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# AN OVERVIEW ON METHODS OF PREPARATION AND CHARACTERIZATION OF NANOEMULSION

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## ABSTRACT

An advanced mode of drug delivery system has been developed to overcome the major drawbacks associated with conventional drug delivery systems. This review gives a detailed idea about a nanoemulsion system. Nanoemulsions are nano-sized emulsions, which are manufactured for improving the delivery of active pharmaceutical ingredients. These are the thermodynamically stable isotropic system in which two immiscible liquids are mixed to form a single phase by means of an emulsifying agent, i.e., surfactant and co-surfactant. The droplet size of nanoemulsion falls typically in the range 20–200 nm. Nanoemulsions are biphasic dispersion of two immiscible liquids: either water in oil (W/O) or oil in water (O/W) droplets stabilized by an amphiphilic surfactant. These come across as ultrafine dispersions whose differential drug loading; viscoelastic as well as visual properties can cater to a wide range of functionalities including drug delivery. However there is still relatively narrow in sight regarding development, manufacturing, fabrication and manipulation of nanoemulsions which primarily stems from the fact that conventional aspects of emulsion formation and stabilization only partially apply to nanoemulsions. This general deficiency sets up the premise for current review. We attempt to explore varying intricacies, excipients, manufacturing techniques and their underlying principles, production conditions, structural dynamics, prevalent destabilization mechanisms, and drug delivery applications of nanoemulsions to spike interest of those contemplating a foray in this field.

**KEYWORDS:** Nanoemulsions, Amphiphilic Surfactant, Entrapment efficiency, Droplet size.

## INTRODUCTION

Nanoemulsion are defined as isotropic, thermodynamically stable transparent or translucent systems of oil and water which stabilize by surfactant with a droplet size usually in the range of 5 to 200 nm. Nanoemulsion having various advantages over the microemulsion are as follows i.e., Nanoemulsions have a much higher surface area and free energy than microemulsion that make them an effective transport system. This system does not show the problems of inherent creaming, flocculation, coalescence and sedimentation, which are commonly associated with microemulsions. Nanoemulsions can be developed by spontaneous emulsification method to enhance the solubility and bioavailability of poorly water soluble drugs. These are non-toxic non-irritant hence can be easily applied to skin and mucous membranes. The use of nanoemulsion as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.[1]

Nanoemulsions consist of fine oil-in-water dispersions, having droplets covering the size range of 100–600 nm. Nanoemulsions, usually spherical, are a group of dispersed particles used for pharmaceuticals biomedical aids and vehicles that shows great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies. The terms sub-micron emulsion (SME), mini-emulsion and ultrafine emulsion are used as synonyms. Nanoemulsion is a heterogeneous mixture of lipid and aqueous phase and stability are achieved by using a suitable material known as emulsifying agents. Nanoemulsion is a translucent system compare to ordinary emulsion or some time microemulsion. It has been demonstrated that with the help of nanoemulsion as a delivery system retention time of a drug in the body can be increased, so low amount of drug is required for the therapeutic action. Past studies shows the utilization of nanoemulsion technology for the enhancement of bioavailability of lipophilic drug.[2,3]

### Methods of preparation of nanoemulsions

#### 1. Solvent evaporation technique/hydrogel method

In this technique, drug solution is prepared and emulsified into another liquid (non-solvent for drug) and then solvent is evaporated, which led to drug precipitation. High speed stirrer can be employed for regulating the crystal growth and particle aggregation. Hydrogel method is very similar the solvent evaporation method. The only difference from the solvent evaporation method is that the drug solution in this case is miscible with the drug antisolvent.[4,5]

**2. High pressure homogenization**

This technique makes use of high-pressure homogenizer/ piston homogenizer to produce nanoemulsions of extremely low particle size (up to 1 nm). During this process, several forces, such as hydraulic shear, intense turbulence and cavitation, act together to yield nanoemulsions with extremely small droplet size. The resultant product can be resubjected to high-pressure homogenization until nanoemulsion with desired droplet size and polydispersity index is obtained. The production of small droplets (submicron) requires application of high energy. Several procedures may be applied to enhance the efficiency of emulsification when producing nanoemulsions. The emulsion is preferably prepared at high volume faction of the disperse phase and diluted afterwards. However, very high phase volume ratios may result in coalescence during emulsification, but more surfactant could be added to create a smaller reduction in effective surface tension and possibly diminishing recoalescence. Surfactant mixtures that show more reduction in surface tension than the individual components could also be used. If possible the surfactant is dissolved in the disperse phase rather than the continuous phase; this often leads to smaller droplets. It may be useful to emulsify in steps of increasing intensity, particularly with emulsions having highly viscous disperse phase.[6,7]

