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Titre / Title :

More efficient emulsification procedure to obtain parenteral emulsions

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Catégories / ***Categories***

-4 New challenges. New ambitions.
4.2 New emulsification technics

Communication / *Paper :*

1. Introduction

Emulsions are encountered in multiple processes which include polymerization, specific extraction of solvents, mineral flotation, crude oil dehydration, biochemical reactors and in a vast variety of products, such as dermocosmetic creams and pharmaceutical products [1]. In recent years, attention has been focused on emulsions with submicrometer droplet size, typically between 20 and 500 nm, also called nanoemulsions. Nanoemulsions are generally obtained by application of high levels of mechanical during emulsification, the so-called dispersion methods, or by low-energy emulsification methods where the system goes through low interfacial tensions values during the mixing process [2, 3, 4]. It has been determined that, in some systems, liquid crystals have an influence in the formation of nanoemulsions in low energy emulsification methods, especially in phase transition emulsification [4]. The method consists in changes from oil external phase dispersion to an intermediate liquid crystalline mesophase and its inversion to an oil-in-water emulsion by adding water to a surfactant-oil-water system (SOW).

Parenteral emulsions are systems formed by a mixture of triglycerides dispersed by phospholipids [5] in aqueous media to be administrated by intravenous route. They have been broadly used as a source of calories and fatty acids essential for patients or neonates whose medical condition makes them unable to eat normally. They are also used in the administration of lyposoluble drugs and in the controlled release of drugs [5, 6, 7]. These emulsions must have a droplet size distribution with diameters below 5 μm to avoid the risk of pulmonary embolism.

The traditional technique to prepare parenteral emulsion requires high-energy input. The procedure can be divided in three stages: pre-emulsification, homogenization and sterilization [5, 6]. In the pre-emulsification stage solubilization of the components is achieved by adding the hydro-soluble constituents in the aqueous phase and those lyposoluble in the oily phase [7]. Then the emulsification of both phases is produced through a high shear mixer, usually adding the oil over the aqueous phase [8], obtaining a coarse emulsion. The resulting coarse emulsion is dispersed rapidly in high pressure homogenizers [8] or microfluidizers [9] at pressures higher than 1000 bar. A fine and monodisperse emulsion is obtained (0.1 to 0.5 μm).

In recent studies, parenteral emulsions have been obtained by low energy emulsification methods, through microfiltration [10] and by formulation engineering using the know-how regarding high internal phase ratio emulsification [12, 13]. In this work a method to obtain

parenteral emulsions composed by lecithin, water, glycerol and soybean oil, using a phase transition low energy emulsification procedure is studied.

2. Experimental

2.1 Materials

Soybean lecithin, a mixture of phospholipids (phosphatidylcholine predominantly) with a purity of 30 % was obtained from Fiorentini, Italy. Among its impurities are fats (41 %) and carbohydrates (8 %). Commercial soybean oil (ρ: 0.919 g/cm^3, viscosity at 25ºC: 57.4 mPa s) was acquired from Branca, Venezuela. Glycerol (98% pure) was obtained from Cer diagnostics, Venezuela. All of the products were used without further purification. Double distilled water was also used.

2.2 Methods

2.2.1 Emulsion formation: the emulsions were obtained dissolving the surfactant in the soybean oil, then the aqueous phase (2.5 % v/v of glycerol) was added slowly to the oil phase maintaining a constant stirring speed of 250 rpm using an Eurostar Power Control Visc stirrer (IKA, Germany). Three methods of aqueous phase addition were evaluated, one at a constant rate of 3 mL/min, another at a rate of 5 mL/min and a third consisting of three steps, the first at a rate of 3 mL/min, the second at 20 mL/min and last at a rate of 3 mL/min. Each batch yielded 50 g of emulsion.

2.2.2 Droplet size: determination of droplet size distribution was done by means of a laser light diffraction analyser LS13320 (Beckman Coulter, U.S.), with a measurement range of 0.04 to 2000 μm.

2.2.3 Viscosity: A rotational rheometer, model SR-5000 (Rheometric Scientifics, U.S.) was used to perform the rheological tests. For high viscosities, parallel plates geometry with a separation of 1 mm was used, while a concentric cylinders arrangement was utilized to evaluate less viscous fluids.

