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Recent Advances In Nanoemulsion Based Drug Delivery System: A Review

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A B S T R A C T

Nanoemulsions are made up of two immiscible liquids combined with emulsifying agents (surfactants and co-surfactants) to generate thermodynamically stable single phase and colloidal dispersion systems. The persuasion method and the Brute force method are both used to create nanoemulsions. Entrapment efficiency, particle size, polydispersity index, zeta potential, and other characterization techniques for nanoemulsions include differential scanning calorimetry, Fourier-transform infrared spectroscopy, and transmission electron microscopy. In vitro drug release, in vitro permeation, stability and thermodynamic stability, shelf life, dispersibility, viscosity, surface tension, refractive index, % transmittance, pH, and osmolarity are all studied further. This review seeks to give collected information on various nanoemulsion formulation and characterization techniques.

Keywords: *Nanoemulsions, nanoemulsion characterization, entrapment efficiency, droplet size*

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INTRODUCTION

Nanoemulsions/Sub-micron emulsions (SMEs)/Mini-emulsions/Ultrafine emulsions are thermodynamically stable transparent (translucent) oil-water dispersions stabilised by an interfacial coating of surfactant and cosurfactant molecules with droplet sizes smaller than 100 nm. Nanoemulsion, also known as multiphase colloidal dispersion, is distinguished by its stability and clarity. The dispersed phase is composed of minute particles or droplets ranging in size from 5 nm to 200 nm and has a very low oil/water interfacial tension. Nanoemulsions are transparent because the droplet size is less than 25% of the wavelength of visible light. Nanoemulsions develop easily and sometimes spontaneously, with little to no high-energy input. In addition to the surfactant, the oil phase, and the water phase, a cosurfactant or cosolvent is frequently utilised. [1,2] Nanoemulsions are now often utilised for a variety of purposes such as vaccine administration, DNA encoded medication delivery, antibiotics, cosmetic and topical treatments, and can be administered by a variety of channels such as oral, pulmonary, ophthalmic, and transdermal. [3,4] It has been established that using nanoemulsion as a delivery mechanism can extend medication retention time in the body, requiring less drug for therapeutic efficacy. Previous research has demonstrated the use of nanoemulsion technology to improve the bioavailability of lipophilic drugs. [5]

Types of Nanoemulsions

Depending on the composition, there are three types of nanoemulsions.

1. Oil in water nanoemulsions where in oil droplets are dispersed in the continuous aqueous phase.
2. Water in oil nanoemulsions where in water droplets are dispersed in the continuous oil phase.
3. Bicontinuous nanoemulsions where in micro domains of oil and water are interdispersed within the system [6].

Components of Nanoemulsion: Main three components of Nanoemulsions are as follows:

1. Oil
2. Surfactant/Cosurfactant
3. Aqueous phase [7]

TABLE.1; List of oils used in nanoemulsions

List of oils used in nanoemulsions name	Chemical name	Manufacture
Captex 355	Glyceryl tricaorylate/caprate	Abitec
Captex 200	Propylene dicaprylate/dicaprate glycol	Abitec
Captex 8000	Glyceryl tricaprylate(tricaprylin)	Abitec

Witepsol	90:10% w/w c12 glyceride tri:diesters	Sasol pharmaceutical excipient
Myritol 318	C8/c10 triglycerides	Russia

TABLE.2; Cosurfactant

S.no.	Solubilizing, surfactants, emulsifying agents adsorption enhancers
1	Capryol 90
2	Gelucire 44/14, 50/13
3	Cremophor RH 40
4	Imwitor 191, 308(1), 380, 742, 780K, 928, 988
5	Labrafil M 1944 CS, M 2125 CS
6	Lauroglycol 90
7	PEG MW>4000
8	Plurol oleique CC 497
9	Poloxamer 124 and 188
10	Softigen 701, 767
11	Tagat TO
12	Tween 8

