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Nanoemulsion- Characterisation Techniques and Formulation Methods

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ABSTRACT

Nanoemulsions are thermodynamically stable colloidal dispersion systems made up of two immiscible liquids combined with emulsifying agents (surfactants and co-surfactants) to produce a single phase. Nanoemulsions have been studied extensively as drug delivery devices. This review attempts to bring together information on the many nanoemulsion formulation and characterization techniques that have been developed. The persuasion approach and the Brute force method are two methods for creating nanoemulsions. Entrapment efficiency, particle size, polydispersity index, zeta potential, and characterization using differential scanning calorimetry, Fourier-transform infrared spectroscopy, and transmission electron microscopy are just a few of the techniques used to characterise nanoemulsions. In vitro drug release, in vitro permeation, stability and thermodynamic stability, shelf life, dispersibility, viscosity, surface tension, friccohesity, refractive index, % transmittance, pH, and osmolarity are all used to assess nanoemulsions.

KEYWORDS: *Formulation study, nanoemulsion characterisation, droplet size, entrapment efficiency*

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INTRODUCTION

Nanoemulsions, also known as submicron emulsions, ultra fine emulsions, and miniemulsions, are submicron sized colloidal particulate systems that are thermodynamically and kinetically stable isotropic dispersions made up of two immiscible liquids, such as water and oil, stabilised by an interfacial lm made up of a suitable surfactant and co-surfactant to form a single phase. With such nanoemulsions, a variety of surfactants with different properties (ionic or non-ionic) have been utilised. Nonionic surfactants (sorbitan esters, polysorbates), anionic surfactants (potassium laurate, sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide), and zwitterions surfactants were the most extensively utilised (quaternary ammonium halide). Oil-in-water (O/W) emulsions with typical droplet diameters ranging from 50 to 1000 nm were the first nanoemulsions. Nanoemulsions are now divided into three types: O/W (oil dispersed in aqueous phase), W/O (water dispersed in oil phase), and bi-continuous (water scattered in both oil and water phases) (microdomains of water and oil are interdispersed within the system). The components of the emulsions can be changed to achieve transformation between these three forms. Multiple emulsions are a sort of nanoemulsion in which both O/W and W/O emulsions are present in the same system at the same time. Both hydrophilic and lipophilic surfactants are utilised simultaneously to stabilise these two emulsions. Nanoemulsions 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 nanoemulsions, (4) delivery of lipophilic drugs after solubilisation, (5) aqueous dosage form for water insoluble drugs, (6) enhanced bioavailability for many drugs, (7) ability to incorporate both lipophilic and hydrophilic drugs, (8) delivery systems to enhance efficacy while reduce total dose and side effects, (9) as non-toxic and non-irritant 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.

FORMULATION OF MICROEMULSION-

For the formulation of nanoemulsions, a variety of procedures have been used, including high pressure homogenization, microfludization, phase inversion, spontaneous emulsification, solvent evaporation, and hydrogel formation [1-4]. The double emulsion-solvent evaporation technique is commonly used to make multiple emulsions. Characterization of nanoemulsions employed as drug delivery devices has been done using a variety of methodologies. Nanoemulsions are formulated mainly using two primary methods, (a) the persuasion method and (b) the Brute force method.

PERSUASION METHOD/PHASE INVERSION TECHNIQUE-

The persuasive method of nanoemulsion preparation does not use any external force; instead, fine dispersions are formed when phase transitions occur by changing either the temperature or the composition while maintaining the other parameter constant. The methods of persuasion can be roughly classified as follows:

1. A phase transition from a near-optimal state can be achieved by changing a single formulation variable, such as temperature or salinity, to a near-optimal value. For a system, such as using a higher temperature to microemulsion, the hydrophilic-lipophilic deviation (HLD) for ideal value is close to the centre level..
2. Changes in numerous variables, or changing more than one formulation variable, are used to move from a near-optimal condition to a phase transition.For example, employing higher temperature and including an additional salt in a microemulsion.
3. Catastrophic inversion is the conversion of the internal phase of a low internal phase emulsion to the exterior phase.
4. Liquid crystal formation stabilised a phase transition, which included nanodroplets stabilisation from a condition near to HLD-0.

