressure homogenization (DissoCubes)

DissoCubes are engineered using piston-gap-type high-pressure homogenizers.[6] 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 (2800-21300 psi) and up to 2000 bars with volume capacity of 40 ml (for laboratory scale). 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 (Figure 7 & 8).

Principle

In piston gap homogenizer, particle size reduction is based on the cavitation principle. A Piston-gap homogenizers like APV Gaulin types has been shown. Particles are also reduced due to high shear forces and the collision of the particles against each other. The dispersion contained in 3cm diameter cylinder; suddenly passes through a very narrow gap of 25µm. The reduction in diameter from 3cm to 25µm leads to increase in dynamic pressure and decrease of static pressure below the boiling point of water at room temperature. Due to this, water starts boiling at room temperature and forms gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure, are reached. The size of the drug nanocrystals that can be achieved mainly depends on factors like temperature, number of homogenization cycles, and power density of homogenizer and homogenization Pressure.[2] DissoCubes technology is an example of this technology developed by R.H. Muller in 1999 e.g. Omeprazole.

Advantages[2]

- It does not cause the erosion of processed materials.
- Very dilute as well as highly concentrated nanosuspensions can be prepared by handling 1mg/ml to 400mg/ml drug quantity.
- It is applicable to the drugs that are poorly soluble in both aqueous and organic media.
- It allows aseptic production of nanosuspensions for parentral administration.

Limitations[2]

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

Media milling (NanoCrystals)

This patent-protected technology was developed by Liversidge *et al.* (1992). Formerly, the technology was owned by the company NanoSystems but recently it has been acquired by Élan Drug Delivery. 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 (Figure 9). 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 <200nm is 30–60 min.[6]

Principle[6]

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 (Figure 9).

Advantages

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

A comparison of the size of naproxen crystals before and after media milling is given in Figure 10.

Limitations

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

Homogenization in non-aqueous media (Nanopure)

Nanopure is suspensions homogenized in water free media or water mixtures i.e. the drug suspensions in the non- aqueous media were homogenized at 0º C or even below the freezing point and hence are called "deep-freeze" homogenization. The results obtained were comparable to DissoCubes and hence can be used effectively for thermolabile substances at milder conditions. The nanocrystals of the drug dispersed in liquid polyethylene glycol (PEG) or various oils can be directly filled as drug suspensions into HPMC capsules or gelatin.

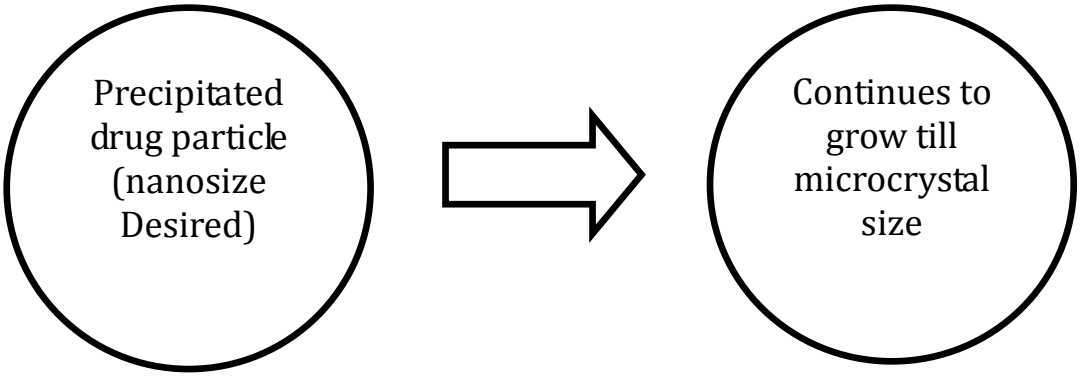
Advantages

- The dispersion medium need not be removed.
- Evaporation is faster and under milder conditions (when water and water miscible liquids are used).

- This is useful for temperature sensitive drugs.
- For i.v. injections, isotonic nanosuspensions are obtained by homogenizing in water-glycerol mixtures.[1]

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. 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.[8]



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.[2]

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 upto 4000 bar at the high velocity of 1000m/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). Dearn prepared nanosuspensions of atovaquone using the microfluidization process. The major limitation of this technique is the high number of passes through the microfluidizer (upto 75 passes) and that the product obtained contains a relatively larger fraction of microparticles[2]. A limitation of this process is the large production time.[7]

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.[1]

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.[1]

Limitations

- Use of hazardous solvents and use of high proportions of surfactants and stabilizers as compared with other techniques.
- Particle nucleation overgrowth due to transient high supersaturation, which may also result in the development of an amorphous form or another undesired polymorph.

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. Itoh *et al.* 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), Hydroxypropyl methylcellulose (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 submicron level and a stable amorphous solid can be obtained.[3]

Emulsion as template[6]

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.

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. 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 di-ultrafiltration in order to make it suitable for 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.

Advantages

- Use of specialized equipment is not necessary.
- Particle size can easily be controlled by controlling the size of the emulsion droplet.
- Ease of scale-up if formulation is optimized properly.

Limitations

- Drugs that are poorly soluble in both aqueous and organic media cannot be formulated by this technique.
- Safety concerns because of the use of hazardous solvents in the process.
- Need for di-ultrafiltration for purification of the drug nanosuspension, which may render the process costly.
- High amount of surfactant/stabilizer is required as compared to the production techniques described earlier.
- The production of drug nanosuspensions from emulsion templates has been successfully applied to the poorly water-soluble and poorly bioavailable anti-cancer drug Mitotane, where a significant improvement in the dissolution rate of the drug (five-fold increase) as compared to the commercial product was observed.

Microemulsion as template/Lipid emulsion[6]

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.

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. 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.

The advantages and limitations are the same as for emulsion templates. The only added advantage is the need for less energy input for the production of nanosuspensions by virtue of microemulsions. Also the limitation will include large amounts of surfactant or stabilizers are required.[9] e.g. Griseofulvin nanosuspension which is prepared by using water, butyl lactate, lecithin and the sodium salt of tauro-deoxycholate, where a significant improvement in the dissolution rate of the drug (three-fold increase) as compared to the commercial product was observed. It was found that the nature of the co-surfactant affected the dissolution rate of the drug nanosuspension, as anticipated. However, this technique is still in its infancy and needs more thorough investigation. For a comparative advantages & limitations with examples, see table 3.

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.

Solidification Techniques[5]

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 pelletization, 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. Microcrystalline cellulose has been successfully used to displace sucrose as a matrix former during freeze-drying of itraconazole nanosuspensions. In addition, the effect of surface hydrophobicity on drug dissolution behavior upon redispersion had been investigated, indicating the more intense hydrophobicity, the more aggregation of the nanoparticles and the slower the drug's dissolution after solidification.

