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REVIEW ARTICLE

Nanoemulsion Preparation, Identification and Evaluation Review

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Abstract

Nanoemulsions consist of two immiscible liquids combined with emulsifying agents (surfactants and co-surfactants) in a thermodynamically stable, colloidal dispersion system, in order to form the same phase. Nanoemulsions have been thoroughly investigated as drug delivery systems. This review is aimed at providing comprehensive information on various techniques of nanoemulsion preparation and identification. There are two different methods for formulating nanoemulsions, the method of persuasion and the method of brute force. Different identification techniques for nanoemulsions include determination of trap efficiency, particle size, polydispersity index, zeta potential as well as characterization by differential scanning calorimetry, Fourier-transform infrared spectroscopy and transmission electron microscopy. Nanoemulsions are further assessed by studying *in vitro* release of drugs, *in vitro* permeation, stability and thermodynamic stability, shelf life, dispersibility, viscosity, surface tension, refractive index, percentage of transmittance, pH and osmolarity.

Key words: *Nanoemulsions, Nanoemulsion preparation, Identification techniques.*

Introduction

Nanoemulsions are submicron emulsions showing thermodynamic and kinetic stability in sub-micron colloidal particulate systems with ultra-finished droplets. Nanoemulsions are isotropic dispersions made up of two immiscible liquids, such as oil and water, stabilized by a suited surfactant and co-surfactant forming interfacial film for a homogeneous single phase [1, 2].

Nanoemulsions, such as ionic or non-ionic, used a variety of surfactants with variable properties. Anionic surfactants (sodium lauryl sulphate; potassium laurate), cationic surfactants (quaternary ammonium halide), nonionic surfactants (polysorbates; sorbitan esters) and zwitterions surfactant (quaternary ammonium halide) were the most commonly used [3].

There are three divisions of nanoemulsion including oil-in-water (O / W) type (oil is dispersed in aqueous continuous phase), water-in-oil (W / O) type (water is dispersed in continuous oil phase), and bi-continuous system consisting of inter-dispersed water and oil within the system.

Nanoemulsion type inversion can be achieved by changing the system components. Multiple emulsions consisting of both O / W and W / O dispersions present simultaneously in one system are another type of nanoemulsion[4]. Two surfactants were used simultaneously to stabilize these two emulsions, one hydrophilic and the other lipophilic.

Nanoemulsions offer various advantages over other dosage forms, some of which include increased absorption rates, more consistent absorption, O/W nanoemulsion protecting the active drug against micro-environmental hydrolysis and oxidation, lipophilic drug solubilization, aqueous preparation for water-insoluble drugs, improved bioavailability, incorporation of both lipophilic drugs, improvement of efficacy by minimizing side effects and reducing total dose, applicability as non-toxic compatible vehicles for skin and mucous membrane delivery, and controlled

release patterns by drug permeation through liquid film by controlling hydrophilicity or lipophilicity and film thickness [5, 6].

Nano emulsions Preparation

Nano emulsions have been formulated using a variety of methods such as micro fluidization, high pressure homogenization, phase inversion, solvent evaporation, spontaneous emulsification, and hydrogel formation. Double emulsion-solvent evaporation technique was used to prepare multiple emulsions [7]. There are two primary nanoemulsion preparation techniques, the method of persuasion and, second, the method of Brute force.

In Persuasion Method/phase Inversion Method

Alternatively, Nano emulsion prepared without any external force involves the formation of fine dispersions as phase transitions occur by altering the temperature or composition while maintaining constant other parameters [8]. Furthermore, the persuasion method can be subdivided into (a) phase transition from a near-optimum state by changing a single variable, which includes altering one formulation variable, such as temperature or salinity, close to the optimum value, (b) Near-optimal phase transition by changing more than one variable, multiple variables, such as applying higher temperatures and adding more salt to a microemulsion. (C) Catastrophic reversal of low internal phase emulsion achieved and converted to external phase. (D) Stabilized phase transition with crystallized liquid, including stabilization of liquid crystal formation nanodroplets from a near-HLD-0 state [9].

