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A Review, Aspects on Nanoemulsion in Drug Delivery

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ABSTRACT: Nanoemulsions have attracted great attention in research, dosage form design and pharmacotherapy. This is a result of a number of attributes peculiar to nanoemulsions such as optical clarity, ease of preparation, thermodynamic stability and increased surface area. This novel technology has overcome the problem of less aqueous soluble drugs and it is a vehicle for aqueous insoluble drugs. This review gives a detailed idea about a nanoemulsion system. Nanoemulsions are nano-sized emulsions, which are manufactured for improving the delivery of active pharmaceutical ingredients. In this review article, the attention is focused to give a basic idea about its formulation, method of preparation, characterization techniques, evaluation parameters, and various applications of nanoemulsion. The nanoemulsion can be prepared by both high energy and low energy methods. High energy methods include high-pressure homogenization, microfluidization, and ultrasonication whereas low energy methods include the phase inversion emulsification method and the self-nano emulsification method. However high energy methods are more favorable for food grade emulsion as they require lower quantities of surfactant than low energy methods. The advantages and disadvantages are also described in this article. The applications of Nanoemulsions are described in three main areas which are food, cosmetics, and drug delivery.

KEYWORD: Nanoemulsion, Novel technology, GRAS, Evaluation parameter, Drug delivery system.

INTRODUCTION

Nanoemulsion is a novel drug delivery system. It is one of the novel approaches of drug delivery systems to enhance the bioavailability of poorly water soluble drugs. It is an inotropic mixture of oil, surfactant, Co-surfactant, water and drug.[18] Nanoemulsion is a colloidal particulate system in the submicron size range acting as carrier of drug molecules.[2] It is oil in water or water in oil emulsion which means droplet diameter ranging from 50 to 1000nm. The particles can exist as oil in water and water in oil form where the core of the particle is either oil or water. They are thermodynamically and kinetically stable, clear and translucent. Nanoemulsions are referred to as "miniemulsion", "ultrafine emulsion", "submicron emulsion". [3] There are three types of nanoemulsion which can be formed:

★ oil in water nanoemulsion in which oil is dispersed in the continuous aqueous phase,
★ water in oil nanoemulsion in which water droplets are dispersed in continuous oil phase, and
★ bi-continuous nanoemulsions.[2]

The federal drug administration (FDA) has utilized the molecularity and function of some emulsifiers as the basis of approval for this use in pharmaceutical and food industries. [25] Nanoemulsions are made from surfactants approved for human consumption and common food substances that are "Generally Recognized as Safe " (GRAS) by the FDA. These nanoemulsions are easily produced in large quantities by mixing a water-immiscible oil phase with an aqueous phase under high shear stress or mechanical extrusion process. The system is devoid of problems like creaming, flocculation, coalescence and sedimentation which are the main problems associated with emulsions. [3]

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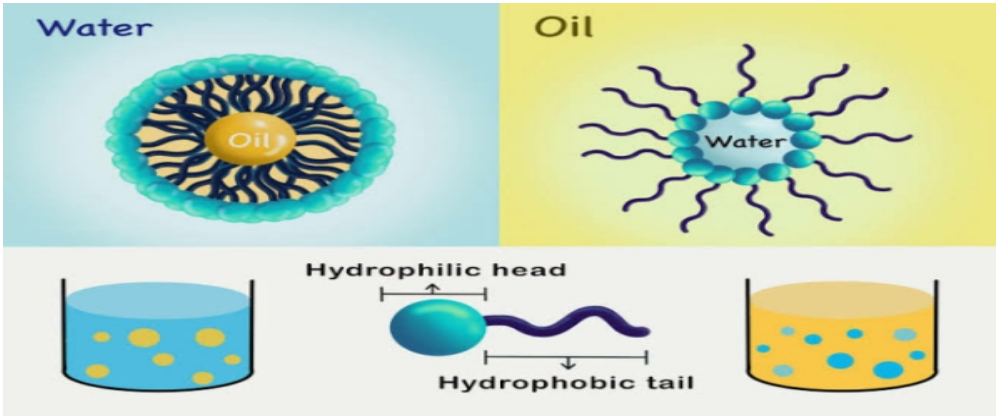

COMPONENTS OF NANOEMULSION

Nanoemulsion is colloidal dispersion made up of two phases,an oil phase and aqueous phase with the help of surfactant and cosurfactant at accurate proportion. [4]

(a) In the formulation of nanoemulsion, the oil phase may include triglycerides like tri, di or mono-acylglycerols, vegetable oil, mineral oil, free fatty acids etc.[5] The formation, stability, and characteristics of nanoemulsion are dependent on the physical and chemical properties of the oil phase such as viscosity, water solubility, density, polarity, refractive index, and interfacial tension as well as chemical stability. [4]

(b) Common surfactant used in the nanoemulsion system for drug delivery and food ingredients are spans (sorbitan fatty acid esters), tween (polyoxyethylene derivatives of sorbitan fatty acid Ester), cremophor EL (polyoxyl-35 castor oil), lauroyl macro glycerides, polysaccharides (gum and starch derivative), phospholipids (egg, soy or dairy lecithin) and amphiphilic proteins. [5] Some of the desirable properties of an emulgent are:

1. it should be able to reduce the surface tension to below 10 dynes/cm.
2. It should be adsorbed rapidly around dispersed phase globules to form a complete and coherent film to prevent coalescence.
3. It should help in building up an adequate zeta potential and viscosity in the system so as to impart optimum stability, and
4. It should be effective in a fairly low concentration. Emulgents form monomolecular, multimolecular or particulate films around the dispersed globules.[3]

(c) Co-surfactants or co-solvent are used along with surfactants. Generally used in formulation of nanoemulsion systems are polyethylene glycol, propylene glycol, ethanol, transcutol-P, ethylene glycol, glycerin and propanol.[5]

(d) To formulate the aqueous phase, water can be mixed with a wide array of polar molecules, carbohydrates, proteins, and others. The formulation, stability, and physicochemical qualities of nanoemulsions are determined by the pH, ionic strength, polarity, density, rheology, refractive index, interfacial tension, and phase behavior of the aqueous phase, which depend on the concentration of the components used and their type. [4]

ADVANTAGES:

- Nanoemulsions have a much higher surface area and free energy than emulsion that make them an effective transport system.
- Nanoemulsions do not show the problems of inherent creaming flocculation, coalescence and sedimentation, which are commonly associated with emulsion.
- It helps to mask taste.
- It is formulated in a variety of formulations such as foams, creams, liquids and sprays.
- It is non-toxic and non-irritant, hence can be easily applied to skin and mucous membrane.
- It is approved for human consumption (for enteric route).
- It doesn't damage healthy human and animal cells. Hence are suitable for human and veterinary therapeutic purposes.
- It protects the drug from hydrolysis and oxidation due to encapsulation in oil droplets.
- It enhances the bioavailability of drugs.

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- Nanoemulsion is the approach to improve water solubility of poorly water soluble drugs (it was applicable in BCS class 2,3,4 drugs) and ultimate bioavailability of lipophilic drugs.
- It may be used as substitute for liposomes and vesicles. [1,3,4,20,25,27]

DISADVANTAGES:

- It is cost expensive operation
- Preparation of nanoemulsion requires in many cases special application techniques, such as the use of high pressure homogenised as well as ultrasonics. microfluidizer became available only in recent years.
- There is a perception in the personal care and cosmetic industry that nanoemulsions are expensive to produce.
- Expensive equipment is required as well as the use of a high concentration of emulsifiers.
- Nanoemulsion has a limited solubilizing capacity for high melting substances.
- The surfactant should be non-toxic for use in pharmaceutical application. [3,4,27]

APPLICATIONS:

1. Use of nanoemulsion in cosmetics.
2. Non-toxic disinfectant cleaner.
3. Nanoemulsion in cell culture technology.
4. Nanoemulsion as a vehicle for transdermal drug delivery systems.
5. Nanoemulsion as a vehicle for an ocular delivery system.
6. Nanoemulsion used for various disease conditions.[1]
7. Antimicrobial nanoemulsion. Based on their antimicrobial activity, research has begun on use of nanoemulsion as a prophylactic medication, a human protective treatment, to treat people exposed to bio-attack pathogens such as anthrax and ebola. A broad spectrum nanoemulsion was tested on surfaces by the US army in Dec 1999 for decontamination of Anthrax spore surrogates. It was tested again by RestOps in March 2001 as a chemical decontamination agent. All tests were successful. The technology has been tested on gangrene and Clostridium botulism spores and can even be used on contaminated wounds to salvage limbs. The nanoemulsion technology can be formulated into a foam, liquid, cream, or spray to decontaminate a variety of materials as has been done by NanoBio Corporation. [6]
8. Nanoemulsion in cancer therapy and targeted drug delivery system. Nanoemulsions are chiefly seen as vehicles for administering aqueous insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents, or diagnostic agents.
9. Nanoemulsion as a mucosal vaccine, Alzheimer's disease, meningitis, Parkinson's disease, migraine, depression, schizophrenia . A vaccine carrier system using nanoemulsions is currently being researched. This medication delivery system uses nanotechnology to vaccinate against human immunodeficiency virus (HIV). There is recent evidence that HIV can infect the mucosal immune system. Therefore, developing mucosal immunity through the use of nanoemulsions may become very important in the future fight against HIV.
10. Nanoemulsion used in intranasal drug delivery systems. There are several problems associated with targeting drugs to the 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.[4,27,21-25]

FORMULATION TECHNIQUE OF NANOEMULSION DRUG DELIVERY SYSTEM (METHOD OF PREPARATION)

❖ High energy methods

1. High pressure homogenization
2. Microfluidization
3. Ultrasonication

❖ Low energy methods

1. Solvent diffusion method (or) solvent displacement method
2. Phase inversion emulsification method

➢ Transitional phase inversion (TPI)

i. Phase inversion temperature (PIT)
ii. Phase inversion composition (PIC)

➢ catastrophic phase inversion (CPI)

i. Emulsion inversion point (EIP)

