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NANOSUSPENSIONS: A PROMISING DRUG DELIVERY SYSTEM

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ABSTRACT: Nanotechnology has emerged as one of the growing field in the pharmacy, where nanosuspension is part of nanotechnology. Nanosuspension is a novel approach for delivery of hydrophobic drugs due to their unique advantage and special features. Nanosuspension not only helps in overcoming the poor solubility but also increases the bioavailability, dissolution rate, and stability. Nanosuspensions are also used for specialized drug delivery system like mucoadhesive hydrogels. It has been reported as that now efforts are been made for extending the application of nanosuspension in site- specific drug delivery. This review article deals with the various methods used in the preparation of nanosuspension & their merits and demerits, characterization and evaluation of nanosuspension, future prospect, marketed products , and case studies.

Keywords: Nanosuspension, Homogenization, Bioavailability, Solubility.

INTRODUCTION:

Nanosuspension is defined as "finely dispersed solid drug particles in an aqueous vehicle, which are stabilized by surfactants, for either topical & oral use or parentral and pulmonary administration, with reduced particle size, leading to increase in surface area, dissolution rate, hence improving bioavailability". Average particle size is between 200 and 600 nm. [1] Industries in pharmacy are constantly searching new approaches to obtain adequate oral bioavailability as most of biological properties exhibiting NCEs are poorly water soluble.[1] Almost 40% Of New chemical entities which are produced through different drug discovery programs are mostly lipohilic or poorly water soluble compounds. It has always been challenging problem to produce poorly water soluble compound. Formulating the nanosized particles can be applied to all drugs belonging to biopharmaceutical classification system (BCS) from class II to IV. [2-7] There are many methods for increasing the solubility of poorly soluble drugs example, precipitation technique, surfactant dispersion, microionization , solubalization ,& other techniques are microemulison, liposomes, inclusion complexation but all this lack in universal acceptability in all drugs.[2]These technique are not applicable to those drugs which are not soluble in aqueous & organic solvents. Thus nanosuspension can be used to solve this problem. Surfactants are used to stabilize the nanosuspension. Nanosuspensions helps in solving the problem of bioavailability and poor solubility but other than this it also alters the pharmacokinetic of the drug which leads in improving the drug safety and efficacy. In case of drugs that are insoluble in both water and in organic media instead of using lipidic systems, nanosuspensions are used as a formulation approach. Formulation of nanosuspension is most appropriate for those compounds which have high melting point, high log P value, and are used as high dose. Nanosuspension has been reported to enhance absorption and bioavailability; it may help to reduce the dose of the conventional oral dosage forms. Drug particle size reduction leads to an increase in surface area & consequently in the rate of dissolution as described by Nernst –Brunner & Levich modification of Noyes Whitney equation. [8]

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METHODS OF PREPARATION OF NANOSUSPENSION:-

Preparing nanosuspension is simple alternative to that of liposomes & other conventional colloidal drug but results to be more cost effective .Nanosuspension can be prepared by two converse methods :-

- Bottom up process technology
- Top down process technology

The top down process involves the disintegration from large particles, microparticles to nanosized particles. The techniques used area's follows:

- High pressure homogenization
- Nanoedge
- Nanopure
- Media milling
- Dry-co-grinding

Bottom up process: conventional precipitation methods are called bottom up process. Techniques used are:

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

TOP DOWN PROCESS:

High Pressure Homogenization:- (Disso cubes)

Principle:-The Piston-gap homoginizer works on the principle of pressure,shearing forces ,and the bombarding of the particles against each other leading to fracture of the drugs . High-pressure homogenization was developed by R.H.Muller. The patent is recently owned by skype pharma plc.It is most generally used method for preparation of nanosuspension of many poorly aqueous soluble drugs. Suspension of the drug is made to pass through the small orifice (cavitation) which results in reduction of static pressure below boiling point of water at room temperature, consequently at room temperature water starts boiling leading to formation of air bubbles, which explodes when suspension leaves the cavitation,and pressure of the air is normal again. The force due to explosion is quite high and sufficient to break down the drug microparticles into nanoparticles. For this method the major concern is the sample particles must be small before loading into the instrument. It is necessary to form pre-suspension of the microsized drug in surfactant solution using high speed stirrer, before loading the drug into homogenization process. It is advisable to start with the micronized drug (particle size<25m) for production of nanosuspensions in order to prevent blocking of the homogenization gap.The instrument can be operated at pressure varying from 100-1500 bars (2800-21300 psi) and up to 2000 bars with volume capacity of 40 ml (for laboratory scale). [9,10]