Brute Force Method

This method involves the use of brute forces to break into the nano range the oil droplets. Instruments used to formulate nanomeulsions include a high-pressure homogenizer, a high-speed mixer, a small pore membrane and an ultrasonic device with high frequency. Nanoemulsion properties such as its small size, optical transparency and high kinetic stability depend not only on variables composition but also on processing variables such as emulsification time, mixing degree, energy input and emulsification path[10].

High-pressure homogenization and micro-fluidization methods are used to achieve very small size of Nano emulsion by using high-pressure equipment at both industrial and laboratory scale. Different other methods are also used to prepare Nano emulsion, such as ultrasonics and emulsification in situ[11].

Micro-fluidization

This method employed a device known as microfluidizer that utilizes high pressure positive displacement pump (500-20 000 psi) that pushes the product out through the interaction chamber consisting of stainless steel microchannels on the impingement area resulting into formation of very small particles of sub-micron range. The mixture is repeatedly circulated through the microfluidizer until the required particle size is achieved. Resultant product is also passed through the filter to separate smaller droplets from larger ones and to obtain a uniform Nano emulsion [12].

High Pressure Homogenization

High-pressure homogenizer or piston homogenizer is therefore used in this strategy to produce nanoemulsions with very small particle size (up to 1 nm). In this technique, at a very high pressure ranging from 500 to 5000 psi, a mixture is forced to pass through an orifice.

The resulting product is subjected to intense turbulence and hydraulic shear with extremely fine particles resulting in emulsion. This has been shown to be the most efficient method for preparing nanoemulsions, but the only drawback associated with this technique is high energy consumption and an increase in emulsion temperature during processing. It also requires larger cycles of homogenization to obtain smaller particle size[13].

Spontaneous Emulsification

This technique involved 3-stage nanoemulsion preparation. The first stage was the formation of an organic solution consisting of oil and lipophilic surfactant in water miscible solvent and hydrophilic surfactant, and then the O / W emulsion is formed by injecting this organic phase under magnetic stirring into the aqueous phase. Evaporation then removed the organic solvent in the third stage [14].

Solvent Evaporation Technique/hydro gel Method

In this technique, the drug solution is prepared and emulsified into another liquid (non-drug solvent) and then evaporated, leading to precipitation of the drug. High speed stirrer can be used to regulate the growth of crystals and the aggregation of particles. The method of hydrogel is very similar to the method of solvent evaporation. The only difference from the method of solvent evaporation is that in this case the antisolvent drug solution is miscible[15].

Ultrasonication

Premixed emulsion is exposed to 20 kHz ultrasonic frequency agitation in this technique which reduces droplets to nanodroplets size. Then the resulting emulsion is passed through high shear region to form droplets with a uniform distribution of the size. In this technique, the water jacket is used to regulate the temperature. During ultrasonic emulsification, sonotrodes also known as sonicator probes consisted of piezoelectric quartz crystals as the energy suppliers.

These sonotrodes contract and expand upon application of alternating electrical voltage. Mechanical vibrations are produced by contacting the liquid resulting in cavitation by the sonicator tip, resulting in the collapse of vapor cavities formed within the liquid. This technique is mainly used when the droplet size requires less than 0.2 µ. [16].

Nano emulsions Identification

Particle Size and Polydispersity Index (PDI)

The particle size and PDI of nanoemulsions are analyzed using photon correlation spectroscopy (PCS) using Malvern Zeta sizer, which monitors the variation in light dispersion as a function of time due to Brownian particle motion[17]. PCS is based on the principle that small-sized particles travel at higher speed compared to large-sized particles. Submicron particles present in solution diffract the laser beam. Rapid fluctuations in laser scattering intensity occur around due to particle diffusion.

