Techniques for Formulation and Characterization of Nanoemulsion : A Review

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Abstract - Nanoemulsions are thermodynamically stable, clear, isotropic liquid mixture of oil, water, surfactant and cosurfactant. the primary difference between emulsion and nanoemulsion is their size of particle dispersed in continuous phase. This system is intended to solve the problems of conventional dosage form. it is the best choice for drug delivery. It is also used for treatment of cancer by targeting the cancer cells. the nanoemulsion has great attraction towards research, drug delivery because of their nano size range. Information on types, surfactants, cosurfactants, building psudoternary phase diagrams, preparation methods, and characterization are all covered in this review.

Index Terms - nanoemulsion, psudoternary phase diagram, preparation methods, characterization.

INTRODUCTION

An ideal drug delivery system fulfils the objective of maximizing therapeutic effect while minimizing toxicity. Nanoemulsions are novel drug delivery systems consisting of emulsified oil and water systems with mean droplet diameters ranging from 50 to 1000 nm. The typical range of droplet sizes is 100 to 500 nm.[1]Miniemulsions, ultrafine emulsions, and submicron emulsions are other names for nanoemulsions. [3][7]

A nanoemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of two immiscible liquid phases such as an oil phase and a water phase. An interfacial tension exists between the two liquids everywhere they are in contact due to differences in attractive interactions between the molecules of the two liquid phases.To lessen this interfacial tension, amphiphilic surface-active molecules or surfactants are added.[4] To aid in the stabilisation process, a co-surfactant or co-solvent may be added in addition to the surfactant. [2] Most widely used surfactants are non-ionic surfactants (sorbitan esters, polysorbates), anionic surfactants (potassium laurate, sodium lauryl sulphate), cationic surfactants (quaternary ammonium halide) and zwitterions surfactants (quaternary ammonium halide).[5] Nanoemulsions are made from surfactants approved for human consumption and common food substances that are "Generally Recognized as Safe" (GRAS) by the FDA.[3]The earliest nanoemulsions were created in the 1940s[6]more recently, K. Landfester has studied the water-in-oil form of nanoemulsion. [7]Nanoemulsions are excellent drug delivery vectors because of their ability to dissolve vast amounts of poorly soluble drugs, their compatibility with one another, and their capacity to shield the drugs from enzymatic and hydrolytic degradation.[1]

The significant property that differentiates the nanoemulsions from other emulsion systems is that, a nanoemulsion shows different pattern in physical and rheological properties with decreasing droplet size. Two major distinguishing advantages of a nanoemulsion are its stability and easy penetration. Due to very fine particle size and less surface tension between the oil and water molecules, it barely has the tendency to agglomerate or precipitate which reduces the possibility of creaming or sedimentation. As a result, a nanoemulsion is much more stable than other emulsion systems and is more translucent compared to microemulsions. [2]

Nanoemulsions are part of a broad class of multiphase colloidal dispersions. Although some lyotropic liquid crystalline phases, also known as "micellar phases", "mesophases", and "microemulsions", may appear to be similar to nanoemulsions in composition and nanoscale structure, such phases are actually quite different. Lyotropic liquid crystals are equilibrium structures comprised of liquids and surfactant, such as lamellar sheets, hexagonally packed columns, and wormlike micellar phases, that form spontaneously through thermodynamic self-assembly.In contrast, external shear is required to split bigger droplets into smaller ones in order for nanoemulsions to occur. [3]

A lot of techniques are available for enhancing absorption of poorly water-soluble drugs, like use of lipid-based systems. Thus enhancement of aqueous solubility in such case is a valuable goal to successfully formulate them into bioavailable dosage forms. A range of novel strategies are currently being developed for efficient delivery of poorly water-soluble drugs, such as the formulation of amorphous solid form, nanoparticles, microemulsions, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes. Among all, the most accepted approach is the lipid-based formulation approach. Lipid-based formulations enhance the absorption by enhancing solubilization, prolonging gastric residence time, stimulating the intestinal lymphatic transport pathway, altering intestinal permeability, reduced activity of efflux transporters and reduced metabolism. Lipid-based formulations present a large range of optional systems such as solutions, suspensions, self-emulsifying systems and nanoemulsions. [1]

In this paper we are trying to understand various aspects related to the advantages, disadvantages, manufacturing of nanoemulsion, characterisation, evaluation and application.

