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A Review on Nanoemulsions: A Recent Drug Delivery Tool

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ABSTRACT

There is a growing interest for using of nano/sub-micron particles in the technology of pharmaceutical, cosmetic and also food. Especially, this interest has been increasing parallel with better emulsification techniques and stabilization mechanisms. There are two main groups of nanoemulsion preparation methods, namely high-energy and low-energy spontaneous emulsification methods. Preparation processes and components used are significant parameters that affect stability from few hours to years. Problems such as creaming, coalescence sedimentation and flocculation are not concern for nanoemulsions due to their small droplet size. However, the main destabilization mechanism is Ostwald ripening for them. In this paper, a comprehensive review is presented to give basic ideas about nanoemulsions, their preparation methods, and evaluations.

Keywords: Nanoemulsion, preparation methods, evaluation

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Introduction

At the present time, a lot of interest has been directed on lipid based formulations to progress the permeability and bioavailability of poorly water soluble drugs. By reminding this in mind a choice of different novel drug delivery system has been used in which nanoemulsion plays an essential role in delivering the active pharmaceutical ingredient at the target organ or site. Among diverse technologies nanoemulsions has been showed better development in drug delivery system. These are considered as an ideal alternative for improving the oral bioavailability of BCS (Biopharmaceutical drug classification system) Class II and IV drugs[1-5]. The term Nanoemulsion is said to a thermodynamically stable clear solution of two non-soluble liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. Nanoemulsions are novel drug delivery system includes an emulsified oil and water systems having mean droplet size which ranges from 50 to 1000 nm. The emulsions and nanoemulsions differ mainly in the size and shape of the particles dispersed in continuous phase[3,6,7]. The particle size in nanoemulsions is (10/200 nm) and those of conventional emulsions are (1/20µm) . A nanoemulsion is kinetically stable liquid which consists an oil phase and water phase with an appropriate surfactant. The dispersed phase mainly comprises small particles having a size range of 5 nm/200 nm, and has less oil/water interfacial tension. Nanoemulsions are colloidal dispersions having an oil phase, aqueous phase, surfactant and co surfactant in correct ratios .Nanoemulsions were shaped both by high energy emulsification methods or low energy emulsification methods. High energy emulsification methods engage high shear mixing, high/pressure homogenization or ultrasonification, while low energy emulsification methods used the advantage of the physicochemical properties of the system which exploits phase transitions to produce nanoemulsion. Nanoemulsion prepared with oil, surfactant and cosurfactant are non-toxic, non/irritant and approved for human consumption that are “generally recognized as safe” by the FDA[8-11].

Methods of preparation of nanoemulsion[12-17]

Phase Inversion Method

Fine dispersion is obtained by chemical energy resulting of phase transitions occur through emulsification method. The adequate phase transitions are produced by changing the composition at constant temperature or by changing the temperature at constant composition, Phase inversion temperature (PIT) method was introduced by Shinoda et al. based on principle of the changes of solubility of polyoxyethylene/ type surfactant with temperature. This

surfactant becomes lipophilic as increase in temperature because of dehydration of polymer chain. At low temperature, the surfactant monolayer has a great positive spontaneous curvature forming oil swollen micellar solution phase.

Table 1:- List of oils, surfactants and cosurfactants used for preparation of nanoemulsion:

Oil (Chemical Name)	Surfactant	Cosurfactant
Captex 355 (GlycerylTricaorylate/Caprate),	Capryol 90;	TranscutolP;
Captex 200 (Propylene Dicaprylate/Dicaprate Glycol),	Gelucire 44/14, 50/13;	Glycerin; Ethylene glycol;
Captex 8000 (GlycerylTricaprylate),	Cremophor RH 40;	Propylene glycol;
Witepsol (90:10 % w/w c12 Glyceride tri: diesters),	Imwitor 191, 308(1), 380, 742, 780 K, 928, 988;	Ethanol;
Myritol 318 (c8/c10 triglycerides),	Labrafil M 1944 CS, M 2125 CS ;	Propanol.
1sopropyel myristate (Myristic acid isopropyl ester).	Lauroglycol 90;	
	PEG MW > 4000;	
	PlurolOleique CC 497;	
	Poloxamer 124 and 188;	
	Softigen 701, 767;	
	Tagat TO;	
	Tween 80.	

