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# Interactions of Pluronic block copolymers with lipid monolayers studied by epi-fluorescence microscopy and by adsorption experiments

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ABSTRACT

The interactions of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) triblock copolymers, i.e. Pluronics F87, F88 and F127, with monolayers composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) were investigated with different monolayer techniques. Surface pressure–area isotherms were recorded of co-spread Pluronic/lipid mixtures with different Pluronic content to determine the influence of the polymers on the monolayer phase transitions. The squeeze-out pressure of the polymers upon film compression was dependent on the PPO block length. The monolayer compression experiments were coupled with fluorescence microscopy to visualize the phase separation into polymer-rich and lipid-rich domains and to monitor morphological changes of the lipid domains in the monolayer. Extensive phase separation was observed in the coexistence region between liquid-expanded (*LE*) and liquid-condensed (*LC*) lipid phases, where pure polymer domains coexisting with round *LE*-domains containing polymer, and polymer-free *LC*-domains were seen. We also investigated the adsorption of Pluronics to a lipid monolayer after injecting a polymer solution underneath a pre-formed lipid monolayer by following the change in pressure at constant area. The results show that polymer adsorption is a superposition of two individual processes with different kinetics. Pluronics with a higher hydrophobicity and with a smaller molecular weight adsorb faster and the type and phase state of the lipid determines the surface pressure where no further Pluronic molecules adsorb to the interface. This critical surface pressure depends on the PPO block length, whereas the strength of the interaction with the lipids is determined by the relative PEO content. This indicates that also interactions between the PEO blocks and the lipid headgroup region are occurring. The interactions with the unsaturated lipid POPC in the liquid-expanded phase turn out to be stronger than for lipids in the liquid-condensed phase, where the polymers are excluded.



## 1. Introduction

Pluronics are amphiphilic triblock copolymers with a hydrophobic middle block containing poly-(propylene oxide) PPO and hydrophilic end blocks of poly-(ethylene oxide) PEO. Other brand names are Symperonics or Poloxamers. The commercially available Pluronics represent a large family of nonionic, water soluble surfactants of 2–20 kDa molecular weight differing in length of hydrophilic PEO and hydrophobic PPO blocks and their ratio [1,2]. Due to the possibility to combine blocks varying in length, different appearances (liquid, paste and flakes (solid)) and variations in the hydrophilic or hydrophobic character of the individual compounds occur.

Pluronics accumulate at the air–water interface due to their amphiphilic structure. Dissolved in water, they form micelles in which a hydrophobic PPO core is surrounded by a hydrophilic PEO corona when the critical micelle concentration (*cmc*) is reached at given temperature. At very high concentrations and/or temperatures the micelles aggregate and gels are formed.

Pluronics are widely used in pharmacological industry, e.g. in chemotherapy [3–8], as cryoprotectants for low-temperature preservation of plant and mammalian tissues [9,10], as emulsifiers of perfluorocarbons in the formulation of artificial blood [11,12], and to inhibit thrombosis [13]. Another application uses the fact that Pluronics in low concentrations can stabilize and seal permeabilized membranes damaged by disease, electric shock, thermal burns, frostbite or radiation [14–20]. F68, for instance, is effective in restoring damaged cell membranes [21–25]. There has been an intense and prolific research activity around this issue. The underlying sealing mechanism is not well understood. It was suggested that Pluronics preferentially interact with damaged membranes over intact ones. However, there exist also reports about the opposite effect, namely bilayer permeabilization and disruption of bilayers above the critical micelle concentration [26–31].

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Little is known about the precise mechanism of the Pluronic–lipid interaction. There have been only few systematical studies determining the influence of the chemical structure of the Pluronic [32–35] and the phase state of the lipid [36] on these interactions. In this study, the interactions of the Pluronics F87, F88 and F127 with monolayers composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were investigated with monolayer techniques, to map the influence of the hydrophobicity and length of the PPO or PEO block of the polymer. The particular polymers F87 ($EO_{60}PO_{40}EO_{60}$), F88 ($EO_{100}PO_{40}EO_{100}$) and F127 ($EO_{100}PO_{60}EO_{100}$) were chosen to form three pairs. The pair F87 and F127 shares the same hydrophobicity and percentage of PEO in the polymer (both polymers have a content of 70 wt.% PEO). F87 and F88 have similar-sized hydrophobic PPO blocks (40 units), whereas F88 and F127 have similar-sized hydrophilic PEO blocks (100 units on each side). The two phospholipids DPPC and POPC were chosen as examples for a lipid with saturated chains (DPPC), showing a monolayer transition from the liquid-expanded (*LE*) to the liquid-condensed (*LC*) phase and a lipid with one unsaturated chain (POPC), showing only a liquid-expanded monolayer phase at room temperature. Monolayers represent half of a lipid bilayer membrane [37–39] and are therefore a convenient model system for the investigation of interactions of Pluronics with lipids. Here we present for the first time surface pressure–area isotherms of co-spread mixtures of a lipid with different amounts of Pluronic to determine phase transitions at the air–water interface upon film compression. The combination with epi-fluorescence microscopy allowed us a simultaneous visualization of the phase separation into polymer-rich and polymer-poor domains and to monitor morphological changes of the domains during film compression. Such $\pi$–*A* isotherms of co-spread mixtures have not been reported yet, only experiments of the adsorption of different Pluronics from the subphase to a pre-formed lipid monolayer at low lipid density and a following compression of these mixed monolayers have been published previously [34,36,40–42]. All pressure–area isotherms published so far show the squeeze-out of the Pluronics at high surface pressure due to their solubility in the subphase and the isotherm reverts to that of the pure lipid. Beside fluorescence microscopy [32–35,42,43] different methods were applied to confirm the squeeze-out of the Pluronics and other types of polymers from the monolayer at high surface pressure e.g. GIXD experiments [41], infrared reflection absorption spectroscopy [44], Monte Carlo simulations [34,45] and atomic force microscopy [40].