Surface Modification Techniques[5]

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

Characterisation of Nanosuspension

Nanosuspensions are characterized for appearance, color, odor, assay, related impurities, particle size, zeta potential, crystalline status, dissolution studies and in vivo studies. Among this, the most important characterization techniques were discussed (Figure 11).[9]

In Vitro Evaluation[1]

Mean particle size and size distribution

Various parameters of nanosuspensions like saturation solubility, dissolution velocity, physical stability, dissolution velocity, physical stability and biological performance depend on the mean particle size and particle size distribution. Mean particle size and particle width (poly-dispersity index) can be determined by Photon Correlation Spectroscopy (PCS), laser diffraction, and coulter current multi-sizer. Poly-dispersity index (PI) should be low for the long-term stability of the nanosuspensions. A PI value of 0.1–0.25 indicates

a narrow size distribution whereas a PI value greater than 0.5 indicates a very broad distribution. Due to low measuring range (3nm to 3 µm) of PCS, determination of the contamination of the nanosuspension (by drugs having particle size greater than 3 µm) is difficult. So, to detect and quantify the microparticles that might have been generated during the production process, laser diffractometry (LD) analysis should be carried out in addition to PCS analysis. Particles ranging from 0.05–80 µm and in certain instruments particle sizes up to 2000 µm can be measured by using LD. Particle size analysis by the Coulter counter technique is essential (in addition to PCS and LD) for nanosuspensions that are intended for intravenous administration. Coulter counter is a more efficient and appropriate technique than LD analysis as it gives the absolute number of particles per volume unit for the different size classes. It quantifies the contamination of nanosuspensions by micro particulate drugs.

Particle charge (Zeta Potential)

Zeta potential determines the stability of the nanosuspension. Both the stabilizer and the drug, govern the zeta potential of a nanosuspension. A zeta potential of minimum ±30mV is required for electrostatically stabilized nanosuspension and ±20mV is required in case of electrostatic and steric stabilization.

Crystalline state and particle morphology

It is important to know the crystal morphology of the drug in the nanosuspension. Polymorphic or morphological changes in drug that occur during nano-sizing can be determined by the knowledge of crystalline state and particle morphology. Amorphous state of the drug formed during preparation of nanosuspension is determined by X-ray diffraction analysis. It gives information about the changes in the physical state of the drug particles as well as the extent of the amorphous fraction. Differential scanning calorimetry can be used additionally. Scanning electron microscopy is also used to get exact information about particle morphology. Effect of high pressure homogenization on the crystalline structure of the drug is estimated by X-ray diffraction analysis in combination with differential scanning calorimetry. Techniques like scanning electron microscopy (SEM), atomic force microscopy (AFM) or transmission electron microscopy (TEM) are preferred for determining the exact size and morphology of nanoparticles in suspension.

Saturation solubility and dissolution velocity

The dissolution velocity and the saturation solubility are enhanced by formulation of nanosuspensions. Reduction in particle size results the increased dissolution pressure and hence the solubility. Change in surface tension occurs as the solubility increases (due to particle size reduction) which lead to increased saturation solubility. Different physiological solutions at different pH and different temperatures are used to carry out the determination of the saturation solubility and dissolution velocity according to the methods reported in the pharmacopoeia. In vivo performance (blood profiles, plasma peaks and bioavailability) of the formulation is assessed by these parameters. Increase in saturation solubility can be explained by the Ostwald-Freundlich equation[16]. Determination of the dissolution velocity of nanosuspensions provides the information about the advantages of nanosuspension over conventional formulations, especially in sustained-release dosage forms.

The Ostwald-Freundlich equation is:

$$C(r) = C(\infty) \exp (2\gamma M / r\rho RT) \text{ ------Equation (2)}$$

Where C(r) and C(∞) are the solubilities of a particle of radius *r* and of infinite size. γ, M, and *ρ* are interfacial tension at the particle surface, the molecular weight of the solute, and the density of the particle, respectively.[16]

Stability

Nanosuspensions Stability depends on the particle size of the suspended particles. Decrease in the particle size to the nano range increases the surface energy of the particles, and the tendency of the particles to agglomerate increases. Therefore the stabilizers are used to decrease the chances of Ostwald ripening and to improve the stability of the suspension by providing a steric or ionic barrier. Stabilizers like cellulosic, Poloxamers, Polysorbates, lecithin, polyoleate and

Povidones are generally used in the nanosuspensions. Lecithin is preferred in the development of parentral nanosuspensions.[1] Nanosuspensions can be stored at different stress conditions like different temperature (15, 25, 35 45°C), thermal cycling, and mechanical shaking and change in their mean particle size can be followed for three months. Different concentrations of small molecule surfactants (like sodium lauryl sulfate (SLS) and dowfax 2A1 (DF)) and polymeric stabilizer (like Hydroxypropyl methylcellulose (HPMC)) can be evaluated to determine the effect of stabilizer type and micellar solubilized drug on Ostwald ripening.[13]

pH

The pH of the nanosuspension can be easily measured by using pH meter.[5]

Osmolarity

Practically, Osmolarity of nanosuspension can be measured by using Osmometer.[5]

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}. The drug content then can be calculated using the calibration curve.[5]

In Vivo Evaluation[1]

Particular drug and route of administration requires the specific in vivo evaluation of the nanosuspensions. Generally the formulations are administered by required route and the plasma drug concentrations are determined by HPLC-UV visible spectrophotometry. Surface hydrophilicity/hydrophobicity (which determines interaction with cells prior to phagocytosis), adhesion properties and the interaction with body proteins are generally evaluated by in vivo parameters. The monitoring of the in-vivo performance of the Nanosuspensions and the establishment of relationship between in-vitro release and in-vivo absorption are required in order to prepare a successful preparation, irrespective of the route of the administration and the delivery systems. Rate of dissolution influences the in-vivo biological performance of oral nanosuspensions. Size of nanoparticle and surface properties of the particles determine the organ distribution for intravenously injected nanosuspensions. The in-vivo organ distribution behavior of the nanosuspension is affected by hydrophilicity/hydrophobicity and interactions of particles with plasma proteins. Surface hydrophobicity is determined by hydrophobic interaction chromatography and absorption of protein is determined by 2-D PAGE quantitatively and qualitatively after intravenous injection of nanosuspensions of drug in animals.

Evaluation of the Surface Modified of Particles

Surface Hydrophilicity[15]

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

Adhesion properties[14]

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 2ml 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.

Interaction with body proteins[14]

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 150µl of each supernatant is placed in a test plate. Micro BCA Protein Assay Reagent Kit (150µl) then added to the supernatants and the plate, is incubated for 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.

Applications

Oral drug delivery[6]

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 GIT. Hence, a drug candidate that exhibits poor aqueous solubility and/or dissolution-rate limited absorption is believed to possess low and/or highly variable oral bioavailability. Owing to low oral bioavailability, such a drug candidate would have to be administered in a larger excess than actually required if it were completely bioavailable in order to achieve a therapeutically active concentration, thus making the therapy costly. Orally administered antibiotics such as atovaquone and bupravaquone reflect this problem very well.

