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Nanosuspensions: A Review

Kamala Kumari, P.V* and Srinivasa Rao, Y.

Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam-530049, India

***Corresponding author e-mail:** kamalaparavastu@gmail.com

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ABSTRACT

The current article cites the importance of the emerging and promising future of new age dosage form, 'nano suspensions'. Particle size reduction, particularly nanonization, is a non-specific, universal approach to improve the bioavailability of poorly soluble drugs. The article emphasizes importance in the preparation, evaluation and the research work going on with various drugs and their appropriate applications. Nano suspensions 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 nano suspensions. The unique features of nano suspensions 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 nano suspensions by parenteral, peroral, ocular and pulmonary routes. Currently, efforts are being directed to extending their applications in site-specific drug delivery.

KEYWORDS: Nanosuspensions, homogenization, Quasi- emulsion solvent technique

INTRODUCTION

Nanosuspensions are defined as the submicron colloidal dispersions of pharmaceutical active ingredient particles in a liquid phase, size below 1μm, without any matrix material which are stabilized by surfactants and polymers.[1] Nanosuspensions differ from nanoparticles and solid lipid nanoparticles with respect to the fact that nanoparticles are polymeric colloidal carriers of drug while solid lipid nanoparticles are lipid carrier of drugs. An increasing number of newly developed drugs are poorly soluble; in many cases drugs are poorly soluble in both aqueous and organic media excluding the traditional approaches of overcoming such solubility factors and resulting in bioavailability problems. An alternative and promising approach is the production of drug nanoparticles (i.e. nanosuspensions) to overcome these problems. Nanosuspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because of their versatile features and unique advantages. The unique features of nanosuspensions have enabled their use in various dosage forms, including specialized delivery systems such as mucoadhesive hydrogels. The major advantages of this technology are its general applicability to most drugs and its simplicity.[2] Preparation of nanosuspension is simple and applicable to all drugs which are water insoluble. Nanosuspensions are prepared by using wet mill, high pressure homogenizer, emulsion solvent evaporation, melt emulsification and supercritical fluid techniques. Nano-suspensions can be delivered by oral, parenteral, pulmonary and ocular routes. Nanosuspensions can also be used for targeted drug delivery when incorporated in the ocular inserts and mucoadhesive hydrogels. Currently, efforts are being directed to extending their applications in site-specific drug delivery. Rapid strides have been made in the delivery of nanosuspensions by parenteral, preoral, ocular and pulmonary routes.

TECHNIQUES FOR PREPARATION OF NANOSUSPENSIONS

Technically preparations of nanosuspensions are simpler alternative than liposomes and other conventional colloidal drug carriers but reported to be more cost effective. It is particularly for poorly soluble drugs and to yield a physically more stable product. For manufacturing nanosuspensions there are two converse methods, "Top-down process technology" and "Bottom-up process technology". The top -down process follows disintegration approach from large particles, microparticles to Nanosized particles [3].

Examples are

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

Bottom-up process is an assembly method forms nanoparticles from molecules[4]. Examples includes

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

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

A. High Pressure Homogenization:

It is most widely used method for preparing nanosuspensions of many poorly aqueous soluble drugs [5]. It involves three steps. First drug powders are dispersed in stabilizer solution to form presuspension, and then the presuspension is homogenized in high pressure homogenizer at a low pressure for premilling, and finally homogenized at high pressure for 10 to 25 cycles until the nanosuspensions of desired size are formed. Different methods are developed based on this principle for preparations of nanosuspensions such as Disso cubes, Nanopure, Nanoedge and Nanojet [6].

Homogenization in aqueous media (Disso cubes): This technology was developed by R.H.Muller using a piston-gap type high pressure homogenizer in 1999 [7]. In this method, the suspension containing a drug and surfactant is forced under pressure through a Nanosized aperture valve of a high pressure homogenizer.

