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Nanocapsules prepared via nanoprecipitation and emulsification–diffusion methods: Comparative study

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ABSTRACT

The encapsulation of hydrophobic drugs has been widely investigated using mainly oil phase in order to enhance the encapsulation efficiency. However, the effect of the starting materials on the colloidal properties of the final nanocapsules irrespective of the elaboration process has been neglected, and the hydrophobic drug location in the disperse media has not been completely elucidated. Therefore, this paper describes the effect of the oil used in the recipe and the preparation method on the behavior of nanocapsules prepared via nanoprecipitation and via emulsification–diffusion. The colloidal stability of the final dispersions, drug location and the drug release are preparation method dependent. In turn, the type of oil governs drug encapsulation efficiency regardless of the method and the size when nanocapsules are prepared by nanoprecipitation.

1. Introduction

In pharmaceutics, nanocapsules based on biodegradable polymers have been widely developed as drug carriers by using different preparation methods [1]. However, few studies have been dedicated to the relationship between the process and the physicochemical properties of the resulting nanocapsules. Consequently, the aim of this work is to emphasize the relationship between the particle elaboration process, the nature of the oil (used in the recipe), the colloidal stability and the encapsulated drug release. To this end, several oils were investigated to identify their possible use in diclofenac nanocapsule preparation by using both the nanoprecipitation and the emulsification–diffusion methods.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL) (Mw: 14 kDa) was obtained from Sigma–Aldrich, poloxamer 188 (PLX) (Lutrol® F68) from Basf, soy lecithin (Lipoid® S75) from Lipoid GmbH, caprylic/capric triglycerides (Miglyol® 810, Miglyol® 812 and Miglyol 829) from Condea Chemie, corn oil from Sigma and almond oil from Fluka. Labrafac PG and Labrafac lipophile WL1349 were kindly given by Gattefossé (France). Diclofenac sodium salt was kindly supplied by O4CP-Institut Villemin, France. Acetone, ethanol, ethyl acetate (EtAc) and all other chemicals and solvents used were analytical grade. Deionised water from Milli-Q system was used in all experiments.

2.2. Methods

2.2.1. Preparation of diclofenac free acid

A 1% diclofenac sodium aqueous solution (100 ml) was treated with 1 N HCl (5 ml). The precipitated was filtered and washed with deionised water to remove chloride ions. It was verified by a specific limit test for chloride, using a silver nitrate solution. Then, precipitate was dried (45 °C) during 72 h and purified by twice recrystallization from ethanol–water solution (80:20, 200 ml) yielding white crystals with fusion range between 173 °C and 182 °C.

2.2.2. Characterization of oil physicochemical properties

Density was measured at 20 ± 2 °C using a pycnometer; weights were known to ±0.1 mg. Interfacial characterization (oil/water and oil/air) was made at 20 ± 2 °C by the pendant drop method using a Drop Shape Analysis System DSA 10Mk2 (Krüss, Germany). For oil/water interfacial tension, the classical sessile drop method (top-to-bottom) or the bottom-to-top sessile drop method was chosen according to the oil density. The viscosity of oils was measured at 20 °C using a viscometer R180 (Lamy, France) equipped with a mobile system No. 11 rotating at 200 s^{-1} shear rate.

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2.2.3. Diclofenac solubility in oils

To approximately 2.5 g of oil contained in a vial, diclofenac acid was added and the sample was hermetically sealed and magnetically stirred in a thermostat at 40 ± 2 °C (Ika-Ret Basic, 500 rpm). Addition of solute was repeated until solid material was remaining after stirring for 24 h. Then, samples were kept in standby at 20 ± 2 °C during 48 h, and supernatant was filtered by 0.45-μm PVDF filter. A known quantity of the supernatant was dissolved in EtOH for UV determination at 270 nm (Varian Cary 50 Probe UV–VIS spectrophotometer) using a validated method ($r = 0.9856$, range 5–20 microgram/ml). The reference solution consisted of an identical amount of the pure oil in EtOH.

2.2.4. Preparation of nanocapsule dispersions

The preparation process of nanocapsules by nanoprecipitation followed the procedure proposed by Fessi et al. [2]. First, 160 mg of PCL and 60 mg of soy lecithin were dissolved in EtOH:acetone 1:3 (20 ml). Then, 0.4 ml of a diclofenac acid-saturated solution in oil was added to the acetonic solution. The resulting organic solution was added (Harvard Apparatus Syringe Infusion Pump 22, 48 ml/h) into an aqueous solution of PLX (0.25%, pH 3.8, 40 ml) magnetically stirred (Ika-Ret Basic, 375 rpm). Particles were instantaneously formed.

