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Interactions of a non-ionic ABA copolymer surfactant with phospholipid monolayers: Possible relevance to emulsion stabilization

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Summary

The π-A isotherms of phospholipid monolayers in the presence of ABA polyoxyethylene, polyoxypropylene block copolymer (poloxamer) in the aqueous subphase exhibit a marked increase in surface pressure, indicating that poloxamer molecules are localized at the air-water interface and are intercalated between phospholipid molecules. While the surface pressure at collapse remained practically constant at poloxamer concentrations lower than the CMC, it increased at poloxamer concentrations above the CMC. The molecular area values were independent of poloxamer concentration. These results suggest that ejection of poloxamer molecules from phospholipid monolayers takes place at high compressions. In independent studies on the stability of emulsions using the same phospholipid-poloxamer combination, it was noted that an optimal concentration of these emulsifiers was necessary to stabilize the emulsion. The emulsion stability data which corroborate the results of surface pressure measurements confirm the existence of an association between poloxamer and phospholipid molecules. At poloxamer concentrations slightly higher than the CMC, poloxamer aggregates appear to form a hydrophilic environment close to the dispersed oily droplets, thus favoring emulsion stabilization. A model of molecular arrangements at the mixed monolayer-water interface is proposed.

Introduction

Although the bioavailability of emulsions designed for oral administration is complex (Wagner et al., 1966; Carrigan and Bates, 1973; Shinkuma et al., 1981; Illum et al., 1989), their potential therapeutic applications are promising (Daeschener et al., 1957; Hori et al., 1977; Friedman et al., 1989).

A stable submicron emulsion containing a hypocholesteremic active agent, clofibride, has recently been developed (Santos Magalhaes et al., 1991b). Optimal emulsion stability was obtained with a mixture of soya phospholipid and poloxamer in a molar ratio of 1 : 1, although it has been clearly shown that each of the two surfac-

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tants is able to reduce the interfacial tension of water (Prasad et al., 1976; Ogino and Onishi, 1981; Magdassi and Siman-Tov, 1990). No study on the surface properties of this emulsifier combination has been reported prior to the recent work of Santos Magalhaes et al. (1991a). The penetration of poloxamer into monolayers of soya phospholipid spread on the air-water interface was followed by the variation in the surface pressure at constant area under equilibrium conditions.

In the present study, attempts were made to determine the conditions necessary for the formation of stable emulsions and to relate them to the surface pressure data of soya phospholipid monolayers in the presence of poloxamer in the aqueous subphase. It was considered that an apolar air phase may be representative of the oil phase.

Materials and Methods

Materials

The phospholipid used was that isolated from soya and contained, according to the manufacturer (Lucas Meyer, Hamburg, Germany), a minimum of 95% phosphatidylcholine (PC), the molecular weight (MW) of which is 775, and a maximum of 4% lysophosphatidylcholine. The ABA polyoxyethylene, polyoxypropylene block copolymer surfactant, poloxamer (POL), under the commercial name Synperonic F68 (ICI, France), was used as received and had an approximate MW of 8350.

Water was triple distilled from permanganate solution using a pyrex apparatus.

Methanol and chloroform used to dissolve phospholipid for spreading at the water interface were pure, analytical grade Merck (Darmstadt, Germany) products.

Surface pressure measurements

The autorecording Langmuir-type film balance was used to measure surface pressure (π)-area (A) isotherms under dynamic conditions (Baszkin et al., 1987).

