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Mixed monolayers made from dipalmitoyl phosphatidylcholine and a fluorinated amphiphile

Hélène M. Courrier [a,b], Thierry F. Vandamme [b], Marie Pierre Krafft [a,*], Shohei Nakamura [c], Osamu Shibata [c,*]

[a] *Centre National de la Recherche Scientifique, Institut Charles Sadron (UPR 022), 6 rue Boussingault, 67 083 Strasbourg Cedex, France*

[b] *Laboratoire de Chimie Thérapeutique et Nutritionnelle: Biodisponibilité Tissulaire et Cellulaire, Faculté de Pharmacie, Université Louis Pasteur, 67401 Illkirch Cedex, France*

[c] *Department of Molecular Bioformatics, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

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Abstract

We have recently reported that water-in-fluorocarbon (FC) reverse emulsions and microemulsions could be used as delivery systems of bioactive materials to the lung, in particular for local administration using pressurized metered-dose inhalers. The surfactant involved in these reverse emulsions is a perfluoroalkylated amphiphile derived from the dimorpholinophosphate polar head group $C_8F_{17}(CH_2)_{11}OP(O)[N(CH_2CH_2)_2O]_2$ (F8H11DMP). The interaction of F8H11DMP with the lung surfactant is unknown, as well as that of reverse FC microemulsions. In this study, based on film balance measurements and fluorescence microscopy, we report on the mixed monolayer behavior of F8H11DMP and dipalmitoylphosphatidylcholine (DPPC) used as a lung surfactant model at the air/water interface. F8H11DMP/DPPC mixtures formed miscible, liquid expanded (LE) mixed monolayers in the whole range of F8H11DMP molar fractions investigated ($X_{F8H11DMP} \geq 0.33$). Repulsive interactions were observed for high surface pressures. Miscible monolayers in LE state were also formed when DPPC/F8H11DMP mixtures were put in contact with perfluorooctyl bromide (PFOB). In this case, however, attractive interactions were observed (for $X_{F8H11DMP} = 0.33$ and 0.5) in the all range of surface pressures investigated. In addition, it was found that spreading a water-in-PFOB microemulsion formulated with F8H11DMP on a DPPC monolayer led to the adsorption of F8H11DMP at the interface and formation of a mixed DPPC/F8H11DMP monolayer.

Keywords: Fluorinated surfactant; Fluorocarbon emulsion; Langmuir monolayer; Perfluorooctyl bromide; Pulmonary drug delivery; Reverse water-in-fluorocarbon microemulsion

1. Introduction

Fluorocarbons (FCs) and the FC moieties of perfluoroalkylated amphiphiles are uniquely char-

* Corresponding authors. Tel.: +33-3-88-414060; fax: +33-3-88-404199; http://www-ics.u-strasbg.fr/ (M.P.K.); tel./fax: +81-92-642-6669 (O.S.)

E-mail addresses: krafft@ics.u-strasbg.fr (M.P. Krafft), osamurcc@mbox.nc.kyushu-u.ac.jp (O. Shibata).

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acterized by very strong intramolecular bonds and very weak intermolecular interactions. This results in a combination of exceptional thermal, chemical and biological inertness, high gas-dissolving capacities, low surface tension, high fluidity, excellent spreading characteristics and low solubility in water [1–4]. An FC, perfluorooctyl bromide ($C_8F_{17}Br$, PFOB, also known as perflubron) is being investigated for use in the form of a FC-in-water emulsion for delivering oxygen to tissues at risk of hypoxia [1,2,4]. In its neat form, PFOB is also being investigated for the delivery of drugs [5] and genes [6] to the lung.

We have shown previously that perfluoroalkylated amphiphiles derived from the dimorpholinophosphate polar head group, $C_nF_{2n+1}(CH_2)_mOP(O)[N(CH_2CH_2)_2O]_2$ (FnHmDMPs), allowed the preparation of water-in-PFOB reverse microemulsions [7]. Stable reverse emulsions were also obtained [8]. These emulsions and microemulsions have potential for controlled release pulmonary delivery of drugs [9]. Water-in-PFOB microemulsions have been shown to deliver homogenous and reproducible doses of a tracer (caffeine) using metered-dose inhalers (pMDI) pressurized with hydrofluoroalkanes (HFAs) [10]. A water-in-PFOB emulsion containing 5% v/v of water, emulsified and stabilized with F8H11DMP (5% w/v), which had been selected as the best emulsifier [7], was found to be harmless towards human lung cultured cells [11].

