

Nanoemulsions

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ABSTRACT: The key difference between emulsions and Nanoemulsions are that the former, whilst they may exhibit excellent kinetic stability, are thermodynamically unstable and will eventually show phase separation. Components of nanoemulsion systems include oils, lip-ids, surfactants, water-soluble co-solvents, and water. Be incorporated are determined with the help of pseudo-ternary phase diagrams. The higher the homogenization cycles the smaller is the particle size obtained. The coarse emulsion is inserted into a micro fluidizer where it is further processed to obtain a stable nanoemulsion. O/w Nanoemulsions are diluted with water whereas w/o are not and thus they undergo phase inversion into o/w Nanoemulsion. Dielectric measurements are a powerful means of probing both structural and dynamic features of Nanoemulsion systems. The apparent pH of the formulation was measured by pH meter. Nanoemulsion formulations offer many advantages over conventional oral formulation for oral administration including increased absorption, improved clinical potency and decreased drug toxicity. The formation and the properties of nanoemulsion can be studied by measuring the interfacial tension. The viscosity of nanoemulsions is a function of the surfactant, water and oil components and their concentrations. Ultra-low negative interfacial tension is required for nanoemulsion formation. The incorporation of potentially irritating surfactants can be avoided by using high energy equipment during manufacturing. The direct delivery and target ability of the drug to affected area of the skin or eyes. Methods for nanoemulsion drug delivery system formulation can be conveniently classified in a rigid fashion based on the energy required, nature of phase inversion and self-emulsification.

Key-words: Component of nanoemulsion, High pressure homogenizer, Micro fluidization, Spontaneous Emulsification, Physicochemical

characterization, Applications of Nanoemulsion, Thermodynamic Stability Studies, Stability of Nanoemulsion.

I. INTRODUCTION

Nanoemulsions/Sub-micron emulsions (SMEs)/Mini-emulsions are thermodynamically stable transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant and co-surfactant molecules having a globule size of less than 100 nm. Recently nanoemulsions are frequently used for delivery of vaccine, DNA encoded drug, antibiotics, cosmetic and topical preparations and are administered via various routes like oral, pulmonary, intranasal, and ocular, and transdermal etc.¹ Nanoemulsions are categorized as multi-phase colloidal dispersion, and are characterized by its stability and clarity. The dispersed phase typically comprises small particles or droplets, and they have very low oil/water interfacial tension. Nanoemulsions are formed spontaneously and readily and sometimes generally without high-energy input. In many cases a co-surfactants or co-solvent is used in addition to the surfactant, the oil phase and the water phase.²⁻⁵

Three types of Nanoemulsions are formed depending on the composition:

Oil in water (o/w): Nanoemulsions wherein oil droplets are dispersed in the continuous aqueous phase;

Water in oil (w/o): Nanoemulsions wherein water droplets are dispersed in the continuous oil phase;

Bi-continuous: Nanoemulsions wherein micro domains of oil and water are interspersed within the system.

In all three types of Nanoemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants. The key difference between emulsions and Nanoemulsions are that the former, whilst they may exhibit excellent kinetic stability, are thermodynamically unstable and will eventually

show phase separation. Another important difference is their appearance; emulsions are cloudy while Nanoemulsions are clear or translucent. In addition, there are differences in their method of preparation.

NANOEMULSION

Nanoemulsion is defined as a colloidal dispersion of two immiscible liquids that is thermodynamically unstable. In nanoemulsion, one of the liquids forms the dispersed phase and other liquid forms the dispersing medium.⁶ Nanoemulsion comprises droplets with diameters ranging from 10~200 nm and each drop let has a protective coating of emulsifier molecules.⁷⁻¹⁰

Self-emulsifying formulation

Self-emulsifying formulations generally comprise of self-emulsifying drug delivery systems (SEDDS) and self-nanoemulsifying drug delivery systems (SNEDDS). SEDDS give coarse emulsion whereas SNEDDS provide nano-size emulsion. These systems are isotropic mixtures of an oil, surfactant, and co-surfactant. Upon in vivo dilution by the aqueous phase, these systems form emulsions (in case of SEDDS) or fine and optical clear nanoemulsions (in case of SNEDDS) under gentle agitation, experienced due to gastrointestinal tract (GIT) motility. SEDDS and SNEDDS are generally described as emulsion or nano-emulsion pre-concentrates because the emulsion or nanoemulsion is formed from dilution in aqueous media in vivo.¹¹

