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Preparation and Optimization of Nanoemulsions for targeting Drug Delivery

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Abstract:
Nanoemulsions have appeared as a novel drug delivery system which allows sustained or controlled release of drug, biological active ingredient and genetic material. Nanoemulsion is a dispersion consisting of oil, surfactant and an aqueous phase, which is a isotropically clear and thermo-dynamically or kinetically stable liquid solution, usually with droplet diameter within the range of 10-500nm. Although interest in nano-emulsions was developed for more than two decades now, mainly for nanoparticle preparation, it is in the last few years that direct applications of nano-emulsions in consumer products are being developed, mainly in pharmacy and cosmetics. These recent applications have made that studies on optimization methods for nano-emulsion preparation be a requirement. The design of effective formulations for drugs has long been a major task, because drug efficacy can severely limited by instability or poor solubility in the vehicle. Nanoemulsion is being applied to enhance the solubility and bioavailability of water insoluble drugs. The nanosized droplets leading to an enormous increase in interfacial areas associated with nanoemulsion would influence the transport properties of the drug [1, 2]. Recently, there has been a considerable attraction for this formulation, for the delivery of hydrophilic as well as hydrophobic drug as drug carriers because of its improved drug solubilization capacity, long shelf life, ease of preparation and improvement of bioavailability of drugs. This review is focused on the most recent literature on developments of nano-emulsions as final application products and on the optimization of their preparation.

Keywords: Nanoemulsion, Applications, Targeting drug delivery, Optimization, Experimental designs.

Introduction:

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 based on low interfacial tension. This is achieved by adding a cosurfactant, which leads to spontaneous formation of a thermodynamically stable nanoemulsion [3]. The term 'Nanoemulsions' is often used to designate emulsions with the internal phase droplets smaller than 1000 nm [4, 5]. The Nanoemulsions are also referred as mini emulsions, ultrafine emulsions and submicron emulsions [6]. Phase behavior studies have shown that the size 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 solubilization of the oil in a microemulsion bicontinuous phase independently of whether the initial phase equilibrium is single or multiphase. Due to their small droplet size, nanoemulsions possess stability against sedimentation or creaming with Ostwald ripening forming the main mechanism of Nanoemulsion breakdown. Nanoemulsions are prepared by the spontaneous emulsification method (titration

method). They can be prepared simply by blending oil, water, surfactant, and cosurfactant, in the right proportion, with mild agitation. The order of mixing the components is generally considered not to be critical since nanoemulsions are formed spontaneously. Although nanoemulsification is a spontaneous process, the driving forces are small and the time taken for these systems to reach equilibrium can be long [7]. To the best of our knowledge, the aqueous titration method used for constructing the phase diagram and the calculations involved for its construction have not been reported in detail. In addition, the basis of selecting different nanoemulsion or microemulsion formulations from the phase diagrams has not been reported, as hundreds of formulations can be prepared from the nanoemulsion region of the phase diagram. The main application of Nanoemulsions is the preparation of nanoparticles using a polymerizable monomer as the disperse phase (the so-called miniemulsion polymerization method) where Nanoemulsion droplets act as nanoreactors [8]. The objective of this technical report is to explain the basis for calculations and construction of pseudoternary phase diagrams and to give an idea for selection of nanoemulsion formulations from the phase diagrams, to avoid metastable formulations in the least possible time. Another focusing on experiencing an active development is the use of Nanoemulsions as formulation for controlled drug delivery and targeting of active drug. As a summary of this point, nano-emulsions are emulsions (non-equilibrium systems, defined according to [9]) with a remarkable small droplet size (in the nanometer range, e.g. 20– 200 nm), regardless of the preparation method. A photography of an oil-in-water (O/W) nano-emulsion with a schematic example of the structure is presented in Fig. 1.