BRUTE FORCE METHOD:

The use of physical force to split the oil droplets into the nano range is part of this procedure. High-pressure homogenizers, high-speed mixers, small-pore membranes, and high-frequency ultrasonic devices have all been used in the creation of nanomeulsions. Nanoemulsion features such as small size, optical transparency, and high kinetic stability are influenced by processing factors such as emulsification time, degree of mixing, energy input, and emulsifying path, as well as the composition of variables. At both the industrial and laboratory scales, high-pressure homogenization and microfluidization procedures are used to achieve very small nanoemulsion sizes using high-pressure equipment. Other methods for preparing nanoemulsions, such as ultrasonication and in situ emulsification, are also being used.Various techniques employed for preparation of nanoemulsion are shown in Table 1[5-20]

HIGH PRESSURE HOMOGENIZATION:

Because nanoemulsion preparation necessitates a high shear force, a high-pressure homogenizer or piston homogenizer is used in this technique to produce nanoemulsions with very small particle sizes (up to 1 nm). A combination is pushed to pass through an aperture at an extremely high pressure, ranging from 500 to 5000 psi, in this technique. The resulting emulsion is then subjected to high turbulence and hydraulic shear, resulting in an extremely tiny particle emulsion. Although this has been proven to be the most effective method for nanoemulsion preparation, the sole disadvantage is the significant energy consumption and emulsion temperature rise during processing. It also necessitates longer runs of homogenization cycles to get lower particle sizes. Yilmaz et al. used a high pressure homogenization approach to create phytosphingosine O/W nanoemulsions and discovered that after 8 homogenization cycles, the droplet size was reduced and the nanoemulsion was stable for over 6 months. [21]

MICROFLUIDIZATION:

This method used a microfluidizer, which is a device that uses a high-pressure positive displacement pump (500-20 000 psi) to push the product out through an interaction chamber with stainless steel microchannels on the impingement area, resulting in the formation of very small particles in the sub-micron range. The mixture is passed through the microfluidizer several times until the desired particle size is attained. The resulting nanoemulsion is also run through a filter to separate tiny droplets from bigger ones and to create a homogenous nanoemulsion. Uluata et al. used a microfluidizer to make octadecane O/W nanoemulsions and found that as the number of passes and homogenization pressure were increased, the droplet size shrank[22]. Goh et al. made tocotrienol-rich fraction nanoemulsions utilising a two-step homogenization procedure in which an initial coarse emulsion was made with a stirrer and then processed with a microfluidizer. They found that after 10 homogenization cycles at a higher pressure, the droplet size was reduced from 120 to 65.1 nm[23].

TABLE 1: TECHNIQUES EMPLOYED FOR PREPARATION OF NANOEMULSION:

TECHNIQUES	FORMULATIONS	CONCLUSIONS	REFERENCES
High pressure homogenization	Oral lipid nano emulsion (primaquine)	Enhance oral bioavailability, 10-200 nm particle size.	[5]
Pseudoternary phase diagram+spontaneous emulsification method Pseudoternary phase diagram+spontaneous emulsification method Pseudoternary phase diagram +spontaneous emulsification method	Ramipril nanoemulsion.	Increased bioavailability, droplet size 80.9	[6]
High pressure homogenization	o/w nano emulsion	Improved skin hydration and elasticity	[7]
spontaneous emulsion	o/w nano eulsion	Nanoemulsion with potential for transdermal delivery of aceclofenac Nanoemulsion with potential for transdermal delivery of aceclofenac Nanoemulsion with potential for transdermal delivery of aceclofenac	[8]
Spontaneous emulsion	Celecoxib nanoemulsion	Enhanced physical and chemical stability of celecoxib in nanoemulsion	[9]
High pressure homogenization	Lecithin-based nano emulsions (progesterone)	Improved permeation rates of progesterone with long-term stability	[10]
High pressure homogenization	Prednicarbate nano emulsion	Increased chemical stability of the drug in formulation	[11]
Phase inversion temperature method.	Acyclovir-loaded multiple W/O/W nano emulsions.	Excellent physicochemical stability for 6 mo at RT, mean droplet size of 100 nm	[12]
Spontaneous nanoemulsification method.	Clotrimazole nano emulsion.	Improved solubility of clotrimazole, mean globule size <25 nm	[13]
Ultrasound emulsidfication method	Basil oil nano emulsion	Nanoemulsions with droplet size of 29.6 nm, for food preservation	[14]
Phase inversion composition method	Efavirenz nanoemulsion	Enhanced bioavailability, globule size <30 nm	[34]
High-pressure homogenizer	Dimethyl silicone dry nanoemulsion inhalation.	Enhanced brain availability of risperidone with a mean particle size of 160 nm Effective in acute lung injury, particle size of 19.8 nm	[15]
High-pressure homogenizer	Parenteral lecithin-based nanoemulsions(risperidone)	Enhanced brain availability of risperidone with a mean particle size of 160 nm	[28]
Microfluidization method	Pitavastatin-containing nanoemulsions	Enhanced permeation	[16]
homogenization+ultra sound	nanoemulsion	Reduced energy demand for emulsification, low particle dimensions and higher stability	[17]
Sonication method	Saponin-stabilized quercetin-loaded o/w nano emulsion	Stable for 45 d at RT, mean particle size of 52±10 nm	[18]