Table 1: Differences between emulsion, nanoemulsion and microemulsion

Name	Chemical name
Captex 355	GlycerylTricaorylate/Caprata
Captex 200	Propylene Dicaprylate/Dicaprate Glycol
Captex 8000	GlycerylTricaprylate (Tricaprylin)
Witepsol	90:10 % w/w c12 Glyceride tri: diesters
Myritol 318	C8/C10 triglycerides
Isopropyl Myristate	Myristic acid isopropyl ester

Advantages of Nanoemulsions over other dosage forms

1. Eliminates variability in absorption
2. Increases the rate of absorption.
3. Helps in solubilizing 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, allowing the total dose to be reduced and thus minimizing side effects.¹²

Disadvantages of Nanoemulsion Based Systems

1. Use of a large concentration of surfactant and co-surfactants necessary for stabilizing the Nano-droplets.
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.¹³

Component of nanoemulsion

Main three components of Nanoemulsions are

1. Oil
2. Surfactant/Cosurfactant
3. Aqueous phase

The examples are mentioned in Table 2, 3, 4. Components of nanoemulsion systems include oils, lipids, surfactants, water-soluble co-solvents, and water. In the formulation of nanoemulsions, the oil phase may include

triglycerides like tri-, di-, or monoacylglycerols, vegetable oils, mineral oils, free fatty acids etc.¹⁴ Oil selection is generally based on the drug solubility. Oil phases which have high drug loading is generally used for development of nanoemulsion.¹⁵ Common surfactants used in the nanoemulsion systems for drug delivery and food ingredients are spans (sorbitan fatty acid esters), tweens [polyoxyethylene (POE) derivatives of sorbitan fatty acid ester],

Table 2: List of oils

Emulsion	Nanoemulsion	Microemulsion
Excellent kinetic stability.	Kinetically unstable.	They possess some kinetic stability
Thermodynamically unstable and will eventually phase separate.	Thermodynamically stable and no phase separation occur.	Thermodynamically stable
Emulsions appear cloudy.	Nanoemulsions are clear or translucent.	Microemulsions are clear
Methods involved in preparation of emulsion require a large input of energy.	Methods of preparation do not require energy input.	Methods of preparation do not require energy input.

Cremophor[®] EL (polyoxyl-35 castor oil), laurylmacroglycerides (Gelucire[®] 44/14), polysaccharides (gum and starch derivatives), phospholipids (egg, soy, or dairy lecithin), and amphiphilic proteins (whey protein isolate and caseinate).^{16,17}

Ultra-low negative interfacial tension is required for nanoemulsion formation. For this

purpose, co-surfactants or co-solvents are used along with a surfactant. Co-surfactants or co-solvents that are generally used in formulation of nanoemulsion systems are polyethylene glycol, propylene glycol, ethanol, transcuto-P (diethylene glycol monoethyl ether), ethylene glycol, glycerin, and propanol.^{17,18}

Table 3: List of surfactants

Name	Chemical name
Tween 20	Polyoxyethylenesorbitanmonolaurate
Tween 80	Polyoxyethylene (20) sorbitanmonooleate
Labrasol	Caprylocaproyl macrogol-8 glycerides
Labrafil 1944	Oleoyl macrogol-6 glycerides
Cremophor RH 40	Polyoxly 40 hydrogenated castor oil
PlurolOleique CC	Polyglyceryl-3 oleate

Factors affecting the Formulation of Nanoemulsion

1. The surfactant is the most important part of the Nanoemulsion. They should not form lyotropic liquid crystalline “micro- emulsions” phases. Systems containing short chain alkanes, alcohols, water, and surfactants form the phases which are generally used with the cosurfactant
2. Appropriate composition is required to avoid Oswald ripening and the dispersed phase should be highly insoluble in the dispersion medium.
3. The presence of excess surfactants enables new surface area of nanoscale to be rapidly coated during emulsification there by inhibiting induced coalescence.¹⁹

Methods of preparation of nanoemulsions

The drug is to be dissolved in the lipophilic part of the nanoemulsion i.e. oil and the water phases and is combined with surfactant and a co-surfactant is then added at slow rate with gradual stirring until the system is transparent. The amount of surfactant and co-surfactant to be added and the percent of oil phase that can

Be incorporated are determined with the help of pseudo-ternary phase diagrams. Ultra sonicators and high pressure homogenizers can then be used so as to achieve desired size range for dispersed globules. It is then being allowed to equilibrate. Gel may be prepared by adding a gelling agent to the above nanoemulsion. Carbomers (crosslinked polyacrylic acid polymers) are the most widely used gelling agent.

