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# FORMULATION AND EVALUATION OF NANOSUSPENSIONS FOR OCULAR ROUTE

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**ABSTRACT**
This study explores the formulation and assessment of nanosuspensions designed for ocular administration. The research focuses on optimizing the nanosuspension composition to enhance ocular drug delivery, considering factors such as particle size, stability, and drug release. Evaluation methods include in vitro and in vivo assessments, aiming to provide valuable insights into the potential application of nanosuspensions for ocular drug delivery.

**KEYWORDS:** Nano-suspension; bioavailability; drug delivery; solubility; ocular routes.

**Ocular drug delivery (odd)**
As in Fig.-1, an eye has various layers/ barriers, which act as barriers, restring the drug to reach the intended site of action. The drugs that are carried through Corneal or Non-corneal routes involves several complicated biological processes such as drug of drug penetration (DP)across the ocular barriers and get transferred to posterior and anterior cavity.[1] Since most of medications used ED which easily drained away from the ocular surface, they leading to low bioavailability and failure in reaching the posterior segments. When this medication is applied through topical methods, only around 1-7% of the drug reaches the aqueous humor.[2]

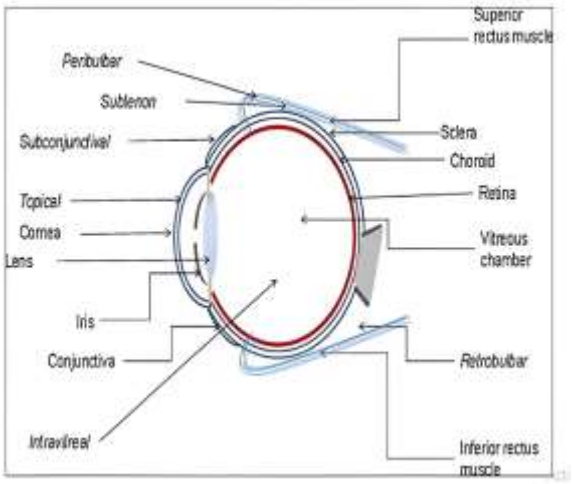


**Fig. 1: Structure of Eye.**

**Nanosuspension**
Nanosuspensions are colloidal dispersions of nanosized drug particles stabilized by surfactants.[3] They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous vehicle in which the diameter of the suspended particle is less than 1µm in size. Reduction of drug particles to nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of saturation solubility.[4]

The increase in the saturation solubility and solution velocity of nanoparticle is due to increase of vapour pressure of the particles

Nanosuspension have disclosed the problems associated with the delivery of poorly water soluble and poorly water-and lipid soluble drugs and are unequalled because of their simplicity and rewards they confer over other strategies.

**Advantages of Nanosuspension[5]**
- Enhance the solubility and bioavailability of drugs
- Suitable for hydrophilic drugs
- Higher drug loading can be achieved
- Dose reduction is possible
- Enhance the physical and chemical stability of drugs
- Provides a passive drug targeting

**Preparation of Nanosuspension**
There are primarily two nanosuspension preparation techniques. The conventional precipitation methods (hydrosols) are termed as "Bottom-Up Technology",

while "Top-Down Technologies" are disintegration methods and favoured excess precipitation. The Top-Down Technologies can be accomplished by several methods including Nanocrystals (media milling), Dissocubes (high-water homogenization), Nanopure (high non-aqueous media homogenization), and Nanoedge (combine precipitation with high-pressure homogenization).

1. Bottom-up technique
2. Top-down technique

**Bottom-Up Technique**

This term means one begin from the molecule level, then upgraded to the solid particle creation by molecular association, meaning that classical precipitation techniques are addressed by reducing the consistency of the solvent, such as pouring a solvent into non-solvent or increasing the temperature or a mixture of both. In pharmaceutical chemistry and technology, precipitation can be a classical technique.[6,7]

**Benefit**[7]

Basic and low-cost equipment is used. Higher solubility in saturation is the advantage of precipitation relative to other nanosuspension preparation methods.