3. The self nano emulsification method [5]

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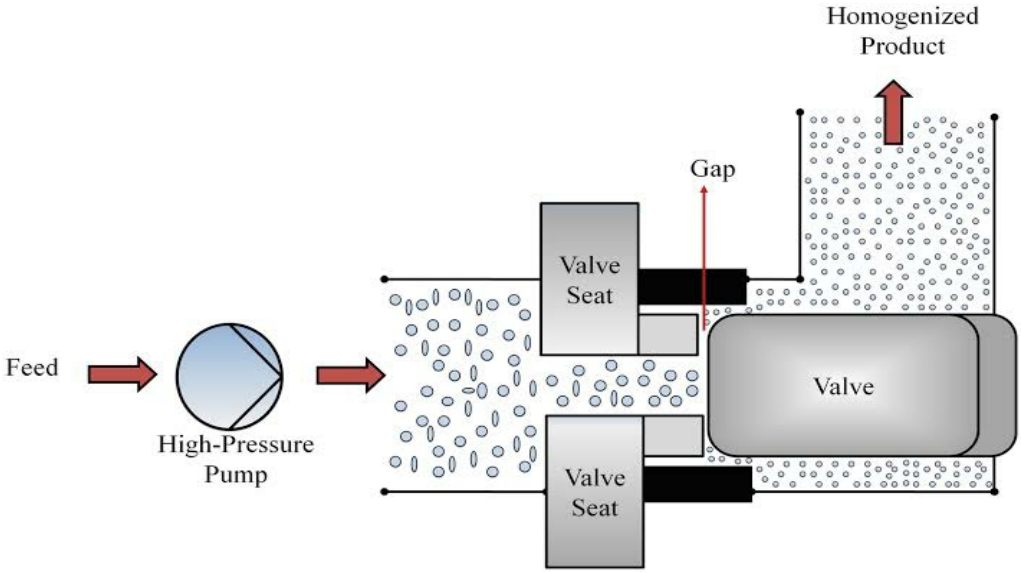

HIGH ENERGY EMULSIFICATION METHOD

Mechanical homogenization was defined as the capacity of producing a homogeneous size distribution of particles suspended in a liquid, by forcing the liquid under the effect of high-pressure through a disruptive valve. It can achieve the energy density needed to produce submicron emulsion (Nanoemulsion). [14] These disruptive forces can be created by using mechanical devices such as HPH, ultrasonication, and microfluidization. The high energy method also provides benefits for stability, rheology and color, consistency, uniformity, viscosity of the nanoemulsion. The high energy method reduces risk of spoilage and inactivation of food components without affecting food safety and nutritional value and sensory attributes. The main disadvantage of the high energy method, continuous homogenization produces heat in the machine, the thermal sensitive products are affected. [28,5,4,21]

➢ **HIGH PRESSURE HOMOGENIZER**

In a high pressure homogenizer, the dispersion of two liquids (oil phase and aqueous phase) or finely divided solids in liquid is achieved by forcing their mixture through a small inlet orifice at very high temperature (500-5000psi). The main principles are turbulence, hydraulic shear and cavitation forces,
which are applied together during this process to give nanoemulsion with very small droplet sizes. HPH are widely used in the pharmaceutical, chemical and food industries. They consist of a high pressure pump and a disruption unit and enable a continuous homogenization. They reduce the particle size up-to 1nm. The most important advantages of HPH for industrial production is that it is scalable, easy tooperate,efficient, and has high reproducibility. [15,5,4,2]

Silverson high shear mixer / homogenizer:

- Much less capital expenditure .
- Much more energy efficient.
- Higher throughput.
- Much simpler to operate, maintain and clean. [7]

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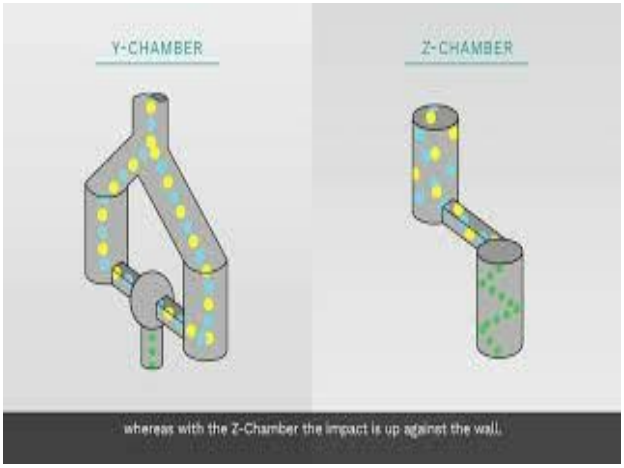
➢ MICROFLUIDIZATION

It is a mixing technology at micro level particles with the help of a device called microfluidizer. It is a direct emulsification method. The aqueous phase and the oily phase are mixed and processed in an inline homogenizer to obtain coarse emulsion. The coarse emulsion undergoes the microfluidizer to obtain stable nanoemulsion. This method forces the product through the interaction chamber with small channels called micro channels inside using a high pressure (500-2000psi) positive displacement pump. The fixed geometry interaction chamber is the core of our technology and comes in different configurations to suit the application. [5,9,4,]

1. Y chamber is used for liquid-to-liquid dispersion. With the Y chamber the two streams of liquid impinge upon each other to create the smaller droplet particle size. It's used in applications such as nanoemulsion, lipid nanoparticles, vaccine adjuvant, encapsulation, liposome, polymer particles.
2. Z chamber is recommended for the suspension or emulsion of solid into a liquid in application such as cell disruption, deagglomeration and particle size reduction. Whereas with the Z chamber the impact is up against the wall. [9]



