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Research paper

Stability of drug-carrier emulsions containing phosphatidylcholine mixtures

Michele Trotta[a,*], Franco Pattarino[b], Terenzio Ignoni[a]

[a]*Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Turin, Italy*
[b]*Dipartimento di Sci. Chim. Alim. Farm., Università di Novara, Novara, Italy*

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Abstract

Lipid emulsion particles containing 10% of medium chain triglycerides were prepared using 2% w/w of a mixture 1:1 w/w of purified soya phosphatidylcholine and 2-hexanoyl phosphatidylcholine as emulsifier mixture, for use as drug carriers. The mean droplet sizes of emulsions, prepared using an Ultra Turrax or a high-pressure homogenizer, were about 288 and 158 nm, respectively, compared with 380 and 268 nm for emulsions containing lecithin, or 325 and 240 nm for those containing 6-phosphatidylcholine. The stability of the emulsions, determined by monitoring the decrease of a lipophilic marker at a specified level within the emulsion, and observing coalescence over time, was also greatly increased using the emulsifier mixture. The emulsion stability did not notably change in the presence of a model destabilizing drug, indomethacin. The use of a second hydrophilic surfactant to adjust the packing properties of the lecithin at the oil–water interface provided an increase in the stability of lipid emulsions, and this may be of importance in the formulation of drug delivery systems.

Keywords: Submicron emulsion; Phospholipid; Indomethacin; Demixing

1. Introduction

Oil-in-water emulsions are heterogeneous systems in which an oil is dispersed as droplets in water; they represent an interesting prospect for the development of formulation for use as vehicle to deliver drugs to the human body. The potential pharmaceutical applications include use as carrier for: poorly water-soluble drugs, sustained-release systems, and site-specific drug delivery by binding ligands for various cell-surface receptors to the particle surface [1]. Such systems are thermodynamically unstable and may be kinetically stabilized by adding a further component or mixture of components that exhibit emulsifying properties.

Most of the known efficient synthetic emulsifiers could be dangerous for health, especially upon parenteral administration, because of their haemolytic effects. Emulsifiers approved by the various pharmacopoeia are phospholipids, block copolymers of polyoxyethylene–polyoxypropylene (poloxamer), fatty acid esters of sorbitans and polyoxyethylene sorbitans. Among them, phospholipids are the emulsifiers most frequently used in parenteral emulsions [2].

To comply with the requirements for parenteral emulsions, the oils must also be carefully selected. The oil phase of the emulsion is based mainly on long-chain triglycerides (LCT) from vegetable sources. Medium-chain triglycerides (MCT) have also been used, alone or in combination with LCT, because of their ability to dissolve high concentrations of liposoluble drugs [3].

Lipid emulsions stabilized with phospholipids have many appealing properties as drug carriers; however, there are also severe problems involved in the use of emulsions for drug administration. The foremost problem regarding the use of emulsion particles as drug carriers is the need to produce small particles, as large colloidal particles administered intravenously are rapidly taken up by the cells of the mononuclear phagocyte system [4]. Moreover, the incorporation of certain drugs in an emulsion formulation with phospholipids as sole emulsifier has been found to reduce stability and cause phase separation [5]. The combination of phospholipids and poloxamer as an emulsifying complex was found to increase emulsion stability, probably because of the formation of a complex interfacial film between poloxamer and the phospholipid molecules at the oil–water interface [6].

* Corresponding author. Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via P. Giuria 9, 10125 Torino, Italy.
Fax: +39-11-670-7687.
E-mail address: trotta@pharm.unito.it (M. Trotta).

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In a previous paper, we described the preparation of a series of modified phospholipids possessing different acyl chains in position 2, from butanoyl to hexadecanoyl, by partial synthesis from soybean lysolecithin [7]. They were used with soybean lecithin and ethanol to prepare transparent oil-in-water emulsions containing MCT with the oil droplet diameter below 50 nm (microemulsions). On decreasing the acyl chain length there was a corresponding increase in the region of existence of the microemulsion systems compared with that containing lecithin alone. This was related to an improvement of the packing parameter of the surfactant mixture at the oil–water interface.

In this study the possibility of using the 2-hexanoyl lysolecithin derivative in formulating emulsions containing MCT to produce small and stable emulsions was explored, and the results compared with those containing lecithin alone.