**Drawbacks**[8,9]

The medicinal substance must, at least, display solubility in one solvent. A solvent with a minimum of one non-solvent must be miscible. Solvent residues have to be eliminated, thus increasing the cost of production. Maintaining the particle character is a little tricky (i.e. size, especially the amorphous fraction).

**Top-Down Technique**

(A) Media-milling
(B) Homogenization with elevated pressure
(C) Melt Emulsification Method

**(A) Milling-Media**

This method was first developed and reported by Liversidge (1992).[10]

The nanosuspensions by this method are prepared by high shear media mill. The milling chamber was charged with the milling media, water, drug and stabilizer and rotated at a very high shear rate under controlled temperature at least 2-7 days.[11] The milling medium is composed of glass, Zirconium oxide or highly cross linked polystyrene resin. The high energy shear forces are formed because of impaction of milling media with the drug which results in breaking of drug micro particles to nanosized particles.

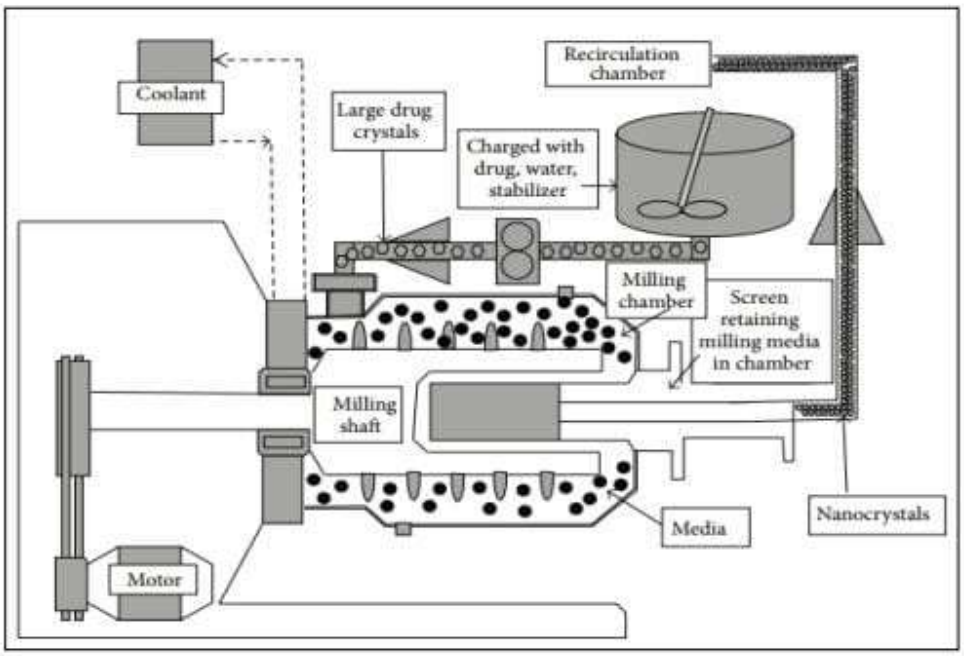


**Fig. 2: Media Milling**

**Advantages**

1. Very dilute as well as highly concentrated nanosuspensions can be prepared by handling 1 mg/ml to 400 mg/ml drug quantity.
2. Nanosized distribution of final nanosized product.

**Disadvantages**

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

**(B) High Pressure Homogenization**

It is most widely used method for preparing nanosuspensions of many poorly aqueous soluble drugs.[12] It involves three steps. First drug powders are dispersed in stabilizer solution to form presuspension,

and then the presuspensionis 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 are Disso cubes, Nanopure, Nanoedge and Nanojet.[13]