One of our general objectives is to investigate the effect of water-in-PFOB microemulsions, hence, also the effect of the fluorinated emulsifier F8H11DMP, on the lung surfactant, and to determine if a bioactive molecule encapsulated in the water droplets of a water-in-FC microemulsion would have a chance to penetrate in the alveolar cells. The lung surfactant that lines up the pulmonary air spaces of mammalians contains several lipids, primarily dipalmitoyl phosphatidylcholine (DPPC) and at least three specific proteins (SP-A, SP-B and SP-C) [12]. The primary function of the lung surfactant is to form a monolayer at the alveolar surface/air interface that is capable of lowering the normal air/water surface tension (72 mN m^{-1}) to near 0. Deficiency or inactivation of lung surfactant, for example in case of premature delivery or cystic fibrosis, may result in a number of severe pulmonary conditions. A monolayer of DPPC that is able to reduce the surface tension of the alveolar surface/air interface to very low values (< 1 mN m^{-1}) was proposed as a possible model for the lung surfactant. However, due to the high cohesive energy of the compressed DPPC monolayer, which is in the liquid condensed (LC) phase, the rapid spreading of DPPC molecules is inhibited [13]. Due to this, DPPC alone can not function as an efficient lung surfactant. DPPC, supplemented by hexadecanol and tyloxapol, which may act as spreading and dispersion agents, respectively [14], is used clinically in the treatment of the infant respiratory distress syndrome [15]. An early report showed that a FC (perfluoromethyldecalin)-in-water emulsion stabilized by DPPC was able to spread rapidly at the air/liquid interface [16].

In this work, based on film balance measurements and fluorescence microscopy, we have studied the miscibility of mixed monolayers made from DPPC and F8H11DMP at the air/water interface. Monolayer behavior of the two latter components put in contact with PFOB was also investigated. Finally, we have explored the behavior of a DPPC monolayer contacted with a water-in-PFOB microemulsion stabilized by F8H11DMP.

2. Materials and methods

The (perfluorooctyl)undecyldimorpholinophosphate F8H11DMP was synthesized as reported previously [17]. It was thoroughly purified by repeated recrystallizations from hexane. Its purity was controlled by ^1H, ^{31}P and ^{13}C NMR (Bruker AC 200), and elemental analysis. DPPC was purchased from Avanti Polar Lipids. PFOB, (99%) was provided by Apollo Scientific Ltd. *n*-Hexane and ethanol came from Merck (Uvasol) and Nacalai Tesque, respectively. NaCl (Nacalai Tesque) was heated at 700 °C for 24 h to remove any surface active organic impurities.

2.1. Preparation of the water-in-PFOB microemulsion

About 0.4 g of F8H11DMP was dispersed in 3.8 ml of PFOB. A 0.2 ml of an aqueous solution of NaCl (0.9% w/v) was added. In order to ensure rapid mixing of the ingredients, the mixture was homogenized by sonication (Branson model 250D, 15 min, pulse 50%). The stem microemulsion, thus, obtained contained 5% v/v of water and 95% v/v of PFOB. The concentration of F8H11DMP was 10% w/v ($\omega = [H_2O]/[F8H11DMP] = 22$). The average diameter of the water droplets was 10 ± 2 nm, as assessed by quasi-elastic light spectroscopy (Zetasizer 3000 HS, Malvern Instruments). This stem microemulsion was further diluted with PFOB (1/4, 1/8 and 1/16) for use. Previous studies have shown that such microemulsions could be diluted, and remained stable for at least 1 year without detectable variation of their average diameter [7].