**3. Spontaneous emulsification**

This technique involved preparation of nanoemulsion in 3 stages. The first stage included formation of an organic solution, comprising of oil and lipophilic surfactant in water miscible solvent and hydrophilic surfactant and then the O/W emulsion is formed by injecting this organic phase into the aqueous phase under magnetic stirring. The organic solvent was then removed in the third stage by evaporation. Sugumar et al. formulated stable eucalyptus oil nanoemulsion by adopting spontaneous emulsification and the mean droplet size was found to be in the range of 50-100 nm.[8,9]

**4. Microfluidization**

Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a high-pressure positive displacement pump (500 - 20,000 psi), which forces the product through the interaction chamber, consisting of small channels called “microchannels”. The product flows through the micro- channels on to an impingement area resulting in very fine particles of submicron range. The two solutions (aqueous phase and oily phase) are combined together and processed in an inline

homogenizer to yield a coarse emulsion. The coarse emulsion is introduced into a microfluidizer where it is further processed to obtain a stable nanoemulsion. The coarse emulsion is passed through the interaction chamber of the microfluidizer repeatedly until the desired particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform nanoemulsion. High- pressure homogenization and microfluidization can be used for fabrication of nanoemulsions at laboratory and industrial scale, whereas ultrasonic emulsification is mainly used at laboratory scale.[10]

### 5. Ultrasonication

In this technique premixed emulsion is exposed to agitation at ultrasonic frequency of 20 kHz reducing the droplets to nanodroplets size. The resultant emulsion is then passed through high shear region to form droplets with uniform size distribution. Water jacket is employed in this technique to regulate the temperature. Sonotrodes also known as sonicator probe consisted of piezoelectric quartz crystals as the energy providers during ultrasonic emulsification. On application of alternating electric voltage, these sonotrodes contract and expand. Mechanical vibrations are produced when the sonicator tip contacted the liquid resulting in cavitation, which leads to collapse of vapour cavities formed within the liquid. This technique is mainly adopted when droplet size less than 0.2 μ is required. Shi et al. formulated emodin-loaded nanoemulsion by using ultrasonic emulsification method at a frequency of 25 kHz and achieved mean diameter of emodinloaded nanoemulsion was found to be in the range of 10-30 nm.[11]

### 6. Phase inversion temperature technique

Studies on nanoemulsion formulation by the phase inversion temperature method have shown a relationship between minimum droplet size and complete solubilization of the oil in a microemulsion by continuous phase independently of whether the initial phase equilibrium is single or multiphase. Due to their small droplet size nanoemulsions possess stability against sedimentation or creaming with Ostwald ripening forming the main mechanism of nanoemulsion breakdown. Phase inversion in emulsions can be one of two types: transitional inversion induced by changing factors which affect the HLB of the system, e.g. temperature and/or electrolyte concentration, and catastrophic inversion, which can also be induced by changing the HLB number of the surfactant at constant temperature using surfactant mixtures. Phase inversion temperature (PIT) method employs temperature dependent

solubility of nonionic surfactants, such as polyethoxylated surfactants, to modify their affinities for water and oil as a function of the temperature. It has been observed that polyethoxylated surfactants tend to become lipophilic on heating owing to dehydration of polyoxyethylene groups. This phenomenon forms a basis of nanoemulsion fabrication using the PIT method. In the PIT method, oil, water and nonionic surfactants are mixed together at room temperature. This mixture typically comprises o/w microemulsions coexisting with excess oil, and the surfactant monolayer exhibits positive curvature. When this macroemulsion is heated gradually, the polyethoxylated surfactant becomes lipophilic and at higher temperatures, the surfactant gets completely solubilized in the oily phase and the initial o/w emulsion undergoes phase inversion to w/o emulsion. The surfactant monolayer has negative curvature at this stage. This method involves heating of the components and it may be difficult to incorporate thermolabile drugs, such as tretinoin and peptides, without affecting their stability. Although it may be possible to reduce the PIT of the dispersion using a mixture of components (surfactants) with suitable characteristics, in order to minimize degradation of thermolabile drugs.[12]