2. Materials and methods

2.1. Materials

Soybean lecithin (phosphatidylcholine > 95%, Epikuron 200) was obtained from Lucas Meyer (Hamburg, Germany) and used without further purification. MCT Myritol 318, a mixture of caprylic and capric triglycerides, was from Henkel (Dusseldorf, Germany). The modified phospholipid, *n*-hexanoyl lysolecithin derivative (6-PC) was prepared by partial synthesis from Epikuron 200 as starting material. The fatty acid was split off in position 2 by enzyme phospholipase A_2 (Novo Nordisk, Bagsvaerd, Denmark) and the resulting lysolecithin was converted to the 2-hexanoyl derivative following the method described by Gupta et al. [8].

Indomethacin and anthracene were from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Emulsion composition

The composition (w/w) of the emulsions was: 10% MCT, 2% emulsifier and 88% distilled water. Lecithin alone, a 1:1 w/w mixture of 6-PC/lecithin or 6-PC alone were used as emulsifiers.

2.3. Selection of the emulsification procedure

To select the operative conditions, a series of emulsions were prepared by dispersing 2 g lecithin in 88 g distilled water at room temperature; the dispersion was then filtered through a 1-μm filter (Millipore, Bedford, MA, USA). Ten grams of MCT was added to this dispersion under a magnetic stirrer and the mixture was then emulsified using a high-shear mixer at 8000 rev/min for 5 min (Ultra Turrax, Janke & Kunkel, Staufen, Germany) or by passing the emulsions through a high-pressure homogenizer (Niro Soavi, Parma, Italy) at a pressure in the 200–1500 bar range for 1–6 cycles. After emulsification with the Ultra Turrax, or after each cycle through the high-pressure homogenizer, the emulsions were cooled at 25°C before reprocessing and the mean droplet diameter determined by a light scattering technique (Brookhaven Inst. Corp., NY, USA).

The criterion for selecting the preparation technique was the reproducible preparation of emulsions that showed the smallest particle size.

2.4. Emulsion preparation

Based on process optimization studies, the emulsions were prepared as described above using a high-shear mixer or a high-pressure homogenizer at 200 bar pressure for six cycles. The emulsions were analysed for size distribution and their surface potential. Each sample was measured in triplicate.

Emulsions containing marker were prepared by dissolving trace amount of anthracene in the MCT before emulsification.

Emulsions containing indomethacin were prepared by suspending 1% drug in the final emulsion containing anthracene. The suspension was stirred at room temperature for 2 h. After 12 h the emulsion was filtered through 0.45-μm filter and used for the stability tests.

2.5. Emulsion stability

The stability of the emulsions was evaluated by monitoring the demixing rate and the coalescence over time.

The emulsion demixing kinetics was determined as described by Le Visage et al. [9] by quantitatively monitoring the decrease of a lipophilic model marker concentration at a specified level within the emulsion. Anthracene was used as model marker. The rate of marker absorption decrease was directly related to emulsion stability; the higher the stability, the lower the demixing rate. Briefly, emulsions containing the minimum amount of marker and prepared under different operative conditions (10 ml) were placed in sealed airtight tubes at 25°C. At different intervals, an aliquot (50 μl) of the emulsion was collected at the same level within the tube containing the emulsion and diluted with methanol. The anthracene concentration was determined by fluorimetric analysis ($\lambda_{ex} = 353$ nm, $\lambda_{em} = 403$ nm; Shimadzu, Japan). To compare the different demixing curves, the absorption values were normalized by dividing the absorption value at each time by the absorption value at time zero. The results reported are the means of three determinations.

Coalescence was determined by monitoring the mean droplet diameter of the emulsions over time. Ten millilitres of the emulsions prepared under different operative conditions were placed in a sealed airtight tube at 25°C. At intervals the tubes were mixed gently in order to obtain homogeneous samples for particle size measurement. Approximately five drops of emulsion were diluted with approximately 50 ml of distilled water. Particle size was measured immediately after dilution. Measurements were

Table 1
Mean droplet diameter (nm) of the emulsions containing lecithin alone prepared under different operative conditions

	200 bar	500 bar	800 bar	1000 bar	1500 bar
Cycle 1	350 ± 23	339 ± 24	340 ± 21	320 ± 22	360 ± 26
Cycle 2	334 ± 20	339 ± 18	330 ± 19	338 ± 22	356 ± 24
Cycle 3	330 ± 16	326 ± 16	320 ± 16	313 ± 20	340 ± 25
Cycle 4	304 ± 17	311 ± 15	315 ± 16	330 ± 20	405 ± 27
Cycle 5	288 ± 15	280 ± 12	310 ± 18	335 ± 16	390 ± 24
Cycle 6	268 ± 15	308 ± 12	325 ± 15	340 ± 14	362 ± 22
Ultra Turrax			380 ± 22		

in triplicate and the average droplet size was determined. The particle size was determined initially and then at intervals until up to 3 months, to evaluate stability.