Principle:

During homogenization, the fracture of drug particles is brought about by cavitation, high-shear forces and the collision of the particles against each other. The drug suspension, contained in a cylinder of diameter about 3mm, passes suddenly through a very narrow homogenization gap of 25µm, which leads to a high streaming velocity. In the homogenization gap, according to Bernoulli's equation, the dynamic pressure of the fluid increases with the simultaneous decrease in static pressure below the boiling point of water at room temperature. In consequence, water starts boiling at room temperature, leading to the formation of gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached again. The implosion forces are sufficiently high to break down the drug microparticles into nanoparticles. Additionally, the collision of the particles at high speed helps to achieve the nano-sizing of the drug. To improve the efficiency of nano-sizing, the addition of viscosity enhancers is advantageous in certain cases as increasing the viscosity increases the powder density within the dispersion zone (homogenization gap).

In order to obtain an optimized formulation, the effect of the following process variables should be investigated.

- **Effect of homogenization pressure:** As the homogenizer can handle varying pressures, ranging from 100 to 1500 bars, the effect of the homogenization pressure on the particle size should be investigated in each case in order to optimize the process parameters. It is expected that the higher the homogenization pressure, the lower the particle size obtained.
- **Number of homogenization cycles:** For many drugs it is not possible to obtain the desired particle size in a single homogenization cycle. Typically, multiple cycles are required. Hence, depending on the hardness of the drug, the desired mean particle size and the required homogeneity of the product, homogenization can be carried out in three, five or 10 cycles. It is anticipated that the higher the number of homogenization cycles, the smaller the particle size obtained. The optimum number of homogenization cycles can be arrived at by analysing the particle size and polydispersity index of the drug after each cycle

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 [8].
- Narrow size distribution of the nanoparticulate drug present in the final product [9].
- Allows aseptic production of nanosuspensions for parenteral administration.
- Flexibility in handling the drug quantity, ranging from 1 to 400mg/mL, thus enabling formulation of very dilute as well as highly concentrated nanosuspensions [10].

Disadvantages

- Prerequisite of micronized drug particles.
- Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.

Homogenization in nonaqueous media (Nanopure):

Nanopure is suspensions homogenized in water-free media or water mixtures like PEG 400, PEG 1000 etc. The homogenization can be done at room temperature, 0^0C and below freezing point (-20^0C), hence it is known as "deep freeze" homogenization [11].

Nanoedge:

Nanoedge technology is the combination of both precipitation and homogenization. The basic principle is same as that of precipitation and homogenization [12]. The major disadvantage of precipitation technique such as crystal growth and long term stability can be overcome by using the Nanoedge technology. Particles of smaller size and better stability in short time can be achieved.

Nanojet:

It is also called as opposite stream technology, uses a chamber where a stream of suspension is divided into two or more parts, which colloid with each other at high pressure, due to the high shear forces produced during the process particle size is reduced[13].

B. Milling Techniques

i) Media Milling:

Principle:

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 unimodal distribution profiles and mean diameters<200nm is 30–60min [14]. 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.

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. A comparison of the size of naproxen crystals before and after media milling
- Flexibility in handling the drug quantity, ranging from1 to 400 mg/mL enabling formulation of very dilute as well as highly concentrated nanosuspensions.

Disadvantages

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

ii) Dry-Co-grinding:

Recently many nanosuspensions are prepared by dry milling technique. Dry- co-grinding can be carried out easily and economically and can be conducted without organic solvents [15]. Physicochemical properties and dissolution of poorly water soluble drugs are improved by Co-grinding because of an improvement in the surface polarity and transformation from a crystalline to an amorphous drug.

Advantages

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

Disadvantages

- Generation of residue of milling media.

C. Emulsification-Solvent Evaporation Technique

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

D. Precipitation

Within the last decade, precipitation has been applied to prepare submicron particles, especially for the poorly soluble drugs [16]. The drug is first dissolved in a solvent, then this solution is mixed with a miscible antisolvent in the presence of surfactants. Rapid

addition of a drug solution to the antisolvent leads to sudden super saturation of drug and formation of ultrafine crystalline or amorphous drug solids [17].

Advantages

- Simple process, Ease of scale up and Economical production.

