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X-Ray Reflectivity Investigations of Fluorinated Amphiphilic Triblock Copolymers on Surfaces

Abstract

*The behaviour of amphiphilic block copolymers at a hydrophilic–hydrophobic interface has a great influence on their potential for pharmaceutical applications. Langmuir Blodgett (LB) films of two types of poly(perfluorohexyl ethyl methacrylate)-*b*-poly(ethylene oxide)-*b*-poly(perfluorohexyl ethyl methacrylate) obtained from the air-water interface at a surface pressure of 35 mN/m are investigated. X-ray reflectivity (XR) measurements give a significant layer thickness of approximately 10 nm. This value is significantly smaller than the expected thickness of at least 15 nm which can be calculated from the transfer ratio and mean molecular area. Using atomic force microscopy (AFM), crystallisation of the sample with the typical finger structure is observed. From the height and surface covering of the fingers, the background height can be estimated to be in the range of 10 nm. The incorporation of copolymers in a lipid monolayer is observed by AFM on LB film and by XR on the Langmuir trough.*

Key words: X-ray reflectivity, Langmuir film, fluorinated copolymer lipid interaction.

■ Introduction

Amphiphilic block copolymers have attracted a great deal of attention for their pharmaceutical and biomedical applications. As with other industrial applications many uses of amphiphilic block copolymers in pharmaceutical formulations are largely related in one way or another to the amphiphilic nature of these materials [1]. For example, the amphiphilic nature of block copolymers is responsible for the formation of self-assembled structures such as micelles in selective solvents, which are attractive for pharmaceutical applications such as hydrophobic drug solubilisation, controlled release of the drug after administration, and so on [2–4]. Furthermore, amphiphilic block copolymers have been widely investigated for their use as steric stabilisers of pharmaceutically important colloidal dispersions [5, 6] for example emulsions, and liposomes [7–9]. For stabilisation, either adsorption or absorption of the molecule can be responsible, but with penetration of the copolymer into the bilayer, a lowered stability can also occur due to channel formation.

Without a membrane to attach, amphiphilic block copolymers are also surface active, and at high grafting densities they are known to form polymer brushes on the water surface [10, 11]. The copolymers are anchored by their water soluble blocks on the water surface with the hydrophobic blocks above the surface. In experiments on a Langmuir trough, the surface densities of polymer brushes and therefore the mean molecular area (mmA,

the average area per polymer molecule on the surface) can be varied easily. X-ray reflectivity (XR) and neutron reflectivity (NR) studies have been found to be very useful techniques to study the developing surface structure at the air-water interface [12–14]. In the case of polyethylene oxide (PEO) copolymer systems, PEO remains at low surface pressure near the surface ('pancake') and only at high grafting densities are the brushes stretched into the subphase due to excluded volume interactions between polymer chains. In such systems, the thickness or height of the brush was found to increase with increasing surface pressure. Measuring the surface pressure π depending on mmA (π -mmA isotherms), this pancake-to-brush transition can be observed at low surface pressure (~ 9 mN/m) [15].

This study is based on water soluble triblock copolymers, consisting of a hydrophilic PEO middle block and poly(perfluorohexyl ethyl methacrylate) (PFMA) as hydrophobic end blocks. The synthesis is described elsewhere [16]. From earlier investigations it was found that PFMA-*b*-PEO-*b*-PFMA amphiphilic triblock copolymers with very short PFMA block (typically below 15 wt%, or, for example, up to two FMA units at each end of a 227 unit long PEO middle block) are water soluble [17, 18]. The π -mmA isotherms of these polymers show beside the typical pseudo plateau of polyethylene oxide (PEO) the beginning of a second plateau [19]. Triblock copolymers with more than 15 wt% PFMA are water insoluble, but they always show this second phase transition

in the isotherms, which can be related to a rearrangement of PFMA [20].