3. Results and discussion

In a series of initial experiments, the optimal operative conditions were evaluated with regard to homogenization pressure and number of cycles, using lecithin alone as emulsifier. The efficiency of the emulsification process was recorded by measuring mean droplet diameter. The homogenization was performed using 1–6 cycles of pressure from 200 bar to 1500 bar, using a one-stage high-pressure homogenizer.

The mean oil droplet diameter of the emulsion prepared using the high-shear mixer and those made by the high-pressure homogenizer in different operative conditions are reported in Table 1, while the graphic representation of the mean droplet size as a function of the two variables, applied pressure and number of cycles in the case of the emulsion containing lecithin, is reported in Fig. 1. The results demonstrate a decrease in particle size using the high-pressure homogenizer compared with the high-shear mixer. A size decrease on increasing the number of cycles was only observed for the system passed at 200 bar. For all the other operative pressures a minimum particle size was observed at the fifth cycle, for pressures of 500 and 800 bar, and at the third cycle for pressures of 1000 and 1500 bar. Moreover, the smallest oil particles, about 268 nm, were obtained using a pressure of 200 bar.

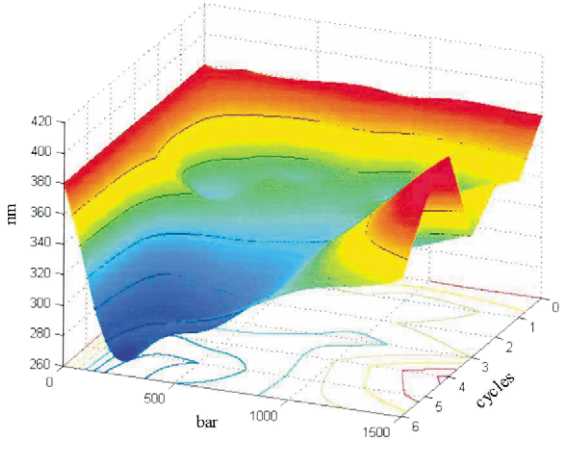

Fig. 1. Mean droplet sizes of the emulsions containing lecithin as a function of the operative pressures and number of cycles.

Duration of processing can affect emulsion stability. Becher [10] showed that the number of times the product was passed through the device affected the mean particle size and the particle size distribution. Repeating the processing or cycling resulted in a decrease in average particle size and a narrowing of the particle size distribution, after which mean particle size and standard deviation both increased as processing continued. This observation is in agreement with reports substantiating that droplet sizes are a result of breakage and coalescence and that, for systems containing relatively high percentages of oil, increasing the operative pressure does not always lead to a reduction of emulsion droplet size [11,12].

From these experiments, the standard operative conditions for all emulsions were set to 200 bar for six cycles.

The efficiency of the emulsification process and the hexanoyl derivative was quantitated by particle sizer measurements. Table 2 gives the mean droplet diameters and polydispersity indices (PI) of emulsions containing lecithin alone, 6-PC/lecithin or 6-PC alone, emulsified using the Ultra Turrax or passed six times through a high-pressure homogenizer at 200 bar. For all systems the results show a decrease in particle size and polydispersity index using high-pressure homogenization, or on increasing the number of cycles. The smallest values were obtained for the emulsion containing 6-PC/lecithin mixture, while the use of 6-PC alone did not lead to better results.

A number of studies have varied surfactant concentration, and generally the droplet size has been found to decrease on increasing concentration. But droplet size also closely depends on the type of surfactant, and even when applying high power input and using sufficient surfactant to give a relatively complete coverage of the interface, droplet size may vary considerably [13]. Size distribution is also quite variable; it is only slightly correlated with interfacial tension, and interpretation of results is difficult.

