A Review On Nanoemulsion

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ABSTRACT
Nanoemulsion are noval drug systems consisting of emulsified oil and water systems with average droplet size of 5-200nm. There are three types of Nanoemulsion that are O/W, W/O and bicontinuous or multipal emulation. Advantages of Nanoemulsion are increase the rate absorption and bioavailability, helpful in taste masking. Components of nanoemulsions are Oil, Surfactant, Co-surfactant, Aqueous phase. Methods of preparations are High-Energy Emulsification Methods, High Pressure Homogenization, High-Shear Stirring, Ultrasonic Emulsification, etc. There are various characterization and evaluation parameters of nanoemulsions are included.

Keywords- Microfluidization, Emulsifier, Ultrasonic emulsification method etc.

INTRODUCTION
Nanoemulsions, also known as submicron emulsions, ultrafine emulsions and miniemulsions, are submicron sized colloidal particulate systems considered as thermodynamically and kinetically stable isotropic dispersions, which consist of two immiscible liquids like water and oil, stabilized by an interfacial film consisting of a suitable surfactant and co-surfactant to form a single phase. A number of surfactants with diverse characteristics (ionic or non-ionic) had been used with such nanoemulsions. Most widely used among them were nonionic surfactants (sorbitan esters, polysorbates), anionic surfactants (potassium laurate, sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide) and zwitterions surfactants (quaternary ammonium halide). Early nanoemulsions were oil-in-water (O/W) type emulsions with average droplet diameter ranging from 50 to 1000 nm. Nanoemulsions more recently are classified into three categories such as O/W type (oil is dispersed in aqueous phase), water-in-oil (W/O) type (water is dispersed in oil phase), and bi-continuous (microdomains of water and oil are interdispersed within the system). Transformation among these three types can be attained by altering the components of the emulsions. Multiple emulsions are also a type of
nanoemulsions, where both O/W and W/O emulsions present simultaneously in one system. For stabilizing these two emulsions, both hydrophilic and lipophilic surfactants are used simultaneously.

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ADVANTAGES

- Increase the rate absorption.
- Eliminates variability in absorption.
- Helps solublize lipophilic drug.
- Provides aqueous dosage form for water insoluble drugs.
- Increases bioavailability.
- Various routes like topical, oral and intravenous can be used to deliver the product.
- Rapid and efficient penetration of the drug moiety.
- Helpful in taste masking.
- Provides protection from hydrolysis and oxidation as drug in oil phase in O/W Nanoemulsion is not exposed to attack by water and air.
- Liquid dosage form increases patient compliance.
- Less amount of energy requirement.
- Nanoemulsions are thermodynamically stable system and the stability allows self- emulsion of the system.
DISADVANTAGES-

• Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.

• Limited solubilizing capacity for high-melting substances.

• The surfactant must be nontoxic for using pharmaceutical applications.

• Nanoemulsion stability is influenced by environmental parameters such as temperature pH.

COMPONENT OF NANOEMULSION

Main three components of nanoemulsions are as follows:
1. Oil
2. Surfactant
3. Co-surfactant
4. Aqueous phase

METHODS OF PREPARATION

Nanoemulsions can be prepared by using high and low energy methods. In high energy methods, mechanical devices deliver required large disruptive forces. On the other hand, in low energy methods, there is no need for an external force. Production of nanoemulsions is achieved by using the intrinsic physiological properties of the system. In this nanoemulsion preparation method, stored energy of the system is utilized by alteration of parameters such as temperature, composition of the system (Setya et al., 2014). At the initial studies of nanoemulsions, the high energy methods were only choice for researches and thus high energy stirring and ultrasonic emulsification were the most widely used methods. Nowadays, low-energy methods have drawn considerable attention since they are ‘soft’, nondestructive and cause no damage to encapsulated molecules.

Several methods have been suggested for the preparation of nanoemulsion. The basic objectives of the nanoemulsion preparation to achieve the droplet size range of 100-600 nm and another is to provide the stability condition. Formation of nanoemulsion system required a high amount of energy. This energy can be provided either by mechanical equipment or the chemical potential inherent within the component. Here some methods are discussed which are freely used for the nanoemulsion preparation.
High-Energy Emulsification Methods

Nanoemulsions are non-equilibrium systems which cannot be formed spontaneously. For this reason, mechanical or chemical energy input is necessary to form them. Nanoemulsions are generally prepared by using high energy methods in which mechanical energy input is applied by high pressure homogenizers, high shear stirring, and ultrasound generators (Sole et al., 2012). These mechanical devices provide strong forces that disrupt oil and water phases to form nanoemulsions. In high energy methods, input energy density is about 10^8 - 10^10 W kg^-1 (Gupta et al., 2016).