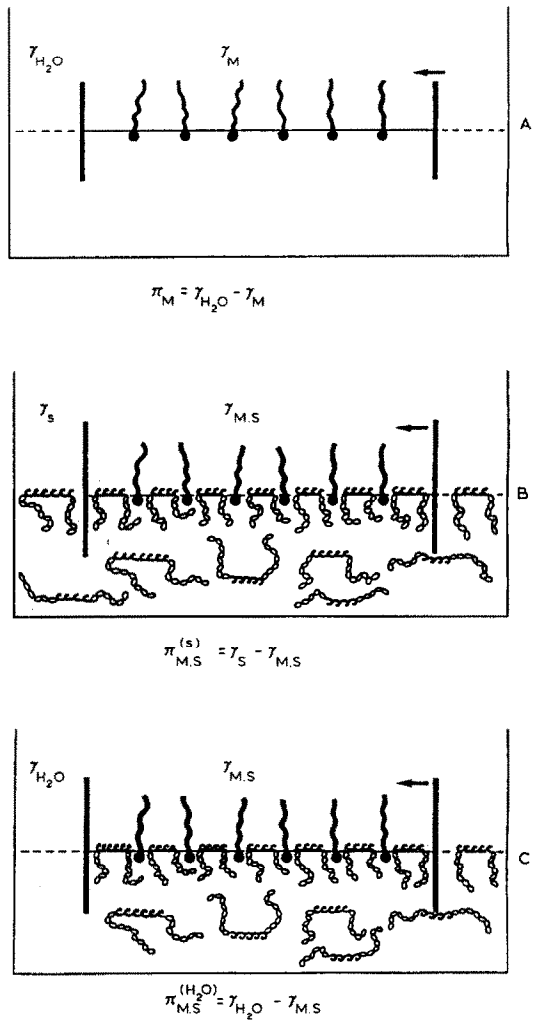

Fig. 1. Schematic representation of the experimental conditions under which the surface pressure was measured for a phospholipid monolayer spread on water (A) and on an aqueous subphase of copolymer surfactant (B); (C) represents the fictive conditions of (B) following correction according to Eqn 3.

PC monolayer spread on the aqueous subphase

The phospholipid was dissolved in a mixture of chloroform and methanol (9:1, v/v) at a concentration of 3.805 mg/ml, equivalent to 2.957×10^{18} molecules/ml. This phospholipid solution was spread on the aqueous substrate over the maximum available area (562 cm^2) by means of a syringe (15 μl) and allowed to evaporate for 15 min prior to compression at a constant rate of 0.4

$cm^2\ s^{-1}$ at room temperature ($20 \pm 2\,^{\circ}C$) (Fig. 1A). The compression of the insoluble films yielded surface pressure (π_M) vs molecular area (A) isotherms. The surface pressure of these films (π_M) is equal to

$$\pi_M = \gamma_{H_2O} - \gamma_M \quad (1)$$

where γ_{H_2O} and γ_M are, respectively, the surface tension of water and of the monolayer.

Mixed PC / POL monolayers at different poloxamer concentrations in the aqueous subphase The same procedure as that described above was used for the compression of phospholipid monolayers spread on the aqueous subphase containing the copolymer surfactant (Fig. 1B). The π-A isotherms at poloxamer concentrations ranging from 10^{-8} to 10^{-5} mol l^{-1} and corresponding to PC/POL molar ratios from 1:0.5 to 1:200 were obtained. The surface tension of the mixed monolayers was calculated from the measured surface pressure ($\pi^S_{M,S}$) according to the equation:

$$\gamma_{M,S} = \gamma_S - \pi^{(S)}_{M,S} \quad (2)$$

The values of γ_S, at different copolymer surfactant concentrations, were measured as a function of time in a separately run experiment and have been reported elsewhere (Santos Magalhaes et al., 1991a).

The surface pressure measurements were replicated, and the mean values are presented.

Emulsion preparation and evaluation

The emulsions were prepared and evaluated according to the procedure described elsewhere (Santos Magalhaes et al., 1991b).

* Symbols used herein: γ_{H_2O}, surface tension of water; γ_S, surface tension of surfactant; γ_M, surface tension of phospholipid monolayer; $\gamma_{M,S}$, surface tension of mixed monolayer; π_S, surface pressure of surfactant; π_M, surface pressure of phospholipid monolayer; $\pi^{(S)}_{M,S}$, surface pressure of mixed monolayer with surfactant in the aqueous subphase; $\pi^{(H_2O)}_{M,S}$, surface pressure of mixed monolayer as if it were on the water subphase, calculated according to Eqn 3.

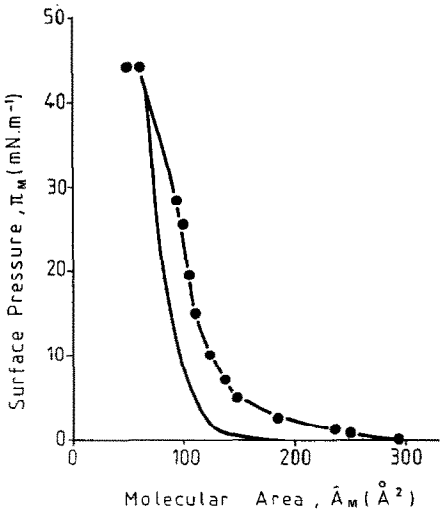

Fig. 2. Isotherms of phospholipid monolayer under dynamic conditions (———) with an initial surface area of 562 cm^2, superficial density of 7.89×10^{13} molecules cm^{-2}, and under equilibrium conditions (●———●) at constant area of 11.7 cm^2.

