Colloid Polym Sci 277:347–353 (1999)

ORIGINAL CONTRIBUTION

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Influence of the non-ionic surfactant PEG-660-12-hydroxy stearate on the surface properties of phospholipid monolayers and their effect on lipid emulsion stability

Received: 25 May 1998
Accepted in revised form: 18 September 1998

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Abstract The effect of the interaction between phospholipid monolayers and PEG-660-12-hydroxy stearate as a non-ionic surfactant on lipid emulsion stability in dynamic and static conditions was studied. The presence of PEG-660-12-hydroxy stearate molecules with phospholipid monolayers (static state) leads to a remarkable increase in the surface pressure (from 5 to 30 mN/m in the initial molecular area), whereas in the dynamic state, when the two emulsifiers are separated and each dissolved in one phase of the two emulsion phases, a sudden decrease in the surface pressures is observed. This indicates that PEG-660-12-hydroxy stearate molecules are intercalated between the phospholipid monolayers forming a molecular mixed film. At the same time, a part of the phospholipid monolayers interacts with the surfactant monomers to form a soluble or partially soluble association complex. This interpretation was also supported by interfacial tension measurements, where the interfacial tension in the dynamic state was lower than that in the static one. This indicates that in static conditions the phospholipids partially interact with PEG-660-12-hydroxy stearate resulting in a non-active association complex. Subsequently there is insufficient utilization of the available surfactants during the emulsification process. In contrast, in dynamic conditions both emulsifiers are available at the free surface from the beginning. This behaviour was substantiated by investigating the stability of emulsions which were prepared either by the static condition or the dynamic one during the autoclaving process.

Key words Surface pressure – Phospholipid monolayers – Dynamic and static conditions – Emulsion stability – Interfacial tension

Introduction

The behaviour of phospholipid monolayers at the air-water interface is of particular interest due to its significant role in stabilizing the dispersed droplets [1–4]. Although numerous reports on interactions between various chemical groups and lipid monolayers have already been published [5–7], little work has been reported on the interaction between non-ionic surfactants and lipid monolayers and their subsequent influence on lipid emulsion (LE) stability. This might be due to the difficulties involved in attempting the interaction between insoluble monolayers and highly surface-active substances that form soluble monolayers.

It is well-established that the stability and the behaviour of LE are affected by the surfactants used and the conditions of emulsion preparation [8, 9]. It is also evident from the literature that charged lipids lead to large repeat-distances depending on the incorporation of water between the lipid bilayer, and thus resulting in

more stable emulsions than for neutral ones [10]. Furthermore, the combination of phospholipids and non-ionic copolymer surfactant (Poloxamer 188) leads to the formation of a resistant close-packed mixed film, which confers improved stability on the dispersed droplets using the steric stability of the non-ionic copolymer surfactant [11, 12]. PEG-660-12-hydroxy stearate (Solutol H15) is a non-ionic non-toxic surfactant that could be used as a substitute for polyoxyethylen-35-ricinoleate (Cremophor EL) in intravenous applications to improve the stability of these systems [13, 14].

Therefore the purpose of this study was to elucidate the interaction between phospholipids and PEG-660-12-hydroxy stearate and the behaviour of phospholipids atthe interface in dynamic and static conditions as a function of PEG-660-12-hydroxy stearate concentration. Moreover, the stability of these emulsions on the basis of the film formed at the interface was also studied in terms of particle size change upon autoclaving, where the interfacial behaviour was evaluated using surface pressure, molecular area and interfacial tension. Consequently this research aimed at optimizing the emulsifier amount used to avoid the biological hazards and toxicity side effects which occur upon using excess emulsifier and which cause high emulsion viscosity [8, 15], and instability problems [9, 16].

Materials and methods

Materials

L-α-Phosphatidylcholinedipalmitoyl (DPPC) was supplied by Sigma (St.Louis, USA) and Lipoid S75 (S75) was isolated from soya and contained, according to the manufacturer (Lipoid, Ludwigshafen, Germany), a minimum of 70% phosphatidylcholine, 10% phos-phatidylethanolamine and 1.7% lysophophatidylcholine. Medium-chain triglycerides were obtained from Hüls (Miglyol 812, Witten/Ruhr, Germany). Polyoxyethylene-660-12-hydroxy stearate (Solutol H15) was supplied by BASF (Ludwigshafen, Germany). Glycerol for parenteral application (99.8%) was purchased from Merck (Darmstadt, Germany). Double-distilled water was used and all other chemicals were of reagent grade.