Disadvantages

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

E. Supercritical Fluid Process

The particle size reduction was achieved more by the solubilization and nanosizing technologies through the super critical fluid process. Super critical fluids (SCF) are noncondensable dense fluids whose temperature and pressure are greater than its critical temperature (Tc) and critical pressure (Tp).This process allows the micronization of drug particles to submicron level. Recent advances in SCF process are to create nanoparticulate suspension of particle size of 5 to 2000nm in diameter [18]. The low solubility of poorly water-soluble drugs and surfactants in supercritical CO_2 and the high pressure required for these processes restrict the utility of this technology in the pharmaceutical industry.

F. Melt Emulsification Method

In this method drug is dispersed in the aqueous solution of stabilizer and heated above the melting point of the drug and homogenized to give an emulsion. During this process, the sample holder was enwrapped with a heating tape fitted with temperature controller and the temperature of emulsion was maintained above the melting point of the drug. The emulsion was then cooled down either slowly to room temperature or on an ice-bath.

Advantages

- Melt emulsification technique relative to the solvent evaporation method is total avoidance of organic solvents during the production process.

Disadvantages

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

G.Lipid Emulsion/Microemulsion Template:

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

Advantages

- High drug solubilization
- Long shelf life
- easy to manufacture

Disadvantages

- Use of hazardous solvent
- Use of high amount of surfactant and stabilizers

H. Solvent Evaporation:

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

FORMULATION CONSIDERATIONS

Stabilizer

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 functions of a stabilizer are to wet the drug particles thoroughly, and to prevent Ostwald's ripening [19, 20] and agglomeration of nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behaviour of nanosuspensions. In some cases, a mixture of stabilizers is required to obtain a stable nanosuspension. The drug-to-stabilizer ratio in the formulation may vary from 1:20 to 20:1 and should be investigated for a specific case. Stabilizers that have been explored so far include cellulosics, poloxamers, polysorbates, lecithins and povidones [21]. Lecithin is the stabilizer of choice if one intends to develop a parenterally acceptable and autoclavable nanosuspension.

Organic solvents

Organic solvents may be required in the formulation of nanosuspensions if they are to be prepared using an emulsion or microemulsion as a template. As these techniques are still in their infancy, elaborate information on formulation considerations is not available. The acceptability of the organic solvents in the pharmaceutical arena, their toxicity potential and the ease of their removal from the formulation need to be considered when formulating nanosuspensions using emulsions or microemulsions as templates. The pharmaceutically acceptable and less hazardous water-miscible solvents, such as ethanol and isopropanol, and partially water-miscible solvents, such as ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate and benzyl alcohol, are preferred in the formulation over the conventional hazardous solvents, such as dichloromethane. Additionally, partially water- miscible organic solvents can be used as the internal phase of the microemulsion when the nanosuspensions are to be produced using a microemulsion as a template.

Co-surfactants

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

Other additives

Nanosuspensions may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant, depending on either the route of administration or the properties of the drug moiety

CHARACTERIZATION OF NANOSUSPENSIONS

The essential characterization parameters for nanosuspensions are as follows.

Mean particle size and particle size distribution: The mean particle size and the width of particle size distribution are important characterization parameters as they govern the saturation solubility, dissolution velocity, physical stability and even biological performance of nanosuspensions. It has been indicated by [22] that saturation solubility and dissolution velocity show considerable variation with the changing particle size of the drug.

Photon correlation spectroscopy (PCS) [23] can be used for rapid and accurate determination of the mean particle diameter of nanosuspensions. Moreover, PCS can even be used for determining the width of the particle size distribution (polydispersity index, PI). The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. A PI value of 0.1–0.25 indicates a fairly narrow size distribution whereas a PI value greater than 0.5 indicates a very broad distribution. No logarithmic normal distribution can definitely be attributed to such a high PI value. Although PCS is a versatile technique, because of its low measuring range (3nm to 3µm) it becomes difficult to determine the possibility of contamination of the nanosuspension by microparticulate drugs (having particle size greater than 3µm). Hence, in addition to PCS analysis, laser diffractometry (LD) analysis of nanosuspensions should be carried out in order to detect as well as quantify the drug microparticles that might have been generated during the production process. Laser diffractometry yields a volume size distribution and can be used to measure particles ranging from 0.05–80 µm and in certain instruments particle sizes up to 2000µm can be measured. The typical LD characterization includes determination of diameter 50% LD (50) and diameter 99% LD (99) values, which indicate that either 50 or