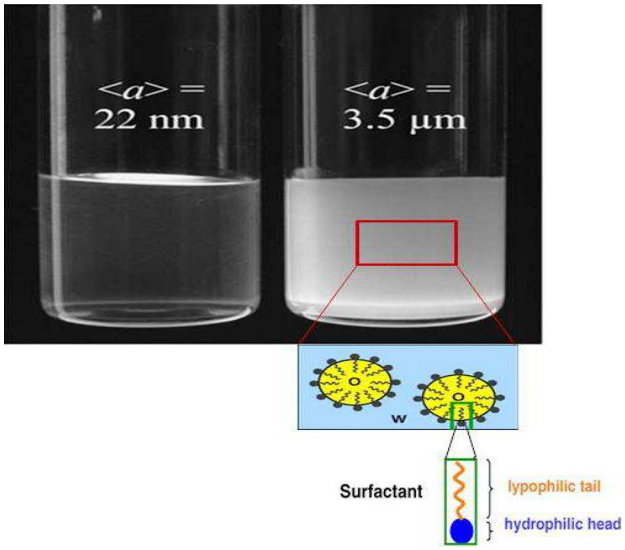

Fig. 1: Visual aspect of an O/W nano-emulsion and structural conformation of the droplets. 1 cm diameter glass vials contain a nanoemulsion having *a* = 22 nm nearly transparent and *a* = *3.5µm* opaque.

Evidently the size range may vary depending on the authors. Some authors consider 500 nm as the upper limit [10]. In any case, the size limit is not a key issue because no qualitative differences are established by droplet size. The formation, properties and stability of nano-emulsions are well established in numerous papers which are reviewed [11]. Regarding applications, nano-emulsions were firstly developed, and used for a long time, to obtain nanoparticles by polymerization [12], the so-called miniemulsion polymerization method, and more recently to obtain solid lipid nanoparticles [13,14], and ceramic particles [15]. At present, new applications are being developed to use nano-emulsions as consumer products. In this review, recent literature on the new applications of nano-emulsions as consumer products is reviewed and classified according to the field of application. This direct application of nano-emulsions requires the optimization with respect to formulation and

preparation variables in order to obtain the desired characteristics. Recent literature on optimization of nano-emulsion preparation is also reviewed and classified according to three approaches: considerations on phase behavior, selective variation of parameters and experimental designs.

Three types of Nanoemulsions are most likely to be formed depending on the composition [16]:

- Oil in water Nanoemulsions wherein oil droplets are dispersed in the continuos aqueous phase
- Water in oil Nanoemulsions wherein water droplets are dispersed in the continuous oil phase
- Bi-continuous Nanoemulsions where in microdomains of oil and water are interdispersed within the system.

In all three types of Nanoemulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants. The key difference between emulsions and nanoemulsions are that the former, whilst they may exhibit excellent kinetic stability, are fundamentally thermodynamically unstable and will eventually phase separate. Another important difference concerns their appearance; emulsions are cloudy while nanoemulsions are clear. In addition, there are distinct differences in their method of preparation since emulsion requires a large input of energy while nanoemulsions do not.

Advantages of Nanoemulsion over other dosage forms

- Increase the rate of absorption.
- Eliminates variability in absorption.
- Helps to solublize lipophilic drug.
- Provides aqueous dosage form for water insoluble drugs.
- Increases bioavailability of active drug.
- Rapid and efficient penetration of the drug moiety.
- Helpful in taste masking.
- Provides protection from hydrolysis and oxidation as drug in oil phase in O/W Nanoemulsion is not exposed to attack by water and air.
- Liquid dosage form increases patient compliance.
- Less amount of energy requirement.
- Nanoemulsions are thermodynamically stable system and the stability allows self-emulsification of the system whose properties are not dependent on the process followed.
- Improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.

Disadvantages of Nanoemulsion based systems

- Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.
- Limited solubilizing capacity for high-melting substances.
- The surfactant must be nontoxic for using pharmaceutical applications.
- Nanoemulsion stability is influenced by environmental parameters such as temperature and pH. These parameters change upon Nanoemulsion delivery to patients.