Formulation of Nanoemulsion Screening of Excipients

The solubility of the drug in various oils, surfactants and co-surfactants is determined by dissolving an excess amount of the drug in small quantities of the selected oils, surfactants and co-surfactants and mixed using a vortex mixer. A combination of oils can also be used for the determination of solubility. The mixtures are allowed to equilibrate at ambient temperature in an isothermal shaker. Samples are removed from the shaker and centrifuged. The supernatant is filtered through a 0.45 µm membrane filter. The concentration of the drug is determined in each oil, surfactant, co-surfactants and combination of oils by HPLC or UV Spectrophotometer at their respective mix

Construction of Pseudo Ternary phase diagram

Pseudo-ternary phase diagrams of oil, water, and S_{mix} are constructed at fixed co-surfactant and surfactant weight ratios. Phase diagrams were obtained by mixing of the ingredients, which shall be pre-weighed into glass vials and titrated with water and stirred well at room temperature. Formation of monophasic/biphasic system is confirmed by visual inspection. In case turbidity appears followed by a phase separation, the samples shall be considered as biphasic. In case monophasic, clear and transparent mixtures are visualized after stirring; the samples shall be marked as points in the phase diagram. The area covered by these points is considered as the nanoemulsion region of existence which is given in the Figure 1. Several methods have been suggested for the preparation of nanoemulsion. Here some methods discussed which are freely used for the nanoemulsion preparation.²⁰

Fig. 1: Construction of pseudo-ternary phase diagram

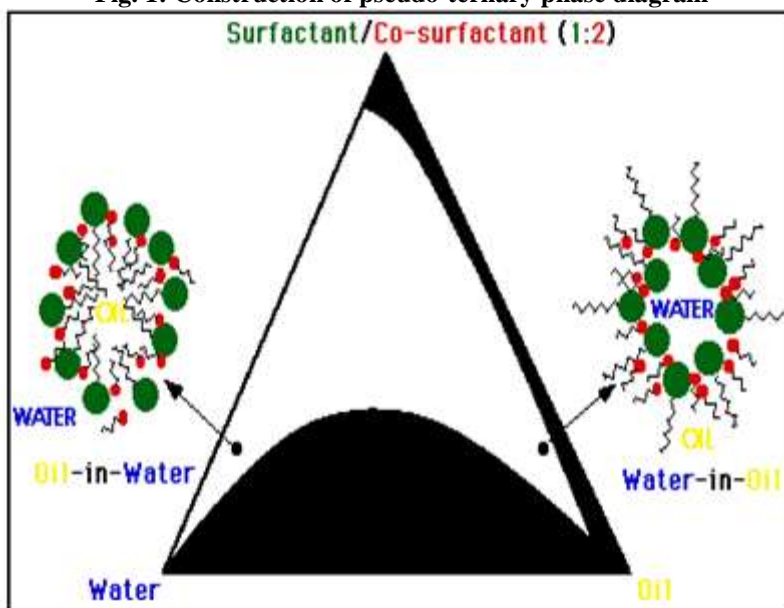


Table 4: List of co-surfactants

Name	Chemical name
Transcutol P	Diethylene glycol monoethyl ether
Ethylene glycol	Ethane 1,2 diol
Propylene glycol	1,2 propanediol

1. High Pressure Homogenization

The preparation of nanoemulsion requires the use of high pressure homogenizer. This technique produces nanoemulsions of low particle size i.e. 10-100nm. The dispersion of (oily and aqueous phase) is achieved by forcing their mixture through a small inlet orifice at a high pressure (500 to 5000 psi), which subjects the product to intense turbulence and hydraulic shear resulting in extremely fine particles of emulsions as shown in Figure 2. The particles which are formed exhibit a liquid, lipophilic core separated from the surrounding aqueous phase by a monomolecular layer of phospholipids.

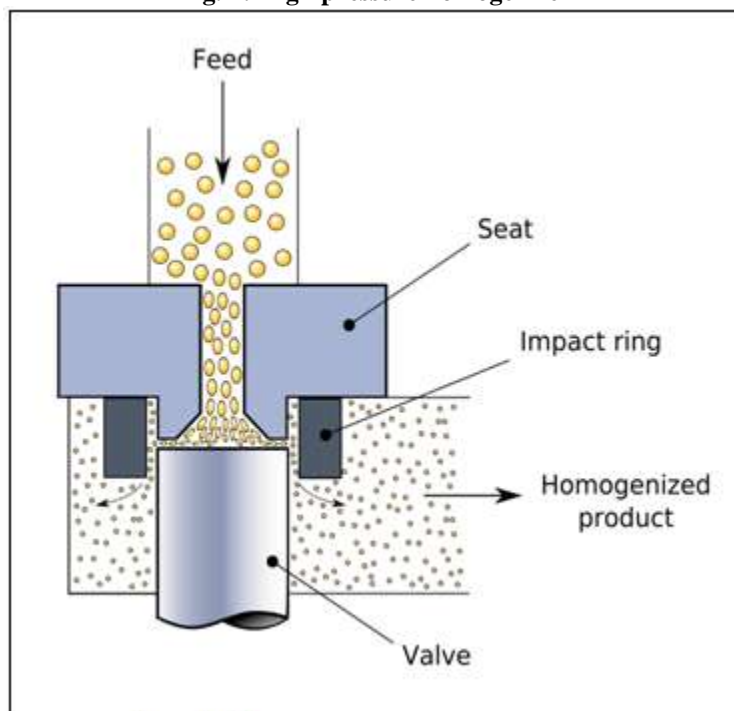
To obtain the optimized formulation following process variables should be investigated:

- **Effect of Homogenization Pressure:** It should be from 100 to 150 bars. The higher the pressure the lower is the particle size obtained.

- **No. of Homogenization cycles:**

The higher the homogenization cycles the smaller is the particle size obtained. The cycles are carried out in 3, 4 or 10 cycles. The number of cycles is analyzed by polydispersity index of drug after each cycle.²⁰

Fig. 2: High pressure homogenizer



Advantages

1. Ease of scale-up and little batch-to-batch variation
2. Narrow size distribution of the nano particulate drug.
3. Flexibility in handling the drug quality.
4. Effectively used for thermolabile substances.

Disadvantages

1. High energy consumption
2. Increase in temperature of emulsion during processing

2. Micro fluidization

Micro fluidization is a mixing technique, which makes use of a device called micro fluidizer. This device uses a high-pressure positive displacement pump (500 to 20000psi), and it forces the product through the interaction chamber, which

consists of small channels called „micro channels‘. The product flows through the micro channels on to an impingement area resulting in fine particles of sub- micron size range. The two solutions (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a coarse emulsion. The coarse emulsion is inserted into a microfluidizer where it is further processed to obtain a stable nanoemulsion. The coarse emulsion is passed through the interaction chamber micro fluidizer repeatedly until desired particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform nanoemulsion. The premixed emulsion is circulated through the micro fluidizer repeatedly until required droplet size is achieved.²¹

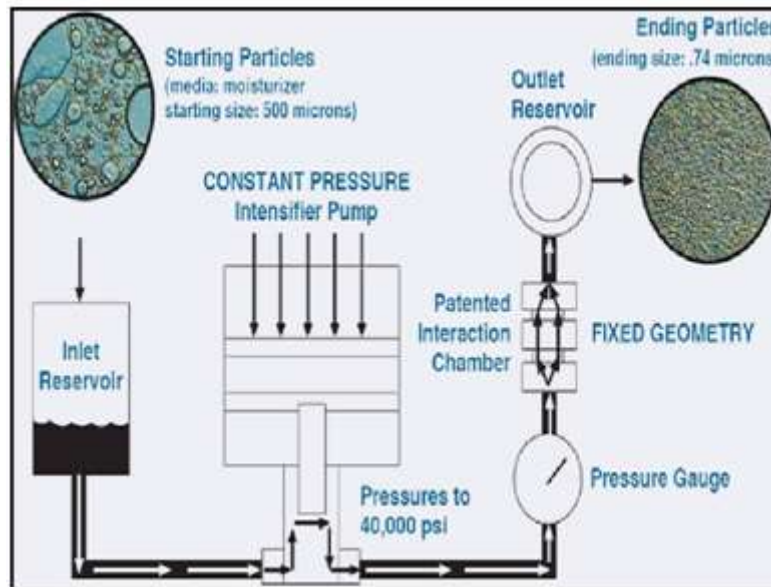


Fig. 3: Micro fluidizer

3. Ultrasonication

Nanoemulsions can be prepared using the ultrasonic sound frequency for the reduction of the globule size. Another approach is the use of a constant amplitude sonotrode at system pressures in excess of the ambient value. It is well known that increasing the external pressure increases the cavitation's threshold within an ultrasonic field and thus fewer bubbles form. However, increasing the external pressure also increases the collapse

pressure of cavitation's bubbles. This means that the col- lapse of the bubbles when cavitation occurs becomes stronger and more violent than when the pressure is at atmospheric conditions. As cavitation is the most important mechanism of power dissipation in a low frequency ultrasonic system, these changes in navigational intensity can be related directly to changes in the power density. The system also uses a water jacket to control the temperature to optimum level.

