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Adsorption of phosphatidylcholine at the benzene-water interface

M. C. R. JOHNSON* AND L. SAUNDERS

*School of Pharmacy, University of London, Brunswick Square, London, W.C.*1, *U.K.*

Interfacial tensions between benzene solutions of egg-phosphatidylcholine and aqueous bovine plasma albumin have been studied. The presence of the protein in the aqueous phase leads to a more rapid adsorption of the phospholipid at the interface, this being a result of interaction between the phospholipid and protein. The most probable mechanism for the interaction is discussed. The interfacial tensions between aqueous dispersions of egg-phosphatidylcholine and pure benzene have also been determined. The results clearly indicate the effect of ultrasonic treatment in producing a stable aqueous phospholipid dispersion.

The adsorption from benzene solution of egg-phosphatidylcholine (egg-PC) at the benzene-water interface is highly time dependent (Johnson & Saunders, 1968). The process is not entirely diffusion controlled but dependent upon the crossing of an energy barrier to adsorption. Mixed protein–egg-PC interfaces have been examined to determine the effect of introducing bovine plasma albumin (BPA) into the aqueous phase upon the adsorption process.

MATERIALS AND METHODS

Interfacial tensions were determined by the modified Wilhelmy plate technique described by Ruyssen (1946), using an electrical balance (Johnson & Saunders, 1968). A thin, depolished platinum plate 1·032 cm in length and approximately 0·5 cm in depth was used. The end correction, l_0, was determined by measuring the surface tension of a sample of water using two plates of different lengths (0·5 cm and 1 cm) but made from the same gauge of platinum (Padday, 1957). The value of l_0 (0·009 cm) was then added to the geometric length of the plate in subsequent calculations of the interfacial tension values. Double-distilled water was used, prepared from a seasoned all-glass still, only the middle third fraction being taken from the final distillate. Analar grade benzene (British Drug Houses) was used, without further purification.

Purified egg-PC was prepared from a commercial sample (Merck A.G.) by chromatographic treatment with alumina and silicic acid, as described by Saunders (1957). Confirmation of purity of the egg-PC was obtained from the phosphorus and nitrogen analysis giving an N:P ratio of 1·04. This value compares favourably with the results of Perrin (1962) and is close to the theoretical value of 1·0.

Bovine plasma albumin (BPA) (Armour; Fraction V) was purified by recrystallization from methanol (Cohn, Hughes & Weare, 1947).

Simple aqueous dispersions of egg-PC were prepared by dissolving the required amount of the material in ether. The ether solution was then added to a weighed

* Present address: Ciba-Geigy Ltd., Basle, Switzerland.

quantity of water, and the ether removed under vacuum at 30°. The resulting milky dispersion was centrifuged to remove any coarse undispersed material, and the dispersion assayed by drying an aliquot to constant weight in a vacuum oven at 30°.

Sonicated dispersions of egg-PC were prepared by the method of Saunders, Perrin & Gammack (1962). A simple aqueous dispersion, after removal of the ether, was subjected to ultrasonic irradiation for 90 min, when the egg-PC had dispersed to give aggregates with a weight average molecular weight of 2×10^6 (see Attwood & Saunders, 1966).

All determinations of interfacial tension were made at 25°.

RESULTS

The interfacial tensions of pure benzene with aqueous solutions of Armour (Fraction V) BPA are plotted as a function of time in Fig. 1. The observed time dependence of the interfacial tensions is a well-known phenomenon with protein films (Cheesman & Davis, 1954).