High-pressure homogenization	Paclitaxel-baicalein nanoemulsion	Strategy to overcome multidrug resistance	[19]
Nanoemulsion templating	PLGA nanoparticles	Imaging agents for biomedical purposes	[20]

ULTRASONICATION-

In this process, a premixed emulsion is agitated at a frequency of 20 kHz, decreasing the size of the droplets to nanodroplets. The resulting emulsion is then pushed through a high shear area, resulting in uniformly sized droplets. This technology uses a water jacket to keep the temperature in check. During ultrasonic emulsification, sonotrodes, also known as sonicator probes, used piezoelectric quartz crystals as energy sources. These sonotrodes shrink and expand when alternating electric voltage is applied. When the sonicator tip makes contact with the liquid, mechanical vibrations are produced, causing cavitation and the collapse of vapour cavities formed inside the liquid. When a droplet size of less than 0.2 is required, this approach is commonly used. Shi et al. used an ultrasonic emulsification process at a frequency of 25 kHz to create an emodin-loaded nanoemulsion with a mean diameter in the range of 10-30 nm[24].

SPONTANEOUS EMULSIFICATION:

This method required three phases of nanoemulsion preparation. The first stage involved creating an organic solution with oil and a lipophilic surfactant in a water miscible solvent and a hydrophilic surfactant, and then injecting this organic phase into the aqueous Sciences784phase under magnetic stirring to create the O/W emulsion. Evaporation was used to remove the organic solvent in the third stage. Sugumar et al. used spontaneous emulsification to create a stable eucalyptus oil nanoemulsion, with the mean droplet size ranging from 50 to 100 nm[25].

SOLVENT EVAPORATION TECHNIQUE/HYDROGEL METHOD:

The drug solution is created and emulsified into another liquid (non-solvent for the drug), after which the solvent is evaporated, resulting in drug precipitation. For controlling crystal development and particle aggregation, a high-speed stirrer can be used. The solvent evaporation approach is quite similar to the hydrogel method. The main difference between this approach and solvent evaporation is that the drug solution is miscible with the drug anti solvent in this situation. a mean value at a fixed angle, which is influenced by particle size. The derived photoelectron time-correlation function produces a histogram of the line width distribution, which can be connected to particle size. To measure particle size, a weighed amount of formulation is dispersed in double-distilled water to achieve a homogeneous dispersion, which must be utilised immediately for particle size and PDI measurements. The PDI can be anywhere between 0 and 1, with 0 (zero) indicating a monodisperse system and 1 indicating a polydisperse particle dispersion[27]. Using this method, orevi et al. determined the particle size and PDI of risperidone nanoemulsion, reporting a mean particle size of 160 nm and a mean size distribution of less than 0.15[28]. Singh et al. used the same method and found that the particle size of primaquine nanoemulsion was in the range of 20-200 nm[5].

CHARACTERIZATION OF NANOEMULSIONS:

DETERMINATION OF ENCAPSULATION EFFICIENCY:

To measure particle size, a weighed amount of formulation is dispersed in double-distilled water to achieve a homogeneous dispersion, which must be utilised immediately for particle size and PDI measurements. The PDI can be anywhere between 0 and 1, with 0 (zero) indicating a monodisperse system and 1 indicating a polydisperse particle dispersion[27]. Using this method, orevi et al. determined the particle size and PDI of risperidone nanoemulsion, reporting a mean particle size of 160 nm and a mean size distribution of less than 0.15[28]. Singh et al. used the same method and found that the particle size of primaquine nanoemulsion was in the range of 20-200 nm[5].

