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Review Article

Nanosizing Approaches: Current Trends in the Solubility Enhancement of Poorly Water-soluble Drugs

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ABSTRACT

Nanosuspension formulation and production techniques are currently undergoing remarkable growth in pharmaceutical industries to address poor aqueous solubility and low bioavailability of active pharmaceutical ingredients. It has some distinct character and benefits over other methodologies used to enhance aqueous solubility. The controlled process including formulation and processing variables provide the requisite shape and size of the drug particles in the form of nanosuspension. Generally, drug particles in the form of nanosuspension can be obtained in the size range of 200 to 1000 nm by manipulating various parameters. The pharmacokinetic parameters of the drugs can also be altered by changing the size of drug particles by developing them in the form of nanosuspension. The current review aims to address the physico-chemical characteristics of nanosuspensions, including various formulation considerations. This review also includes potential applications and recent advancements in the field of nanosizing technology.

Keywords: Nanoparticles, Nanosuspension, Nanosizing approaches, Saturation solubility, Pharmacokinetics.

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INTRODUCTION

The pharmaceutical market comprises various therapeutic classes of drugs being developed in large capacity by pharmaceutical industries, however poor aqueous solubility and low GI permeability are the biggest hurdles leading to poor bioavailability and compromised efficacy. The current formulation challenges cannot be addressed by conventional formulation approaches because they are unable to enhance the dissolution of poorly water-soluble drugs. The conventional solubility-enhancing approaches suffer from known limitations such as undesirable drug release patterns, some unwanted effects as well as toxicity.[1] Nanotechnology, a potential tool for various novel and smart drug delivery systems with uniform and required shapes and sizes as well as acceptable release patterns, can solve the problem encountered with conventional drug delivery systems. It helps in the development of safe and effective nanomedicines.[2] The solubility, release pattern, and undesirable side effects or toxicity can be easily altered, and targetability can be achieved after using nanotechnology in formulation development. In the last two decades, outstanding progress has been noticed in the drug delivery and imaging area using various nano vectors in the pharmaceutical field; for example, liposomes, micro and nanoparticles have been the subjects of intense research and development. In the current era, nanosizing is hastily growing and becoming a potential approach due to the continuous efforts of scientists and researchers from different fields.

Nanosizing is an approach in which the particle size of drug substances is reduced to the submicron or nano range. The particles (nano or micron range) thus obtained can be further stabilized with the application of different surfactants and polymers, which help to retard the regrowth of the particles. Thus, it can be formulated in the form of any dosage form, such as tablets, capsules, and suspensions for oral administration. The resulting formulations show high dissolution and solubility of drug substances.[3] Among various nanosizing techniques, the probability to formulate a stable suspension of the plain drug with a uniform nano-size range of particles using different new approaches and equipment is more. It minimizes the use of other excipients in the formulation in very high concentration.[4] It requires a very minimum concentration of suitable surfactants

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or suspending agents to stabilize the in aqueous or non-aqueous media.[5] A conventional pharmaceutical suspension may be a coarse dispersion in which uniform particles are dispersed in any vehicle. The absorption of the medicine from GIT or extracellular fluid to cells of different organs particularly depends upon their solubility within the extra and intracellular fluid, their partitioning within the cell membrane, and developed concentration gradients with very low drug concentration. Varieties of formulations of nano- or microsized drugs have been developed to overcome these problems which are shown in Figure 1.

Formulation development with poorly water-soluble drugs is a challenging task for pharmaceutical industries, and this challenging task is further augmented when drugs are also insoluble in nonaqueous media (Figure 2). In the condition where the drug substances are insoluble in both aqueous and nonaqueous media, nanosuspension technology is very simple and easy and plays an important role in enhancing the solubility of drugs in both media.[6-8]

Nanosuspension is a dispersion of nanosized (below 1 µm) or colloidal range drug particles preferably below 200 nm that are dispersed and stabilized by a stabilizer in any liquid vehicle. It can be prepared by different methods and delivered by different routes of administration, mainly oral, nasal, ocular, and parenteral routes (Figure 2).[9] This review highlights the physico-chemical background of nanosuspensions. This review also illustrates potential applications and recent advancements in the field of nanosizing technology.

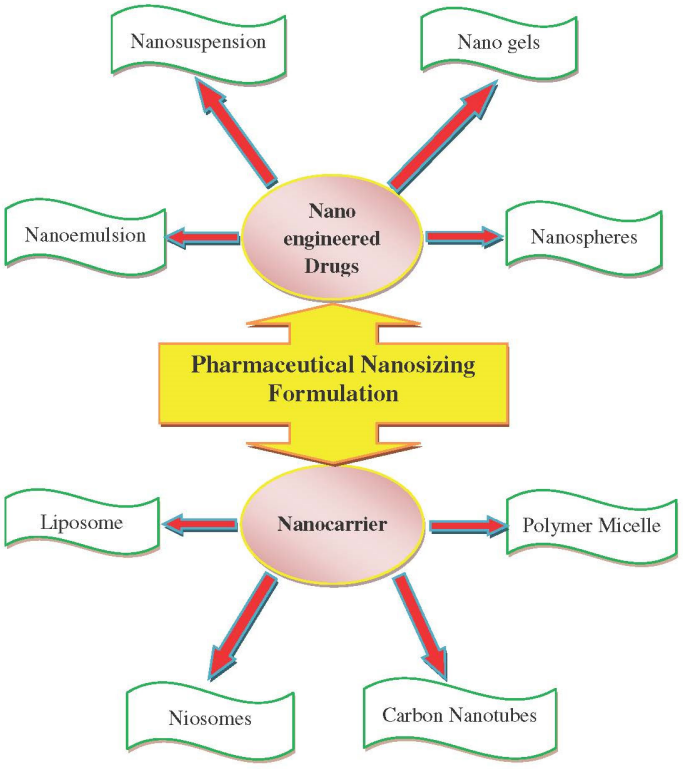

Figure 1: The types of pharmaceutical nano-formulations.

PHYSICO-CHEMICAL BACKGROUND OF NANOSUSPENSIONS

Nanosuspensions were developed to enhance the dissolution or solubility of the drug to increase the bioavailability and reduce the dose and cost of the formulation[10] It is a new strategy that can help to overcome the drawbacks of conventional drug delivery systems, such as biocompatibility, toxicity, and stability.[11,12] Some theoretical aspects should be applied to design an ideal nanosuspension. The reason behind solubility or dissolution enhancement through nanosuspension is that the increase in the surface of drug substances upon size reduction leads to an enhanced dissolution rate of drug particles, principally from microsizing to nanosizing, as per the Noyes-Whitney equation with slight modification according to Nerst-Brunner and Levic[13] the rate of dissolution can be expressed as given equation-1.

$$\frac{dX}{dt} = [(\frac{DA}{h}) \times (Cs - \frac{X}{V})] \text{----------(1)}$$

Where dx/dt is the rate of dissolution, 'A' is particle surface area, 'D' is the diffusion coefficient, 'h is' the thickness of the diffusion layer, 'Cs' concentration of the drug at saturable level, 'X' is the concentration of drug in bulk media and 'V' is the total volume of the dissolution media.

Moreover, saturation solubility can be enhanced by minimizing the particle size of the drug. The saturation solubility of the drug is limited in the case of coarse powders, which is affected by the concentration of the drug substance, the volume of dissolution media, and temperature. The saturation solubility is size-dependent, as postulated by the Ostwald-Freundlich equation. On decreasing the particle size of drug substances, the increase in the saturation solubility concept is clearly explained by the theory of Ostwald-Freundlich's equation-2.

$$\log(\frac{Cs}{C}) = (\frac{2V}{2.303}) \times RTr \text{----------(2)}$$

where Cs represents the saturated drug concentration, 'C' and 'V' is the solubility and particle volume of large particles, R is the gas constant, T is the absolute temperature and r is the radius.

The Kelvin equation generally expresses the changes in the physical form of drug substances from liquid to gas and this principle is utilized to describe the phase transition of drug molecules from solid nanocrystal to solution form with a suitable dissolution media.[14] Enhancement in saturation solubility may lead to enhanced concentration gradients across the GI membrane, resulting in improved drug transport from the GI lumen to systemic circulation for a drug that is otherwise poorly absorbed in the gastrointestinal tract.[15]