Nano-sizing of such drugs can lead to a dramatic increase in their oral absorption and subsequently bioavailability. The amelioration in oral bioavailability can be attributed to the adhesiveness of the drug nanosuspension, increased surface area (due to reduction in particle size by 10–50 fold), and increased saturation solubility, leading to an increased concentration gradient between the gastrointestinal tract lumen and blood, and increased dissolution velocity. This enhancement in bioavailability will lead to a subsequent reduction in drug dose, rendering the therapy cost-effective and obliterating any undue drug dumping in the body. e.g. Danazole, a poorly bioavailable gonadotropin inhibitor, showed a drastic improvement in bioavailability when administered as a nanosuspension as compared to the commercial Danazole macrosuspension Danocrine. Danazole nanosuspension led to an absolute bioavailability of 82.3%, whereas the marketed Danazole suspension Danocrine was 5.2% bioavailable. In addition, Danazole nanosuspension resulted in a reduction in the inter-subject variability and fed/fasted ratio of danazole.

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

- Improved dose proportionality
- Reduced inter-subject variability.
- Reduced fed/fasted state variability (Figure 12)

Many poorly water-soluble molecules whose bioavailability is dissolution-rate limited, are not dose proportional. Atovaquone when formulated as suspension, shows 10-15% bioavailability in high dose (750 mg t.i.d.), while its nanosuspension shows 2.5 fold increase in bioavailability. Another important advantage in

oral drug delivery is the quick onset of action, which is seen in case of naproxen, an NSAID.

In spite of the tremendous potential of nanosuspensions in oral delivery, formulating compounds as nano-crystalline dispersions is not of value when metabolic and/or permeation-related issues affect bioavailability. However, in future, it should be possible to engineer nanosuspensions by using the agents that enhance permeation and/or minimize gut-related metabolic issues. This amalgamated approach would facilitate delivery of the compounds belonging to BCS Class IV that exhibit poor water solubility and poor membrane permeability.

Parentral drug delivery[1]

Parentral route of administration is used when rapid onset of action is required, when drug has extensive first pass metabolism or it is not absorbed by gastrointestinal tract. Parentral administration of a drug requires the drug in solubilized form or the drug with particle size less than 5 µm so that blockage of capillaries does not occur. As Nanosuspensions have size of particles in nano range so they are suitable candidates for parentral drug delivery. Various routes of parentral administration like intra-articular, intraperitonal, intravenous injection allow the administration of the drug by means of nanosuspensions suitably. Nanosuspensions overcome the problems of solubilization capacity, parentral acceptability, physical instability, high manufacturing cost and difficulties in scale-up as in other parentral formulations. Due to direct nano-sizing of the drug particles almost all drugs can be easily processed for parentral administration. They show the increased efficacy of the drug. Nanosuspensions reduce the cost of therapy and improve therapeutic performance of drug by improving the parenterally tolerable dose of drug. Enhancement in the stability of drugs has been noticed in Nanosuspensions. e.g. Clofazimine nanosuspension shows improved stability and efficacy when compared to the liposomal Clofazimine in M. avium-infected female mice. Pharmacokinetic profile and bio-distribution of the drug in nanosuspension after parenteral administration is influenced by a number of factors like physical properties of the drug particles, dose of the drug, the infusion time, the intrinsic solubility of the drug in blood, the interaction of the drug with plasma proteins, pattern of the plasma protein interaction and the phenomenon of natural targeting.

Pulmonary drug delivery[1]

Drugs that are poorly soluble in pulmonary secretions can be administered by the formulation of the nanosuspensions. These drugs are delivered as suspension aerosols or as dry powders by means of dry powder inhalers. Nebulized form of the aqueous nanosuspensions is used for the delivery of drugs to lung. Nebulization is generally done by using mechanical or ultrasonic nebulizers. Nanosuspensions could be used in all available types of nebulizer. Nanosuspensions provide following advantages over the conventional pulmonary formulations.

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

Because of the microparticulate nature and wide particle size distribution of the drug moiety present in suspension aerosols and dry powder inhalers, the following disadvantages are encountered[6]:

- Limited diffusion and dissolution of the drug at the site of action because of its

poor solubility and microparticulate nature, which may affect the bioavailability of the drug.

- Rapid clearance of the drug from the lungs because of cilliary movements.
- Less residence time for the drugs, leading to absence of prolonged effect.
- Unwanted deposition of the drug particles in pharynx and mouth.

Lung infections can be treated by nanosuspensions. e.g. Bupravaquone nanosuspensions formulated by nebulization. Nanosuspension of Budesonide has also been prepared successfully for pulmonary delivery. It shows a good relationship between the drug concentration in the formulation and the number of micrograms of drug delivered per actuation.

Ocular drug delivery[1]

Nanosuspensions can be explored for the drugs that exhibit poor solubility in lachrymal fluids. Nanosuspensions provide the following benefits for ocular drug delivery.

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

The efficacy of the nanosuspensions depends on the intrinsic solubility of the drug in lachrymal fluids. Thus the release and ocular bioavailability of the nanosuspension is governed by intrinsic dissolution rate of the drug in lacrimal fluids. Suspensions may not give the consistent performance as the intrinsic dissolution rate of the drug varies because of the constant inflow and outflow of lachrymal fluids. Nanosuspension is an ideal approach for ocular delivery of hydrophobic drugs as they improve the saturation solubility of the drug.

Nanosuspensions can be formulated using various types of polymers e.g. polymeric nanosuspension of Ibuprofen for ophthalmic controlled delivery which has been prepared using Eudragit RS100 by a quasi-emulsion and solvent diffusion method. Nanosuspensions of glucocorticoid drugs; hydrocortisone, prednisolone and dexamethasone show improved shelf-life and the bioavailability after ophthalmic application due to enhanced rate of drug absorption.

Topical formulations[1]

Creams and water-free ointments can be formulated by the incorporation of the drug nanoparticles into the formulations. In the topical dosage form, saturation solubility can be enhanced by the use of nano-crystalline form of the drug. It enhances the diffusion of the drug into the skin.

Drug targeting[1]

Nanoparticulate systems have shown great potential in targeting of the drugs, especially to the brain targeting. As the surface properties and in-vivo behavior of nanosuspensions can be altered easily by changing either the stabilizer or the milieu, so they are good candidates for targeted delivery. Commercially viable nanosuspensions for targeted delivery are developed due to versatility of the nanosuspension, easy scale-up and commercial production of the nanosuspensions. Targeting of the drug to the brain can be achieved by modifying the surface of nanoparticles by using suitable polymers. e.g. Brain targeting of peptide dalargin has been done successfully by the modification of the nanoparticle surface using poly-isobutyl cyanoacrylate. Active or passive targeting of the desired site by using various surface coatings (the engineering of stealth nanosuspensions) is the future of targeted drug delivery systems. Various types of targeting has been achieved successfully like targeting of *Cryptosporidium parvum* (the organism responsible for cryptosporidiosis) by using surface modified mucoadhesive nanosuspensions of bupravaquone, and pulmonary aspergillosis can easily be targeted by amphotericin B, in the form of pulmonary nanosuspensions instead of using stealth liposomes. Gastrointestinal bacteria and parasitic infections can be targeted due of enhanced adhesion properties.