99% of the particles are below the indicated size. The LD analysis becomes critical for nanosuspensions that are meant for parenteral and pulmonary delivery. Even if the nanosuspension contains a small number of particles greater than 5–6µm, there could be a possibility of capillary blockade or emboli formation, as the size of the smallest blood capillary is 5–6µm. It should be noted that the particle size data of a nanosuspension obtained by LD and PCS analysis are not identical as LD data are volume based and the PCS mean diameter is the light intensity weighted size. The PCS mean diameter and the 50 or 99% diameter from the LD analysis are likely to differ, with LD data generally exhibiting higher values. The nanosuspensions can be suitably diluted with deionized water before carrying out PCS or LD analysis.

For nanosuspensions that are intended for intravenous administration, particle size analysis by the Coulter counter technique is essential in addition to PCS and LD analysis. Since the Coulter counter gives the absolute number of particles per volume unit for the different size classes, it is a more efficient and appropriate technique than LD analysis for quantifying the contamination of nanosuspensions by microparticulate drugs.

Crystalline state and particle morphology: *The* assessment of the crystalline state and particle morphology together helps in understanding the polymorphic or morphological changes that a drug might undergo when subjected to nanosizing. Additionally, when nano suspensions are prepared drug particles in an amorphous state are likely to be generated. Hence, it is essential to investigate the extent of amorphous drug nanoparticles generated during the production of nanosuspensions. The changes in the physical state of the drug particles as well as the extent of the amorphous fraction can be determined by X-ray diffraction analysis [20, 24] and can be supplemented by differential scanning calorimetry [25]. In order to get an actual idea of particle morphology, scanning electron microscopy is preferred [20].

Particle charge (zeta potential): The determination of the zeta potential of a nanosuspension is essential as it gives an idea about the physical stability of the nanosuspension. The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself. In order to obtain a nanosuspension exhibiting good stability, for an electrostatically stabilized nanosuspension a minimum zeta potential of ± 30mV is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of ±20mV is desirable [26].

Saturation solubility and dissolution velocity: The determination of the saturation solubility and dissolution velocity is very important as these two parameters together help to anticipate any change in the in-vivo performance (blood profiles, plasma peaks and bioavailability) of the drug. As nanosuspensions are known to improve the saturation solubility of the drug, the determination of the saturation solubility rather than an increase in saturation solubility remains an important investigational parameter. The saturation solubility of the drug in different physiological buffers as well as at different temperatures should be assessed using methods described in the literature. The investigation of the dissolution velocity of nanosuspensions reflects the advantages that can be achieved over conventional formulations, especially when designing the sustained-release dosage forms based on nanoparticulate drugs. The dissolution velocity of drug nanosuspensions in various physiological buffers should be determined according to methods reported in the pharmacopoeia.

In-vivo biological performance: The establishment of an in-vitro/in-vivo correlation and the monitoring of the in-vivo performance of the drug is an essential part of the study, irrespective of the route and the delivery system employed. It is of the utmost importance in the case of intravenously injected nanosuspensions since the in-vivo behaviour of the drug depends on the organ distribution, which in turn depends on its surface properties, such as surface hydrophobicity and interactions with plasma proteins [27, 28]. In fact, the qualitative and quantitative com position of the protein absorption pattern observed after the intravenous injection of nanoparticles is recognized as the essential factor for organ distribution [27, 28, 29]. Hence, suitable techniques have to be used in order to evaluate the surface properties and protein interactions to get an idea of in-vivo behaviour. Techniques such as hydrophobic interaction chromatography can be used to determine surface hydrophobicity , whereas 2-D PAGE [27] can be employed for the quantitative and quailtative measurement of protein adsorption after intravenous injection of drug nanosuspensions in animals.