The preparation of particles by emulsification–diffusion was performed as reported by Quintanar et al. [3]. Briefly, PCL (0.1 g) was dissolved in 10 ml of water-saturated EtAc. Then, 0.4 ml of a diclofenac acid-saturated solution in oil was added to the polymer solution. The resulting organic phase was emulsified with 40 ml of a solvent-saturated aqueous phase containing PLX (1%), by using a high speed homogenizer (Ultraturrax stirrer T25 IKA; 6500 rpm for 10 min). The emulsion was added in one shot to water (200 ml) under mechanical stirring (500 rpm, Heidolph RZR 2102), leading to the formation of the particles.

The solvent and part of the water of the particle dispersions were removed by evaporation under reduced pressure and 40 °C (Büchi Rotavapor R-124) until a final volume of 10 ml.

2.2.5. Size and zeta potential of submicron particles

The nanocapsule size was measured by photon correlation spectroscopy (Zetasizer Nanoseries, Malvern Instruments; 5 measures/sample, 5 runs of 10 s/measure at 25 °C), after adequate dilution of a suspension aliquot in deionised water (water pH between 6 and 7). Zeta potential was deduced from the electrophoretic mobility measurement by using the Zetasizer Nanoseries (5 measures/sample, 5 runs/measure at 25 °C). The particle dispersion was highly diluted in pH 6.0 1 mM NaCl solution.

2.2.6. Evaluation of the colloidal stability

The colloidal stability of the nanocapsules was evaluated by induced aggregation by adding 0.05 ml of nanocapsule dispersion to 2.5 ml of 10 mM NaCl solution adjusted at pH 3 with HCl. Particle size was measured at time zero, and samples were kept in standby at room temperature for 24 h. Then, particle size was measured once again.

2.2.7. Quantitative assay for diclofenac

Diclofenac was assayed by high-performance liquid chromatography (Thermo Separation, Spectra System SCM1000 pump, AS3000 autosampler and UV6000 LP detector). A C18 column (Nova-Pak®, Waters, 4 μm, 3.9 × 300 mm) was used, and the mobile phase consisted of acetonitrile:water (65:35% v/v) adjusted to pH 4 with glacial acetic acid. The work conditions were as follows: sample volume injected: 20 μl, mobile phase flow: 1 ml/min, and wavelength of diclofenac detection: 280 nm. The range of linear response was 0.2–20 μg/ml with $r = 0.9992$. Free drug was determined in the clear supernatant following separation of nanocapsules from aqueous medium by a combined ultrafiltration and centrifugation technique (Amicon® Ultra, Millipore, regenerated cellulose, 10 kDa MWCO). Total diclofenac was measured following complete dissolution of the nanocapsules in acetonitrile.

2.2.8. In vitro drug release

For investigating the diclofenac release, 0.5 ml of nanocapsule dispersion was added to 9 ml of phosphate buffer (pH 6.8) maintained at 37 ± 2 °C with slow magnetic stirring (≈25 rpm). Samples of 0.5 ml were collected at 3, 15, 30 and 60 min, submitted to ultrafiltration/centrifugation and assayed for diclofenac by HPLC as mentioned above. Sample volume was replaced immediately after each sampling.

All described experiments in the method subhead were carried out at least in triplicate.

3. Results and discussion

A critical aspect of formulating drug containing nanocapsules is appropriate selection of the oil used. In fact, this starting material limits the quantity of active substance carried and can have a considerable impact on particle characteristics. Table 1 summarizes the composition and the physicochemical properties of the oils investigated.

Generally, particles prepared by emulsification–diffusion are larger than those obtained by the nanoprecipitation (Fig. 1). Likewise, the type of oil has a more significant effect on size when nanocapsules are prepared by nanoprecipitation. None of the physicochemical properties of oil (density, viscosity, surface tension or polarity) correlates with the size of nanocapsules, in either the nanoprecipitation or the emulsification–diffusion. It appears that oil composition has some effect on nanocapsule size as the highest particle sizes were obtained by using almond oil and corn oil, which contain low percentage of long-chain saturated fatty acids (8–13%) and significant quantities of mono- and di-long-chain unsaturated fatty acids (85–95%) [5]. The smallest nanocapsule sizes were obtained by using medium-chain triglycerides containing not less than 95% saturated fatty acids [5]. Thus, it might occur that certain fatty acids in corn oil and almond oil crystallize during the nanoprecipitation process, leading to bigger particle sizes.