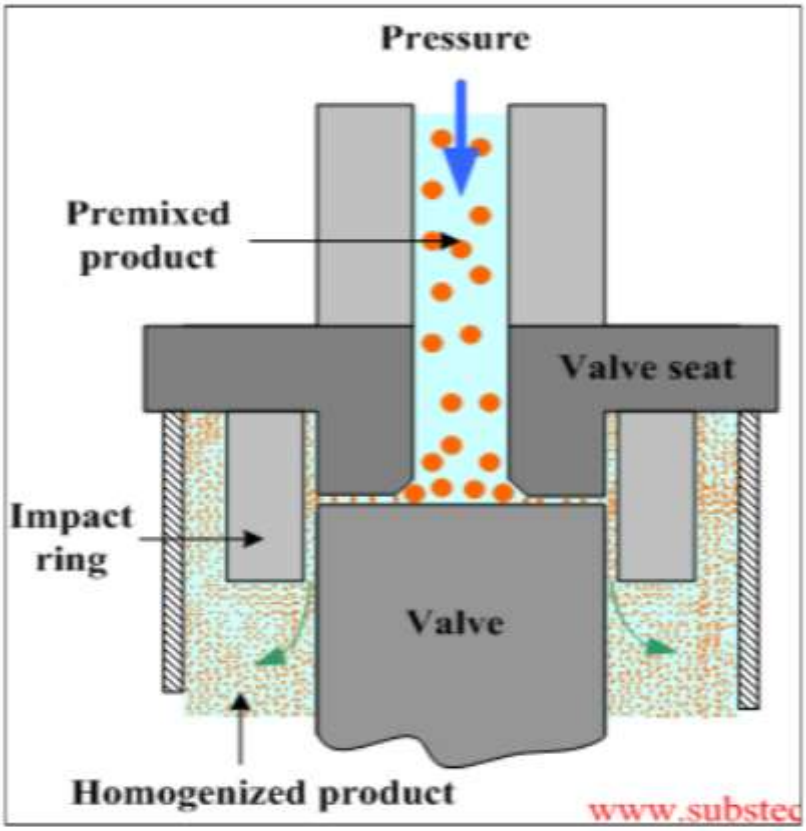


**Fig. 3: Homogenization at High Pressure.**

a. Homogenization in aqueous media (Disso cubes)
b. Homogenization in non-aqueous media (Nanopure)
c. Combined precipitation and homogenization (Nanoedge)
d. Nanojet

**a. Homogenization in aqueous media (disso cubes)**

Using a piston-gap type high-pressure homogenizer, R.H. Muller created this technology in 1999. The basic idea is high pressure with a volume capacity of 40 ml and pressures between 100 and 1500 bar and up to 2000 bar (for laboratory scale). We can easily change micronsized particles into nanosized particles by applying this pressure. We must obtain the sample from the jet mill so we can utilize it to lower the particle size down to 25 microns,[14] which is what it initially requires as a micron range particle. In addition, we may perform batch and continuous operations with this equipment. Here, we must first transform the particles into a presuspension state.[15]

**Principle**

The cavitation principle is the main foundation of this technique.[16] The 3 cm diameter cylinder's dispersion is suddenly forced into a 25 m-wide opening. The drift volume of liquid in a closed system per crosssection is constant, according to Bernoulli's law. Due to a decrease in diameter from 3 cm to 25 m, it causes an increase in dynamic pressure and a decrease in static pressure below the boiling point of water at ambient temperature. Then, as the suspension leaves the gap (a process known as cavitation) and normal air pressure is reached, water begins to evolve boiling at room temperature and generates gas bubbles that implode. The drug nanoparticles are created when the particle cavitation forces are sufficiently high.

**b. Nanopure**

Nanopure is homogenized suspensions in water-free media or mixes of water like oils, while the Disso-cubes technology the determining factor of the technique is cavitation caused by boiled water. Oils have minimum vapor-pressure and higher boiling-point than water.[17] The decrease in static pressure would therefore not be sufficient to cause cavitation. Polymeric material disintegration by high-pressure homogenization suggest that disintegration has been promoted by higher temperatures of around 80˚C, while at 0˚C the suspended drug particles inside the non-aqueous medium have been homogenized and thus called deepfreeze" homogenization.[18]

**c. Combined precipitation and homogenization (nanoedge)**

To precipitate the medication, the organic solvent in which it is dissolved is mixed with a miscible anti-solvent. The medication precipitates due to the low solubility in the water-solvent mixture. High-shear processing has also been combined with precipitation.[19] Rapid precipitation and high-pressure homogenization are used to accomplish this. To fragment materials, the nanoedge patented technique through Baxter relies on the precipitation of friable materials under conditions of highshear and/or thermal energy. When a medication solution is added quickly to an antisolvent, the blended solution unexpectedly becomes supersaturated and produces fine crystalline or amorphous particles.