Results and Discussion

Surface pressure of phospholipid monolayer

The π-A isotherm of the phospholipid monolayer is shown in Fig. 2. Its molecular area at collapse is 60 ± 4 Å^2 at a film pressure of 41.8 mN m^{-1}. The surface tension at collapse, γ_M, is 72.2–41.8 = 30.4 mN m^{-1} and confirms the surfactant nature of soya phosphatidylcholine, which is widely used as the sole emulsifier in fat emulsions for intravenous administration (Hansrani et al., 1983). These values, obtained under dynamic conditions, are similar to those reported by De Bernard (1958) for a monolayer of egg phosphatidylcholine, for which a molecular area of 59 Å^2 at collapse was reported. They are also in agreement with the π-A isotherms obtained at constant area (11 cm^2) with successive additions of phospholipid aliquots until collapse was achieved.

Comparison of the π-A isotherms obtained under dynamic conditions with those at constant area reveals some differences. The mean molecular area of the phospholipid is 90 Å^2 under compression of the monolayer and 134 Å^2 in the latter case. A possible explanation would involve

differences in the sensitivity of measuring techniques. The Wilhemy plate method used for the π-A measurements yields more precise values at low pressures.

For the purpose of approaching as closely as possible the experimental conditions occurring at equilibrium, film compressions were carried out at a very low rate of 0.02 $cm^2\ s^{-1}$. The results were not significantly different from those obtained at a compression rate of 0.4 $cm^2\ s^{-1}$.

Hendrikx (1973) carried out similar types of experiments and compared the π-A isotherms of egg phosphatidylcholine obtained by addition of lipid molecules with those obtained under compression. She also observed a certain discrepancy between the two isotherms. According to Hendrikx, this discrepancy should be attributed to the inaccuracy in delivering exact volumes of phospholipid solutions, an error difficult to circumvent when the spreading surface is small. However, irrespective of the experimental conditions, the value of the film pressure at collapse (44–45 mN m^{-1}) was close to that observed in the present study for soya phospholipid.

Surface pressure of mixed PC / POL monolayers at different poloxamer concentrations in the aqueous subphase

The π-A isotherms of phospholipid monolayers spread on the air-water interface in the presence of poloxamer in the aqueous subphase are represented in Fig. 3. In all cases, an immediate and marked increase in the initial surface pressure is observed. This pressure increase, prior to film compression, shows that the surfactant copolymer molecules are localized at the air-water interface and are intercalated between the phospholipid molecules. Such an increase in surface pressure has also been noted by others who observed the penetration of surfactants (Alexander et al., 1986) or drugs such as antihistamine (Atwood and Udeala, 1975), insulin (Birdi, 1976), hydrocortisone (Cleary and Zatz, 1977) or indomethacin (Sicre and Cordoba, 1989) into phospholipid monolayers.

If the surface pressures of the mixed films spread on the surfactant subphase were to be compared with that spread on the water subphase, they should be recalculated according to Eqn 3, deduced from Eqns 1 and 2:

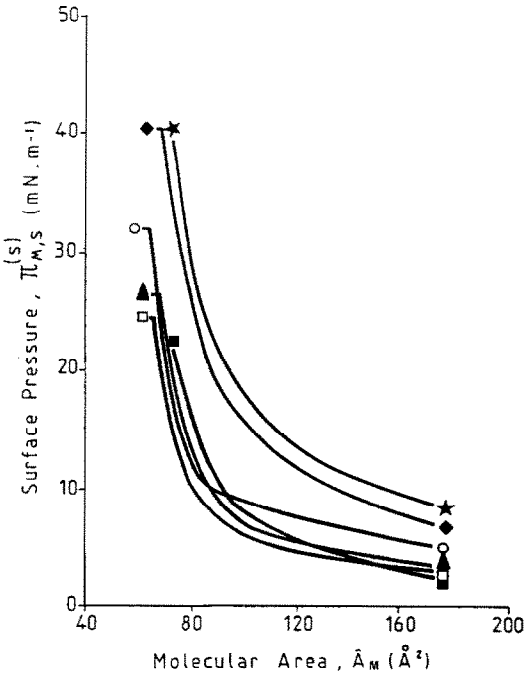

Fig. 3. π-A isotherms of mixed phospholipid monolayers at various copolymer surfactant concentrations in the subphase: (◆) 5.40×10^{-8}; (★) 1.08×10^{-7}; (○) 5.40×10^{-7}; (▲) 1.08×10^{-6}; (□) 5.36×10^{-6}; (■) 2.14×10^{-5} mol l^{-1}.