Required energy is supplied in a shortest time to the system in order to obtain homogeneous small sized particles. High-pressure homogenizers are capable of doing this and therefore they are the most widely used devices for preparing nanoemulsions (Solans et al, 2005). Moreover, producing emulsions using ultrasound is a cost-effective process which needs less surfactant use (Kaltsa et al., 2013). Therefore, considering conventional mechanical processes more homogeneous batches are achieved.

High Pressure Homogenization

It is the most popular method used for the production of nanoemulsions. This method benefits from the high pressure homogenizer or the piston homogenizer to manufacture nanoemulsions that particle sizes are up to 1 nm. During the method, the macroemulsion is forced to pass through in a small orifice at an operating pressure between 500 to 5000 psi. Extremely small droplet sized nanoemulsions are achieved because during the process several forces like hydraulic shear, intense turbulence and cavitation act together. This process can be repeated until the final product reaches the desired droplet size and polydispersity index (PDI). The uniformity of droplet size in nanoemulsions is specified by PDI. Higher PDI means lower uniformity of droplet size in nanoemulsions. Monodisperse samples have PDI lower than 0.08, PDI between 0.08 and 0.3 states a narrow size distribution, whereas PDI greater than 0.3 indicates broad size distribution. However, obtaining of small droplets that are in submicron levels requires large amount of energy (Lovelyn&Attama, 2010). This amount of energy and increasing temperatures during high pressure homogenization process might cause deterioration of the components. Thermolabile compounds such as proteins, enzymes and nucleic acids may be damaged.
High-Shear Stirring

In this method, high-energy mixers and rotor-stator systems are used for the preparation of nanoemulsions. Droplet sizes of the internal phase can be significantly decreased by increasing the mixing intensity of these devices. However, obtaining emulsions with the average droplet size less than 200-300 nm is rather difficult.

Ultrasonic Emulsification

There are two mechanisms which take part in ultrasonic emulsification. Firstly, acoustic field creates interfacial waves that makes oil phase to disperse in the continuous phase as droplets. Secondly, ultrasound provokes acoustic cavitation which provides formation and collapse of microbubbles respectively due to pressure fluctuations of a single sound wave. In this way, enormous levels of highly localized turbulence is generated and this causes micro implosions which disrupt large droplets into sub-micron size (Zhang, 2011). In this method, premixed macroemulsion is agitated by vibrating solid surface at 29 kHz or larger frequencies. High-power ultrasonic devices such as focusing horns and pointed tips cause extreme shear and cavitation that result in breaking up of droplets. It has been observed that in most of the ultrasonic systems emitted sound field is inhomogeneous. For this reason, in order to have all droplets to experience highest shear rate, recirculation of the emulsion through the region of high power must be provided. Moreover, by doing this type of recirculation many times it is possible to obtain emulsions with uniform droplet size at dilute concentrations. Emulsifier type, the amount emulsifier, and viscosity of phases are the most critical parameters that affect homogenization efficiency. Thus, optimization of these parameters is necessary to prepare nanoemulsions having fine droplets. However, there are some concerns about sonication methods due to fact that they have possibility to induce protein denaturation, polysaccharide depolymerization and lipid oxidation.
Microfluidization

It is most widely employed in the pharmaceutical industry in order to acquire fine emulsions. In this method, a device called microfluidizer is used which provides high pressures. During the process, high pressure forces the macroemulsion to go through to the interaction chamber and thus nanoemulsions with submicron ranged particles can be produced. Uniform nanoemulsion production can be achieved by repeating the process many times and varying the operating pressure in order to get desired particle size. There is a collision between crude emulsion jets from two opposite channels in the nozzle of microfludizer which is also called as the interaction chamber. The mobility of crude emulsion is provided by a pneumatically powered pump that has capability of compressing air up to pressures between 150 to 650 MPa. This high pressure forces the crude emulsion stream to go through microchannels and after the collision of two opposite channels enormous level of shearing force is obtained. Therefore, by the help of this force fine emulsions are produced.

