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PREPARATION AND CHARACTERIZATION OF NANOEMULSION: AN OVERVIEW

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ABSTRACT

Nano emulsions are colloidal dispersion systems that are thermodynamically stable, composed of two immiscible liquids mixed along with emulsifying agents (surfactants and co-surfactants) to form a single phase. Nano emulsions have extensively been investigated as drug delivery systems. This review aims to provide consolidated information regarding various formulation and characterization techniques developed for nano emulsions. Nano emulsions are formulated using two different methods, the persuasion method and the Brute force method. Various characterization techniques for nano emulsions include determination of entrapment efficiency, particle size, polydispersity index, zeta potential as well as characterization through differential scanning calorimetry, Fourier-transform infrared spectroscopy and transmission electron microscopy. Nano emulsions are further evaluated by studying in vitro drug release, in vitro permeation, stability and thermodynamic stability, shelf life, dispersibility, viscosity, surface tension, friccohesity, refractive index, percent transmittance, pH and osmolarity. Nano emulsions are submicron sized emulsions that are under investigation as drug carriers for improving the delivery of therapeutic agents. In this review attention is focused to give the brief regarding formulation aspect, method of preparation characterization techniques. These are applicable in drug targeting. Nano emulsions are colloidal dispersion systems that are thermodynamically stable, composed of two immiscible liquids mixed along with emulsifying agents (surfactants and co-surfactants) to form a single phase. Nano emulsions have extensively been investigated as drug delivery systems. This review aims to provide consolidated information regarding various formulation and characterization techniques developed for nano emulsions. Nano emulsions are formulated using two different methods, the persuasion method and the Brute force method. Various characterization techniques for nano emulsions include determination of entrapment efficiency, particle size, polydispersity index, zeta potential as well as characterization through differential scanning calorimetry, Fourier-transform infrared spectroscopy and transmission electron microscopy. Nano emulsions are further evaluated by studying in vitro drug release, in vitro permeation, stability and thermodynamic stability, shelf life, dispersibility, viscosity, surface tension, friccohesity, refractive index, percent transmittance, pH and osmolarity.

KEYWORDS: Nano emulsion, preparation methods, evaluation.

INTRODUCTION

Nano emulsions, also known as submicron emulsions, ultrafine emulsions and mini emulsions, are submicron sized colloidal particulate systems considered as thermodynamically and kinetically stable isotropic dispersions, which consist of two or more 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 Nano emulsions. Most widely used among them were nonionic surfactants (sorbitol esters, polysorbates), anionic surfactants (potassium laurate, sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide) and zwitterions surfactants (quaternary ammonium halide). Early nano emulsions were oil-in-water (O/W) type emulsions with average droplet diameter ranging from 50 to 1000 nm. Nano emulsions 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 inter dispersed within the system). Multiple emulsions are also a type of nano emulsions, where both O/W and W/O emulsions present simultaneously in one system. Nano emulsions offer various advantages over other dosage forms and these advantages are, (1) increased rate of absorption, (2) reduced variability in absorption, (3) protection from oxidation and hydrolysis in O/W nano emulsions, (4) delivery of lipophilic drugs after solubilization, (5) aqueous dosage form for water insoluble drugs, (6) enhanced bioavailability for many drugs, (7) ability to incorporate both lipo. and hydrophilic drugs, (8) It enhance efficacy while reduce total dose and side effects, (9) as non-toxic and nonirritant vehicles for skin and mucous membrane

delivery and (10) release control by permeation of drug through liquid film, whose hydrophilicity or lipophilicity as well as thickness can be precisely controlled. The main application of Nano emulsions is the preparation of nanoparticles using a polymerizable monomer as the disperse phase (the so-called mini emulsion polymerization method) where Nano emulsion droplets act as nanoreactors. And other important application which is experiencing an active development is the use of Nano emulsions as formulations, namely, for controlled drug delivery and targeting. The main application of nano emulsions is the preparation of nanoparticles using a polymerizable monomer as the disperse phase where nano emulsion droplets act as nanoreactors.

Formulation of Nanoemulsion

A number of techniques had been adopted for formulation of nano emulsions such as high-pressure homogenization, micro fluidizations, phase inversion, spontaneous emulsification, solvent evaporation and hydrogel formation. A variety of techniques had been utilized for characterization of such nano emulsions used as drug delivery systems. Nano emulsions are formulated mainly using two primary methods, (a) the persuasion method and (b) the Brute force method.[1-4]

Advantages of Nanoemulsion[5]

1. Nano emulsions have higher surface area and free energy that make them an effective transport system.
2. They avoid problems like inherent creaming, flocculation, coalescence and sedimentation.
3. It formulated in variety of formulations such as foams, creams, liquids and sprays.
4. Nano emulsion is non-toxic, non-irritant hence can be easily applied to skin and mucous membranes.
5. It can be administered orally if the formulation contains surfactants which are biocompatible.
6. It does not damage healthy human and animal cells hence are suitable for human and veterinary therapeutic purposes.
7. Nano emulsion is the provides better uptake of oil-soluble supplements in cell cultures technology to improve growth of cultured cells and allows toxicity studies of oil-soluble drugs.
8. It may be applied as a substitute for liposomes and vesicles and it is possible to build lamellar liquid crystalline phases around the nano emulsion's droplets.
9. Their small size is responsible, for penetrate through the "rough" skin surface and this enhances penetration of actives.
10. It constitutes the primary step in nano capsules and nanospheres synthesis using nano precipitation and the interfacial poly-condensation