Preparation of LE

The LE was prepared as follows. Different S75/PEG-660-12-hydroxy stearate ratios were used at a total concentration of 1.5% in all formulations. The emulsifiers were dissolved either together in one phase (static condition) or separately in two emulsion phases (dynamic condition). The oil phase and the 2.5% aqueous solution of glycerol (for adjustment of isotonicity) were heated separately to about 50–55 °C. The oil phase was added to the aqueous solution and this mixture was pre-emulsified using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany) at 8000 rpm for 3 min. Final emulsification was carried out by passing 40 ml of the coarse emulsion through a high-pressure homogenizer (Micron Lab 40, APV Gaulin, Lübeck, Germany). After homogenization, the pH of the emulsions was adjusted to about 7.5 using 0.1 N aqueous sodium hydroxide. The batches of emulsions were poured into 15 ml vials, the vials were sealed and the emulsions were sterilized using a steam autoclave (K15T, Keller, Weinhein, Germany) at 121 °C for 15 min.

Measurements

Particle size analysis

The mean diameter of the bulk population was determined by photon-correlation spectroscopy covering the size range 5 nm to approximately 3 μm (Malvern spectrometer RR 102, Malvern, UK, with a helium-neon laser $\lambda = 632.8$ nm, Siemens, Germany). For size analysis approximately 1 μl fat emulsion was added to 1 ml distilled water in order to obtain the optimum scattering intensity.

Large particles (range 0.18–35 μm) were detected by laser diffractometry (Helos, Sympatec, Claushal-Zellerfeld, Germany) which yielded the particle size distribution using a lens with a 20 mm focal length. The emulsions were characterized by their volume diameters D_{50}, D_{99} and D_{max}, that means 50%, 99% or all of the particles are below the given size.

Surface pressure measurements

The π-A isotherms (pressure versus area curves) were taken on a Langmuir-type film balance (Krüss, Hamburg, Germany) equipped with a continuous measuring system. The balance has a Teflon barrier and the edges of the trough were rendered hydrophobic with paraffin wax in order to obtain high purity during measurements. Purity of the surface was confirmed by measurement of the surface tension at several positions of the barrier, a pure water surface exhibiting the same tension at all positions. All measurements were carried out at room temperature (20 ± 1 °C).

Phospholipid monolayers spread on the aqueous subphase. The phospholipids DPPC and S75 were dissolved in chloroform at a concentration of 0.734 and 0.78 mg/ml (1 mmol) equivalent to 6.1×10^{17} and 5.7×10^{17} molecules/ml. The phospholipid solution was spread on the aqueous subphase over the maximum available area (562 cm^2) by a means of a Hamilton syringe (25 μl) and was allowed to equilibrate for 15 min. The films were compressed at a constant rate of 0.32 cm s^{-1}. The surface pressure of the insoluble films (π_M) is equal to

$$\pi_M = \gamma_{H_2O} - \gamma_M$$

where γ_{H_2O} and γ_M are the surface tensions of water and the monolayers.

Mixed phospholipid/PEG-660-12-hydroxy stearate monolayers (static and dynamic conditions) at different PEG-660-12-hydroxy stearate concentrations. The same procedure as described above was used for the compression of phospholipid monolayers spread on the aqueous subphase containing different concentrations (0.217, 0.437 and 0.875 mmol) of the non-ionic surfactant (dynamic condition) or for phospholipid monolayers/PEG-660-12-hydroxy stearate (using the same concentrations as in the dynamic condition and dissolved together in one phase) spread on a freely aqueous phase (static condition). The π-A isotherms at PEG-660-12-hydroxy stearate concentrations ranging from 0.2175 to 0.875 mmol and corresponding to phospholipid/PEG-660-12-hydroxy stearate molar ratios 1:0.25, 1:0.5 and 1:1, respectively, were obtained. The surface pressure measurements were repeated, and the mean values were used.