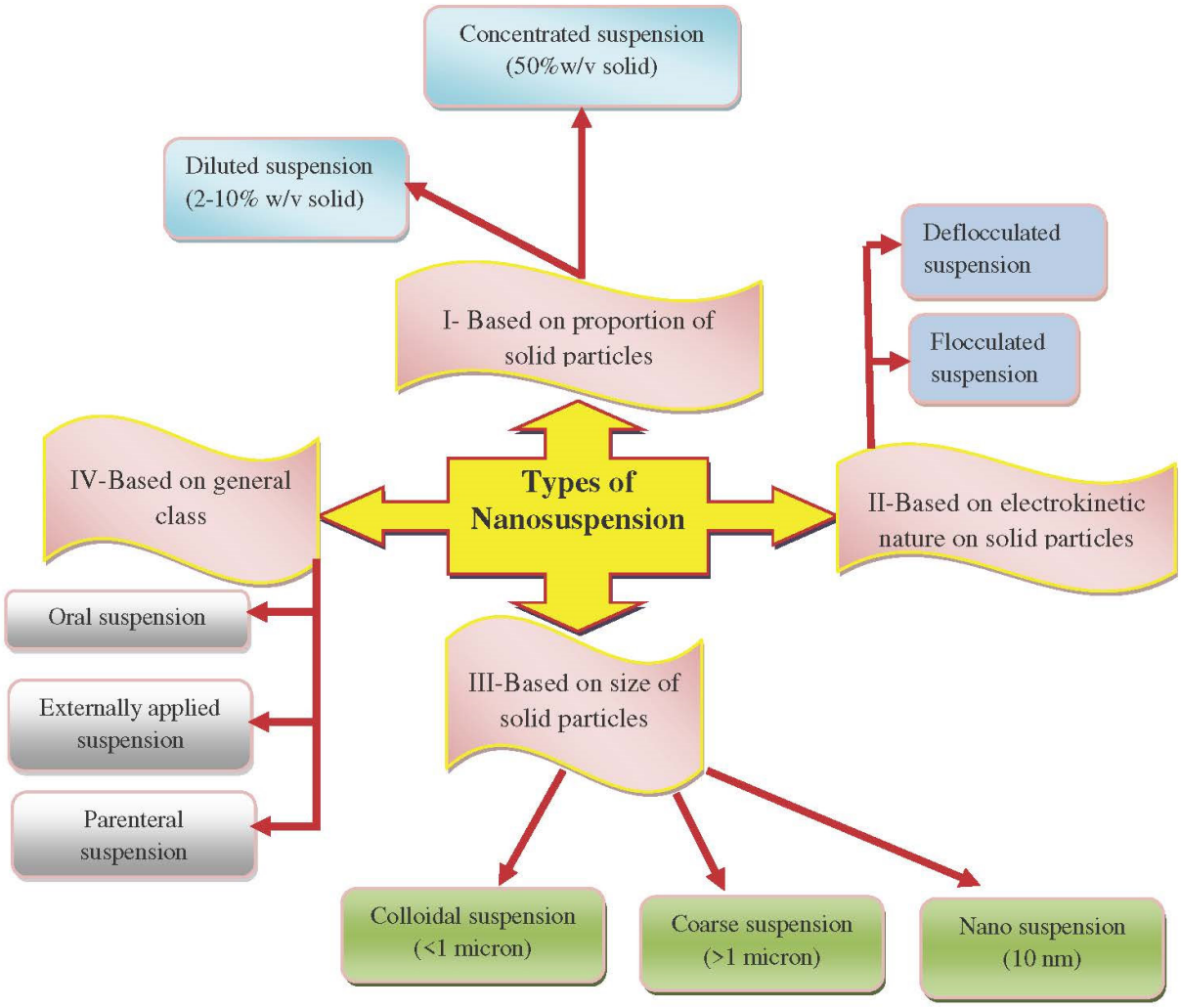

Figure 2: Types of nanosuspensions.

PROPERTIES OF NANOSUSPENSION

Nanosuspensions show interesting properties that can be described as (i) Upon nanonization of drug substances, a large surface area is available for solvent action, resulting in high dissolution and saturation solubility of the drug, (ii) The use of stabilizers (polymers or surfactants) with the amorphous form of active ingredient further improves the stability of drug particles and minimizes the chances of Ostwald ripening, (iii) A change in the physical state from crystalline to amorphous after the nanonization process gives a better solubility profile, (iv) The use of stabilizers (polymers or surfactants) with the amorphous form of active ingredient further improves the stability of drug particles and minimizes the chances of Ostwald ripening, and (v) Surface modification of drug particles of nanosuspension can be easily achieved by conjugating a targeting moiety directly on the particle surface with the help of a suitable linker, and site-specific delivery can be achieved.[17]

Merits of Nanosuspensions

Nanosuspension technology is a potential tool not only for solubility enhancement but also offers the hope of developing formulations of potent drugs that cannot be formulated in conventional dosage forms by conventional methods. Nanosuspension provides some meritorious attributes, as shown in Figure 3.[18-20] It provides ease of manufacturing due to its simple production method, lower fed/fasted variability, provide a high dissolution rate and high saturation solubility for both poorly aqueous and nonaqueous soluble drugs. It also improved the biological compatibility of the drug by minimizing toxicity and undesirable side effects and improved the physiochemical stability of drug substances due to the presence of stabilizers. Nanosuspension has the highest adsorption to epithelial cells or tissue. It can incorporate and formulate nanosuspensions in the form of tablets, capsules, suspensions, hydrogels, etc… It may enhance retention time on the mucus membrane of the GI tract because its nanosized particles increase the likelihood of absorption and augment bioavailability. In addition, it improves

dose proportionality or increased drug loading and provides better patient compliance.

Demerits of Nanosuspensions

Some of the disadvantages of nanosuspension formulations are (i) In the form of a suspension, cake formation after the sedimentation of drug particles is a common problem, and (ii) Nanosuspension formulation is massive. In addition, Uniformity and accuracy in dose cannot be achieved unless the suspension is stabilized.

FORMULATION CONSIDERATION

It is necessary to have the proper knowledge to select excipients for the formulation of nanosuspensions. The important excipients that are generally used in the formulation of nanosuspensions are suitable stabilizers, such as polymers, surfactants, cosurfactants, and solvents (Table 1).[16,17]

Stabilizers

Stabilizers play a very significant role in nanosuspension formulation. This helps to reduce the surface free energy of particles and prevent particles from agglomerating. It works on three mechanisms: the wetting of drug particles, the development of a steric or an ionic barrier, and the retardation of Ostwald's ripening. The physical stability and *in vivo* behavior of the nanosuspension depend on the selected stabilizer type and quantity for formulation. Some stabilizers most preferably used for the formulation development of nanosuspensions are poloxamers, polysorbates, povidones, some types of biocompatible cellulose, and lecithins. Lecithin is a chemically stable excipient upon autoclaving and is also suitable for the development of nanosuspensions for parenteral administration.[18]

Figure 3: Advantages of drug nanosuspensions.

Solvents

If the drug is a potential target for the development of drug nanosuspensions and is not soluble in water, then organic solvents are the second choice. It is required for nanosuspension that the drug should be soluble in any of the solvents, either aqueous or nonaqueous. Organic solvents may be required if nanosuspensions are prepared by the emulsification method. Solvents are usually selected based on their toxicity and evaporation rate. Water-miscible and poorly water-miscible solvents, such as isopropanol, ethanol, ethyl acetate, and benzyl alcohol, are generally preferred in these techniques to prepare nanosuspensions.[18,19]

Cosurfactants

The selection of a cosurfactant is important in the case of the microemulsion method used to formulate nanosuspensions of drugs. It influences the phase behavior of the microemulsion. Commonly employed cosurfactants, such as bile salts and dipotassium glycyrrhizinate, and a variety of surfactants, including transcutol, glycofurol, ethanol, and isopropyl alcohol (are widely used).[20]

MANUFACTURING TECHNIQUES FOR NANOSUSPENSIONS

Nanosuspension can be produced by two inverse processing techniques: the 'bottom-up' and 'top-down' technologies and both methods can also be used in combination (Figure 4). Bottom-up techniques include precipitation, Microemulsification, and melt emulsification, while top-down technologies may be a breakdown process from giant particles and include media milling (nanocrystals) techniques, high-pressure homogenization techniques (Disso 6 cubes), high-pressure homogenization techniques in nonaqueous media (Nanopure) and combined precipitation with high-pressure homogenization (Nanoedge).

Bottom-up Processes

In these techniques, nanosized drug particles are developed by the aggregation of drug molecules in a suitable vehicle. In bottom-up technology, it is required to dissolve drugs in organic solvents followed by nonsolvent (water) mixing, which results in the precipitation of drugs that can be controlled by the homogenization process. Rapid precipitation by adding an anti-solvent to the drug solution followed by high-speed homogenization is preferred to obtain small particles.

This technique involves crystal growth after nuclei formation under suitable temperature conditions, and drug substances precipitate in amorphous form from a supersaturated solution of drugs (Figure 5).[21] This method is very simple, cost-effective, and provides a high saturation solubility of the product. However, it is necessary for drug substances to be soluble in at least a solvent,

either aqueous or non-aqueous. The solvent residue present in the final product is another issue with this technique.

Controlled precipitation or microprecipitation

The precipitation method is a general method used for nanosizing poorly soluble drugs. Nanosuspension developed by this method using a surfactant as a stabilizer in which drug substances are required to dissolve in a suitable solvent in the presence of a stabilizer followed by the addition of another solvent (non-solvent for drug) that leads to the sudden precipitation of drug in the form of very fine particles. The precipitation of the drug occurs in this method, followed by temperature-dependent nuclear development and crystal growth. An ideal nanosuspension with a minimum and uniform particle size requires a high rate of nucleolus formation with slow crystal growth, and this process depends only on temperature.[22]

Microemulsion technique

The microemulsion technique is another way of manufacturing nanosuspensions. The microemulsion technique is employed in the case of drug substances that are soluble either in aqueous or nonaqueous solvents (highly volatile solvents are preferable). In this technique, the drug is generally dissolved in the organic solvent, and this drug solution is emulsified in aqueous media in the presence of a surfactant. The internal phase (organic solvent) is allowed to evaporate under reduced pressure, leaving behind an aqueous suspension of fine drug particles. A nanosuspension of griseofulvin was prepared using the same technique with butyl acetate as the internal phase and lecithin sodium taurodeoxicholate as the emulsifier.[23]

Melt Emulsification Methodology

The melt emulsification method is another technique for preparing nanosized formulations. Kocbek *et al.* 2006 first prepared ibuprofen nanosuspension by using this technique.[24] In this technique, ibuprofen was dispersed or dissolved in double distilled water and then heated over the melting point of the drug. Under the same temperature conditions, the solution was further homogenized at high-speed using a high-speed homogenizer to obtain a fine dispersion of the drug. Then, the fine dispersion of melted ibuprofen was allowed to cool, after which ibuprofen was precipitated in the form of very fine particles, and finally, a nanosuspension of finely divided particles of the drug in water was obtained. The size of the particles depends on the concentration of the drug and stabilizer, temperature and speed of homogenization. The organic solvent is not used in this technique, and it is the advantage of this technique over other techniques.