Advantages of Nanoemulsions over other dosage forms

1. Eliminates variability in absorption
2. Increases the rate of absorption.
3. Helps in solublizing lipophilic drug.
4. Provides aqueous dosage form for water insoluble drugs.
5. Increases bioavailability.
6. Various routes like topical, oral and intravenous can be used to deliver the product.
7. Rapid and efficient penetration of the drug molecule.
8. Helps in taste masking.
9. Provides protection from hydrolysis and oxidation as drug in oil phase in o/w emulsion
10. Less amount of energy required.
11. Liquid dosage form increases patient compliance
12. Nanoemulsions are thermodynamically stable systems and the stability allows self-emulsification of the system whose properties are not dependent on the process followed.
13. Nanoemulsions carry both lipophilic and hydrophilic compounds.
14. Use of Nanoemulsion as delivery systems improves the efficacy of a drug, al-lowing the total dose to be reduced and thus minimizing side effects.[8]

Disadvantages of Nanoemulsion

1. Use of a large concentration of surfactant and cosurfactants necessary for stabilizing the Nanodroplets.
2. Limited solubilizing capacity for high melting substances.
3. The surfactant must be nontoxic for pharmaceutical applications.
4. Nanoemulsion stability is influenced by environmental parameters such as temperature and pH. These parameters change upon Nanoemulsion delivery to patients.[9]

Formation of Nanoemulsion (Theory):

In Nanoemulsion which is categorized as multiphase colloidal dispersion which is generally characterized by its stability and clarity. There is an application of high shear generally obtained by micro fluid or ultrasonic approach generally used to reduce the droplet size to nanoscale. There is a marginal difference between the terms Nanoemulsion and microemulsion also known as micellar phase or mesophase. The microemulsion generally forms through thermodynamic self assembly whereas nanoemulsion requires external shear for rupturing the droplets. In retrospect, the historical choice of the word "microemulsion" to describe the nanoscale is unfortunate since they are structurally between 1 to 100 nm as for Nanoemulsion. Micro emulsions are not the emulsions of micro scale droplets. They are formed by self assembled equation phase in which the surface tension dose not play a significant role. The Nanoemulsions underlines the basic principle in its formulation. They generally comprises of two immiscible phase with an interfacial tension between them reduced by addition of surfactant.[10]

Factors affecting the Formulation of Nanoemulsion:

1. Appropriate composition is required to avoid Oswald ripening the dispersed phase should be highly insoluble in the dispersed medium.
2. The surfactant is an essential part of the Nanoemulsion. They should not form lyotropic liquid crystalline "microemulsion" phases. Systems containing short chain alkanes, alcohols, water, and surfactants form the phases which are generally used with the co surfactant.

3. The presence of excess surfactants enables new surface area of nanoscale to be rapidly coated during emulsification there by inhibiting induced coalescence.
4. Extreme share must be applied to rupture microscale droplets to nanoscale by providing the stress level to reach above the Laplace pressure of the droplets with a pressure of 10- 100 atm. Out of various methods ultrasonication is widely used in laboratory. [11]

Methods of Preparation of Nanoemulsion

Numerous methods have been optional to practice nanoemulsion. Development of nanoemulsion system needs an elevated amount of energy. This energy can be obtained either by mechanical equipment or by the chemical potential inherent within the component. Some methods used for the preparation of nanoemulsion are:

1. Sonication Method:

In this technique, the droplet size of usual emulsion is compact with the help of sonication mechanism. Only fewer amounts of batches of nanoemulsion can be produced by this method [12].

2. High Pressure Homogenizer:

This method is based on by applying a large pressure over the system consisting an oil phase, aqueous phase and surfactant or co-surfactant. The high pressure is applied with the help of homogenizer. Some problems allied with homogenizer are poor productivity, component deterioration due to production of much heat. From this method, only oil in water (o/w) liquid nanoemulsion of less than 20% oil phase can be formed and cream nanoemulsion of high viscosity or hardness having a mean droplet diameter less than 200 nm cannot be prepared [13].