The ability of surfactant molecules to give the necessary curvature of the interfacial film required to form fine emulsions or microemulsions has been related to the packing geometry, which is the ratio between hydrocarbon volume,

Table 2
Mean droplet diameter (nm) and polydispersity index (PI) of emulsions containing different emulsifiers passed at 200 bar

	Lecithin	6-PC/lecithin	6-PC
Cycle 1	350 ± 23 (0.15)	248 ± 21 (0.12)	302 ± 20 (0.12)
Cycle 2	334 ± 20 (0.12)	235 ± 18 (0.10)	284 ± 18 (0.10)
Cycle 3	330 ± 16 (0.10)	212 ± 18 (0.08)	271 ± 15 (0.08)
Cycle 4	304 ± 17 (0.08)	192 ± 14 (0.07)	258 ± 14 (0.08)
Cycle 5	288 ± 15 (0.08)	170 ± 15 (0.06)	250 ± 11 (0.07)
Cycle 6	268 ± 15 (0.06)	158 ± 12 (0.04)	240 ± 12 (0.06)
Ultra Turrax	380 ± 22 (0.14)	288 ± 19 (0.12)	325 ± 24 (0.12)

optimum head group area and tail length [14] of the molecule at the interface.

Lecithin has a packing parameter around 0.8 [15] and this value is further increased if the oil phase penetrates into the alkyl chains of the lecithin molecule [16]. In order to produce fine oil-in-water emulsions or microemulsions, it is necessary to reduce this parameter by using co-surfactants, generally short-chain alcohols [17], thus allowing the interfacial film sufficient flexibility to take up the curvature required to form fine emulsions or microemulsions.

A possible way to reduce the packing parameter could be the partial substitution of lecithin by a more hydrophilic emulsifier [18].

The hexanoyl derivative used in these experiments is more hydrophilic than lecithin, having the same large hydrophilic head and a relatively smaller hydrocarbon volume. Incorporation of this molecule at the oil–water interface could form a mixed monolayer with lecithin. The flexibility of this mixed film is greater than that of lecithin, because the different structures of the two molecules prevent close packing at the interface.

Emulsion systems are thermodynamically unstable. Following emulsification, the particles dispersed in the internal phase constantly seek to cream, to agglomerate into a single kinetic unit (i.e. floccule), to form larger droplets (coalescence), and eventually to separate as a second continuous phase.

In further experiments, the efficiency of the high-pressure emulsification process was quantitatively compared with the emulsification using an Ultra Turrax, and the efficiency of the hexanoyl derivative by measuring the oil droplet migration rate by monitoring the decrease of a model marker concentration at a specified level within the emulsion. The rate of this absorption decrease was directly related to the emulsion stability, i.e. the higher the stability, the lower the demixing rate.

The demixing measurements were also performed on emulsions added of a model destabilizing drug, indomethacin. Submicron emulsions are considered to be very sensitive to any modification in content and only some drugs have

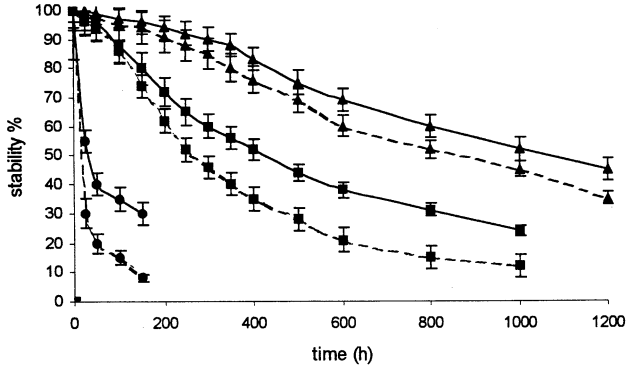

Fig. 2. Demixing kinetics of emulsions prepared using a high-shear mixer (Ultra Turrax) in the absence (solid line) and in the presence of indomethacin (dotted line). ●, lecithin; ■, 6-PC; ▲, 6-PC/lecithin (1:1 w/w).

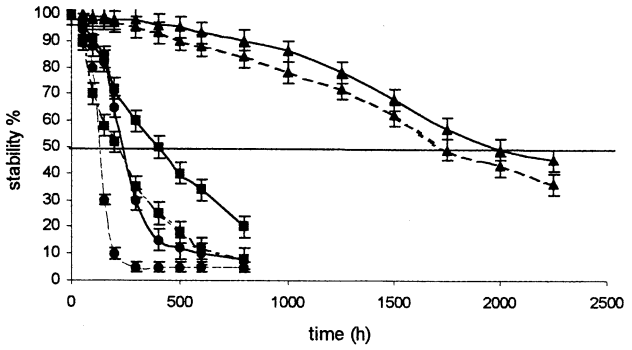

Fig. 3. Demixing kinetics of emulsions prepared using a high-pressure homogenizer (200 bar, six cycles) in the absence (solid line) and in the presence of indomethacin (dotted line). ●, lecithin; ■, 6-PC; ▲, 6-PC/lecithin (1:1 w/w).

successfully been introduced into such systems. If destabilization occurs it is maximal at saturated concentration [5].