Low-Energy Emulsification Methods

Nanomulsification can also be achieved with low energy methods which provides small size and more uniform droplets. These methods such as phase inversion temperature and phase inversion component provide smaller and more uniform droplets by using physicochemical properties of the system (Caldero et al., 2011). Although low energy procedures are generally more effective to produce small droplet sizes than high energy procedures, there are some limitations for them about the using of some types of oils and emulsifiers like proteins and polysaccharides. In order to overcome this problem high level of synthetic surfactant concentrations are used to produce nanoemulsions in low energy techniques but this narrows down their application area, especially for many food process.
Spontaneous Nanoemulsification

It benefits from the chemical energy releasement based upon dilution process with the continuous phase which occurs usually at constant temperature without any phase transitions in the system during the emulsification process. This method can produce nanoemulsions at room temperatures and no special devices are required. It basically subjected to interfacial tension, viscosity of interfacial and bulk, phase transition region, surfactant structure, and surfactant concentration. In the pharmaceutical industry, systems prepared by using this method are usually called as selfemulsifying drug-delivery systems (SEDDS) or selfnanoemulsifying drug-delivery systems (SNEDDS). When an oil phase with a water soluble substance is mixed with water, oil droplets spontaneously forms. The mechanism depends on the movement of water dispersible substance from the oil phase to the water phase, indicated as red arrows in Figure 3. This leads to interfacial turbulence and thus formation of spontaneous oil droplets.

Phase Inversion Methods

These methods utilize the chemical energy that is released because of the phase transitions during emulsification process. Required amount of phase transitions are achieved by changing the composition at constant temperature or by changing the temperature at constant composition.

Phase Inversion Temperature (PIT)

In this method, temperature is changed at constant composition. Non-ionic surfactants which have temperature dependent solubility like polyethoxylated surfactants play important role. Emulsification is achieved by modifying affinities of surfactants for water and oil as a function of temperature (Lovelyn & Attama, 2010; Chime et al., 2014). During heating of polyethoxylated surfactants they become lipophilic due to dehydration of polyoxyethylene groups. Therefore, this circumstance establishes the principle of producing nanaoemulsions by PIT method. In order to prepare nanoemulsions by using PIT method, it is necessary to bring sample temperature to its PIT level or hydrophile–lipophile balance (HLB) level (Anandharamakrishnan, 2014). In the PIT method, the droplet sizes and the interfacial tensions reach their minimum value. This method promotes emulsification by benefiting from the extremely low interfacial tensions at the HLB temperature. Nevertheless, it has been observed that although emulsification is spontaneous at the HLB temperature, coalescence rate is greatly fast and emulsions are highly unstable. It has
been reported that stable and fine emulsion droplets can be produced by rapid cooling of the emulsion near the temperature of PIT.

**Phase Inversion Composition (PIC)**

In this method, composition is changed at constant temperature. Nanoemulsions are obtained by consistently adding water or oil to the mixture of oil-surfactant or water-surfactant. The PIC method is more suitable for a large scale production than the PIT method since adding one component to an emulsion is easier than to generate abrupt change in temperature (Solans & Sole, 2012). By adding water to the system, volume of water increases and this result to reach a transition composition. In other words, the level of hydration of the polyoxyethylene chains of the surfactant increases and thus spontaneous curvature of the surfactant goes to a change from negative to zero. As in the HLB temperature, in the transition composition a balance is obtained for the surfactant hydrophilic–lipophilic properties. When this transition composition is exceeded, small sized metastable oil in water droplet are composed due to the separation of the structures that have zero curvature.

**Solvent Displacement Method**

This method of nanoemulsion has been adopted from the nano precipitation method used for polymeric nanoparticles. In this the oily phase is dissolved in water-miscible organic solvents like ethanol and acetone. Then this organic solvent is poured into aqueous phase containing surfactant which leads to the formation of nanoemulsion by the rapid diffusion of organic solvent.
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CHARACTERIZATION OF NANOEMULSIONS
Determination of encapsulation efficiency

For determining the amount of drug entrapped in the formulation, weighed amount of formulation is dispersed in organic solvent by ultrasonication and the drug is extracted into suitable buffer. Drug content is estimated by analysing the extract spectrophotometrically at \( \lambda_{\text{max}} \) of drug after making suitable dilutions against suitable blank. The entrapment efficiency and loading efficiency (LE) of the drug can be calculated by using the following Eqns., 
\[
\text{drug EE} = \frac{\text{drug content in the product obtained (mg)}}{\text{total amount of drug added (mg)}} \times 100
\]
\[
\text{drug LE} = \frac{\text{drug content in the product obtained (mg)}}{\text{total product weight (mg)}} \times 100
\]
Drug content could also be determined using reverse phase high-performance liquid chromatography (HPLC) techniques. Singh et al. employed this technique for finding primaquine concentration and reported 95 % encapsulation efficiency of formulated nanoemulsion.

