Evidence for the Formation of Amphotericin B-phospholipid Complexes in Langmuir Monolayers

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Purpose. To study the interaction of the polyene antifungal amphotericin B with phospholipid Langmuir monolayers and to correlate with stability of phospholipid-stabilized drug emulsions.

Methods. Pressure—area isotherms of mixed monolayers of amphotericin B (0–20 mol%) and different phospholipid types were recorded using conventional Langmuir trough methods. Emulsion stability of amphotericin B-containing lipid emulsions was measured using dynamic light scattering.

Results. Incorporation of amphotericin B into monolayers composed of saturated phospholipids (Lipoid E80-3) had a profound effect on the shape of the isotherm. This effect was directly related to the concentration of amphotericin B in the monolayer. At high drug concentrations, the shape of the isotherms became progressively similar to that of pure DPPC, thus exhibiting regions attributable to phospholipid in different phase states. This effect on isotherm shape was not observed following incorporation of the drug into monolayers composed of the equivalent unsaturated lecithin (Lipoid E80).

Conclusions. These results are interpreted as indicating the formation of an amphotericin B-phospholipid complex, resulting in phase separation within the monolayer. The extent and nature of this phase separation was dependent on both the concentration of drug in the system, and the saturation state of the phospholipid component. The relevance of these observations to the stability of amphotericin B drug emulsions stabilised by saturated and unsaturated phospholipid emulsifiers is discussed. These observations may also be relevant to the toxicity of these, and other novel amphotericin B formulations.

KEY WORDS: amphotericin B; lecithin; emulsion; stability; monolayer; low-dimensional structures.

INTRODUCTION

Amphotericin B is a membrane-active polyene antibiotic which is the drug of choice for the treatment of disseminated fungal infections, particularly in immunocompromised patients (1). It is administered as a solubilizate in sodium deoxycholate (Fungizone, Bristol-Myers Squibb), due to its insolubility in most solvents. Unfortunately, a wide range of adverse effects are produced by this formulation. These include infusion-related reactions, such as fever and chills, rigors, nausea and vomiting, and general malaise (2), as well as more serious complications, the most important of which is nephrotoxicity (3). Consequently, attempts have been made to reduce the toxicity of the drug whilst maintaining antifungal efficacy. The most common approach involves reformulation using a variety of novel delivery systems. These include liposomal (AmBisome, Vestar Ltd) and lipid complex (Amphocil, Zeneca Ltd, and Abelcet, The Liposome Company, Inc.) formulations which have recently been launched commercially (4–6). All three products demonstrate good efficacy, but with a greatly reduced toxicity.

It is generally acknowledged that the reduced toxicity of novel lipid carriers of amphotericin B is primarily dictated by the nature of the phospholipid component (7–9). Many studies, particularly with liposomes, indicate that the degree of saturation (and hence phase state) of the phospholipid component is of central importance in determining the toxicity of the formulation (10–12).

We have developed novel formulations of amphotericin B in emulsion form. A method has been developed whereby the drug can be intercalated into the stabilising layer of the phospholipid at the surface of a preformed carrier emulsion. This method does not involve the use of solvents, and is based on a knowledge of the pH-solubility characteristics of the drug (and a temperature jump for saturated emulsions) (13). In the resulting drug emulsion, the amphotericin is intercalated in the phospholipid emulsifier monolayer on the surface of the oil droplet (14). Studies have demonstrated that when the drug is correctly incorporated into the surface of the oil droplet, the resulting emulsion formulations are less toxic than the conventional Fungizone product, as demonstrated by tests such as red cell lysis (15), potassium efflux (16) and damage to confluent layers of cultured kidney cells (17).

The relationship between the in-vitro toxicity of amphotericin B emulsion formulations and phospholipid composition and method of manufacture has been examined using an erythrocyte potassium leakage assay (16). Using this model, the toxicity of the emulsion formulations was compared with that of Fungizone and liposomal amphotericin B (AmBisome), which were found to exhibit high and low toxicity respectively. Emulsion toxicity was found to be dependent on the transition temperature of the phospholipid emulsifier and the thermal history of the emulsion. Emulsions made using unsaturated phospholipids (including those made using Intralipid 20% as the carrier) showed a low toxicity irrespective of the method of manufacture. Emulsions made using phospholipids with a phase transition temperature above the maximum process temperature displayed a toxicity comparable to Fungizone. This toxicity was significantly reduced when such formulations were heated to a temperature above the phase transition temperature for the phospholipid. These data were interpreted as demonstrating that the amphotericin B molecule must be fully inserted into the phospholipid emulsifier in order to produce a low toxicity emulsion, and that this insertion is critically dependent on the composition of the emulsifier and its phase transition properties.