2.2.4 Conductivity: Changes in emulsion morphology from W/O to O/W were detected by monitoring the conductivity by means of a digital conductivity meter model CDM210 (Radiometer Analytical, Copenhagen) equipped with a platinized-platinum cell (model XE130), which was connected to a computerized data acquisition system.

3. Results and Discussion

Figure 1 shows droplet size distribution of emulsions obtained at a constant temperature of 25ºC and a lecithin/oil ratio (R) equal to 0.35. The emulsion prepared at 0.33 mL/min has a Sauter diameter of 3.15 μm and a $d_{(90)}$ of 16.91 μm. When flow was increased to 3 mL/min under similar mixing conditions, the system showed a higher viscosity and a droplet size distribution similar to that of the emulsion formed at a water addition rate of 0.33 mL/min. The emulsion obtained at a constant rate of 5 mL/min presented a particular behaviour. During the first stages of emulsification, the aqueous phase solubilized in the mixture of soybean oil and lecithin, formed a viscous phase. Some studies have reported enbranched reverse cylindrical micelles (organogel), which is a mesophase characteristic of W/O systems dispersed with lecithin. The lecithin organogel is a translucent isotropic phase [13, 14], which looks like the initial solution before water is added, but for the increased viscosity of the mesophase. When the composition of aqueous phase in the system was increased between 13 to 41 wt % a substantial increase of viscosity was observed. At this point, a change in the physicochemical behaviour of the system occurred, a viscoelastic mesophase was formed which slowed the mass transfer of aqueous phase to the interior of the dispersion. When a sample of this dispersion was added in water (or oil) no redispersion in water (nor in oil) was observed. This behaviour is characteristic of microemulsion or liquid crystal mesophases [14]. Finally, the viscosity of the system diminished as a function of water content (up to 68 wt%). The emulsion obtained

showed a less polydisperse distribution compared to those obtained at rates of 0.33 and 3 mL/min of aqueous phase.
The viscoelastic behaviour presented at 41 wt % of aqueous phase is related to the formation of association structures in the lecithin/water/fatty acid systems. Lamellar liquid crystals have been detected in the lecithin/water/isopropil miristeate [14] and lecithin/water/isopropylpalmitate systems [15], also multilayer vesicles (spherulites) dispersed in water mesophase have been found in the lecithin/water/isopropylpalmitate system.