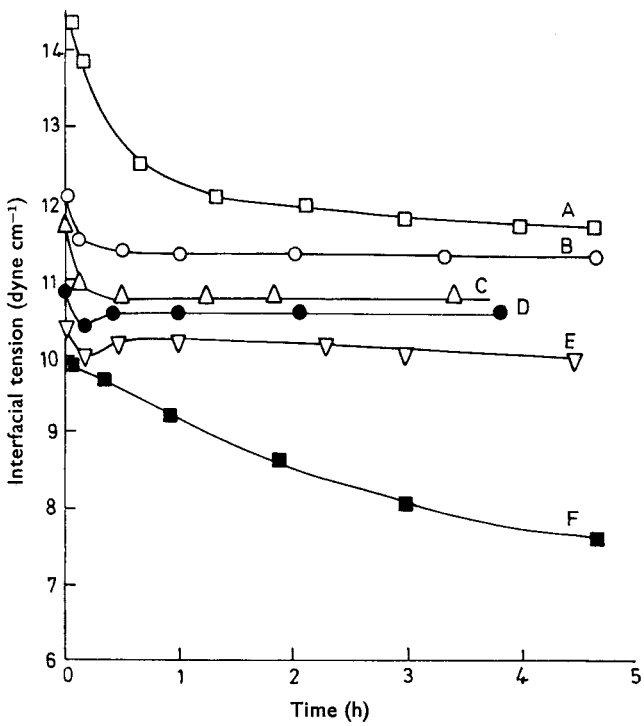

FIG. 1. Interfacial tensions of pure benzene with aqueous solutions of BPA (Armour) at 25°. A, $5{\cdot}0 \times 10^{-4}$% w/v; B, $5{\cdot}0 \times 10^{-3}$% w/v; C, $1{\cdot}0 \times 10^{-2}$% w/v; D, $5{\cdot}0 \times 10^{-2}$% w/v; E, $1{\cdot}0 \times 10^{-1}$% w/v; F, 1·0% w/v.

The occurrence of minima in some of the curves in Fig. 1 suggested competitive adsorption of some impurity in the protein solution, most probably fatty acid. Hence, the system was re-examined using the recrystallized protein, the results being given in Fig. 2.

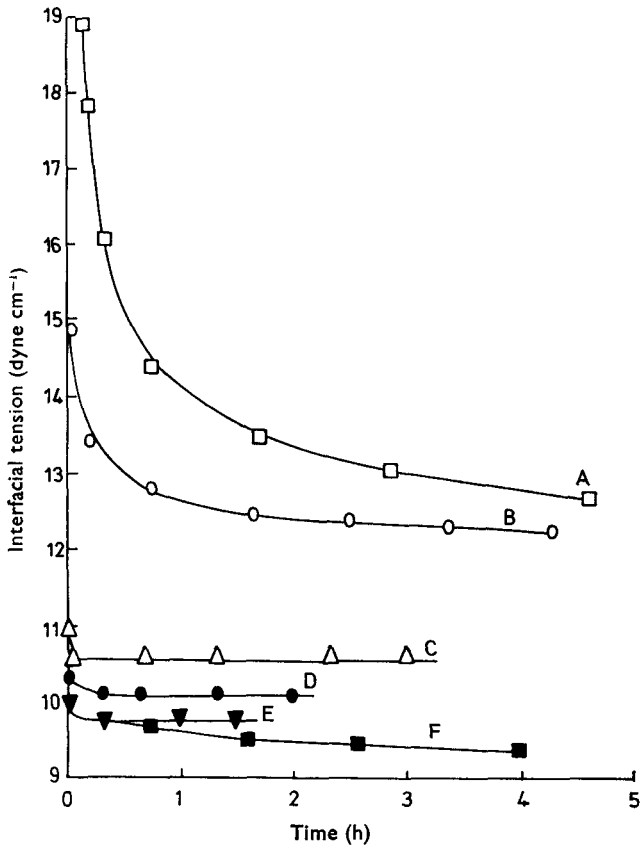

FIG. 2. Interfacial tensions of pure benzene with aqueous solutions of purified BPA at 25°. A, $1{\cdot}0 \times 10^{-3}$% w/v; B, $5{\cdot}0 \times 10^{-3}$% w/v; C, $1{\cdot}0 \times 10^{-2}$% w/v; D, $5{\cdot}0 \times 10^{-2}$% w/v; E, $1{\cdot}0 \times 10^{-1}$% w/v; F, 1·0% w/v.