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METHOD OF PREPARATION:

Various methods for their preparation including the high-energy and low-energy emulsification methods and the combined methods are reviewed. Among the high-energy methods, the emphasis is placed on high-energy stirring, ultrasonic emulsification, high pressure homogenization including micro fluidics and membrane emulsification. Among the low- energy emulsification methods, the attention is focused on the phase inversion temperature method, the emulsion inversion point method and the spontaneous emulsification. Using a combined method, which includes the high-energy and low-energy emulsification, it is possible to prepare reverse nanoemulsions in highly viscous systems. Main advantages and limitations off different methods of nanoemulsion preparation are discussed and the potential fields of nanoemulsion applications are considered [17].

Phase Inversion Method: Fine dispersion is obtained by chemical energy resulting of phase transitions occur through emulsification method. The adequate phase transitions are produced by changing the composition at constant temperature or by changing the temperature at constant composition. The phase inversion temperature (PIT) method was introduced based on the principle of changes of solubility of polyoxy ethylene type surfactant with temperature. This surfactant becomes lipophilic with increase in temperature because of dehydration of polymer chain. At low temperature the surfactant monolayer has a great positive spontaneous curvature forming oil swollen micellar solution phase.

Sonication Method: In this method the droplet size of conventional emulsion are reduced with the help of sonication mechanism. Only small batches of nanoemulsion can be prepared by this method.

High Pressure Homogenizer: This method is performed by applying a high pressure over the system having oil phase, aqueous phase and surfactant or co-surfactant. The pressure is applied with the help of homogenizer. Some problems associated with homogenizer are poor productivity, component deterioration due to generation of much heat. With this method only Oil in water (O/W) liquid nanoemulsion of less than 20% oil phase can be prepared and cream nanoemulsion of high viscosity or hardness with a mean droplet diameter lower than 200 nm cannot be prepared.

Microfluidizatiion: Microfluidization technology makes use of a device called 'MICRO FLUIDIZER". This device uses a high pressure positive displacement pump (500-200 PSI) which forces the product through the interaction chamber, consisting of small channels called micro channels. The product flows through the micro channels on to an impingement area resulting in very fine particles of submicron range. The two 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 is further processed to obtain a stable nano emulsion.

Production with high amplitude ultrasound: This method is a viable alternative to high pressure homogenization. Intense shear forces necessary

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for the nanoemulsification are generated by ultrasonic cavitation which produces violently and asymmetrically imploding vacuum bubbles and break up particles down to the nanometer scale. This method is successfully used in small scale production of nanoemulsions.[18]

Components of Nanoemulsion

Main three components of Nanoemulsions are as follows:

- Oil
- Surfactant/Co-surfactant
- Aqueous phase

Optimization of Nano-Emulsion Preparation

The properties of nano-emulsions, as non-equilibrium systems, depend not only on composition variables but preparation variables such as emulsifying path, agitation or emulsification time. These variables can have a significant influence on the nano-emulsion final properties. Direct application of nanoemulsions requires optimization studies for achieving the best properties for specific applications. The most frequent aim for optimization is to exploit the advantages of nano-emulsions with respect to conventional emulsions (i.e. macroemulsion): small size and low polydispersity. Therefore, in general, optimization is directed to obtain minimum droplet size and/or minimum polydispersity. Another aim in nano-emulsion optimization is to improve the stability because, as stated above, stability is the main problem to overcome to find practical applications for nano-emulsions. Optimization is also directed to obtain an optimum in the function for which the nano-emulsions are used (e.g. drug delivery).The properties to be optimized, for example droplet size and polydispersity, will depend, of course, on composition variables, and could depend on preparation variables, so optimization can be carried out with respect to these two types of variables. Concerning optimization methods, sometimes the characteristics of emulsification path allow predicting optimum properties of nanoemulsions, so optimizations are carried out by studying the phase behaviour of the systems. In other occasions, optimization is experimentally carried out by selective variation of one variable. Finally, given the high number of variables that can influence the final properties of nano-emulsions, optimization is carried out by experimental designs which allow reducing the number of experiments needed. Review of papers about optimization is presently classified according to these three types of methods.