DETERMINATION OF PARTICLE SIZE AND POLYDISPERSITY INDEX (PDI):

The particle size and PDI of nanoemulsions are investigated using Malvern Zetasizer and photon correlation spectroscopy (PCS), which measures the variation in light scattering caused by Brownian motion of particles as a function of time. PCS is based on the idea that particles with a smaller size travel at a faster rate than particles with a larger size. The laser beam is diffracted in solution by sub-micron particles. Rapid fluctuations in laser scattering intensity occur around a mean value at a constant angle due to particle diffusion, and this is dependent on particle size. A histogram of the line width distribution is generated by the estimated photoelectron time-correlation function, which can be connected to particle size. To measure particle size, a weighed amount of formulation is dispersed in double-distilled water to achieve a homogeneous

dispersion, which must be utilised immediately for particle size and PDI measurements. The PDI can be anywhere between 0 and 1, with 0 (zero) indicating a monodisperse system and 1 indicating a polydisperse particle dispersion[27]. Using this method, orevi et al. determined the particle size and PDI of risperidone nanoemulsion, reporting a mean particle size of 160 nm and a mean size distribution of less than 0.15[28]. The similar procedure was used by Singh et al., who obtained particle sizes of primaquine nanoemulsion in the range of 20-200 nm[5].

MORPHOLOHGICAL STUDY OF NANOEMULSION:

Transmission electron microscopy is used to investigate the morphology of nanoemulsion (TEM). In TEM, an electron beam is incident on and passes through a thin foil specimen. When these incident electrons interact with the specimen, they become unscattered electrons, elastically scattered electrons, or inelastically scattered electrons. The magnification is controlled by the distance between the objective lens and the specimen, as well as the distance between the objective lens and its image plane. The unscattered or scattered electrons were concentrated by electromagnetic lenses and cast onto a screen, resulting in an amplitude-contrast image, a phase-contrast image, electron diffraction, or a phantom picture of discrete darkness, depending on the density of unscattered electrons. For revealing the size and shape of nanoemulsion droplets, bright field imaging at increasing magnification in combination with diffraction modes was used. A few drops of nanoemulsion or a suspension of lyophilized nanoparticles are produced in double-distilled water and deposited onto a holey film grid and immobilised for TEM. Following immobilisation, excess solution must be whipped off the grid and stained. After that, the dyed nanoparticles are inspected at a specific voltage[30]. Singh et al. used TEM to investigate the surface morphological properties of primaquine nanoemulsion and found that it has a spherical form with a smooth surface[5].

ATOMIC FORCE MICRROSCOPE (AFM):

AFM is a relatively recent technology that is being used to investigate the surface morphology of nanoemulsion formulations these days. AFM is performed by diluting nanoemulsions with water and then drop coating the diluted nanoemulsion onto a glass slide. The coated drops are then dried in an oven and scanned at a speed of 100 mV/s[31]. Drais et al. conducted an AFM investigation on carvedilol nanoemulsion and discovered that the size ranged from 42 to 83 nm with good formulation stability[32].

IN VITRO DRUG RELEASE STUDY:

In vitro drug release studies aid in predicting medication formulation efficacy in vivo. A USP dissolving device is used to determine a drug's in vitro release rate. The medication equivalent to 10 mg was disseminated in nanoemulsion or dried nanoparticles, which were then put into dialysis membrane pouches and placed in a flask containing buffer. This experiment is conducted at 370.5°F at a stirring speed of 50 rpm. The sample is taken out at regular intervals and replaced with the same volume of new dissolving medium each time. The absorbance of the sample is then measured spectrophotometrically at a certain wavelength after the samples have been diluted appropriately. Using a calibration curve, the absorbance of the collected sample is utilised to calculate percent drug release at various time intervals. Using a dissolution apparatus type-II, Kotta et al. investigated the in vitro drug release profile of an antiHIV medication nanoemulsion and found that 80 percent of the drug was released in 6 hours[33].