Mucoadhesion of the nanoparticles[1]

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

Bioavailability enhancement[1]

Poor solubility, poor permeability or poor stability of a drug in the gastrointestinal tract (GIT) renders the poor oral bioavailability of the drug. Nanosuspensions enhance the bioavailability by increasing the solubility and permeability of the drug across the membrane. e.g. Bioavailability of oleanolic acid (poorly soluble drug), a hepatoprotective agent, has been improved by formulating a nanosuspension which was proven by the significantly enhanced therapeutic effect. The enhanced bioavailability was found due to the faster dissolution (90% in 20 min) of the lyophilized nanosuspension powder when it was compared with the dissolution from a coarse powder (15% in 20 min).

Patents on Nanosuspensions

Because of such a versatile technology of nano-sizing, there are many patents on this technology (Table 4).

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.

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 5 summarizes current marketed nanosuspension formulations while table 6 & 7 summarizes the New Drug Application Based on Nanosuspensions Technique Reported and Marketed by now.

Case Studies

Case 1

Polymeric nanosuspensions were prepared from Eudragit RS100 and RL100 polymer resins and loaded with Flurbiprofen (FLU), with the aim at improving the availability of the drug at an intra-ocular level for the prevention of the miosis induced during extracapsular cataract surgery. Nanosuspensions were prepared by a quasi-emulsion solvent diffusion technique using different formulation parameters (drug to polymer ratio, initial polymer concentration, agitation speed, etc.). The resulting nanoparticles showed mean sizes around 100 nm. The drug was incorporated with very high yields in the polymer matrices. One formulation was tested in the rabbit compared to a commercial eye-drop product containing an equivalent amount of FLU sodium salt. The incorporation of the drug in the polymer system enhanced FLU antagonizing activity against the miosis induced by a surgical trauma to the eye anterior chamber and increased its active

concentration in the aqueous humour. For the possibility of modulating the preparation conditions and the stability shown upon storage, as the original suspensions or after freeze-drying, as well as for the very good tolerability, the described formulations may be useful in clinical practice to maintain mydriasis during cataract or other eye surgical treatments.[19, 20]

Case 2

Paclitaxel is a diterpenoid isolated from *Taxus brevifolia*. It is effective for various cancers, especially ovarian and breast cancer. Due to its aqueous insolubility, it is administered dissolved in ethanol and Cremophor® EL (BASF, Ludwigshafen, Germany), which can cause serious allergic reactions. In order to eliminate Cremophor® EL, Paclitaxel was formulated as a nanosuspension by high-pressure homogenization. The nanosuspension was lyophilized to obtain the dry Paclitaxel nanoparticles (average size, 214.4 ± 15.03 nm), which enhanced both the physical and chemical stability of Paclitaxel nanoparticles. Paclitaxel dissolution was also enhanced by the nanosuspension. The pharmacokinetics and tissue distribution of Paclitaxel were compared after intravenous administration of Paclitaxel nanosuspension and Paclitaxel injection. Paclitaxel injection showed reduced area under the concentration, greater clearance, and shorter elimination half-life compared with the Paclitaxel solution, while In contrast, the Paclitaxel nanosuspension resulted in a significantly greater $AUC_{0-\infty}$ in liver, lung, and spleen, but not in heart or kidney.[21, 22, 23]

Case 3

Omeprazole is a proton pump inhibitor, which is used for the treatment of peptic ulcers, reflux esophagitis and Zollinger-Ellison syndrome. It is a poorly soluble, chemically labile drug with a high degradation rate in aqueous media. Of course, to yield an intravenously injectable product, a nanosuspension rather than a macrosuspension needs to be produced. The possibility of protecting Omeprazole from degradation by using the DissoCubes technique was investigated. This technique is suitable for producing particulate drug formulations in order to protect chemical labile drugs from degradation. The drug nanosuspensions are easy to produce and, show excellent chemical stability compared to drug solutions. It is possible to produce highly concentrated nanosuspensions, which are chemically stable and protected from degradation. The high performance liquid chromatography analysis has proved the predominance of the nanosuspension produced by high pressure homogenization in comparison to an aqueous solution. Even 1 month after production no discoloration or drug loss was recognizable when the nanosuspension was produced at 8° C. As a result it can be stated that the production of nanosuspensions by high pressure homogenization is suitable for preventing degradation of labile drugs[24] (Figure 13).

CONCLUSION

Nanosuspensions appear to be a unique and yet commercially viable approach to combating problems such as poor bioavailability that are associated with the delivery of hydrophobic drugs, including those that are poorly soluble in aqueous as well as organic media. Production techniques such as media milling and high-pressure homogenization have been successfully employed for large-scale production of nanosuspensions. The advances in production methodologies using emulsions or microemulsions as templates have provided still simpler approaches for production but with limitations. Further investigation in this regard is still essential. Attractive features, such as increased dissolution velocity, increased saturation solubility, improved bioadhesivity, versatility in surface modification and ease of post-production processing, have widened the applications of nanosuspensions for various routes. The applications of nanosuspensions in parentral and oral routes have been very well investigated and applications in pulmonary and ocular delivery have been realized. However, their applications in buccal, nasal and topical delivery are still awaiting exploration. The development of stealth nanosuspensions laced with functionalized surface coatings capable of

eliciting passive or active targeting as per the requirement can be regarded as the future step in the nanosuspension research.

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Table 1: Advantages of nanosuspensions over conventional formulations[2]

Route of Administration	Disadvantages of Conventional Formulations	Benefits of Nanosuspensions
Oral	Slow onset of action/ poor absorption	Rapid onset of action/ improved solubility so improved bioavailability Reduced fed/fasted ratio
Ocular	Lacrimal wash off/ low bioavailability	Higher bioavailability/ dose consistency Lesser irritation
Intravenous	Poor dissolution/ non-specific action	Rapid dissolution/ tissue targeting Prolonged retention time in systemic circulation
Intramuscular	Low patient compliance due to pain	Reduced tissue irritation High bioavailability Rapid onset of action
Inhalations	Low bioavailability due to low solubility	Rapid dissolution/ high bioavailability/ dose regulation

Table 2: Various examples of solvents used in nanosuspension formulation

Type of solvent	Name	Remarks
Water-miscible solvents	Ethanol, Iso-propanol	Pharmaceutically acceptable, less hazardous
Partially water-miscible solvents	ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate, benzyl alcohol	Preferred in the formulation over the conventional hazardous solvents, such as dichloromethane.