Classification of Nanoemulsion:

On the basis of composition of oil and water portions nanoemulsion are classified into three types:

a) Oil in water (O/W) nanoemulsions in this type the oil droplets are dispersed in continuous aqueous phase

b) Water in oil (W/O) nanoemulsions here the water droplets are dispersed in continuous phase which are oil.

c) Bi-continuous nanoemulsions in this type the microdomains of oil and water are inter-dispersed within the system.

The O/W nanoemulsions further classified into three types based on the type of surfactants used which are as follows:

• Neutral O/W nanoemulsions, in this type the neutral surfactant are used

• Cationic O/W nanoemulsion, Here cationic surfactants are used

• Anionic O/W nanoemulsions, the anionic surfactants are used[6]

Advantages of nanoemulsions:

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, allowing the total dose to be reduced and thus minimizing side effects.[8]

15. Nanoemulsions have higher surface area and free energy that make them an effective transport system.

16. They do not show the problems of inherent creaming, flocculation, coalescence and sedimentation.

17. It do not damage healthy human and animal cells hence are suitable for human and veterinary therapeutic purposes.

18. It provides better uptake of oil-soluble supplements in cell cultures technology to improve growth of cultured cells and allows toxicity studies of oil-soluble drugs. [9]

19. It may be used as substitute for liposomes and vesicles. [10]

20. Properties of fluidity at optimum concentration of oil and their optically transparent behaviour gives the subject a pleasant feel when applied on skin

21. Alcohol base in perfumes can be avoided moreover the fragrance enhancers are easily administered through nanoemulsions formation because of their easy formulations. [12]

Disadvantages:

1. It requires large concentration of surfactant and cosurfactant for stabilizing the nanodroplets.

2. It generally shows a limited solubilizing capacity for high-melting substances.

3. There is lacuna for understanding the interfacial chemistry which is involved in production of nanoemulsions.

4. For use in pharmaceutical applications the nature of surfactant must be nontoxic.

5. It requires the use of high concentrations of emulsifiers. [6]

6. Recently it is a drug delivery system which has attracted the interest of researchers because

 the facility was not there is past years.

7. Its production is expensive to the industry [12]

I. Surfactants:

The surfactant should capable of micro emulsification of the oily phase and should also possess the good solubilizing potential for the hydrophobic drug compounds. The choice of the surfactant is critical for the nanoemulsion formulation. [4] In preparation of W/O nanoemulsion Surfactants with HLB values3-6 are useful where for the preparation of O/W nanoemulsion surfactants with higher HLB values8-18 are useful. [6] The hydrophobic core enhances the entrapment of drug, thus increasing its solubility. When the oil content is high, surfactant concentrate on the oil/water interface forming emulsions, wherein the drug is solubilized in the internal oil phase. On the other hand, when the oil content is low, minute oil entrapped surfactant globules are produced, which are known as nanoemulsions. The surfactant used in nanoemulsion formation could be ionic or non-ionic but ionic surfactants are not preferred due to toxicological effects. Various surfactants are mostly used such as lecithins, poloxamers and polysorbate 80. It is very important to determine the surfactant concentration properly as large amounts of surfactants may cause GI irritation. There is a relationship between the droplet size and the concentration of the surfactant being used. In some cases, increasing the surfactant concentration could lead to smaller droplets such as in the case of a mixture of saturated C8-C10 polyglycolized glycerides. On the other hand, in some cases, the mean droplet size may increase with increasing surfactant concentrations. [4]

II. Co-surfactant:

Many times, surfactant alone cannot lower the oil-water interfacial tension adequately to yield a nanoemulsion which necessitates the addition of a co-surfactant to bring about the surface tension close to zero. Co-surfactants penetrate into the surfactant monolayer providing additional fluidity to interfacial film and thus distracting the liquid crystalline phases which are formed when surfactant film is too rigid.[4] Co surfactants raises the fluidity of the interface due to presence of fluidizing groups like unsaturated bonds, then demolishes liquid crystalline or gel structure and alters the HLB value in such way to cause spontaneous formation of micro emulsion. [11]Usually, a very low HLB co-surfactant is used with a high HLB surfactant to modify the overall HLB of the system. Unlike surfactant, the co-surfactant may not be capable of forming self-associated structures like micelles on its own. Hydrophilic cosurfactants preferably alcohols of intermediate chain length such as hexanol, pentanol and octanol, which are known to reduce the oil/water interface and allow the spontaneous formation of nanoemulsion. [4]

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.[8]

3. Surfactant must be selected carefully such that an ultralow interfacial tension may be achieved which is a primary requirement to produce nanoemulsion.