### 7. Brute force method

This method includes utilization of brute forces for breaking the oil droplets into the nano range. Instruments that have been utilized for formulation of nanomeulsions include high pressure homogenizer, high speed mixer, small pore membrane and high frequency ultrasonic device. Nanoemulsion properties like its small size, optical transparency and high kinetic stability is not only dependent upon the composition of variables but also on the processing variables like emulsification time, degree of mixing, energy input and emulsifying path. High-pressure homogenization and microfluidization methods are employed at both A number of techniques had been adopted for formulation of nanoemulsions such as high pressure homogenization, microfludization, phase inversion, spontaneous emulsification, solvent evaporation and hydrogel formation. Multiple emulsions are usually prepared using the double emulsion-solvent evaporation technique. A variety of techniques had been utilized for characterization of such nanoemulsions used as drug delivery systems. Nanoemulsions are formulated mainly using two primary methods, (a) the persuasion method and (b) the Brute force method. Persuasion method/phase inversion technique: Nanoemulsion preparation by persuasion method doesn’t require any external force, but instead it involves formation of fine dispersions when phase transitions occur by changing either the temperature or composition while keeping the alternate parameter constant. Persuasion method can be

broadly categorized as, (i) phase transition from near-optimum state via change in single variable, which includes altering one variable of formulation such as temperature or salinity close to optimal value. Hydrophilic-lipophilic deviation (HLD) for optimal value is close to centre level for a system, for example, employing higher temperature to microemulsion. (ii) Phase transition from near optimal state via change in multiple variables, meaning altering more than one variable of formulation. For example, employing higher temperature and including an additional salt in a microemulsion. (iii) Catastrophic inversion, an inversion of low internal phase emulsion so that the internal phase converts to external phase. (iv) Phase transition stabilized by liquid crystal formation, which includes nanodroplets stabilization from a state close to HLD-0 by liquid crystal formation. Brute force method: This method includes utilization of brute forces for breaking the oil droplets into the nano range. Instruments that have been utilized for formulation of nanomeulsions include high pressure homogenizer, high speed mixer, small pore membrane and high frequency ultrasonic device. Nanoemulsion properties like its small size, optical transparency and high kinetic stability is not only dependent upon the composition of variables but also on the processing variables like emulsification time, degree of mixing, energy input and emulsifying path. High-pressure homogenization and microfluidization methods are employed at both industrial and laboratory scale for attaining very small size of nanoemulsion by utilizing high pressure equipment. Various other methods are also being employed for preparation of nanoemulsion such as ultrasonication and in situ emulsification.[13,14,15]

**Characterization of nanoemulsions**

**1. pH**

Important parameter of nanoemulsion is pH. The excipients used in the formulation decide the pH of the final preparation and hence the route of administration. The change in the pH may affect the zeta potential of the formulation which in turn can affect the stability of preparation. The pH of the formulations can be measured using digital pH meter. Results should be taken in triplicate and the average should taken in to consideration.[16]

**2. Determination of encapsulation efficiency**

For determining the amount of drug entrapped in the formulation, weighed amount of formulation is to dispersed in organic solvent by ultrasonication and the drug should extracted into suitable buffer. Drug content is estimated by analysing the extract spectrophotometrically at λmax of drug after making suitable dilutions against suitable blank.

The entrapment efficiency (EE) and loading efficiency (LE) of the drug can be calculated by using the following Eqns, drug EE = drug content in the product obtained (mg)/total amount of drug added (mg)×100 and drug LE = drug content in the product obtained (mg)/total product weight (mg)×100. Drug content could also be determined using reverse phase high-performance liquid chromatography (HPLC) techniques.[17,18]

### 3. Viscosity

The viscositiy can be measured to determine rheological properties of formulations. Brookfield Rheometer viscometer can be used to serve this purpose. Results should be taken in triplicate and the average should be taken in to consideration.[19,20]

### 4. Determination of particle Size and Polydispersity index (PDI)

The particle size and PDI of nanoemulsions can be analyzed employing photon correlation spectroscopy (PCS) using Malvern Zetasizer, which monitors the variation in light scattering because of Brownian motion of particles as function of time. PCS is based on the principle that the particles with small size travels with higher velocity as compared to particles with large size. The laser beam gets diffracted by sub-micron particles present in solution. Due to diffusion of particles, rapid fluctuations in laser scattering intensity occur around was then removed in the third stage by evaporation. Sugumar et al. formulated stable eucalyptus oil nanoemulsion by adopting spontaneous emulsification and the mean droplet size was found to be in the range of 50-100 nm. Solvent evaporation technique/hydrogel method: In this technique, drug solution is prepared and emulsified into another liquid (non-solvent for drug) and then solvent is evaporated, which led to drug precipitation. High speed stirrer can be employed for regulating the crystal growth and particle aggregation. Hydrogel method is very similar the solvent evaporation method. The only difference from the solvent evaporation method is that the drug solution in this case is miscible with the drug antisolvent. Characterization of nanoemulsions Determination of encapsulation efficiency: For determining the amount of drug entrapped in the formulation, weighed amount of formulation is to be dispersed in organic solvent by ultrasonication and the drug is to be extracted into suitable buffer. Drug content is estimated by analysing the extract spectrophotometrically at λmax of drug after making suitable dilutions against suitable blank. The entrapment efficiency (EE) and loading efficiency (LE) of the drug can be calculated by using the following Eqns, drug EE = drug content in the product obtained (mg)/total amount of drug added (mg)×100 and drug LE = drug content in the product obtained (mg)/total product

weight (mg)×100. Drug content could also be determined using reverse phase high-performance liquid chromatography (HPLC) techniques.[21,22,23,24]