Determination of particle size and polydispersity index (PDI)

The particle size and PDI of nanoemulsions are analysed 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 a mean value at a fixed angle and this is dependent upon particle size. The calculated photoelectron time correlation function generates a histogram of the line width distribution that can be related to the size of particle. For measuring particle size, weighed amount of formulation is dispersed in double-distilled water for obtaining homogenous dispersion and that has to be used instantly for measuring the particle size and PDI. The PDI can range from 0 to 1, where 0 (zero) stands for monodisperse system and 1 for a polydisperse particle dispersion. Đorđević et al. evaluated the particle size and PDI of risperidone nanoemulsion by using this method and reported mean particle size around 160 nm with mean size distribution less than 0.15. Singh et al. has also adopted the same technique and reported particle size of primaquine nanoemulsion in the range of 20-200 nm.
**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 is measured by Malvern Zeta sizer instrument. For measuring zeta potential, nanoemulsion is 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 Zeta sizer for risperidone nanoemulsion.

**Fourier-transform infrared spectroscopy (FTIR) spectral analysis**

FTIR analysis can be carried out for the assessment of drug excipient interaction, polymerization, crosslinking as well as drug loading in the formulation. It is also used for identifying the functional groups with their means of attachment and the fingerprint of the molecule. At low temperature a molecule exists in ground state and on absorbing the radiant energy, they get excited to higher energy states. IR spectroscopy is based on determining this energy difference (ΔE) between the excited and ground states of the molecule. For performing FTIR, sample can be prepared by employing suitable method such as potassium bromide pellet method, Nujol mulls and then sample is scanned in FTIR at moderate scanning speed between 4000 - 400 cm⁻¹. Srilatha *et al.* conducted FTIR studies on pure drug and glipizidenanoemulsion and reported absence of drug excipient interactions (hence compatibility of drug and excipient) as all the characteristics peaks of drug appeared at same point in formulation.

**Morphological study of nanoemulsion**

The morphological study of nanoemulsion is carried 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 doubledistilled 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.

**Atomic force microscope (AFM)**

AFM is comparatively a new technique being used these days for exploring the surface morphology of nanoemulsion formulations. AFM is carried out by diluting nanoemulsions with water followed by drop coating of the diluted nanoemulsion on a glass slide. Further the coated drops are dried in oven and scanned at of 100 mV/s. Drais et al. performed AFM study on carvedilol nanoemulsion and found that the size varied from 42 to 83 nm with good stability of the formulation.

**In vitro drug release study**

*In vitro* drug release studies help to estimate the *in vivo* performance of drug formulation. The *in vitro* release rate of a drug is usually studied on a USP dissolution apparatus. Nanoemulsion or dried nanoparticles containing drug equivalent to 10 mg were dispersed in buffer and then it is introduced into dialysis membrane pouches and placed in a flask containing buffer. This study is carried out at 37±0.5° and a stirring speed of 50 rpm. Sample are withdrawn at periodic intervals and each time replaced by the same volume of fresh dissolution medium. Samples are then diluted suitably and the absorbance of sample is measured spectrophotometrically at a particular wavelength. Absorbance of the collected sample is used for calculating % drug release at different time intervals using calibration curve. Kotta et al. studied the *in vitro* drug release profile of anti-HIV drug nanoemulsion using dissolution apparatus type-II and reported 80 % drug release in 6 h.
In vitro skin permeation studies