Advantage:-

1. Major advantage of this is that it can be applied to the drugs that are poorly soluble in aqueous as well as in organic media.
2. Prevents the erosion of processed materials.
3. It can be used for diluted as well as concentrated suspensions and also allows aseptic production.
4. It allows aseptic production for parental administration. [11]

Disadvantage: -

1. Pre-processing is required before loading the drug.
2. High cost instruments are required which ultimately results in increasing the cost of dosage form. [11]

NANOPURE (HOMOGENIZATION IN NON – AQUEOUS MEDIA):

Suspensions homogenized in water-free media or water mixtures like PEG 400, PEG 1000 etc. In nanopure technology the drug suspensions in non aqueous media were homogenized at 0°Celsius or even below the freezing point and hence are called "deep – freeze"homogenizations. It can be used for thrmolabile substances at milder conditions. The nanocrystals of the drug dispersed in liquid polyethylene glycol (PEG) or various oils can be directly filled as drug suspensions into HPMC capsules or gelatin. [1,9]

NANOEDGE:-

The principle involved in Nanoedge is same as that of precipitation and homogenization .A combination of these techniques gives much smaller particle size and improved stability in short period of time. In this technique drug is dissolved in an organic solvent and this solution is mixed with the miscible anti-solvent for precipitation .Drug precipitates due to low solubility in the water solvent mixture. Precipitation is coupled with high shear processing, which is accomplished by combination of rapid precipitation and high pressure homogenization. The major drawback of the precipitation technique is that it leads to crystal growth and long-term stability, can be solved using the Nanoedge technology.

NANOJET: -

It is also known as opposite stream technology ,in this stream of suspension is divided into two or more parts with the use of chamber , where suspension colloid with each other at high pressure up to 4000 bar at high velocity of 1000m/s. Due to high shear forces produced in this process leads to reduction in particle size . The major limitation is that high numbers of passes are required through the microfluidizer, nearly about 75 passes and the product obtained contains a relatively large fraction of microparticles. This process also requires large production time. [9]

MEDIA MILLING:-

This technology was brought by Liversidge Etal(1992). Recently this technology was developed by company Elan Drug Delivery.

Principle: -In this media, nanosuspensions are produced using high –shear media mills or pearl mills. This technology works on the principle of shear forces generated as the result of impaction of the milling media where the drug provide the energy input to break the microparticulate drug into nanosized particles.

Construction: -The Milling media consist of a milling chamber, recirculation chamber, and a milling shaft,motor. The Milling chamber is charged with milling media, water, drug, and stabilizer. Milling medium is composed of glass, zirconium oxide or highly cross linked polystyrene resin.

Working: - Crude slurry consisting of drug, water, and stabilizer is fed into the milling chamber then milling mills or pearls are rotated at a very high shear rate. Milling process is executed under controlled temperature. Process can be completed in batch or in recirculation mode.

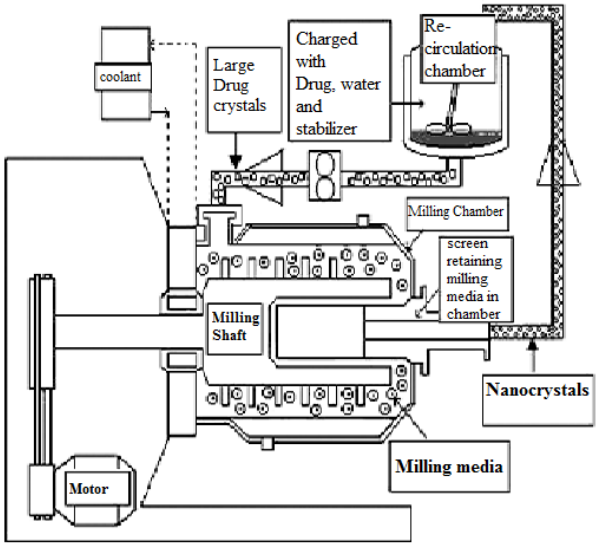

Figure 1: Schematic representation of the media milling process. [1,10]

Dry-co-grindig:-

Dry-co-grinding can be carried it's quite easy and economicalit needs to be conducted without organic solvents. This technique helps in enhancing the physicochemical properties and dissolution of poorly water soluble drugs due to improvement in surface polarity and transformation from a crystalline to an amorphous drug.