The demixing behaviour of the emulsions prepared using an Ultra Turrax or a high-pressure homogenizer in the absence and in the presence of an excess of indomethacin was observed during storage at 25°C, and the results are reported in Figs. 2 and 3, respectively. The results indicate a very different behaviour depending on the emulsifier used, while the homogenization procedures appeared important to define the demixing behaviour for the systems containing lecithin or the emulsifier mixture. Anyway, in the absence of drug, for both the emulsions prepared with lecithin, a demixing was very rapid and an almost lipid phase was observed on the bottom of the tube after a few days. Demixing was less pronounced for emulsions containing 6-PC. Of the emulsions containing lecithin/6-PC mixture, a big increase in stability was observed for that prepared with the high-pressure homogenizer (Fig. 3), in which a lipid phase was observed after 4 months.

The addition of indomethacin had a detrimental effect on the stability of all emulsions except that containing the emulsifier mixture. The emulsions containing indomethacin had about the same particle size as drug-free emulsions; thus no correlation existed between droplet size and destabilizing effect.

The emulsion stability in terms of half-life ($t_{1/2}$), the time required to halve the marker concentration at a specified level within the emulsion, is reported in Table 3. The $t_{1/2}$

Table 3
Time (h) required to halve the marker concentration at a specified level within the emulsions ($t_{1/2}$) in the absence and in the presence of indomethacin

	Ultra Turrax $t_{1/2}$		High-pressure homogenization (200 bar, six cycles) $t_{1/2}$	
		+Indomethacin		+Indomethacin
Lecithin	30	20	250	140
6-PC/lecithin	1050	740	1900	1750
6-PC	420	260	410	220

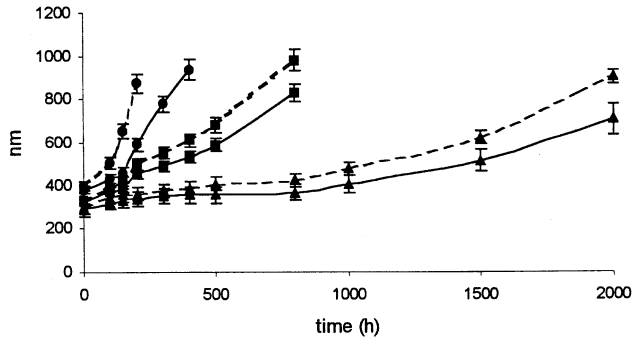

Fig. 4. Mean droplet size over time of emulsions prepared using a high-shear mixer (Ultra Turrax) in the absence (solid line) and in the presence of indomethacin (dotted line). ●, lecithin; ■, 6-PC; ▲, 6-PC/lecithin (1:1 w/w).

values clearly show that in the case of emulsions containing lecithin or 6-PC/lecithin mixture, high-pressure homogenization produced more stable emulsions than high-shear homogenization, and 6-PC/lecithin mixture appeared more effective than lecithin or 6-PC in producing a stable emulsion. For the emulsion containing 6-PC no marked difference was observed between the two operative methods, indicating that the use of high power input did not lead to a decrease of emulsion demixing.

Small particle size promotes good physical stability, because creaming is prevented by Brownian movement, but the marked difference in $t_{1/2}$ values among the emulsions could not be explained by the difference in the initial droplet size alone; other factors, such as coalescence, might affect the demixing behaviour.

Coalescence is reflected in particle size increase of the emulsion droplets. The direct measurements of particle size, as a function of time, can help to characterize emulsion stability.

The physical stability of emulsions prepared with a high-shear mixer in the absence and in the presence of indomethacin was observed during storage at room temperature; the mean particle size of the different emulsions over time is reported in Fig. 4.

The intensity of the process was very pronounced for the emulsions containing lecithin. Measurement of the droplet size indicated a marked increase after 1 week, and after about 1 month oily drops could be seen on the surface due to coalescence. The coalescence was faster in the same formulation containing indomethacin, in which there was a large increase in measured droplet size immediately, in spite of the satisfactory initial particle size. Droplets as large as 20–30 μm in diameter were present, as confirmed by inspection under the microscope, and oily drops could be seen on the surface after a few days. It may be concluded that indomethacin causes changes at the interface boundary leading to an increase in coalescence.