The mean value at a fixed angle depends on the size of the particle. The calculated time correlation function of the photoelectron generates a line width distribution histogram that can be associated with particle size. For particle size measurement, weighed amount of formulation is dispersed in double-distilled water to obtain homogeneous dispersion and must be used instantly to measure particle size and PDI. The PDI may vary from 0 to 1, where 0 (zero) is a monodisperse system and 1 is a dispersion of polydisperse particles[18].

Zeta Potential

The zeta potential is a method of measuring particle surface load when placed in liquid. Zeta potential is used to predict dispersion stability and its value depends on drug, polymer, vehicle, electrolyte presence and adsorption physicochemical properties. It is measured by the instrument of Malvern Zetasizer. Nanoemulsion is diluted to measure zeta potential and its value is estimated from oil droplets ' electrophoretic mobility. It is believed that zeta potential of ±30 mV is sufficient to ensure Nano emulsion's physical stability[19].

Encapsulation Efficiency (EE) and Loading Efficiency (LE)

Drug content is estimated after making suitable dilutions against suitable blank by analyzing the extract spectrophotometrically at λmax of the drug. The drug's interception efficiency (EE) and loading efficiency (LE) can be calculated using the following equations, drug EE = drug content of the product obtained (mg)/total quantity of drug added (mg) x100 and drug LE= drug content of the product obtained (mg)/total product weight (mg) x100. Drug content could also be determined using high-performance liquid chromatography (HPLC) techniques in reverse phase[20].

Fourier-Transform Infra-Red Spectroscopy (FTIR)

For the evaluation of drug excipient interaction, polymerization, crosslinking and drug loading in the formulation, FTIR analysis can be performed. It is also used to identify functional groups with their attachment means and the molecule's fingerprint [21]. At low temperature, there is a molecule in the ground and they get excited to higher energy states when they absorb the radiant energy.

IR spectroscopy is based on determining the energy difference (ED) between the molecule's excited and ground conditions.

Sample can be prepared for FTIR performance by using suitable methods such as potassium bromide pellet method, and then scanned in FTIR at moderate scanning speeds between 4000-400 cm-1 [22].

Atomic Force Microscope (AFM)

AFM is comparatively a new technique used these days to explore nanoemulsion formulations ' surface morphology. AFM is performed by diluting water nanoemulsions followed by drop coating on a glass slide of the diluted nanoemulsion. The coated drops are further dried in the oven and scanned at 100 mV / s[23].

Morphological Imaging

The morphological Nano emulsion study is conducted using electron microscopy (TEM) transmission. In TEM, an electron beam is incident and passed through a thin foil specimen. These incident electrons are transformed into unscattered electrons, elastically dispersed electrons or inelastically dispersed electrons when interacting with the specimen. The magnification is regulated by the distance between the objective lens and the specimen and between the objective lens and its image plane[24].

In dual distilled water, a few drops of nanoemulsion or a suspension of lyophilized nanoparticles are prepared and placed on the holey film grid and immobilized for performance of TEM. After immobilization, the excess solution must be wicked off the grid and stained. At specific voltage, the stained nanoparticles are then examined [25].

Vitro Release Profile Study

***In Vitro* Drug Release Study**

Studies of drug release *in vitro* help estimate *in vivo* drug formulation performance. A drug's rate of *in vitro* release is usually studied on a USP dissolution device. In buffer, nanoemulsion or dried nanoparticles containing a drug equivalent to 10 mg were dispersed and then placed in dialysis membrane pouches and placed in a buffer-containing flask. This study is conducted at a speed of 37±0.5 ° and 50 rpm. At regular intervals, the sample is removed and replaced by the same volume of fresh dissolution medium each time. Samples are then diluted appropriately and at a particular wavelength the sample absorbance is measured spectrophotometrically. Using calibration curve, absorbance of the collected sample is used to calculate percentage of drug release at different time Intervals[26].

***In Vitro* Skin Permeation Studies**

Franz-diffusion cell is used to investigate studies of permeation *in vitro* and ex vivo. Usually the abdominal skin of adult male rats weighing 250±10 g is used for permeation studies. The rat skin is placed between the diffusion cell donor and receiver chambers.