Interfacial tension measurements

The interfacial tension was measured by the spinning drop method [17]. The system is thermostatically controlled and an accurate temperature of 40 ± 0.5 °C (corresponding to the production temperature) was obtained by means of an oil bath, which is also responsible for the lubrication of the ball bearings. Furthermore, the surface of the droplet is closed and no question of contact angle arises.

The interfacial tension was measured in the dynamic and static conditions as explained above and it was calculated according to the equation

$$Q = e(vd)^3 n^2 \Delta P$$

where Q is the interfacial tension (mN/m), e is a constant and has the value 3.427×10^{-7}, v is a magnification factor (mm/scale division), d is the diameter of the drop (scale division), n is the number of revolutions per minute and ΔP is the difference in the density of the two phases (g/cm^3).

Results and discussion

Changes in particle sizes upon autoclaving

The influence of the autoclaving process on the particle sizes of the emulsions prepared by static and dynamic conditions is summarized in Tables 1 and 2.

Table 1 shows the changes in particle sizes of LE induced by autoclaving in the static condition. The data demonstrate that the increase in the PEG-660-12-hydroxy stearate ratio above 40% of the total emulsifier concentration resulted in a remarkable change in particle size (D_{99}, D_{max}) upon autoclaving, whereas lower PEG-660-12-hydroxy stearate ratios showed stable preparations. In contrast to the static condition, if the emulsifiers dissolved separately in the two emulsion phases (dynamic condition) stable formulations could be achieved without a noticeable change in particle sizes upon autoclaving using a ratio of PEG-660-12-hydroxy stearate up to 60% (as shown in Table 2). In the static condition this difference could be attributed in most instances to interaction between PEG-660-12-hydroxy stearate micelles and phospholipid monolayers. This leads to a lack of free emulsifier which is very important for covering the oil droplets and consequently for stabilizing the system [8, 18]. However, in the dynamic condition both emulsifiers were dissolved separately in the two emulsion phases and were present at the free surface from the beginning of the experiment [1, 19]. It should be kept in mind that PEG-660-12-hydroxy stearate has a cmc concentration of about 0.021% (w/v) [13], which is lower than the concentrations that are usually used to stabilize the LE.

Surface pressure of mixed phospholipid/PEG-660-12-hydroxy stearate monolayers in different conditions (dynamic or static)

The π-A isotherms of the phospholipid monolayers (DPPC and S75) are shown in Fig. 1. The results were obtained using a subphase of pure water at room temperature. The DPPC molecular area at collapse is 46 ± 4 Å^2 at a film pressure of 44 ± 1 mN/m. These results agree with those previously published [20, 21]. In the case of S75, there is a little difference (molecular area at collapse is 50 ± 4 Å^2 at a film pressure of 35 ± 1 mN/m). This difference could be attributed to the presence of the other phospholipids (phosphatidylethanolamine and lysophosphatidylcholine), which are contained in S75 [1, 5].

The π-A isotherms of phospholipid monolayers with PEG-660-12-hydroxy stearate (static condition) spread on the free air-water interface are shown in Fig. 2. In all cases, an immediate and noticeable increase in the initial surface pressure is observed. This pressure increase, prior to film compression, shows that the surfactant molecules are localized at the air-water interface and are intercalated between the phospholipid monolayers. Such an increase in surface pressure has been noted by others who studied the penetration of surfactants [22, 23] or drugs [24] into phospholipid monolayers. Isotherms of phospholipid monolayers spread on the aqueous subphase containing different concentrations of PEG-660-12-hydroxy stearate (Fig. 3) show totally different

Table 1 Effect of PEG-660-12-hydroxy stearate emulsifier ratio on emulsion droplet diameters upon autoclaving (the emulsifiers were dissolved together). The total concentration of both emulsifiers is 1.5% (w/w) and these are the ratios of PEG-660-12-hydroxy stearate to phospholipids