On the other hand, the nanocapsule zeta potential depends on the preparation method. Thus, particles obtained by nanoprecipitation always have more negative surface charge values (around 50 mV) than those prepared by the emulsification–diffusion (around 10 mV). Initially, it is possible to explain that behavior may be based on the recipe used by each method. Unlike the emulsification–diffusion, the nanoprecipitation involves an amphoteric without surfactant for preparing nanocapsules (soybean lecithin, isoelectrical point: ≈3.5 [5]), which could be imbibed in the polymeric membrane [6]. Consequently, a significant surface charge is exhibited on the particle surface.

However, our previous research works performed by our group on the zeta potential behavior of PCL nanospheres prepared by the nanoprecipitation and by the emulsification–diffusion showed differences in particle zeta potentials depending on the preparation technique (zeta potential values of ≈15 and ≈5 for nanospheres prepared by nanoprecipitation and emulsification–diffusion, respectively) [7]. Our investigation suggests that this behavior could be associated with the polymer conformation as re-precipitates, which in turn depends on the nature of the aqueous phase, the dissociation grade of carboxylic groups as soon as precipitation occurs and with the methodological aspects specific to each procedure. In short, when nanoprecipitation is used, the PCL polar groups are located on the particle surface, and by using the emulsification–diffusion, the hydrophobic moiety of the polymer

Table 1
Physicochemical properties of the oils.

Oil	Composition	Density (g/ml; 20 ± 2 °C)	Surface tension (niN/m; 20 ± 2 °C)	Oil/water interfacial tension (niN/m; 20 ± 2 °C)	Viscosity (mPa s, 20 °C)	Acid number (mg KOH/g)	Diclofenac solubility (%w/w, 20 ± 2 °C)
Corn oil	Triglycerides of linoleic acid (58.9%), oleic acid (25.8%), palmitic acid (11.0%), stearic acid (1.7%), linolenic acid (1.1%)	0.918 (0.03)	23.1 (3.5)	25.4 (1.3)	53.6 (0.8)	Max. 0.5[a]	0.34 (0.1)
Almond oil	Glycerides of oleic acid (64–82%), linoleic acid (8–28%), palmitic acid (6–8%)	0.914 (0.01)	30.2 (1.8)	23.5 (1.3)	69.0 (1.1)	Max. 2.0[a]	0.41 (0.1)
Miglyol 810	Medium-chain triglycerides: caprylic C8 70–80%, capric C10 18–28%	0.947 (0.03)	27.4 (1.0)	25.8 (0.9)	28.6 (1.2)	Max. 0.1[b]	0.82 (0.1)
Miglyol 812	Medium-chain triglycerides: caprylic C8 50–65%, capric C10 30–45%	0.944 (0.01)	25.0 (1.2)	25.2 (1.7)	29.1 (1.9)	Max. 0.1[b]	0.73 (0.1)
Miglyol 829	Caprylic/capric/succinic triglycerides: caprylic C8 45–55%, capric C10 30–40%, succinic acid 15–20%	1.008 (0.02)	25.5 (1.2)	5.4 (17.2)	149.9 (2.6)	Max. 1.0[b]	1.15 (0.1)
Labrafac PG	Propylene glycol dicaprylate/dicaprate	0.918 (0.01)	27.4 (1.1)	21.7 (1.0)	11.8 (0.5)	Max. 0.2[b]	0.93 (0.1)
Labrafac lipophile	Medium-chain triglycerides: caprylic C8 50–80%, capric C10 20–50%	0.945 (0.02)	24.6 (2.0)	24.1 (1.6)	25.5 (1.8)	Max. 0.2[b]	0.85 (0.1)

*In parenthesis RSD. nd.: Nondetermined measure.
[a] PhEur 2005 [4].
[b] Supplier quality control specification.