3. Phase Inversion Method:

Fine dispersion can be obtained by chemical energy resulting in phase transitions through emulsification method. The sufficient phase transitions are produced by changing the composition at constant temperature or by changing the temperature at constant composition. The phase inversion temperature (PIT) method was introduced based on the principle of changes of solubility of polyoxyethylene type surfactant with temperature. This surfactant becomes lipid soluble with increase in temperature because of dehydration of polymer chain. At low temperatures the surfactant monolayer has a great positive spontaneous curvature forming oil swollen micellar solution phase [14].

4. Production with high amplitude ultrasound

This method is an alternative for high pressure homogenization. High shear forces are necessary for the nanoemulsification are produced by ultrasonic cavitations which produces violently and asymmetrically imploding vacuum bubbles and reduce the particle size to the nanometer scale. This method is successfully used in small scale production of nanoemulsions [15].

5. Solvent Displacement Method

In this method, oily phase is dissolved in water miscible organic solvents such as acetone, ethanol. The organic phase is mixed into an aqueous phase containing surfactant to produce nanoemulsion by rapid diffusion of organic solvent. Organic solvent is removed from nanoemulsion by vacuum evaporation [16].

6. Microfluidizatiion

Microfluidization technology makes use of a device called 'MICRO FLUIDIZER". This device consist of high pressure positive displacement pump (500-200 PSI) which forces the product through the interaction chamber, consisting of small channels called micro channels. The product moves through the micro channels on to an impingement area which results in very fine particles of submicron range. The two solutions (aqueous phase and oily phase) are mix together and processed in an inline homogenizer to get a coarse emulsion. The coarse emulsion is into a micro fluidizer where it is further processed to get a stable nanoemulsion [17].

Characterization of Nanoemulsion

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. [18], 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 [19].

Determination of particle size and polydispersed index (PDI):

The particle size and PDI of nanoemulsions are analyzed employing photon correlation spectroscopy (PCS) using Malvern Zetasizer, which monitors the variation in light scattering because of Brownian motion of particles as function

of time. PCS is based on the principle that the particles with small size travels with higher velocity as compared to particles with large size. The laser beam gets diffracted by sub-micron particles present in solution. Due to diffusion of particles, rapid fluctuations in laser scattering intensity occur around a mean value at a fixed angle and this is dependent upon particle size. The calculated photoelectron time correlation function generates a histogram of the line width distribution that can be related to the size of particle. For measuring particle size, weighed amount of formulation is dispersed in double distilled water for obtaining homogenous dispersion and that has to be used instantly for measuring the particle size and PDI. The PDI can range from 0 to 1, where 0 (zero) stands for monodisperse system and 1 for a polydispersed particle dispersion [20]. Đorđević et al. evaluated the particle size and PDI of risperidone nanoemulsion by using this method and reported mean particle size around 160 nm with mean size distribution less than 0.15[21]. Singh et al. has also adopted the same technique and reported particle size of primaquine nanoemulsion in the range of 20-200 nm [19].

Determination of zeta potential:

The zeta potential is a method for measuring surface charge of particles when it is placed in liquid. Zeta potential is used for predicting dispersion stability and its value depends on physicochemical property of drug, polymer, vehicle, presence of electrolytes and their adsorption. It is measured by Malvern 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 Zetasizer for risperidone nanoemulsion [21].

Fourier-transform infrared spectroscopy (FTIR) spectral analysis:

FTIR analysis can be carried out for the assessment of drug excipient interaction, polymerization, crosslinking as well as drug loading in the formulation. It is also used for identifying the functional groups with their means of attachment and the fingerprint of the molecule. At low temperature a molecule exists in ground state and on absorbing the radiant energy, they get excited to higher energy states. IR spectroscopy is based on determining this energy difference (ΔE) between the excited and ground states of the molecule. For performing FTIR, sample can be prepared by employing suitable method such as potassium bromide pellet method, Nujol mulls and then sample is scanned in FTIR at moderate scanning speed between 4000- 400 cm-1. Srilatha et al. conducted FTIR studies on pure drug and glipizide nanoemulsion and reported absence of drug excipient interactions (hence compatibility of drug and excipients) as all the characteristics peaks of drug appeared at same point in formulation [22].