$$\pi^{(H_2O)}_{M,S} = \gamma_{H_2O} - \gamma_S + \pi^{(S)}_{M,S} \qquad (3)$$

These isotherms, shown in Fig. 4, correspond to the situation illustrated in Fig. 1C. Two distinct types of π-A curves are observed: type A, which corresponds to concentrations much lower than the CMC of the copolymer surfactant (5.3×10^{-6} mol l^{-1}) and type B, which corresponds to concentrations of the order of or greater than the copolymer surfactant CMC. Both types of isotherms exhibit similar behaviour for expanded and mid-compression states, but differ markedly at collapse.

An increase in the initial surface pressure with increasing copolymer surfactant concentration was observed for expanded and mid-compression states (Fig. 5). In the expanded state of the phospholipid monolayer, for example, corresponding

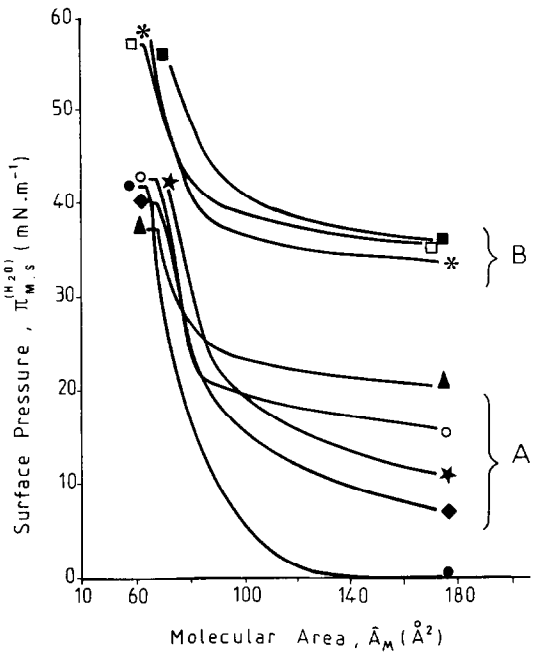

Fig. 4. π-A isotherms of mixed phospholipid monolayers as a function of copolymer surfactant concentrations in the subphase: (●) 0.0; (◆) 5.40×10^{-8}; (★) 1.08×10^{-7}; (○) 5.40×10^{-7}; (▲) 1.08×10^{-6}; (☆) 3.78×10^{-6}; (□) 5.36×10^{-6}; (■) 2.14×10^{-5} mol l^{-1} (isotherms recalculated according to Eqn 3).

to a molecular area of 176 Å^2, and at a copolymer surfactant concentration of 5.4×10^{-7} mol l^{-1}, the surface pressure increase was 15.5 mN m^{-1}.

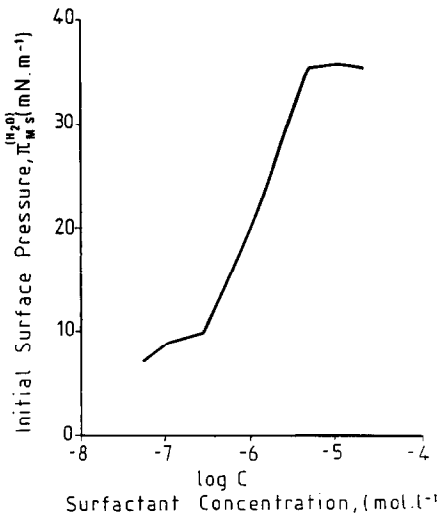

Fig. 5. Influence of copolymer surfactant concentration on the initial film surface pressure in the expanded state.