APPLICATION OF NANOSUSPENSIONS

Nanosuspensions are used as oral, parenteral, ocular, and pulmonary drug delivery systems.

- **Oral Administration**

Oral administration is the first patient choice because of painless and noninvasive administration [30, 31]. In addition, oral formulations have several advantages

for the pharmaceutical industry such as easy manufacturing, short production time, and reasonable production cost [30]. Oleanolic acid, which has many applications such as hepatoprotective, antitumour, antibacterial, anti-inflammatory, and antiulcer effects, has low aqueous solubility which results in erratic pharmacokinetics after oral administration. Applying oleanolic acid in the form of nanosuspension increases dissolution rate to about 90% in the first 20 min compared to just 15% for micronized drug powder [32]. Reduction of drug particle size to the nanoscale leads to an increased dissolution rate and can improve adhesion of the drug particles to the mucosa. Better contact with intestinal cells (bioadhesive phase) and a greater concentration gradient between blood and GIT increase drug intestinal absorption [32, 33, 34]. Nanosuspensions are also used to control infections. Atovaquone and buparvaquone for the treatment of leishmaniasis and opportunistic Pneumocystis carinii infections in HIV patients are effective in high doses due to low bioavailability. A comparative study of atovaquone in the form of micronized particles and nanosuspensions showed that the latter decreased infectivity from 40% to 15%. In another example, buparvaquone nanosuspensions reduced infection from 2.0 to 1.02 and micronized particles only to 1.47.

- **Parenteral Administration**

In emergency cases such as cardiac arrest and anaphylactic shock parenteral administration is the first choice [35]. Parenteral administration includes administration of dosage forms by subcutaneous, i.v., intramuscular, and intra-arterial methods [36]. Advantages of this type of administration include avoidance of first-pass metabolism, reliable doses, and higher bioavailability. Control over the dose and rate allows more predictable pharmacodynamic and pharmacokinetic profiles after i.v. administration compared to oral administration [37]. Administered drug particles are required to be smaller than 5 μm to prevent blockage of capillaries [39]. A study on mice investigated tumour growth inhibition rate and showed that oridonin in the form of nanosuspension decreased considerably the volume and weight of the tumour. Oridonin in the form of nanosuspension raised the rate of tumour inhibition to 60.23% compared to 42.49% for the conventional form [38]. Nanosuspensions improve therapeutic efficiency and reduce the cost of therapy through improved dosing efficiency and smaller injection volumes.

- **Pulmonary Drug Delivery**

Pulmonary drug delivery aims at treating several respiratory conditions such as asthma and chronic obstructive pulmonary diseases [40, 41]. Advantages of pulmonary drug delivery over oral and parenteral drug administration include direct delivery to the site of action which leads to decreased dosage and side effects [42]. Conventional pulmonary delivery systems provide only rapid drug release, poor residence time, and lack of selectivity [43]. Nanosuspensions can solve problems of poor drug solubility in pulmonary secretions and lack of selectivity through direct delivery to target pulmonary cells. Adhesiveness of nanosuspensions to mucosal surfaces leads to improved selectivity because of minimal drug loss and prolonged residence time at target site. Pulmonary nanosuspensions improve drug diffusion and dissolution rate and consequently increase bioavailability and prevent undesirable drug deposition in the mouth and pharynx. Surface engineered nanosuspensions may provide quick onset followed by controlled drug release which is optimal drug delivery pattern for most pulmonary diseases. Moreover, nanosuspensions for treating lung infections have demonstrated good proportion between actual and delivered drug concentrations in each actuation [44]. The internalisation rate for nanoparticles of 0.5 μm diameter into the pulmonary epithelial cell has been reported to be 10 times higher compared to particles of 1 μm and 100 times higher compared to particles of 2-3 μm [45, 46].