IN VITRO SKIN PERMEATION STUDIES:

In vitro and ex vivo permeation experiments are conducted using the Keshary Chien-diffusion cell. The abdomen skin of adult male rats weighing 250–10 g is commonly used in permeation studies. The rat skin is sandwiched between the diffusion cell's donor and receiver chambers. The temperature of receiver chambers holding fresh water and 20% ethanol is kept constant at 37°, and the contents of the chamber are continually agitated at 300 rpm. In the donor chamber, the formulas are maintained. A small amount (0.5 ml) of the solution from the receiver chamber was taken at certain time intervals (e.g., 2, 4, 6, 8 h) for gas chromatographic analysis and promptly replaced with an equivalent volume of fresh solution. Every sample is repeated three times. The total amount of drug permeated through rat skins at each time interval is calculated using cumulative adjustments and shown as a function of time. The slope of the figure is used to calculate drug permeation rates in a steady-state[34]. Harwansh et al. employed the Franz diffusion cell to test the transdermal permeability of glycyrrhizin through human cadaver skin and found that nanoemulsion formulations had higher permeability than traditional gel formulations[35].

STABILITY STUDIES:

Stability studies are carried out to determine the drug substance's stability under the effect of various environmental conditions such as temperature, humidity, and light. Nanoemulsion stability tests are carried out after storing the formulation for 24 months

in a dispersed and freeze-dried state, as per the standards of the International Conference on Harmonisation. The following storage conditions were used: ambient (252°/605% RH), refrigerated (53°), and freeze (–205°). The required amount of nanoemulsion is kept in glass vials that are hermetically sealed. The samples are taken at predetermined intervals and analysed for particle size, loading, and EE, as well as the in vitro drug release profile[25]. When the formulation was held for 3 months at 25°/60 percent RH and 30°/65 percent RH, Singh et al. found no change in viscosity, drug content, or particle size[5].

DETERMINATION OF ZETA POTENTIAL:

When particles are submerged in liquid, the zeta potential is used to determine their surface charge. The zeta potential is a physicochemical property of a medication, polymer, or vehicle that is used to predict dispersion stability. Its value is determined by the presence of electrolytes and their adsorption. The Malvern Zetasizer equipment is used to measure it. Nanoemulsion is diluted to determine zeta potential, which is calculated based on the electrophoretic mobility of oil droplets. A zeta potential of 30 mV is thought to be sufficient for ensuring nanoemulsion physical stability. Using the Malvern Zetasizer, orevi et al. reported a zeta potential of roughly –50 mV for risperidone nanoemulsion[28].

FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR) SPECTRAL ANALYSIS:

Drug excipient interaction, polymerization, crosslinking, and drug loading in the formulation can all be assessed using FTIR analysis. It's also utilised to determine the functional groups and their attachment methods, as well as the molecule's fingerprint. A molecule exists in its ground state at low temperatures, and as it absorbs radiant energy, it is stimulated to higher energy levels. The energy difference (E) between the excited and ground states of the molecule is determined via IR spectroscopy. Samples can be prepared for FTIR using appropriate methods such as potassium bromide pellets or Nujol mulls, and then scanned in FTIR at a modest scanning speed of 4000-400 cm-1. Srilatha et al. performed FTIR experiments on pure drug and glipizide nanoemulsion and found no drug excipient interactions (therefore drug and excipient compatibility) because all of the drug's distinctive peaks occurred at the same position in the formulation[29].

SHELF LIFE DETERMINATION:

Accelerated stability experiments are carried out to determine the shelf life of a nanoemulsion. The formulations are kept for approximately three months at three different temperatures and humidity levels (30°, 40°, and 500.5°). After a certain amount of time has passed (0, 30, 60, and 90 days), samples are extracted and analysed using HPLC at max to determine the remaining drug content. As controls, samples taken at zero time are taken. This determines the sequence of the reaction, and then the reaction rate constant (K) for deterioration is computed from the slope of the lines using the equation below at each raised temperature: The logarithm values of K are displayed against the reciprocal of absolute temperature at different high temperatures using slope = K/2.303 (Arrhenius plot The plot value of K at 25° is derived from this, and the value is then used to calculate shelf life using the following Eqn.: t0.9=0.1052/K25. Where t0.9 denotes the time necessary for a medicine to degrade by 10% and is referred to as shelf life [30]. Ali et al. found that clobetasol propionate-loaded nanoemulsions have a shelf life of roughly 2.18 years at room temperature (25°) and concluded that nanoemulsions can improve clobetasol propionate stability[36]. When stored in a refrigerator, a silymarin nanoemulsion has a shelf life of roughly 3.8 years, according to Parveen et al.[37].