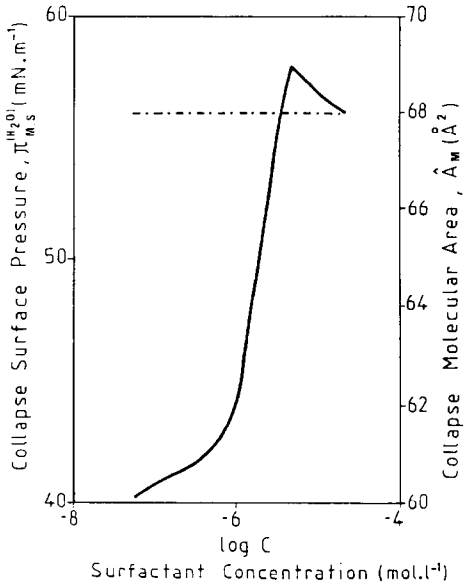

Fig. 6. Influence of copolymer surfactant concentration on the surface pressure and molecular area of the film at collapse.

At mid-compression, (molecular area 120 Å^2), and at a copolymer concentration equal to 1.08×10^{-7} mol l^{-1}, the increase was 17.5 mN m^{-1}. This surface pressure increase clearly indicates that an interaction between the copolymer surfactant and the phospholipid took place.

At collapse, the surface pressure remained practically constant at copolymer surfactant concentrations below the CMC (Fig. 4A), but increased above the CMC (Fig. 4B). In the latter case, this increase was also observed for monolayers in low compressional states. However, the molecular area was constant irrespective of the copolymer surfactant concentration (Fig. 6B). The constant value of the molecular area, which is comparable to that of the phospholipid alone (68 Å^2), could be attributed to the ejection of copolymer surfactant molecules from the phospholipid monolayer at high compression.

One possible explanation of the increased collapse surface pressures at copolymer surfactant concentrations higher than the CMC is that the copolymer surfactant, although ejected from the monolayer, remains associated with the hydrophilic groups of the phospholipid.

The results obtained in this study are in good agreement with those previously obtained in penetration experiments with phospholipid monolayers spread at constant area. For instance, at similar phospholipid film density (7.89×10^{13} molecules cm^{-2} and 8.22×10^{13} molecules cm^{-2} under dynamic and equilibrium conditions, respectively), and at copolymer surfactant concentrations in the subphase respectively equal to 2.4×10^{-7} and 5.0×10^{-7} mol l^{-1}, the surface pressure values are very similar, i.e. 13.6 and 18.0 mN m^{-1} in the present study and 14.7 and 15.8 mN m^{-1} measured at equilibrium at constant area.

Relevance to emulsion stabilization

In order to determine whether any relationship between the monolayer studies and emulsion formulation existed, the values of the surface pressure were converted into those of surface tensions (Table 1). These data, when combined with those summarized in Table 2 and characterizing emulsion properties, lead to the following conclusions:

(i) Both emulsifiers ensure emulsion stability, since the combination of the two yields emulsions that are much more stable than those obtained with only one emulsifier.

(ii) An optimal concentration of copolymer surfactant is required to stabilize the emulsion. Low or high concentrations of poloxamer lead to de-emulsification. However, a small excess of copolymer surfactant concentration as compared to the optimal value (5.36×10^{-6} mol l^{-1}) is still acceptable.

According to Table 1, to achieve an increase in surface pressure and consequently a reduction in surface tension, the copolymer concentration should be higher than the CMC. If the copolymer concentration is too low, the surface pressure will be insufficient. Conversely, at copolymer surfactant concentration much higher than the CMC, solubilization of the lipophilic emulsifier will take place, and would trigger off the ejection of some copolymer molecules from the interface. However, if the copolymer surfactant concentration slightly exceeds that corresponding to the optimal concentration (5.36×10^{-6} mol l^{-1}), solubilization of the phospholipid seems to be unlikely. It is believed that at these concentrations, poloxamer aggregates form a hydrophilic environment close to the dispersed oily droplets, favouring emulsion stabilization.