Top Down Technology

This technique is preferable over the precipitation method and is also known as the disintegration (size reduction) method. It includes media milling and high-pressure homogenization techniques. High-pressure homogenization was developed in the mid-1990 and is preferably used in the pharmaceutical industry. The different names of this technique depend upon the material or facility used; for example, nanopure is a technique in which the whole process is carried out in nonaqueous media, and in dissocubes, the whole process is carried out in aqueous media with high-pressure homogenization.[25,26] It is a very simple and cost-effective technique that can be utilized for large-scale production. The major disadvantage of this technique is that erosion occurs from the milling equipment which leads to decontamination of the final product.

High-pressure homogenization

It is the most preferable method utilized to prepare nanosuspensions of poorly aqueous soluble drugs to increase their solubility.[26] In this technique, drug substances are dispersed in the aqueous phase and homogenized at high speed at low pressure in the presence of a suitable stabilizer. This presuspension is further homogenized at high speed and high pressure (10-25 cycles) until nanosuspensions of uniform shape and size are obtained.[27]

Dissocube technique

The dissocube method for nanosuspension formation was invented by Muller *et al.* in 1998. In this method, the drug suspension is allowed to pass at high pressure through a small aperture of nozzles. Under this condition, static pressure is reduced below the boiling pressure of water, and boiling of water and gas bubble formation occurs.[28] Upon leaving the gap, the pressure is normalized, and all the bubbles shrink, leading to the association of particles in the center and particle size reduction. Some drug particles vary in hardness and require multiple cycles of size reduction to reduce the size of the particles. Nanosuspensions of atovaquone were prepared by Scholar *et al.* using the same method.[29] This method can be used for nanosuspension formulations with both low and high concentrations of drug and provides aseptic conditions during nanosuspension formation.[30-32]

Nanopure technology

In this technique, a nanosuspension is formulated in water-free media such as oils and fatty acids. This type of oily substance has a very low vapor pressure and higher boiling point. For such material, breakdown by high-pressure homogenization at 800°C promoted disintegration, but the technique should not be used for heat-unstable substances. In this technology, drug particles within water-free media are homogenized at 800°C or below to freeze. Homogenization with freezing technology is suitable for heat-sensitive materials or drug substances.[33]

Table 1: Formulation aspects of nanosuspension.

Excipient	Stabilizer	Co-surfactant	Solvent		Other additives
			Organic solvent	Partially water-miscible solvents	
Remarks	Wet the drug particle, prevent Ostwald ripening.	Influence phase behavior when micro-emulsion is used to formulate nano-suspension.	Pharmaceutically acceptable, less hazardous.	Preferred in the formulation over conventional hazardous solvents, such as dichloromethane.	According to the need of the route of administration.
Examples	poloxamer, cellulosic, povidone, lecithin, PVP K30, SLS.	Bile salt, glycofurol, tween-80, span, transcutol.	Ethanol, iso-propanol methanol, chloroform.	Ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate, benzyl alcohol.	Buffer, salt, polyols, osmogens.

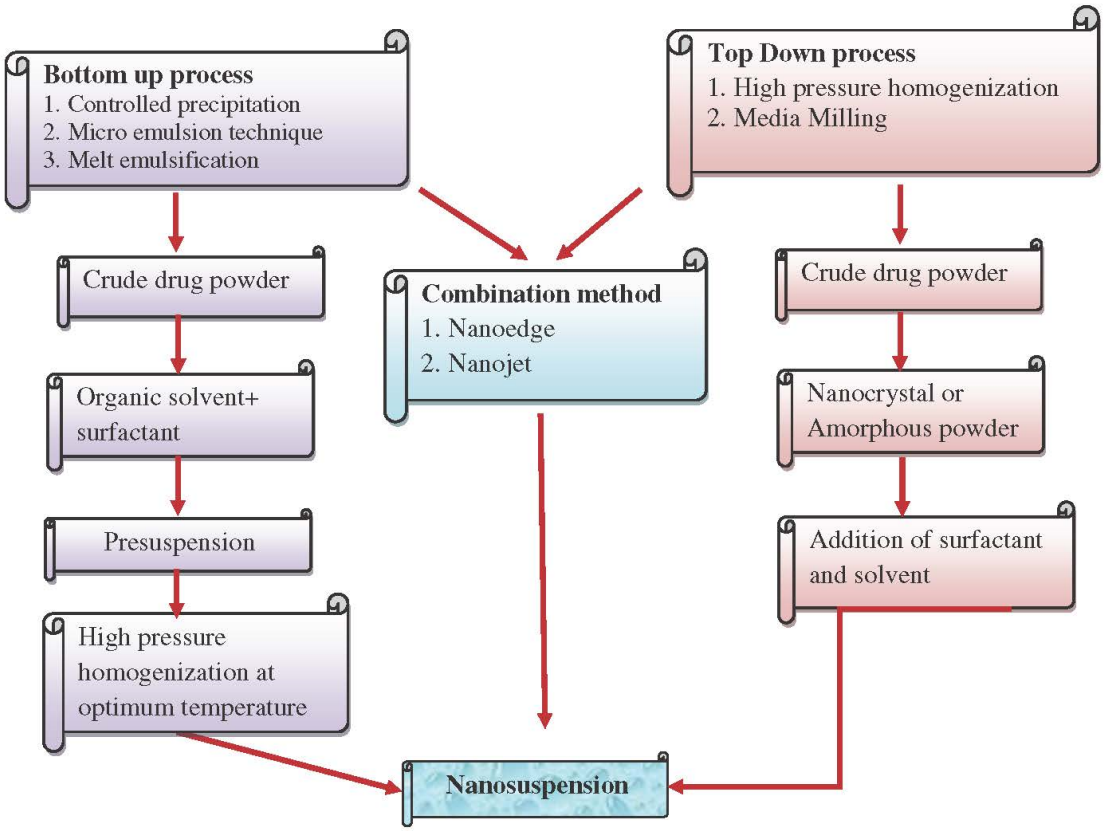

Figure 4: Nanosizing techniques for drug particles.

Piston gap homogenization

The cavitation principle generally works to reduce the particle size of drugs in piston gap homogenization. In piston gap homogenizers, the high force of shear and collision of the particle is responsible for the reduction in particle size. Large-bore cylinder pistons develop pressures up to 2000 bar. Generally, the dispersion medium of the suspension is water. The large drug particles with liquid passed by a very slim ring gap of approximately 3-25 µm with 1500-100 bar pressure resulted in enhanced dynamic pressure and reduction in static pressure on the liquid. Under this condition, boiling with gas bubbles starts, which generates cavitations or gaps upon the collapse of bubbles, resulting in an increase in high air pressure that results in size reduction. The process of size reduction depends upon the temperature, homogenization time, and power density of the homogenizer and homogenization pressure.[33]

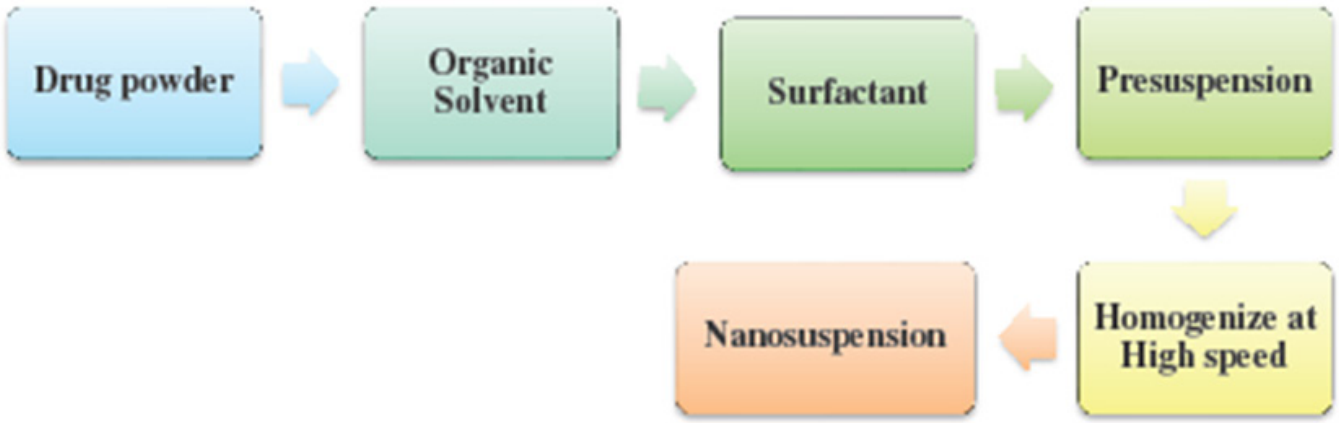

Figure 5: Bottom-up techniques process to formulate nanosuspension.