Amphotericin B emulsions stabilised by unsaturated phospholipids have been shown to be both chemically and physically stable. In contrast, in preliminary studies, emulsions stabilised by saturated phospholipids demonstrated poor stability, and a tendency to flocculate, following drug incorporation (18). The difference in the stability of the two emulsion systems may be due to the interaction of the drug with the phospholipid component of the formulation. It is also likely that such amphotericin B-lipid interactions are important in determining the toxicological properties of some of the other novel lipid formulations currently under development.

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In this paper, we report studies of the interaction of amphotericin B with phospholipid monolayers spread on an aqueous subphase in a Langmuir trough. We also report studies of the influence of phospholipid emulsifier structure on the stability of amphotericin B lipid emulsions. We propose that differences in the interaction of the drug with saturated and unsaturated phospholipids, as highlighted by the monolayer studies, may explain the instability of amphotericin B emulsions stabilised by saturated phospholipids. Implications of this work on the molecular toxicology of these and other novel amphotericin B formulations are discussed.

MATERIALS AND METHODS

Materials

Amphotericin B BP was a gift from Dumex, Denmark. The phospholipids and lecithins used were dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC), obtained from Natterman Chemie, Cologne, Germany, and Lipoid E80 and Lipoid E80-3 purchased from Lipoid KG, Ludwigshafen, Germany. DMPC and DPPC are synthetic phospholipids whose fatty acid chains are fully saturated and are composed of 14 and 16 carbon atoms respectively. The manufacturers' stated purity of these phospholipids was over 99%. Lipoid E80 is an unsaturated egg lecithin consisting of approximately 80% phosphatidylcholine, 7% phosphatidylethanolamine, 2% sphingomyelin, as well as trace quantities of lysophosphatidylcholine, lysophosphatidyl ethanolamine, and cholesterol. Lipoid E80-3 is equivalent in composition except that the fatty acids in this lecithin are fully hydrogenated. These two lecithins were selected for study since they are used to stabilise oil-in-water (O/W) emulsions intended for parenteral administration. Cholesterol (grade I) was purchased from Sigma Chemical Co, and had a stated purity of over 99%. All other reagents used were either of Analar (chemicals) or HPLC (solvents) grade; all were used as received.

Emulsion Preparation

Soya oil (20% w/w) emulsions stabilized by the appropriate phospholipid (1.2% w/w) were prepared by microfluidizer as previously described (19). Amphotericin B was incorporated at a concentration of 1 mg/ml (27). Briefly, amphotericin B was dissolved in sodium hydroxide solution (1 M), added to the emulsion, and 1 M hydrochloric acid added to pH 5, above the phase transition temperature of the emulsifier.

Emulsion Characterization and Stability Measurement

Emulsion stability was assessed by measuring z-average droplet diameter, and polydispersity, using a Malvern 4700 photon correlation spectrometer (PCS) (Malvern S4700, Malvern Instruments, Malvern, UK), as described previously (20). Zeta potentials were measured in 1 mM HEPES, pH 7.4, using a Zetasizer 4 (Malvern Instruments). Emulsion stability was quantified as the change in these parameters over a 7 day period immediately after manufacture.

Langmuir Trough Studies

Monolayers were spread at the air-water interface in a Joyce-Loebl Langmuir mini-trough (Joyce-Loebl Instruments, Sunderland, UK). The trough was interfaced to a BBC B microcomputer for data collection and isotherm plotting. For calibration purposes the area contained within the constant perimeter barrier was calculated at maximum and minimum surface area, and at eight points equally spaced between these two positions. Film pressure was measured using a Wilhelmy plate arrangement attached to a microbalance, calibrated using weights of known mass (0–125 mg).

All glassware used was ultrasonically cleaned in Decon 90 solution for 5 minutes, followed by three rinses in high purity water (Elga Ltd, High Wycombe, UK). For the final rinse glassware was sonicated in high purity water for 30 minutes. Volumetric flasks were first rinsed with chloroform before being ultrasonically cleaned and rinsed as for glassware. Following this initial clean, volumetric flasks were soaked in chromic acid for one week, before finally rinsing five times with high purity water. Between isotherm recordings the trough was rinsed with clean water and Decon 90, washed with chloroform, and finally rinsed with high purity water. The barrier was cleaned by sonication in chloroform for 30 minutes, followed by sonication in high purity water for a further 30 minutes. Following these cleaning procedures, the surface remained clean and free from ghost isotherms for at least 30 minutes.