4. The surfactant must be flexible or fluid enough to promote the formation of nanoemulsion. [10]

Properties of Nanoemulsion:

The properties of the nanoemulsions can be listed as follows.

1. They provide a larger surface area and the free energy in them helps them to be useful in transportation.

2. Creaming, sedimentation, flocculation and coalescence are never observed in nanoemulsions with time duration because of their small droplet size.

3. Therapeutically they are very important as they cause no damage to human and animal cells.

4. Transdermal penetration is seen because of their small droplet sizes.

5. In comparison to microemulsions the nanoemulsions need surfactants in less amount.

6. Due to their very small droplet sizes the nanoemulsions flocculation does not occurred due to which they remain dispersed in system. [12]

Construction of psudoternary phase diagram:

The surfactant with the maximum emulsifying ability for oils was selected for phase behavior studies for a specific combination of the Smix phase. The ratio of the surfactant and co-surfactant mixture (Smix) was 1:1 based on pre-experimental trials. The phase diagrams of the pseudo-ternary system (oil phase, Smix phase, and aqueous phase) were constructed using the aqueous titration (spontaneous emulsification) method. An adequate quantity of Smix was dissolved in different oil phases in glass vials at room temperature. Each oil–Smix mixture was titrated drop-wise continuously with double distilled water using a micropipette by vortex mixing until it turned turbid. The changes in the clarity of the preparation during the titration were diligently observed. [13] The percentage composition of the component in each ternary system was determined and the observed results were plotted on triangular co-ordinates to construct the phase diagrams. [14]

The calibration plot method was used to determine the region of the nanoemulsification in the developed phase diagram. The calibration plot was constructed between the weight (mg) of graph paper versus its area (cm2).[13]
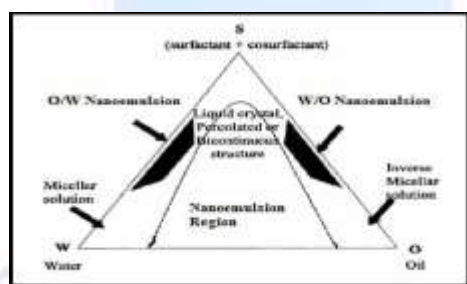

Fig.1 Construction of pseudoternary phase diagram

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 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, cosurfactant and combination of oils by HPLC or UV Spectrophotometer at their respective λmax. [8]

HIGH ENERGY EMULSIFICATON METHODS:

I. High-pressure homogenization

The high-pressure homogenization method is one of the high-energy emulsification methods, which uses special equipment to produce shear, impact, cavitation, turbulence and eddy, etc., to break the droplets. The technique relies on the powerful cavitation phenomenon to disrupt and produce smaller sizes oil droplets. Other factors such as homogenisation pressure and number of cycles can profoundly influence the mean droplet size and particle distributions[19] In the process of high pressure homogenization, the working pressure range of traditional high pressure homogenizer is usually 50-100 MPa, and the newly developed homogenizer is up to 350 MPa. The mixture of oil, water and surfactant is pushed through the narrow slit of the 5-10 mm homogenizer under high pressure. Due to the relatively small gap size, as the pressure increased, a tremendous shear force formed, and make the droplet to deform and break into smaller droplets.[15] A droplet size of the approximately 1 nm can be prepared. [12][7] In general, the mixture has been treated several times before the droplets become homogenized. During this process, the temperature and pressure also affect the droplet size, which can become small with the increase of temperature and pressure. [15]

II. Microfluidization:

Microfluidization is a mixing technology at micro size level that uses a device called microfluidizer. In microfludization, fluids are forced to pass through the microchannels under high-pressure (500~20,000 psi). Microchannels are generally micro size channels which allow mixing at micro size level. The phases of macroemulsion (aqueous and oil phases) are mixed together and then passed through the microfluidizer. The macroemulsion is guided through the microchannels under high pressure towards the interaction chamber. In the interaction chamber, two streams of macroemulsions strike each other at high velocity. This collision creates forces like shearing, cavitation, and impact, which produce stable nanoemulsions.