BOTTOM UP PROCESS:-

Super critical fluid method:-

It is one of the finest methods which produce nanoparticles from drug solution. The various methods used are rapid expansion of supercritical solution (RESS), super critical anti solvent process(PCA). There is expansion of drug solution in super critical fluid through a nozzle, which leads loss of solvent power of the supercritical fluid resulting in precipitation of the drug as fine particles. In the PCA method, there is atomization of drug solution into a chamber containing compressed Co2. The solution leads to supersaturation & thus precipitates as fine crystals as there is removal of solvent system.

Limitations:

1. Use of high ratio of stabilizers and surfactants and use of hazardous solvents as compared to other techniques.
2. Supersaturation leading to nucleation growth resulting in development of amorphous form or any undesired polymorph. [2]

LIPID EMULSION / MICROEMULSION TEMPLATE:-

Different way to produce nanosuspension is to use an emulsion formed by conventional method using partially water miscible solvent as dispersed phase. Just by diluting the emulsion nanosuspension can be produced. Nanosuspensions can also be obtained by microemulsion as template. Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids such as oil and water stabilized by an interfacial film or surfactant & co-surfactant. Suitable microemulsion yields the drug nanosuspension .Example is Grisofulvin nanosuspension.

EMULSIFICATION SOLVENT EVAPORATION TECHNIQUE:

In this technique there is preparation of solution of drug followed by its emulsification in/ another liquid that is nonsolvent for the drug. There is evaporation of solvent forming precipitation of drug, particle agglomeration and crystal growth can be controlled using high speed stirrer.

Precipitation technique:

Precipitation has been used from many years to prepare submicron particles especiallyfor poorly soluble drugs.[13,14] Drug is first dissolved in solvent. This solution is mixed with miscible antisolvent in presence of

surfactant. Addition of drug solution to antisolvents forms supersaturation of drug in mixed solution, and leading to generation of crystals or amorphous drug solids. It involves two phases; nuclei formation and crystal growth.

Advantage:

1. Economic production and simple process.

Disadvantages:

1. Crystal growth can be limited by addition of surfactant.
2. Drug must be soluble compulsorily in one solven

FORMULATION CONSIDERATIONS:-

Stabilizer: -Stabilizers have very crucial role in formulation of the nanosuspension. They are mainly used to wet the surface of the solute or drug particle to delay the Ostwald ripening and agglomeration of nanaosuspension to give good physical stability leading to stable formulation by providing steric or ionic barriers. The type and amount of stabilizer have pronounced effect on physical stability and in vivo behavior of nanosuspension.[12] Examples of stabilizers used in preparation of nanosuspension are polysorbates, lecithin, povidones. [15]

Organic solvent: -If nanosuspension are prepared by using emulsion or microemulsion template then organic solvents are used in formulation .Organic solvents can be hazardous in physiologic and environmental means. Ethyl acetate, ethyl formate, propylene carbonate, benzyl alcohol are used in formulation.

Co-surfactant:-The selection of co-surfactant is important when using microemulsion to formulate. The co-surfactant have effect on phase behavior , the effect of co-surfactant on uptake of the internal phase for selected micro emulsion composition and on drug loading should be investigated. Even solublizers such as Transcutol, glycofurol, ethanol, and isopropanol can be safely used as co surfactant in formulation of microemulsion.[2,16]

Other additives :- Choice of other ingredients depend on either the physiochemical properties of candidate drug or route of administration, but few additives such as buffers, salts, polyols osmogent are normally used.

Post production processing:

When drug candidate is highly susceptible to hydrolytic cleavage or chemical degradation post production of nanosuspension becomes important. Processing may be required when nanosuspensions are not stabilized by stabilizer for longer period of time or when there are restrictions with respect to the desired route. Considering this aspect the techniques like lyophillization or spray drying may be used to produce a dry powder of nanosized particles. Mostly spray drying is selected as it is more economic than lyophillization.

