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Review Article

NANOEMULSIONS- A REVIEW

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ABSTRACT

Nanoemulsions are submicron sized emulsions which act as drug carriers for improving the delivery of therapeutic agents. They are the most advanced nanoparticle systems for the systemic delivery of active pharmaceutical for controlled or sustained drug delivery and targeting. These are thermodynamically stable isotropic systems in which two immiscible liquids (water and oil) are mixed to form a single phase by means of appropriate surfactants. Nanoemulsion globule size falls typically in the range of 10-100 nm and shows a narrow size distribution. Nanoemulsions show great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies. Thus the aim of this review is focused on nanoemulsion advantages, disadvantages, various methods of preparation, characterization techniques and the various applications of sub-micron size emulsion in different areas such as various route of administration, in chemotherapy, in cosmetic, etc.

Keywords: Nanoemulsion, globules, sustained, surfactant, pharmaceutical.

INTRODUCTION

Nanoemulsions/Sub-micron emulsions (SMEs)/Mini-emulsionsare thermodynamically stable transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant 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 administrated via various routes like oral, pulmonary,intranasal,and ocular, and transdermaletc.[1] Nanoemulsions are categorized as multiphase colloidal dispersion,and are characterized by its stability and clarity. The dispersed phase typically comprises small particles or droplets, andthey have very low oil/water interfacial tension.Nanoemulsions are formed spontaneously and readily and sometimes generally without high-energy input. In many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase[2-5]

Three types ofNanoemulsions 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 cosurfactants. 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, since emulsions require a large input of energy while Nanoemulsions do not as described in Table 1.

Advantagesof 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/wemulsion
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. Nanoemulsionscarry 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 [6].

Disadvantages of Nanoemulsion Based Systems

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[7].

Components of Nanoemulsion

Main three components of Nanoemulsions are

1. Oil
2. Surfactant/Co surfactant
3. Aqueous phase[8,9]

The examples are mentioned in Table 2, 3, 4.

Nanoemulsions are colloidal dispersions composed of an oil phase, aqueous phase, surfactant and cosurfactants at appropriate ratios. Unlike coarse emulsions micronized with external energy nanoemulsions are based on low interfacial tension. This is achieved by adding cosurfactants, which leads to spontaneous formation of a thermodynamically stable nanoemulsion. The droplet size in the dispersed phase is small, usually below 140 nm in diameter, which makes the nanoemulsions transparent liquids.They are used to deliver drugs to the patients via several routes, but the topical application of nanoemulsions has gained much interest. The three main factors determining the transdermal permeation of drugs are: mobility of drug in the vehicle, release of drug from the vehicle, and permeation of drug into the skin. Thusthey improve the transdermal delivery of drugs over the conventional topical preparations such as emulsions and gels. Mobility of drugs in nanoemulsions is more facile as compared to the nanoemulsion with gel which will increase its viscosity and further decrease the permeation into the skin. The superior transdermal flux from nanoemulsions hasbeen shown to be mainly due to their high solubilization potential for lipophilic and hydrophilic drugs. This creates an increased thermodynamic activity towards the skin.They may affect the permeability of drug into the skin. In this case, the components of nanoemulsions serve as permeation enhancers. Several compounds used in nanoemulsions have been mentioned to improve the transdermal permeation by altering the structure of the stratum cornea. For example, short chain alkanols are widely used as permeation enhancers. It is known that oleic acid, a fatty acid with one double bond in the chain structure, perturbs the lipid barrier in the stratum corneum by forming separate domains which interfere with the continuity of the multilamellar stratum corneum and may induce highly permeable pathways in the stratum corneum.[9-11]

Factors affecting the Formulation of Nanoemulsion

1. The surfactant is the most important part of the Nanoemulsion. They should not form lyotropic liquid crystalline “microemulsions” phases. Systems containing short chain alkanes, alcohols, water, and surfactants form the phases which are generally used with the co surfactant
2. Appropriate composition is required to avoid Oswald ripening andthe 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[12].