The temperature of the receiver chambers containing 20% ethanol fresh water is set at 37° and the chamber content is continuously stirred at 300 rpm. The formulations are stored in the chamber of the donor. A certain amount (0.5 ml) of the solution from the receiver chamber was removed at specific time intervals such as 2, 4, 6, 8 h for gas chromatographic analysis and immediately replaced by an equivalent volume of fresh solution.

Three times each sample is performed. Cumulative corrections are performed at each time interval to obtain total amount of drug permeated by rat skins and are plotted against time function. Plot slope is used to calculate a steady-state drug's permeation rates [27].

Stability Studies

Stability studies are performed under the influence of various environmental factors such as temperature, humidity and light to assess the stability of the drug substance.

Storing Stability Studies

Nano emulsion stability studies are performed after 24 mo formulations have been stored in dispersed and freeze-dried state. The following storage conditions are ambient (25±2°/60±5% RH), cooling (5±3°) and freezing (–20±5°).

The required nanoemulsion volume is stored in glass bottles and is sealed tightly. Samples are removed at predefined time intervals and analyzed for features such as particle size, loading, EE and drug release profile *in vitro* [28].

Thermodynamic Stability Studies

Studies of thermodynamic stability are usually conducted in three steps. Firstly, the heating-cooling cycle, which is performed by different temperature, conditions to observe any effect on the stability of nanoemulsion. Nanoemulsion is exposed to six cycles between 4° (refrigeration temperature) and 40° by storing the formulation for no less than 48 h at each temperature.

For centrifugation studies, formulations that are stable at these temperatures are further selected. Second, a centrifugation study in which the formulated nanoemulsions are centrifuged for 30 min at 5000 rpm and observed for separation of phase or creaming or cracking. Those who have shown no signs of instability are subject to thaw cycle freezing.

Third, the freeze-thaw cycle in which formulations of nanoemulsion are exposed to three freeze-thaw cycles with temperatures ranging from -21 ° to+25 °. Formulations that do not show any signs of instability pass this test and are considered to have good stability. These formulations then undergo dispersibility studies to assess the self-emulsification efficiency[29, 30].

Shelf Life Determination

Accelerated stability studies are performed to determine the shelf-life of a nanoemulsion. The formulations are stored for nearly 3 mo at three distinct temperatures and conditions of ambient humidity (30 °, 40 ° and 50±0.5 °). Samples are removed and analyzed after a specific time interval (0, 30, 60 and 90 d) using HPLC at πmax to estimate the remaining drug content. Checks are used for samples withdrawn at zero time.

This determines the order of the reaction and after calculating the reaction rate constant (K) for the degradation from the line slope using the following equation at each elevated temperature: slope = −K/2.303, the logarithm values of K are plotted against the reciprocal of absolute temperature at different elevated temperatures (Arrhenius plot).

It is determined from this plot value of K at 25 ° and is further used to calculate shelf life by placing the value in the following Eqn: t0.9=0.1052/K25. Where t 0.9 is the time required to degrade the drug by 10 percent and it is called shelf life[31].

Evaluation Studies

Determination of viscosity

Viscosity evaluation is an important parameter for Nano emulsion's physicochemical characterization. Various instruments are used to measure viscosity such as Ostwald viscometer, falling ball viscometer from Hoeppler, viscometer from Stormer, viscometer from Brookfield and viscometer from Ferranti-Shirley.

Brookfield is the preferred one among all these viscometers to measure nanoemulsion viscosity. Viscosity determination asserts whether the system is emulsion O / W or W / O. Low system viscosity shows that it is type O / W and high viscosity shows that in the oil type system it is water [32].

Dispersibility Studies

Dispersibility studies to evaluate the efficiency of nanoemulsion self-emulsification are performed using a standard USP XXII dissolution device. 2 ml of each formulation is incorporated into 500 ml of 37±0.5 ° distilled water. For gentle agitation, a standard stainless-steel dissolution paddle rotates at 50 rpm. Nanoemulsion formulations performance *in vitro* is visually evaluated using a grading system. Where, grade A nanoemulsions appear to be clear or bluish quickly within 1 min.