### 5. Drug content

The drug content of Drug nanoemulsion formulation can be measured using UV visible spectroscopic method. The 2 µg/ml of aliquot preparation using nanoemulsion formulation using diluting solvent. The samples can be measured as maximum wavelength using UVVIS spectroscopic method. Results should be taken in triplicate and the average was taken in to consideration.[25]

### 6. Centrifugation

This parameter was characterized to check the physical stability. The nanoemulsion system should centrifuged at 5000 rpm for 10 minutes to determine whether the system shows signs of creaming or phase separation. The system should observed visually for appearance.[26]

### 7. Determination of zeta potential

The zeta potential is a method for measuring surface charge of particles when it is placed in liquid. Zeta potential is used for predicting dispersion stability and its value depends on physicochemical property of drug, polymer, vehicle, presence of electrolytes and their adsorption. It can be measured by Malvern Zetasizer instrument. For measuring zeta potential, nanoemulsion should be diluted and its value is estimated from the electrophoretic mobility of oil droplets. Zeta potential of ±30 mV is believed to be sufficient for ensuring physical stability of nanoemulsion. Đorđević et al. obtained zeta potential around –50 mV by using Malvern Zetasizer for risperidone nanoemulsion.[27,28]

### 8. Morphological study of nanoemulsion

The morphological study of nanoemulsion is can be carried out by using transmission electron microscopy (TEM). In TEM, a beam of electron is incident on a thin foil specimen and passed through it. On interacting with the specimen, these incident electrons transform into unscattered electrons, elastically scattered electrons or inelastically scattered electrons. The distance among the objective lens and the specimen and among the objective lens and its image plane regulates the magnification. The electromagnetic lenses concerted the unscattered or scattered electrons and cast them onto a screen that produce amplitude-contrast picture, a phase-contrast image, electron diffraction, or a phantom picture of distinct darkness, which is dependent upon the density of unscattered electrons. Bright field imaging

at increasing magnification in combination with diffraction modes used for disclosing the size and form of nanoemulsion droplets. For performing TEM, few drops of nanoemulsion or a suspension of lyophilized nanoparticles is prepared in double distilled water and are placed onto holey film grid and immobilized. Excess solution has to be wicked off from the grid following immobilization and stained. The stained nanoparticles are then examined at particular voltage. Singh et al. studied surface morphology characteristics of primaquine nanoemulsion by TEM analysis and reported spherical shape of primaquine nanoemulsion with smooth surface.[29,30]

### 9. Conductivity

Electrical conductivity of formulated samples can be measured using a digital conductometer at ambient temperature. Results should be taken in triplicate and the average was taken in to consideration.[31]

### 10. Dilution test

If the continuous phase is added in nanoemulsion, it will not crack or separate into phases. Maximum amount of water and oil were added to o/w and w/o formulations respectively and then inspected visually for clarity and phase separation. 50 and 100 times aqueous dilution of the formulation can be visually checked for phase separation and clarity. Results should be taken in triplicate and the average was taken in to consideration.[32]

### 11. Stability studies

Stability studies can be performed for assessing stability of the drug substance under the influence of a various environmental factors like temperature, humidity and light. The stability studies of nanoemulsion can carried out after storing the formulation for 24 mo in dispersed and freeze-dried state as per International Conference on Harmonisation guidelines. The storage conditions followed are ambient (25±2°/60±5 % RH), refrigeration (5±3°) and freeze (–20±5°). The requisite volume of nanoemulsion is stored in glass bottles and is tightly sealed. Samples should be withdrawn at predefined time interval and analysed for the characteristics such as particle size, loading and EE and in vitro drug release profile.[33,34]

## CONCLUSION

An advanced mode of drug delivery system can be developed to overcome the major drawbacks associated with conventional drug delivery systems. This review gives a detailed idea about a nanoemulsion system. Nanoemulsions are nano-sized emulsions, which are manufactured for improving the delivery of active pharmaceutical ingredients. These are the thermodynamically stable isotropic system in which two immiscible liquids are mixed to form a single phase by means of an emulsifying agent, i.e., surfactant and co-surfactant. The droplet size of nanoemulsion falls typically in the range 20–200 nm. The main difference between emulsion and nanoemulsion lies in the size and shape of particles dispersed in the continuous phase. In this review, the attention is focused to give a basic idea about its formulation, method of preparation, characterization techniques, evaluation parameters, and various applications of nanoemulsion.

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**CONFLICT OF INTEREST**

No conflict of interest.

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