2.2. Surface pressure–area isotherms

The surface pressure, π, was measured using an automated home-made Wilhelmy film balance. The pressure-measuring system (accuracy ± 0.1 mN m^{-1}) was equipped with a filter paper (Whatman 541, periphery 4 cm). The trough (effective area 715.5 cm^2) was made from Teflon-coated brass. The surface pressure–molecular area (π–A) isotherms were recorded at 25 °C. The compression speed was 0.70×10^{-1} nm^2 per molecule per min. No influence of the compression rate (0.70×10^{-1} vs. 2.00×10^{-1} nm^2 per molecule per min) was detected within the limits of the experimental error. Standard deviation for area measurements was approximately 1.00×10^{-2} nm^2. In all cases, the substrate solution was a 0.15 M NaCl solution prepared using thrice distilled water (surface tension: 72.7 mN m^{-1} at 20.2 °C; resistivity: 18 MΩ cm).

2.3. Monolayers at the air–water interface

Spreading solutions of DPPC or F8H11DMP (1 mM) were prepared in *n*-hexane/ethanol (9:1 v/v). 50 μl of the DPPC (or F8H11DMP) solution were spread on the substrate solution. For each DPPC/F8H11DMP molar ratio, DPPC and F8H11DMP were first co-solubilized in *n*-hexane/ethanol (9:1 v/v), and 50 μl of the resulting solutions were spread on the substrate solution. The spreading solvent was allowed to evaporate for 15 min before compression.

2.4. Monolayers in contact with an excess of PFOB

60 μl of a 0.5 mM solution of DPPC (or F8H11DMP) in an *n*-hexane/ethanol/PFOB mixture (4.5:0.5:5) were spread on the substrate solution. Compression of the resulting DPPC (or F8H11DMP) monolayer in contact with PFOB was achieved after evaporation of the spreading solvent (15 min). Various DPPC/F8H11DMP molar ratios (2:1, 1:1, 1:2) were obtained by mixing appropriate volumes of a solution of F8H11DMP (1 mM) in PFOB and a solution of DPPC (1 mM) in *n*-hexane/ethanol (9:1). Fifty microliters of these solutions were spread on the substrate solution. Compression of the mixed monolayers in contact with PFOB was achieved after evaporation of the spreading solvent (15 min). The molecular areas were calculated using the amounts of DPPC and F8H11DMP, without taking PFOB into account.

2.5. DPPC monolayers contacted with diluted water-in-PFOB microemulsions stabilized by F8H11DMP

We needed here to develop a protocol that would mimic, at least to some extent, the behavior of PFOB microemulsion droplets on the DPPC monolayer. Therefore, we first formed a DPPC monolayer (by spreading 50 μl of the 1 mM DPPC solution in *n*-hexane/ethanol (9:1)) and compressed it to 1 nm^2 per molecule, which corresponds to a surface pressure of 1-2 mN m^{-1}. At this pressure, the DPPC monolayer is in the liquid expanded state (LE) phase. Our attempts to spread the stem microemulsion, i.e. containing 5% v/v of water and 95% v/v of PFOB, on the DPPC monolayer were unsuccessful. Satisfactory results, in terms of reproducibility, were obtained, however, by spreading microemulsions diluted in PFOB (see above). 0.4, 0.8 and 1.6 μl of the 1/4,

1/8 and 1/16 diluted microemulsions were deposited on the DPPC monolayer in order to keep the DPPC/F8H11DMP molar ratio constant (equal to 1:0.24). After 15 min, the DPPC monolayer in contact with the microemulsion was compressed.

2.6. Fluorescence microscopy

Fluorescence was observed using an automated home-made Langmuir film balance (Chan RG Langmuir float type; resolution 0.01 mN m^{-1}) equipped with a BM-1000 fluorescence microscope (USI System) [18]. The trough (effective area 750 cm^2) was made from Teflon-coated brass. Compression speed was 0.70×10^{-1} nm^2 per molecule per min. Surface pressure and fluorescence microscopy were recorded simultaneously upon compression. The fluorescent probe (1 mol%) was 3,6-bis(diethylamino)-9-(2-octadecylcarbonyl)phenyl chloride (R18, Molecular Probes). A 300 W Xenon lamp (XL 300, Pneum) was used for fluorescence excitation. Excitation and emission wavelengths were selected by an appropriate beam splitter/filter combination (Mitutoyo band path filter 546 nm, cut filter Olympus 590 nm). The monolayer was observed using a 20 magnification long-distance objective lens (Mitutoyo $f = 200$/focal length 20 mm). Micrographs were recorded with a video camera (757 JAI ICCD camera, Denmark), connected to the microscope, directly into computer memory via an online image processor (Vaio PCV-R53 Sony: Video Capture Soft). The entire optical set-up was placed on an active vibration isolation unit (Model-AY-1812, Visolator, Japan). All measurements were performed at 25 °C.