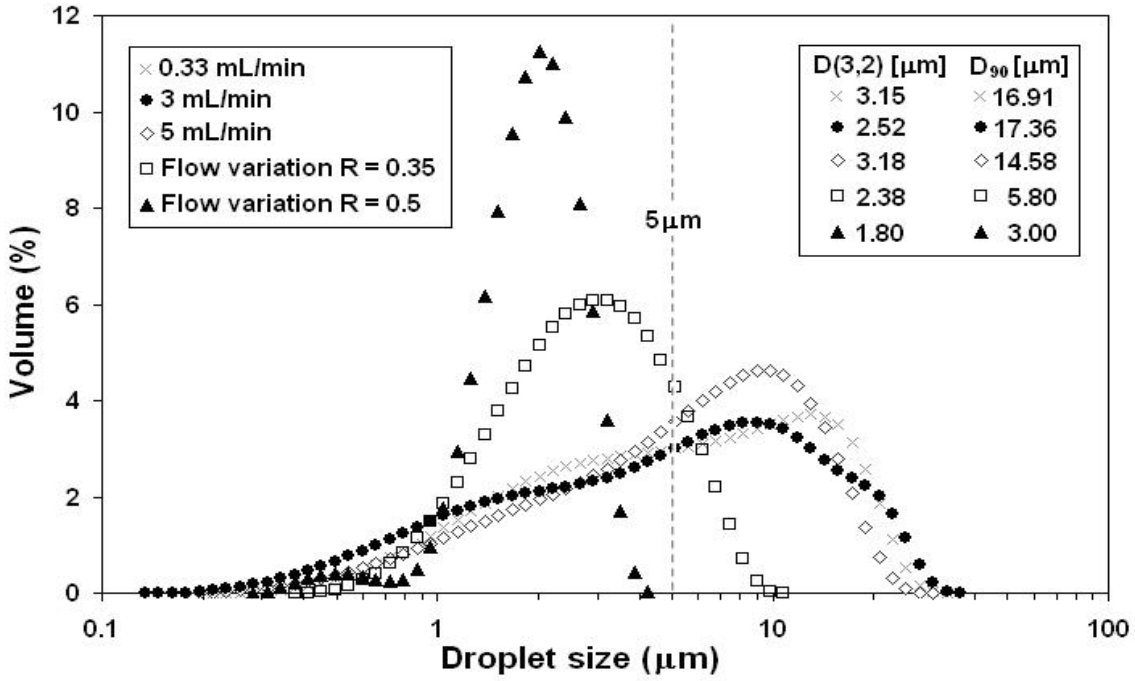

Figure 1. Effect of aqueous phase rate of addition on emulsions droplet size, for R = 0.35 and 0.5, 25 ºC.

The morphology of the dispersion was studied by means of conductivity measurements. The rate of aqueous phase addition was increased up to 20 mL/min in an interval of compositions where the strong changes of viscosity were observed (between 13 to 41 wt% of aqueous phase content in the dispersion). The emulsification process was performed in three stages: initially the rate of water addition was maintained at 3 mL/min until a 13 wt % of water content in the system, then the rate of addition was increased to 20 mL/min up to 41 wt % of water (at this point a viscoelastic phase is formed) and finally the remaining water was poured at 3 mL/min in orden to obtain the required emulsion. The emulsion produced by this method has a small droplet size with a narrow distribution. Ninety percent of the dispersion volume, or $d_{(90)}$, had diameters below 5.80 μm. In the present work the dispersions formed in the system lecithin/aqueous phase/soybean oil generate a viscoelastic mesophase that has a considerable effect on the decrease of droplet size distribution of emulsions depending on the kinetic of the emulsification process. This indicates that the emulsification occurs through a process of phase transition, being the formation of a liquid crystal mesophase the controlling stage on droplet size [4].
To reach the requirement for intravenous administration (less than 5 μm), the lecithin – oil ratio was increased to R = 0.5. The three steps emulsification procedure (flow variation) was used for these tests. The emulsion obtained had a droplet size distribution with diameters below 5 μm, as shown in Figure 1. In this case, a concentrated emulsion having 12.5 wt % of soybean oil and 12.5 wt % of lecithin was prepared. This emulsion was then diluted to the final concentration required, which is 10 % w/w of soybean oil, 10 % w/w of soybean lecithin, 78 % w/w of water and 2 % w/w of glycerol, which corresponds to 20 % of lipids and carbohydrates that can be biodegraded and metabolized by a human being. This emulsion requires further processing stages to be adequate for parenteral administration, membrane filtration and sterilization in an autoclave. Previous studies have shown that the membrane filtration process produces a decrease of droplet size of parenteral emulsions [10].
Conductivity measurements were also made during the emulsification of the system having a R

= 0.35, using the three methods of aqueous phase addition. Figure 2 shows conductivity measurements as a function of the mass fraction of aqueous phase. When using the emulsification methods involving dilution rates of 5 mL/min and flow variation, there is initially a very low conductivity due to a predominantly external oily phase. Then, there is a gradual change in conductivity (0.31 to 0.66 mass fraction for 5 ml/min and 0.29 to 0.56 for flow variation), which corresponds to phase inversion and the formation of an O/W emulsion. As a contrast, when the emulsification process is made at 3 mL/min, there is a sharp increase of conductivity (0.52 to 0.56 aqueous mass fraction), indicating the W/O to O/W inversion.
The inversion process that occurs in a wide interval of compositions is characteristic of dispersions when oil is initially the external phase (low electrical conductivity), followed by a gradual phase transition to a mesophase with intermediate conductivity, such as a microemulsion or liquid crystal. Eventually, the system ends up as an aqueous external phase exhibiting a high conductivity.

It has to be noted that the inversion onset for the flow variation method occurred at the lowest aqueous mass fraction. In fact, the emulsion obtained by way of this process exhibits the smaller droplet size. This phenomenon is related to the rate of addition of aqueous phase during the initial stage of emulsification and to the formation of a liquid crystal mesophase, which produces the emulsification by phase transition.