CHARACTERIZATION OF NANOSUSPENSION:

Nanosuspension are characterized on basis of colour, odour, assay, zeta potential , related impurities ,ph, particle size , in vivo studies and dissolution studies. Few major techniques are discussed below;

IN VITRO EVALUATION:

1. **Mean particle size and particle size distribution:**
   The mean particle size and distribution are the two main factors which affect the physical stability, dissolution rate, saturation solubility, even in-vivo behavior of nanosuspensions.
   Due to changing particle size of the drug there is considerable variation in the saturation solubility and dissolution velocity. Particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) coutler counter multiliser.[17,18]
   PI is an important parameter that determines the physical stability of nanosuspension and it should be as low as possible as it determines the stability of nanosuspension. The coulter-countergives the absolute number of particles pervolume unit for the different size classes, and itis a more efficient and appropriate technique than LD for quantifying the contamination ofnanosuspensions by microparticulate drugs [19]
2. **Surface charge (zeta potential):**
   Zeta potential provides information about the surface charge and further long term physical stability of the nanosuspensions. A minimum zeta potential of ±30 mV is essential, where as in case of a combined electrostatic and steric stabilizer, a zeta potentialof ±20 mV wouldbe sufficient to stabilize the suspension.[2]
3. **Crystalline state and particle morphology :**
   Due to high pressure homogenization nanosuspension undergo a change in their crystalline structure,which may be to a polymorphic form or to amorphous forms. The determination of the crystalline state and particle morphology helps in understanding the polymorphic or morphological changes that a drug may undergo when subjects to nanosizing. The changes in the crystalline structure can be identified by X-ray diffraction analysis and supplemented by DSC. to get an actual idea of particle morphology scanning electron microscopy can be used.[2,12]

4. **Saturation solubility and dissolution velocity :-**
   The assessment of saturation solubility and dissolution velocity helps in determining the in vitro behavior of theformulation [12, 18] Nanosuspensions have an important advantage that it can increase the dissolution velocity as well as the saturation solubility.
5. **pH Value:**
   The ph of aqueous formulation should be taken at given temperature or when the equilibrium is attained, so as to minimize the "pH drift" & electrode surface coating with suspended particles.Addition of electrolyte must be avoided in the external phase of formulation to stabilize the pH.
6. **Stability :**
   Stability of nanosuspensions is directly proportional to particle size, decrease in particle size leads to increase in surface area & thus increase in surface energy leading to increase in tendency of particles to agglomerate. Hence stabilizers are used so as to prevent the Ostwald ripening improvesthe stability of nanosuspension. Nanosuspensions can be stored at different stressconditions like different temperature (15, 25, 35,45°C), thermal cycling, and mechanical shaking and change in their mean particle size can be followed for three months. [20]
7. **Drug Content :**
   Drug content of nanosuspension formulation is achieved by extracting nanosuspension by appropriate solvent mixture like Methanol:THF(1:1) mixture,shake well & centrifuge.
   The supernatants are separated and diluted with same solvent mixture and absorbance is measured at suitable λ_{max}. The drug content is calculated using the calibration curve.[21]

In Vivo Evaluation:

The monitoring of in-vivo performance of the drugs is important part of the study,regardless of route and the delivery system employed. Formulations are generally administered by required route and plasma drug concentration is determined by HPLC- UV visible spectrophotometry. In-vivo parameters are generally used to evaluate, surface hydrophobicity /hydrophilicity, interaction with body proteins and adhesion properties. Dissolution rate influences in-vivo biological performance of the oral nanosuspensions. Surface properties and the size of nanosuspension determine the organ distribution of intravenously injected nanosuspensions. Surface hydrophobicity is determined by hydrophobic interaction chromatography and absorption of protein is determined by 2-D PAGE quantitatively and qualitatively after intravenous injection of nanosuspensions of drug in animals.[10]

EVALUATION OF THE SURFACE MODIFIED PARTICLES:

Surface hydrophilicity:

Surface hydrophobicity/hydrophilicity it's a critical parameter that affects the in vivo organ distribution after intravenous injection. As adsorption of plasma proteins is key factor for organ distribution it is also determined by surface hydrophobicity and the interaction with cells before phagocytosis is also determined by surface hydrophobicity. To prevent artifacts, the surface hydrophobicity must be determined in the aqueous dispersion medium. The best technique to be used is hydrophobic interaction chromatography (HIC),earlier used to determine the hydrophobicity of bacteria,& then shifted to the characterization of nanoparticulate drug carriers. [1,8]

Adhesion properties:

For in-vivo bioadhesive studies Male Wistar rats can be used. Generally each rat receives single dose containing 10 mg of nanoparticles which are combined with drug (approx.45 mg particles/kg body weight). Animal is cut,abdominal cavity is opened and the stomach, small intestine and cecum is removed they are rinsed with phosphate saline buffer. The stomach , small intestine and cecum is cut into 2cm length and digested in alkali for 24hr. further there is addition of 2ml methanol, and then centrifuged. 1 ml sample of supernatant is to be assayed for drug by spectroflurimetry to estimate the number of nanoparticles adhered to mucosa. If necessary standard curves can be prepared for calculation. [22]

Interaction with body proteins:

In-vitro interaction between mucin and the nanoparticles can be studied by incubating nanoparticles and mucin (4:1 weight ratio) either in neutral or acidic medium. The incubation is processed at 37^0C temperature with stirring. The dispersion is then centrifuged and in test plate 150µl of each supernatant is placed. Then the plate is incubated for 2 h at 37^o C after addition of BCA Protein Assay Reagent Kit to the supernatants. By following this procedure absorbance of mucin is measured at λmax of the drug. Total amount of mucin absorbed to nanoparticles is determined by taking the difference between its initial concentration and the concentration in dispersion after incubation and centrifugation. [1,22]

FUTURE PROSPECTS:

Nanosuspension technology is considered as the unique andnovel drug delivery system to surmount the drug problems such as those drugs which are poorly soluble in aqueous as well as organic media. Currently there is large scale production of nanosuspensions using productions methods like high pressure homogenizer and media milling. Nanosuspension can be combined with various

dosage forms like pallets, capsules, tablets, and can be used for parenteral products. Nanosuspensions will keep interest in developing the oral and non-oral formulations.

PATENTS ON NANOSUSPENSION:

As nanosuspension have versatile properties so there are many patents on this technology.

MARKETED PRODUCTS:

Preferred dosage forms of nanosuspensions:

Non-aqueous or aqueous drug nanosuspensions having long term physical stability can be placed in the market as liquid products. Drug nanosuspensions in pure water or in water containing mixtures, are generally used as wetting agents for the extrusion mass to produce pellets or it is used as granulation fluid in granulation process for production of tablets. Spray drying is possible, the produced powder is used for pellet or tablet production or else it is filled in hard gelatin capsules. Thus there are many different ways transfer drug nanocrystals to final dry oral dosage form for patient. With regard to parenteral products, the drug nanosuspensions can be used.

CASE STUDIES:

CASE 1: PACLITAXEL

Paclitaxel is a diterpenoid obtained from Taxus brevifolia. It is used for different cancers, specially ovarian and breast cancers. Due to its aqueous insolubility it is dissolved in ethanol and Cremophor EL, which can cause serious allergic reactions. To prevent this allergic reactions paclitaxel was formulated as nanosuspension with the high pressure technology. The paclitaxel nanosuspension was lyophilized to obtain dry paclitaxel nanoparticles, which increased the chemical stability and dissolution rate of nanosuspension. It is found that paclitaxel has greater AUC0–∞ in spleen, in liver, and lung, but not in heart or kidney. [1, 21-25]

CASE 2: ZALTOPROFEN

Zaltoprofen is non-steroidal anti-inflammatory drug. It has low solubility and high permeability. It falls under BCS class-II classification. Zaltoprofen is used as analgesic for the treatment of post trauma, chronic inflammation, and rheumatoid arthritis. Zaltoprofen acts by inhibiting through peripheral mechanism by inhibition of bradykinninB_2, receptor mediated responses in primary afferent neurons .[29,30] As Zaltoprofen has low solubility so as to increase its solubility and dissolution rate it is formulated as nanosuspension. Zaltoprofen is formulated as nanosuspension by using solvent/antisolvent precipitation method. The ratio of solvent to antisolvent was kept constant i.e. 1:20 and stirring speed 8000 rpm and stirring time 9 hrs was also constant. [26-31]

Zaltoprofen which is available was compared and evaluated for its particle size and in-vitro dissolution study. The average particle size was observed to be 237 nm.The rate of dissolution of the optimized nanosuspension was enhanced (89 % in 50 min) mainly due to the formulation of nanosizedparticles. Stability study revealed that nanosuspension was more stable at room and refrigerator condition with nosignificant change in particle size distribution.