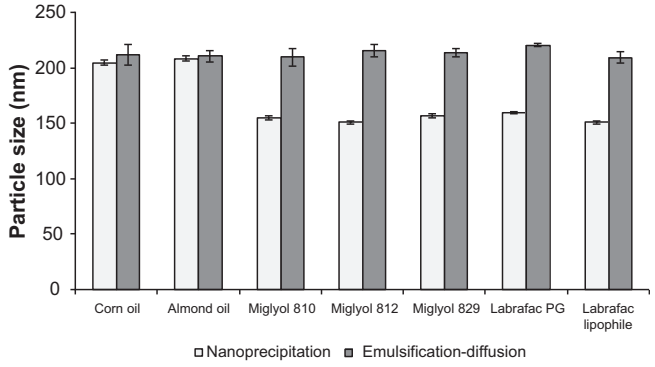

Fig. 1. Size of nanocapsules prepared by nanoprecipitation and emulsification–diffusion methods by using different oils.

predominates on the particle surface, which contributes to explain the zeta potential differences according to the preparation method.

The former interpretation on the zeta potential results suggests that the interaction between the stabilizing agent (PLX) and the nanocapsule surface could be different. The key difference between nanocapsules obtained by the two preparation methods could be their hydrophobic/hydrophilic degree at the surface (i.e., the ratio of hydrophobic to hydrophilic groups on the particle surface). On the other hand, the stabilizing agent PLX is a block copolymer of structure POE–PPO–POE.

Then, from a theoretical standpoint, for nanocapsules prepared by emulsification–diffusion, a high density of PPO segments anchored on the particle would be expected because the hydrophobic moieties of PCL predominate on the surface, favoring hydrophobic interactions between PCL and the stabilizing agent. Consequently, the overlap of the adsorbed PLX layers when two particles approach results in strong repulsion due to the solvated POE end chains because they are subject to good solvency conditions (steric stabilization) [8,9].

In turn, the low hydrophobic/hydrophilic degree at the surface of nanocapsules prepared by nanoprecipitation will hinder the adsorption of PLX on the particle, rendering a low density of anchored PLX molecules and a high surface charge density [9,10]. Thus, particles are stabilized via the electrosteric effect.

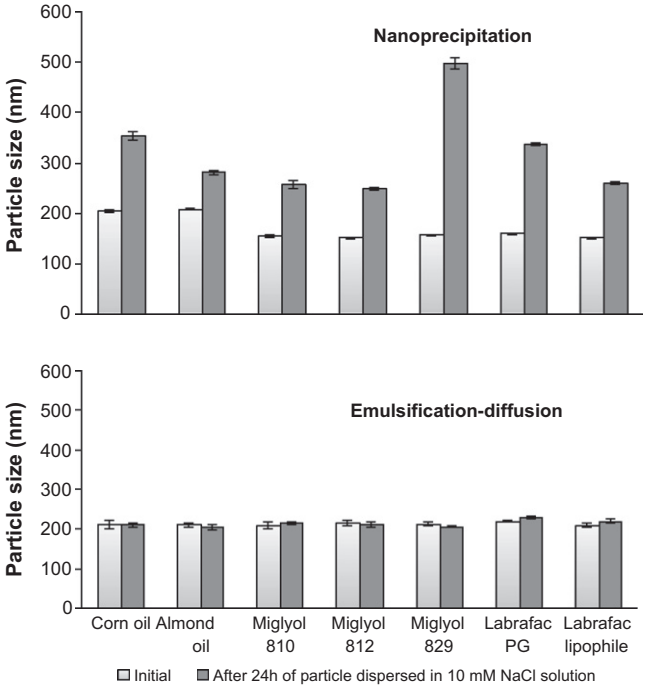

Fig. 2. NaCl induced aggregation of nanocapsules prepared by nanoprecipitation and emulsification–diffusion methods using different oils.

Considering the high zeta potential values exhibited by nanocapsules prepared by nanoprecipitation, the electrostatic effect might predominate in this case.

These particle stabilization mechanisms were confirmed by the behavior of dispersions in the presence of electrolytes. As shown in Fig. 2, monovalent ions from NaCl cause the aggregation of nanocapsules prepared via nanoprecipitation, which is underlined by the significant change in particle mean size. In turn, the size of nanocapsules prepared via emulsification–diffusion is not influenced by monovalent ions in spite of their low zeta potential.