PEG-660-12-hydroxy stearate concentration	Mean particle size (nm)		D_{50} (μm)		D_{99} (μm)		D_{max} (μm)	
	before sterilization	after sterilization	before sterilization	after sterilization	before sterilization	after sterilization	before sterilization	after sterilization
0%	176 ± 4.5	184 ± 5.0	0.71 ± 0.01	0.71 ± 0.02	1.59 ± 0.01	1.6 ± 0.01	1.8	1.8
20%	158 ± 2.5	161 ± 5.1	0.63 ± 0.02	0.64 ± 0.02	1.47 ± 0.02	1.48 ± 0.03	1.8	1.8
30%	157 ± 4.2	162 ± 4.7	0.64 ± 0.01	0.64 ± 0.01	1.47 ± 0.02	1.48 ± 0.01	1.8	1.8
40%	155 ± 2.6	163 ± 3.5	0.63 ± 0.01	0.64 ± 0.02	1.46 ± 0.01	1.47 ± 0.02	1.8	1.8
45%	146 ± 4.5	148 ± 4.9	0.63 ± 0.02	0.64 ± 0.02	1.45 ± 0.02	2.24 ± 0.04	1.8	3.6

Table 2 Effect of PEG-660-12-hydroxy stearate emulsifier ratio on emulsion droplet diameters upon autoclaving (the emulsifiers were dissolved separately)

PEG-660-12-hydroxy stearate concentration	Mean particle size (nm)		D_{50} (μm)		D_{99} (μm)		D_{max} (μm)	
	before sterilization	after sterilization	before sterilization	after sterilization	before sterilization	after sterilization	before sterilization	after sterilization
0%	176 ± 4.5	184 ± 5	0.71 ± 0.01	0.71 ± 0.02	1.59 ± 0.01	1.6 ± 0.1	1.8	1.8
20%	158 ± 2.2	165 ± 5.2	0.65 ± 0.01	0.66 ± 0.02	1.48 ± 0.01	1.49 ± 0.02	1.8	1.8
30%	161 ± 4.3	168 ± 3.2	0.66 ± 0.02	0.66 ± 0.01	1.48 ± 0.01	1.5 ± 0.02	1.8	1.8
40%	156 ± 2.6	162 ± 3.5	0.65 ± 0.02	0.66 ± 0.01	1.49 ± 0.01	1.49 ± 0.01	1.8	1.8
50%	148 ± 3.5	153 ± 3.9	0.63 ± 0.01	0.63 ± 0.02	1.48 ± 0.01	1.48 ± 0.01	1.8	1.8
55%	148 ± 2.1	154 ± 3.7	0.64 ± 0.01	0.65 ± 0.02	1.46 ± 0.01	1.47 ± 0.02	1.8	1.8
60%	159 ± 3.4	160 ± 4.3	0.66 ± 0.02	0.66 ± 0.01	1.5 ± 0.02	1.52 ± 0.02	1.8	1.8
70%	152 ± 2.8	154 ± 6.5	0.64 ± 0.02	0.65 ± 0.01	1.47 ± 0.02	2.32 ± 0.06	1.8	3.6

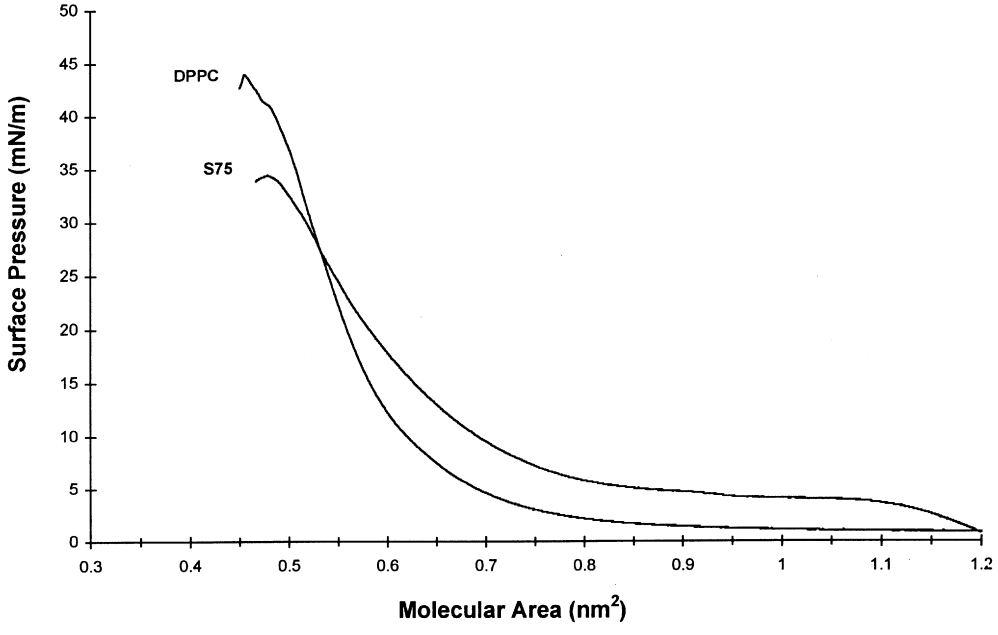

Fig. 1 Continuously recorded isotherms of L-α-phosphatidylcholinedipalmitoyl and Lipoid S75 on a pure water substrate