➢ ULTRASONICATION

In ultrasonic emulsification, sonotrodes called a sonicator probe provide the energy to break down the particle size as it contains piezoelectric quartz crystal, which can expand and contrast according to an alternating electric voltage. High amount of power supply needs to produce high frequency. In the process the sonicator requires alternating current power supply, generally of 50-60 Hz, it produces maximum 20-40kHz. Electromechanical transducers convert this frequency energy into mechanical vibration. Ultrasonic amplitude is used to increase the vibration amplitude. It is better than other high energy methods in terms of operation and cleaning. The tip of the sonicator probe forms a mechanical vibration when it comes into contact with the liquid and this causes cavitation (it is the formation and collapse of vapor cavities in liquid) to occur. A two step mechanisms of ultrasonic emulsification are:

- Interface waves are produced in an acoustic field to break the dispersed phase into a continuous phase.
- Then, formation of acoustic cavitation collapses microbubbles into smaller droplets through the pressure fluctuations.

The collapse of microbubbles causes intense turbulence that causes formation of nano-sized droplets. Through ultrasonication, nanoemulsions can be produced in the absence of surfactants. It doesn't produce heat in between the process, so thermally sensitive substances are also used to convert Nanoemulsion in the ultrasonication process.[4,5,8]

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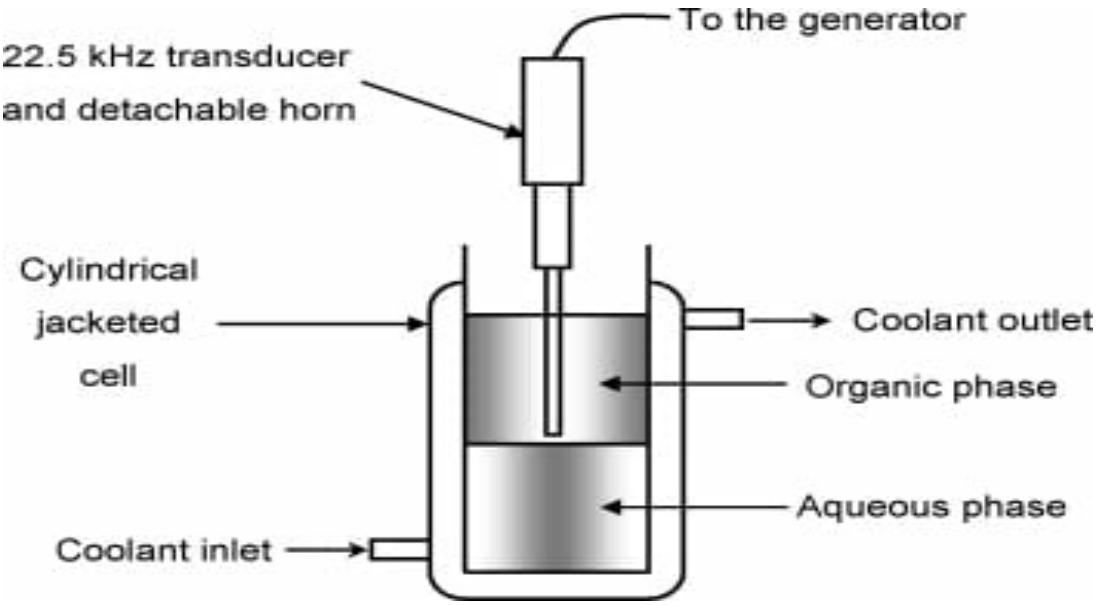
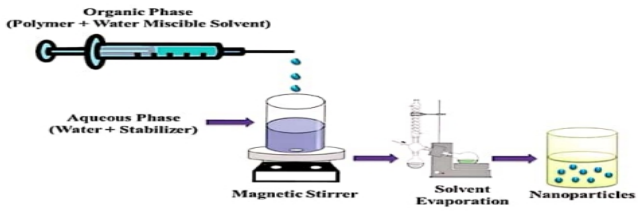


LOW ENERGY EMULSIFICATION METHODS

The low energy method, or condensation method, can be classified into two categories. One is based on phase inversion taking place during the emulsification process where stored chemical energy is used to obtain a small droplet-size distribution. The other low energy emulsification method is the spontaneous emulsification method. Mechanisms involved in this method are diffusion, dilution and phase inversion. The lower energy technique is better because it has simple implementation and use of expensive (or) sophisticated manufacturing equipment is not required. Low energy methods are known as the physicochemical approach, these methods require low energy for production of nanoemulsion systems. This low energy method is not considered for formulation of food grade nanoemulsion as they require high concentration of surfactant,which adversely affect food formulation taste and safety.[16]