METHOD OF PREPRATION OF NANOEMULSION[6-11]

1) Phase Inversion Method

Fine dispersion is obtained by 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 polyoxymethylene / type surfactant with temperature. This surfactant becomes lipophilic as increase in temperature due to dehydration of polymer chain. At coldness, the surfactant monolayer features a great positive spontaneous curvature forming oil swollen micellar solution phase.

2) Phase Inversion Temperature (PIT)

In this method, temperature is changed at constant composition. Nonionic 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 polyoxymethylene groups. Therefore, this circumstance establishes the principle of producing nano emulsions by PIT method. In order to prepare nano emulsions 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 taking advantage of the extremely low interfacial tensions at the HLB temperature. Never the less, 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 are often produced by rapid cooling of the emulsion near the temperature of PIT.

3) Phase Inversion Composition (PIC)

In this method, composition is changed at constant temperature. Nano emulsions 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. 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 polyoxymethylene chains of the surfactant increases and thus spontaneous curvature of the surfactant goes to a change from negative to zero. As within the HLB temperature, within 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 is composed due to the separation of the structures that have zero curvature**.**

4) Sonication Method[12]

Sonication method is best way to prepare nano emulsions. 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 nano emulsions can be prepared by this method. Ultrasound are often used on to produce emulsion, but since breaking an interface requires an outsized amount of energy, it's better to organize coarse emulsion before applying sound pressure level. Due to small product throughput the ultrasound emulsification process mainly applied in laboratories where emulsion droplet size as low as 0.2 micrometer are often obtained.

5) Ultrasonic System

In ultrasonic emulsification, the energy input is provided through so called sonotrodes (sonicate probe) containing piezoelectric quartz crystals that can be expand & contract in response to alternating electrical voltage. As the tip of sonicate probe contacts the liquid, it generates mechanical vibration and therefore cavitation's occurs, which is the main phenomenon responsible for ultrasonically induced effects. Cavitation is the formation and collapse of vapors cavities in a flowing liquid. Such a vapors 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 those cavities causes powerful shock waves to radiate throughout the answer in proximity to the radiating face of the tip, thereby breaking the dispersed droplets. Within the ultrasound range, the facility available varies inversely with the frequency and only powerful ultrasound (0/200kHz) is in a position to supply physical and chemical changes like emulsification.

6) Microfluidizer[13]

It is possible to produce emulsion at much higher pressures up to approximately 700 Mpa, in the nozzle of microfluidizer that is the heart of this device (the interaction chamber), 2 jets of crude emulsion from two opposite channels collide with one another. Stream process of microfluidizer 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. Flow by the force of stream by high pressure through microchannels toward an impingement area creates a tremendous shearing action, which can provide an exceptionally fine emulsion.

7) High/Energy Emulsification Method[14-16]

Nano emulsions are non/equilibrium systems which cannot be formed spontaneously. For this reason, mechanical or chemical energy input is necessary to form them. Nano emulsions 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. These mechanical devices provide strong forces that disrupt oil and water phases to form nano emulsions. Necessary 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 nano emulsions.

8) High Pressure Homogenizer

This method benefits from the high-pressure homogenizer or the piston homogenizer to manufacture nano emulsions that particle sizes are up to 1 nm. Within 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 nano emulsions are achieved because during the process several forces like hydraulic shear, intense turbulence and cavitation act together. The uniformity of droplet size in nano emulsions is specified by PDI. Higher PDI means lower uniformity of droplet size in nano emulsions. Samples of Monodisperse 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. 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.

9) High/Shear Stirring

In this method, high/energy mixers and rotor/stator systems are used for the preparation of nano emulsions. Internal phase droplet size 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 Nano emulsification 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. 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 nano emulsions in low energy techniques but this narrows down their application area, especially for many food processes.

10) Spontaneous-Nano-emulsification

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 nano emulsions 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-nanoemulsifying drug/delivery systems (SNEDDS). When an oil phase with a water-soluble substance is mixed with water, oil droplets spontaneously form. 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 nano emulsion

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 nano-technology, and begin to seek commercial applications for their inventions, securing valid and defensible patent protection will be vital to their long-term survival.

Determination of Encapsulation Efficiency

This method is applied for the 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 analyzing the extract Spectro-photometrically at λ max of drug after making suitable dilutions against suitable blank. The drug entrapment efficiency and loading efficiency of the drug can be calculated by using the following Equations- 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. The most important and accurate method of determination of drug content also 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 nano emulsion**.**

Determination of Zeta Potential[17]

Zeta potential method is used for measure the surface charge of particles when it is placed in liquid. It also predicts 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, nano emulsion 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 nano emulsion. Dardic et al. obtained zeta potential around –50 mV by using Malvern Zeta sizer for risperidone nano emulsion.