On the other hand, Fig. 3 shows the results for diclofenac release from PCL based nanocapsules using Miglyol 810 and labrafac PG as oil models. As can be seen, different behaviors are highlighted as a

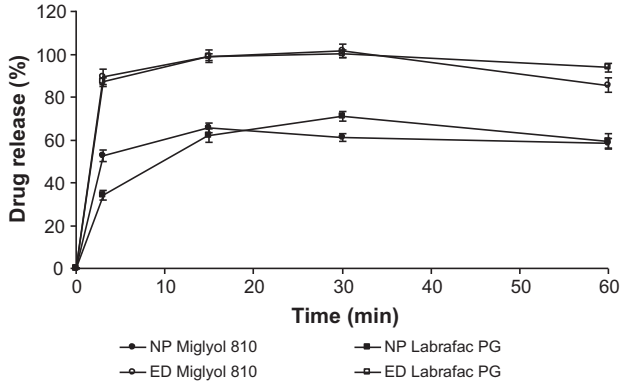

Fig. 3. The drug release behavior of nanocapsules prepared by the nanoprecipitation technique (NP) and by the emulsification–diffusion method (ED) using Miglyol 810 and Labrafac PG as oils.

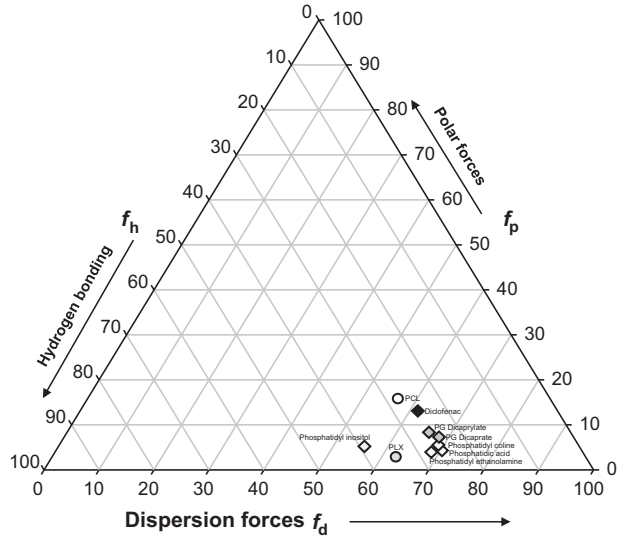

Fig. 4. Teas graph for the materials used in the preparation of nanocapsules by the nanoprecipitation technique and the emulsification–diffusion method. The Hansen solubility parameters (δ_d, δ_p and δ_h) were calculated by using the group contribution (van Krevelen method). Then, they were expressed as fractional cohesion parameters f_d, f_p and f_h by $f_i = 100\delta i/(\delta_d + \delta_p + \delta_h)$, where i corresponds to δ_d, δ_p or δ_h. Propyleneglycol dicaprylate and propyleneglycol dicaprate are the main components of Labrafac PG; phosphatidylcholine, phosphatidylethanolamine, phosphatidyl inositol and phosphatidic acid are the main components of soy lecithin [5].

function of the preparation method. The total amount of diclofenac encapsulated by emulsification–diffusion is released within 15 min, whereas about 60% of the active substance encapsulated

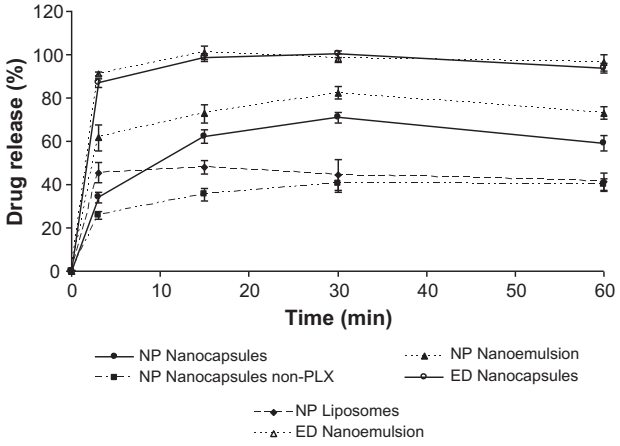

Fig. 5. Drug release behavior of different nanocarriers: Labrafac PG nanocapsules prepared by nanoprecipitation (NP) and by emulsification–diffusion (ED) methods, labrafac PG nanoemulsions prepared by nanoprecipitation and by emulsification–diffusion methods, liposome prepared by nanoprecipitation and labrafac PG nanocapsules prepared by nanoprecipitation without stabilizing agent (PLX).

by nanoprecipitation is available in the release medium for as long as 48 h after the beginning of the experiment.