The interfacial tension-time curves for the purified protein no longer exhibit minima, indicating that the impurity has been removed by the precipitation procedure.

In the concentration range 0·01 to 0·1% w/v the interfacial tensions of the purified BPA solutions exhibited only a small time dependence, equilibrium values being attained within 10 min of forming the interface (Fig. 2). Hence, for the study of mixed egg-PC-BPA interfaces, a constant protein concentration of 0·1% w/v BPA was used, thus avoiding superimposition of a large time effect due to the protein, upon that resulting from the adsorption of egg-PC from the benzene phase. The interfacial tension results between solutions of egg-PC in benzene and 0·1% w/v BPA aqueous solutions are given in Fig. 3.

For the interfacial tensions between simple aqueous dispersions of egg-PC and benzene, large time effects were observed although no clear dependence upon bulk concentration was found. In the case of ultrasonicated aqueous dispersions, a clear dependence upon the egg-PC concentration was observed. The interfacial tensions between pure benzene and the ultrasonicated dispersions are plotted as a function of time in Fig. 4.

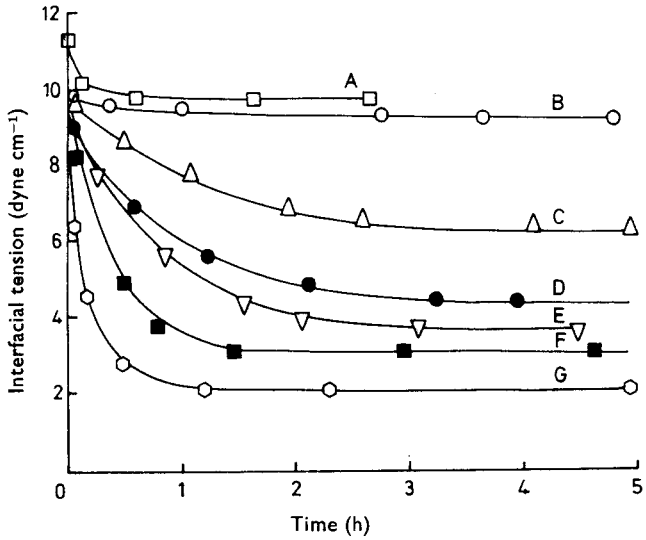

FIG. 3. Interfacial tensions for egg-PC in benzene with 0·1% w/v purified BPA aqueous solution at 25°. A, $1{\cdot}02 \times 10^{-5}$% w/w; B, $5{\cdot}52 \times 10^{-5}$% w/w; C, $8{\cdot}49 \times 10^{-5}$% w/w; D, $9{\cdot}84 \times 10^{-5}$% w/w; E, $1{\cdot}18 \times 10^{-4}$% w/w; F, $1{\cdot}68 \times 10^{-4}$% w/w; G, $2{\cdot}34 \times 10^{-4}$% w/w.