- **Ocular Administration**

Major problems in ocular therapy include(i)poor drug solubility in lachrymal fluids,(ii)repeated instillation of conventional eye drops due to drainage through the nasolacrimal duct,(iii)repeated instillation and systematic drug absorption often causing side effects [47].

Nanosuspensions as ocular drug delivery systems offer several advantages.

(i)Nanoparticle modified surface by appropriate bioerodible polymer causes prolonged residual time in cul-de-sac desired for effective treatment. Commonly reported polymers in ocular nanosuspensions are poly(alkyl cyanoacrylates), polycaprolactone, and poly(lactic acid)/poly(lactic-co-glycolic acid) [48]. Employing polymers in ocular drug delivery significantly prolongs drug ocular residence time and improves bioavailability [49]

(ii)Positively charged nanoparticles have strong adhesion to negatively charged mucin which extends the drug release. For example, polymer Eudragit RS 100 was used in ibuprofen nanosuspensions to increase drug residence time by creating positively charged surface which resulted in improved corneal adhesion [50]. Flurbiprofen nanosuspensions covered by Eudragit polymers RS 100 and RL 100 exhibited prolonged drug release [51]. Chitosan is another

mucoadhesive cationic polymer used in ocular drug delivery to bond with negatively charged mucin and enhance drug residence time.

(iii)Reduced drug loss because of the natural adhesiveness of drug nanoparticles.

(iv) Enhanced rate and extent of drug absorption: for instance, in a study by Kassem et al., nanosuspensions of hydrocortisone, prednisolone, and dexamethasone were prepared by high pressure homogenisation. Measured intraocular pressure of normotensive Albino rabbits demonstrated that glucocorticoid drugs in the form of nanosuspensions unlike conventional dosage forms significantly increase the absorption rate and the therapeutic efficiency [52].

Employing polymers with the ability of in situ gelling (instilled in a liquid form and transformed to a gel in the cul-de-sac) controls the drug release. Study by Gupta et al. suggested that formulating forskolin nanoparticles in conjunction with in situ gel forming polymers noveon AA-1 polycarbophil/poloxamer 407 controls drug release through increased corneal contact time and slower drug diffusion within the viscous polymer medium [51].

RESEARCH WORK AND SUCCESSFUL FORMULATIONS BASED ON NANOSUSPENSIONS

- A suspension containing 20 nm silica particles in ethylene glycol was subjected to electrohydrodynamic atomization (EHDA) in the stable cone-jet mode using a ring-shaped ground electrode. The droplets produced were sized by laser diffraction and were in the range 0.5-20 mum. Immediately after deposition, droplet relics were analysed by optical microscopy and were found to be in the size range 1-80 mum. Subsequently, using a pointed rod-electrode (rather than a ring), and by increasing the intensity of the electric field and by reducing the flow rate of suspension subjected to EHDA, relics of similar to 50 mum in size were deposited using a patterning device. In both of the above instances, the relics contained two distinct zones, an outer ring of ethylene glycol and a much smaller dense inner region of silica nanoparticles. These results show that, by using EHDA, a novel controlled deposition method of nanosuspensions has been developed [53].
- All-Trans Retinoic Acid (ATRA) nanosuspensions were prepared with a modified precipitation method. The ATRA solution in acetone was injected into pure water by an air compressor under the action of ultrasonication. Photon correlation spectroscopy results showed that the mean particle size of ATRA nanoparticles in nanosuspensions reduced from 337 nm to 155 nm as the injection velocity increased and the polydispersity index was 0.45-0.50. The morphology of ATRA nanoparticles varied with the different concentration of ATRA solution in acetone. ATRA nanoparticles showed an amorphous state and stable in 6 months. It could be concluded that this modified precipitation method could produce stable and controllable ATRA nanosuspension to a certain extent, thus benefit for higher saturation solubility [54].
- Albendazole, an anthelmintic drug belonging to BCS class II, has poor bioavailability. Bioavailability is dissolution rate dependent and hence needs novel approach for enhancement of bioavailability. The aim of the study was to develop nanosuspension of albendazole by using various techniques like nanoprecipitation, emulsion template and sonication. Nanosuspensions were prepared using polyvinylpyrrolidone K30 as a stabilizer and Tween 80 as a surfactant. Average particle size, zeta potential, particle size distribution, pH, viscosity, photomicrography, sedimentation, redispersibility and % drug content were determined to characterize prepared nanosuspensions. In vitro release study was performed in 0.1N HCl using cellophane membrane and with marketed product. Residual solvent compared determination was carried out by gas chromatography for nanosuspensions prepared by nanoprecipitation and emulsion template techniques. All the results obtained for characterization were satisfactory. The prepared nanosuspensions showed particle size 673±9.18 nm to 893±21.6 nm, zeta potential -8.70±0.5mV to -8.96±0.8, polydispersity index 0.204±0.04 to 0.644±0.07. In vitro release study of the nanosuspensions showed 33.80% to 42.92% drug release in first hour which was higher than the marketed suspension (16.19% release in first hour). The optimized nanosuspensions showed up to 97.05 % drug release within 6-8 hours while marketed product showed up to 91.03% drug release within 10 hours[55].
- Oleanolic acid is a naturally derived triterpene used clinically in the treatment of hepatitis in China, but its poor solubility often leads to poor bioavailability. In the present study, oleanolic acid nanosuspensions were prepared by the nanoprecipitation method and then systematically characterized. The average particle size of the obtained nanosuspensions was 284.9 nm, with a polydispersity index of 0.216. Transmission