When the solubility of the amorphous state is exceeded, precipitation of an amorphous material may also be observed at high supersaturation. Precipitation and homogenization have the same fundamental principles as nanoedge. Combining these techniques yields faster improvement in stability and lower particle sizes. The nanoedge technology can address the primary drawbacks of the precipitation method, such as crystal development and long-term stability.[20]

**d. Nanojet**

This process, also known as opposite stream or nanotechnology, makes use of a chamber, in which a stream of suspension is split into two or more components that collide under high pressure. Particle size reduction is a result of the process's strong shear force.[21] The M110L and M110S microfluidizers (Microfluidics), which are used in the preparation of atovaquone nanosuspensions, operate on this concept.[22] The main drawback of this method is the high volume of passes through the microfluidizer and the correspondingly higher percentage of microparticles found in the final product.[23]

**(C) Melt Emulsification Method**

The material is dispersed in the stabilizer's aqueous solution and heated overhead the drug's melting point and homogenized to produce an emulsion. Throughout this procedure the sample container was encased with a heating tape tailored with a temperature regulator and the emulsion temperature was detained above the drug's melting point.[24] The emulsion was then gradually cooled down to room temperature or on an ice bath

**(D) 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.[25]

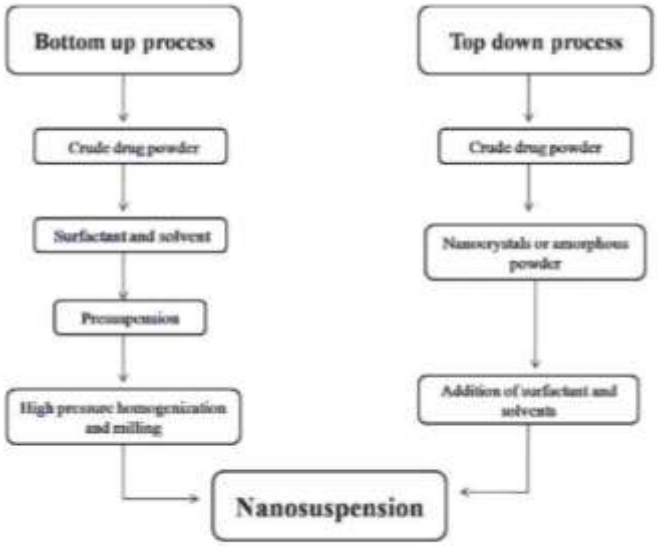


**Fig 3:** Preparation of nanosuspension

**Formulation Consideration**

**i. Stabilizer**

A stabilizer is used to wet the drug particles systematically and to prevent the maturing and agglomeration of nanosuspension by providing a stearic or ionic barrier to yield a physically stable formulation.[26] Some of the stabilizers are poloxamers, polysorbate, cellulosic, povidones, and lecithin. Drug particles dispersed within a liquid continuous medium are stabilized by steric, electrostatic mechanisms, or by a combination of both via polymers and/or Surfactants. Steric stabilization is usually imparted by nonionic polymers and nonionic surfactants, e.g., cellulose derivatives, poloxamers (also considered as polymeric surfactants), polysorbates, and povidones, preventing particles from getting into the range of attractive Vander Waals forces. Electrostatic stabilization is usually imparted by ionic surfactants, e.g., sodium dodecyl

sulfate (SDS), dioctyl sulfosuccinate sodium salt (DOSS) and benzethonium chloride (BKC) providing mutual repulsion of similar charged particles.[27]

**ii. Organic Solvents**

Toxicity potential and the ease of their removal after formulation are the two vital aspects that decide the suitability of organic solvents in the pharmaceutical area during the formulation of nanosuspension by using emulsion or microemulsion as templates. Ethanol and isopropanol are watermiscible solvent, whereas ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate and benzyl alcohol are partially water-miscible, less hazardous and pharmaceutically acceptable.[28]