In this paper, the focus is on water soluble species of our block copolymers with a PFMA content of 9–13 wt%. For some of these samples (e.g. PEO₂₀F9; see the experimental section for details of the sample), the beginning of a second phase transition was observed in the π -mmA isotherms, indicating that at least for these polymers an enrichment of PFMA units at the water surface occurs during compression. On the other hand, a PEO₁₀F9 sample, which showed no second transition in the isotherm, also did not show a significant enrichment of PFMA at the water surface during compression measured by X-ray reflectivity. For a simultaneous water surface covering of polymer (PEO₁₀F11) and monolayer forming lipid (1,2-diphytanoyl-sn-glycero-3-phosphocholine, DPhPC), an incorporation of the polymer in the lipid film can be observed for surface pressures below ~ 26 mN/m using infrared reflection absorption spectroscopy. But with further compression to ~ 35 mN/m, the copolymer is totally squeezed out of the lipid monolayer. This indicates that these copolymers might not be able to be absorbed into a membrane consisting of that lipid, which is confirmed by measuring the transmembrane current under voltage-clamp conditions [21]. The adsorption of these copolymers at membranes was investigated by measuring the ζ -potential and hydrodynamic size of the liposomes as a function of added copolymer concentration. It was found that triblock-copolymers, being still water soluble but having higher PFMA content, lead to a significant

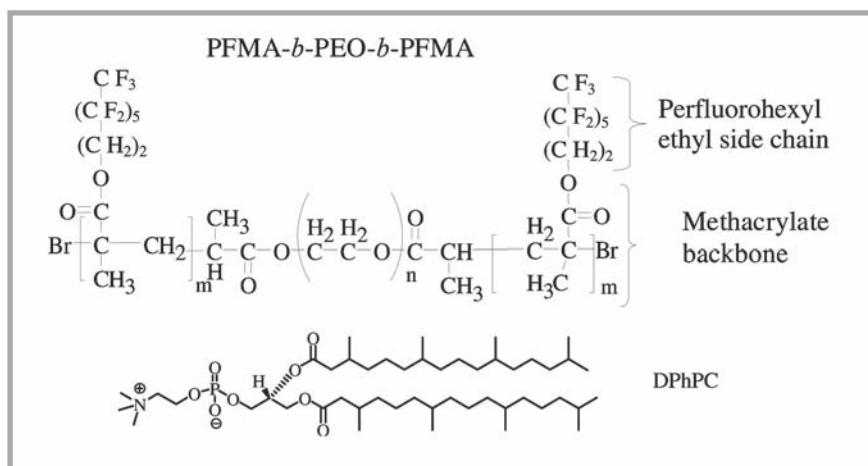


Figure 1. Chemical structure of PFMA-*b*-PEO-*b*-PFMA triblock copolymer and 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine, DPhPC.

Table 1. Characterisation of the block copolymers.

Copolymer	M_w/M_n^a	$n(\text{EO})^b$	PFMA ^c wt%	$n(\text{FMA})^d$	M_n^e kg/mol
PEO ₁₀ F9	1.33	227	9	~2	10.9
PEO ₁₀ F11	1.9	227	11	~3	11.2
PEO ₂₀ F9	1.3	455	9	4–5	22.0

^a SEC results measured in THF using PEO standards

^b number of EO units per chain obtained from initial macroinitiator mass (10 and 20 kg/mol)

^c ¹H NMR results

^d number of FMA units per polymer chain obtained from PFMA wt% and PEO macroinitiator molar mass

^e molar mass obtained from PFMA wt% and PEO macroinitiator molar mass.

change in the ζ -potential and are therefore much more effective than diblock copolymers of that kind [21]. From cytotoxicity measurements, no acute toxic effect of the block copolymers on living cells was found, [22] indicating the potential for pharmaceutical applications as liposome stabilisers.

Therefore, there are two limiting factors for the PFMA content of the triblock copolymers for pharmaceutical applications. On the one hand, there must be at

least two PFMA units in the molecule to form a triblock copolymer, which is necessary for good liposome adsorption. On the other hand, water solubility limits the PFMA content to about 15 wt%. In order to understand the behaviour of these copolymers at the air–water interface at the relevant pressure of ~35 mN/m, when the polymer was squeezed out of the lipid monolayer, XR measurements on the Langmuir trough and XR and AFM measurements on Langmuir-Blodgett (LB) films were performed and compared.