Monolayer Material Preparation

Phospholipid solutions were prepared in chloroform, to a concentration of approximately 2 mM. Solutions containing both phospholipid and amphotericin B (5–20 mol%) (binary systems) were prepared using a mixture of chloroform and methanol (60:40 v/v). The solubility of the drug in anhydrous methanol is 0.5–0.6 mg/ml, therefore the maximum concentration of amphotericin B easily attained in these mixtures was approximately 20 mol%. Similarly, a solution of cholesterol in chloroform was prepared, to a final concentration of approximately 3 mM. All solutions were freshly prepared using solvents of HPLC grade on the day of the experiment.

Monolayer Spreading and Film Compression

A known quantity (40–50 μl) of the material being studied was added to the surface of cleaned subphase using a micrometer controlled syringe. The quantity to be added was calculated to give a compressed monolayer with the barrier positioned approximately half way between its maximum and minimum limits. For all experiments, the subphase consisted of highly purified water (Elga Ltd) buffered to pH 7.4 with sodium phosphate buffer (1 mM). The temperature of the subphase was maintained at 21°C (±0.5) by circulating water from a thermostated water bath (Grant Instruments Ltd, Cambridge, UK) through the trough.

After addition of the sample, a fixed time (typically 10 minutes) was allowed for the carrier solvent to evaporate. The monolayer was then slowly compressed (30 mm/minute) such that the total time taken to record an isotherm was approximately 10 minutes. Changes in film pressure with decreasing film area were recorded to construct a pressure—area isotherm. Isotherms for each material studied were recorded at least in duplicate. The steeply sloping linear section of each isotherm was extrapolated back to zero surface pressure, to determine the area per molecule ($Å^2$) of the material (or average area per molecule for the binary

systems). The slope of this linear extrapolation was used to calculate the compressibility (m/mN) of the monolayer (21). The measurable compression range of each monolayer (Å^2), defined as the difference in area from zero surface pressure to collapse pressure, was also determined.

The behaviour of cholesterol monolayers has been much studied (22), therefore this molecule was selected for initial experiments to validate the operation of the Langmuir trough and associated data collection system. The resulting isotherm (Figure 1) had an area per molecule of 36.4 Å^2, and an extremely low compressibility. These values compare favourably with those quoted in the literature (Table I). Subsequent experiments examined the isotherms of monolayers of DMPC, DPPC and mixed monolayers of DPPC, Lipoid E80 and Lipoid E80-3 containing amphotericin B (0–20 mol%).

It should be noted that these lecithins are composed of a mixture of phospholipids (see materials). The exact proportions of the different phospholipid components in mixed lecithins is poorly defined, particularly with respect to the precise composition of the phospholipid fatty acid chains. It is therefore impossible to assign a precise 'molecular weight' to these materials. Therefore, in order to construct pressure–area isotherms for Lipoid E80 and Lipoid E80-3, the molecular weights were assumed to be 700 and 704 respectively.

RESULTS

Table II summarizes stability data for the emulsions studied; i.e., those made with Lipoid E80 (unsaturated) and Lipoid E80-3 (saturated) emulsifiers, over a 7 day period.

Both control emulsions (i.e. those without incorporated amphotericin B) were stable over the period of the study. Incorporation of amphotericin B into the Lipoid E80 emulsion resulted in a small increase in droplet size and polydispersity,

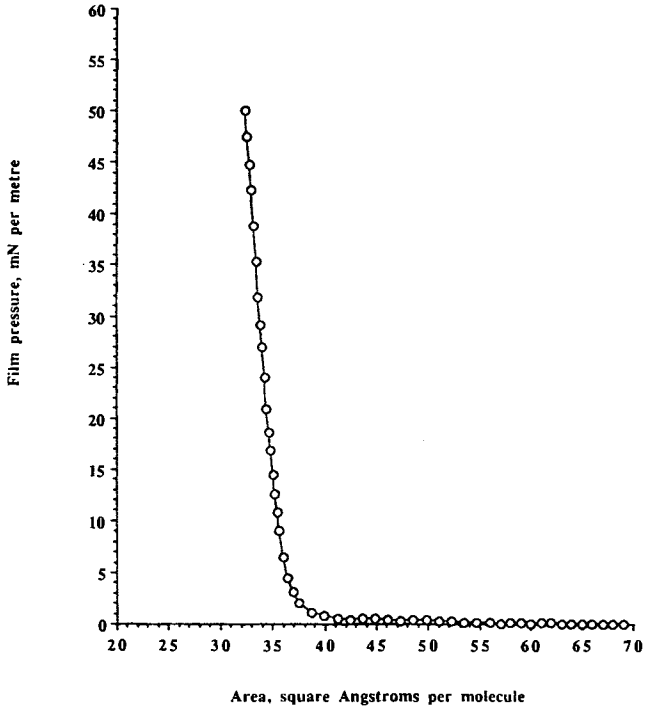

Fig. 1. Pressure–area isotherm of cholesterol at 21°C.