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. 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 .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 oilsurfactant 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. 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 thepolyoxyethylene 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 .

Sonication Method[18]

Sonication method is best way to prepare nanoemulsions. In this method the droplet size of conventional emulsion or microemulsions were reduced with the help of sonication mechanism. This method is not applicable for large batches, but only small batches of nanoemulsions can be prepared by this method. Ultrasound can be used directly to produce emulsion, but since breaking an interface requires a large amount of energy, it is better to prepare coarse emulsion before applying acoustic power. Due to small product throughput the ultrasound emulsification process mainly applied in laboratories where emulsion droplet size as low as 0.2 micrometer can be obtained .

Ultrasonic System

In ultrasonic emulsification, the energy input is provided through so called sonotrodes (sonicator probe) containing piezoelectric quartz crystals that can be expand & contract in response to alternating electrical voltage. As the tip of sonicator probe contacts the liquid, it generates mechanical vibration and therefore cavitations occurs, which is the main phenomenon responsible for ultrasonically induced effects. Cavitation is the formation and collapse of vapour cavities in a flowing liquid.. Such a vapour cavity forms when the local pressure is reduced to that of at the temperature of the flowing liquid because of local velocity changes. The collapse of these cavities causes powerful shock waves to radiate throughout the solution in proximity to the radiating face of the tip, thereby breaking the dispersed droplets. Within the ultrasound range, the power available varies inversely with the frequency and only powerful ultrasound (0/200kHz) is able to produce physical and chemical changes such as emulsification.

Microfluidizer[20]

It is possible to produce emulsion at much higher pressures up to approximately 700 Mpa, in the nozzle of microfludizer that is the heart of this device (the interaction chamber), two jets of crude emulsion from two opposite channels collide with one another. The process stream is delivered by a pneumatically powered pump that is capable of pressurizing the in/house compressed air (150/650 Mpa) up to about 150 Mpa. Forcing the flow stream by high pressure through microchannels toward an impingement area creates a

tremendous shearing action, which can provide an exceptionally fine emulsion .

High/Energy Emulsification Method[21-23]

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, highshear stirring, and ultrasound generators . These mechanical devices provide strong forces that disrupt oil and water phases to form nanoemulsions. In high energy methods, input energy density is about 108 /1010 W kg/1. 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. Moreover, producing emulsions using ultrasound is a cost/effective process which needs less surfactant use. Therefore, considering conventional mechanical processes more homogeneous batches are achieved.

High Pressure Homogenizer

It is the most popular method used for the production of nanoemulsions. This method benefits from the highpressure 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 . 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 .

Low/Energy Emulsification Method

Nanoemulsification can also be achieved with lowenergy 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. 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 foodprocess .

Spontaneous Nanoemulsification

It benefits from the chemical energy replacement 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 self emulsifying drug/delivery systems (SEDDS) or self Nano/emulsifying 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. This leads to interfacial turbulence and thus formation of spontaneous oil droplets .

Patents related to nanoemulsion

Patents are the strongest form of intellectual property protection and are essential to the growth of a nanotechnology company. Similar to their importance to the development of the biotechnology and informational technology industries, patents will also play a critical role in the success of the global nanotechnology revolution; in fact patents are already shaping the nascent and rapidly evolving field of nanoscience and small technologies. As companies develop the products and processes of nantechnology, and begin to seek commercial applications for their inventions, securing valid and defensible patent protection will be vital to their long term survival.