KesharyChien-diffusion cell is used for investigating in vitro and ex vivo permeation studies. For performing permeation studies, abdominal skin of adult male rats weighing 250±10 g is usually employed. The rat skin is positioned between the donor and the receiver chambers of diffusion cells. Temperature of receiver chambers containing fresh water with 20 % ethanol is fixed at 37° and the contents of the chamber are continuously stirred at 300 rpm. The formulations are kept in the donor chamber. At specific time intervals such as 2, 4, 6, 8 h, a certain amount (0.5 ml) of the solution from the receiver chamber was removed for performing gas chromatographic analysis and each time replaced with an equivalent volume of fresh solution immediately. Each sample is performed three times. Cumulative corrections are done for obtaining total amount of drug permeated through rat skins at each time interval and are plotted against function of time. Slope of plot is used for calculating the permeation rates of drug at a steady-state. Harwansh et al. used Franz diffusion cell for assessing transdermal permeability of glycyrrhizin through human cadaver skin and reported increased permeability with nanoemulsion formulation as compared to conventional gel.

Stability studies

Stability studies are 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 are 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 are withdrawn at predefined time interval and analysed for the characteristics such as particle size, loading and EE and in vitro drug release profile. Singh et al. performed stability studies on nanoemulsion and observed that no change in viscosity, drug content and particle size when the formulation was stored for 3 mo at 25°/60 % RH and 30°/65 % RH.
Shelf life determination

For determining shelf life of a nanoemulsion, accelerated stability studies are performed. The formulations are stored at three distinct temperatures and ambient humidity conditions (30°, 40° and 50±0.5°) for almost 3 mo. After a particular time interval (0, 30, 60 and 90 d) samples are withdrawn and analysed using HPLC at λ_{max} for estimating the remaining drug content. Samples withdrawn at zero time are used as controls. The order of the reaction is determined by this and after that the reaction rate constant (K) for the degradation is calculated from the slope of the lines by using following equation at each elevated temperature: slope = \(-K/2.303\), the logarithm values of K are plotted at different elevated temperatures against the reciprocal of absolute temperature (Arrhenius plot). From this plot value of K at 25° is determined and it is further used for calculating shelf life by putting the value in following Eqn.: t_{0.9}=0.1052/K_{25}. Where t_{0.9} stands for time required for 10% degradation of the drug and it is termed as shelf life. Ali et al. determined the shelf life of clobetasol propionate-loaded nanoemulsion around 2.18 y at room temperature (25°) and concluded that the stability of clobetasol propionate can be augmented by incorporating in a nanoemulsion. Parveen et al. reported that the shelf life of a silymarinnanoemulsion to be around 3.8 y when stored in a refrigerator.

Thermodynamic stability studies

Thermodynamic stability studies are usually carried out in three steps. Firstly heating-cooling cycle, which is performed for observing any effect on the stability of nanoemulsion by varying temperature conditions. Nanoemulsion is exposed to six cycles between 4° (refrigeration temperature) and 40° by storing the formulation at each temperature for not less than 48 h. The formulations which are stable at these temperatures are further chosen for centrifugation studies. Secondly, centrifugation study in which the formulated nanoemulsions are centrifuged at 5000 rpm for 30 min and observed for phase separation or creaming or cracking. Those which did not show any sign of instability are subjected to freeze thaw cycle. Thirdly, the freeze-thaw cycle, in which nanoemulsion formulations are exposed to three freeze-thaw cycles with temperature varying between -21° and +25°. Formulations that show no signs of instability pass this test and deemed to have good stability. These formulations are then subjected to dispersibility studies for evaluating the efficiency of self-emulsification. Srilatha et al. performed thermodynamic studies on glipizide.
nanoemulsion by subjecting it to three cycles of stability and reported good physical stability of nanoemulsion with no appearance of phase separation, creaming or cracking.

**Dispersibility studies**

Dispersibility studies for evaluating the efficiency of self-emulsification of nanoemulsion are carried out by using a standard USP XXII dissolution apparatus. 2.1 ml of each formulation is incorporated into 500 ml of distilled water maintained at 37±0.5°. A standard stainless steel dissolution paddle rotates at 50 rpm for providing gentle agitation. In vitro performance of the nanoemulsion formulations is evaluated visually by using a grading system described below (Shafiq S.et.al.2007). Grade A nanoemulsions form rapidly within 1 min and appear to be clear or bluish. Grade B nanoemulsions form rapidly but are slightly less clear emulsions appear to be bluishwhite. Grade C nanoemulsions are fine milky emulsion that form within 2 min. Grade D are those dull, greyishwhite emulsions that has a little oily appearance and are slower to form (>2 min). Grade E nanoemulsions display either poor or negligible emulsification with large oil globules present on the surface.