Media milling techniques

In media milling, a high rate of shear force generated by pearl or media mills is responsible for the size reduction process.[34] At a controlled temperature and high rotation speed of pearls, the drug suspension enters the chamber, and then the particles are reduced due to impact and attrition with the walls of the two opposite jars. The high efficiency of particle size reduction in this technique is due to the simultaneous force of impact and attrition. High energy and shear forces give the required force that breaks the microparticles into a nano range of particles. The main drawback of this technique is that the residue from the mill may contaminate the end product and cause undesirable effects.[31,35]

Combination Technology

Nanojet technology

It is also known as stream technology because a stream of particulate suspension is entered into a chamber in which it divides into multiple streams and further colloids to each other at very high pressure. The collision of the stream generates a high shear force, which breaks the particles into nanosized particles.[36] Patented nanosuspensions of atovaquone preparation using the same technique.[37]

Nanoedge

The nanoedge production technique is a combination of microprecipitation and high-pressure homogenization techniques. This method is considered a combination of precipitation techniques that creates fragmentation under high shear and thermal energy. This method is applicable to the size reduction of friable materials. The suspension obtained by this method is again homogenized to reduce the size to prevent further growth of particles, as the case may be. In the microprecipitation method, there are chances of particle growth that may lead to stability issues, which are overcome by the combination of nanoedge methods. Water-miscible solvents are generally used in precipitation methods, such as methanol, ethanol, and isopropanol. It is necessary to completely remove the solvent from the final product, which is achieved by an evaporation step followed by HPH. Thus, an evaporation technique is also included in nanoedge technology for the better production of nanosuspensions, which will result in solvent-free modified starting material.[38,39]

CHARACTERIZATION OF NANOSUSPENSIONS

Characterization of nanosuspensions was carried out by determining their average particle size, appearance, color, odor, assay, and related impurities. In addition, the nanosuspensions were also evaluated for zeta potential, crystalline status, dissolution, *in vivo* efficacy, saturation solubility, surface hydrophobicity, hemolytic activity, and cell and protein interaction.

In vitro Evaluations

Mean particle size and size distribution

Particles in the nano range are the main basic property and attribute of nanosuspension formulation. It can affect the saturation solubility of nanosuspension particles, the rate of its dissolution, stability, GI permeability, and biological performance of nanosuspensions. Muller *et al.*, 2001 demonstrated that all the above parameters show considerable variation with the changing size of drug particles. The particle size and size distribution are generally measured by particle size analyzers based on photon correlation spectroscopy, laser diffraction, and a Coulter counter.[40]

The Polydispersity Index (PI) is an important parameter that represents the uniformity in size that is directly correlated with the stability and dose of the drug. The PDI value should be less than or near zero, which means that it is monodisperse and uniform in size.

If the PI of the nanosuspension is very high, then it is difficult to determine the presence of foreign particles and any pathogen or part of the pathogen as impurities other than drug nanocrystals. These contaminations can be easily detected if drug particles of nanosuspension show a low PI. Particles more than 3 μm in size are more difficult to determine by PCS. In this case, where the particles are more than 3 μm, Laser Diffractometry (LD) analysis is a choice of analysis to analyze the size of particles. The LD method also measures the size of particles on the basis of particle volume and is capable of measuring particles ranging from 0.05 to 2000 μm. Coulter counter is one of the methods for particle size determination that gives the absolute number of particles size per volume; thus, it is a more preferable and suitable technique than LD, and it can also be used to detect and quantify the contamination present in the nanosuspension of microparticulate drugs. Three-dimensional visualization of particle shapes can be made possible by atomic force microscopy.[31,40]

Surface charge (Zeta Potential)

Zeta potential provides information on the charge present on particles and may directly affect and be responsible for the stability of nanosuspensions and interactions with biological or blood components in the body. Surface charges can arise from (i) ionization of drug molecules on the particle surface or (ii) adsorption of ions present in a solvent onto the particle surface. A preferred zeta potential plays a major role in the stability of the formulation and provides sufficient electric repulsion to prevent the particles from aggregating.[41] Zeta potential is the potential at the hydrodynamic shear plane and can be determined from the particle mobility under an applied electric field, and its movement due to its electric potential. Usually, the zeta potential value should be less than ±40 mV, which represents long-term physical stability as well as non-toxic or low biological interactions.[40]

Crystalline state and its morphology

In a nanosuspension system, it is important to know the crystal morphology of the drug. The amorphous forms (high-energy metastable form) generally convert back to crystalline forms (stable form). Although the amorphous form is not desired when considering the stability of a nanosuspension system, the high internal energy of the amorphous form of the drug can enhance its solubility and vapor pressure.[42] The existence of the amorphous state of a drug is possible if the amorphous material is stabilized using appropriate stabilizers. The stabilizers should be selected according to the thermodynamic and kinetic properties of the amorphous systems. The chances of polymorphic changes may occur during formulation development, and it is necessary to determine the crystal habit and morphology for better solubility and stability.

Differential Scanning Calorimetry (DSC) is generally used to determine the crystalline structure of any substance. When the nanonization of a crystalline drug is carried out, the prepared crystalline drug converts to a non-crystalline habit; hence, it is necessary to identify the particle morphology during the preparation of nanosuspensions.[43-45] X-ray Diffraction (XRD) is another important technique to determine the changes in physical state morphological changes in drug particles.[29] Scanning electron microscopy is also used to obtain the shape and surface morphology of drug particles. High-pressure homogenization may affect the crystalline structure of the drug, as identified by XRD and DSC. Highly efficient techniques, such as Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Transmission Electron Microscopy (TEM), are ideal techniques used to characterize the exact particle morphology.

Saturation solubility and rate of dissolution

Nanosized drug particles show enhancement in solubility and rate of dissolution. It is well known that increasing the temperature and similar nature of solvent enhances the solubility of the solutes. Reduction in particle size leads to a large surface area interacting with the dissolution medium, resulting in a high dissolution rate and solubility. Different physiological solutions with varying pH values are reported in standard monographs, which are used to carry out dissolution studies with the Kelvin equation, and the Ostwald-Freundlich equations can explain the increase in saturation solubility.

Stability

Nanosuspension stability depends on the size of the suspended particles. A decrease in the size resulted in a high surface energy that increased the chances of agglomeration of particles followed by crystal growth. Therefore, stabilizers such as poloxamers, polysorbates, lecithin, polyoleate, and povidones are used, which develop steric barriers to decrease crystal growth (Ostwald ripening) with suspended particles. The stabilizer can improve the stability of nanosuspensions under different temperature conditions or thermal cycling (15, 25, 35 45°C) and mechanical shaking.[12,31,35] The combination of surfactants and hydrocolloids provides long-term stability in nanosuspensions due to the formation of a barrier that may be ionic and widen electrostatic repulsion between particles.[46,47]

Drug content

Drug content was determined by extracting the drug from the nanosuspension in a suitable solvent mixture followed by high-speed centrifugation. Then, the supernatant samples were collected and analyzed using spectrophotometry or chromatography. The calibration curve was used to calculate the drug content.[48,49]

In vivo Evaluation

The bioequivalence, bioavailability, and plasma drug profile study was performed to check the *in vivo* performance of the prepared formulation. It can be affected by the nature of the drug, and

its interactions with plasma proteins are generally considered important *in vivo* evaluation parameters.[50] *In vivo* evaluation of the nanosuspension involves the determination of plasma drug concentration, its pharmacokinetics and pharmacodynamic profile, drug interaction with cell adhesion, and interaction with body protein cells and other blood components.[50,51] The *in vivo* and *in vitro* correlation studies are necessary to make an ideal preparation.

Evaluation of the Surface Modified Particles

Surface hydrophilicity

Surface hydrophilicity/hydrophobicity is a significant constraint that can affect organ distribution. The surface hydrophobicity responsible for cell interactions earlier in phagocytosis is relevant to plasma protein adsorption. Thus, it is necessary to determine the hydrophilicity of particles to overcome this problem. A suitable technique is Hydrophobic Interaction Chromatography (HIC), previously used to measure the surface hydrophobicity of bacteria, which can be utilized to characterize nanosuspension crystal hydrophilicity. The bioavailability of protein and peptide drugs was analyzed qualitatively by 2-D PAGE.[52]

Adhesion properties

The adhesion property of the nanosuspension formulation can be determined in an animal model using male Wistar rats. In this study, 1.0 mL suspension equivalent to 10 mg of drug was administered orally, and then the animals were sacrificed by cervical dislocation at 1hr and 3 hr after administration. Organs such as the stomach, intestine, and cecum were removed and washed twice with PBS pH 7.4. The 2 cm length of each organ was immersed into an alkali solution mixed with methanol for one day to extract the drug from the formulation followed by vortexing and then centrifugation at 3000 rpm for 15 min. The supernatant was quantified for drug content using an aliquot (1 mL) of the supernatant, which was analyzed by using any suitable method of drug estimation.[53]

Interaction with body proteins[54]

The *in vitro* interaction between the nanosuspension and mucin can be studied by incubating the mucin and nanosuspension (in a 1:4 ratio) in the acidic or neutral medium at 37°C with stirring. The suspension was then centrifuged, its supernatant (150 µL) was placed in a 96-well plate, and 150 µL of BCA protein assay reagent was added. The plate was incubated at 37°C±2°C for 120 min. The samples were analyzed to quantify mucin by colorimetry using λ_{max} of the drug. The amount of mucin adsorbed with drug particles can be used to quantify the differentiation of the concentration at zero and a suitable time interval after incubation.[54]

NANOSUSPENSION DELIVERY STRATEGIES

Most of the potent drugs discovered by pharmaceutical companies fail in the development stage due to their low/poor solubility.[55] The solubility and bioavailability of some hydrophobic drugs can be enhanced with the use of nanosuspension technology.[56] Active Pharmaceutical Ingredients (APIs) are being developed as nanosuspensions or nanocrystals, and this technique has led to the production of several nanosuspension products that are currently on the market as well as some innovative nanosuspensions that are currently in the development stage.[57]