The process was slower in emulsions prepared using 6-PC alone, where destabilization occurred not immediately but within 3 weeks and oily drops appeared after 2 months. The presence of indomethacin increased coalescence to a lesser extent than did lecithin.

For the emulsion containing lecithin/6-PC mixture, besides creaming, no other visual changes were observed. After 3 months two separated phases formed: the upper phase was still emulsion and the lower an almost clear solution. However, no breakage of the emulsion occurred and after brief mixing, the system showed a moderate increase in droplet size.

Coalescence of the emulsions prepared using the high-pressure homogenizer is reported in Fig. 5. Compared with the emulsions prepared using the high-shear mixer, a marked decrease in coalescence was observed only for the emulsions containing lecithin, whereas there was no difference between the emulsions containing 6-PC or 6-PC/lecithin prepared with the two different technique.

Emulsifying agents are used to slow down this inevitable separation. Occasionally it will be found that a single emulsifier can yield the desired emulsion. More often, however, in the case of oil/water emulsions, mixed surfactants have been reported to have a synergistic effect on emulsion stability in terms of coalescence rate. The enhanced stability has been attributed to the formation of intermolecular complexes at the oil–water interface. It would seem that the maximum effect is obtained when a water-soluble surfactant and an oil-soluble surfactant, capable of interacting at the oil–water interface, are used in combination. This combination appears to produce mixed surfactant films of high surfactant coverage, as well as of sufficient viscosity to prevent creaming and promote stability [19].

Although numerous studies addressed enhancing stability by using mixed surfactants, the mechanism involved is not yet fully understood. However, the importance of this concept in industrial emulsions, where it is the rule rather than the exception to use mixed surfactants, would seem to justify further research in this area.

Emulsifiers can stabilize the emulsion droplets not just by formation of a mechanical barrier, but also by producing an electrical barrier or surface charge.

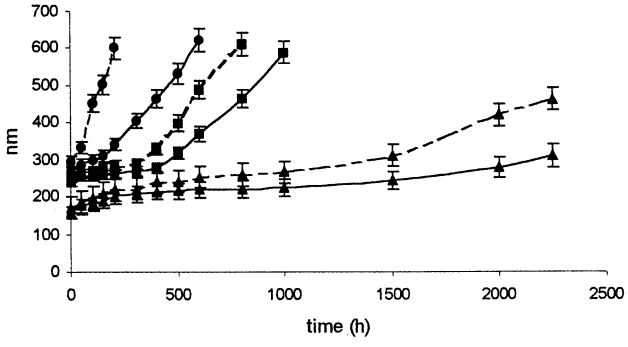

Fig. 5. Mean droplet size over time of emulsions prepared using a high-pressure homogenizer (200 bar, six cycles) in the absence (solid line) and in the presence of indomethacin (dotted line). ●, lecithin; ■, 6-PC; ▲, 6-PC/lecithin (1:1 w/w).

Table 4
Z-potential values (mV) of the emulsions in the absence and in the presence of indomethacin

	Ultra Turrax		High-pressure homogenization (200 bar, six cycles)	
		+Indomethacin		+Indomethacin
Lecithin	−27 ± 2	−19 ± 3	−29 ± 2	−20 ± 2
6-PC/lecithin	−40 ± 3	−36 ± 4	−45 ± 3	−42 ± 4
6-PC	−31 ± 3	−22 ± 3	−33 ± 3	−26 ± 3

The *Z*-potentials of the emulsions are reported in Table 4. It is interesting to note that the highest *Z*-potential value was measured for the emulsion containing 1:1 6-PC/lecithin prepared using a high-pressure homogenizer and that this value did not change in the presence of drug. The increase in the *Z*-potential may presumably be attributed to the incorporation of polar compounds, present in the purified soya lecithin, in the mixed interfacial film when 6-PC is present. This interfacial film acts as a stabilizer by forming a high-energy barrier that repels adjacent droplets and leads to the formation of stabilized emulsified droplets.

In conclusion, the combination of lecithin and hexanoyl derivative, as a second hydrophilic surfactant, in the formation of emulsions containing medium chain triglycerides, together with a highly efficient emulsification process, provided a decrease in oil droplet size and an increase of the stability of the emulsions, which is maintained in the presence of destabilizing drug.

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