Grade B Nano emulsions appear to be bluish-white, but are slightly less clear emulsions. Nanoemulsions of grade C are a fine milky emulsion forming within 2 minutes. Grade D is the dull, grayish-white emulsions that look a little oily and form slower (> 2 min). Grade E nanoemulsions show either poor or negligible emulsification on the surface with large oil globules [33].

Refractive Index

Refractive index shows how light propagates through the nanoemulsion medium and transparency. The medium refractive index (n) can be defined as the wave (c) velocity ratio in reference medium to the wave (vp) phase velocity in medium: n= c / vp.

The Nano emulsion refractive index can be determined at 25±0.5 ° by Abbes type refractometer by placing a drop in nanoemulsion on the slide and comparing it with the water refractive index (1.333) [34].

If the Nano emulsion refractive index has the same refractive index as that of water, then it is considered that the nanoemulsion is transparent in nature.

PH and Osmolarity Measurements

The pH meter is used to measure a Nano emulsion's pH, and the micro-osmometer is used to determine emulsion's osmolarity based on the freezing point method. To accomplish this, micro tube transfers 100 µl of nanoemulsion and measurements are taken[35].

Percent Transmittance

The percentage transmission of a formulated nanoemulsion is estimated using a UV spectrophotometer with distilled water as a blank at a specific wavelength. If it is found that the percentage transmittance of a nanoemulsion exceeds 99 percent, it is considered transparent in nature[36].

Dye Solubilization

In an O/W globule, a water-soluble dye is dispersible whereas it is soluble in the W/O globule's aqueous phase. Similarly, in the W/O globule, an oil-soluble dye is dispersible but soluble in the O/W globule's oily phase. When adding water-soluble dye to O/W Nano emulsion, the color will be evenly absorbed whereas if it is a W/O emulsion, the dye will only remain in dispersed phase and the color will not evenly spread. This can be seen with emulsion microscopic examination[37].

Conductance Measurement

The O / W nanoemulsions are highly conducting as they have water in the external phase, whereas W / O nanoemulsions do not perform as they have water in the internal or dispersal phase. Measurements of electrical conductivity are very beneficial in determining the nature of the continuous phase and in detecting phenomena of phase inversion.

Increased conductivity of certain W / O nanoemulsion systems was observed at low volume fractions and such behavior is deduced as an indicator of percolative behavior or ion exchange between droplets prior to the development of bi-continuous structures. Conductometer is used to determine nanoemulsion conductance.

A pair of electrodes is attached to a lamp for conductance measurement and an electrical source is immersed in an emulsion. When the emulsion is O / W type, the current will be conducted by water and the lamp will shine through the connecting electrodes due to the passage of the current.

If it is water in oil emulsion, the lamp will not glow as oil does not conduct the current in the external phase[38].

Dilutability Test

The reason for the dilution test is that it is possible to add continuous phase to a nanoemulsion in greater proportion without causing any problems in its stability. O/W Nano emulsions can therefore be diluted with water, but W/O Nano emulsions are not and go through a phase inversion into O/W Nano emulsion. Only oil can dilute the W/O Nano emulsion [39].

Fluorescence Test

There are numerous oils under UV light showing fluorescence. If a W/O Nano emulsion under a microscope is subjected to a fluorescence light, the entire field will fluoresce and the fluorescence will be in spots if it is an O / W[19].

Interfacial Tension

It is possible to investigate the formation and properties of nanoemulsion by measuring the interfacial tension. Ultra-low interfacial tension values are consistent with phase behavior, primarily the coexistence of surfactant phase or mid-phase nanoemulsions with equilibrium aqueous and oil phases.

Spinning-drop device is used to determine ultralow interfacial tension. Interfacial tensions are obtained by measuring a low-density phase drop shape, rotating it in a high-density phase cylindrical capillary [40].

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