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# Communications to the Editor

## Fluorescence Spectroscopy on Polyelectrolyte Free Standing Films

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**1. Introduction.** A foam consists of free-standing surfactant films connected by so-called Plateau borders. To obtain more information about the mechanisms of foam stability, single free-standing aqueous films (foam films) have been studied by many groups during the last 50 years (refs 1−4 and references therein). The film stability is determined by an interplay of electrostatic repulsion, van der Waals attraction and steric repulsion. The sum of all the interactions between the two film interfaces is called disjoining pressure, which is measured in a thin film balance, where the film thickness is measured as a function of the external pressure. Pure surfactant films below the critical micellization concentration (cmc) thin in a continuous way with increasing pressure. After the addition of polyelectrolytes, the films show a stepwise drainage which is due to structural forces arising from a mesoscopic ordering of chains in the film core above the critical overlap concentration.[5−8] This behavior is also predicted by theoretical models.[9,10] These steps in film thickness are irreversible, prompting the question: what happens during a jump in film thickness? Recent studies showed that the surfactant used affects the film thickness, but not the jump size. Therefore, the film stratification is attributed to the polyelectrolyte structuring and not to surfactant/polyelectrolyte interactions. The effect of the interactions between surfactant and polylectrolyte on the film properties is the subject of current experiments, and it is not described in the present paper. The aim of the work presented in this paper is to find out if the polyelectrolyte is pressed out of the film stepwise or if it is collapsed in the film. A possible method to answer this question is to label the polyelectrolytes. In the present work, a fluorescent label is used.

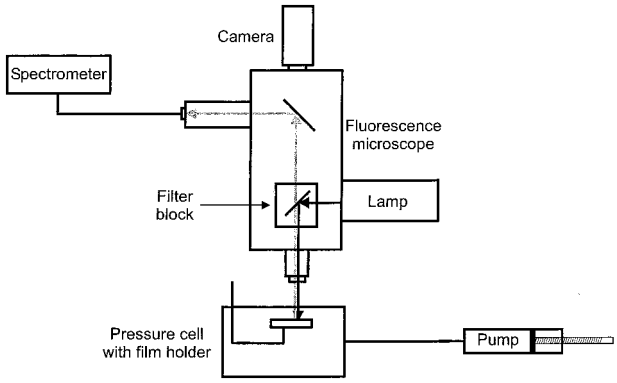


**Figure 1.** Experimental setup of the fluorescence thin film balance: a presure cell, including the film holder combined with a fluorescence microscope, a CCD camera, and a multichannnel spectrometer.

Fluorescence has been a useful tool to investigate structural aspects of aqueous systems containing surfactants both in bulk solution and near interfaces. Fluorescence measurements are able to give more detailed information about the aggregation and microstructure of surfactants[11] and the interaction between surfactants and polyelectrolytes.[12−14] At interfaces, a series of fluorescence experiments deals with the phase transition of phospholipid monolayers.[15] In free-standing films, FRAP (fluorescence recovery after photobleaching) and fluorescence microscopy have been applied to understand the diffusion and the structure of phospholipid films.[16−18] In the present paper, mixed surfactant/polyelectrolyte films are investigated in a thin film balance combined with a fluorescence microscope and an optical multichannel spectrometer. To avoid a separate expulsion of the dye without the polyelectrolyte chains, the dye is covalently bound to the polyelectrolyte.

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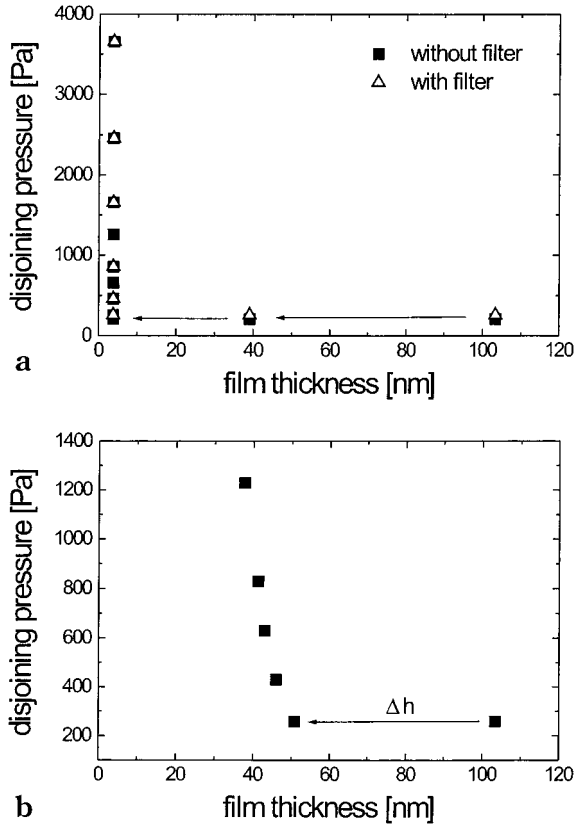


**Figure 2.** Disjoining pressure isotherm (disjoining pressure as a function of film thickness) of a free-standing (foam) film formed from an aqueous solution containing (a) $5 \times 10^{-3}$ monomol/L FITC−PAH and $2 \times 10^{-5}$ mol/L $C_{12}E_5$ and (b) $5 \times 10^{-3}$ monomol/L FITC−PAH and $8 \times 10^{-5}$ mol/L $C_{16}$TAB.