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 cosurfactant is then added at slow rate with gradual stirring until the system is transparent. The amount of surfactant and cosurfactant to be added and the percent of oil phase that can

be incorporated are determined with the help of pseudo-ternary phase diagrams. Ultrasonicators and high pressure homogenizerscan 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 (crosslinkedpolyacrylic 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 cosurfactants is determined by dissolving an excess amount of the drug in small quantities of the selected oils, surfactants and cosurfactants and mixed using a vortexmixer. 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, cosurfactant and combination of oils by HPLC or UV Spectrophotometer at their respective λmax

Construction of Pseudo Ternary phase diagram

Pseudo-ternary phase diagrams of oil,water, and Smix are constructed at fixed cosurfactant 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 nanoemulsionpreparation.[12]

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[12].

Advantages

1. Ease of scale-up and little batch-to-batch variation
2. Narrow size distribution of the nanoparticulate 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. Microfluidization

Microfluidization is a mixing technique, which makes use of a device called microfluidizer. 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 submicron 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 microfluidizer 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 microfluidizer repeatedly until required droplet size is achieved[13]

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 cavitations threshold within an ultrasonic field and thus fewer bubbles form. However, increasing the external pressure also increases the collapse pressure of cavitations bubbles. This means that the collapse 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.

4. Phase inversion method

In this method, fine dispersion is obtained by the use of chemical energy resulting of phase transitions produced by emulsification pathway. The phase transition is produced by varying the composition of the emulsion and keeping temperature constant or vice versa.

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 stirring giving o/w emulsion.
- The water-miscible solvent was removed by evaporation under reduced pressure[14].

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 forcesusing high-speed stirrer[15].

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[16.]

Physicochemical characterization of nanoemulsions

1. DyeSolubilization

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/wNanoemulsions are diluted with water whereas w/o are not and thus they undergo phase inversion into o/w Nanoemulsion[17, 18]

3. Conductance Measurement

Ino/wNanoemulsion 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/oNanoemulsion systems was observed at low volume fractions and such behaviour was interpreted as an indication of a 'percolativebehaviour' or exchange of ions between droplets before the formation of bicontinuous structures. Dielectric measurements are a powerful means of probing both structural and dynamic features of Nanoemulsion systems.

4. Dynamic Light-Scattering measurements

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.

5. Polydispersity index

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

6. Phase analysis

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

7. Particle Size Analysis

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

8. InterfacialTension

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[19].

9. 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 andcosurfactant increases interfacial tension between water and oil resulting in increased viscosity. Viscosity is very important for stability 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.

10. pH

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

11. Refractive Index

The refractive index, n, of a medium is defined as the ratioof the speed, c, of a wave such as light or sound in a reference medium to the phase speed, vp, of the wave in the medium. n=c/vp; It was determined using an Abbes type refractrometerat 25±0.5°C.

12. 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 notedas; a drop of the nanoemulsion was directly deposited on the holey film grid and observed after drying[20].

13. *In Vitro* Skin Permeation Studies

In vitro skin permeation studies were performed using KesharyChiendiffusion 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^{o}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.

14. Thermodynamic Stability Studies

During the thermodynamic stability of drug loaded Nano-emulsions 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°C kept under standard laboratory conditions. These studies were performed for the period of 3 months.[21]

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 drugssuch as steroids, hormones, diuretic and antibiotics. Pharmaceutical drugs of peptides and proteins are highly potent and specific in their physiological functions[22].

Primaquine when incorporated into oral lipid nanoemulsion showed effective antimalarial activity against Plasmodium bergheii infection in mice at a 25% lower dose level as compared to conventional oral dose. Lipidnanoemulsion of primaquine improved oral bioavailability by the liver with drug concentration higher by at least by 45% as compared with the puredrug.

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 targetability 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)[23].

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[24].

5. In Cosmetic

The aesthetic properties, i.e. low viscosity and transparent visual aspects of nanoemulsionwith 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 miniemulsion from oil-in water concentrate suited to minimizing transepidermal 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 [25].

6. Transdermal

Indomethacin a potent NSAID, the anti-inflammatory effects of true optimized nanoemulsion formulation were compared with marketed 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 aceclofenacnanoemulsion (82.2%) as compared to nanoemulsion gel formulation (71.4%) and conventional gel (41.8%).