**iii. Surfactants**

To illuminate the dispersion, it is incorporated into a formulation surfactant which performs its action as wetting or deflocculating reducing the tension of the interfaces. Commonly used surfactants are polysorbate (Tween/Span series), povidone, cellulosic, poloxamers and lecithin.[29]

**iv. Co-Surfactants**

This describes other co-surfactants for specific stabilizers that can be used safely in microemulsion formulation, cosurfactants such as salts (dipotassium glycyrrhizinate) can be used safely with stabilizers such as glycerol, ethanol, and isopropanol.[30]

**v. Other Additives**

The composition of nanosuspensions such as osmogene, cryoprotectant, polyols, buffers and salts depend on either the route of administration or the properties of the product moiety.[31]

**Table 2:** List of solvents and polymers used

| Solvents | Polymers |
|---|---|
| Ethyl acetate | Ethyl Cellulose |
| Methylene Chloride | Cellulose Acetate Butyrate |
| Chloroform | Poly methyl methacrylate |
| Ethanol | Eudrgit RS 100, Eudrgit RL 100, Hydroxy propyl methyl cellulose E-5 (HPMC E-5), Poloxamer 407 |

***Newer Method**

**A. Supercritical fluid method**

To create nanoparticles, a variety of techniques are employed, including the rapid expansion of supercritical solution (RESS) process, the supercritical antisolvent process, and the precipitation with compressed antisolvent (PCA) process. In the RESS technique, drug solution is expanded through a nozzle into supercritical fluid, causing the supercritical fluid to lose some of its solvent power, precipitating the drug as small particles.[32] Young et al. created cyclosporin nanoparticles with a diameter of 400–700 nm using the RESS technique. The medication solution is atomized into the CO2 compressed chamber while using the PCA method. The solution becomes oversaturated when the solvent is removed, which leads to precipitation. When a drug solution is injected into a supercritical fluid during a supercritical antisolvent procedure, the solvent is removed, and the drug solution is transformed into supersaturated.

**B. Dry-co-grinding**

Many nanosuspensions are being made using the dry milling process. Dry co-grinding can be done quickly, affordably, and without the need of organic solvents. Due to an improvement in surface polarity and a change from a crystalline to an amorphous drug, co-grinding improves the physicochemical characteristics and dissolving of poorly watersoluble medicines.

**Characterization of Nanosuspensions**

Nanosuspensions are characterized by appearance, color, odor, assay, related impurities, particle size, zeta potential, crystalline morphology status, dissolution studies and in vivo studies. Among these the essential characterization techniques were discussed.

**1. Mean particle size and particle size distribution**

The mean particle size and particle size distribution affect the saturation solubility, dissolution rate, physical stability, even invivo behavior of nanosuspensions. The particle size distribution can be determined by photon correlation spectroscopy (PCS), laser diffraction (LD) and Coulter counter multisizer.[33] PCS can also be used for identifying the width of particle size distribution (polydisperity index, PI).A PI value of 0.1-0.25 indicates a fairly narrow size distribution, if PI value greater than 0.5 indicates a very broad distribution.[34] The coulter-counter gives the absolute no of particles per volume unit for the different size classes, and it is more efficient and appropriate technique than LD for quantifying the contamination of nanosuspensions by micro particulate drugs.

**2. Surface Charge (Zeta Potential)**

Surface charge properties of the nanosuspensions are studied through zeta potential. The value of particle surface charge indicates the stability of nanosuspensions at the macroscopic level. A minimum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions.[35,36] and a minimum of ±20 mV for steric stabilization.[37] The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential.[38] Electroacoustic technique is also used for the determination of the zeta potential in the areas of material sciences.[39]

**3. Crystalline State and Particle Morphology**

Polymorphic or morphological changes of nanosized particles can be checked by assessing the crystalline state and particle morphology.[40] As nanosuspension requires high-pressure homogenization, change in crystalline structure of formulation occurs which may be converted to either amorphous or other polymorphic forms.[41] Alteration in the solid state of the drug particles and the extent of the amorphous portion is determined by X-ray diffraction analysis and supplemented by differential scanning calorimetry analysis.[42]