CASE 3: AZITHROMYCIN [32]

It is macrolide antibiotic and its active against gram negative and gram positive organisms. [33] Azithromycin is ophthalmic preparation used for Conjuctivitis also known as "Pink eye".Ophthalmic nanosuspensions are colloidal dispersions of nanosized drug particles which are prepared by suitable method and can be stabilized by surfactant as they are helpful for those drugs which have poor solubility in lacrimal fluids. [34, 35] Azithromycin is formulated as nanosuspension as it is insoluble. Therefore preparation of azithromycin nanosuspension will lead to colloidal dispersion with drug solution having increased retention time. [36] To prepare Azithromycin nanosuspension Solvent diffusion method is used so as to increase its solubility.

The average particle size is found to be 100 to 400 nm. Viscosity of prepared nanosuspension is 48 cps which is sufficient for better retention with cornea.

Table1:- The New Drug Application Based on Nanosuspensions Technique Reported and Marketed by Now. [1]

SR. NO	Drug	Route	Indication
1.	Clofazimine	Intravenous	Anti-mycobacterial
2.	Omeprazole	Intravenous	Proton pump Inhibitor
3.	Naproxen	Oral/parenteral	Anti-inflammatory
4.	Cilostazol	Oral	Anti-platelet agent
5.	Busulfan	Intrathecal	Anticancer

APPLICATIONS OF NANOSUSPENSION:-

Oral drug delivery:-

Oral route has many advantages over others. Generally absorption through GIT and solubility decide the efficacy of the orally administered drug. Nano-sizing of the drug can lead to increase in the oral absorption and bioavailability. Increase in the bioavailability will lead to the subsequent reduction in drug dose, leading to cost-effective therapy. [1,10]

Pulmonary drug delivery:-

Drugs that are inadequately dissolvable in pulmonary secretions may be formulated with the help of the nanosuspensions. These medications are conveyed as suspension aerosols or as dry powders by method for dry powder inhalers. Nebulization is generally achived with the use of ultrasonic s or mechanical nebuliz

Advantages of nanosuspension over conventional pulmonary formulations:-

- Increase in diffusion and dissolution rate at the site of action leading to increase in bioavailability of the drug.
- Drug has affinity to mucosal surfaces.
- Drug gets evenly distributed in the lungs as all the droplets of aerosols contains nanopaticles as compared to the macroparticulate form of the drug. [10]

- **Ocular drug delivery :-**

Nanosuspensions are also used for the drugs that have poor solubility in lachrymal fluid.

Benefits of nanosuspension in ocular drug delivery:-

- Can stay for longer period in cul-de-sac.
- Avoids the tonicity created by the water soluble drugs.
- Nanosuspension incorporated with the suitable hydrogel base or mcoadhesive base so as to provide sustained release of the drug. Intrinsic solubility of the drug in lachrymal fluid is related to the effect of nanosuspension.

- **Drug Targeting:-**

Nanosuspensions have great potential for drug targeting especially in the brain targeting.

- By changing the stabilizer the surface properties or the in-vivo behavior of the nanosuspensions can be altered, thus they are good candidates for drug delivery program.[10]

- **Bioavailability enhancement:-**

Nanosuspensions increase the solubility and permeability of the drug across the membrane leading to increase in the bioavailability. [10]

CONCLUSION:

Nanosuspension is a promising drug delivery system which has been under research for several years. Its ability to deliver insoluble drugs has a remarkable effect in the drug delivery process. When prepared and characterized properly nanosuspensions deliver drug efficiently and form a useful system in treating various diseases. Drugs like paclitaxel (anticancer drug), zaltoprofen (analgesic) and azithromycin (antibiotic) have shown improved ability when administered as nanosuspension. Nanosuspensions have various advantages like ocular drug delivery, bioavailability enhancement and specific drug targeting is under extensive research.

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