



DESIGN AND DEVELOPMENT OF NANOEMULSION FORMULATION: A REVIEW

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ABSTRACT:

Nanoemulsion are relatively stable physically, because the droplet doesn't collide as frequently as in ordinary emulsions and their small droplets sizes enable them to penetrate deep into the tissues through fine capillaries. Thus, such emulsion is being investigated extensively as drug carriers and for its ability to specialize in specific sites within the body. A completely unique Drug Delivery System for enhancement of water solubility of poorly soluble drugs and high first pass metabolism. Nanoemulsion have small droplets size 10-200 nm, high solubilization capacity, high interfacial area, low viscosity, transparent or translucent appearance, and high kinetic stability, and used for various applications. A thermodynamically stable, longer self-life nanoemulsion and remain uniformly dispersed throughout the continuous phase. During this review aim to supply information regarding selection of excipients, Methods for Formulation, Optimization Parameters, Instabilities in Formulation, and thus the numerous applications utilized within the formulation of nanoemulsion.

Keywords: Nanoemulsion, Types, Methods, Optimization, Instabilities, Applications.

1. INTRODUCTION:

In pharmaceutical observation, nanoemulsion is one among the chief dosage forms in delivering active ingredients to the target which features a superb attention in recent years for its application in various fields. As nanoemulsions are used as a drug delivery system through various systemic routes like oral, topical and parenteral.ⁱ

The term Nanoemulsion is said to a thermodynamically stable clear solution of two non-soluble liquids, like oil and water, stabilized by an interfacial film of surfactant molecules. Nanoemulsions are novel drug delivery system includes an emulsified oil and water systems having mean droplet size which ranges from 50 to 1000 nm. The emulsions and nanoemulsions differ mainly in the size and shape of the particles dispersed in continuous phase. The particle size in nanoemulsions is (10-200 nm) and of conventional emulsions are (1-20 μ m).ⁱⁱ

Nanoemulsions, also mentioned as submicron emulsions, ultrafine emulsions and mini emulsions, are submicron sized colloidal particulate systems considered as thermodynamically and kinetically stable isotropic dispersions, which contains of two immiscible liquids like water and oil, stabilized by an interfacial film consisting of an appropriate surfactant and co-surfactant to make one phase. A kind of surfactants with diverse characteristics (ionic or non-ionic) had been used with nanoemulsions.ⁱⁱⁱ

High energy and low energy methods are used for formulation of nanoemulsion. Optimization of nanoemulsion by change in various parameters. During or after the formulation chemical and physical instabilities also are observed and application in various fields are discussed during this review.

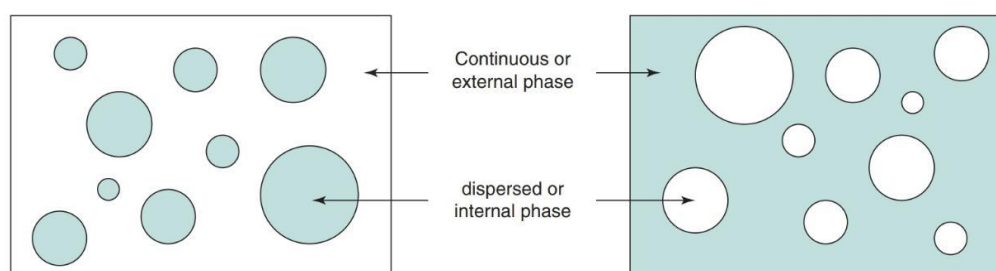


Figure 1 : Oil in Water(O/W) and Water in Oil(W/O) Emulsion

1.1 Advantages of Nanoemulsion:

- It's an approach to strengthen water solubility and bioavailability of lipophilic drugs.
- Helps in stabilizing lipophilic drugs and taste masking.
- Provides protection from hydrolysis and oxidation of drug due to encapsulation in oil droplets.
- Enhance the permeation of drug through the skin.
- Droplet size are nano, so area is large thus increase the speed of absorption and reduce variability, thus enhance bioavailability of drug.
- They need potential to deliver peptides that are susceptible to enzymatic hydrolysis in GIT.
- The utilization of Nanoemulsion as delivery systems can improve the efficacy of a drug, allowing the entire dose to be reduced and thus minimizing side effects.^{iv}

1.2 Disadvantages of Nanoemulsion:

- Use of giant concentration of surfactant and co-surfactant necessary for stabilizing the Nano droplets.
- Require specialized equipment for preparation.
- Limited solubilizing capacity for top melting substances.
- Surfactant must be nontoxic for pharmaceutical application.
- NE stability is influenced by environmental parameters like temperature and ph.^v

1.3 Advantages Over Other Dosage Forms:

- Increased rate of absorption, and reduced variability in absorption.
- Protection from oxidation and hydrolysis in O/W nanoemulsions.
- Delivery of lipophilic drugs after solubilisation.
- Aqueous dosage form for water insoluble drugs.
- Enhanced bioavailability for several drugs.
- Ability to include both lipophilic and hydrophilic drugs.
- Delivery systems to strengthen efficacy while reduce total dose and side effects.
- As non-toxic and non-irritant vehicles for skin and mucosa delivery and release control by permeation of drug through liquid film, whose hydrophilicity or lipophilicity also as thickness are often precisely controlled.
- Nanoemulsions are thermodynamically stable system and thus the stableness allows self-emulsification of the system whose properties aren't enthusiastic to the tactic followed.
- Improve the efficacy of a drug, allowing the entire dose to be reduced and thus minimizing side effects.