➢ **SOLVENT DIFFUSION METHOD (OR) SOLVENT DISPLACEMENT METHOD**

Oily phase is dissolved in water-miscible organic solvent such as acetone, ethanol and ethyl methyl ketone. The organic phase is poured into an aqueous phase containing surfactant to yield spontaneous Nanoemulsion by rapid diffusion of organic solvent. The organic solvent is removed from the nanoemulsion by vacuum evaporation. This method produces Nanoemulsions using only simple stirring at room temperature and is used for parenteral preparation. The major drawback for this method is using organic solvent in the process such as acetone, which requires additional input for their removal from Nanoemulsion. The preparation of monodisperse, small-sized polymeric nanoparticles, this technique is a totally convenient, reproducible, fast, and economicone-step manufacturing process . In addition, the process of solvent removal may appear simple at laboratory scale but can pose several difficulties during scale-up. [1,4,3]



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➢ **PHASE INVERSION EMULSIFICATION METHOD**

In this phase inversion method releases chemical energy that is used to form the nanoemulsion, and can be achieved,

(i) by changing temperature at constant composition, the so-called Phase Inversion Temperature (PIT) .

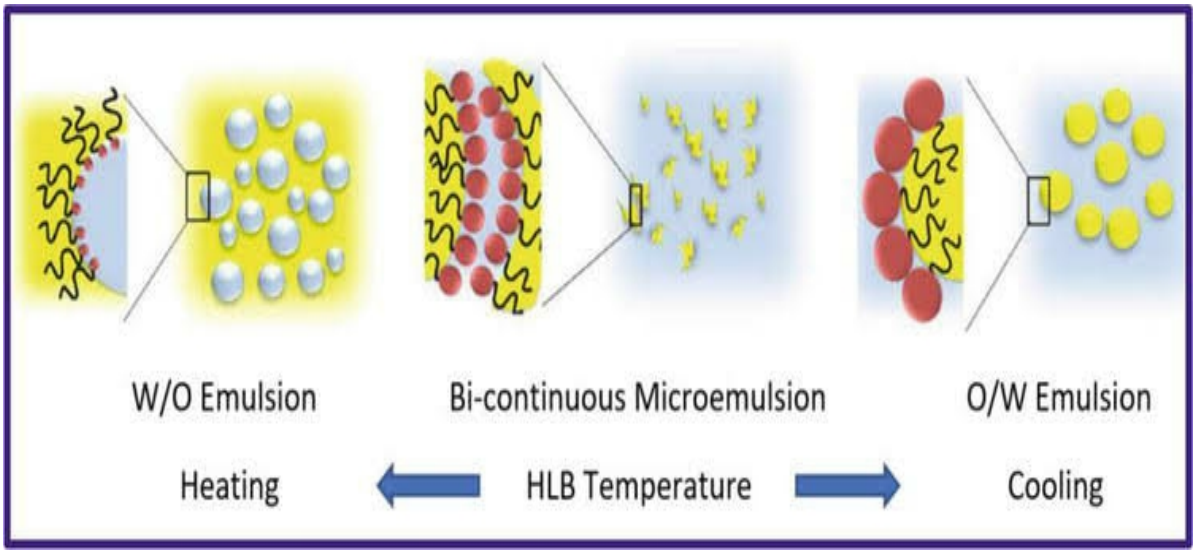
(ii) by varying the composition of the system at constant temperature, the so-called Emulsion Inversion Point (EIP) method or Phase Inversion Composition (PIC) method. [16]

TRANSITIONAL PHASE INVERSION

In this method, spontaneous curvature of surfactant causes phase transition during the emulsification process. Transitional phase inversion takes place due to the changes in spontaneous curvature or affinity of the surfactant due to changes in parameters like temperature and composition. During transitional phase inversion, spontaneous curvature or surfactant affinity is changed.[5]

PHASE INVERSION TEMPERATURE (PIT)

The PIT emulsification method was introduced by Shinoda and Saito in 1968 . PIT emulsification method is based on the change in surfactant curvature induced by temperature, or on the change in lipophilic-hydrophilic balance of some nonionic surfactants with temperature (HLB-temperature emulsification method). The PIT method is based on the alterations in the dispersion of polyoxyethylene-type nonionic surfactants with temperature. With increasing temperature, these kinds of surfactants will turn lipophilic due to the dehydration of the polyoxyethylene chains. At low temperatures, the surfactant monolayer has a large positive spontaneous curvature forming oil-swollen micellar solution phases (or O/W Microemulsions), which may coexist with an excess oil phase. At high temperatures, the spontaneous curvature becomes negative and water-swollen reverse micelles (or W/O microemulsions) coexist with an excessive amount of the water phase. In intermediate temperature, the affinity of amphiphiles for each phase is similar, interfacial curvature is very low, (almost close to zero) and a thermodynamically stable planar structure with zero curvature appears, and lamellar liquid crystals, or bicontinuous microemulsions are formed. The PIT method cannot be applied when ionic surfactants are used as the temperature will not modify the spontaneous curvature of these systems.[5,19,4]



➢ **PHASE INVERSION COMPOSITION (PIC)**

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 (polyoxyethylene) type nonionic surfactants are generally used in PIC method to formulate nanoemulsions, although other types can also be

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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. Similarly, other composition parameters, such as the addition of salt and pH changes, also cause nano-size emulsion droplets by transitional phase inversion. The PIC method, ionic surfactants can be used for low-energy emulsification while only nonionic surfactants can be used for the PIT method. A few potential advantages include low costs and the simplicity of apparatus. The smallest size of the droplets (30 nm) has been produced using this technique.[5,16,17,26]