behaviour. In this case, there is a marked decrease in the surface pressure as well as in the molecular area, which indicates a monolayer disruption at the air-water interface.

These results show that the monolayers at the interface exhibit different behaviour. The surface pressure at constant molecular area and the molecular areas at constant surface pressure are shown as a function of surfactant concentration in Fig. 4. A remarkable increase in the surface pressures with increasing PEG-660-12-hydroxy stearate concentration is observed at an expanded and mid-area per molecule of 1.1 and 0.8 nm^2, respectively. For example, in the expanded state the surface pressure increased from about 5 to about 30 mN/m. At the same time, the molecular area at constant surface pressure (25 mN/m, which is the mid-surface pressure value) showed the same pattern, i.e. increasing the amount of PEG-660-12-hydroxy stearate leads to a marked increase in molecular area (Fig. 4). This increase in surface pressure and molecular area clearly indicates that interaction between the non-ionic surfactant and the phospholipid took place.

Conversely, Figs. 2 and 5 show that under the dynamic condition the increase in PEG-660-12-hydroxy stearate concentration leads to a great decrease in the molecular area as well as in the surface pressure. This decrease is dependent on the amount of PEG-660-12-hydroxy stearate, where the lowest values in surface pressure and molecular area were achieved at the highest PEG-660-12-hydroxy stearate concentration. The latter decrease could be attributed in most instances to the solubilization of phospholipids within the surfactant micelles in the bulk phase [23].

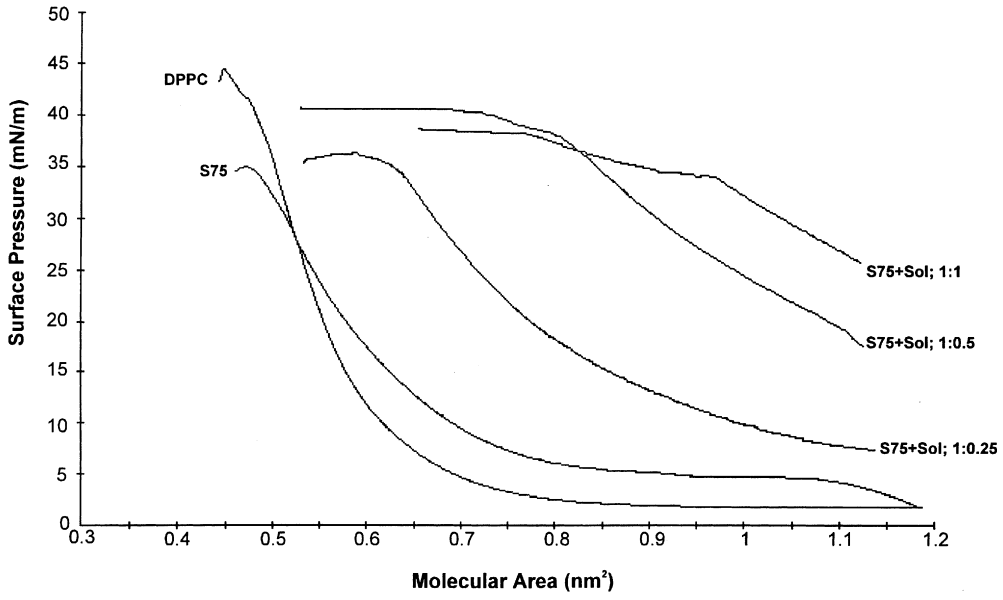

Fig. 2 Isotherms of phospholipid monolayers mixed with various PEG-660-12-hydroxy stearate concentrations (together in one phase) on a pure water substrate