electron microscopy and atomic force microscopy showed that the drug existed as spherical or near-spherical nanoparticles in the nanosuspensions. Differential scanning calorimetry and X-ray diffraction studies indicated that oleanolic acid was present in an amorphous state in the lyophilized nanosuspensions. At 25ºC, the saturation solubility of oleanolic acid was increased by about 6 times after nanoation (25.72 microg mL(-1) vs 4.37 microg mL(-1)). In the in-vitro drug release experiments, the lyophilized nanosuspensions showed a faster drug dissolution rate than that of the coarse drug powder (approx. 90% vs 15% during the first 20 min), and nearly 95% of the oleanolic acid was released by 120 min. As evidenced by the lower serum alanine aminotransferase activity and liver malondialdehyde content, pre-treatment with oleanolic acid nanosuspensions significantly enhanced the hepatoprotective effect of oleanolic acid against carbon tetrachloride-induced liver injury [56].

- A study was performed to investigate potential of Eudragit RLPO-based nanosuspension of glimepiride (Biopharmaceutical Classification System class II drug), for the improvement of its solubility and overall therapeutic efficacy, suitable for peroral administration. Nanoprecipitation method being simple and less sophisticated was optimized for the preparation of nanosuspension. Physicochemical characteristics of nanosuspension in terms of size, zeta potential, polydispersity index, entrapment efficiency (% EE) and *in vitro* drug release were found within their acceptable ranges. The size of the nanoparticles was most strongly affected by agitation time while % EE was more influenced by the drug/polymer ratio. Differential scanning calorimetry and X-ray diffraction studies provided evidence that enhancement in solubility of drug resulted due to change in crystallinity of drug within the formulation. Stability study revealed that nanosuspension was more stable at refrigerated condition with no significant changes in particle size distribution, % EE, and release characteristics for 3 months. *In vivo* studies were performed on nicotinamide–streptozotocin-induced diabetic rat models for pharmacokinetic and antihyperglycaemic activity. Nanosuspension increased maximum plasma concentration, area under the curve, and mean residence time values significantly as compared to aqueous suspension. Oral glucose tolerance test and antihyperglycaemic studies demonstrated plasma glucose levels were efficiently controlled in case of nanosuspension than glimepiride suspension. Briefly, sustained and prolonged activity of nanosuspensions could reduce dose frequency, decrease drug side effects, and improve patient compliance. Therefore, glimepiride nanosuspensions can be expected to gain considerable attention in the treatment of type 2 diabetes mellitus due to its improved therapeutic activity [57].