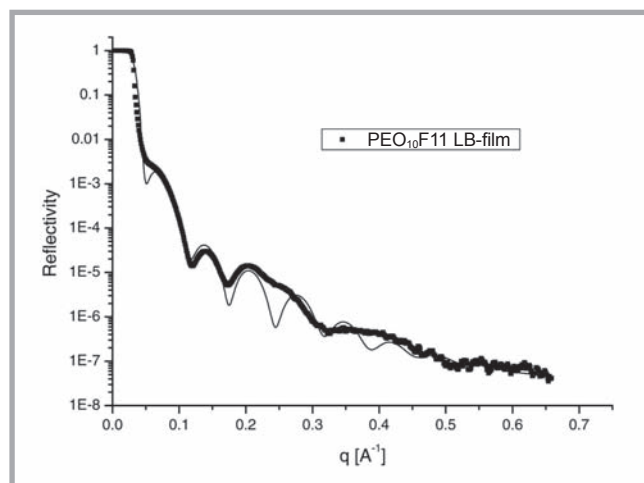


Figure 2. XR measurement of a Langmuir-Blodgett film of PEO₁₀F11, obtained at a surface pressure of 35 mN/m. The line indicates the best fit with an 8 nm thick layer, with a 2 nm thick rough surface part on it.

Experimental section

Materials

The PFMA-*b*-PEO-*b*-PFMA diblock and triblock copolymers used in this study (see **Figure 1**) were synthesised and characterised in accordance with previously reported procedures [16]. In the abbreviation scheme PEO_xF_y *x* represents the molecular weight of the PEO block (in kg/mol, according to the supplier) and *y* represents the PFMA content in wt%, based on NMR measurements. Polydispersity of the polymerisation products was measured using size exclusion chromatography (SEC), with the calibration being carried out using PEO standards. Due to the well known effect that modified polymers can show a lower mass in SEC caused by a contraction of the chain, values for M_n were calculated via the PFMA content obtained from NMR measurements. The characteristic data of the polymers are given in **Table 1**. From homopolymer samples, the bulk densities were measured in a helium pycnometer (PEO: 1.22 g/cm³; PFMA: 1.69 g/cm³). Measurements of surface pressure-area isotherms on the water surface are described elsewhere [19]. In short, copolymers are dissolved in chloroform and spread on deionised water in a Langmuir trough. After evaporation of the solvent, the isotherms were measured. To obtain the complete isotherm, the copolymer solutions were spread on water surfaces with different initial pressures and thus different parts of the isotherm were recorded. After combining them into one plot they are seen to overlap within the experimental error. The experimental setup was enclosed in a box for constant humidity and minimisation of surface contamination. For monolayer penetration experiments, the lipid monolayer was prepared as described above, and before compression, the polymer solution in water was injected through the film into the subphase (equivalent to infrared reflection-absorption spectroscopy (IR-RAS) measurements [18]). LB films were obtained by vertical dipping of a silicon wafer out of the compressed monolayer at a surface pressure of 35 mN/m for polymer films and at 10 mN/m for penetrated lipid films.

The compression and pulling velocities were chosen to obtain a transfer ratio of 1.

X-ray reflectivity measurements

XR measurements of LB films were carried out by a Kratky compact camera

modified for the reflection experiments. The X-ray source is a rotating anode generator with a copper anode (CuK_α radiation). XR measurements on the Langmuir trough were carried out at the BW1 beam line at HASYLAB (DESY, Hamburg, Germany) using a liquid surface diffractometer with an incident wavelength of $\lambda = 1.3037 \text{ \AA}$. A thermostated Langmuir trough equipped with a Wilhelmy film balance to measure surface pressure and a single barrier to change the surface area was mounted on the diffractometer. The instrumental details are given in an article by Als-Nielsen [23]. The data were corrected for background scattering and the obtained reflectivity curves were fitted using the Parratt algorithm [24] embedded in a program by Braun ('Parratt - The Reflectivity Tool', kindly provided by HMI, Berlin [25]).

Atomic force microscopy (AFM)

A nanoscope multimode AFM in tapping mode (Digital Instruments, Santa Barbara, USA) was used for AFM of LB films. The samples were dried for at least 24 h in a desiccator at room temperature before measurement.