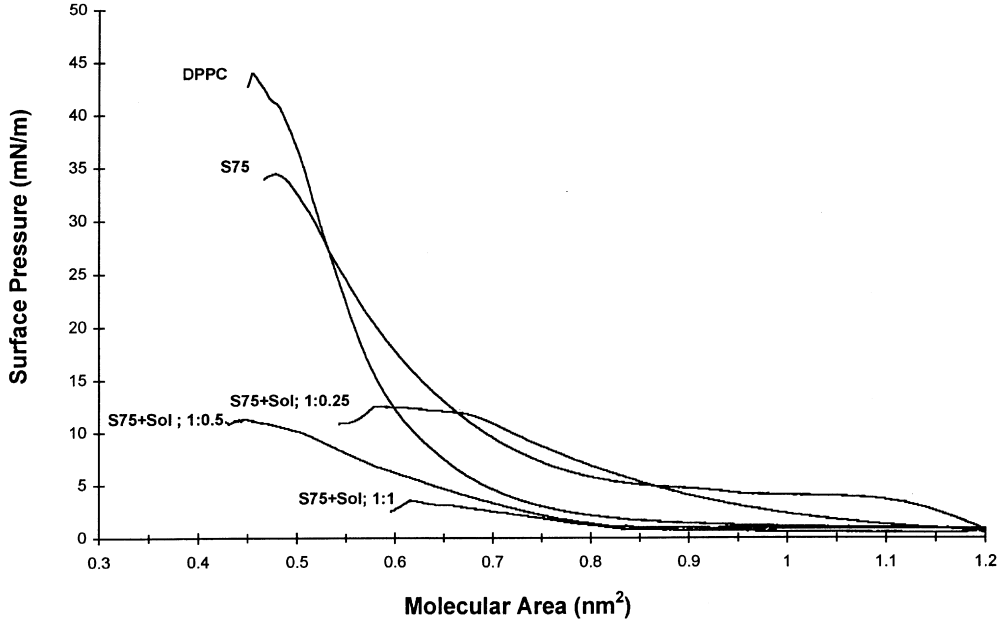

Fig. 3 Isotherms of phospholipid monolayers as a function of PEG-660-12-hydroxy stearate concentrations in the subphase

It should be borne in mind that in the dynamic condition the balance was left to equilibrate for 15 min, which is enough time for mobilization of the phospholipid monolayers from the interface to interact or partially dissolve within the surfactant micelles which are available in the bulk phase. Interestingly, this interaction took place more easily and effectively in the static condition where both emulsifiers dissolved in one phase. These arguments are supported by the hypothesis suggested by other workers [23, 25].

It could be deduced from the surface pressure results combined with those summarized in Tables 1 and 2 (characterizing emulsion physico-chemical properties) that PEG-660-12-hydroxy stearate molecules are intercalated between the phospholipid monolayers. At the same time, phospholipids interact or partially dissolve within the surfactant micelles, which are present in the bulk phase, thus resulting in a lack of free emulsifier. This, consequently, affects the physico-chemical properties of the system. This unfavourable interaction could

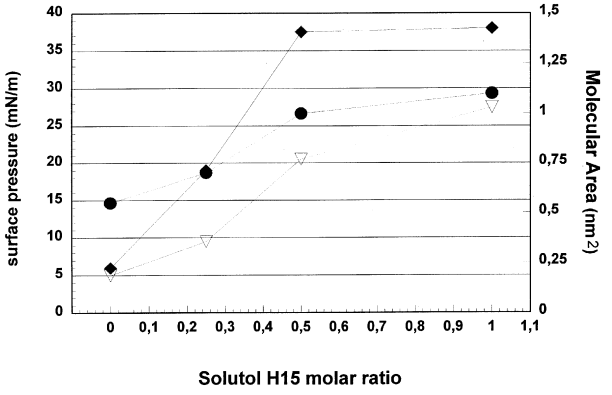

Fig. 4 Plots of surface pressure at constant molecular area (▽ 1.1 and ◆ 0.8 nm^2) and molecular area at constant surface pressure (● 25 mN/m) against surfactant concentration (static state)