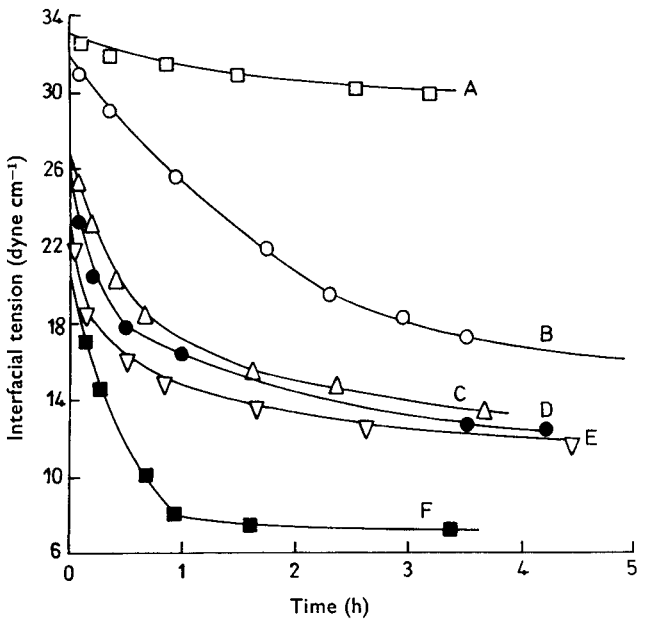

FIG. 4. Interfacial tensions for pure benzene with sonicated aqueous dispersions of egg-PC at 25°. A, $1{\cdot}10 \times 10^{-3}$% w/w; B, $5{\cdot}94 \times 10^{-3}$% w/w; C, $1{\cdot}87 \times 10^{-2}$% w/w; D, $2{\cdot}42 \times 10^{-2}$% w/w; E, $3{\cdot}17 \times 10^{-2}$% w/w; F, $4{\cdot}70 \times 10^{-2}$% w/w.

DISCUSSION

Previous experiments (Johnson & Saunders, 1968) have shown that the interfacial tensions between benzene solutions of egg-PC and pure water are highly time dependent, equilibrium values not being achieved even after 5 h. In contrast, the interfacial tensions of the egg-PC-BPA systems, while exhibiting a time dependence, attained equilibrium values after about 3 h in most cases (see Fig. 3).

Whilst adsorption of PC molecules from the benzene phase is not entirely diffusion controlled, it is an activated process, the phospholipid molecules requiring a certain activation energy for adsorption at the interface. The more rapid attainment of equilibrium at the interface in the presence of protein suggests that the interfacial protein is, in some way, reducing the adsorption activation energy of the phospholipid. This is probably the result of interaction between the protein and phospholipid.

Interactions between proteins and lipid monolayers at the air-water interface have been studied by injecting protein beneath the monolayer (Doty & Schulman, 1949; Eley & Hedge, 1956; Colaccio, 1969). The resulting increase in film pressure has been taken as a measure of the degree of interaction of the lipid and protein.

It has been found by Eley & Hedge (1956) and Colaccio (1969) that with phosphatidylcholine films, initially at low surface pressure, interaction with injected BPA and γ-globulin occurred, a large increase in film pressure being observed. Eley & Hedge (1956) concluded that the interaction of phosphatidylcholine with BPA was predominantly electrostatic between the charged groups of the lipid and the protein. By studying aqueous dispersions of egg-PC and BPA, O'Keeffe (1967) concluded that a complex of indefinite composition was formed, the most plausible mechanisms of binding being between the hydrophobic portions of the two species and electrostatic forces between the respective charged groups of the two compounds.

For the benzene-water interface, forces between the hydrophobic portions of the lipid and any protein protruding into the benzene phase, will be much reduced owing to the surrounding non-polar environment. The most probable interaction therefore is provided by electrostatic forces between the polar groups of the zwitterionic phospholipid protruding into the aqueous phase, and the corresponding oppositely charged groups of the protein amino-acid residues.

In the aqueous phase at pH 7, the protein carboxyl groups will be ionized, thus the interaction of these groups with the quaternary amino-groups of the PC is most probable.

In addition to a reduction in the adsorption activation energy, such interaction would result in the adsorbed phospholipid molecule achieving a lower potential energy at the interface due to the presence of the protein. The tendency of the phospholipid to diffuse back into the benzene phase would thus be reduced compared with the protein free interface; this would favour a more rapid equilibration.

For the aqueous dispersions of egg-PC at the benzene water interface, the effect of sonication which produces a stable aqueous dispersion of egg-PC, was clearly reflected in the observed concentration dependence of the interfacial tension results. The lack of dependence upon bulk concentration in the case of simple aqueous dispersions, is attributed to the unstable, highly polydisperse nature of these non-sonicated preparations.

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