Phase Behaviour Studies for Optimization

Studies on phase behaviour for optimization of nano-emulsion properties can be important when the so-called condensation or low-energy emulsification methods are used, because the phases involved during emulsification are determinant in order to obtain nano-emulsions of small droplet size and low polydispersity. In contrast, if shear methods are used, there is not a composition emulsification path and only phases at the final composition are important. The importance of the phase behavior, namely crossing microemulsion (bicontinuous, D) or lamellar liquid crystalline phase regions during emulsification is described in detail in recent reviews [19, 20, 10]. Some recent original works in which this conclusion is experimentally proved are

[21–24] for nano-emulsions obtained by the phase inversion temperature method (PIT); [25–27] for nano-emulsions obtained by phase inversion composition method (PIC), or [28-30] for nano-emulsions prepared by a self-emulsifying method. Only bicontinuous (D) or O/W microemulsions are considered appropriate for self-emulsifying while lamellar liquid crystal compositions do not self-emulsify by dilution, probably due to viscosity of the lamellar phase [28]. Comparing results from Refs. [31] and [32] with results from Ref. [28], it can be 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). In Ref. [33], nano-emulsions with a very 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 nano-emulsions with a minimum in droplet size and consequently low polydispersity can be 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 final two phase region where the nano-emulsions form". These are composition conditions necessary but not sufficient, because the kinetics of incorporation of oil to this water continuous phase or the coalescence can make that nano-emulsion droplet size also depends on preparation variables such as aqueous phase addition rate for PIC method or cooling rate for PIT method.

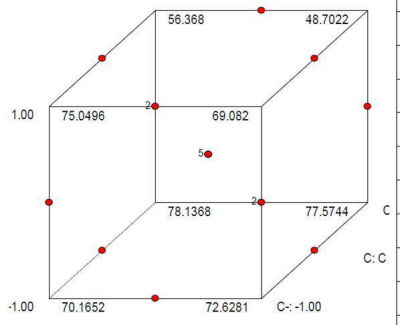
OPTIMIZATION BY SELECTIVE VARIATION OF PARAMETERS

Parameters whose influence on nano-emulsion characteristics can be studied may be classified as composition or preparation variables. For emulsification by low-energy methods composition variables will have a much higher influence than preparation variables, however for shear emulsification, the influence of preparation variables will be determinant. Examples of recent literature about optimization of nanoemulsions obtained by shear include the study of the influence of different variables and the correlation of droplet size with them [34]. In Ref. [35] a food system is studied with a high pressure microfluidizer to emulsify and using a surfactant and different polymers for stabilizing the emulsions. The competing phenomena of breaking and coalescence are discussed taking into account the effect of stabilizers. In Ref. [36], optimization of nano-emulsion preparation by submitting a coarse emulsion to subcritical water conditions is presented. The optimization was studied by selective variation of composition parameters (surfactant and oil concentration), and preparation parameter (temperature). For this system small sizes, 40 nm, are obtained. For other condensation methods, variables whose effect is commonly studied are the surfactant oil ratio and 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 [24, 37] variation of droplet size is studied with respect to oil surfactant ratio with the obvious result that the higher the oil surfactant ratio the greater the droplet size, and in [23]

variation of droplet size with surfactant mixing ratio is studied with the remarkable result that droplet size does not 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 [38] optimization with respect to preparation method and variation of droplet size with oil surfactant ratio are presented. In [27] different routes for emulsification are studied and droplet size variation with HLB, water fraction and surfactant concentration is also reported. In Ref. [39], effect of variables HLB and oil surfactant ratio are separately studied with the expected result that there is an optimum HLB and that the higher the oil surfactant ratio the greater the droplet size. In Ref.[40] optimization of W/O nano-emulsion preparation is presented. For different combinations of Span-Tween surfactants, an optimum surfactant composition presenting a water solubility maximum is chosen, and droplet size variation is studied with respect to water concentration. Also with W/O nano-emulsions, the result is, as expected and coinciding with Ref. [31], that the higher the water concentration the greater the droplet size. For nano-emulsions prepared by self-emulsification, there is a detailed work on optimization [41]. 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, in Ref. [26] the influence of sucrose surfactants on percutaneous penetration is studied, and in Ref. [35] the efficacy of a schistosomicidal agent is improved by incorporating the agent in nano-emulsions.