3. Results and discussion

3.1. Monolayers of DPPC, F8H11DMP and their mixtures at the air/water interface

Fig. 1 shows surface pressure/molecular area (π/A) isotherms for DPPC, F8H11DMP, and DPPC/F8H11DMP (2:1, 1:1, and 1:2) monolayers. The DPPC monolayer is characterized by a sharp transition between the liquid expanded (LE) and liquid condensed (LC) phases that occurs at 25 °C

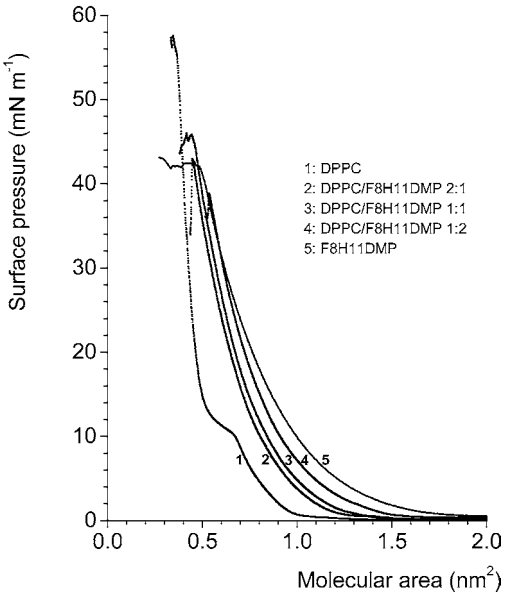

Fig. 1. Surface pressure vs. molecular area isotherms of DPPC, F8H11DMP and their mixtures.

at $\sim$10.5 mN m^{-1} (0.65 nm^2). Fluorescence micrographs, recorded upon DPPC compression (Fig. 2), showed domains of the LC phase that appeared in the LE phase at the beginning of the LE/LC coexistence region, indicated by a plateau on the isotherm [19]. The number of these domains increased with π, and these domains eventually coalesce to form an uniform LC phase. The extrapolated area (A_0) in the LC phase and pressure of collapse (p^c) were 0.52 nm^2 and 54 mN m^{-1} (0.39 nm^2), respectively.

The F8H11DMP isotherm was monotonous and expanded, indicating that the monolayer is in a LE state. A_0 and π^c were $\sim$0.95 nm^2 and $\sim$ 43 mN m^{-1} (0.50 nm^2), respectively. Variation of the surface pressure as a function of time for DPPC and F8H11DMP monolayers (Fig. 3) indicated that the F8H11DMP monolayer was more stable than the DPPC monolayer, with no significant loss of molecules, as compared with $\sim$ 20% for the latter. Fluorescence microscopy of the F8H11DMP monolayer did not show any domains upon compression, as expected for a uniform, fluid LE phase (not shown).

In order to assess the miscibility of DPPC and F8H11DMP, the π–A isotherms of DPPC/