Results and Discussion

A monolayer (Langmuir film) of $\text{PEO}_{10}\text{F11}$ was transferred at a surface pressure of 35 mN/m, corresponding to a mean molecular area (mMA) of 90 \AA^2 , onto a flat hydrophilic silicon wafer using the LB technique. Assuming that all water was removed during drying, the density ρ of the PEO phase is between 1.1 g/cm^3 (amorphous) and 1.23 g/cm^3 (crystalline) [26]. Therefore, the thickness $h = M_n / (N_A * \rho * \text{mMA})$ of the PEO layer can be expected to be in the range of 15-17 nm. The additional PFMA can increase this value by only $\sim 8 \%$, that is 1-2 nm (obtained from density and amount of PFMA). It is known that crystallisation of thin PEO films is strongly suppressed for films thinner than $\sim 15 \text{ nm}$, [27] but inhomogeneities in thickness or the presence of PFMA or other nucleation sites can also induce crystallisation in these samples. In **Figure 2**, the XR trace of a $\text{PEO}_{10}\text{F9}$ LB film is depicted. Using a two layer system on top of a silicon wafer to describe the data, the best fit was obtained by an electron density profile with an 8 nm thick PEO layer covered by a 2 nm thick rough surface. Applying a three layer model to the fit, better fitting results can be obtained, but the fundamental characteristic thickness

remains the same and is definitely very different from the expected 15-17 nm.

In the case of crystallisation, a finger-like morphology of the LB film can be expected, [19, 27] and is indeed observed by AFM of a similar copolymer ($\text{PEO}_{10}\text{F9}$), as depicted in **Figure 3**. An XR measurement will therefore have contributions of thick (fingers) and thin (background) reflections. The overall covering of the surface by the finger structure is in the range of 66%. A part of the finger structure is depicted in **Figure 4** as a 3D AFM image. The thickness is about 6 nm, measured from the background level. In combination with the expected thickness of 15 nm for the total film, the height h_B of the background can be estimated as $h_B = 15 \text{ nm} - (0.66 * 6 \text{ nm}) = 11 \text{ nm}$. The error of this estimation is expected to be quite large, as due to the AFM tip (a tip with radius $< 10 \text{ nm}$ was used), the error in determination of height and surface covering can be 20% or more. Nevertheless, the obtained value is near to the XR height.

For pure PEO samples it was observed [27] that a uniform height of the finger structure was obtained only after recrystallisation at elevated temperatures or for long periods, which was not performed on our samples. Therefore, the amorphous background is significantly smoother than the hard crystalline fingers, which leads to a prominent contribution to the XR curve, although the corresponding area is smaller.

To investigate the copolymer penetration of a DPhPC monolayer, LB films of a lipid monolayer with incorporated $\text{PEO}_{10}\text{F9}$ were prepared. A surface pressure of 10 mN/m was chosen so the co-

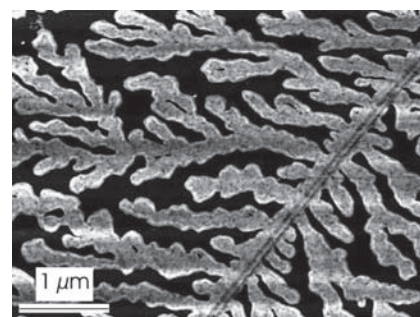


Figure 3. AFM picture of a Langmuir-Blodgett film of $\text{PEO}_{10}\text{F9}$, obtained at a surface pressure of 35 mN/m. The scale bar is 1 μm . A typical height of the finger structure is 5 nm and the total covering is approximately 66%.

polymer is not squeezed out. The AFM picture (**Figure 4b**), shows a smooth DPhPC film covering the silicon wafer totally, with single 'droplets', or surface micelles, on it. The micelles have a typical diameter of approximately 50 nm and height of 1 nm. They can be assigned to copolymer chains or micelles attached to the lipid monolayer in the Langmuir film. It cannot be distinguished by AFM whether the micelles are above, below, or embedded into the lipid film, but from IRRAS measurements at this compression it can be assumed that the copolymer is incorporated. It should be noted that on the water surface the PEO reaches the water subphase, and therefore the observed micelles are originally mainly below the lipid.

XR studies have also been performed directly on the Langmuir trough to avoid the transfer process. It was found that the reflectivity undergoes nearly no change during compression for a $\text{PEO}_{10}\text{F9}$ sample [20]. The PEO is continuously suppressed in the water subphase and no significant enrichment of PFMA at the