Oral Drug Delivery

The oral route of administration is most common and is propitious to patients because of safety, painlessness, and non-invasive administration.[58,59] In addition, oral formulations can easily be manufactured, take less time to produce, and have low manufacturing costs. The efficacy of drugs administered by the oral route mainly depends on their solubility and absorption through the GIT. Drug candidates that have low bioavailability problems require higher doses to achieve therapeutic concentrations, which threatens chances for untoward side effects and toxicity, as well as enhancing the treatment cost. The same problem was observed with atovaquone and bupravaquone antibiotic drugs that are generally administered in high doses by the oral route to achieve the desired therapeutic concentration. Nanosizing this type of drug creates drastic changes in blood plasma concentrations. Furthermore, this amelioration in bioavailability may ultimately reduce the dose, increase the therapeutic effectiveness, decrease the cost of the final product, and reduce the side effects. Nanosuspension of amphotericin B (poorly water-soluble drug) was prepared by Jacobs *et al.*, 2003, who reported a substantial increase in bioavailability when compared to its conventional formulation.[60-62]

Albendazole nanosuspension prepared by High-pressure homogenization technique using different stabilizers shown promising drug action in comparison to pure drug products.[63] Ibuprofen nanosuspensions were prepared using the ultra-homogenization method. It was shown in the study data that the drug particles were significantly reduced by adding Tween and Polyvinylpyrrolidone. The C_{max} value was found that five-fold high in the case of nanosuspension when compared with pure ibuprofen untreated suspension. The T_{max} value was found at 14.8 min which was found to three times decrease to achieve C_{max}.[63]

Bioavailability Enhancement

Nanosuspensions can improve oral bioavailability by enhancing the aqueous solubility as well as the permeability of the GIT. The bioavailability and therapeutic effect of oleanolic acid (hepatoprotective agent) were enhanced by formulating it in nanosuspension form. A high rate of dissolution (90% in 5 min)

was observed for the lyophilized nanosuspension formulation, while its coarse powder counterpart showed only 15% dissolution after 20 min.[64] Rahim *et al.*, 2019 prepared nanosuspension of glimepiride by precipitation ultrasonication method to enhance the dissolution and oral bioavailability. The study revealed that 85% of the drug in nanosuspension form was dissolved within 10 min. The *in vivo* study found that the bioavailability (AUC_{0-24}) of the drug in nanosuspension form was increased two times in 24 hr in comparison to plain drug particles. The above study said that the nanosuspension of glimepiride can improve oral bioavailability by enhancing solubility and dissolution rate and nanosuspension can be utilized for the effective management of diabetes by effectively inhibiting α-glucosidase enzymes.[65] Hedaya *et al.*, 2021 prepared ibuprofen nanosuspensions by ultra homogenization method and characterized *in vivo* drug absorption and compare with unhomogenized drug particle suspension and marketed product by giving a dose of drug equivalent to 25 mg/kg of ibuprofen to Rabbit. The group of the animal model was administered 5mg/kg of Ibuprofen by the I.V. route. Blood samples were taken at a suitable time interval and analyzed by HPLC. The result was found that the C_{max} values were 14.8±1.64, 9.01±0.761, 7.03±1.38, and 3.23±1.03 μg/mL respectively for nano suspension, unhomogenized suspension, marketed drug suspension, and untreated drug suspension in water. It was found 5 times enhancement of the bioavailability with ibuprofen nanosuspension in comparison to untreated drug suspension in water and two-time bioavailability enhancement was found in comparison to marketed ibuprofen suspension.[66]

Young-Guk *et al.*, 2020 prepared nanosuspension of ticagrelor to enhance its dissolution and oral bioavailability. It is a BCS class second drug and has P2Y12 receptor antagonist properties. Drug nanosuspension was formulated with D-α-Tocopherol Polyethylene Glycol 1000 Succinate (TPGS) and Poly Vinyl Alcohol (PVA) to obtain stable nanosuspension with uniform size of drug particles. It was found that these techniques enhanced the 2.2-fold increase in the bioavailability of ticagrelor.[67] Nitrendipine is a beta blocker used as a vasodilator but has poor water solubility and bioavailability. Their nanosuspension was prepared by precipitation–ultrasonication method to increase the oral bioavailability. The utilized method was capable to produce the drug particles below 200nm and the obtained drug powder has no evidence of the crystal form of the drug which was confirmed by the P-XRD. The dissolution rate was found to increase due to increased surface area upon particle size reduction. The bioavailability study of prepared nanosuspension was performed on rats and found more than sixfold and fivefold more in comparison to the marketed product of tablets respectively.[68] Revaprazan hydrochloride is a proton pump inhibitor having low oral bioavailability due to poor aqueous solubility. The nanosuspension of revaprazan hydrochloride prepared by high-pressure homogenization provides high AUC, $C_{max,}$ and a decrease in T_{max} and MRT.[69]

Target Drug Delivery

Target drug delivery to a specific site in the body is a novel approach to enhance therapeutic effectiveness and reduce the dose of the drug and dose-associated side effects. Targeting can be achieved by changing the surface properties (conjugating the targeting moiety) of suspended drug particles. The surface-engineered mucoadhesive nanosuspensions of buparvaquone were explored to specifically target *Cryptosporidium parvum*, the causative organism for cryptosporidiosis.[70-74] Similarly, amphotericin B nanosuspension was used against pneumonic aspergillosis targeting in place of stealth liposomes.[72] Nanosuspension surface properties and the use of stabilizers can simply alter the *in vivo* behavior. In a previous study, surface engineering was used to target anti-mycobacterial and leishmanial drugs.[73] Shegokar *et al.*, 2011 prepared surface-engineered nanosuspension of the poorly water-soluble drug Nevirapine. It is a non-nucleoside reverse transcriptase inhibitor used in case of HIV infection. It was found that its accumulation was significantly enhanced in the spleen, brain, and other soft organs when its surface was modified with albumin, Polyethylene glycol, and other polysaccharides. Its accumulation in these organs was found significantly enhanced in comparison with without surface-modified Nevirapine suspension. The surface modification of drug nanosuspension increases its retention in the systemic circulation and enhanced the bioavailability o the drug.[74]

Ophthalmic Drug Delivery

The eye is an organ that needs high protection against any pathogenic contaminated material. Scientists have put in hard work to design safe and effective drug delivery by the ocular route. Well-established conventional dosage forms for the ocular route suffer from poor ocular bioavailability. To overcome the problem of conventional dosages, many nanosizing approaches/techniques have been developed, such as polymeric *in situ* gels, nanoparticulate formulations, microemulsions, nanosuspensions, nanoparticles, liposomes, and niosomes, for the treatment of ocular diseases.[75] Scientific literature advocates that nanosuspensions have some benefit among all other nanosizing techniques. Moreover, the adhesiveness of nanocrystals (due to their nano size) can help to reduce the loss of drugs with the outflow of lachrymal fluids.[76] Poorly water-soluble drugs in nanosuspension form are beneficial for delivery through lachrymal fluids. Suspensions provide benefits such as prolonged duration that are fascinating for the effective treatment of many ocular diseases. The intrinsic rate of dissolution for nanocrystalline drugs in lachrymal fluids controls ocular bioavailability and performance and depends on the outflow of lachrymal fluids. Nanosuspensions of nonsteroidal anti-inflammatory drugs supposed for controlled delivery have developed that release the drug in a much-sustained manner.[77,78] Nanosuspensions of hydrocortisone, prednisolone, and dexamethasone were developed, which provide sustained

release for a longer period and enhance the absorption of the drug.[79] Sustained release can be easily achieved by incorporating nanocrystals in an appropriate hydrogel or ointment base or may be incorporated into any ocular inserts. NSAIDs formulation was developed for ophthalmic drug delivery using polymers such as Eudragit RS 100 and Eudragit RL 100.[80,81]

Parenteral Administration

The purpose of developing a parenteral drug delivery system is to avoid the first-pass effect and achieve high bioavailability and fast and sustained action.[82-84] Similar to other formulations, nanosuspensions are appropriate for parenteral administration due to some special characteristics, such as high drug loading with a small volume of injection, low toxicity because of the low concentration of additives and passive (by RES uptake) or active (after surface modification with a targeting moiety) targeting can be easily achieved.