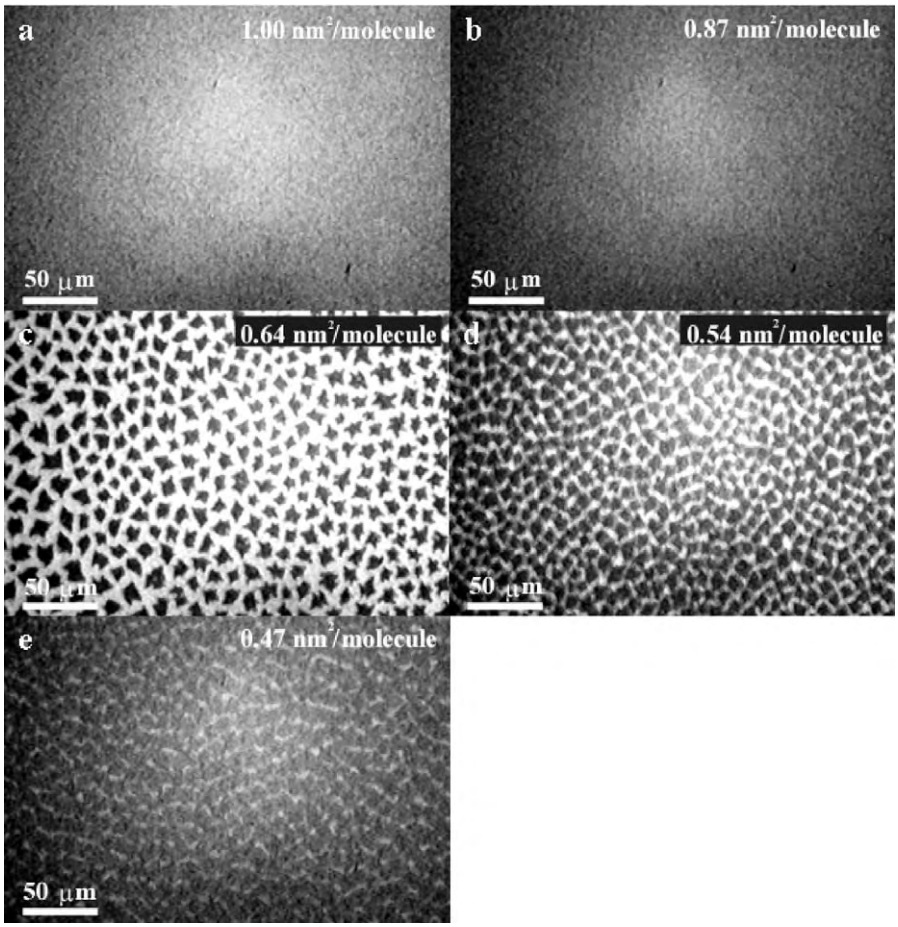

Fig. 2. Fluorescence micrographs of DPPC monolayer for various molecular area.

F8H11DMP mixed monolayers were recorded for various F8H11DMP molar fractions ($X_{F8H11DMP} = 0.33$, 0.50, 0.67; Fig. 1). All isotherms were monotonous and expanded, strongly indicating LE phases. The suppression of the characteristic LE–LC transition of the DPPC in the mixed DPPC/F8H11DMP monolayers was a first indication that the two components were at least partially miscible. The fact that the collapse surface pressures of the mixed monolayers varied with $X_{F8H11DMP}$ ($\pi^c = 43$, 40 and 38 mN m^{-1} for $X_{F8H11DMP} = 0.33$, 0.50 and 0.67, respectively) also indicated partial miscibility. Miscibility of two components in a monolayer can be assessed by determining whether the variation of the mean molecular area as a function of the molar fraction of one of the components satisfies or not the additivity rule. A linear relationship over the composition range indicates ideal mixing or total phase separation, while deviations between the theoretical and experimental curves reflect attractive or repulsive interactions between the two components. Fig. 4 plots the variation of mean molecular areas as a function of $X_{F8H11DMP}$ for various surface pressures. At low pressures (5 and 10 mN m^{-1}) no deviations were observed, indicating the absence of specific interactions between DPPC and F8H11DMP, the two components being either ideally miscible or totally phase-separated within the monolayer. Since the mixed monolayers did not present the DPPC phase transition, we concluded that the two components