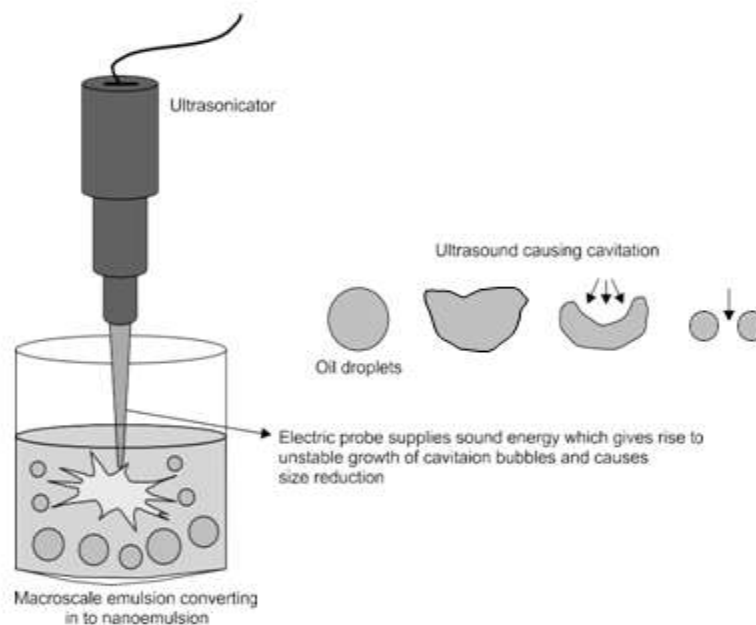


Fig. 4. Ultra sonication techniques

4. Phase inversion method

In this method, fine dispersion is obtained by the use of chemical energy resulting of phase transitions produced by emulsification path- way.

The phase transition is produced by varying the composition of the emulsion and keeping temperature constant or vice versa.

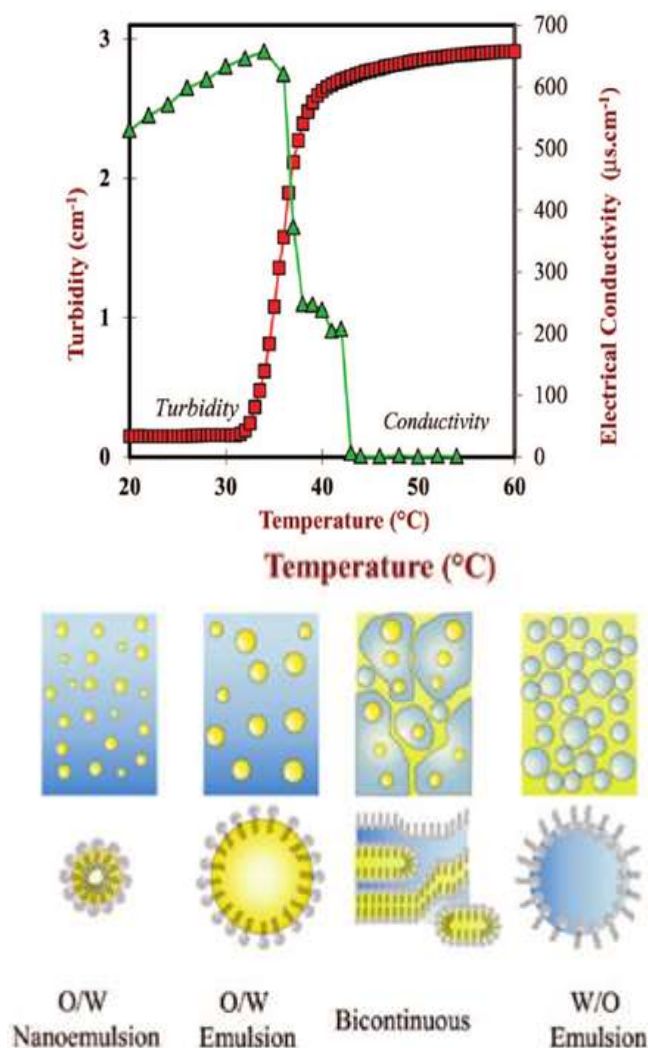


Fig. 5. Phase Inversion

5. Spontaneous Emulsification

It involves three main steps

- Preparation of homogeneous organic solution composed of oil and lipophilic surfactant in water miscible solvent and hydrophilic surfactant.
- The organic phase was injected in the aqueous phase under magnetic stir- ring giving o/wemulsion.
- The water-miscible solvent was re- moved by evaporation under reduced pressure.²²

6. Solvent Evaporation Technique

This technique involves preparing a solution of drug followed by its emulsification in another liquid that is non-solvent for the drug. Evaporation of the solvent leads to precipitation of the drug. Crystal growth and particle aggregation can be controlled by creating high shear forces using high-speed stirrer.²³

7. Hydrogel Method

It is similar to solvent evaporation method. The only difference between the two methods is that the drug solvent is miscible with

the drug anti-solvent. Higher shear force prevent crystal growth and Ostwald ripening.²⁴

Physicochemical characterization of nanoemulsions

1. Dye Solubilization

A water soluble dye is solubilized within the aqueous phase of the w/o globule but is dispersible in the o/w globule. An oil soluble dye is solubilized within the oil phase of the o/w globule but is dispersible in the w/o globule.