Stability Studies

The stability studies of nano emulsion are carried out after storing the formulation for 24 Mo in dispersed and freeze/dried state as per International Conference on Harmonization guidelines. The storage conditions followed are ambient (25±2°/60±5 % RH), refrigeration (5±3°) and freeze (–20±5°). The requisite volume of nano emulsion is stored in glass bottles and is tightly sealed. Samples are withdrawn at predefined time interval and analyzed 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 nano emulsion 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 nano emulsion, 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 analyzed using HPLC at λ-max for estimating the remaining drug content. 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 nano emulsion around 2.18 y at room temperature (25°) and concluded that the stability of clobetasol propionate can be augmented by incorporating in a nano emulsion. Parveen et al. reported that the shelf life of a silymarin nano emulsion to be around 3.8 y when stored in a refrigerator.[18]

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 nano emulsion by varying temperature conditions. Nano emulsion is exposed to six cycles between 4°C (refrigeration temperature) and 40°C 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 nano emulsions are centrifuged at 5000 rpm for 30 min and observed for phase separation or creaming or cracking. Thirdly, the freeze/thaw cycle, in which nano emulsion 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. Srilatha et al. performed thermo-dynamic studies on glipizide nano emulsion by subjecting it to three cycles of stability and reported good physical stability of nano emulsion with no appearance of phase separation, creaming or cracking.[19]

Dispersibility Studies[20-26]

Dispersibility studies for evaluating the efficiency of self/emulsification of nano emulsion 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 nano emulsion formulations is evaluated visually by using a grading system described below. Grade nano emulsions form rapidly within 1 min and appear to be clear or bluish. Grade B nano emulsions form rapidly but are slightly fewer clear emulsions appear to be bluish white. Grade C nano emulsions are fine milky emulsion that form within 2 min. Grade D are those dull, greyish white emulsions that has a little oily appearance and are slower to form (>2 min). Grade E nano emulsions 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 nano emulsion. Various instruments are employed for measuring viscosity such as Ostwald viscometer, Hopper falling ball viscometer, Stormer viscometer, Brookfield viscometer and Ferranti/Shirley viscometer. Among all these viscometers, Brookfield is the preferred one for measuring the viscosity of nano emulsion. 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 curvimeter 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 nano emulsions. Shafiq et al. has determined viscosity of ramipril nano emulsion 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 nano emulsion. 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 nano emulsion can be determined by Abbes type refractometer at 25±0.5°C by placing a drop of nano emulsion on slide and comparing it with refractive index of water (1.333). If refractive index of nano emulsion has equal refractive index as that of water, then the nano emulsion is considered to have transparent nature. Harika et al. measured the refractive index of amphotericin B nano emulsion 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 nano emulsion and micro osmometer is used for determining the osmolarity of emulsion, which is based upon freezing point method. For performing this, 100 µl of nano emulsion is transferred in microtube and measurements are taken. Morsi et al. measured the pH of the acetazolamide nano emulsion 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 Solubilization

Water-soluble dye is dispersible in O/W globule where as it is soluble in the aqueous phase of the W/O globule. The 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 nano emulsion, it will evenly take up the color whereas if it is a W/O emulsion, dye will remain in dispersed phase only and the color will not spread evenly. This can be seen with microscopic examination of emulsion. Laxmi et al. carried out this test on artemether nano emulsion 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 unlabeled therefore confirming the formed nano emulsion as O/W type.

Dilutability Test

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

Application of Nanoemulsion[27-33]

1. Use of nano emulsions in cosmetics
2. Antimicrobial Nano emulsions
3. Prophylactic in Bio-Terrorism Attack
4. Nano emulsions as Mucosal Vaccines
5. Nano emulsion as Non-Toxic Disinfectant Cleaner
6. Nano emulsions in Cell Culture Technology
7. Nano emulsion formulations for improved oral delivery of poorly soluble drug
8. Self-nanoemulsifying drug delivery systems
9. Nano emulsions as a vehicle for transdermal delivery
10. Nano emulsion in the treatment of various other disease conditions like diclofenac cream, a potential treatment for osteoarthritis.
11. Self-nanoemulsifying delivery systems like solid as a platform technology for formulation of poorly soluble drugs.
12. Nano emulsion in cancer therapy and in targeted drug delivery.

CONCLUSION

Nano emulsion formulations offer several advantages for the delivery of drugs, biologicals, or diagnostic agents and able to protect labile drug, control drug release, increase drug solubility, increase bioavailability and reduce patient variability. Traditionally, Nano emulsions have been used in clinics for more than four decades as total parenteral nutrition fluids. Nano emulsions are chiefly seen as vehicles for administering aqueous insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents, or diagnostic agents. Additional, targeting moiety has opened new avenues for targeted delivery of drugs, genes, photosensitizers, and other molecules to the tumor area. The expectation that furthers research and development work will be carried out in the near future for clinical realization of these targeted delivery vehicles.

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