Morphological study of nanoemulsion:

The morphological study of nanoemulsion is carried by using transmission electron microscopy (TEM). In TEM, a beam of electron is incident on a thin foil specimen and passed through it. On interacting with the specimen, these incident electrons transform into unscattered electrons, elastically scattered electrons or inelastically scattered electrons. The distance among the objective lens and the specimen and among the objective lens and its image plane regulates the magnification. The electromagnetic lenses concerted the unscattered or scattered electrons and cast them onto a screen that produce amplitude-contrast picture, a phase-contrast image, electron diffraction, or a phantom picture of distinct darkness, which is dependent upon the density of unscattered electrons. Bright field imaging at increasing magnification in combination with diffraction modes used for disclosing the size and form of nanoemulsion droplets. For performing TEM, few drops of nanoemulsion or a suspension of lyophilized nanoparticles is prepared in double distilled 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 [23]. Singh et al. studied surface morphology characteristics of primaquine nanoemulsion by TEM analysis and reported spherical shape of primaquine nanoemulsion with smooth surface [19].

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[24]. Drais et al. performed AFM study on carvedilol nanoemulsion and found that the size varied from 42 to 83 nm with good stability of the formulation [25].

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 [23]. Kotta et al. studied the in vitro drug release profile of antiHIV drug nanoemulsion using dissolution apparatus type-II and reported 80 % drug release in 6 h[26].

In vitro skin permeation studies:

Keshary Chien-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 [27]. 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 [28].

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 [26]. 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 [19].

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 [23]. 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 [30]. Parveen et al. reported that the shelf life of a silymarin nanoemulsion to be around 3.8 y when stored in a refrigerator [31].

Thermodynamic stability studies:

Thermodynamic stability studies are usually carried out in three steps. Firstly heating and 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 studies 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 [32]. These formulations are then subjected to dispersibility studies for evaluating the efficiency of self-emulsification. Srilatha et al. performed thermodynamic studies on glipizide nanoemulsion by subjecting it to three cycles of stability and reported good physical stability of nanoemulsion with no appearance of phase separation, creaming or cracking[22].

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 [32]. Grade A nanoemulsions form rapidly within 1 min and appear to be clear or bluish. Grade B nanoemulsions form rapidly but are slightly less clear emulsions appear to be bluish white. Grade C nanoemulsions are fine milky emulsion that form within 2 min. Grade D are those dull, grayish white 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 [20]. 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[33]. Shafiq et al. has determined viscosity of ramipril nanoemulsion 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[32].

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 [20,34]. 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 [35].

Percent transmittance:

Percent transmittance of a formulated nanoemulsion is estimated using UV spectrophotometer at a particular wavelength with distilled water as a blank. If percent transmittance of a nanoemulsion is found to be greater than 99 %, then it is considered as transparent in nature [23]. Harika et al. reported percent transmittance of >97 % for a amphotericin B nanoemulsion formulated [27].

pH and osmolarity measurements:

The pH meter is used for measuring the pH of a nanoemulsion and micro osmometer 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 micro tube and measurements are taken [36]. 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 [37].

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 [38]. 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 [39]. Laxmi et al. carried out this test on artemether nanoemulsion by adding eosin yellow, a water soluble dye to the formulation and examined it under a microscope. They discovered that the aqueous continuous phase was labelled with dye while the oily dispersed phase remained unlabelled therefore confirming the formed nanoemulsion as O/W type[40].

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 [38,39]. 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 [40].