THERMODYNAMIC STABILITY STUDIES:

Thermodynamic stability tests are typically performed in three steps. The first is a heating-cooling cycle, which is used to see if changing temperature conditions has any effect on nanoemulsion stability. Nanoemulsion is subjected to six temperature cycles ranging from 4° (freezer temperature) to 40° by storing the formulation at each temperature for at least 48 hours. Centrifugation investigations are then conducted on the formulations that are stable at these temperatures. Second, a centrifugation research is conducted in which the formed nanoemulsions are centrifuged at 5000 rpm for 30 minutes and phase separation, creaming, and cracking are observed. Those that showed no signs of instability were put through a freeze-thaw cycle. Finally, the freeze-thaw cycle, which involves exposing nanoemulsion formulations to three freeze-thaw cycles at temperatures ranging from –21° to +25°. Formulations that pass this test and exhibit no symptoms of instability are considered to have good stability[6]. These formulations are then put through dispersibility tests to see how effective they are at self-emulsification. Srilatha et al. conducted thermodynamic investigations on glipizide nanoemulsion by subjecting it to three stability cycles and found that the nanoemulsion had good physical stability with no signs of phase separation, creaming, or cracking[29].

DISPERSIBILITY OF VISCOSITY:

Using a conventional USP XXII dissolving apparatus, dispersibility studies for evaluating the efficacy of self-emulsification of nanoemulsions are carried out. In 500 mL of distilled water kept at 370.5°, 2.1 mL of each formulation is combined. For gentle agitation, a typical stainless steel dissolving paddle moves at 50 rpm. The performance of the nanoemulsion formulations in vitro is assessed visually using the grading method provided below[6]. Within one minute, Grade A nanoemulsions form and appear transparent or bluish. Grade B nanoemulsions form quickly but are slightly less clear, with a bluish-white appearance. Nanoemulsions of Grade C are fine milky emulsions that form in less than 2 minutes. Grade D emulsions are dull, greyish-white emulsions with a slight greasy look and a longer formation time (>2 minutes). With big oil globules on the surface of Grade E nanoemulsions, emulsification is either weak or non-existent.

DETERMINATION OF VISCOSITY:

The determination of viscosity is an important metric in the physicochemical characterisation of nanoemulsions. Viscosity is measured using a variety of devices, including the Ostwald viscometer, Hoeppler falling ball viscometer, Stormer viscometer, Brookfield viscometer, and Ferranti-Shirley viscometer. Brookfield is the preferred viscometer for measuring nanoemulsion viscosity among all of these viscometers. The viscosity of the system determines whether it is an O/W or a W/O emulsion. Low viscosity indicates an O/W type system, whereas high viscosity indicates a water in oil type system[27]. Survismeter, on the other hand, is currently the most extensively used instrument for measuring surface tension, viscosity, interfacial tension, contact angle, dipole moment, particle size, and hydrodynamic volumes of nanoemulsions[38]. Shafiq et al. used a Brookfield cone and plate rheometer to test the viscosity of ramipril nanoemulsion formulations and found that the viscosity was less than 21 cP, with a minimum viscosity of 10.68 cP[6].

REFRACTIVE INDEX:

The refractive index describes how light travels through a material and how transparent a nanoemulsion is. The refractive index (n) of a media is defined as the ratio of the wave speed (c) in the reference medium to the wave phase speed (vp) in the medium: n=c/vp. An Abbes type refract to meter set to 250.5° can be used to determine the nanoemulsion's refractive index by placing a drop of nanoemulsion on a slide and comparing it to the refractive index of water (1.333). When the refractive index of a nanoemulsion equals that of water, the nanoemulsion is said to be transparent[2,27]. Harika et al. used an Abbe refractometer to measure the refractive index of amphotericin B nanoemulsion, and the refractive index of the formulation was found to be similar to that of water.[39]

PRERCENT TRANSMITTANCE:

Using a UV spectrophotometer set to a specific wavelength and distilled water as a blank, the percent transmittance of a created nanoemulsion is calculated. A nanoemulsion is deemed transparent in nature if its percent transmittance is greater than 99 percent[30]. Harika et al. found that an amphotericin B nanoemulsion prepared by Harika et al. had a percent transmittance of >97%.

PH AND OSMOLARITY MEASUREMENT:

A pH metre is used to determine the pH of a nanoemulsion, while a microosmometer is used to determine the osmolarity of the emulsion using the freezing point method. This is done by transferring 100 l of nanoemulsion into a microtube and taking measurements[40]. Morsi et al. used a pH metre to assess the pH of the acetazolamide nanoemulsion and found it to be in the range of 4.9 to 5.5, claiming it to be adequate and non-irritant for eye application[41].