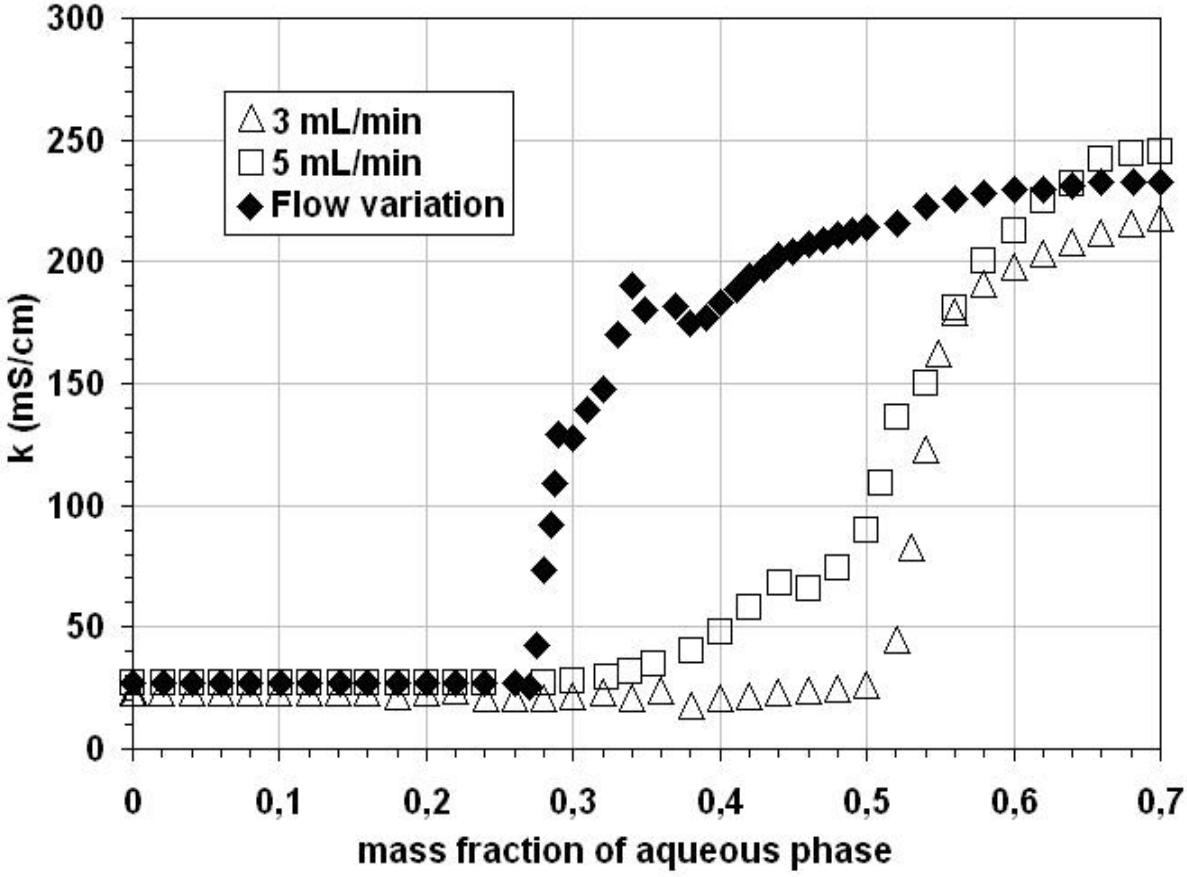

Figure 2. Dispersions conductivity for aqueous phase rate of addition 3 mL/min, 5 mL/min and by flow variation

4. Conclusions

Parenteral emulsions that comply with size restrictions for intravenous administration (largest diameters less than 5 μm), have been manufactured using soybean lecithin and a low energy emulsification protocol that produces significant changes in the physicochemical behaviour of the system. The principle is based on the transition from a molecular structured phase (lamellar liquid crystals) to an O/W type emulsion, seemingly controlled by the rate of aqueous phase addition and the formation of a viscoelastic mesophase.

5. References

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