which was, however, still within the range which could be considered clinically useable. However, incorporation of amphotericin B into the Lipoid E80-3 emulsion caused immediate aggregation, as demonstrated by the initial size of 569 nm, and high (0.64) polydispersity. By the 7th day of the trial, this sample showed extensive aggregation and free oil. The PCS diameter (445 nm) in fact does not reflect the true state of the sample because a major fraction of the oil droplets had grown to a size which was above the detection range of the technique. In all cases the zeta potentials were unchanged over the 7 day period, and the incorporation of amphotericin B did not make a significant change to the zeta potentials. This suggests that the observed variations in stability were not mediated by simple DLVO-type colloid stability mechanisms. (We should add that the instability observed using Lipoid E80-3 has been observed for other saturated lecithin emulsifiers in our studies).

Figure 2 shows the pressure-area isotherm of DPPC at 21°C. This isotherm shows several characteristic features (23). Region I represents a homogenous liquid-phase monolayer with a decreasing compressibilty as the area per molecule is decreased. At its high molecular area limit, a mixed fluid and gaseous phospholipid phase is observed (24). As the area per molecule is reduced, monolayer condensation leads to a coexisting region (Region II) of solid and fluid phospholipid (25), where solid phospholipid domains form periodic patterns (26). In region III these solid phospholipid domains interconnect and coexist with fluid phospholipid. Long-range lateral diffusion is therefore slow in this region. In region IV the molecular area is a minimum and all the phospholipid is in a solid state. In this region, lateral compressibility is low, and the aliphatic chains are thought to be densely packed. At the same temperature DMPC (Figure 2) exhibits an isotherm characteristic for one single phase at all measured pressures. This is a homogenous fluid phase, similar to that in region I of the DPPC curve.

Figure 3 shows the pressure—area isotherms of the unsaturated egg lecithins Lipoid E80 and Lipoid E80-3. At the temperature of the experiment, both lecithins exhibit isotherms that are similar in nature to that of DMPC, i.e., the isotherms are not composed of distinct regions (DPPC). As expected the saturated material occupies a smaller area per molecule than the unsaturated lecithin, at any given surface pressure (46.3 and 69.0 Å^2 respectively). In addition the saturated lecithin monolayer was fully compressed over a significantly smaller area than the equivalent unsaturated material. The saturated lecithin therefore has a lower compressibility (0.00564 m/mN) than its unsaturated equivalent (0.00884 m/mN). The unsaturated lecithin also exhibited a significantly lower surface pressure when fully compressed (collapse pressure; 48 mN/m), compared with the saturated equivalent, Lipoid E80-3.

Figures 4 and 5 show the effect of amphotericin B (0–15 mol%) on the pressure—area isotherms of Lipoid E80 and Lipoid E80-3 respectively. When amphotericin B was incorporated into Lipoid E80 monolayers there was little change in the overall shape of the isotherm, even at high drug concentration (15 mol%). However, at higher drug concentrations (10 and 15 mol%) the position of the isotherm was altered towards a smaller area per molecule at high film pressures, and a larger area per molecule at lower film pressures. This shift was reflected in a lower area per molecule for films containing 10 (61.4 Å^2) or 15 mol% (62.2 Å^2) of amphotericin B, compared with an area per molecule of 69.0 Å^2 for films containing either 5 mol% or

Table I. Area Per Molecule and Compressibility of Phospholipid and Lecithin Monolayers, and Mixed Monolayers of Amphotericin and Phospholipid/Lecithin

Phopsholipid/Lipid Component	Amphotericin Concentration (mol%)	Area per Molecule (Å^2)	Area per Molecule Literature Value (Å^2)	Compressibility (m/mN)
Cholesterol	0	36.4	Val	0.00225
DPPC	0	53.0	Val	0.00577
	5	46.9	—	0.00474
	10	43.5	—	0.00562
	15	45.6	—	0.00667
	20	40.0	—	0.00729
DMPC	0	70.1	Val	0.00901
Lipoid E80	0	69.0	—	0.00884
	5	69.0	—	0.00882
	10	61.4	—	0.00869
	15	62.2	—	0.00982
Lipoid E80-3	0	46.3	—	0.00564
	5	40.8	—	0.00461
	10	40.7	—	0.00492
	15	40.5	—	0.00502

no amphotericin B. The compressibility of films containing 5 or 10 mol% amphotericin B (0.00882 and 0.00869 m/mN respectively) remained similar to that of the pure lecithin (0.00884 m/mN), but was increased at the highest drug concentration studied (15 mol%) (0.00982 m/mN). However, despite these small changes in compressibilty, incorporation of the drug into the monolayer had a significant effect on the compression range of the mixed monolayers. Thus, there was an increase in compression range from 50 to 66 Å^2 as the concentration of amphotericin B was increased from 0 to 15 mol% (Table I).