Microfluidizers produce narrower and smaller nanoemulsion particle size distributions of than homogenizers. Also, microfluidizers produce stable nanoemulsions at low surfactant concentrations. Microfluidization methods have been used to produce food ingredient nanoemulsions. Microfluidization techniques produce food grade nanoemulsions with uniform droplet size distributions and greater stabilities. [16]

III. Ultrasonic homogenization:

Ultrasonic emulsification is very efficient in reducing droplet size. In ultrasonic emulsification, the energy is provided through sonotrodes called as sonicator probe. It contains piezoelectric quartz crystal which can expand and contract in response to alternating electric voltage. As the tip of sonicator contacts the liquid, it produces mechanical vibration and cavitation occurs. Cavitation is the formation and collapse of vapour cavities in liquid. Thus, ultrasound can be directly used to produce emulsion; it is mainly used in laboratories where emulsion droplet size as low as 0.2 micrometer can be obtained. [10]

Phacoemulsification is the use of ultrasonic wave to generate strong mechanical vibration and cavitation to break the oil-water interface, which can achieve mixture at molecular level, resulting in smaller emulsion particle size and more uniform particle size distribution. Phacoemulsification can reduce the size of nanoemulsion droplet effectively. Phacoemulsification technology has these following advantages.

- Compared with other high-energy emulsification methods, it requires less emulsifier and energy, which produces less pollution.
- Moreover, operation of phacoemulsification is not complicated.

In terms of disadvantages, the active substance degrades when the macromolecules are broken by ultrasonic waves.
Besides, phacoemulsification is more suitable for small scale production because effective emulsification only occurs near the waveguide radiator and large production will affect the droplet size distribution. [15]

LOW ENERGY EMULSIFICATON METHODS:

I. Solvent evaporation method:

In this method, a mixed drug with organic solvent utilizes reasonable surfactant and gets ready O/W emulsion by blending in a consistent phase. At that point, organic solvent is evaporated under vacuum or warming or at atmospheric conditions to obtain microspheres stacked with drugs, followed by centrifugation or filtration. [17]

II. PIT method:

In the PIT method, surfactant spontaneous curvature is inversed by changing temperature. Nonionic surfactants, such as polyethoxylated surfactants, undergo dehydration of POE groups of polyethoxylated surfactant, which makes it more lipophilic and leads to changes in curvature of the surfactant. Thus, phase inversion occurs and nanoemulsion is produced. In this method, oil, water, and nonionic surfactants are mixed at room temperature to form oil-in-water (O/W) emulsions. Then, as the temperature gradually increases, dehydration of surfactant POE groups occurs that makes surfactant more lipophilic and surfactant start showing a higher affinity towards the oily phase. This cause phase inversion from the initial O/W emulsion to water-in-oil (W/O) nanoemulsion through intermediate liquid crystalline or bi-continuous structures (e.g., lamellar phase). At hydrophile-lipophile balance (HLB) temperatures (an intermediate temperature) the non-ionic surfactant has zero curvature and shows a similar affinity to the aqueous and oily phases. For efficient phase inversion, rapid cooling or heating of HLB (for obtaining O/W or W/O emulsions, respectively) is required. Rapid cooling or heating produces kinetically stable nanoemulsions.

III. PIC Method:

The phase inversion composition or PIC method is similar to the PIT method; however, in PIC, phase inversion is achieved by changing the system composition rather than the system temperature.

In PIC, one of the components such as water is added to a mixture, and oil-surfactant or oil is added to the water-surfactant mixture. POE type nonionic surfactants are generally used in PIC method to formulate nanoemulsions, although other types can also be used. When water is added slowly to the oil phase and as the volume of the water fraction increases, surfactant POE chain hydration occurs. The surfactant hydrophilic-lipophilic properties of the water phase will become balanced and spontaneous curvature of surfactant will change to zero, similar to at the HLB temperature in the PIT method. During this transition, a bi-continuous or lamellar structure is formed. When additional water is added the transition composition is exceeded, and the structures of the surfactant layer with zero curvature change to having high positive curvature. This change in curvature leads to phase inversion and causes nano-size droplet formation. Thus, changing the composition of the system causes phase inversion. Similarly, other composition parameters, such as the addition of salt and pH changes, also cause nano-size emulsion droplets by transitional phase inversion. [20]