1.4 Types of Nanoemulsion:

1. Water in oil (W/O) Nanoemulsion: During which droplet of Water was dispersed in continuous phase oil.^{vi}
2. Oil in water (O/W) Nanoemulsion: During which Oil droplet was dispersed in continuous phase Water.
3. Bi-continuous Nanoemulsion: During which Surfactant was soluble in both oil as well as water Phase, and droplet was dispersed in both oil also as water phase.^{vii}

2. Components of Nanoemulsion:**2.1 Oil:**

The oil is critical for max solubilizing ability for selected drug candidate for Nanoemulsion Formulation. This is often most vital approach having the high drug loading ability. The naturally also as synthetically occurring the mixture of oils and fats are triglycerides contain in long chain fatty acids. The Triglycerides are classified as short chain Triglycerides (12 carbons) is critical to decrease the degree of unsaturation and is critical to stop oxidative degradation. The selection of oil phase is counting on the power of the solubilized drugs and it's vital to from nanoemulsion. The oil is critical to increases friction to maneuver of drug into intracellular compartment is critical to increases water solubility of less water-soluble drug.

As an example, the mixture of fatty oil and medium chain triglycerides is critical to need care appropriate balance between loading capacity of drug and emulsification or Nanoemulsification. The long chain and medium chain triglyceride oils under different degrees of saturation is critical to use in designing of SMEDDS. Triglycerides are highly lipophilic oily molecules and thus the solvent capacity of medicine is common function of effective concentration in ester groups, the medium chain triglycerides (MCT) molecules having higher solvent capacity and skill for resistance to oxidation as compare to long chain triglycerides molecules. Now days, the MCT are replaced by novel semi-synthetic MCT is critical to influencing water solubility of poorly soluble drugs and oil phases are modified by oils, digestible or non-digestible oils and fats like olive oil, palm oil, corn oil, oleic acid, sesame oil, soybean oil, hydrogenated oil for better solubility.^{viii}

2.2 Surfactant:

Surfactant are defined as molecules and ions are adsorbed at interface i.e., surfactant. It's having ability to stop the interfacial nature phenomenon and supply interfacial tension. It's major component for preparation of nanoemulsion. Its act has self-Nanoemulsifying, self-emulsifying and self-Micro emulsifying agent is ability to solubilized poorly water-soluble drug. Most of the compounds can existing the properties of surfactants for designing of emulsifying system. The limited surfactant unit is orally acceptable. Non-ionic surfactants are having high Hydrophilic and Lipophilic Balance (HLB). Optimum amount of surfactant unit is employed for preparation nanoemulsion but great quantity of surfactant can chemical toxicity. Hence the security is major considerable parameter for selection of Surfactant molecule. The molecule of surfactant is obtained in natural also as synthetic origin. Surfactant having limited capacity of Self Emulsification.^{ix} The Non-ionic surfactant having more stable as compared to Ionic surfactant molecule which they're nontoxic and thermodynamically stable molecule. The surfactant concentration is especially supported the dimensions of droplet Molecule for preparation of emulsification and Nano emulsification. This is often important for stabilization of oil Droplet under an area of surfactant system. The surfactant concentration is especially counting on size of

droplet the surfactant concentration was increases ultimately size of droplet was increases. It's vital component of preparation of Nanoemulsion system for improving the solubility of poorly water-soluble drugs.^x

2.3 Co-surfactant:

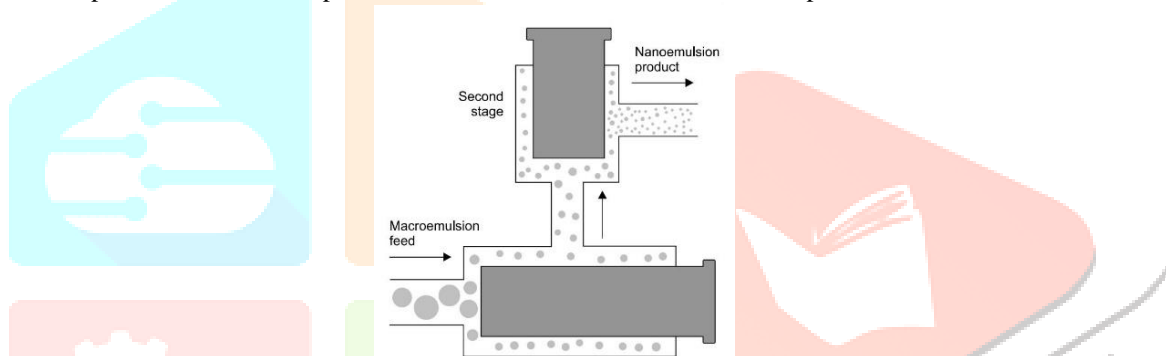
Co-surfactant is analogous function to surfactant unit. Co-surfactant was added in conjunction with surfactant unit or combination of surfactant unit to ready to increases the power of surfactant to improving water solubility of poorly water-soluble drug. The co-surfactant is single chain surfactant unit are ready to prevent the interfacial fluidity. The co-surfactant molecule is inheriting contact with surfactant, oil and water it can separated by Monomolecular Layer of surfactant molecule. The Monomolecular Layer of Surfactant molecule is understood as Liquid Crystal formation layer. The foremost important application of co-surfactant in self-Nanoemulsifying Drug Delivery system (SNEDDS) is to stop interfacial natural phenomenon between oil and water interface. Co-surfactant like Ethanol, Methanol, Pentanol, Glycol, Propylene Glycol.^{xi}

Following Table Describes Formulation Components of Nanoemulsion With Their Examples:

Sr No	Components	Examples
1	Oil	Castor oil, Corn oil, Coconut oil, linseed oil, Mineral oil, olive oil, groundnut oil
2	Surfactant	Polysorbate20, Polysorbate80, Polyoxy 60, DGME, Sorbitan monooleate, Caprylic glyceride
3	Co-surfactant	Ethanol, glycerine, PEG300, PEG400, Polyene glycol, Poloxamer
4	pH stabilizer	Sodium hydroxide or hydrogen chloride, Triethanolamine
5	Preservatives	Methyl Paraben, Propyl Paraben, Benzalkonium Chloride (0.01% w/v), Potassium Sorbate