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 know which variables have a significant influence, and to correlate desired response with variables by polynomial equations. In Fig. 2 an example of experimental design is shown, and in Fig. 3 there is an example of response surface.



Experiment	Speed (rpm)	Flow rate (ml/min)
1	342	0.76
2	**456**	**1.34**
3	250	2.45
4	**456**	**1.34**
5	650	3.76
6	546	4.28
7	**456**	**1.34**
8	318	5.68
9	235	3.76
10	435	4.79
11	638	6.26

Fig.3. Example of experimental design for the preparation variables agitation (rpm) and addition rate (ml/min)

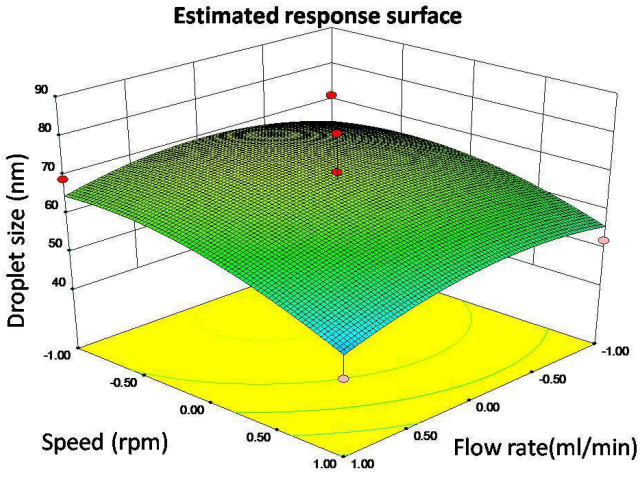


Fig.3. Example of surface response from the preparation variables agitation (rpm) and addition rate (ml/min).

Not many papers present optimization of nano-emulsion preparation by experimental designs, and most of them deal about pharmaceutical

formulations for self-emulsification [27, 42–45]. In Ref. [27] experimental design was used to determine the influence of two qualitative independent variables: type of oil and type of lipophilic emulsifier. The other four references correspond to the same research group. In Refs. [42,43] the incorporation of retinol to a self nanoemulsifying formulation is studied, being oil, surfactant and cosurfactant amounts in the formulation the three independent variables, and mean droplet size, turbidity, and dissolution rate at 10 and 30 min, the four response variables studied. Response equations are presented, and system is optimized for dissolution rate at 30 min using the other three responses as restrictions. In Ref. [44] the surface response technology explained in a more detailed way and six response variables are analyzed. In Ref. [45], authors apply the same methodology to evaluate ultrasonic technique in characterization of nano-emulsions. In Ref. [31] a complete explanation of experimental design application to study the preparation of nano-emulsions is presented. Methodology is applied to low-energy emulsification by phase inversion composition method, and effects of composition variables and preparation variables were all together evaluated. Droplet size as response surface was minimized separately, first with respect to composition variables, and afterwards with respect to preparation variables. The results confirm that the higher the oil surfactant ratio the greater the droplet size, and that there is an optimum surfactant mixing ratio or, what is the same, an optimum HLB. Concerning the preparation variables, addition and agitation rate have little but significant influence and an optimum agitation rate is found. In Ref. [46], optimization methodology by experimental design is applied to nano-emulsions in an ionic surfactant system obtained by the phase inversion composition method. Again, the higher the oil surfactant ratio the greater the droplet size, and there is an optimum ratio of surfactants in 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 such as cooling rate or agitation do not have a significant influence on droplet size. A general conclusion of papers using experimental designs is that this methodology constitutes a very good tool for studying preparation of nano-emulsions.