When the drug was incorporated into monolayers of Lipoid E80-3, the equivalent hydrogenated phospholipid, there was a profound effect on the shape of the isotherm (Figure 5). As the concentration of drug was increased the shape of the isotherms became progressively similar to that of pure DPPC (Figure 2). Thus the isotherms began to show characteristic regions corresponding to phospholipid in different phase states (27). At the highest concentration studied, 15 mol% amphotericin B, the isotherm closely resembled that of DPPC (at 21°C). Despite the observed changes in the shape of the isotherms, the area per molecule for monolayers containing the drug remained virtually constant (40.5–40.8 Å^2), but were significantly lower than those for the pure lecithin (46.3 Å^2). These changes in the shape and position of the isotherms were accompanied by changes in the compressibilty of films containing the drug. As for Lipoid E80 monolayers, the effect of drug incorporation on the Lipoid E80-3 isotherm could be more easily quantified by considering the compression range of each isotherm (Table I). Again there was an increase in compression range from 26 to 52 Å^2 as the concentration of amphotericin B was increased from 0 to 15 mol%, largely due to the emergence of the new phase at higher molecular areas.

Incorporation of low concentrations of amphotericin B (5–15 mol%) into monolayers of DPPC again resulted in changes in the appearance of the isotherm (Figure 6). In this case, the actual shape of the isotherm was unaltered, although its position was progressively shifted to smaller areas per molecule, as the concentration of the drug in the system was increased (Table I). The observed shift in the position of the isotherm was again accompanied by a decrease in compressibility at lower drug concentrations (5 and 10 mol%), followed by an increase in compressibility at higher drug concentration (15 mol%) (Table I). However, in contrast to the two lecithins studied, drug incorporation did not have a significant effect on the compression range of the DPPC isotherm. At the highest drug concentration studied in DPPC (20 mol%), the position

Table II. Particle Size (z Average), Polydispersity, and Surface Charge (Zeta Potential) of Amphotericin Emulsions

Emulsifier	Lipoid E80				Lipoid E80-3			
	Control		AmB		Control		Amb	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Size (nm)	247.7	253.8	380.9	377.2	256.3	259.5	569.4	445.7[a]
(+/−)	5.3	4.5	7.8	12.2	4.0	3.9	97.0	62.3
Polydispersity	0.088	0.118	0.362	0.345	0.102	0.085	0.641	0.466
(+/−)	0.045	0.025	0.043	0.053	0.053	0.039	0.143	0.199
Zeta potential (mV)	−33.9	−33.3	−31.5	−33.7	−38.8	−39.7	−39.0	−38.9
(+/−)	0.5	0.5	0.7	0.5	0.4	0.5	0.5	0.4

[a] Significant oil separation had occurred by day 7 of the trial with this sample.

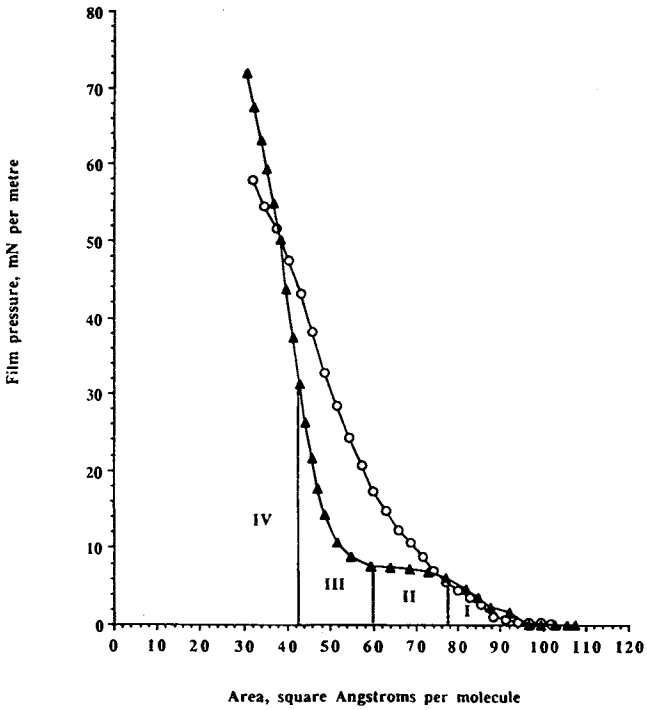

Fig. 2. Pressure–area isotherms of dipalmitoylphosphatidylcholine (DPPC) (▲) and dimyristoylphosphatidylcholine (DMPC) (○) at 21°C. The different regions of the DPPC isotherm (I–IV) are described in the text.