3. Method of Preparations:

Various methods for preparation of nanoemulsion including the high-energy and low-energy emulsification methods and therefore the combined methods are reviewed. Among the high-energy methods, the stress is placed on high-energy stirring, ultrasonic emulsification, high homogenization including micro fluidics and membrane emulsification. Among the low-energy emulsification methods, the phase inversion temperature method, the emulsion inversion point method and therefore the spontaneous



emulsification. Employing a combined method, which incorporates the high-energy and low energy emulsification, it's possible to organize reverse nanoemulsions in highly viscous systems. Main advantages and limitations off different methods of nanoemulsion preparation are discussed and therefore the potential fields of nanoemulsion applications are considered.^{xii}

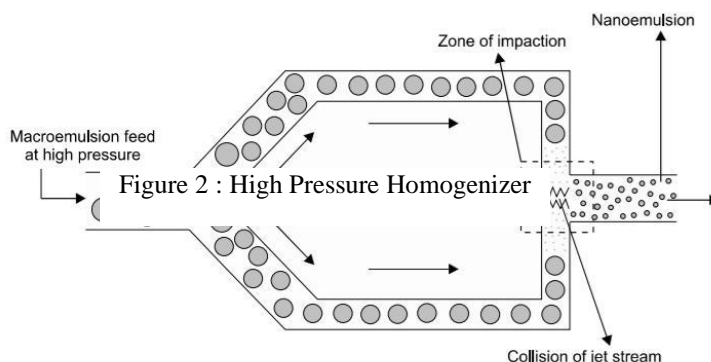
3.1 High Energy Methods:

3.1.1 High-Pressure Homogenization:

This method is performed by applying a high over the system having oil phase, aqueous phase and surfactant or co-surfactant. The pressure is applied with the assistance of homogenizer. Some problems related to homogenizer are poor productivity, component deterioration leads to generation of much heat. With this method only Oil in Water (O/W) liquid nanoemulsion of but 20% oil phase are often prepared and cream nanoemulsion of high viscosity or hardness with a mean droplet diameter less than 200 nm can't be prepared.^{xiii}

3.1.2 Micro fluidization:

Micro fluidization technology makes use of a tool called 'MICRO FLUIDIZER'. This device uses a high-pressure positive displacement pump (500-200 PSI) which forces the merchandise through the interaction chamber, consisting of small channels called micro channels. The merchandise flows through the micro channels on to an impingement area leading to very fine particles of submicron range. the 2 solutions (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a course emulsion. The course emulsion is into a micro fluidizer where it's further processed to get a stable nano emulsion^{xiv}



3.1.3. Ultra-sonication:

Ultra-sonication is best than other high energy methods in terms of operation and cleaning. In ultrasonic emulsifications, ultrasonic waves provide cavitation forces that break the macroemulsion to nanoemulsion. During this method, ultrasonicators are used, which contains a search that emits ultrasonic waves. By varying ultrasonic energy input and time, It will achieve the specified particle size and stability of the nanoemulsion. In ultrasonic emulsification, physical shear is especially provided by the method of acoustic cavitation. Cavitation is that the phenomenon of formation and growth of microbubbles then collapse of microbubbles, which are caused by the pressure fluctuations of the sound wave.^{xv} The collapse of microbubbles causes intense turbulence that causes formation of nano-sized droplets. Irradiation of an oil and water system by ultrasound causes cavitation forces and supply excess energy for brand spanking new interface formations, forming nano-sized emulsion droplets. Through ultrasonication, nanoemulsions are often produced within the absence of surfactants. During a recent study, it had been shown that efficiency of

Figure 3 : Micro fluidizer

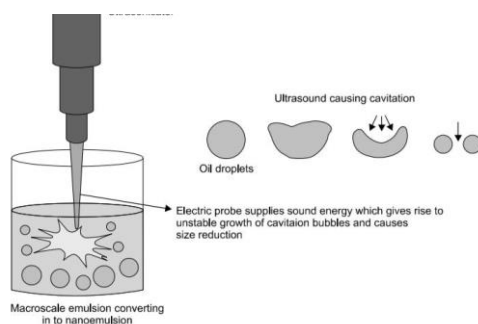


Figure 4 : Ultrasonication

ultrasonic emulsification depends on ultrasonication intensity, time, and nature of the surfactant. Ultrasonication has been used extensively for producing nanoemulsions of medicine and food ingredients. Food grade ultrasonication nanoemulsion shows greater stability and smaller droplet size, and requires less energy input than other high energy method.^{xvixvii}

3.2. Low Energy Methods:

3.2.1. Phase Inversion Emulsification Method:

In this method, spontaneous curvature of surfactant causes phase change during the emulsification process. Modification in spontaneous curvature of the surfactant occur by changes in parameters like temperature, composition, etc. There are two sorts of phase inversion emulsification methods: TPI methods, which involve PIT and PIC, and CPI methods, which involves EIP. Transitional phase inversion takes place to the changes in spontaneous curvature or affinity of the surfactant to changes in parameters like temperature and composition. However, CPI occurs when dispersed particles added continuously until the dispersed particles drops are aggregated with one another to make bicontinuous/lamellar structural phases.^{xviii} The catastrophe means a sudden change within the behaviour of a system, thanks to changing conditions. For catastrophic phase inversion to occur, it's important that the surfactant is chiefly presented within the dispersed particles, thus the speed of coalescence is high, which results in rapid phase inversion. During transitional phase inversion, spontaneous curvature or surfactant affinity is modified, whereas in catastrophic phase inversion spontaneous curvature or surfactant affinity doesn't change.^{xix}