Table 2: Patents on nanoemulsion

Patent Application title	Patent App. No.	Date
Nanoparticles and nanoemulsions	14/893,123	2017/12/05
Nanoemulsion therapeutic compositions and methods of using the same	12/567571	2015/02/24
Antimicrobial nanoemulsion compositions and methods	8236335	2012/08/07
Antimicrobial nanoemulsion compositions and methods	8232320	2012/07/31
Topical compositions and methods of detection and treatment	20120039814	2012/02/16
Cancer vaccine compositions and methods of using the same	20110280911	2011/11/17
Methods of using nanoemulsion compositions having anti/ inflammatory activity	20110200657	2011/08/18
Stable nanoemulsions for ultrasound/mediated of drug delivery and imaging	20110177005	2011/07/21
Method for the preparation of nanoparticles from nanoemulsion	20110135734	2011/06/09
Antimicrobial nanoemulsion compositions and methods	20110070306	2011/03/24
Nanoemulsion formulations for direct delivery	20110045050	2011/02/24
Lyophilized nanoemulsion	20110015266	2011/01/20
Nanoemulsion vaccines	20100316673	2010/12/16
Nanoemulsion of resveratrol/phospholipid complex and method for preparing the same and applications thereof	20100297199	2010/11/25
Per fluorocarbonnanoemulsion containing quantum dot nanoparticles and method for preparing the same	20100233094	2010/09/16
Compositions for treatment and prevention of acne, methods for making the compositions, and methods of use thereof	20100226983	2010/09/09
Nanoemulsion therapeutic compositions and methods of using the same	20100092526	2010/04/15
Stable mixed emulsions	20100069511	2010/03/18
Antimicrobial nanoemulsion compositions and methods	7655252	2010/02/02
Antimicrobial nanoemulsion compositions and methods	20100003330	2010/01/07
Nanoemulsion influenza vaccine	20090304799	2009/12/10
Nanoemulsion adjuvants	20090291095	2009/11/26
Oil/in/water nanoemulsion, a cosmetic composition and a cosmetic product comprising it, a process for preparing said nanoemulsion	20090208541	2009/08/20
Nanoemulsion therapeutic compositions and methods of using the same	20080317799	2008/12/25
	PCT/AU2008/001714	2008/11/18
Nanoemulsion vaccines	20080254066	2008/10/16
Nanoemulsion vaccines	20080181905	2008/07/31
Nanoemulsion vaccines	7314624	2008/01/01
Nanoemulsion compositions having anti/inflammatory activity	20070036831	2007/02/15
Nanoemulsion formulations	20020155084	2002/10/24
Nanemulsion based on non ionic and cationic amphilic lipid and uses there of	6039936	2000/03/21
Solid fat nanoemulsions as vaccine delivery vehicles	5716637	1998/02/10
Solid fat nanoemulsions as drug delivery vehicles	5576016	1996/11/19

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 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. [27], 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. 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 timecorrelation 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 risperidonenanoemulsion 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 primaquinenanoemulsion in the range of 20/200 nm .

Determination of Zeta Potential[28]

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 Zetasizer 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 Zetasizerfor risperidonenanoemulsion .

Morphological Study of Nanoemulsion[29-31]

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 studied surface morphology characteristics of primaquinenanoemulsion by TEM analysis and reported spherical shape of primaquinenanoemulsion 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 carvedilolnanoemulsion 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 studied the in vitro drug release profile of antiHIV drug nanoemulsion using dissolution apparatus type/II and reported 80 % drug release in 6 h .

In Vitro Skin Permeation Studies[32-36]

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 (Sugumar et al., 2015). 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 λmax 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.: t0.9=0.1052/K25. Where t0.9 stands for time required for 10 % degradation of the drug and it is termed as shelf life (Bali et al., 2010). 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[37]. 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[6]. These formulations are then subjected to dispersibility studies for evaluating the efficiency of self/emulsification. Srilatha et al. performed thermodynamic studies on glipizidenanoemulsion 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. Grade Ananoemulsions 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, Hoeppler 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=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 .

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. On adding water soluble dye to O/W nanoemulsion, it will evenly takes 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.. Laxmi et al. carried out this test on artemethernanoemulsion 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. 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

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