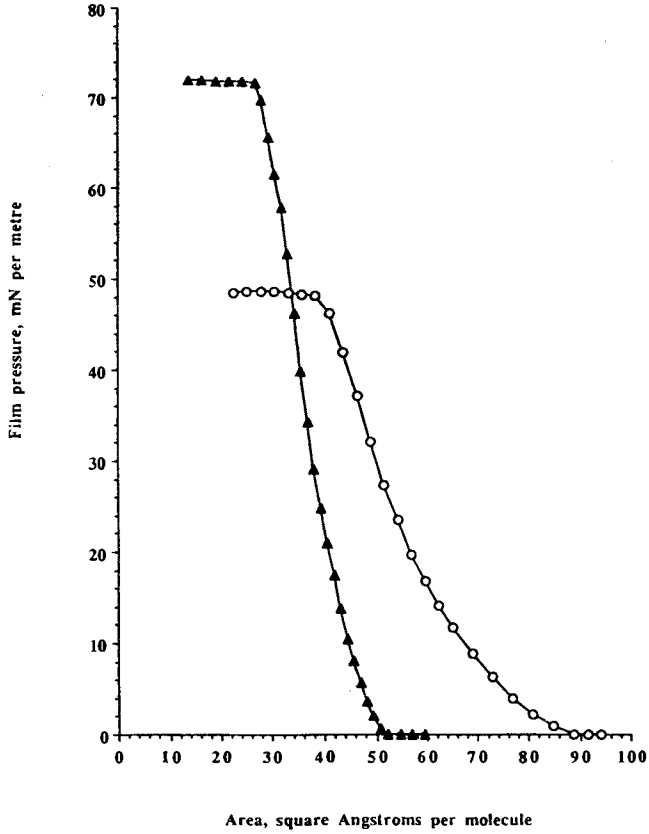

Fig. 3. Pressure—area isotherms of the unsaturated egg lecithin Lipoid E80 (○), and its hydrogenated equivalent, Lipoid E80-3 (▲), at 21°C.

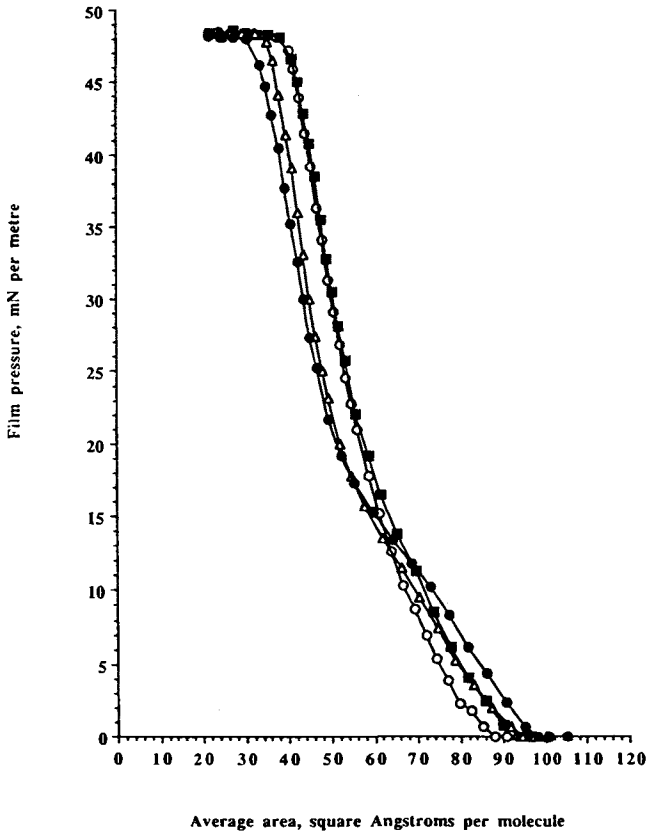

Fig. 4. Pressure–area isotherms of Lipoid E80 (unsaturated) (○), and mixed monolayers of Lipoid E80 and amphotericin B 5 mol% (■), 10 mol% (△), and 15 mol% (●). All isotherms were recorded at 21°C.

of the isotherm was further shifted to a smaller area per molecule, but in addition the overall shape of the isotherm was significantly altered (Figure 6). Thus, at this drug concentration the extent of the solid/fluid regions of the isotherm (areas I and II) were significantly enlarged. The shape of this isotherm appears to indicate the formation of two distinct solid phases.