A. Phase Inversion Temperature (PIT):

In Hell method, surfactant spontaneous curvature is inverted by changing temperature. Non-ionic surfactants, like polyethoxylated surfactants, undergo dehydration of POE groups of polyethoxylated surfactant, which makes it more lipophilic and results in changes in curvature of the surfactant. Thus, phase inversion occurs and nanoemulsion is produced. During this method, oil, water, and non-ionic surfactants are mixed at temperature to make oil-in water (O/W) emulsions. Then, because the temperature gradually increases, dehydration of surfactant POE groups occurs that creates surfactant more lipophilic and surfactant start showing a better affinity towards the oily phase. This cause phase inversion from the start of 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 an identical affinity to the aqueous and oily phases. For efficient phase inversion, fast cooling or heating of HLB (for obtaining O/W or W/O emulsions, respectively) is required. Rapid cooling or heating produces kinetically stable nanoemulsion.^{xx}

B. Phase Inversion Composition (PIC):

The phase inversion composition or PIC method is analogous to PIT method; however, in PIC, phase inversion is achieved by changing the system composition instead of the system temperature. In PIC, one among the components like water is added to a mix, and oil-surfactant or oil is added to the water-surfactant mixture. POE type non-ionic surfactants are generally utilized in PIC method to formulate nanoemulsions, although other types also can be used. When water is added slowly to the oil phase and because 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, almost like at the HLB temperature within the PIT method. During this transition, a bi-continuous or lamellar structure is made. When additional water is added the transition composition is exceeded, and therefore the structures of the surfactant layer with zero curvature change to having high positive curvature. This alteration in curvature results in phase inversion and causes nano-size droplet formation. Thus,

changing the composition of the system causes phase inversion. Similarly, other composition parameters, like the addition of salt and pH. Figure 5 Phase Inversion Emulsification Techniques changes, also cause nano-size emulsion droplets by transitional phase

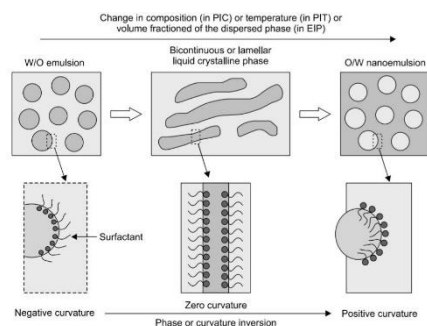


Figure 5 : Phase Inversion Emulsification Techniques

inversion.^{xxi}

C. Emulsion Inversion Points (EIP):

In the EIP method, phase inversion occurs through CPI mechanisms. The Catastrophic Phase Inversion (CPI) is induced by changing the fractioned volume of the dispersed particles instead of the surfactant properties. Because the water phase is added to the oil-surfactant mix, the system starts acting as a W/O nanoemulsion. When increasing amounts of water is added to above a critical water content with continuous stirring, water droplets merge with one another and therefore phase inversion point is reached; this causes bi-continuous or lamellar structures to be formed. Further dilution with water causes phase inversion from a W/O to an O/W system through intermediate bi-continuous microemulsion. The sizes of the nanoemulsion droplets formed depend upon the method variables, like the speed of water addition and therefore the stirring speed. For catastrophic phase inversion to occur, the surfactant should primarily present within the dispersed particles, therefore the rate of coalescence is high and rapid phase inversion occurs. Small molecule surfactants are often utilised in catastrophic phase inversion. These surfactants are ready to stabilize both W/O emulsions and O/W emulsion. Initially in catastrophic phase inversion, the surfactant is especially present within the dispersed particles, thus it behaves as an abnormal emulsion (unstable emulsion) which doesn't obey Bancroft's rules. Consistent with Bancroft's rules, for a stable emulsion (normal emulsion) emulsifier should predominantly present within the continuous phase. Therefore, catastrophic phase inversion occurs from the abnormal emulsion to make a more stable normal emulsion.^{xxii}

3.3. The shelf-nano 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 continual phase, which causes turbulence and creates nano-sized emulsion droplets. The self-emulsification method is additionally mentioned because the spontaneous emulsification method. SNEDDS are supported the self-emulsification phenomenon and contain more hydrophilic surfactants or co-surfactants (co-solvents), and a lower lipid content.^{xxiii} SNEDDS are often defined as isotropic mixture of an oil, surfactant, co-surfactant, and drug. When this mixture is diluted by aqueous fluids *in vivo*, it forms fine and optical clear O/W nanoemulsion, aided by gentle agitation provided by digestive motility of the stomach and intestine. The two most ordinarily reported mechanisms of nanoemulsion formation from SNEDDS are diffusion of the hydrophilic co-solvent or co-surfactant from the organic phase into the aqueous phase, and formation of nanoemulsion negative free energy at transient negative or ultralow interfacial tensions. SNEDDS also are the foremost popular and promising tool for delivery of hydrophobic drugs with low bioavailability. SNEDDS have also been used for delivery of bioactive food components.^{xxiv}

4. Optimizations of Nano-Emulsion Preparation:

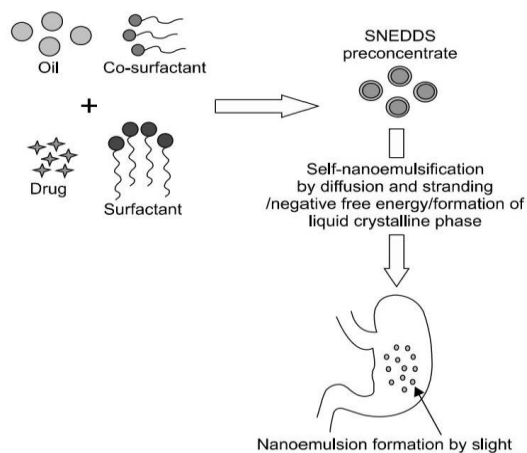


Figure 6 : Self Nano Emulsification Drug

The properties of nano-emulsions, as nonequilibrium systems, depend not only on composition variables but preparation variables such as emulsifying path, agitation or emulsification time. These variables can have a big influence on the nano-emulsion final properties. Direct application of nanoemulsions requires optimization studies for achieving the simplest properties for specific applications. The foremost frequent aim for optimization is to take advantages of nano-emulsions with respect to conventional emulsions (i.e., macroemulsion): small size and low polydispersity. Therefore, generally, optimization is directed to get minimum