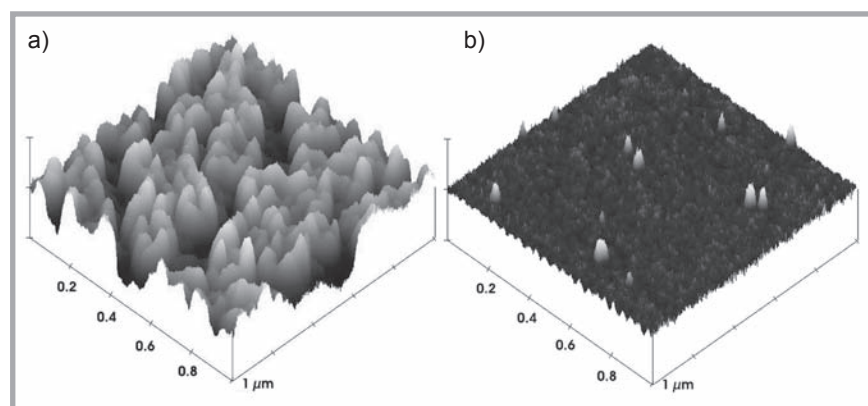


Figure 4. (a) 3D AFM image of $\text{PEO}_{10}\text{F9}$ obtained at a surface pressure of 35 mN/m. The vertical scale unit is 5 nm. (b) 3D AFM image of a Langmuir-Blodgett film of DPhPC with incorporated $\text{PEO}_{10}\text{F9}$ obtained at a surface pressure of 10 mN/m. The vertical scale unit is 3 nm.

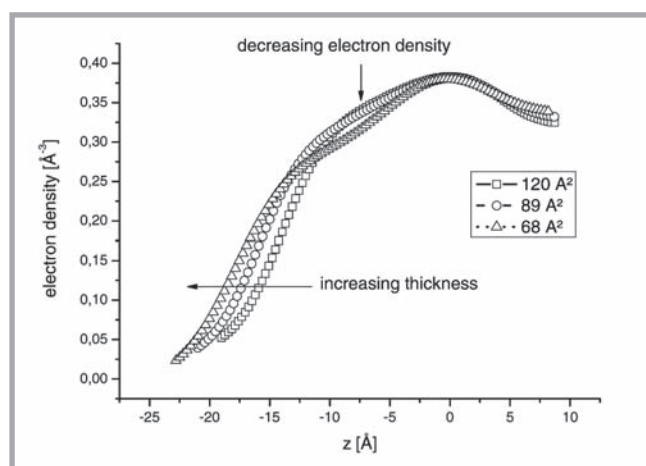


Figure 5. Electron density profiles for a DPhPC monolayer with incorporated PEO₂₀F9 at various surface pressures.

water surface occurs. To increase the signal strength for XR measurements on the Langmuir trough, the polymer PEO₂₀F9 was chosen for further investigation. Due to its larger PFMA group, it is more strongly attached to the air-water interface, which leads to an observation of the second plateau in the π -mMA isotherm [19]. Similarly to the way in which IR-RAS measurements were taken, [18] an expanded lipid monolayer on the Langmuir trough was formed and the copolymer – dissolved in chloroform – was injected into the subphase. The copolymer emerges and occupies the free area at the air-water interface after some minutes. During compression, XR measurements were performed at different surface pressures. The electron density curves fitted in **Figure 5**. A significant difference from pure lipid measurements is the decrease of the electron density during compression (indicated by an arrow in **Figure 5**). In the case of pure lipid, the free area of the expanded film is reduced during compression, and the electron density increases during compression. When the free area is occupied by the copolymer, the electron density at the surface is already high, and during the compression of the lipid, the polymer is pressed back into the subphase. The electron density of the final compression (mMA = 68 Å², π = 35 mN/m) cannot be distinguished from a pure lipid measurement (not shown here) at this surface pressure. This coincides with the IRRAS results at this surface pressure and indicates that the polymer is squeezed out of the lipid monolayer.

Conclusions

When investigating the behaviour of PEO-containing copolymers at the air-water interface, the sample preparation

must be carried out carefully, as PEO tends to crystallise. The behaviour of amphiphilic copolymers at the air-water interface is interesting for pharmaceutical applications, but investigations on 15 nm thick LB films have shown significant crystallisation destroying the original structure. The change in thickness of the PEO layer was observed by XR. The incorporation of PEO-containing copolymers in a lipid monolayer can be observed by AFM on LB films, as the PEO is separated in single chains or micelles, preventing crystallisation. The incorporation of copolymers into the lipid monolayer can also be measured directly on the Langmuir trough by XR. For relatively small surface pressures the copolymer increases the electron density at the water surface, but with increasing compression the polymer is pressed into the subphase until only the electron density of pure lipid can be observed. The results coincide with IRRAS measurements.

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