DISCUSSION

This series of experiments allowed direct comparison of the effects of amphotericin B on saturated and unsaturated phospholipid and lecithin monolayers. For each phospholipid/lecithin studied, incorporation of amphotericin B into the monolayer resulted in a decrease in the average area per molecule at higher surface pressures. When amphotericin B was incorporated into DPPC monolayers there was a progressive decrease in the area per molecule, as the concentration of drug in the mixture was increased. Incorporation of the drug at 5 mol% into Lipoid E80 had no measurable effect on the area per molecule, but at higher concentrations (10 and 15 mol%) the area was again reduced to a near constant value (61.4–62.2 $Å^2$). A slightly different trend was seen following drug incorporation into monolayers of Lipoid E80-3. For this hydrogenated lecithin, the area per molecule was decreased to a constant value, for all drug/lecithin mixtures studied. Despite these minor differences, the overall effect of amphotericin B was to condense the phospholipid monolayer in all cases.

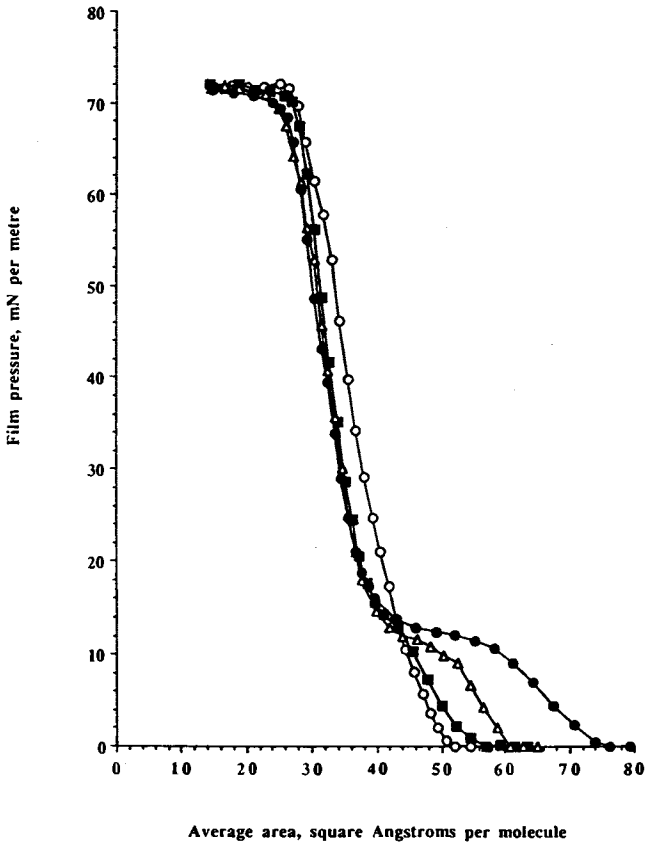

Fig. 5. Pressure–area isotherms of Lipoid E80-3 (hydrogenated) (○), and mixed monolayers of Lipoid E80 and amphotericin B 5 mol% (■), 10 mol% (△), and 15 mol% (●). All isotherms were recorded at 21°C.

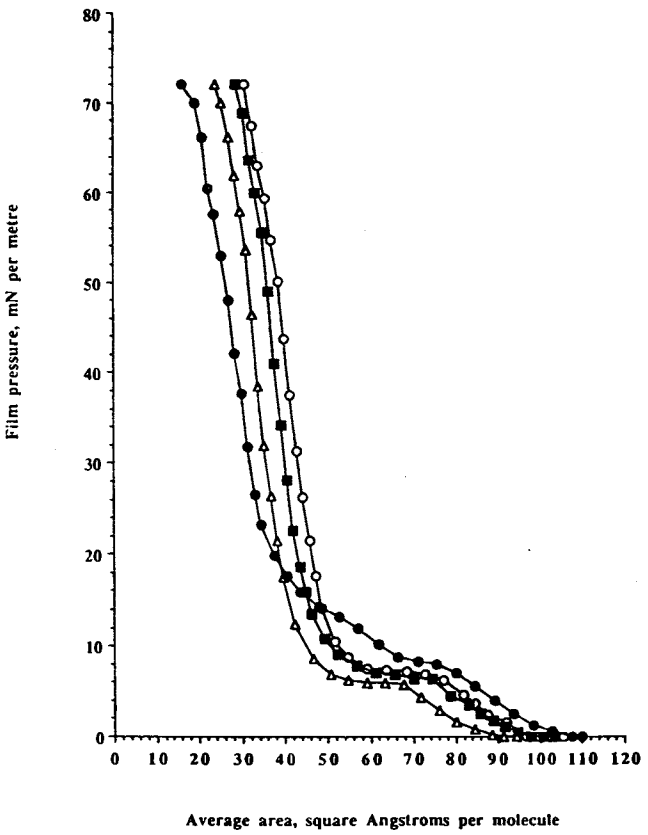

Fig. 6. Pressure–area isotherms of DPPC (○), and mixed monolayers of DPPC and amphotericin B 5 mol% (■), 10 mol% (△), and 20 mol% (●). All isotherms were recorded at 21°C.