CONCLUSION AND PROSPECTIVE

This review presents the recent progress in therapeutic nanosuspensions produced by various techniques such as high pressure homogenisation, media milling, and emulsification. However, in early stages, several in vivo studies clearly demonstrate the potential of these drug delivery vehicles in parenteral, oral, ocular, and pulmonary administration, where not only a controlled release but also an appropriate bioadhesion is required. The research on drug nanosuspensions is in its infancy. However, these systems carry flexibility and opportunity for further tailoring particles, surface properties to optimise in vivo responses, and generation of new clinical approaches for treating a number of diseases (heart, cancer, diabetes, Parkinson's, Alzheimer's, etc.) are required. Considering that nanoparticle uptake is size dependent, working on the size optimization of drug nanosuspension can help us prepare an appropriate nanosuspension formulation with better diffusion through the mucus gel layer. In addition, incorporation of polymers on the particle surface and size reduction can be regarded as the future step in nanosuspension research.

To summarise future research directions include

- increasing in vivo bioavailability and correlating in vitro and in vivo bioavailability data;
- achieving controlled and sustained drug release over extended period of time using biocompatible matrix polymers;
- development of stimuli-responsive systems such as magnetic field, light, temperature, and pH, which is particularly important for highly toxic drugs;
- further studies that are necessary to understand the behaviour of nanosuspensions in vivo, including interactions with cells and different biological barriers such as the blood-brain barrier; surface engineering of nanosuspensions for active or passive targeting in order to enhance their ability to reach the target.

Table 1: List of drugs and other information

S.No	Model drug	Route	Polymer used	Method of preparation	Purpose	Reference
1.	Ibuprofen	lyophilized powder or granules	Tween 80 and PVP K25	Melt emulsification method	Enhancing dissolution rate	Kocbek *et al* [58]
2.	Ibuprofen	Topical application	Eudragit RS100	Quasi-emulsion solvent diffusion technique	Improving the bioavailability	Rosario Pignatello *et al* [59]
3.	Flurbiprofen (FLU),	Eye-drop formulation	Eudragit RS100 and RL100	Quasi-emulsion solvent diffusion technique	Improving the availability	Pignatello *et al*[60]
4.	Spironolactone	Oral and i.v. formulations	Nanosized, micronized, and coarse drug material and surfactant.	Disso Cubes	Bioavailability	Langguth *et al*[61]
5.	Piposulfan (alkylating agent), Etoposide (topoisomerase II inhibitor), Camptothecin (topoisomerase I inhibitor) and	Intravenous injection.	2% w/v solids suspension containing 1 % w/v surfactant	Wet milling technology	Retain biological effectiveness	Merisko Liversidge *et al*[62]
6.	Diclofenac sodium	Ophthalmic application	Poly(lactide-co-glycolide) and poly(lactide-co-glycolide-leucine) {poly[Lac(Glc-Leu)]} biodegradable polymers	Emulsion and solvent evaporation technique	Improving the ocular availability	Sagar *et al*[63]
7.	Celecoxib	Dry powder suitable for tabletting.	Tween 80, PVP K-30 and SDS	Emulsion-diffusion method	Increase drug dissolution rate	Andrej Dolenc *et al*[64]
8.	Risperidone	Parenteral drug delivery	Poly (D, L-Lactide	Nanoprecipitation method	High encapsulation efficiency	Muthu and Singh[65]
9.	Asulacrine	Intravenous (i.v.) administration	Lyophilized to obtain the dry ASL	High pressure homogenization	Dissolution and saturation solubility were enhanced	Srinivas Ganta *et al*[66]
10.	Azithromycin	Freeze-dried powder		High pressure homogenization	Increasing its saturation solubility and dissolution velocity	Dianrui Zhang *et al*[67]
11.	Atorvastatin calcium	Anhydrous form		High pressure homogenization technique	Enhance its solubility and dissolution characteristics	Arunkumar *et al*[68]
12.	Oridonin	Crystalline state		High-pressure homogenization	Increased drug saturation solubility and dissolution velocity.	Lei Gao *et al*[69]

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