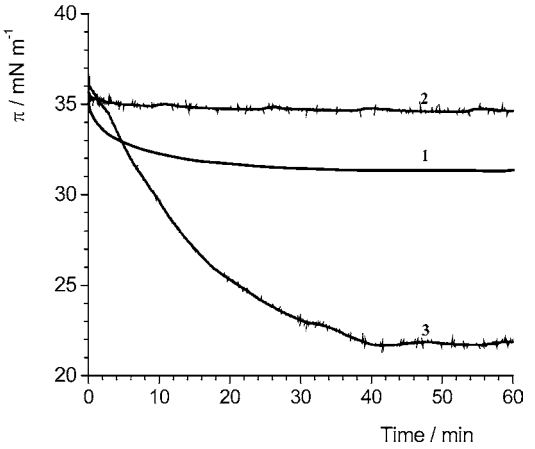

Fig. 3. Variation of the surface pressure of monolayers compressed at 35 mN m^{-1} as a function of time. DPPC monolayer (1), F8H11DMP monolayer (2) and F8H11DMP monolayer in contact with PFOB (3).

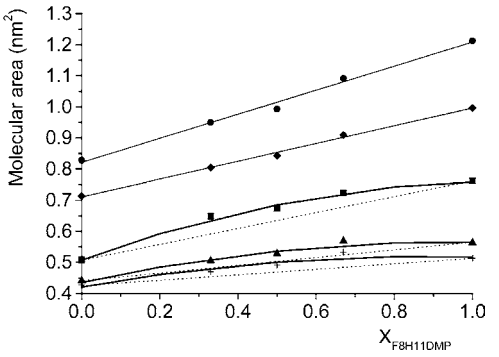

Fig. 4. Variation of the molecular area as a function of the molar fraction of F8H11DMP at various surface pressures: ●, 5 mN m^{-1}; ◆, 10 mN m^{-1}; ■, 20 mN m^{-1}; ▲, 30 mN m^{-1},+40 mN m^{-1} (dotted lines, theoretical curves).

were miscible. At high pressures (20, 30 and 40 mN m^{-1}) positive deviations were observed, reflecting repulsive interaction between DPPC and F8H11DMP, and, thus, partial miscibility. These results concur with previous results that showed that DPPC or hydrogenated carboxylic acids were miscible with fluorinated carboxylic acids in monolayers [20,21].

Fluorescence micrographs (Fig. 5) recorded during the compression of the (1:2) DPPC/F8H11DMP monolayer confirmed the disappearance of the DPPC LE/LC phase transition. No condensed domains were observed throughout the

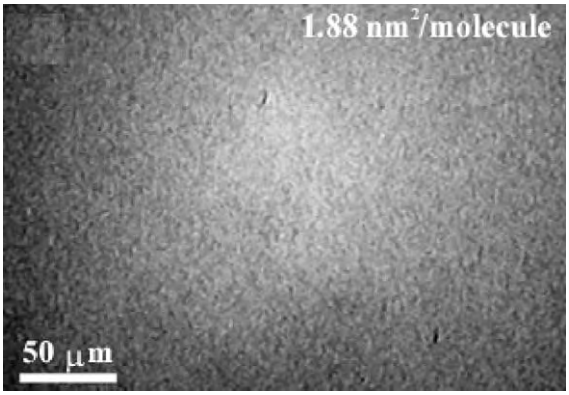

Fig. 5. Fluorescence micrograph of the DPPC/F8H11DMP (1:2) monolayer at a molecular area of 1.88 nm^2.

range of surface pressure investigated; the monolayer remained in the LE state. For the (2:1) and (1:1) monolayers, the transition was suppressed for surface pressures up to 30 mN m^{-1}, small domains appeared, however, at higher pressures.

3.2. Monolayers of F8H11DMP and DPPC/F8H11DMP mixtures in contact with PFOB

We have shown in the above section that DPPC and F8H11DMP were miscible in monolayers at the air/water interface. Before testing the complex microemulsion system on the DPPC monolayer, we have investigated the effect of neat PFOB (i.e. the continuous phase of the microemulsions) on DPPC, F8H11DMP and DPPC/F8H11DMP monolayers. Results are shown on Fig. 6.