➢ CATASTROPHIC PHASE INVERSION (CPI) :

The catastrophe means a sudden change in the behavior of a system, due to changing conditions. For catastrophic phase inversion to occur, it is important that the surfactant is chiefly presented in the dispersed phase, thus the rate of coalescence is high, which leads to rapid phase inversion. It occurs when dispersed phase is added continuously until the dispersed phase drops are aggregated with each other to form bi-continuous/lamellar structural phases. In catastrophic phase inversion spontaneous curvature or surfactant affinity does not change.[5]

EMULSION INVERSION POINT (EIP) :

In the EIP method, phase inversion occurs through CPI mechanisms . The catastrophic phase inversion is induced by changing the fractioned volume of the dispersed phase rather than the surfactant properties. In the EIP method, the addition of water to a system of water/oil/surfactant forming a lamellar phase increases the hydration degree of the surfactant polar head thereby increasing its spontaneous curvature. The sizes of the nanoemulsion droplets formed depend on the process variables, such as the rate of water addition and the stirring speed. Small molecule surfactants can be used in catastrophic phase inversion. These surfactants are able to stabilize both W/O emulsions and O/W emulsions. Initially in catastrophic phase inversion, the surfactant is mainly present in the dispersed phase, thus it behaves as an abnormal emulsion (unstable emulsion) which does not obey Bancroft's rules. According to Bancroft's rules, for a stable emulsion (normal emulsion) emulsifiers should predominantly present in the continuous phase. catastrophic phase inversion occurs from the abnormal emulsion to form a more stable normal emulsion.[5,16,17,26]

➢ SELF NANOEMULSION (OR) SPONTANEOUS EMULSIFICATION METHOD

In the self-emulsification method, nanoemulsion formation is achieved without changing the spontaneous curvature of the surfactant. Surfactant and/or co-solvent molecules rapidly diffuse from the dispersed phase to the continuous phase, which causes turbulence and creates nano-sized emulsion droplets. The self-emulsification method is also referred to as the spontaneous emulsification method. Three stages are involved in this method, namely,

- A homogeneous lipid phase consisting of oil and a lipophilic surfactant and another phase of a water-miscible solvent and hydrophilic surfactant were prepared;
- The O/W emulsion is formed when the lipid phase is injected in the aqueous phase under nonstop magnetic stirring; and finally,
- Evaporation under reduced pressure is employed to remove the aqueous phase.

In the food and pharmaceutical industries, high levels of surfactants and cosurfactants are not allowed due to regulatory, cost, and sensory reasons. Some studies have been undertaken to decrease the quantity of co-surfactants as well as in reducing the surfactant to dispersed phase ratio by using the spontaneous emulsification method. [16,5]

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FORMULATION AVAILABLE IN MARKET :

DRUG	BRAND	INDICATION
Propofol	Diprivan®	Anesthetic
Dexamethasone	Limethason®	Steroid
Flurbiprofen	axetil Ropion®	Nonsteroidal analgesic
Vitamin A, D, E & K	Vitalipid®	Parenteral nutrition
Clopidogrel	Plavix®	antiplatelet drug
Cyclosporin	Restasis®	ophthalmic drop for dry-eye syndrome.
palmitate, alprostadil (liple)		vasodilator platelet inhibitor.

IN COSMETICS,

nanoemulsion used as:

- Body moisturizer
- Face lotion with vitamin
- Face cream for night use with vitamins.

*Pureology shampoo has been working with nanoemulsion since 2000, for the color treated hair.

*In 2005 Procter and gamble 's olay brand was developed with nanoemulsion products.

*some other companies using nanotechnology in their skin products: Neutrogene from Johnson and Johnson ,Mary Kay and Clinique from Lauder.

Nano ZnO based sunblock cream

RevitaLift : nanotechnology based antiwrinkle product

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EVALUATION PARAMETER OF NANOEMULSION

1)Droplet size analysis:

Droplet size analysis of nanoemulsion is measured by a diffusion method using a light-scattering, particle size analyzer counter, LS 230. It is also measured by correlation spectroscopy that analyzes the fluctuation in scattering of light due to Brownian motion.

i. The formulation (0.1ml) is dispersed in 50ml of water.
ii. Gently mix by inverting the flask.
iii. Measurement is done using zetasizer 1000HS.
iv. Light scattering is monitored at 25°C at a 90° angle.[2]

(a) Transmission electron microscopy

Droplet size analysis of nanoemulsion can also be performed by Transmission electron microscopy (TEM).

i. It is used to identify the morphology and structure of the nanoemulsion.
ii. The nanoemulsion formulation is diluted with water (1/100).
iii. A drop of the diluted Nanoemulsion is directly deposited on the holey film grid and observed after drying. [20,2]

2)viscosity determination :

The viscosity of nanoemulsion is measured by using Brookfield-type rotary viscometer at different shear rates at different temperatures. Determination of viscosities affirms whether the system is O/W or W/O emulsion. Low viscosity of systems shows that it is O/W type and high viscosity shows that it is water in oil type systudie. However, currently the survismeter has been the most widely employed equipment as it measures surface tension, viscosity, interfacial tension, contact angle, dipole moment and particle size and hydrodynamic volumes of the nanoemulsions.[11,2]