DYE SOLUBILISATION:

In an O/W globule, a water soluble dye is dispersible, whereas it is soluble in the aqueous phase of a W/O globule. In the same way, an oil soluble dye is dispersible in the W/O globule but soluble in the O/W globule's oily phase[3]. If you add water soluble dye to an O/W nanoemulsion, the colour will spread uniformly, however if you use a W/O emulsion, the dye will stay in the dispersion phase and the colour will not spread equally. This can be seen by looking at the emulsion under a microscope[4]. Laxmi et al. performed this test on artemether nanoemulsion by mixing in eosin yellow, a water soluble dye, and examining the results under a microscope. They noticed that the aqueous continuous phase was dyed while the oily dispersed phase was left unlabeled, indicating that the produced nanoemulsion was of the O/W type[42].

DILUTABILITY TEST:

The purpose of the dilution test is to see if a continuous phase can be added in increasing proportions to a nanoemulsion without affecting its stability. As a result, O/W nanoemulsions can be diluted with water, whereas W/O nanoemulsions cannot and must undergo phase inversion to become O/W nanoemulsions. Only oil can be used to dilute the W/O nanoemulsion[3,4]. Laxmi et al. conducted a dilutability test on nanoemulsion by diluting it with water and found no evidence of phase inversion or

precipitation, stating that their nanoemulsion formulation is stable[42].

CONDUCTANCE MEASUREMENT:

Because they have water in the external phase, O/W nanoemulsions are strongly conducting, but W/O nanoemulsions are not conducting because they have water in the interior or dispersal phase. Electrical conductivity measurements are quite helpful in understanding the nature of the continuous phase and detecting phase inversion occurrences. Increases in conductivity of some W/O nanoemulsion systems were seen at low volume fractions, and this behaviour was interpreted as an indicator of percolative behaviour or ions exchange among droplets prior to the creation of bicontinuous structures. Dielectric measurements can be used to investigate the structural and dynamic properties of nanoemulsion systems[3]. To determine the conductivity of nanoemulsion, a conductometer is used. A pair of electrodes is coupled to a lamp and an electric source is immersed in an emulsion to perform conductance measurements. When the emulsion is of the O/W type, water conducts the current, and the lamp glows as a result of the current flowing between the connecting electrodes. If the lamp is made of water in an oil emulsion, the lamp will not light because oil in the exterior phase does not carry current[4]. Harika et al. used an electroconductometer to examine the conductivity of amphotericin B nanoemulsion. On the basis of an electroconductivity research, they determined that the system is O/W and reported conductivity of the formulations in the range of 454.2-552.3 S/cm.

INTERFACIAL TENSION:

The production and properties of nanoemulsion can be studied by measuring the interfacial tension. Phase behaviour, primarily the coexistence of surfactant phase or middle-phase nanoemulsions with aqueous and oil phases in equilibrium, leads to very low values of interfacial tension. The spinning-drop apparatus is used to determine ultra-low interfacial tension. Interfacial tensions are measured by rotating a drop of the low-density phase in a cylindrical capillary filled with the high-density phase and measuring the form of the drop[3].

FLUORESCENCE TEST:

Many oils glow when exposed to ultraviolet light. When a W/O nanoemulsion is exposed to fluorescence light under a microscope, the entire field fluoresces, whereas an O/W nanoemulsion fluoresces in spots[4].

IN VIVO STUDIES:

In vivo investigations can be carried out by using the appropriate animal model for the specified activity. Srilatha et al. tested the anti-diabetic activity of glipizide nanoemulsion using a hyperglycemia model in which they first induced diabetes in rats using an intraperitoneal injection of streptozotocin solution, then gave the formulation to diabetic rats and performed pharmacodynamic studies on them. Blood glucose levels were reduced for up to 12 hours, according to the researchers[29]. Chouksey et al. investigated the in vivo performance of atorvastatin nanoemulsion by conducting pharmacokinetic tests on nanoemulsion and found that the nanoemulsion formulation had a higher bioavailability than the pure drug[43].

Nanoemulsions have a lot of potential as a medication delivery technique, but they need to be properly harnessed to reach their full potential. With such a precise delivery system, quality assurance and quality control are critical, so the evaluation tests must be carried out thoroughly.

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