Pulmonary Drug Delivery

Drug nanosuspensions can be administered directly by the pulmonary route for effective and fast therapeutic effects. The pulmonary route is beneficial if any trace amount of organic solvent remains with nanoparticulate formulation, as organic solvents can be easily expelled from pulmonary excretion and protect the body from any potential toxicological effects of organic solvents *in vivo*. Another advantage of this route is that a large quantity or dose of the drug can be administered this route. In addition, the adhesive features of nanosized drug crystals reduce drug loss through clearance by cilia movement.[85] Nanosuspensions of the drug in an aqueous vehicle base can be nebulized because of the tiny size of the particles. Several drugs with poor water solubility have been administered via the respiratory route in the form of nanosuspensions for the management of a variety of diseases; examples of such drugs are budesonide, ketotifen, ibuprofen, Indocin, nifedipine, antimycotic agents, interleukin-2, p53 gene, leuprolide, antibiotics, etc.[86]

OTHER APPLICATIONS OF NANOSUSPENSION

Use in cosmetics

Nanoparticles are widely utilized in cosmetics, as active constituents that are absorbed effectively to provide effectual action because of the small size of the formulation and scale back the water loss from the skin. Nanosized preparations are used as moisturizers and creams. Further nanosized preparations resulted in elegant and stable products.[87-89] Use as anti-microbial Agent: Antimicrobial nanosizings are O/W preparations that contain a broad spectrum of activity against microorganisms, engulfed viruses (e.g., HIV), fungi, and spores. Nanosizings are droplets consolidated with lipid-containing organisms and unleash a part of the energy cornered inside the emulsion. The lipid membrane of infectious organisms is destabilized by active ingredients, and therefore, the free energy results in microbic cell lysis.[90]

Nanosuspensions in Cancer Therapy

Nanosuspensions are used as vehicles in cancer therapy to prolong the rate of drug release with contractile organs and intratumoral injection (W/O systems). It additionally enhances the transport of anticancer drugs through the vascular system.[91] Prophylactic in bioterrorism attack: Nanoemulsions are used as a prophylactic drug and are used against bioattack pathogens such as anthrax and viral hemorrhagic fever. In the broad spectrum, it can be used as a chemical decontamination agent for anthrax, gangrene, etc.[92]

Nanosuspension as Mucosal Vaccines

Nanosuspensions can be used for needle-free immunization by delivering recombinant macromolecules and inactivated organisms to the membrane surface. Nanosuspensions themselves work like an inert adjuvant along with the protein moiety, thus facilitating uptake by antigen-presenting cells and thus finding applications in cell culture.[92]

Marketed Product

There are some marketed nanosuspension formulations that were formulated and patented by different manufacturing companies and are listed in Table 2.

CONCLUDING REMARK AND FUTURE PERSPECTIVES

Poorly water-soluble drugs in nanosuspension form offer a large surface area, surface modification, and controlled size that make nanosuspensions suitable for targeting and drug delivery. Nanosuspension-based formulation approaches offer potential benefits for solubility enhancement and delivery of drugs with low aqueous solubility. The therapeutic concentration of the drug can be controlled by changing the size of the drug particles as well as surface modification of the drug particles. Nanosuspension can change the pharmacokinetics and pharmacodynamics parameters of the drugs. Hydrophilic and hydrophobic drugs can be converted into the form of nanosuspensions and can be administered in the form of suspensions, tablets, and capsules. The high rate of dissolution, high saturation solubility, and ease of modifying its surface properties make nanosuspension formulation appropriate for administration through various routes. This has necessitated the exploration of nanosuspension applications in the buccal, nasal, and topical delivery fields. Hence, for the next few years, there have been various promising areas of research, which need more emphasis, particularly on the preparation of stealth nanosuspensions fastened with modification of surface properties and adaptable features to elicit passive or active targeting. The difficulty of dealing with the inherent instability and the use of nanosuspensions to meet the requirements for increasing its

Table 2: Nanosuspension Drug Products in the Market.

Product	Drug compound	Indication	Company	Dosage form	Nanosuspension technology	Route	Patent no./ references
Rapamune	Sirolimus	Immuno-suppressant	Wyeth	Tablet	Media Milling	Oral	US 8,053,444 b2[93]
Emend	Aprepitant	Anti-emetic	Merck	Capsule	Media milling	Oral	US 8,133,994[94]
MEGACE ES®	Megestrol Acetate	Appetite stimulant	Par Pharmaceutical	Liquid nanosuspension	Media Milling / Élan Drug Delivery Nanosystems	Oral	US2007/0196498[95]
Focalin®XR	Dexmethylphenidate Hydrochloride	Attention deficit hyperactivity disorder (ADHD)	Novartis	Capsules	Elan's Nanosystems	Oral	US 2004/0180928 A1[96]
Ritalin®LA	Methylphenidate Hydrochloride	Treating attention deficit hyperactivity disorder	Novartis	Capsules	Elan's systems	Oral	US 6,344,215 B1[97]
Zanaflex	Tizanidine Hydrochloride	Short acting muscle relaxer	Acorda	Capsule	Media Milling/ ElanNanosystems	Oral	US 2010/0298305A1[98]
Abraxane	Paclitaxel	Anticancer	American Pharmaceutical Partners	Lyophilized powder for injectable suspension	Nab™	Intravenous	EP2481 405A1[99]
Gris-PEG®	Griseofulvin	Antifungal	Novartis	Tablet	Co-precipitation	Oral	US 4,151,273[100]
Cesamet®	Nabilone	Antiemetic	Lilly	Capsule	Co-precipitation	Oral	US US9433601 B2[101]
Invega Sustenna®	Paliperidone palmitate	Schizophrenia	Johnson and Johnson	Liquid nanosuspension	High pressure homogenization	Parenteral	US 8,758,780 B2[102]

stability will encourage the advancement of nanosuspension technology. Most likely, pharmaceutical nanosuspension can be considered a resurgence in formulation technologies in the future that could potentially have a major impact on human health. Nanosuspension technology is able to fetch massive applications and transform research in the drug delivery field.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

ABBREVIATIONS

APIs: Active pharmaceutical ingredients; **ADHD:** Attention deficit hyperactivity disorder; **TPGS:** D-α-Tocopherol polyethylene glycol 1000 succinate; **GIT:** Gastrointestinal Tract; **PVA:** Polyvinyl alcohol.