**4. Crystal morphology**

To characterize the changes in polymorphic due to the impact of high-pressure homogenization in the crystalline structure of the drug, techniques like. Scanning electron microscopy (SEM), X-ray diffraction analysis (XRD) in combination with differential scanning calorimetry of Differential thermal analysis (DSC) can be utilized. NS can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms because of high pressure homogenization.[43]

**5. Dissolution velocity and saturation solubility[44]**

NS havean significant advantage over other techniques, as NS increases the dissolution velocity as well as the saturation solubility. These two parameters need to be determined in various physiological solutions. The above two assessmenti.e. the saturation solubility and dissolution velocity helps in determining the In-vitro behavior of the formulation. Reduction in size leads to an increase in the dissolution pressure and an increase in solubility Muller explained that the energy introduced during the particle size reduction process leads to an increase in the surface tension and a related increase in the dissolution pressure.

**6. Physical stability of NS[44]**

The small particle size of NS, which inbuilt to their success, which is also responsible for their physical instability. NS consist of hydrophobic particles dispersed in a hydrophilic medium (usually water). The enormous surface area coupled with the nano-sized particles results in high interfacial tension and increased free energy, NS are basically are thermodynamically unstable systems to decrease their free energy nano-particles tend to reduce interaction with water via flocculation, aggregation or crystal growth. However, these processes affect the central characteristics of NS (i.e., small size and high surface area) and consequently the benefits of the NS formulations, as discussed above, are lost. Stabilizers are added to reduce the free energy of the system by decreasing interfacial tension and to prevent NP aggregation by electrostatic or steric stabilization. Stabilizers can be

- Surfactants,
- Polymers or
- A mixture of both.

Examples of some of the commonly used surfactants include tween 80, sodium lauryl sulfate and poloxamer 188. polyvinylpyrrolidone (PVP), hydroxypropyl methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), and polyvinyl alcohol (PVA) are examples of polymeric stabilizers.[43]

**7. The value for pH**

To attenuate the drift of pH and suspended particles surface coating with electrodes, pH value for aqueous formulations must taken at a certain temperature and after equilibrium settling is achieved. The external phase of the formulation should be free from electrolyte to establish pH stabilization.[44]

**8. The Size of Droplet**

Light scattering or microscopic techniques also calculate the distribution of the droplet size for micro-sized emulsion carriers. A dynamic light dispersion spectrophotometer that uses a 632 nm wavelength neonlaser.[46]

**9. Nanosuspension Stability**

The excited nanosized particles due to elevated surface energy causes drug crystals to agglomerate. The stabilizer's most significant purpose is to thoroughly wet the drug particles to avoid the nanosuspension Ostwald ripening and/or agglomeration, which form a chemically stable preparation by supplying a steric and/or ionic barrier. Stabilizers like cellulosics, polysorbates, and lecithin are commonly used for nanosuspensions. In the production of. parenteral nanosuspension, Lecithin may also be favored.[47]

**10. The Density**

A critical parameter is the real gravity or density of the formulation. Density depletion indicates air-entrapment inside the formulation structure. A well-mixed, uniform formulation should be used to measure density at a given temperature; such measurements are supported by the precision hydrometer.[48]

**11. In Vivo Biological Efficiency**

An essential part of the analysis is to launch an in vitro/in vivo correlation and monitorhe drug's in vivo output irrespective of the route and hence the delivery system used. In the case of intravenously injected nanosuspension, it is of vital importance.[49] Since the drug's in vivo behavior depends on the distribution of the organ, which successively depends on drug surface properties like surface hydrophobicity and plasma protein binding. To quantify the surface properties and protein interactions to promote a thought of in vivo behaviour, effective techniques must therefore be used.[50]

**Evaluation on eye suspension[51]**

**1. Description**

A qualitative narration of the drug product shall be provided. The description part shall include the

acceptance criteria of the final drugs acceptable appearance which shall also include clarity and color, of the dosage form.