The DPPC LE/LC transition becomes hardly visible after that PFOB has been put in contact with the DPPC monolayer. The isotherm is slightly more expanded than the DPPC isotherm (A_0 is 0.65 and 0.52 nm^2 for the former and the latter, respectively), indicating that only a small quantity of PFOB penetrates in the DPPC monolayer. π_c remained essentially unchanged ($\sim$43 mN m^{-1}). The excess PFOB molecules likely form a film on the surface of the monolayer due to the positive spreading coefficient of PFOB [1,2]. The isotherm of the F8H11DMP monolayer in contact with PFOB was not significantly different from that of the F8H11DMP monolayer in contact with air. The stability of the F8H11DMP monolayer in

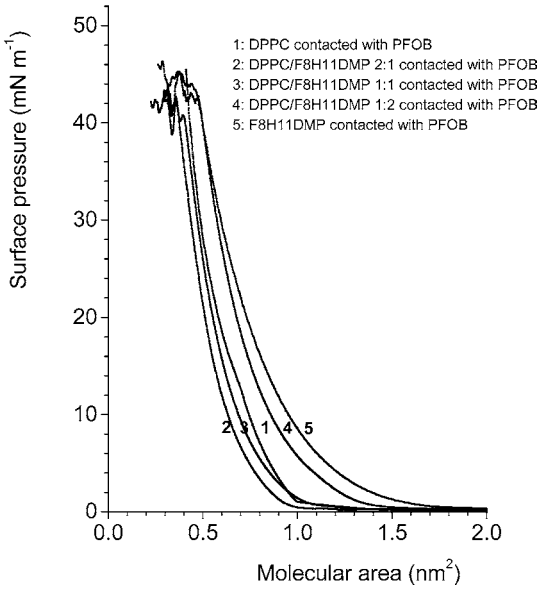

Fig. 6. Compression isotherms of DPPC, F8H11DMP and mixed DPPC/F8H11DMP monolayers in contact with PFOB.

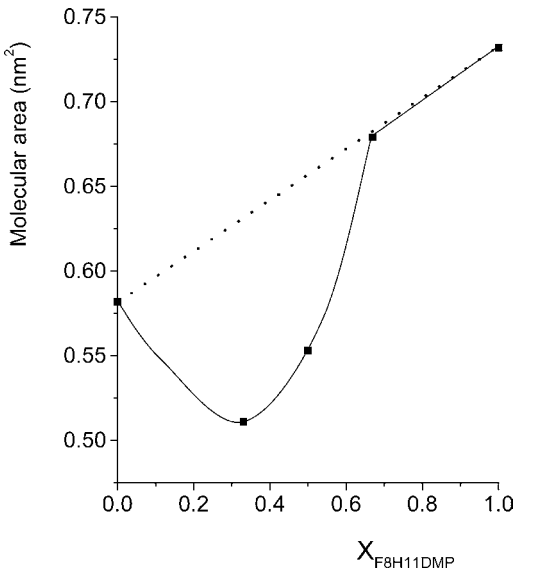

Fig. 7. Variation of the molecular area as a function of the molar fraction of F8H11DMP at 20 mN m^{-1} (dotted line, theoretical curve; ■, experimental points).

contact with PFOB is significantly lower than that of F8H11DMP at the air/water interface. After 1 h, the monolayer that had been previously compressed to 35 mN m^{-1}, lost ~40% of its molecules (Fig. 3). It is likely that PFOB dissolves F8H11DMP molecules and drains them as droplets into the subphase; a reorganization of film molecules may also occur without loss of molecules.

The isotherms of all the DPPC/F8H11DMP monolayers were monotonous and expanded, showing that the monolayers were in the LE state. The variations of the molecular area as a function of $X_{F8H11DMP}$ were plotted for a range of surface pressures (5, 10, 20, 30 mN m^{-1}; Fig. 7). Results showed deviations between the theoretical and experimental curves for $X_{F8H11DMP} = 0.33$ and 0.5, for all π values. It is noteworthy that in this case the deviations between DPPC and F8H11DMP are negative. This reflects attractive interactions, likely due to the presence of PFOB. Fluorescence microscopy did not reveal the formation of condensed domains, indicating that the DPPC/F8H11DMP mixed monolayers in contact with PFOB were in the LE phase (not shown).