REFERENCES

1. Uehara T, Ishii D, Uemura T, Suzuki H, Kanei T, Takagi K, *et al*. γ-glutamyl PAMAM Dendrimer as Versatile Precursor for Dendrimer-Based Targeting Devices. Bioconjug Chem. 2010 Jan 20;21(1):175-81. doi: 10.1021/bc900410q, PMID 20000792.
2. Duncan R, Vicent MJ. Polymer therapeutics-prospects for 21st century: the end of the beginning. Adv Drug Deliv Rev. 2013;65(1):60-70. doi: 10.1016/j.addr.2012.08.012, PMID 22981753.
3. Lipinski C. Poor aqueous solubility – an industry wide problem in drug discovery. Am Pharm Rev. 2002;5(3):82-5.
4. Ranjita S. Nanosuspensions: A new approach for organ and cellular targeting in infectious diseases. J Pharm Investig. 2013;43(1):1-26. doi: 10.1007/s40005-013-0051-x.
5. Sutradhar KB, Khatun S, Luna IP. Increasing possibilities of nanosuspension. J Nanotechnol. 2013;2013:1-12. doi: 10.1155/2013/346581.
6. Liebermann AH. Oral aqueous suspension pharmaceutical dosage forms: dispersed systems; 1989.
7. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci. 2003;18(2):113-20. doi: 10.1016/s0928-0987(02)00251-8, PMID 12594003.
8. Vedaga SB, Gondkar SB, Saudagar RB. Nanosuspension: an emerging trend to improve solubility of poorly water soluble drugs. J Drug Deliv Ther. 2019;9(3):549-53.
9. Rahim H, Sadiq A, Ullah R, Bari A, Amin F, Farooq U, *et al*. Formulation of aceclofenac tablets using nanosuspension as granulating agent: an attempt to enhance dissolution rate and oral bioavailability. Int J Nanomedicine. 2020;15:8999-9009. doi: 10.2147/IJN.S270746, PMID 33235448.
10. Wei Y, Li L, Xi Y, Qian S, Gao Y, Zhang J. Sustained release and enhanced bioavailability of injectable scutellarin-loaded bovine serum albumin nanoparticles. Int J Pharm. 2014;476(1-2):142-8. doi: 10.1016/j.ijpharm.2014.09.038, PMID 25269007.
11. Joshi G, Kumar A, Sawant K. Enhanced bioavailability and intestinal uptake of gemcitabine HCl loaded PLGA nanoparticles after oral delivery. Eur J Pharm Sci. 2014;60:80-9. doi: 10.1016/j.ejps.2014.04.014, PMID 24810394.
12. Ji N, Li X, Qiu C, Li G, Sun Q, Xiong L. Effects of heat moisture treatment on the physicochemical properties of starch nanoparticles. Carbohydr Polym. 2015;117:605-9. doi: 10.1016/j.carbpol.2014.10.005, PMID 25498677.
13. Hörter D, Dressman JB. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv Drug Deliv Rev. 2001;46(1-3):75-87. doi: 10.1016/s0169-409x(00)00130-7, PMID 11259834.
14. Junghanns JU, Müller RH. Nanocrystal technology, drug delivery and clinical applications. Int J Nanomedicine. 2008;3(3):295-309. doi: 10.2147/ijn.s595, PMID 18990939.
15. Jinno JI, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, *et al*. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J Control Release. 2006;111(1-2):56-64. doi: 10.1016/j.jconrel.2005.11.013, PMID 16410029.
16. Jacob S, Nair AB, Shah J. Emerging role of nanosuspensions in drug delivery systems. Biomater Res. 2020;24(1):3. doi: 10.1186/s40824-020-0184-8, PMID 31969986.
17. Dhiman S, Singh TG. Nanosuspension: a recent approach for Nano drug delivery system. Int J Curr Pharm. 2011;3(4):96-101.
18. Nagaraju P, Krishnachaithanya K, Srinivas VD, Padma SV. Nanosuspensions: A promising drug delivery systems. Int J Pharm Sci Nano. 2010;2(4):679-84.
19. RM S, Anup N, Madhavan N, Koteshwara K, Professor A. Nanosuspensions: a novel drug delivery approach. Int J Res Ayurveda Pharm. 2011;2(1):162-5.
20. Mudgil M. Nanotechnology: A new approach for ocular drug delivery system. Int J Pharm Pharm Sci. 2012;4(2):105-12.
21. Kumar AN, Deecaraman M, Rani C. Nanosuspension technology and its applications in drug delivery. Asian J Pharma. 2009;3:168–73.
22. Pu X, Sun J, Li M, He Z. Formulation of nanosuspensions as a new approach for the delivery of poorly soluble drugs. Curr Nanosci. 2009;5(4):417-27. doi: 10.2174/15734131309789378177.
23. Soumya M, Gupta S, Jain R, Mazumder R. Solubility enhancement of poorly water soluble drug using nanosuspension technology. Int J Res Dev Pharm Life Sci. 2013;2(6):642-9.
24. Kocbek P, Baumgartner S, Kristl J. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. Int J Pharm. 2006;312(1-2):179-86. doi: 10.1016/j.ijpharm.2006.01.008, PMID 16469459.
25. Yadav GV, Singh SR. Nanosuspension: A promising drug delivery system. Pharmacophore. 2012;3(5):217-43.
26. Müller RH, Jacobs C, Kayser O. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv Drug Deliv Rev. 2001;47(1):3-19. doi: 10.1016/s0169-409x(00)00118-6, PMID 11251242.
27. Liversidge GG, Cundy KC. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. International Journal of Pharmaceutics. 1995;125(1):91-7. doi: 10.1016/0378-5173(95)00122-Y.
28. Muller RH, Bohm BH, Grau J. Nanosuspensions: a formulation approach for poorly soluble and poorly bioavailable drugs. Handbook of pharmaceutical controlled release technology. 2000:345-57.
29. Schöler N, Krause K, Kayser O, Müller RH, Borner K, Hahn H, *et al*. Atovaquone nanosuspensions show excellent therapeutic effect in a new murine model of reactivated toxoplasmosis. Antimicrob Agents Chemother. 2001;45(6):1771-9. doi: 10.1128/AAC.45.6.1771-1779.2001, PMID 11353624.
30. Kumar P, Krishna KG. Nanosuspensions: the solution to deliver hydrophobic drugs. Int J Drug Deliv. 2011;3(4):546-57.
31. Müller RH, Gohla S, Keck CM. State of the art of nanocrystals–special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm. 2011;78(1):1-9. doi: 10.1016/j.ejpb.2011.01.007, PMID 21266197.
32. Bhowmik D, Harish G, Duraivel S, Pragathi B, Raghuvanshi V, Sampath KP. Nanosuspension: a novel approaches in drug delivery system. Pharm Innov. 2013;1:12.
33. Kamble VA, Jagdale DM, Kadam VJ. Nanosuspension a novel drug delivery system. Int J Pharm Biol Sci. 2010;1(4):352-60.
34. Bartos C, Jójárt-Laczkovich O, Katona G, Budai-Szűcs M, Ambrus R, Bocsik A, *et al*. Optimization of a combined wet milling process in order to produce poly(vinyl alcohol) stabilized nanosuspension. Drug Des Devel Ther. 2018; 12:1567-80. doi: 10.2147/DDDT.S159965. PMID: 29910603.
35. Muller RH, Keck CM. Challenges and solutions for the delivery of biotech drugs—a review of drug nanocrystal technology and lipid nanoparticles. J Biotechnol. 2004;113(1-3):151-70. doi: 10.1016/j.jbiotec.2004.06.007, PMID 15380654.
36. Dearn AR, Glaxo Wellcome Inc. Atovaquone pharmaceutical compositions. U.S Patent 6. 2000;18:80.
37. Chingunpituk J. A Review on nanosuspension Technology for drug delivery. Walailak J Sci Technol. 2011;4(2):139-53.
38. Kipp JE, Wong JCT, Doty MJ, Rebbeck CL, Baxter International Inc. Microprecipitation method for preparing submicron suspensions. U.S Patent 6. 2003;607:784.
39. Kipp JE, Wong JCT, Doty MJ, Werling J, Rebbeck CL. Brynjelsen. Method for preparing submicron particle suspensions. Baxter International Inc; 2005. U.S Patent. 6,884,436.
40. Zhang J, Xie Z, Zhang N, Zhong J. Chapter 13 - Nanosuspension drug delivery system: preparation, characterization, postproduction processing, dosage form, and application. In Micro and Nano Technologies. Nanostructures for Drug Delivery. Elsevier. 2017:413-43. doi: org/10.1016/B978-0-323-46143-6.00013-0. ISBN 9780323461436.
41. Gao L, Zhang D, Chen M, Zheng T, Wang S. Preparation and characterization of an oridonin nanosuspension for solubility and dissolution velocity enhancement. Drug Dev Ind Pharm. 2007;33(12):1332-9. doi: 10.1080/03639040701741810, PMID 18097807.
42. Fakes MG, Vakkalagadda BJ, Qian F, Desikan S, Gandhi RB, Lai C, *et al*. Enhancement of oral bioavailability of an HIV-attachment inhibitor by nanosizing and amorphous

formulation approaches. Int J Pharm. 2009;370(1-2):167-74. doi: 10.1016/j.ijpharm.2008.11.018, PMID 19100319.