**2. Identification (IDT)**
IDT shall establish the identity (ID) of the drug or drugs present and should separate the compounds of closely related structures that are likely to be present. IDT should be specific for the drug substance(s) [e.g., IR- infrared spectroscopy, NIR-Near-infrared or Raman spectro-photometric methods] shall be used for identification of the drug product. Chromatographic procedures are the most widely used IDT for drug substance(s).

**3. Assay**
Assay is performed to determine the strength (content) of the drug product.

**4. Impurities**
Impurites such as synthetic byproducts/ inorganic/ organic impurities may be present in the drugs and excipient's which are used in the manufacture of the drug products. These impurities are can be deducted by the help of drug substance and excipients monographs.

**5. pH**
Normal pH of eye tears about 7.4. The eye can tolerate products over a range of pH values from about 3.0 to about 8.6, depending on the buffering capacity of the formulation. The pH value of the formulation should be the one where the drug product is the most stable.

**6. Osmolarity**
OP may be tolerated over a wide range of tonicity (0.5%–5% sodium chloride, equivalent to about 171–1711 mOsm/kg). Hypotonic solutions are better tolerated than hypertonic solutions. Precautions shall be taken to ensure that the osmolarity of the product is maintained throughout its shelf life. Any possible contributions or interferences from the packaging system shall be taken into consideration.

**7. Particulate and foreign matter**
All OP shall be checked for package integrity and, to the extent possible, for the presence of foreign/particulate matter (visible particles). These unwanted particles arise from two sources: extrinsic (i.e., foreign matter); and intrinsic (i.e., product-related matter). Extrinsic matter is not associated with the product or process. Intrinsic particles are added during assembly of the product or result from a change over time. A 3rd category, inherent matter, describes the physical state or particles that are anticipated matters of the product.

**8. Sterility**
OP must meet the requirements of sterility test. The immediate container i.e., Primary Packing Material for OP shall be sterile at the time starting from filling and closing. It is mandatory that the immediate containers for OP shall be sealed and tampers proof so that sterility is ensured at the time of first use.

**9. Antimicrobial preservatives**
Antimicrobial agents must be added to formulations that are packaged in containers which allow withdrawal or administration of multiple doses, the preservative need not be added unless one of the following conditions prevails:

- If the OP contains a radio-nuclide with a physical halflife of <24 hr.
- If the OP, without additional agents, is sufficiently microbicidal to meet the requirements of antimicrobial.

**10. Uniformity of dosage units**
Uniformity of dosage units is applicable to dosage forms which are packaged in single-unit containers. It includes both the mass and the content of the drug substance(s) in the dosage form. This can be performed by either content uniformity or weight variation.

**11. Container contents**
Container contents of OP shall be determined.

**12. Leachables and extractables**
The OP packaging system especially the primary packing material shall not interact physically or chemically with the product in any manner to alter the strength, quality, or purity of the OP. The evaluation of possible leachables and/or extractables shall be taken into the account the risk assessment of the product, its indication, and its packaging system. Furthermore, it should be noted that a risk assessment of extractable/leachable impact on topical or intraocular route of administration is a challenging undertaking. Toxicological or safety assessments of primary or secondary packaging component extractable and leachable are not typically available for ophthalmic routes of administration. A risk assessment may include evaluation of toxicology and safety from other routes of administration and an assessment of the Total Daily Intake of the extractable/leachable being evaluated. The preponderance of such assessments leads to an estimate of extractable/leachable risk via ODD to the patient.

**13. Container–Closure integrity**
The packaging system of OP shall be closed or sealed in such a manner as to prevent any kind of contamination or loss of contents and shall provide supporting information of being tamper proof.

## CONCLUSION
From the above literature survey which has been conducted it can be concluded that, NS solved the problem of poor solubility of the drugs. Media Milling, Freeze-drying and high-pressure homogenizer production methods, are being widely used for large scale production of NS. NS really plays a very important role for betterment of human as the production method is simple compare to other type of NDDS formulation, NS

required less additives/ excipients and also increases dissolution velocity and saturation solubility. By emphasizing this technology, our society will be surely be benefited. Thus NS technology is able enough to bring enormous immediate benefits and will revolutionize the research and practice of medicine in the field of pharmacy.

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