3.3. DPPC monolayers in contact with a water-in-PFOB microemulsion stabilized by F8H11DMP

We have developed a protocol that intends to model the practical case of drug delivery to the lung via a water-in-FC emulsion. To this aim, we first formed a DPPC monolayer and compressed it to 1 nm^2 per molecule, which corresponds to a surface pressure of 1–2 mN m^{-1}. At this pressure, the DPPC monolayer was in the LE phase. When droplets of a water-in-PFOB microemulsion diluted by 1/4, 1/8 or 1/16 were deposited on the DPPC monolayer, the droplets were seen to spread rapidly on the surface due to positive spreading coefficient of PFOB. Surface pressure increased and plateaued after 15 min at 3.6 mN m^{-1} for the 1/4 dilution and at 5 mN m^{-1} for 1/16 and 1/8 dilutions (Fig. 8). The extrapolated molecular areas were measured to be 0.66, 0.69 and 0.72 nm^2. For all dilutions, the DPPC/F8H11DMP molar ratio remained constant (1:0.24), while the proportion of PFOB was 30.5, 61 and 122 for the 1/4, 1/8 and 1/16 dilutions, respectively. The theoretical molecular area of a 1:0.24 DPPC/F8H11DMP monolayer was 0.75 nm^2, assuming additivity of the molecular areas of DPPC (0.52

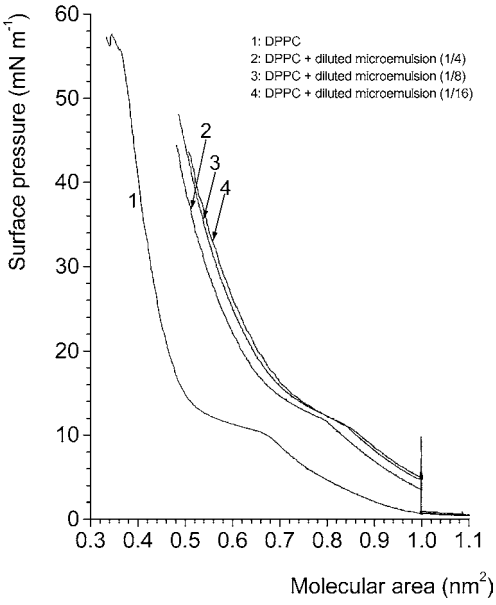

Fig. 8. Compression isotherms of a DPPC monolayer in contact with water-in-FC microemulsions.

Å^2) and of F8H11DMP (0.95 Å^2). This means that the F8H11DMP molecules involved in the microemulsions were virtually quantitatively adsorbed at the air–water interface and formed spontaneously a mixed monolayer with DPPC. In addition, it can be seen that increasing the concentration of PFOB did not lead to a significant variation of the isotherm. Fluorescence microscopy showed no evidence of LC domains in the DPPC monolayer in contact with the PFOB microemulsion, that appeared to remain in the LE state throughout the range of surface pressure investigated.

4. Conclusions and perspectives

This study demonstrates that DPPC and F8H11DMP, a fluorinated amphiphile derived from the dimorpholinophosphate polar head group, can form miscible monolayers (ideally miscible for low surface pressures, and miscible with repulsive interactions for high surface pressures) at the air/water interface. Interestingly, the mixed monolayers are in the LE state throughout the range of surface pressures investigated. When put in contact with PFOB, the mixed DPPC/F8H11DMP monolayers are also in the LE state and the two components, DPPC and F8H11DMP, are miscible. However, attractive interactions were observed (for $X_{\mathrm{F8H11DMP}} = 0.33$ and 0.5) in the all range of surface pressures investigated, likely due to the presence of PFOB. These results indicate that fluorinated amphiphiles or water-in-FC emulsions may facilitate the spreading of DPPC, which is the main component of the pulmonary surfactant, by preventing the formation of LC domains. This observation may be of importance for lung surfactant substitute design. In addition, it was found that contacting a DPPC monolayer with a water-in-PFOB microemulsion formulated with F8H11DMP led to the formation of a mixed DPPC/F8H11DMP monolayer. This is an indication that the water droplets of the FC microemulsion may fuse with the lung surfactant and facilitate drug delivery.

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