Conductance measurement:

The O/W nanoemulsions are highly conducting because they have water in external phase whereas W/O nanoemulsions are not conducting as they have water in internal or dispersal phase. Electrical conductivity measurements are very much beneficial for determining the nature of the continuous phase and for detecting phase inversion phenomena. At low volume fractions, increase in conductivity of certain W/O nanoemulsion systems was observed and such kind of behaviour is deduced as an indicator of a percolative behaviour or ions exchange among droplets prior to the development of bicontinuous structures. Dielectric measurements are a great means of exploring the structural and dynamic features of nanoemulsion systems [38]. Conductometer is employed for determining the conductance of nanoemulsion. For carrying out conductance measurement, a pair of electrodes is attached to a lamp and an electric source is immersed into an emulsion. When the emulsion is O/W type then water will conduct the current and lamp will glow because of passage of current among connecting electrodes. The lamp will not glow if it is water in oil emulsion as oil in external phase does not conduct the current [39]. Harika et al. performed conductivity test on amphotericin B

nanoemulsion using an electroconductometer. They reported conductivity of the formulations in the range of 454.2-552.3 µS/cm and concluded the system to be O/W on the basis of electroconductivity study [35].

Interfacial tension:

By measuring the interfacial tension, the formation and the properties of nanoemulsion can be investigated. Ultra low values of interfacial tension corresponds to phase behaviour, mainly the coexistence of surfactant phase or middle-phase nanoemulsions with aqueous and oil phases in equilibrium. For determining ultralow interfacial tension spinning-drop apparatus is used. Interfacial tensions are obtained by measuring the shape of a drop of the low-density phase, rotating it in cylindrical capillary filled with high-density phase [38].

Fluorescence test:

There are numerous oils that show fluorescence under UV light. If a W/O nanoemulsion is subjected to a fluorescence light under a microscope, the whole field will fluoresces and if it is an O/W the fluorescence will be in spots[39].

In vivo studies:

In vivo studies can be performed by adopting suitable animal model according to the activity chosen. Srilatha et al. has performed antidiabetic activity on glipizide nanoemulsion by choosing hyperglycaemia model in which they first induce diabetes in rats by intraperitoneal injection of streptozotocin solution and then the formulation was given to diabetic rats and the pharmacodynamic studies were performed on them. They reported the reduction in blood glucose levels for up to 12 h [22]. Chouksey et al. has evaluated in vivo performance of atorvastatin nanoemulsion by performing pharmacokinetic studies on nanoemulsion and they reported better bioavailability of nanoemulsion formulation as compared to pure drug [41]. Nanoemulsions hold great potential as an efficient drug delivery tool that could be effectively harnessed to realise the complete potential. Quality assurance and quality control shall be of paramount importance with such a precise delivery system and hence the evaluation tests are to be performed rigorously.

Applications of Nanoemulsions

1. Parenteral Delivery

Nanoemulsion has advantages in intravenous administration, due to the strict requirement of this route of administration, particularly the necessity for the formulation droplet size lower than 1 micrometer. Parenteral (or Injectable) administration of nanoemulsion is employed for a variety of purposes, namely nutrition e.g. Fats, Carbohydrates, Vitamins etc. Nanoemulsion formulations have distinct advantages over macroemulsion systems when delivered parenterally because of the fine particle Nanoemulsion is cleared more slowly than the coarse particle emulsion and, therefore, have a longer residence time in the body. Both O/W and W/O Nanoemulsion can be used for parenteral delivery.

2. Oral Delivery

Nanoemulsion formulations offer many advantages over conventional oral formulation for oral administration including increased absorption, improved clinical potency and decreased drug toxicity. Thus, Nanoemulsion proves to be ideal in delivering of drugs such as steroids, hormones, diuretic and antibiotics. Pharmaceutical drugs of peptides and proteins are highly potent and specific in their physiological functions [42]. Primaquine when incorporated into oral lipid nanoemulsion showed effective antimalarial activity against Plasmodium bergheii infection in mice at a 25% lower dose level as com-pared to conventional oral dose. Lipid nanoemulsion of primaquine improved oral bioavailability by the liver with drug concentration higher by at least by 45% as compared with the pure drug.