Table 3: Advantages and disadvantages of various preparation techniques of nanosuspensions[8]

Method	Advantages	Limitations	Drug
High-pressure Homogenization	widely applying regions, ease of scale-up and little batch to batch variation, narrow size distribution in the final product, allowing aseptic production of nanosuspensions for parenteral administration and flexibility in handling the drug quantity	pretreatment of micronized drug particles and pre-suspending materials before subjecting it to homogenization	Albendazole Amphotericin B Aphidicolin Atovaquone Azithromycin Bupravaquone Clofazamine Fenofibrate Glucocorticoid drugs
Milling	the same as those for high-pressure	potential erosion of	Cilostazol

	homogenization	material from the milling pearls	Danazol Naproxen
Microprecipitation	low need of energy, stable products and simple process	narrowly applying space, wide size distribution and potential toxicity of non-aqueous solvents	Carbamazepine Cyclosporine Griseofulvin Retinoic acid
Emulsion and Microemulsion	low need of energy, stable products, simple process, small size of particles and uniform particle distribution	high concentration undesired surfactants and residual solvents	Breviscapine Griseofulvin Ibuprofen Mitotane
Microprecipitation -high pressure homogenization	much smaller, more uniform and more stable compared to that by the Microprecipitation; less mechanical force and energy compared with the high-pressure homogenization	The manufacturing process is complicated	___
Dry Co-grinding[5]	Easy process No organic solvent Require short grinding time	Generation of residue of milling media	Clarithromycin Glibenclamide Glisentide Griseofulvin Naproxen Nifedipine Phenytoin Pranlukast

Table 4: Overview of the technologies and patents / patent applications on which the various homogenization processes are based[6]

Nanocrystal	Company	Patent/patent application examples
Hydrosol	Novartis (Prev. Sandoz)	GB 22 69 536 GB 22 00 048
Nanomorph™	Soligs/Abbott	D 1963 7517
NanoCrystal™	Élan NanoSystems	US 5,145,684
DissoCubes®	SkyePharma	US 5,858,410
Nanopure	PharmaSol	PCT/EP00/0635
Nanoedge™	Baxter	US 6,884,436

Table 5: Current Marketed Pharmaceutical Products Utilizing Nano-crystalline Formulation[5]

Product	Drug compound	Indication	Company	Nanoparticle technology
RAPAMUNE®	Sirolimus	Immunosuppressant	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

Table 6: The New Drug Application Based on Nanosuspensions Technique Reported and Marketed by Now[5]

Drugs	Indication	Author or company	Route	Status
Danazol	Hormone	Rogers T.L.	Oral	Reported
Paclitaxel	Anticancer	American Bioscience	Intravenous	Marketed
Naproxen	Anti-inflammatory	Anchalee Ain-Ai	Oral/parentral	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	Intravenous	Reported
Buparvaquone	Antibiotic	Muller R. H.	Oral	Reported
Oridonin	Anticancer	Lei Gao	Intravenous	Reported
Ascorbyl palmitate	Ascorbyl palmitate	Veerawat T.	Intravenous	Reported
Prednisolone	Glucocorticoid	M.A. Kassem	Ophthalmic	Reported
Dihydro-artemisinin	Antimalarial	Jiraporn C.	Intravenous	Reported
Cilostazol	Anti-platelet agent	Jun-ichi Jinno	Oral	Reported
Carbamazepine	Psychotolytic	D. Douroumis	Oral	Reported
Omeprazole	Proton pump inhibitor	Jan Moschwitzer	Intravenous	Reported
Mitotane	Adrenal cortex hormone	M. trotta	Oral	Reported
Griseofulvin	Antifungal	Boris Y. Shekunov	Oral	Reported

Table 7: The New Drug Application Based on Nanosuspensions Technique Reported and Marketed by Now[12]

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

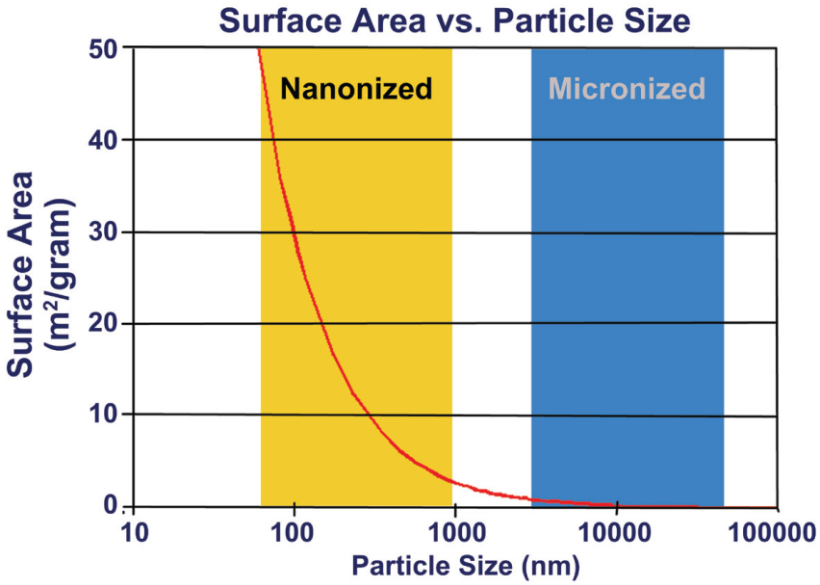

Figure 1[4]: the plot demonstrates the increase in surface area obtained when solids are fractured from the micron-size range (microparticles) to the nanometer-size particles used in the various nanoparticle formulations to improve the performance of poorly water-soluble compounds.

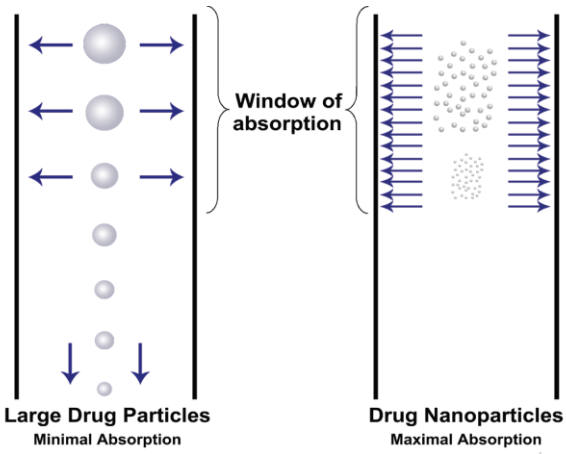

Figure 2[4]: the diagram demonstrates one of the primary issues associated with poorly water-soluble molecules whose bioavailability is dissolution-rate limited. On the left, large drug particles cannot adequately dissolve, which results in the inability to be absorbed. On the right, nanometer drug particles are rapidly dissolved during transit through the gut, thus maximizing absorption and improving bioavailability.

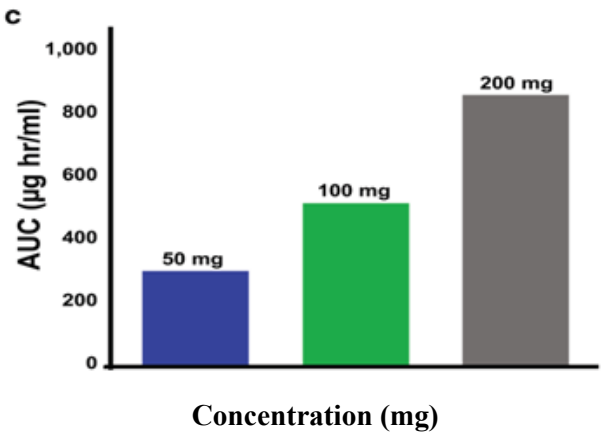

Figure 3[4]: A dose-escalation study is shown demonstrating dose proportionality for a nanoparticle formulation of a poorly water-soluble compound.