7. Nanoemulsions in Cancer Therapy

Nanoemulsions can beused 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 itis alsonon-irritant system[24, 25.]

8. Nanoemulsions in intranasal drug delivery

Intranasal drug delivery system has now been recognized as a reliable route for the administrationof drugs next to parenteral and oral routes. Nasal mucosa has emerged as a therapeuticallyviable channel for the administration of systemic drugs and also appears to be afavourable 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 mostefficient sites because of its reduced enzymatic activity, high availability of immunoactive sitesand its moderately permeable epithelium. There are several problems associated withtargeting 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 systemiccirculation and barrier between the blood and brain. The olfactory region of the nasalmucosa 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 ofnanoemulsions 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 intravenousroute. Another application of intranasal drug delivery system in therapeutics is their usein development of vaccines. Immunity is achieved by the administration of mucosal antigen.Currently, the first intranasal vaccine has been marketed. Among the possible deliverysystems, the use of nano based carriers hold a great promise to protect the biomolecules, promotenanocarrier 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 aboutsignificant results in targeting of the drugs to the brain in treatment of diseases related to the centralnervous system. Bhanushali*et al* developed intranasal nanoemulsion and gel formulationsfor rizatriptan benzoate for prolonged action. Various mucoadhesive agents weretried out to form thermo-triggered mucoadhesivenanoemulsions.

9. Nanoemulsions in pulmonary drug delivery

The lung is the most important target for drug delivery due to noninvasive administration viainhalation aerosols, avoidance of first-pass metabolism, direct delivery to the site of action forthe treatment of respiratory diseases, and the availability of a huge surface area for local drugaction and systemic absorption of drug. Colloidal carriers (ie, nanocarrier systems) in pulmonarydrug delivery offer many advantages such as the potential to achieve relatively uniformdistribution 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 dosingfrequency, improves patient compliance, decreases incidence of side effects, and the potentialof 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 delivery (non-pulmonary route) have shown that binding ofthe emulsion/DNA complex is stronger than liposomal carriers. This stable emulsionSystem delivered genes more efficiently than liposomes. Silva *et al* evaluated factors thatinfluence DNA compaction in cationic lipid nanoemulsions [cationic nanoemulsions containingstearylamine (a cationic lipid that presents a primary amine group when in solution, is ableto compact genetic material by electrostatic interactions, and in dispersed systems such asNanoemulsions this lipid anchors on the oil/water interface conferring a positive charge tothem. The influence of the stearylamine incorporation phase (water or oil), time ofcomplexation, 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 zetapotential of lipoplexes was lower than the results found for basic nanoemulsions, the granulometrydid not change. Moreover, it was demonstrated that lipoplexes are suitable vehicles for gene delivery[26].

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 regu-

lar 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's 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. Vander wall'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 ofnanoemulsion 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.[27-29]

Table 1: Differences between emulsion, nanoemulsion and microemulsion

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.	Microemulsion 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.

Table 2: List of oils

Name	Chemical name
Captex 355	GlycerylTricaorylate/Caprate
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