EVALUATION PARAMETERS OF NANOEMULSION:

Thermodynamic Stability Studies: Nanoemulsion was subjected to various storage conditions of temperature and humidity to assess their stability as per ICH guidelines Q1A (R2) [47]. Physical and chemical stability of nanoemulsion were evaluated for six months by storing them at 30 ± 2^{0}C / 65 ± 5% RH and 40 ± 2^{0}C / 75 ± 5% RH To overcome the problem of metastable formulation, thermodynamic stability tests were performed. Formulations centrifuges at 3500 rpm for 30 minutes. Those formulations that does not show any phase separations take for the heating and cooling cycle. Six cycles between refrigerator temperatures of 4°C and 45°C for 48 hours. The formulations stables at these temperatures are subjected to the freeze-thaw cycle test. Three freeze-thaw cycles do for the formulations between –21°C and +25°C. Those formulations that survive thermodynamic stability tests are select for the further studies.

Droplet Size Analysis: The droplet size of the nanoemulsion was determined by photon correlation spectroscopy. The formulation (0.1 mL) was dispersed in 50 mL of water in a volumetric flask and gently mixed by inverting the flask. Measure by using a Zetasizer and light scattering monitor at 25°C at 90°or 180° angle. [48]

Transmission Electron Microscopy: Morphology and structure of the nanoemulsion usually determined by transmission electron microscopy (TEM). A combination of bright-field imaging at increasing magnification and of diffraction modes use to reveal the form and size of the nanoemulsion. To perform the TEM observations, the nanoemulsion formulation dilute with water (1/100). A drop of the diluted nanoemulsion directly deposited on the film grid and observed after dry [49, 50]

Refractive Index: The refractive index, n, of a medium is defined as the ratio of the speed, c, of a wave such as light or sound in a reference medium to the phase speed, vp, of the wave in the medium represented by equation 1

n=c/vp ---------- 1

It was determined using an Abbes type refractrometer [48-49]

Drug Content: Drug content determine by reverse phase HPLC method using different columns of appropriate porosity [49, 51]

CONCLUSION:

Nano-emulsions are proposed for numerous applications in pharmacy as drug delivery systems because of their capacity of solubilizing non polar active compounds. Nanoemulsion formulations offer several advantages for the delivery of drugs, biologicals, or diagnostic agents and able to protect labile drug, control drug release, increase drug solubility, increase bioavailability and reduce patient variability. Due to the stability problems, most of proposed formulations are selfemulsifying systems and the nano-emulsions are produced just before their application. Although there have not been reported too many applications in other fields, there is a great potential for nano-emulsion applications if Oswald-ripening destabilization mechanism is limited by using very insoluble oils. Concerning optimization in preparation of nano-emulsions by shear, an optimum shear or time shearing can exist if breaking and coalescence are competing phenomena during the process.

Concerning optimization in the preparation of nano-emulsions by low-energy methods, recent literature confirms that crossing bicontinuous or aqueous continuous phases during emulsification allows obtaining O/W nano-emulsions of small droplet size and low polydispersity. Optimizations by selective variation of parameters or experimental designs allow to conclude that, with respect to composition variables, generally there is an optimum surfactant mixture composition, or HLB, and that the higher the oil surfactant ratio the greater the droplet size. The preparation variables, as addition, agitation or cooling rate, generally do not have a significant influence if the system is optimized with respect to composition.

This last conclusion has a very important derivation: if preparation variables do not have influence, the system can be scaled-up, from lab to industrial, and similar results can be expected.

Traditionally, Nanoemulsions have been used in clinics for more than four decades as total parenteral nutrition fluids. Targeting moiety has

opened new avenues for targeted delivery of drugs, genes, photosensitizers, and other molecules to the tumor area. As a final comment, judging from the most recent literature, the interest in nano-emulsion preparation and application is growing, It is expected that further research and development work will be carried out in the near future for clinical realization of these targeted delivery vehicles.

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