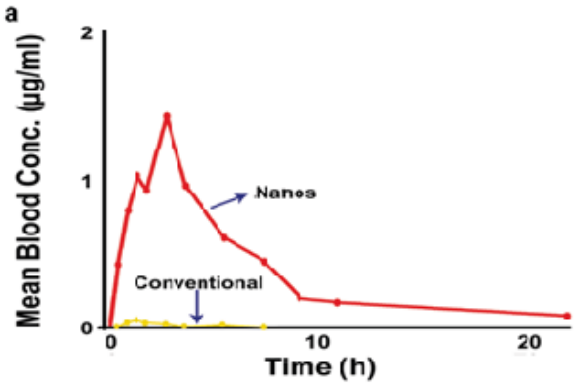

Figure 4[4]: The bioavailability of a poorly water-soluble model compound formulated as a nanoparticle dispersion (red) or as a conventional crude suspension (yellow).

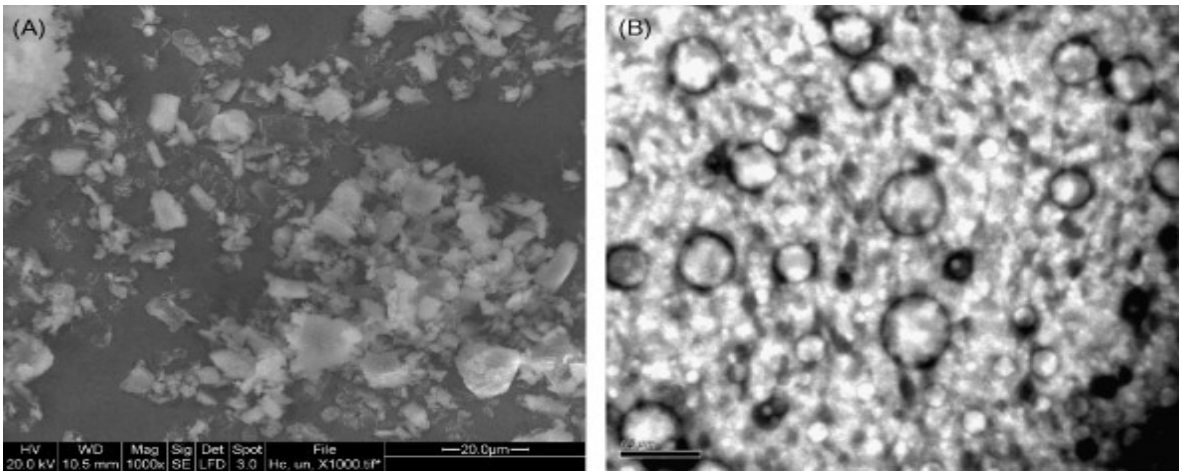

Figure 5[17]: (A) SEM micrograph of unprocessed hydrocortisone.
(B) TEM micrograph of nanosuspension.

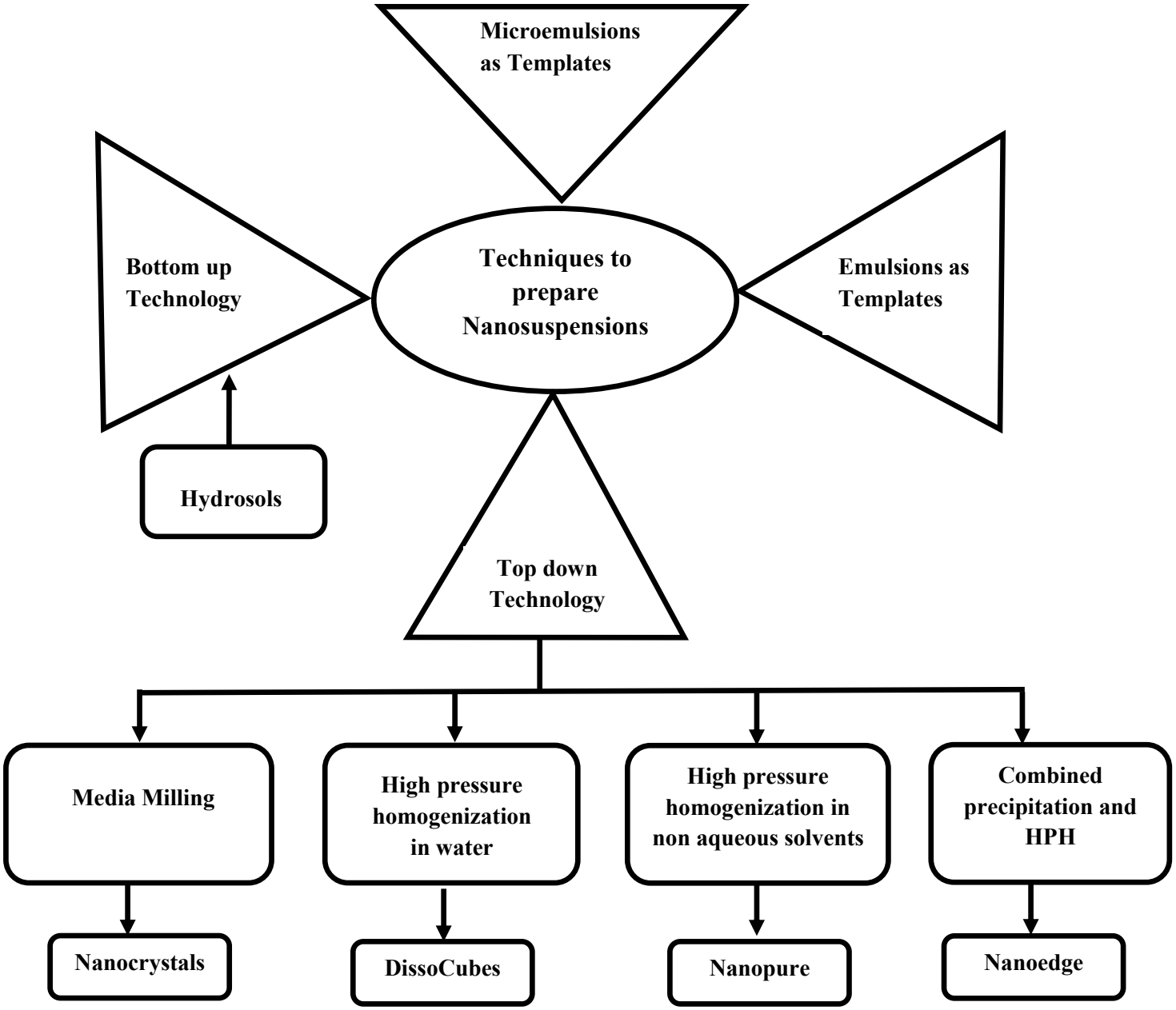

Figure 6: Various methods for preparation of nanosuspensions[1]