We interpret these results as indicating the formation of an amphotericin B-phospholipid complex, resulting in phase separation. Phase separation is associated with an initial decrease in monolayer compressibility (albeit slight for Lipoid E80), followed by an increase in compressibility at higher drug concentrations.

Differences in the effect of the drug on isotherms of Lipoid E80 (unsaturated) and Lipoid E80-3 (hydrogenated) suggest that the phase state of the resulting amphotericin B-phospholipid complex is different in each case. We propose that condensation in the Lipoid E80 monolayer is primarily into homogenous fluid regions, resulting in little change to the overall appearance of the isotherm. However, in the Lipoid E80-3 monolayer, condensation is primarily into coexisting solid and fluid regions (see DPPC isotherm, Figure 2) accounting for the observed changes in the appearance of the isotherm, particularly at high drug concentrations. The appearance of distinct plateaux in the isotherm is characteristic of the separation of a solid phase, which we suggest is an amphotericin B-phospholipid complex.

If incorporation of amphotericin B into the phospholipid monolayer results in condensation (or structuring) of the phospholipid around the drug molecule, we would expect the compressibility of the monolayer to alter, relative to that of the pure lecithin. In the case of the unsaturated lipid, the monolayer is present in the fluid phase, and solid domains due to complex formation would be expected to float freely in the monolayer. On the other hand, domain formation in the more rigid saturated lecithin monolayer would result in the formation of defects. Such defects would be expected to increase the compressibility of the monolayer. If, in the corresponding unsaturated film, condensation results in formation of fluid regions, the formation of these defects is likely to have less effect on the compressibilty of the monolayer, since the dynamic mobility of the fluid phase lipid will 'fill in' the defects (or their lifetime will be shorter). We would also expect the complex formed from the unsaturated lipid to be less stable than that formed from the saturated lipid, as its formation is opposed by thermal diffusion. Consequently it is reasonable that the most noticeable changes in the isotherms occur when using saturated lipids.

Attempts have been made in recent years to identify the conditions needed for the formation of stable O/W emulsions by studying the interactions occurring between the surfactants at the oil-water interfacial film of the dispersed oil droplets (28). The stability of the emulsions studied in the present work is clearly dependent on both the phospholipid and the presence of amphotericin B, and the variations in stability can be understood by reference to the behaviour of the corresponding monolayers. We should note that the amphotericin B mole fractions studied in monolayers are directly comparable to those achieved in emulsion formulations. In practise it is difficult to assign an accurate value to the drug mole fraction in the emulsifier, since a fraction of free emulsifier is normally present in the continuous phase; however values of 5–10% are typical for 20% lipid emulsions containing 1 mg ml^{-1} of amphotericin B.

When the drug condenses in a monolayer composed of an unsaturated emulsifier, homogenous solid regions are formed in a pre-existing fluid matrix. In a monolayer composed of a saturated emulsifier condensation primarily forms solid domains in an existing solid monolayer. In both cases condensation of phospholipid around the drug molecule may lead to the formation of defects (or 'holes') in the monolayer. Because of these defects, the resulting monolayer for each phospholipid type is more compressible. However, because in an unsaturated phospholipid film (fluid phase) the dynamic mobility of the phospholipid is able to 'fill in' the defects, the effect of drug incorporation on compressibility is less than for the corresponding saturated material.

If this effect occurs in monolayers spread on an aqueous subphase, it is possible that a similar disruptive effect occurs when the drug is incorporated into the interfacial film of a fat emulsion. In this case, condensation of the emulsifying phospholipid around the drug molecule disrupts the homogeneity of the interfacial film. This disruptive effect may lead to the formation of defects in the emulsifier film, through which 'naked' disperse phase (soya oil) will be accessible to the continuous phase. In this disrupted state, the oil droplets can flocculate (as observed) by contact between these hydrophobic defects. As discussed above, because of the difference in phase state between saturated and unsaturated phospholipids, the effect of drug incorporation on those emulsions stabilised by an unsaturated emulsifier is less than on those stabilised by saturated materials. Thus, emulsions stabilised by a saturated emulsifier flocculate, whilst those stabilised by unsaturated phospholipid emulsifiers do not, and retain stability.

It is possible to speculate on the significance of these results to the behaviour of some of the low-toxicity amphotericin B formulations currently being developed. A notable feature of these formulations is that they use predominantly saturated phospholipids; AmBisome uses DSPC and hydrogenated PC, and Abelcet (ABLC) uses DMPC and DMPG. It is possible that the complex of amphotericin B with saturated phospholipids is more strongly bound, and consequently less toxic. Alternatively, the 2-phase solid nature of the saturated lipid/amphotericin B membrane may be of importance in determining the detailed interaction between the delivery system and the cellular membrane.

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