As in co-spread monolayers the polymers might not be in equilibrium with the subphase, we also performed the reverse experiment, where the incorporation of Pluronics into the monolayers was followed after injection of the polymers into the subphase. We studied the kinetics of incorporation as a function of pressure of the pre-formed lipid monolayer and determined the influence of the Pluronic structure on the adsorption kinetics. During the adsorption process of polymer molecules to a DPPC monolayer in the liquid-expanded (*LE*) state, the surface pressure increases and the formation of typical bean-shaped liquid-condensed (*LC*) domains can be observed by epi-fluorescence microscopy [33]. The dependence of the adsorption behavior of Pluronics on the lipid and its monolayer state was determined by replacing the DPPC monolayer with a POPC monolayer. Our monolayer experiments showed that not only the PPO block of Pluronics provides the driving force for the interaction with the lipid monolayer by hydrophobic interactions, but that also the PEO blocks interact with the lipid monolayers, probably intercalating in between the lipid headgroup regions.

## 2. Experimental

### 2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was a gift from Lipoid GmbH (Ludwigshafen, Germany) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from Genzyme Pharmaceuticals LLC (Liestal, Switzerland). 1,2-Dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rh–DHPE) was purchased from Invitrogen (Karlsruhe, Germany). Pluronic F127 ($EO_{100}PO_{60}EO_{100}$), MW = 12,600 $g \cdot mol^{-1}$), Pluronic F88 ($EO_{100}PO_{40}EO_{100}$), MW = 11,400 $g \cdot mol^{-1}$) and Pluronic F87 ($EO_{60}PO_{40}EO_{60}$), MW = 7700 $g \cdot mol^{-1}$) were obtained from BASF (Ludwigshafen, Germany). Chloroform (high performance liquid chromatography grade) was purchased from Carl Roth GMBH + CO KG (Karlsruhe, Germany). All chemicals were used as received without further purification. Aqueous solutions were prepared with ultrapure water from the Milli-Q Advantage A10 (Millipore S.A.S., Molsheim Cédex, France). Conductivity was lower than 0.055 $\mu S \cdot cm^{-1}$ (25 °C) and TOC below 5 ppb.

### 2.2. Monolayer pressure–area isotherms

Surface pressure vs. molecular area isotherms were recorded using a film balance with a maximal area of 536 $cm^2$, equipped with two symmetrically moveable barriers and a Wilhelmy plate (Riegler and Kirstein GmbH, Berlin, Germany). Both, lipid and Pluronics, were dissolved separately in $CHCl_3$ and mixed directly before measurement giving molar ratios of 100:1, 10:1 and 1:1. The phospholipids and the polymers are probably not molecularly dissolved in chloroform but may form micellar aggregates. When the organic solution is spread onto the water surface, these micellar aggregates dissociate and monolayers are formed. Prior to each experiment the trough was carefully and thoroughly rinsed. Pure and mixed lipid/Pluronic solutions were spread onto the water surface using a microsyringe (Hamilton Bonaduz AG, Bonaduz, Switzerland). After evaporation of the solvent the film was compressed with a rate of 2 $Å^2$ $molecule^{-1}$ $min^{-1}$. The trough was thermostatted at 20 ± 0.1 °C using an external circulating water bath (Thermostat F3, Haake, Karlsruhe, Germany).

### 2.3. Epi-fluorescence microscopy of lipid monolayers

Fluorescence microscopy imaging of monolayers at the air–water interface was performed using an Axio Scope A1 Vario epi-fluorescence microscope (Carl Zeiss MicroImaging, Jena, Germany). Underneath the microscope a Langmuir Teflon trough with a maximal area of 264 $cm^2$ and two symmetrically moveable computer-controlled Teflon barriers (Riegler & Kirstein, Berlin, Germany) was positioned on an x–y stage (Märzhäuser, Wetzlar, Germany) to be able to move the film surface with respect to the objective lens to any desired position. The x–y–z motion control was managed by a MAC5000 system (Ludl Electronic Products, Hawthorne, NY, USA). The trough was enclosed by a homebuilt Plexiglas hood to ensure a dust-free environment. The temperature of 20 ± 0.1 °C was maintained with a circulating water bath and the whole setup was placed on a vibration-damped optical table (Newport, Darmstadt, Germany). The air–water surface was illuminated using a 100 W mercury arc lamp, a long-distance objective (LD EC Epiplan-NEOFLUAR 50x) was used and the respective wavelengths were selected with a filter/beam splitter combination, which is appropriate for the excitation and detection of Rh–DHPE (Zeiss filter set 20: excitation band-pass BP 546/12 nm, beam splitter FT 560 nm, emission band-pass BP 575–640 nm). Images were