IV. 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.[9]

Characterization of Nanoemulsion:

i. Droplet size:
In this case either by using light scattering technique or by using electron microscopy, the droplet size distribution of Nanoemulsion vesicles can be determined. However, to predicting the stability of nanoemulsion this method is considered as best method. [21]

ii. pH:
The apparent pH of the formulation is measured by pH meter.[22]

iii. Transmission Electron Microscopy (TEM):
Morphology and structure of the nanoemulsion are studied using transmission electron microscopy. Combination of bright field imaging at increasing magnification and of diffraction modes is used to reveal the form and size of nanoemulsion droplets. Observations are performed as a drop of the nanoemulsion is directly deposited on the holey film grid and observed after drying [22]

iv. Drug contain:
This method is employed to determine the amount of drug contained in the formulation. Various methods (especially Western Blot method) are utilized in this order.[23]
Drug content is determined by reverse phase HPLC method using C18 column.

v. Particle charge (zeta potential):
Particle charge determines the physical stability of the nanoemulsion. Particle charge is quantified as zeta potential value which is measured via electrophoretic mobility of particles in an electrical field. Zeta potential of optimized formulation was measured using Beckman coulter Delsa Nano C particle analyzer, USA [24]

vi. Thermodynamic Stability Studies
In this case of characterization for studying the thermodynamic stability of formulation the drug loaded Nanoemulsions following are the stress tests are reported:
• Heating Cooling Cycle: This is the first step in this method the Nanoemulsion formulations were subjected to six cycles which are between refrigerator temperature (4 °C) and 45 °C. After that the stable formulations were then subjected to next centrifugation test.
• Centrifugation: This are second step in this case stable formulation from first step were centrifuged at 3500 rpm and after that the formulation which did not show any phase separation were taken for the next step of freeze thaw stress test.
• Freeze Thaw Cycle: In this step the formulation which are stable at second step are then subjected to three freeze thaw cycles between 21 °C and+25 °C. And this are then kept under standard laboratory conditions. For period of 3 months this study is performed. At accelerated temperature of 30 °C, 40 °C, 50 °C and 60°Cat ambient humidity the three batches of formulations were kept. After that at regular intervals of 0, 1, 2 and 3 months the samples were withdrawn and this sample is then analysed for drug content by stability indicating HPLC method[21]

vii. Dynamic Light Scattering Spectrophotometer:
Dynamic light scattering measurements are done at 90° using a neon laser of wavelength 632nm. The particle size and particle size distribution are determined by dynamic light scattering spectrophotometer.[22]

viii. Dye solubility test:

In this test water soluble dye eosin yellow was added to the nanoemulsion and observed under the microscope. It was found that the continuous aqueous phase was labeled with eosin yellow dye while the dispersed oily phase remained unlabeled. This test confirmed that nanoemulsion formed was o/w type.[24]

ix. Dilutability test:

The W/O type is not dilutable and undergoes phase inversion into O/W type. The O/W type nanoemulsion is dilutable with water. [25]

x. Phase Analysis:

To determine the type if nanoemulsion that has formed the phase system (o/w or w/o) of the nanoemulsions is determined by measuring the electrical conductivity using a conductometer [22]

xi. Viscosity measurement:

Viscosity is an important criterion for efficient drug release and stability. It is measured by using a viscometer. A Brookfield type rotary viscometer measures viscosity at different shear rates at various temperatures.[25]

xii. In Vitro skin permeation studies:

The Franz diffusion cell is used for the drug release profile of nanoemulsions. The drug release is studied by dispersing a known amount of the formulation in the donor compartment of a Franz cell containing a membrane barrier, and then monitoring the appearance of the encapsulated drug in the receptor compartment (containing phosphate buffered saline, pH 7.4; 100 rpm; $37 \pm 1°C$). About 2 ml of the sample is withdrawn from the receptor medium at regular intervals and is replaced with an equal amount of medium. This withdrawn sample is then filtered using 0.22- 50 μm filter and the drug released is analysed using UV-Vis or HPLC spectroscopy at wavelength of peak absorption of the drug.[25]

Conclusion:

Nanoemulsions are widely used in pharmaceutical systems. Nanoemulsion formulation offers several advantages such as delivery of drug, diagnostic agent. Nanoemulsion also protect the drug. it can also provide prolonged action of the medicaments. Overall all nanoemulsion formulation may be considered as effective, safe and with increased bioavailability.

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