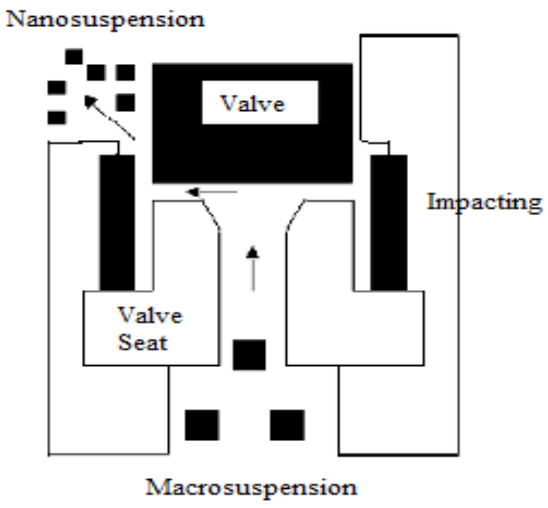

Figure 7:

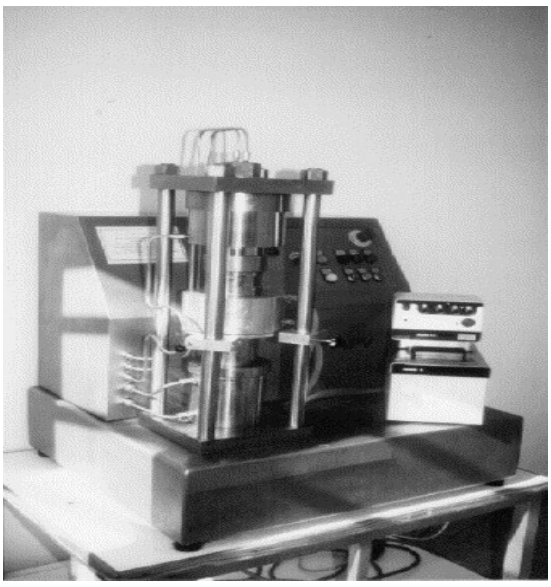

Figure 8:

Figure 7: Schematic representation of the high-pressure homogenization process.

Figure 8: Piston-gap homogenizers like APV Gaulin types - APV LAB 40 (APV Deutschland GmbH, Lubeck, Germany).[15]

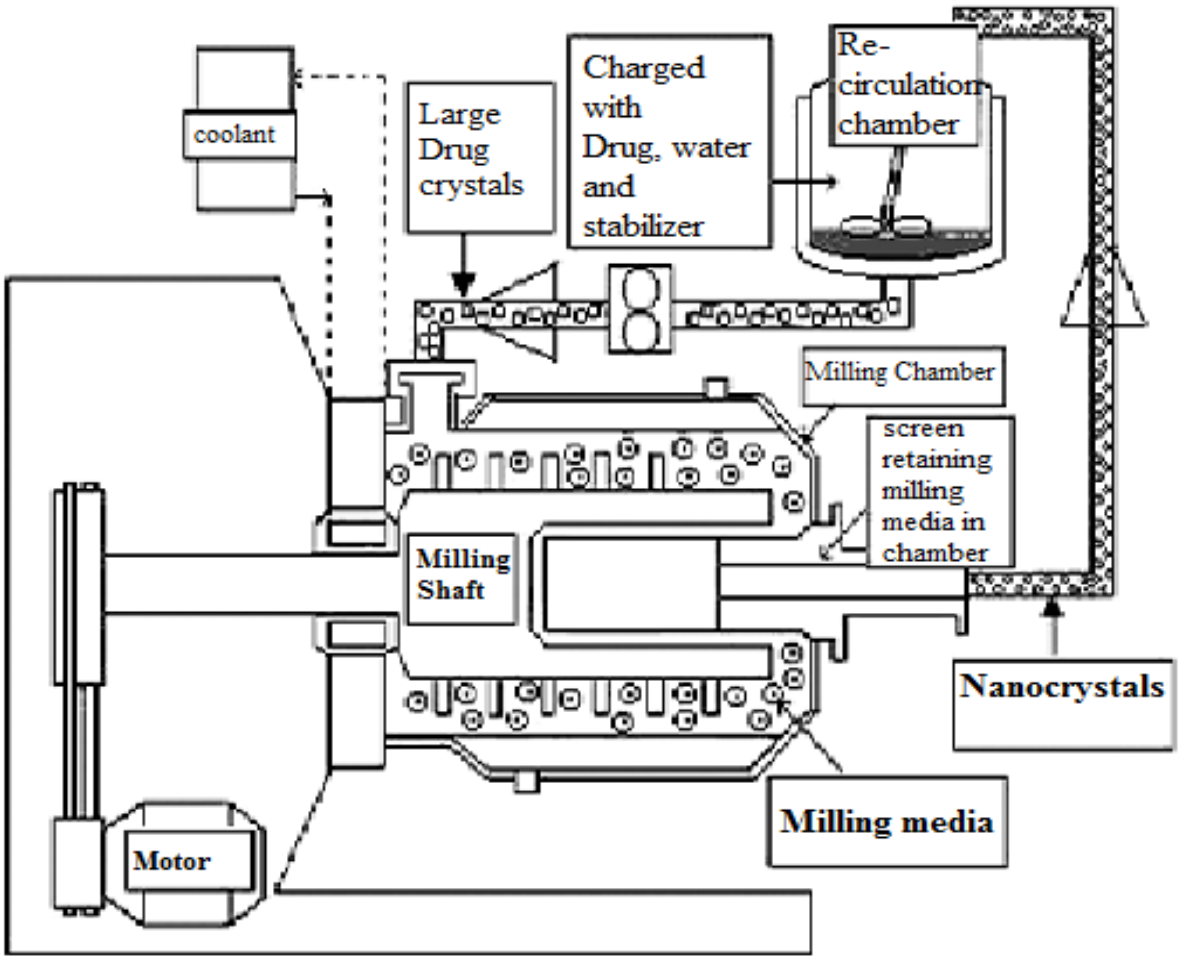

Figure 9: Schematic representation of the media milling process.[1]