**Determination of viscosity**

Viscosity assessment is an important parameter for physicochemical characterization of nanoemulsion. Various instruments are employed for measuring viscosity such as Ostwald viscometer, Hoyerler falling ball viscometer, Stormer viscometer, Brookfield viscometer and Ferranti-Shirley viscometer. Among all these viscometer, Brookfield is the preferred one for measuring the viscosity of nanoemulsion. Determination of viscosities affirms whether the system is O/W or W/O emulsion. Low viscosity of systems shows that it is O/W type and high viscosity shows that it is water in oil type system. However, currently survismeter has been the most widely employed equipment as it measures surface tension, viscosity, interfacial tension, contact angle, dipole moment and particle size and hydrodynamic volumes of the nanoemulsions. Shafiq et al. has determined viscosity of ramiprilnanoemulsion formulations by using Brookfield cone and plate rheometer and reported the viscosity of formulations as less than 21 cP with the minimum viscosity of 10.68 cP.

**Refractive index**

Refractive index tells how light propagates through the medium and transparency of nanoemulsion. Refractive index (n) of medium can be defined as ratio of speed of wave (c) in reference medium to the phase
speed of wave (vp) in medium: \( n = \frac{c}{vp} \). Refractive index of the nanoemulsion can be determined by Abbes type refractometer at 25±0.5° by placing a drop of nanoemulsion on slide and comparing it with refractive index of water (1.333). If refractive index of nanoemulsion has equal refractive index as that of water, then the nanoemulsion is considered to have transparent nature. Harika et al. measured the refractive index of amphotericin B nanoemulsion by Abbe refractometer and the value of refractive index of the formulation was found to be similar to that of the water.

**Percent transmittance**

Percent transmittance of a formulated nanoemulsion is estimated using UV spectrophotometer at a particular wavelength with distilled water as a blank. If percent transmittance of a nanoemulsion is found to be greater than 99 %, then it is considered as transparent in nature. Harika et al. reported percent transmittance of >97 % for a amphotericin B nanoemulsion formulated.

**pH and osmolarity measurements**

The pH meter is used for measuring the pH of a nanoemulsion and microosmometer is used for determining the osmolarity of emulsion, which is based upon freezing point method. For performing this, 100 μl of nanoemulsion is transferred in microtube and measurements are taken. Morsi et al. measured the pH of the acetazolamide nanoemulsion by pH meter and found pH in the range of 4.9 to 5.5 thus claiming it to be adequate and non-irritant for application to the eye.

**Dye solubilisation**

A water soluble dye is dispersible in an O/W globule whereas it is soluble in the aqueous phase of the W/O globule. Similarly an oil soluble dye is dispersible in the W/O globule but soluble in the oily phase of the O/W globule (Bhosale RR, et.al. 2014). On adding water soluble dye to O/W nanoemulsion, it will evenly take up the colour whereas if it is a W/O emulsion, dye will remain in dispersed phase only and the colour will not spread evenly. This can be seen with microscopic examination of emulsion (Jaiswal M, et.al. 2015). Laxmi et al. carried out this test on artemether nanoemulsion by adding eosin yellow, a water soluble dye to the formulation and examined it under a microscope. They discovered that the aqueous continuous phase was
labelled with dye while the oily dispersed phase remained unlabelled therefore confirming the formed nanoemulsion as O/W type.

**Dilutability test**

The rationale of dilution test is that continuous phase can be added in larger proportion into a nanoemulsion without causing any problem in its stability. Thus O/W nanoemulsions are dilutable with water but W/O nanoemulsions are not and go through a phase inversion into O/W nanoemulsion. The W/O nanoemulsion can be diluted with oil only (Bhosale RR et.al. 2014). Laxmi et al. performed dilutability test on nanoemulsion by diluting it with water and observed no sign of phase inversion and precipitation thus claiming their nanoemulsion formulation to be stable.