TABLE 1

Main characteristics of the mixed film at the air-water interface, at collapse surface pressure

Molar ratio of PC/POL Poloxamer concentration (mol l^{-1})	Mixed film surface tension [a] $\gamma_{M,S}$ (mN m^{-1})	Mixed film surface pressure [b] $\pi_{M,S}^{(H_2O)}$ (mN m^{-1})
1:0.5 (5.40×10^{-8})	31.8	40.2
1:1.0 (1.08×10^{-7})	29.8	42.2
1:2.5 (2.70×10^{-7})	31.3	40.7
1:5.0 (5.40×10^{-7})	29.3	42.7
1:10 (1.08×10^{-6})	28.0	44.0
1:35 (3.78×10^{-6})	14.2	57.8
1:45 (4.86×10^{-6})	14.1	57.9
1:50 (5.36×10^{-6})	15.0	57.0
1:100 (1.07×10^{-5})	15.0	57.0
1:200 (2.14×10^{-5})	16.0	56.0
1:350 (3.78×10^{-5})	20.5	51.5

[a] Calculated according to Eqn 2.
[b] Calculated according to Eqn 3.

A model for the molecular arrangements of the emulsifier at the air-water interface is proposed in Fig. 7.

(iii) The most stable emulsion required a molar ratio of phospholipid to copolymer of 1:1. However, as shown in Table 1, no marked increase in surface pressure was noted for a molar ratio of 1:1, and the surface tension of the mixed film was similar to that of the phospholipid film. This is not surprising, since the concentrations of surfactant at the interfaces in the two studies were quite different. Moreover, the presence of

TABLE 2

Main properties of a clofibride soybean emulsion stabilized with a mixed interfacial film of phospholipid and poloxamer at different molar ratios

PC/POL molar ratio PC/POL concentration (mol l^{-1})	Microscopic aspect	Mean droplet size ± S.D. (nm)	Effect of aging			Accelerated test on emulsion stability			Long-term stability assessment (storage at 25 °C)
			4 °C	25 °C	40 °C	Centrifugation (2000 × *g*/60 min)	Freeze-thaw cycle (16 h–18 °C/ 8 h + 25 °C)	Oscillation movement (48 h) (350 strokes/min, 25 °C)	
1:0 (6.450×10^{-4})	Unstable	–	–	–	–	–	–	–	–
109:1 (5.871×10^{-4}/ 5.398×10^{-6})	Unstable	–	–	–	–	–	–	–	–
10:1 (3.065×10^{-4}/ 2.994×10^{-5})	Stable	820 ± 87	–	+	+	–	–	+	2 days
2:1 (1.022×10^{-4}/ 4.989×10^{-5})	Stable	750 ± 120	–	+	+	–	–	+	8 days
1.5:1 (7.661×10^{-5}/ 5.240×10^{-5})	Stable	420 ± 87	–	+	–	–	–	+	1 month
1:1 (5.516×10^{-5}/ 5.240×10^{-5})	Stable	166 ± 120	–	+	+	+	+	+	18 months
0.7:1 (3.831×10^{-5}/ 5.614×10^{-5})	Stable	516 ± 58	–	+	+	–	–	+	8 days

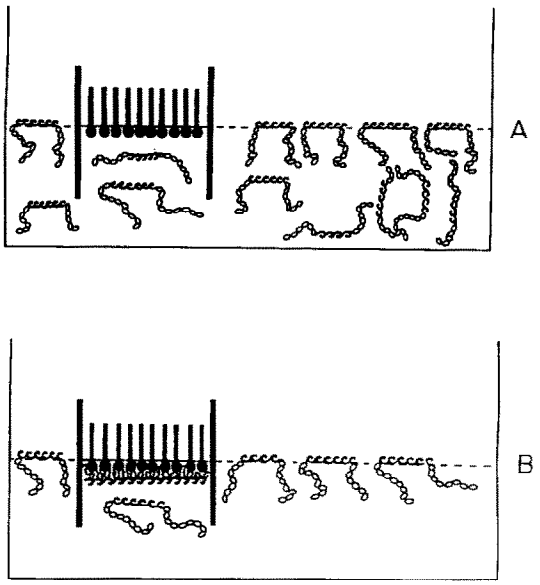

Fig. 7. Schematic representation of the phospholipid-poloxamer interaction hypothesis, at film collapse and at surfactant concentrations: (A) below the CMC and (B) higher than or equal to the CMC.

oil in the real emulsion systems may alter the partitioning of the phospholipid at the interface, leading to an increase in molar ratio in favour of the poloxamer.

Conclusion

The results of surface pressure measurements of phospholipid monolayers in the presence of poloxamer dissolved in the subphase have demonstrated the existence of an association between these two emulsifiers.

An increase in surface pressure was observed with increasing copolymer concentration. An optimal copolymer concentration was also identified. These results can be partially related to the stabilization of emulsions.

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