Density gradient studies have demonstrated that the nanoprecipitation [11] and the emulsification–diffusion [12] yield nanocapsules exclusively. However, according to the experimental evidence provided by Ammoury et al. [13], the slow and incomplete drug release from colloidal suspensions prepared by using nanoprecipitation could be attributed to the retention capacity of other nanocarriers, such as liposomes and nanoemulsions formed at the same time as when nanocapsules are prepared. Accordingly, we assume that nanoemulsions may also be formed when nanocapsules are prepared by emulsification–diffusion. In addition, there is possible that PLX micelles/aggregates are formed by the two preparation methods, because the PLX concentrations used (0.25% and 1% for nanoprecipitation and for emulsification–diffusion, respectively) are above the critical PLX micelle concentration (0.1%) [14]. Thus, the quantity of diclofenac encapsulated by each one of these possible colloidal carriers depends on the multiple drug partition coefficients between the different phases occurring during nanocarrier formation.

Indeed, it is difficult to advance an opinion on which nanocarriers are formed when preparing nanocapsules by the nanoprecipitation and emulsification–diffusion methods. Speculation can be made from a physicochemical point of view. Consequently, we use the Teas graph to deduce the interactions between molecules. As can be seen in Fig. 4, each molecule involved in nanocapsule preparation, i.e., drug, polymer, oil and surfactant, is represented by a single point in the Teas graph that reflects the contribution

Table 2
Characterization of different nanocarriers prepared via the nanoprecipitation technique and via the emulsification–diffusion method.

	Starting materials[a]					Size (nm)	Zeta potential (mV)	Drug in supernatant[b] (%)
	Diclofenac	PCL	Labrafac PG	Soy lecithin	PLX			
Nanocarriers prepared via the nanoprecipitation technique								
Liposomes	x			x	x	42	−45	2.72 ± 0.42
Nanoemulsion	x		x	x	x	137	−37	0.19 ± 0.09
Nanocapsules without PLX	x	x	x	x		152	−50	0.13 ± 0.08
Nanocarriers from typical recipe	x	x	x	x	x	160	−46	0.26 ± 0.05
Nanocarriers prepared via the emulsification–diffusion method								
Nanoemulsion	x		x		x	186	−10	0.08 ± 0.02
Nanocarriers from typical recipe	x	x	x		x	220	−8	0.04 ± 0.01

[a] Acetone, ethyl acetate and water were the used solvents according to the procedures previously described in the method subhead.
[b] Supernatant obtained after the ultrafiltration/centrifugation of samples.

of the dispersion, polar and hydrogen forces according to its chemical structure. The closer the relative position of the points in the ternary diagram, the higher the affinity between the molecules. Fig. 4 illustrates the case of nanocarriers prepared by using labrafac PG as oil. Considering that labrafac PG and soy lecithin are mixtures of various substances, their main components are depicted in the diagram. As can be seen, high affinity is predicted between the different molecules, and perhaps, nanoemulsion formation might be slightly favored with respect to the formation of nanocapsules.

On the other hand, to verify drug release behavior as a function of the nanocarrier formed, some diclofenac nanocarriers were prepared via nanoprecipitation and via emulsification–diffusion by modifying our typical recipes as indicated in Table 2. Fig. 5 shows that any of the colloids obtained via nanoprecipitation leads to the complete release of diclofenac. Regarding the emulsification–diffusion, nanoemulsions and nanocapsules exhibited the same dissolution pattern, releasing 100% of the diclofenac during the first 15 min of the dissolution study. It was not possible to separate the different carriers by ultrafiltration/centrifugation because nanocapsules and nanoemulsions have similar sizes; also, liposomes could form agglomerates. Moreover, the amount of active substance in the aqueous supernatant of the different colloidal systems suggests that diclofenac can be efficiently captured by any of the carriers (Table 2).

4. Conclusions

This investigation describes a systematic study on the preparation of diclofenac nanocapsules via the nanoprecipitation and via the emulsification–diffusion, with emphasis placed on the incidence of the type of oil used in the recipe. Our results show the effect of the preparation method on the size and zeta potential of the nanocapsules. Thus, the smallest particle size and the largest absolute values of zeta potential were obtained by the nanoprecipitation. The particle zeta potential determines the stabilization mechanism of the colloidal dispersion, as demonstrated by the induced aggregation experiment carried out. Thus, the nanoparticles obtained by nanoprecipitation were mainly stabilized via an electrostatic effect, while those prepared by emulsification–diffusion exhibited steric stabilization. Regarding the studies on the *in vitro* release of diclofenac, it is clear that the different behaviors of the nanoparticle dispersions depend on the method used. Our investigation revealed interesting evidence on the efficiency of nanocapsule formation using recipes that could simultaneously lead to the formation of nanoemulsions, liposomes and micelles.

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