droplet size and/or minimum polydispersity. Another aim in nano-emulsion optimization is to enhance the steadiness because, as stated above, stability is that the main problem to beat to seek out practical applications for nano-emulsions. Optimization is additionally directed to get an optimum within the function that the nano-emulsions are used (e.g., drug delivery). The properties to be optimized, for instance droplet size and polydispersity, will depend, of course, on composition variables, and will depend on preparation variables, so optimization is often administered with reference to these two sorts of variables. Concerning optimization methods, sometimes the characteristics of emulsification path allow predicting optimum properties of nanoemulsions, so optimizations administered by studying the phase behaviour of the systems. In other occasions, optimization is experimentally administered by selective variation of one variable. Finally, given the high number of variables which will influence the ultimate properties of nanoemulsions, optimization is administered by experimental designs which permit reducing the number of experiments needed. During this review of papers about optimization is presently classified consistent with these three sorts of methods.

4.1 PHASE BEHAVIOUR STUDIES FOR OPTIMIZATION:

Studies on phase behaviour for optimization of nano-emulsion properties are often important when the so-called condensation or low-energy emulsification methods are used, because the phases involved during emulsification are determinant so as to get nano-emulsions of small droplet size and low polydispersity. In contrast, if shear methods are used, there's not a composition emulsification path and only phases at the ultimate composition are important. The importance of the phase behaviour, namely crossing microemulsion (bicontinuous, D) or lamellar liquid crystalline phase regions during emulsification is described intimately in recent reviews.^{xxv} Some recent original works during which this conclusion is experimentally proved are for nano-emulsions obtained by the phase inversion temperature method (PIT); for nano-emulsions obtained by phase inversion composition method (PIC),^{xxvi} or for nanoemulsions prepared by a self-emulsifying method. Only bicontinuous (D) or O/W microemulsions are considered appropriate for self-emulsifying while lamellar liquid crystal compositions don't self-emulsify by dilution, probably thanks to viscosity of the lamellar phase.^{xxvii} Comparing results from and with results from, it are often concluded that by slow addition of water to a lamellar liquid crystalline phase nano-emulsions can be obtained, while emulsions with higher droplet size are obtained by rapid dilution (as in self-emulsifying methods).^{xxviii} In, nanoemulsions with a really small droplet size are obtained in an ionic surfactant system by adding aqueous phase through an emulsification path crossing a micellar cubic liquid crystalline phase. Actually, conditions for obtaining O/W nanoemulsions with a minimum in droplet size and consequently low polydispersity are often summarized as follows: "In emulsification by phase inversion temperature or composition methods an aqueous continuous phase, O/W or bicontinuous, with all the oil solubilized must be crossed immediately before reaching the ultimate two-phase region where the nano-emulsions form". These are composition conditions necessary but not sufficient, because the kinetics of incorporation of oil to the present water continuous phase or the coalescence can make that nano-emulsion droplet size also depends on preparation variables like aqueous phase addition rate for PIC method or cooling rate for PIT method.^{xxix}

4.2 OPTIMIZATION BY SELECTIVE VARIATION OF PARAMETERS:

Parameters whose influence on nano-emulsion characteristics are often studied could also be classified as composition or preparation variables. For emulsification by low-energy methods composition variables will have a higher influence than preparation variables, however for shear emulsification, the influence of preparation variables are going to be determinant. Samples of recent literature about optimization of nanoemulsions obtained by shear include the study of the influence of various variables and therefore the correlation of droplet size with them.^{xxx} During a food system is studied with a high pressure microfluidizer to emulsify and employing a surfactant and different polymers for stabilizing the emulsions. The competing phenomena of breaking

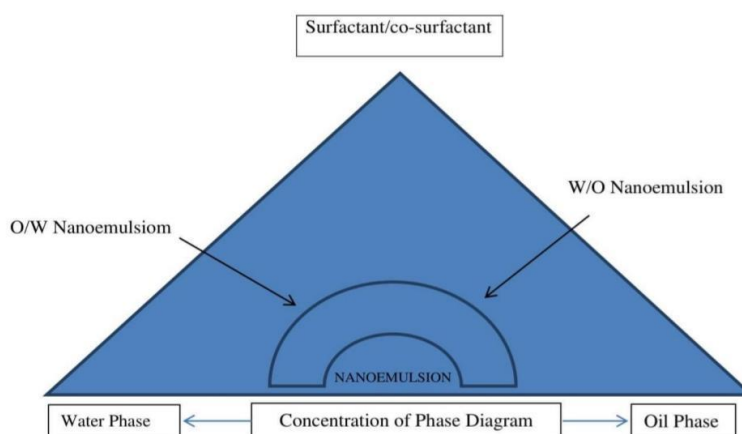


Figure 7 : Ternary Phase Diagram

and coalescence are discussed taking under consideration the effect of stabilizers.^{xxxi} In, optimization of nano-emulsion preparation by submitting a rough emulsion to subcritical water conditions is presented. The optimization was analysed by selective variation of composition parameters (surfactant and oil concentration), and preparation parameter (temperature). For this technique small sizes, 40 nm, are obtained. For other condensation methods, variables whose effect is usually studied are the surfactant oil ratio and therefore the ratio between surfactants when a surfactant mixture is used. For nano-emulsions prepared by the phase inversion temperature method, optimization by selective variation parameters is presented in several cited references of recent bibliography. In variation of droplet size is studied with respect to oil surfactant ratio with the apparent result that the upper the oil surfactant ratio the greater the droplet size, and in variation of droplet size with surfactant mixing ratio is studied with the remarkable result that