2. Dilutability Test

O/w Nanoemulsions are diluted with water whereas w/o are not and thus they undergo phase inversion into o/w Nanoemulsion.^{25, 26}

Conductance Measurement

Ino/w Nanoemulsion where the external phase is water, are highly conducting whereas w/o are not, since water is the internal or dispersed phase. To determine the nature of the continuous phase and to detect phase inversion phenomena, the electrical conductivity measurements are highly useful. A sharp increase in conductivity in certain w/o Nanoemulsion systems was observed at low volume fractions and such behaviour was interpreted as an indication of a percolative behaviour or exchange of ions between droplets before the formation of bi-continuous structures. Dielectric measurements are a powerful means of probing both structural and dynamic features of Nanoemulsion systems.

3. Dynamic Light-Scattering measurements

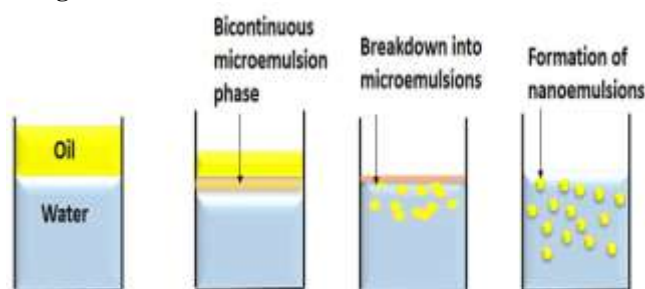


Fig. 6. Interfacial Tension

8. Viscosity measurement

Viscosity is measured using a viscometer. The viscosity of nanoemulsions is a function of the surfactant, water and oil components and their concentrations. Increasing the water content lowers the viscosity, while decreasing the amount of surfactant and co-surfactant increases interfacial tension between water and oil resulting in increased viscosity. Viscosity is very important for stability

The DLS measurements are taken at 90° in a dynamic light scattering spectrophotometer which uses a neon laser of wavelength 632 nm. The data processing is done in the built-in computer with the instrument.

4. Polydispersity index

The average diameters and polydispersity index of samples were measured by Photon Correlation Spectroscopy. The measurements were performed at 25°C using a He-Ne laser.

5. Phase analysis

Phase analysis of nanoemulsion is determined by measuring the electrical conductivity using a conductometer.

6. Particle Size Analysis

A Photon Correlation Spectrometer is used to monitor the particle size of nanoemulsions. Light scattering monitor 90° angle at 25°C.

7. Interfacial Tension

The formation and the properties of nanoemulsion can be studied by measuring the interfacial tension. Ultra-low values of interfacial tension are correlated with phase behavior, particularly the existence of surfactant phase or middle-phase nanoemulsions in equilibrium with aqueous and oil phases. Spinning-drop apparatus can be used to measure the ultra-low interfacial tension. Interfacial tensions are derived from the measurement of the shape of a drop of the low-density phase, rotating it in cylindrical capillary filled with high-density phase.²⁷

and efficient drug release. Nanoemulsion carrier formulations are basically oil-in-water and so in addition to being less greasy than water-in-oil formulations, often possess lower apparent viscosities. They are therefore expected to exhibit faster release of active ingredients and wash out easily after application on the skin surface. Various equipment and methods are available for assessment of rheological properties of

nanoemulsion carriers. Monitoring of viscosity change is a method of assessing stability of liquid and semi-solid preparations including nanoemulsion formulations. The viscosity of Nanoemulsions of several compositions can be measured at different shear rates at different temperatures using Brookfield type rotary viscometer.

9. pH

The apparent pH of the formulation was measured by pH meter.

10. Refractive Index

The refractive index, n , of a medium is defined as the ratio of the speed, c , of a wave such as light or sound in a reference medium to the phase speed, v_p , of the wave in the medium. $n=c/v_p$; It was determined using an Abbes type refractometer at $25\pm 0.5^\circ\text{C}$.