3. Topical Delivery

Topical administration of drugs can have ad-vantages over other methods for several reasons, one of which is the avoidance of hepatic first pass metabolism of the drug and related toxicity effects. Another is the direct delivery and target ability of the drug to affected area of the skin or eyes. The nanoemulsion can achieve a high level of topical antimicrobial activity that has only been previously achieved by systemic antibiotics. The nanoemulsion has broad spectrum activity against bacteria (e.g. *E.coli, S. aureus*) fungi (e.g. Candida, Dermatophytes).[43]

4. Ocular Delivery

For the treatment of eye diseases, drugs are essentially delivered topically Nanoemulsions have been investigated for ocular administration, to dissolve poorly soluble drugs, to in-crease absorption and to attain prolong re-lease profile [44].

5. In Cosmetic

The aesthetic properties, i.e. low viscosity and transparent visual aspects of nanoemulsion-with droplet sizes below 200nm, its high surface area allowing effective transport of the active ingredient to the skin make them especially attractive for their application in cosmetics. Nanoemulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation or coalescence that is observed with macro emulsion. The incorporation of potentially irritating surfactants can be avoided by using high energy equipment during manufacturing. Nanogel technology to create mini emulsion from oil-in water concentrate suited to minimizing trans epidermal water loss,

enhanced skin protection and penetration of active ingredient. It would be useful for sun care products, moisturing and antiageing creams. It helps to give skin care formulations a good skin feels [45]

6. Transdermal

Indomethacin a potent NSAID, the anti-inflammatory effects of true optimized nanoemulsion formulation were compared with mar-keted gel in carragenan induced paw edema in rats. The %inhibition value was significant for developed Nanoemulsion, so huge potential for transdermal application of indomethacin. Nanoemulsions for transdermal delivery of celecoxib, formulation which consisted of 2% celecoxib 10% oil phase (Sefsol 218 and Triacetin) 50% surfactant mixture (Tween 80 and Transcutol-P) and 40% water. The anti-inflammatory effect and percent inhibition value after 24h administration was found to be high for nanoemulsion formulation (81.2%) as compared to celecoxib gel (43.7%) and nanoemulsion gel (64.5%). The *in vitro- in vivo* studies revealed a significant increase in the anti-inflammatory effects of aceclofenac nanoemulsion (82.2%) as compared to nanoemulsion gel formulation (71.4%) and conventional gel (41.8%).

7. Nanoemulsions in Cancer Therapy

Nanoemulsions can be used as vehicle in cancer chemotherapy for prolonging the rate of drug release after intramuscular and intratu-moral injection (W/O systems). It also enhances the transdermal drug delivery due to increase in the transport of anti-cancer drugs via lymphatic permeation through the skin and it is also non-irritant system [44,45].

8. Nanoemulsions in intranasal drug deli-very

Intranasal drug delivery system has now been recognized as a reliable route for the administration of drugs next to parenteral and oral routes. Nasal mucosa has emerged as a therapeutically viable channel for the administration of systemic drugs and also appears to be a favourable way to overcome the obstacles for the direct entry of drugs to the target site. This route is also painless, non-invasive and well tolerated. The nasal cavity is one of the most efficient sites because of its reduced enzymatic activity, high availability of immune active sites and its moderately permeable epithelium. There are several problems associated with-targeting drugs to brain, especially the hydrophilic ones and those of high molecular weight. This is because of the impervious nature of the endothelium, which divides the systemic circulation and barrier between the blood and brain. The olfactory region of the nasal-mucosa provides a direct connection between the nose and brain and by the use of nanoemulsions loaded with drugs, conditions such as Alzheimer‟s disease, migraine, depression, schizophrenia, Parkinson‟s diseases, meningitis, etc. can be treated. Preparation of nanoemulsions containing risperidone for its delivery to the brain via nose has been reported. It is inferred that this emulsion is more effective through the nasal rather than intravenous route. Another application of intranasal drug delivery system in therapeutics is their use in development of vaccines. Immunity is achieved by the administration of mucosal antigen. Currently, the first intranasal vaccine has been marketed. Among the possible delivery-systems, the use of nano based carriers hold a great promise to protect the biomolecules, promote nanocarrier interaction with mucosa and to direct antigen to the lymphoid tissues. Therefore the use of nanoemulsions in intranasal drug delivery system is set to bring about significant results in targeting of the drugs to the brain in treatment of diseases re-lated to the central nervous system. Bhanushali *et al* developed intranasal nanoemulsion and gel formulations for rizatriptan benzoate for prolonged action. Various mucoadhesive agents were tried out to form thermo-triggered mucoadhesives nanoemulsions.