droplet size doesn't depend on surfactant mixing ratio if nano-emulsions are prepared by cooling from the HLB temperature. For nano-emulsions prepared by the phase inversion composition method, there are also several studies in recent bibliography. In optimization with reference to preparation method and variation of droplet size with oil surfactant ratio are presented.^{xxxii} In several routes for emulsification are studied and droplet size variation with HLB, water fraction and surfactant concentration is additionally reported. In, effect of variables HLB and oil surfactant ratio are separately studied with the expected result that there's an optimum HLB which the upper the oil surfactant ratio the greater the droplet size. In optimization of W/O nano-emulsion preparation is presented. For various combinations of Span-Tween surfactants, an optimum surfactant composition presenting a water solubility maximum is chosen, and droplet size variation is studied with reference to water concentration.^{xxxiii} Also, with W/O nano-emulsions, the result's, needless to say and coinciding with, that the upper the water concentration the greater the droplet size. For nano-emulsions prepared by self-emulsification, there's an in-depth work on optimization. Droplet size variation with oil, surfactant HLB, and solvents, was studied. The results indicated that there are optimum values for HLB and proportions of solvents. As an example of optimization of nano-emulsion function, within the influence of sucrose surfactants on percutaneous penetration is studied, and in the efficacy of a schistosomicidal agent is improved by incorporating the agent in nanoemulsions.^{xxxiv}

4.3. EXPERIMENTAL DESIGNS FOR OPTIMIZATION:

Experimental designs allow to experimentally studying the influence of several variables with a limited number of experiments. Statistical analysis of results will allow to understand which variables have a big influence, and to correlate desired response with variables by polynomial equations. Experimental design wants to determine the influence of two qualitative independent variables: sort of oil and sort of lipophilic emulsifier. The opposite four references correspond to an equivalent research group. Within the incorporation of retinol to a self-nanoemulsifying formulation is studied, being oil, surfactant and cosurfactant amounts within the formulation the three independent variables, and mean droplet size, turbidity, and dissolution rate, the four response variables studied^{xxxv}. Response equations are presented, and system is optimized for dissolution rate at 30 min using the opposite three responses as restrictions. Within the surface response technology explained during a more detailed way and six response variables are analysed.^{xxxvi} In, an equivalent methodology to judge ultrasonic technique in characterization of nano-emulsions. During a complete explanation of experimental design application to review the preparation of nanoemulsions is presented. Methodology is applied to low-energy emulsification by phase inversion composition method, and effects of composition variables were all at ones evaluated. Droplet size as response surface was minimized separately, first with reference to composition variables, and afterwards with reference to preparation variables. The results confirm that the upper the oil surfactant ratio the greater the droplet size, which there's an optimum surfactant mixing ratio or, what's equivalent, an optimum HLB. Concerning the preparation variables, addition and agitation rate have little but significant influence and an optimum agitation rate is found. In, optimization methodology by experimental design is applied to nano-emulsions in an ionic surfactant system obtained by the phase inversion composition method. Again, the upper the oil surfactant ratio the greater the droplet size, and there's an optimum ratio of surfactants within the mixture used. Concerning the preparation variables, they present again no or low influence on droplet size. Other not published results of the authors on nano-emulsions prepared by the phase inversion temperature confirm that preparation variables like cooling rate or agitation don't have a big influence on droplet size. A general conclusion of papers using experimental designs is that this methodology constitutes a really good tool for studying preparation of nano-emulsions.^{xxxvii}

5. Instabilities in Nanoemulsion: ^{xxxviii}

5.1. Physical Instabilities:

5.1.1. Creaming:

Creaming may be a process which occurs when the dispersed droplets separate under the influence of gravity to make a layer of more concentrated emulsion, the cream. Creaming occurs inevitably in any dilute emulsion containing relatively large droplets (~1 μm) if there's a density difference between the oil and water phases. Most oils are less dense than water, in order that the oil droplets in an o/w emulsion rise to the surface to make an upper layer of cream, whereas water droplets sediment to make a lower layer in w/o emulsions. Although a creamed emulsion is often restored to its original state by gentle agitation, this is often considered undesirable because the emulsion is inelegant and, more seriously, the patient may receive an inadequate dose if the emulsion isn't agitated sufficiently before use. The foremost effective way in practice to scale back creaming is to organize emulsions with small droplet sizes, and to thicken the external phase by the addition of viscosity modifiers. Density adjustment to decrease the density difference between the two phases has received little attention.

5.1.2. Flocculation:

Flocculation may be a weak, reversible association between emulsion droplets which are separated by trapped continuous phase. Each cluster of droplets (floccule) behaves physically as one kinetic unit, although every droplet within the floccule retains its individuality. Floccules are often redispersed by mild agitation, like shaking of the container. Thus, the tendency for flocculation can be reduced by the utilization of an appropriate emulsifier. Although the timescale between flocculation and coalescence are often extended almost indefinitely by the adsorbed emulsifier, flocculation is usually considered undesirable because floccules cream sooner under the influence of gravity than individual emulsion droplets.

5.1.3. Coalescence:

Coalescence describes the irreversible process during which dispersed particles droplets merge to make larger droplets. The method will continue until the emulsion breaks (cracks) and there's complete separation of the oil and water phases. Coalescence occurs when the emulsion droplets are ready to overcome the repulsive energy barrier and approach the first minimum. Once during this minimum, they're in very close proximity to every other, so stability against coalescence is decided essentially by the resistance of the interfacial film to rupture. Coalescence begins with the drainage of liquid films of continuous phase from between the oil droplets as they approach each another and become distorted, and ends with the rupture of the film. Rigid close-packed elastic films formed by specific emulsifier mixtures and thick multi-layered films provided by many polymers protect droplets against coalescence as they're highly immune to film rupture.