11. Transmission Electron Microscopy (TEM)

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of nanoemulsion droplets. Observations were noted as; a drop of the nanoemulsion was directly deposited on the holey film grid and observed after drying.²⁸

12. In Vitro Skin Permeation Studies

In vitro skin permeation studies were performed using Keshary-Chien diffusion cell. It was performed on abdominal skins and was obtained from male rats weighing 250 ± 10 gm with a recirculating water bath and 12 diffusion cells. The skins were placed between the donor and the receiver chambers of vertical diffusion cells. The receiver chambers were filled with fresh water containing 20% ethanol. The receiver chambers were set at 37°C and the solution in the receiver chambers was stirred continuously at 300 rpm. The formulations were placed in the donor chamber. At 2, 4, 6, 8 h, 0.5 ml of the solution in the receiver chamber was removed for analysis and replaced immediately with an equal volume of fresh solution. Each sample was performed three times. The cumulative corrections were made to obtain the total amounts of drugs diffused at each time interval. The cumulative amounts of drug permeated through rat skins were plotted as a function of time. The permeation rates of drug at a steady-state through rat skins were calculated from the slope of linear portion of the cumulative

amount permeated through the rat skins per unit area versus time plot.

Thermodynamic Stability Studies During the thermodynamic stability of drug loaded Nanoemulsions following stress tests are reported

a. Heating Cooling Cycle Nanoemulsion formulations were subjected to six cycles between refrigeration temperature 4°C and 45°C . Stable formulations were then subjected to centrifugation test.

b. Centrifugation

Nanoemulsion formulations were centrifuged at 3500 rpm and those that did not show any phase separation were taken for the freeze thaw test.

c. Freeze Thaw Cycle

In this the formulation were subjected to three freeze thaw cycles between 21°C and $+25^\circ\text{C}$ kept under standard laboratory conditions. These studies were performed for the period of 3 months.²⁹

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.³⁰

Primaquine when incorporated into oral lipid nanoemulsion showed effective antimalarial activity against Plasmodium berghei infection in mice at a 25% lower dose level as compared 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 thepuredrug.

3. Topical Delivery

Topical administration of drugs can have advantages 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).³¹

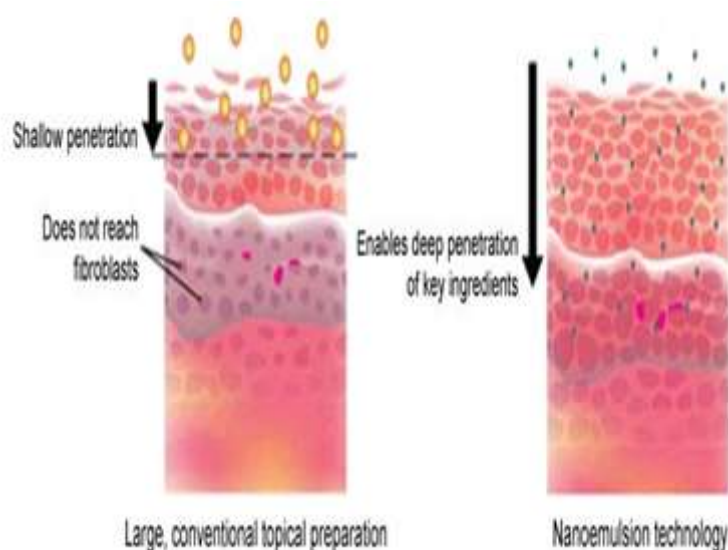


Fig. 7. Topical Delivery

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 increase absorption and to attain prolong release profile.³²

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. Nano-gel technology to create miniemulsion 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, moisturizing

and anti-ageing creams. It helps to give skin care formulations a good skin feels.³³

6. Transdermal

Indomethacin a potent NSAID, the anti-inflammatory effects of true optimized nanoemulsion formulation were compared with marketed gel in carrageenan 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%).

Nanoemulsions in Cancer Therapy

Nanoemulsions can be used as vehicle in cancer chemotherapy for prolonging the rate of drug release after intramuscular and intratumoral 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 nonirritant system^{24,25}.

7. Nanoemulsions in intranasal drug delivery

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 favorable 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 mucosae 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 related 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 mucoadhesive nanoemulsions.

8. 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, Nano carrier systems) 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.