43. Rahim H, Sadiq A, Khan S, Amin F, Ullah R, Shahat AA, *et al*. Fabrication and characterization of glimepiride nanosuspension by ultrasonication-assisted precipitation for improvement of oral bioavailability and *in vitro* α-glucosidase inhibition. Int J Nanomedicine. 2019;14:6287-96. doi: 10.2147/IJN.S210548. PMID 31496686.
44. Vedaga SB, Gondkar S, Saudagar R. Nanosuspension: an emerging trend to improve solubility of poorly water soluble drugs. Drug Discov Ther. 2019;9(3):549-53.
45. Zhao YX, Hua HY, Chang M, Liu WJ, Zhao Y, Liu HM. Preparation and cytotoxic activity of hydroxycamptothecin nanosuspensions. Int J Pharm. 2010;392(1-2):64-71. doi: 10.1016/j.ijpharm.2010.03.027, PMID 20302926.
46. Pilli GD, Elumalai K, Muthukumar VA, Sundaram PS. A revised analysis of current and emerging nano suspension technological approaches for cardiovascular medicine. Beni-Suef Univ J Basic Appl Sci. 2022;11(1):10. doi: 10.1186/s43088-022-00193-4.
47. Guo J, Ping Q, Chen Y. Pharmacokinetic behavior of cyclosporin A in rabbits by oral administration of lecithin vesicle and Sandimmun Neoral. Int J Pharm. 2001;216(1-2):17-21. doi: 10.1016/s0378-5173(00)00680-3, PMID 11274802.
48. Chakravorty R. Nanosuspension as an emerging nanotechnology and techniques for its development. Res J Pharm Technol. 2022;15(1):489-93. doi: 10.52711/0974-360X.2022.00079.
49. Yadav M, Dhole S, Chavan P. Nanosuspension: a novel techniques in drug delivery system. World J Pharm Pharm Sci. 2014;3(2):410-33.
50. Choi YH, Han HK. Nanomedicines: current status and future perspectives in aspect of drug delivery and pharmacokinetics. J Pharm Investig. 2018;48(1):43-60. doi: 10.1007/s40005-017-0370-4. PMCID: PMC6244736.
51. Yuan H, Li X, Zhang C, Pan W, Liang Y, Chen Y, *et al*. Nanosuspensions as delivery system for gambogenic acid: characterization and *in vitro/in vivo* evaluation. Drug Deliv. 2016;23(8):2772-9. doi: 10.3109/10717544.2015.1077294. PMID: 26292058.
52. Ponchel G, Montisci MJ, Dembri A. Mucoadhesion of colloidal particulate systems in the gastro-intestinal tract. Eur J Pharm Biopharm. 1997;44(1):25-31.
53. Bi Y, Liu J, Wang J, Hao J, Li F, Wang T, *et al*. Particle size control and the interactions between drug and stabilizers in an amorphous nanosuspension system. J Drug Deliv Sci Technol. 2015;29:167-72. doi: 10.1016/j.jddst.2015.07.012.
54. Nagarwal RC, Kumar R, Dhanawat M, Das N, Pandit JK. Nanocrystal technology in the delivery of poorly soluble drugs: an overview. Curr Drug Deliv. 2011;8(4):398-406. doi: 10.2174/156720111795767988, PMID 21453258.
55. Pınar SG, Canpınar H, Tan Ç, Çelebi N. A new nanosuspension prepared with wet milling method for oral delivery of highly variable drug cyclosporine A: development, optimization and *in vivo* evaluation. Eur J Pharm Sci. 2022;171:106123. doi: 10.1016/j.ejps.2022.106123, PMID 35017012.
56. Fuhrmann K, Gauthier MA, Leroux JC. Crosslinkable polymers for nanocrystal stabilization. J Control Release. 2010;148(1):e12-3. doi: 10.1016/j.jconrel.2010.07.006, PMID 21529591.
57. Kayaert P, Van Den Mooter G. Is the amorphous fraction of a dried nanosuspension caused by milling or by drying? A case study with naproxen and cinnarizine. Eur J Pharm Biopharm. 2012;81(3):650-6. doi: 10.1016/j.ejpb.2012.04.020, PMID 22579733.
58. Sastry SV, Nyshadham JR, Fix JA. Recent technological advances in oral drug delivery - a review. Pharm Sci Technol Today. 2000;3(4):138-45. doi: 10.1016/s1461-5347(00)00247-9, PMID 10754543.
59. Jacobs C, Kayser O, Müller RH. Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. Int J Pharm. 2000;196(2):161-4. doi: 10.1016/s0378-5173(99)00412-3, PMID 10699709.
60. Gabor F, Fillafer C, Neutsch L, Ratzinger G, Wirth M. Improving oral delivery. 2010:345-98.
61. Kayser O, Olbrich C, Yardley V, Kiderlen AF, Croft SL. Formulation of amphotericin B as nanosuspension for oral administration. Int J Pharm. 2003;254(1):73-5. doi: 10.1016/s0378-5173(02)00686-5, PMID 12615413.
62. Rao Y, Kumar M, Apte S. Formulation of nanosuspensions of albendazole for oral administration. Curr Nanosci. 2008;4(1):53-8. doi: 10.2174/157341308783591807.
63. Shid RL, Dhole SN, Kulkarni N, Shid SL. Nanosuspension: a review. Res Rev J Pharm Nanotechnol. 2016;4(3):1-9.
64. Jakubowska E, Bielejewski M, Milanowski B, Lulek J. Freeze-drying of drug nanosuspension– study of formulation and processing factors for the optimization and characterization of redispersible cilostazol nanocrystals. J Drug Deliv Sci Technol. 2022;74:103528. doi: 10.1016/j.jddst.2022.103528.
65. Hedaya M, Bandarkar F, Nada A. *In vitro* and *in vivo* evaluation of ibuprofen nanosuspensions for enhanced oral bioavailability. Med Princ Pract. 2021;30(4):361-8. doi: 10.1159/000516299, PMID 33823524.
66. Na YG, Pham TMA, Byeon JJ, Kim MK, Han MG, Baek JS, *et al*. Development and evaluation of TPGS/PVA-based nanosuspension for enhancing dissolution and oral bioavailability of ticagrelor. Int J Pharm. 2020;581:119287. doi: 10.1016/j.ijpharm.2020.119287, PMID 32243963.
67. Xia D, Quan P, Piao H, Piao H, Sun S, Yin Y, *et al*. Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability. Eur J Pharm Sci. 2010;40(4):325-34. doi: 10.1016/j.ejps.2010.04.006, PMID 20417274.
68. Li W, Yang Y, Tian Y, Xu X, Chen Y, Mu L, *et al*. Preparation and *in vitro/in vivo* evaluation of revaprazan hydrochloride nanosuspension. Int J Pharm. 2011;408(1-2):157-62. doi: 10.1016/j.ijpharm.2011.01.059, PMID 21295124.
69. Jacobs C, Kayser O, Müller RH. Production and characterisation of mucoadhesive nanosuspensions for the formulation of bupravaquone. Int J Pharm. 2001 Feb;214(1-2):3-7. doi: 10.1016/s0378-5173(00)00622-0, PMID 11282227.
70. Müller RH, Jacobs C. Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability. Int J Pharm. 2002;237(1-2):151-61. doi: 10.1016/s0378-5173(02)00040-6, PMID 11955813.
71. Kohno S, Otsubo T, Tanaka E, Maruyama K, Hara K. Amphotericin B encapsulated in polyethylene glycol-immunoliposomes for infectious diseases. Adv Drug Deliv Rev. 1997;24(2-3):325-9. doi: 10.1016/S0169-409X(96)00474-7.
72. Kayser O, Lemke A, Hernández-Trejo N. The impact of nanobiotechnology on the development of new drug delivery systems. Curr Pharm Biotechnol. 2005;6(1):3-5. doi: 10.2174/1389201053167158, PMID 15727551.
73. Shegokar R, Singh KK. Surface modified nevirapine nanosuspensions for viral reservoir targeting: *in vitro* and *in vivo* evaluation. Int J Pharm. 2011;421(2):341-52. doi: 10.1016/j.ijpharm.2011.09.041, PMID 21986114.
74. Sahoo SK, Dilnawaz F, Krishnakumar S. Nanotechnology in ocular drug delivery. Drug Discov Today. 2008;13(3-4):144-51. doi: 10.1016/j.drudis.2007.10.021, PMID 18275912.
75. Gao L, Zhang D, Chen M. Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. J Nanopart Res. 2008;10(5):845-62. doi: 10.1007/s11051-008-9357-4.
76. Pignatello R, Bucolo C, Ferrara P, Maltese A, Puleo A, Puglisi G. Eudragit RS100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen. Eur J Pharm Sci. 2002;16(1-2):53-61. doi: 10.1016/s0928-0987(02)00057-x, PMID 12113891.
77. Pignatello R, Bucolo C, Puglisi G. Ocular tolerability of Eudragit RS100 and RL100 nanosuspensions as carriers for ophthalmic controlled drug delivery. J Pharm Sci. 2002 Dec;91(12):2636-41. doi: 10.1002/jps.10227, PMID 12434408.
78. Kassem MA, Abdel Rahman AA, Ghorab MM, Ahmed MB, Khalil RM. Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. Int J Pharm. 2007;340(1-2):126-33. doi: 10.1016/j.ijpharm.2007.03.011, PMID 17600645.
79. Bucolo C, Maltese A, Puglisi G, Pignatello R. Enhanced ocular anti-inflammatory activity of ibuprofen carried by an Eudragit RS100 nanoparticle suspension. Ophthalmic Res. 2002;34(5):319-23. doi: 10.1159/000065608, PMID 12381895.
80. Patel VR, Agrawal YK. Nanosuspension: an approach to enhance solubility of drugs. J Adv Pharm Technol Res. 2011;2(2):81-7. doi: 10.4103/2231-4040.82950, PMID 22171298.
81. Pignatello R, Bucolo C, Spedalieri G, Maltese A, Puglisi G. Flurbiprofen-loaded acrylate polymer nanosuspensions for ophthalmic application. Biomaterials. 2002;23(15):3247-55. doi: 10.1016/S0142-9612(02)00080-7.
82. Ahire E, Thakkar S, Darshanwad M, Misra M. Parenteral nanosuspensions: a brief review from solubility enhancement to more novel and specific applications. Acta Pharm Sin B. 2018;8(5):733-55. doi: 10.1016/j.apsb.2018.07.011. PMID: 30245962.
83. Mansour HM, Rhee YS, Wu X. Nanomedicine in pulmonary delivery. Int J Nanomedicine. 2009;4:299-319. doi: 10.2147/ijn.s4937, PMID 20054434.
84. Sonneville-Aubrun O, Simonnet JT, L'Alloret F. Nanoemulsions: A new vehicle for skincare products. Adv Colloid Interface Sci. 2004;108-109:145-9. doi: 10.1016/j.cis.2003.10.026, PMID 15072937.
85. Prabhakar C, Bala Krishna K. A review on nanosuspensions in drug delivery. Int J Pharm Biol Sci. 2011;2(1):549-58.
86. Shah P, Bhalodia D, Shelat P. Nanoemulsion: A pharmaceutical review. Syst Rev Pharm. 2010;1(1):24-32. doi: 10.4103/0975-8453.59509.
87. Chang Y, McLandsborough L, McClements DJ. Physicochemical properties and antimicrobial efficacy of electrostatic complexes based on cationic ε-Polylysine and anionic pectin. J Agric Food Chem. 2011;59(12):6776-82. doi: 10.1021/jf201162g, PMID 21574554.
88. Souto EB, Nayak AP, Murthy RSR. Lipid nanoemulsions for anti-cancer drug therapy. Pharmazie. 2011;66(7):473-8. PMID 21812320.
89. Gupta V, Mohapatra S, Mishra H, Farooq U, Kumar K, Ansari MJ, *et al*. Nanotechnology in Cosmetics and Cosmeceuticals-A Review of Latest Advancements. Gels. 2022;8(3):173. doi: 10.3390/gels8030173. PMID: 3532328.
90. Pagar KR, Darekar AB. Nanoemulsion: A new concept of Delivery System. Asian J Res Pharm Sci. 2019;9(1):39. doi: 10.5958/2231-5659.2019.00006.7.

91. Karthick G, Akiladevi D, Ahamed MI. A Comprehensive review of a new nanosuspension for Improving the oral bioavailability of poorly soluble drugs. J Pharm Res Int;34(20B):16-21.
92. Ramesh Y, Sarayu B, Hari Chandana GH, Neelima O, Sana S. Formulation and evaluation of lamivudine nanosuspension. J Drug Delivery Ther. 2021;11(4-S):71-7. doi: 10.22270/jddt.v11i4-S.4961.
93. Reven A, Legen I, JeralaStrukelj Z. Sirolimus formulation. United States US8053444B2.
94. Vankawala PJ, Elati RRC, Kolla NK, Chlamala SR, Gangula S. Preparation of a prepitant. US8133994.
95. Sherman CB. Megestrol acetate suspension. United States Patent. 20070196498.
96. Gutman A, Zaltsman I, Shalimov A, Sotrihin M, Nisnevich G, Yudovich L, *et al*. Process for the preparation of dexmethylphenidate hydrochloride. 2004. United States Patent. US20040180928A1.
97. Bettman MJ, Percel PJ, Hensley DL, Vishnupad KS, Venkatesh GM. Methylphenidate modified release formulations. Eurand America Inc; 2002. United States Patent. 6,344,215.
98. Capehart B, Hil C. Tizanidine for the treatment of Post-traumatic stress disorder and nightmares. United States Government, 2010. United States Patent. US 2010/0298305A1.
99. Desai NP. Los Angeles C.A. Nanoparticles of paclitaxel and albumin in combination with bevacizumab against cancer. Abraxis BioScience; 2012. PCT/US2007/023446.
100. Riegelman S, Chiou WL. Increasing the absorption rate of insoluble drugs. regents of the University of California; 1979. 4,151,273.
101. Damme PAV, Anastassov GE, Sanammad S. Chewing gum compositions comprising cannabinoids. 2016. United States Patent. 9,433,601.
102. Sprogoe K, Hersel U, Rau H, Maitro G, Wegge T. Subcutaneous paliperidone composition. Ascendis pharma as; 2014. United States Patent. 8,758,780.

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