9. Nanoemulsions in pulmonary drug delivery

The lung is the most important target for drug delivery due to noninvasive administration via inhalation aerosols, avoidance of first-pass metabolism, direct delivery to the site of action for the treatment of respiratory diseases, and the availability of a huge surface area for local drug action and systemic absorption of drug. Colloidal carriers (ie, nanocarrier system) in pulmonary drug delivery offer many advantages such as the potential to achieve relatively uniform distribution of drug dose among the alveoli, achievement of improved solubility of the drug from its own aqueous solubility, a sustained drug release which consequently reduces dosing frequency, improves patient compliance, decreases incidence of side effects, and the potential of drug internalization by cells.

10. Nanoemulsions in gene delivery vector

Emulsion systems have been emerged as alternative gene transfer vectors to liposomes. Other emulsion studies for gene deli-very (non-pulmonary route) have shown that binding of the emulsion/DNA complex is stronger than liposomal carriers. This stable emulsion System delivered genes more efficiently than liposomes. Silva *et al* evaluated factors that influence DNA compaction in cationic lipid nanoemulsions [cationic nanoemulsions containing stearyl amine (a cationic lipid that presents a primary amine group when in solution, is able to compact genetic material by electrostatic interactions, and in dispersed systems such as Nanoemulsions this lipid anchors on the oil/water interface conferring a positive charge to them. The influence of the stearyl amine incorporation phase (water or oil), time of complexation, and different incubation temperatures were studied. Characterization was done by dynamic light scattering (DLS). The results demonstrate that thebest DNA compaction process occurs after 120 min of complexation, at low temperature (4 ±1 °C), and after incorporation of the cationic lipid into the aqueous phase. Although the zeta potential of lipoplexes was lower than the results found for basic nanoemulsions, the granulometry did not change. Moreover, it was demonstrated that lipoplexes are suitable vehicles for gene deli-very [46].

TABLE.3; Marketed product of nanoemulsion [47]

Drug/bioactive	Brand name	manufacturer	indication
Alprostadil palmitate	Liple	Mitsubishi pharmaceutical	Vasodilator, platelet inhibitor
Dexamethason	Limethason	Mitsubishi pharmaceutical	Steroid
Propofol	Diprivan, troypofol	Astra zaneca, troika	Anaesthetic
Flurbiprofen axtil	Ropion	Kaken pharmaceutical	NSAID
Vitamins A,D,Eand K	Vitalipid	Fresenius kabi	Parenteral nutrition
Etomidate	Etomidat-lipuro	B.Braun melsungen	Anaesthetic
cyclosporine	Restasis, gengraf	Allergen,abott	Immnuosupressant
ritonavir	norvir	abbott	antiretoviral

CONCLUSION

Nanoemulsion formulations have various advantages for medication, biological, or diagnostic agent delivery, including the potential to protect labile pharmaceuticals, control drug release, increase drug solubility, boost bioavailability, and decrease patient variability. The purpose of this review is to concentrate on nanoemulsion as a medication delivery mechanism. Lipophilic molecules are the primary components that dissolve the medicine; this entire mixture is gaining popularity as colloidal carriers (size less than 100 nm). The various nanonization technologies offer versatile choices for developing tailor-made nano therapies for various medications and administration routes. These strategies can also be utilised to improve the clinical efficacy of hazardous medications or to speed up the clinical translation of therapeutic candidates that have been judged ineffective due to a lack of solubility.

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