5.1.4. Ostwald Ripening:

Ostwald ripening is an irreversible process which involves the expansion of huge droplets at the expense of smaller ones. Ostwald ripening occurs in emulsions containing small sub-micrometre droplets (smaller than ~600 nm), as long as the dispersed particles also features a significant solubility within the continuous phase. Ostwald ripening may be a direct consequence of the Kelvin effect, which explains how the solubility of a partially miscible droplet increases markedly as its radius decreases. Thus, small emulsion droplets have a better solubility than larger droplets. So as to succeed in the state of equilibrium, the tiny droplets dissolve and their molecules diffuse through the continual phase and redeposit onto larger droplets, which grow bigger (ripen), leading to an overall increase in average droplet size. Ostwald ripening differs from coalescence therein it doesn't need any contact between the droplets. Ostwald ripening, instead of coalescence, is that the underlying mechanism of instability in many o/w fat emulsions and in perfluorocarbon emulsions. Although flocculation and coalescence are inhibited by the properties of surfactant interfacial films, Ostwald ripening may be enhanced if micelles are also present to further solubilize the oil. Ostwald ripening are often prevented by the addition of a little quantity of an immiscible second oil to the main partially miscible oil to scale back molecular diffusion of this major component. Fat emulsions containing local anaesthetics or local analgesics show enhanced stability within the presence of less soluble hydrophobic oils, and perfluorodecalin contrast media emulsions are more stable within the presence of small quantities of insoluble perfluorotributylamine. Ostwald ripening additionally inhibited by the addition of the surfactant Pluronic F68®, which is strongly adsorbed at the o/w interface and doesn't form micelles within the continuous phase. Polymers that increase the viscosity of the emulsion external phase also inhibit Ostwald ripening as they hamper the molecular diffusion process.

5.1.5. Emulsion Inversion:

Emulsion inversion occurs occasionally in emulsions under specific conditions. A change in emulsifier solubility from water soluble at coldness to grease soluble at heat (e.g., some non-ionic surfactants) causes phase inversion at a specific temperature from an o/w emulsion to a w/o emulsion, and this phenomenon is employed within the low-energy preparation of nanoemulsions. Emulsion inversion can also occur by specific interactions with other additives. For instance, if a sodium salt is employed to stabilize an o/w emulsion, the emulsion may invert to a w/o emulsion by the addition of divalent ions, like as Ca^{2+} ions, to make the calcium salt, which stabilizes a w/o emulsion.

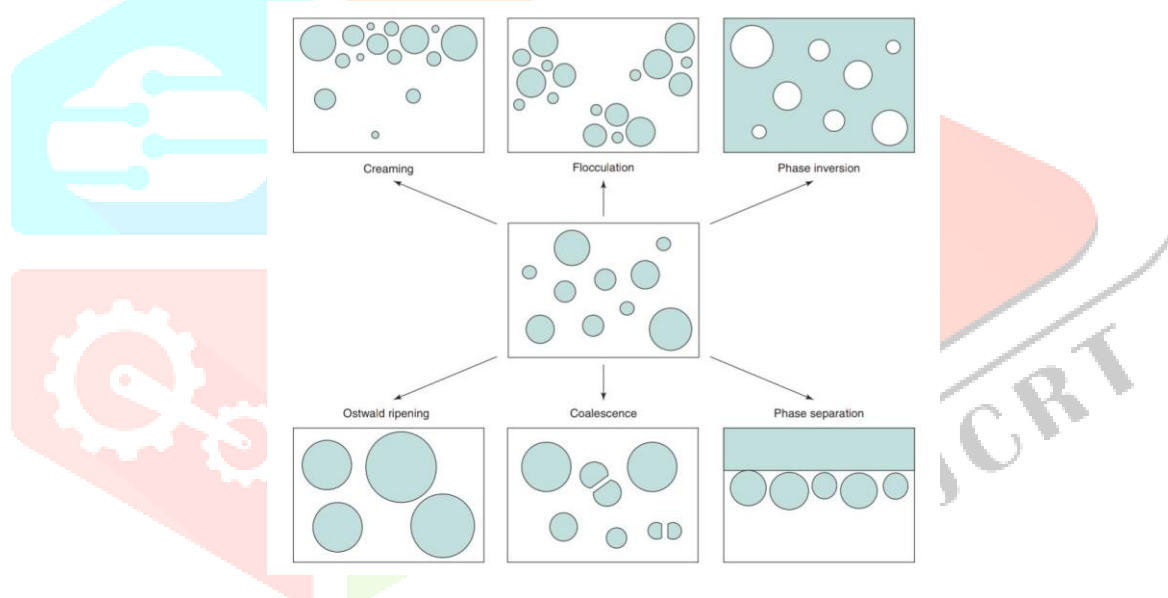


Figure 8 : Summary of Instabilities in Nanoemulsion

5.2 Chemical Instabilities:

Chemical instability ideally, all emulsion components should be chemically inert under the conditions of emulsification. Unfortunately, this is often not always the case, so it's important to know the chemical nature of all the emulsion components before a variety is made. Particular care has got to be taken within the selection of pharmaceutical oils as they'll be vulnerable to oxidation by atmospheric oxygen or microbial contamination, developing an unpleasant odour and taste as they become rancid. Antioxidants and preservatives could also be incorporated into the emulsion to attenuate these effects. Polymeric emulsifiers may undergo depolymerization by hydrolysis or microbial degradation, with loss of emulsification power and consistency. Interactions between the emulsifying agent and other components are of particular concern because emulsifying properties could also be destroyed, causing the emulsion to interrupt. For instance, POE non-ionic emulsifiers form hydrogen bonds with phenolic preservatives, resulting in poor preservation also as loss of emulsifying power. Ionic emulsifying agents are usually incompatible with materials of the other charge. This happens when cationic materials like surfactants or drugs (e.g., cetrimide, neomycin sulphate) are added to a cream containing an anionic emulsifying agent like sodium lauryl sulphate. The cream loses consistency on storage because lamellar structures within the continuous phase are destroyed by the resultant suppression of repulsive forces.