Table 3: List of surfactants

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

Table 4: List of cosurfactants

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

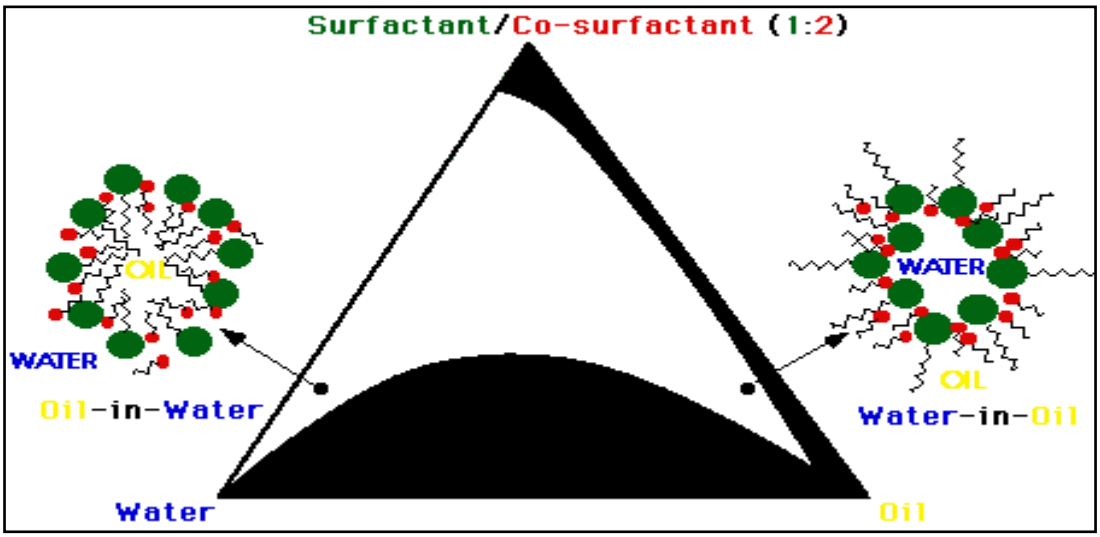

Fig. 1: Construction of pseudo-ternary phase diagram

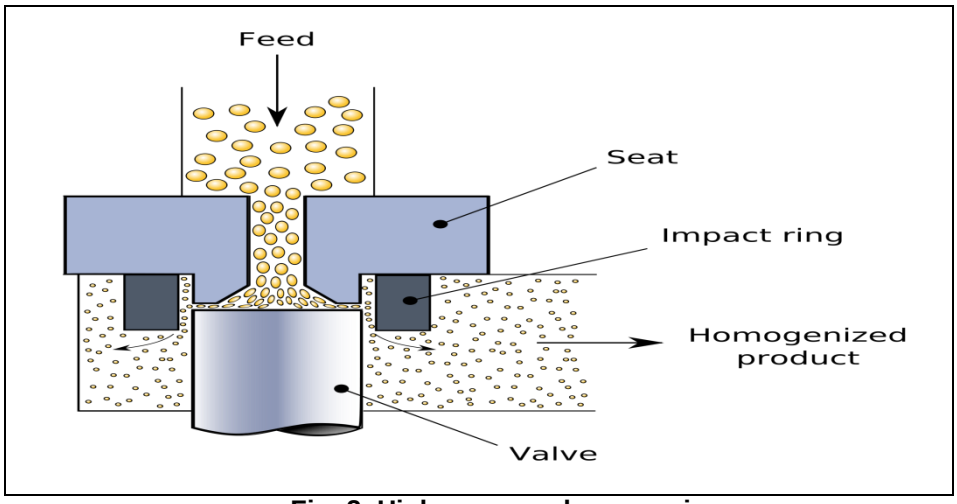

Fig. 2: High pressure homogenizer

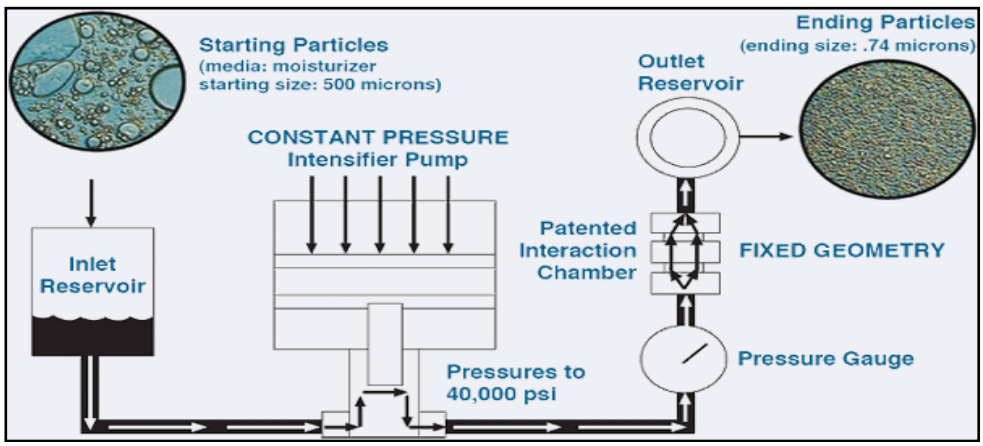

Fig. 3: Microfluidizer

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