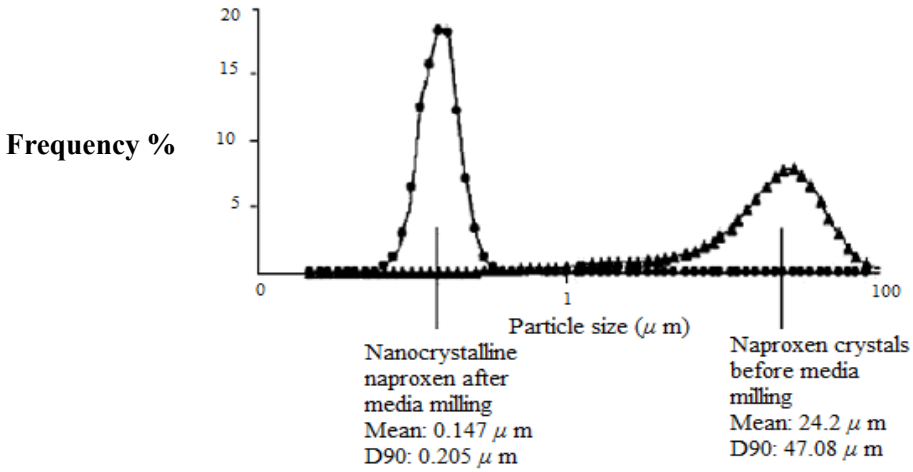

Figure 10: The particle size distribution of naproxen crystals before (▲) and after (●) milling. Before milling the drug crystals had a mean particle size of 24.2 µm. After being processed for 30 min in a media mill, the mean particle size of the nano-crystalline dispersion was 0.147 µm with D_{90} = 0.205 µm. The particle size measurements were generated using laser light diffraction in a Horiba LA-910 using polystyrene nanospheres ranging from 0.1 to 10 µm as standards.

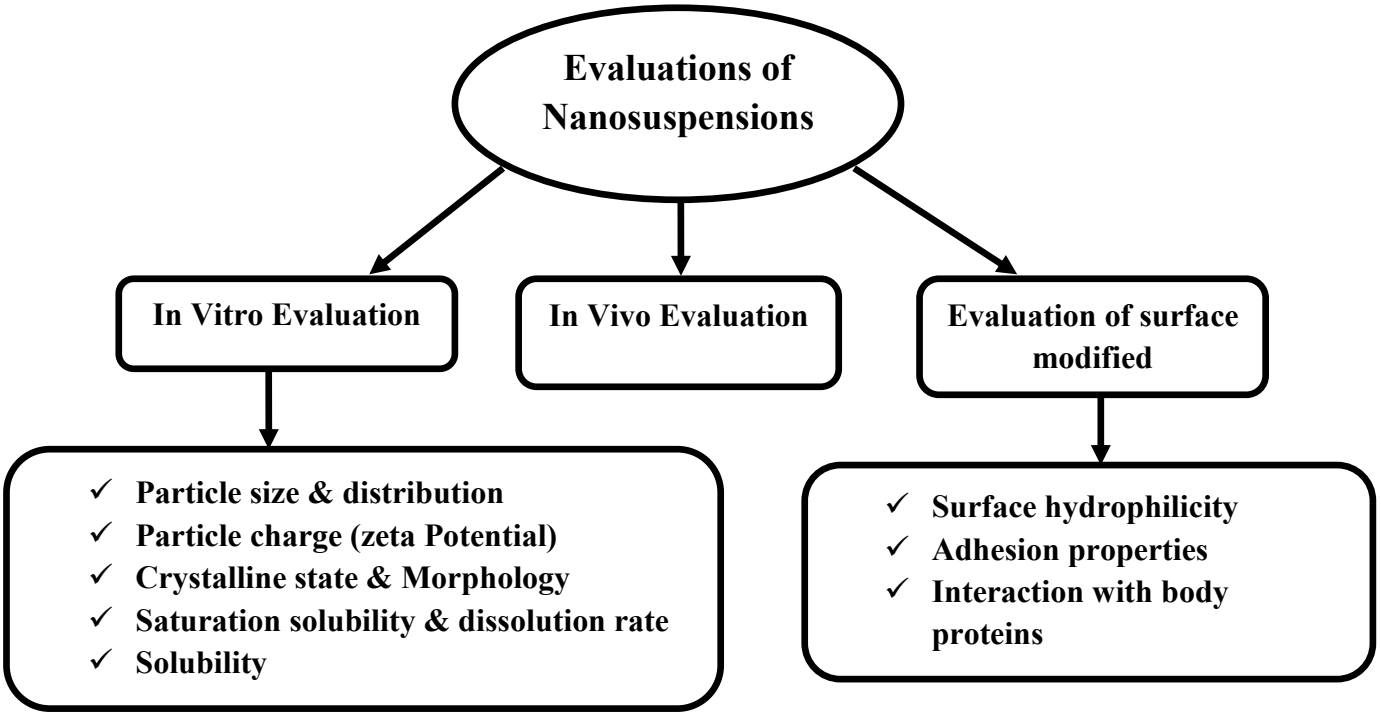

Figure 11: Flowchart showing various methods for characterization of nanosuspensions

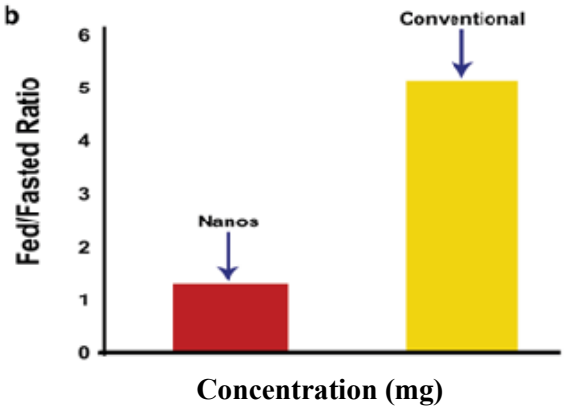

Figure 12[4]: The bar graphs show the comparison in the fed/fasted variation in bioavailability of a model compound when formulated as nanoparticle dispersion (red) or as a crude suspension (yellow).

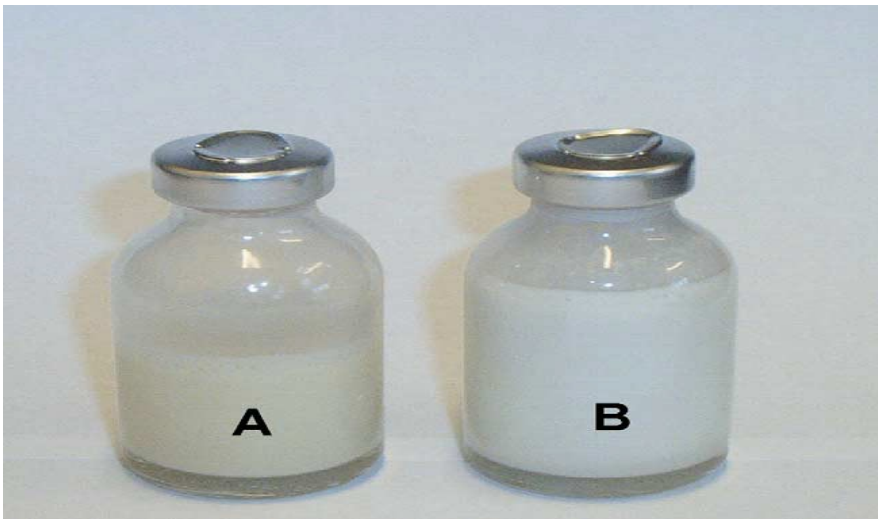

Figure 13: Discoloration during production process.
(A) Production at room temperature,
(B) Production at 8ºC.

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