6. Thermodynamic Stability Studies:

During the thermodynamic stability of drug loaded Nanoemulsions following stress tests as reported.

6.1. Heating Cooling Cycle:

Nanoemulsion formulations were subjected to six cycles between refrigerator temperature (4°C) and 45°C. Stable formulations were then subjected to centrifugation test.

6.2. Centrifugation:

Nanoemulsion formulations were centrifuged at 3500 rpm and people that didn't show any phase separation were taken for the freeze thaw stress assay.

6.3. Freeze Thaw Cycle:

The formulation was 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. Three batches of formulations were kept at accelerated temperature of 30°C, 40°C, 50°C and 60°C at ambient humidity. The samples were withdrawn at regular intervals of 0, 1, 2 and 3 months and were analysed for drug content by stability-indicating UV or HPLC method.^{xxxix}

7. Applications:

The delivery of therapeutics in a cell-specific manner may be highly promising application of nanotechnology. Delivery vehicles composed of smart materials having tuneable physical and biological properties will improve current therapeutic strategies by encapsulating toxic agents thereby limiting off-target interactions; improving the bioavailability of poorly soluble drugs imparting tissue or cell specificity and improving or enabling intracellular delivery.^{xi} Nanoemulsions are colloidal dispersions composed of an oil phase, aqueous phase, surfactant and co-surfactant at appropriate ratios. Unlike coarse emulsions micronized with external energy. Nanoemulsions are supported low interfacial tension. This is often achieved by adding a co-surfactant, which results in spontaneous formation of a thermodynamically stable nanoemulsion.

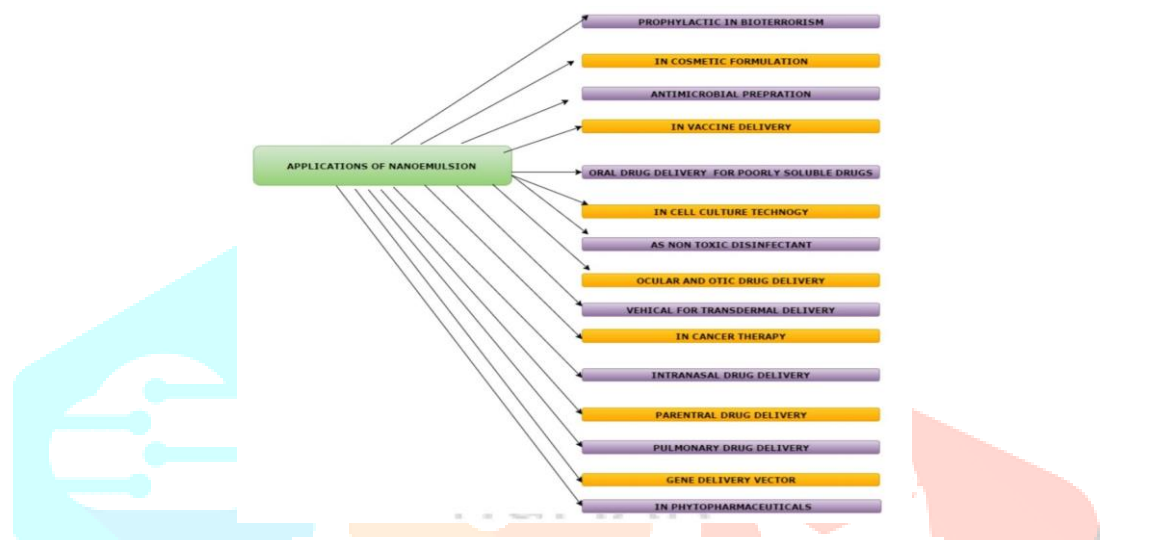


Figure 9 : Application of Nanoemulsion

The term Nanoemulsions is usually wont to designate emulsions with the interior phase droplets smaller than 1000 nm. The nanoemulsions also are referred as mini emulsions, ultrafine emulsions and submicron emulsions. Phase behaviour studies have shown that the dimension of the droplets is governed by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition. Studies on nanoemulsion formation by the phase inversion temperature method have shown a relationship between minimum droplet size and complete solubilisation of the oil during a microemulsion bicontinuous phase independently of whether the initial phase equilibrium is one or multiphase. Thanks to their small droplet size, nanoemulsions possess stability against sedimentation or creaming with ostwald ripening forming the most mechanism of Nanoemulsion breakdown.^{xii} The major difference between emulsion and nanoemulsion albeit emulsion is kinetically stable but thermodynamically unstable, emulsion is cloudy and nanoemulsion is extremely clear in physical appearance. Nanoemulsions might be and are used as effective drug delivery system vaccine delivery, prophylactic in bioterrorism attack, non-toxic disinfectant cleaner, cell culture technology, formulations for improved oral delivery of poorly soluble drug, ocular and optic drug delivery, intranasal drug delivery, parenteral drug delivery cosmetics and transdermal delivery of drug, cancer therapy and pulmonary drug delivery.^{xiii}

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9. Conclusion:

Nanoemulsion is colloidal dispersion of two or more immiscible phase i.e., oil and water. They need more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents, or diagnostic agents. Nanoemulsion drug delivery systems effectively overcome the low bioavailability drawback related to drugs and food components which are hydrophobic, and having high first pass metabolism. High energy methods are employed by researchers to enhance delivery of drugs and bioactive food components. Optimizations by selective variation of parameters or experimental designs allow to conclude that, with reference to composition variables, generally there's an optimum surfactant mixture composition, or HLB, which the upper the oil surfactant ratio the greater the droplet size. Stability of formulation could be enhanced by controlling various factors such as type and concentration of surfactant and co surfactant, sort of oil phase, methods used, process variables and addition of additives used over the inter phases of nanoemulsion formulation. The applications of nanoemulsion are limited by the physical and chemical instability. During this review, the new strategies and considerations for successful nanoemulsion formulation have been presented with the hope that it will function the inspiration for several more success in the field.

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