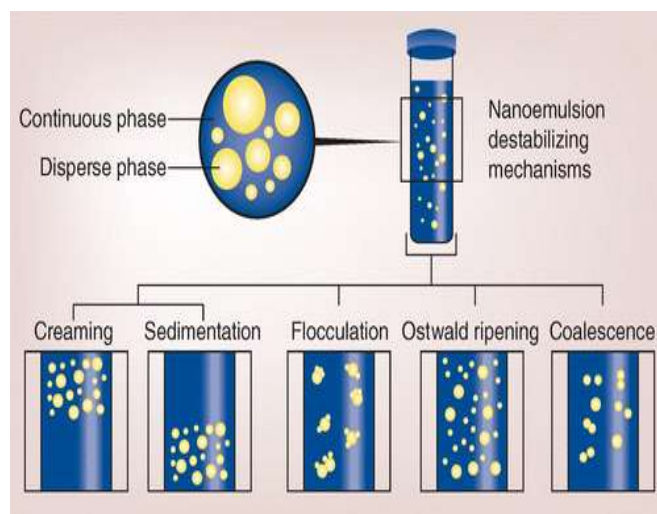


Fig. 8. Nanoemulsions in pulmonary drug delivery

Nanoemulsions in gene delivery vector

Emulsion systems have been emerged as alternative gene transfer vectors to liposomes. Other emulsion studies for gene delivery (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 stearylamine (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 stearylamine 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 the best 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 delivery.³⁴

Stability of Nanoemulsion

Stability of a dosage form refers to the physical and chemical integrity of the dosage unit and when appropriate, the ability of the dosage unit to maintain protection against microbiological contamination. Stability of drug product is one of

the problems associated with the development of emulsions, microemulsions and nanoemulsions. They enhance physical as well as chemical stability of drugs.

Stability factors of Nanoemulsion

Stability studies are performed on nanoemulsions by storing them at refrigerator and room temperatures over a number of months. The viscosity, refractive index and droplet size are determined during this period of storage. Insignificant changes in these parameters indicate for stability. Accelerated stability studies can also be performed. In this instance, nanoemulsion formulation are kept at accelerated temperatures and samples withdrawn at regular intervals and analyzed for drug content by stability indicating HPLC methods. The amount of drug degraded and remaining in nanoemulsion formulation is determined at each time interval. As a general consideration the stability of nanoemulsion largely depends upon the following factors

- a. Coalescence of the two droplets of dispersed phase due to the surface tension and intermolecular attractions. This is mainly reduced by addition of suitable surfactants.
- b. If the dispersed phase has high solubility in the dispersed medium. This results in diffusive migration of smaller droplets with low Laplace pressure to larger droplets of high Laplace pressure also known as Ostwald ripening. The dispersed phase should be selected such that it should have minimum or no solubility in the continuous phase. The instability of nanoemulsion is due to some main factors including creaming, flocculation, coalescence and Ostwald ripening. Among them, Ostwald

ripening is the main mechanism of nanoemulsion instability because rest of the problem is minimized by the small size of nanoemulsion and use of nonionic type of surfactant. Creaming of nanoemulsion is prevented by the faster diffusion rate of smaller droplets. Vanderwall's force is responsible for the attraction of droplets and leads to the flocculation of emulsion. But in case of nanoemulsion, nonionic surfactant does not create any kind of attractive force, hence no flocculation occurs.

The droplet size of nanoemulsion also prevents the flocculation because these small droplets show high curvature and Laplace pressure opposes the deformation of large droplets. Coalescence of droplets of nanoemulsion can be prevented by a thick multilamellar surfactant film adsorbed over the interface of droplets. The only problem of instability of nanoemulsion can arise by the Ostwald ripening. In Ostwald ripening small droplets with high radius of curvature converted into large droplets with low radius of curvature. Another method to prevent the effect of Ostwald ripening is addition of polymeric surfactant on the interface which increases the elasticity of droplets and further decreases the effect of Ostwald ripening.³⁵⁻³⁷

II. CONCLUSION

Nanoemulsion drug delivery systems effectively overcome the low bioavailability drawback associated with drugs and food components which are hydrophobic, and having high first pass metabolism. Methods for nanoemulsion drug delivery system formulation can be conveniently classified in a rigid fashion based on the energy required, nature of phase inversion and self-emulsification. High energy methods for nanoemulsion drug delivery system formulation have greater control over dispersion of particle sizes and are more flexible for the choice of composition. High energy methods have been used by researchers to improve delivery of drugs and bioactive food components. For providing high energy, sophisticated instruments are required; high energy methods are therefore more costly than low energy methods as these methods require low energy and are more efficient. High energy methods are more useful for delivery of nanoemulsions containing bioactive food components, as these methods require low concentrations of surfactant. For delivery of hydrophobic drugs with low bioavailability,

SNEDDS are the most popular methods used by researchers. However, further research is required to uncover the potential of phase inversion emulsification methods in effective drug loading and delivery.

Authors Contributions

All the authors have contributed equally

Conflicts of interest:

There are no conflicts of interest among the authors.

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