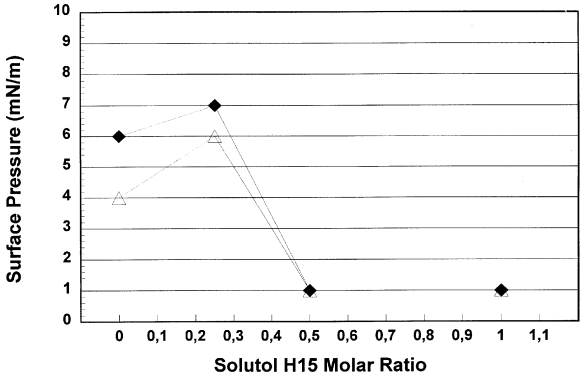

Fig. 5 Plot of surface pressure at constant molecular area (△ 1.1 and ◆ 0.8 nm^2) as a function of PEG-660-12-hydroxy stearate concentration (dynamic state)

be mostly avoided by dissolving the emulsifiers separately in the two emulsion phases and mixing them directly before homogenization.

Interfacial tension in the static and in the dynamic state

Interfacial tension measurements were applied to the LE prepared by both static and dynamic conditions to support the hypothesis aiming at reflecting the interfacial properties of the emulsifier mixture.

As shown in Fig. 6, a sudden decrease in interfacial tension is observed in the dynamic state after adding PEG-660-12-hydroxy stearate in the water phase. This indicates, as might be expected, that the greater amount of the two emulsifiers is participated in the film formation which results in a lower interfacial tension. Contrasting behaviour is observed when the two emulsifiers are dissolved together (static state). After the initial addition of PEG-660-12-hydroxy stearate the

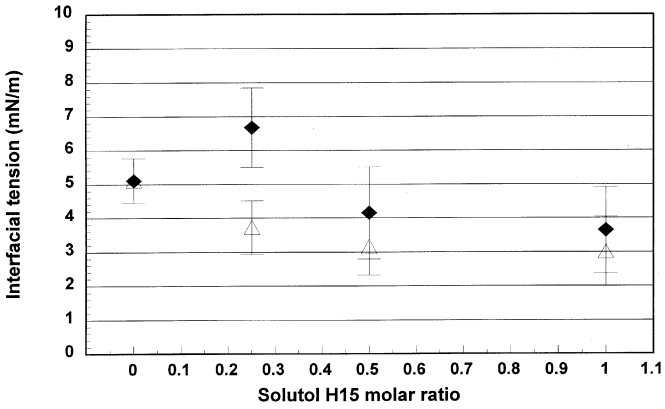

Fig. 6 Effect of increasing PEG-660-12-hydroxy stearate concentrations on the interfacial tension in static (◆) and dynamic conditions (△)

interfacial tension increases noticeably (Fig. 6). Further addition of PEG-660-12-hydroxy stearate leads to a predictable decrease in interfacial tension, but the interfacial tension is still higher than in the dynamic state. This different behaviour could be explained by the ability of the phospholipids to dissolve (partially) in the PEG-660-12-hydroxy stearate micelles contained in the bulk phase [23] or by the ability of the phospholipids to interact to some extent with PEG-660-12-hydroxy stearate molecules [19, 25]. As a result, this leads to the formation of a non-surface-active association compound and consequently, the complete amount of the emulsifier could not be used.

Furthermore, it is evident from the literature that amphiphilic molecules associate into micellar aggregates and this leads to a reduction in the energetically unfavourable contact between water and the apolar parts of the amphiphilic molecules while the polar groups are still solvated by the water [25, 26]. Moreover, the presence of such micellar aggregates affects the adsorption rate of the emulsifier molecules into the interface [27, 28].

Conclusion

The results of surface pressure measurements of phospholipid monolayers in the presence of PEG-660-12-hydroxy stearate have demonstrated the existence of an association between these two emulsifiers resulting in a complex-film formation. Moreover, it could be deduced that in the static state there is an interaction between the emulsifier molecules, whereas in the dynamic state both emulsifiers are present at the free surface from the beginning of the experiment. By virtue of these findings it could be concluded that dissolving the emulsifiers separately, one in the oil phase and the other in the aqueous phase, and mixing them directly before the

homogenization process can minimize the unfavourable interaction between the molecules. Simultaneously, this results in an optimum use of the available emulsifier amount.

Acknowledgement The authors are indebted and grateful to Kirsten Elschner, Department of Pharmaceutical Chemistry, for valuable technical support in surface pressure measurements and stimulating discussions during the work.

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