**Conductance measurement**

The O/W nanoemulsions are highly conducting because they have water in external phase whereas W/O nanoemulsions are not conducting as they have water in internal or dispersal phase. Electrical conductivity measurements are very much beneficial for determining the nature of the continuous phase and for detecting phase inversion phenomena. At low volume fractions, increase in conductivity of certain W/O nanoemulsion systems was observed and such kind of behaviour is deduced as an indicator of a percolative behaviour or ions exchange among droplets prior to the development of bicontinuous structures. Dielectric measurements are a great means of exploring the structural and dynamic features of nanoemulsion systems. Conductometer is employed for determining the conductance of nanoemulsion. For carrying out conductance measurement, a pair of electrodes is attached to a lamp and an electric source is immersed into an emulsion. When the emulsion is O/W type then water will conduct the current and lamp will glow because of passage of current among connecting electrodes. The lamp will not glow if it is water in oil emulsion as oil in external phase does not conduct the current (Jaiswal M, et.al. 2015). Harika et al. performed conductivity test on amphotericin B nanoemulsion using an electroconductometer. They reported conductivity of the formulations in the range of 454.2-552.3 μS/cm and concluded the system to be O/W on the basis of electroconductivity study.
Interfacial tension

By measuring the interfacial tension, the formation and the properties of nanoemulsion can be investigated. Ultra low values of interfacial tension corresponds to phase behaviour, mainly the coexistence of surfactant phase or middle-phase nanoemulsions with aqueous and oil phases in equilibrium. For determining ultralow interfacial tension spinning-drop apparatus is used. Interfacial tensions are obtained by measuring the shape of a drop of the low-density phase, rotating it in cylindrical capillary filled with high-density phase.

Fluorescence test

There are numerous oils that show fluorescence under UV light. If a W/O nanoemulsion is subjected to a fluorescence light under a microscope, the whole field will fluoresces and if it is an O/W the fluorescence will be in spots (Jaiswal M, et al.2015).

In vivo studies

In vivo studies can be performed by adopting suitable animal model according to the activity chosen. Srilatha et al. has performed antidiabetic activity on glipizidenanoemulsion by choosing hyperglycaemia model in which they first induce diabetes in rats by intraperitoneal injection of streptozotocin solution and then the formulation was given to diabetic rats and the pharmacodynamic studies were performed on them. They reported the reduction in blood glucose levels for up to 12 h (Srilatha R, et.al.2013). Chouksey et al. has evaluated in vivo performance of atorvastatin nanoemulsion by performing pharmacokinetic studies on nanoemulsion and they reported better bioavailability of nanoemulsion formulation as compared to pure drug.

Nanoemulsions hold great potential as an efficient drug delivery tool that could be effectively harnessed to realise the complete potential. Quality assurance and quality control shall be of paramount importance with such a precise delivery system and hence the evaluation tests are to be performed rigorously.

EVALUATION PARAMETERS OF NANOEMULSION
1. Thermodynamic Stability Studies: The various formulations were subjected to different thermodynamic stability tests.

2. Droplet Size Analysis: The droplet size is analysed by photon correlation spectroscopy. In this the formulation is dispersed in 50 mL of water in a volumetric flask and gently mixed by inverting the flask.


4. Refractive Index: The refractive index, n, of a medium is defined as the ratio of the speed, c, of a wave such as light or sound in a reference medium to the phase speed, vp, of the wave in the medium represented by equation

\[ n = \frac{c}{v_p} - 1 \]

5. It is determined using an Abbes type refractrometer.

6. Drug Content: Drug content determine by reverse phase HPLC method using different columns of appropriate porosity.

Table 3: MARKETED NANO EMULATION FORMULATIONS

<table>
<thead>
<tr>
<th>Drug/Bioactive</th>
<th>Brand Name</th>
<th>Manufacturer</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitatealprostadil</td>
<td>Liple</td>
<td>Mitsubishi Pharmaceutical, Japan</td>
<td>Vasodilator, platelet inhibitor</td>
</tr>
<tr>
<td>Dexamethason</td>
<td>Limethason</td>
<td>Mitsubishi Pharmaceuticals, Japan</td>
<td>Steroid</td>
</tr>
<tr>
<td>Propofol</td>
<td>Diprivan</td>
<td>Astra Zeneca</td>
<td>Anesthetic</td>
</tr>
<tr>
<td>Flurbiprofenaxtil</td>
<td>Ropion</td>
<td>KsakenPharmaceutics, Japan</td>
<td>NSAID</td>
</tr>
<tr>
<td>Vitamins A, D, E and K</td>
<td>Vitalipid</td>
<td>Fresenius Europe</td>
<td>Kabi Parenteral nutrition</td>
</tr>
</tbody>
</table>

REFERENCES