3)Refractive index:

Refractive index tells how light propagates through the medium and transparency of nanoemulsion. Refractive index (n) of medium can be defined as ratio of speed of wave (c) in reference medium to the phase speed of wave (vp) in medium:

$$n=c/vp$$

Refractive index of nanoemulsion is measured by Abbe's refractometer. By placing a drop of nanoemulsion on a slide and comparing it with the refractive index of water (1.333). If refractive index of nanoemulsion has equal refractive index as that of water, then the nanoemulsion is considered to have transparent nature.[11]

4)Drug content:

Preweighed nanoemulsion is extracted by dissolving in a suitable solvent, extract is analyzed by spectrophotometer or HPLC against standard solution of drug.[2]

5)Polydispersity:

It indicates the uniformity of droplet size in nanoemulsion. PCS is based on the principle that the particles with small size travels with higher velocity as compared to particles with large size. The higher the value of polydispersity, the lower will be the uniformity of droplet size of nanoemulsion. It can be defined as the ratio of standard deviation to mean droplet size. It is measured by a spectrophotometer.[11]

6)Dilution test:

Dilution of a nanoemulsion either with oil or with water can reveal this type. The test is based on the fact that more of the continuous phase can be added into a nanoemulsion without causing the problem of its stability. Thus, an o/w nanoemulsion can be diluted with water and a w/o nanoemulsion can be diluted with oil.[2]

7)Dye test:

If a water-soluble dye is added in an o/w nanoemulsion the nanoemulsion takes up the colour uniformly. Conversely, if the emulsion is w/o type and the dye being soluble in water, the emulsion takes up the colour only in the dispersed phase and the emulsion is not uniformly coloured. This can be revealed immediately by microscopic examination of the emulsion. [2]

8)pH and similarity measurements:

The pH of nanoemulsion can be measured by pH meter.[2] A nanoemulsion and micro osmometer is used for determining the osmolarity of emulsion, which is based upon the freezing point method. For performing this, 100 µl of nanoemulsion is transferred in a microtube and measurements are taken.[11]

9)Zeta potential:

Zeta potential is measured by electrophoretic mobility in an instrument known as Zeta PALS, Malvern nanosizer/ zetasizer. It is used to measure the charge on the surface of droplets in nanoemulsion.[2] Zeta potential is used for predicting dispersion stability and its value depends on physicochemical property of drug, polymer, vehicle, presence of electrolytes and their adsorption. For measuring zeta potential,

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nanoemulsion is diluted and its value is estimated from the electrophoretic mobility of oil droplets.[11]

10)Fluorescence test:

Many oils exhibit fluorescence when exposed to UV light. When a w/o nanoemulsion is exposed to a fluorescence light under a microscope, the entire field fluorescences. If the fluorescence is spotty, the nanoemulsion of o/w type. Emission light of nanoemulsion is measured by a spectrofluorometer. [2]

11)Conductance measurement:

The conductance of nanoemulsion is measured by a conductometer. In this test a pair of electrodes connected to a lamp and an electric source is dipped into an emulsion. If the emulsion is o/w type, water conducts the current and the lamp gets lit due to passage of current between the electrodes. The lamp does not glow when the emulsion is w/o: oil being in external phase does not conduct the current.[2]

12)Filter paper test:

This test is based on the fact that an o/w nanoemulsion will spread out rapidly when dropped onto filter paper. In contrast, a w/o nanoemulsion will migrate only slowly. This method should not be used for highly viscous creams.[11]

13)Thermodynamic stability studies :

Thermodynamic stability studies are usually carried out in three steps. Firstly heating-cooling cycle, which is performed for observing any effect on the stability of nanoemulsion by varying temperature conditions. Nanoemulsion is exposed to six cycles between 4° (refrigeration temperature) and 40° by storing the formulation at each temperature for not less than 48 h. The formulations which are stable at these temperatures are further chosen for centrifugation studies. Secondly, centrifugation study in which the formulated nanoemulsions are centrifuged at 5000 rpm for 30 min and observed for phase separation or creaming or cracking. Those which did not show any sign of instability are subjected to a freeze thaw cycle. Thirdly, the freeze-thaw cycle, in which nanoemulsion formulations are exposed to three freeze-thaw cycles with temperature varying between –21° and +25°. Formulations that show no signs of instability pass this test and are deemed to have good stability.[1,2,11]

14)Determination of Entrapment Efficiency:

Entrapment efficiency (EE %) was determined by measuring the concentration of free drug (unentrapped) in aqueous medium. This is of prime importance, as it influences the release characteristics of drug molecules. The amount of drug encapsulated per unit weight of nanoparticles is determined after separation of the entrapped drug from the nanoemulsion formulation:

EE = Weight of total drug in formulation – Weight of drug in aqueous phase × 100 / Weight of total drug in formulation.[10]

15)Fourier-transform infrared spectroscopy (FTIR) spectral analysis:

FTIR analysis can be carried out for the assessment of drug excipient interaction, polymerization, crosslinking as well as drug loading in the formulation. It is also used for identifying the functional groups with their means of attachment and the fingerprint of the molecule. At low temperature a molecule exists in ground state and on absorbing the radiant energy, they get excited to higher energy states. IR spectroscopy is based on determining this energy difference (ΔE) between the excited and ground states of the molecule.[12]

16)In vitro drug release studies:

In vitro drug release studies help to estimate the in vivo performance of drug formulation. The in vitro release rate of a drug is usually studied on a US dissolution apparatus. Nanoemulsion or dried nanoparticles containing drug equivalent to 10 mg were dispersed in a buffer and then it is introduced into dialysis membrane pouches and placed in a flask containing buffer. This study is carried out at 37±0.5° and a stirring speed of 50 rpm. Samples are withdrawn at periodic intervals and each time replaced by the same volume of fresh dissolution medium. Samples are then diluted suitably and the absorbance of the sample is measured spectrophotometrically at a particular wavelength. Absorbance of the collected sample is used for calculating % drug release at different time intervals using a calibration curve.[11]

17)In vitro skin permeation studies :

Keshary Chien-diffusion cell is used for investigating in vitro and ex vivo permeation studies. For performing permeation studies, abdominal skin of adult male rats weighing 250±10 g is usually employed. The rat skin is positioned between the donor and the receiver chambers of diffusion cells. Temperature of receiver chambers containing fresh water with 20 % ethanol is fixed at 37° and the contents of the chamber are continuously stirred at 300 rpm. The formulations are kept in the donor chamber. At specific time intervals such as 2, 4, 6, 8 h, a certain amount (0.5 ml) of the solution from the receiver chamber was

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removed for performing gas chromatographic analysis and each time replaced with an equivalent volume of fresh solution immediately. Each sample is performed three times. Cumulative corrections are done for obtaining the total amount of drug permeated through rat skins at each time interval and are plotted against the function of time. Slope of plot is used for calculating the permeation rates of drugs at a steady-state.[11]

18)Stability studies

Stability studies are performed for assessing stability of the drug substance under the influence of various environmental factors like temperature, humidity and light. The stability studies of nanoemulsion are carried out after storing the formulation for 24 mo in dispersed and freeze-dried state as per International Conference on Harmonisation (ICH) guidelines. The storage conditions followed are ambient (25±2°/60±5 % RH), refrigeration (5±3°) and freeze (–20±5°). The requisite volume of nanoemulsion is stored in glass bottles and is tightly sealed. Samples are withdrawn at predefined time intervals and analysed for the characteristics such as particle size, loading and EE and in vitro drug release profile.[11]

19)Shelf life determination:

For determining shelf life of a nanoemulsion, accelerated stability studies are performed. The formulations are stored at three distinct temperatures and ambient humidity conditions (30°, 40° and 50±0.5°) for almost 3 mo. After a particular time interval (0, 30, 60 and 90 d) samples are withdrawn and analysed using HPLC at λmax for estimating the remaining drug content. Samples withdrawn at zero time are used as controls. The order of the reaction is determined by this and after that the reaction rate constant (K) for the degradation is calculated from the slope of the lines by using following equation at each elevated temperature: slope = −K/2.303, the logarithm values of K are plotted at different elevated temperatures against the reciprocal of absolute temperature (Arrhenius plot). From this plot value of K at 25° is determined and it is further used for calculating shelf life by putting the value in following Eqn.: t0.9=0.1052/K25. Where t0.9 stands for time required for 10 % degradation of the drug and it is termed as shelf life.[11]

20)In vivo studies:

In vivo studies can be performed by adopting a suitable animal model according to the activity chosen. Srilatha et al. has performed antidiabetic activity on glipizide nanoemulsion by choosing hyperglycaemia model in which they first induce diabetes in rats by intraperitoneal injection of streptozotocin solution and then the formulation was given to diabetic rats and the pharmacodynamic studies were performed on them. They reported the reduction in blood glucose levels for up to 12 h.[12]

CONCLUSION

Nanoemulsion drug delivery systems can be prepared by simple technology. They offer efficient targeting and controlled drug delivery. They also offer efficient protection of the encapsulated bioactive materials and enhanced delivery relative to most conventional dosage forms. We recommend more research efforts to exploit the potentials of emulsion nanotechnology in drug delivery of small molecule drugs and novel phytopharmaceuticals. The above study leaves a future scope for refining technology which can further be used for preparation of various other nano systems in pharmaceutical products Selecting the accurate method and optimizing the conditions for the improved stability of NEs will help in the development of high-throughput production and their widespread application in food, beverage, and pharmaceutical industries based on their specific needs. Based on their physicochemical and functional properties, nanoemulsions have very promising multisectorial uses in healthcare, food, polymer manufacturing and cosmetics industries. High energy methods are therefore more costly then 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. Overall all nanoemulsion formulations may be considered as effective, safe and with increased bioavailability. Nanoemulsion may also protect the drugs, which are susceptible to hydrolysis and oxidation.The importance of design and development of emulsion nanocarrier systems aimed at controlling and/or improving required bioavailability levels of therapeutic agents cannot be overemphasized. This article has highlighted development of the various nanoemulsion carrier formulations developed so far have been identified. It is expected that further research and development will be carried out in the future regarding nanoemulsion.

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