**2. Experimental Section. 2.1. Materials.** Poly(allylamine hydrochloride) (PAH, MW = 50 000−65 000 g/mol) and fluorescein isothiocyanate (FITC) were purchased from Aldrich and used without further purification. PAH was labeled in our lab with FITC following the procedure described by Nargessi.[19] The absorption spectrum shows that the degree of labeling was between one and two chromophores per PAH chain. The concentration of FITC−PAH was $5 \times 10^{-3}$ monomol/L (concentration of monomer units). $C_{16}$TAB was purchased from Aldrich and used without further purification. The concentration of $C_{16}$TAB in solution was $8 \times 10^{-5}$ mol/L (cmc: $10^{-3}$ mol/L).

Double distilled water purified by a Millipore-Q desktop (pH 5.5, specific resistance 18 MΩ cm) was used for the preparation of the solutions.

**2.2. Apparatus. 2.2.1. Thin Film Balance (TFB).** The disjoining pressure isotherms of free-standing films were measured in a thin film balance using the porous plate technique.[20−21] With this technique, it is possible to measure the disjoining pressure in the film as a function of film thickness (measured by an interferometric method[22]). Initially, a drop of aqueous polymer solution is located in a hole (1 mm diameter) in the porous plate. The film holder is enclosed in a cell and the internal pressure is changed by a syringe pump. After application of an external pressure, the drainage process starts. The foam film will be in equilibrium when the applied pressure equals the disjoining pressure between the film surfaces and simultaneously a local minimum in the free energy is achieved. At this moment, the intensity of the reflected light will remain constant with time. The film is observed by a microscope and the film thickness is measured by an interferometric method:[22] white light incident perpendicular to the film surface is reflected at the upper and lower film interfaces. The superposed reflected light is detected by a photomultiplier after being filtered by an interference filter at 550 nm.

**2.2.2. Fluorescence Thin Film Balance (FTFB).** The setup of a fluorescence thin film balance is shown in Figure 1. It is a standard thin film balance, but combined with a fluorescence microscope and a spectrometer. The fluorescence microscope (E 600 FN, Nikon) is equipped with a filter block for FITC excitation (interference filter (450−480), dichroitic mirror ($\lambda$ > 505 nm) and a band-pass filter for emission (($\lambda$ > 515 nm). A further block provides the alternative possibility of observing the film with white light. The fluorescence images are captured by a high-resolution CCD camera (HR 1 UV, Poxitronic Funk GmbH 6 Co., Bensheim, Germany) and recorded on video VHS (VR100, Philips). They are digitized with a Flashpoint 3D frame grabber (Integral technologies, Inc., Indianapolis, IN) and processed with commercial Software (NIH−Image, Adobe PhotoShop). Further, the fluorescence signal can be analyzed by a spectrometer (Triax 180, Jobin Yvon-Spex Instruments S. A., Cedex) coupled to the microscope.

Before we present disjoining pressure and fluorescence results, some remarks on two possible sources for errors should be added:

(1) The maximum of the absorption spectrum of FITC is between 450 and 480 nm, and the maximum of the emission spectrum is around 520 nm (Figure 4). Since the film thickness is determined for reflected light, detected at 550 nm in a TBF (working with white light), the low-frequency edge of the emission spectrum could enhance the light intensity reflected from the film. Therefore, the light was filtered by a cutoff filter (transmission at $\lambda$ > 515 nm) before illuminating the film to avoid the fluorescence excitation of the dye (at ∼480 nm). Isotherms measured for $C_{12}E_5$/FITC−PAH coincide with and without filter (Figure 2a). So, the main contribution to the detected light intensity comes from perpendicular light reflected at the film surfaces and the determination of film thickness is not affected by the fluorescence.

(2) Vice versa, it was tested if the light reflected from the film has an influence on the fluorescence intensity. This was checked with unlabeled polyelectrolytes in the FTBF with a filter block for FITC excitation. No intensity was detected in the case of the dye-free film, which means that the interferometric properties of the film do not influence the fluorescence results. [This result was expected before since the incident (excitation) light intensity which is enhanced by interferometric effects is filtered out by the dichroitic mirror and the emission filter located in the filter block of the fluorescence microscope.]

**3. Results and Discussion.** Figure 2b shows the disjoining pressure isotherm for foam films prepared from $C_{16}$TAB/FITC−PAH solutions at 23 °C. They exhibit a fast transition (after 10 s) at low pressure (about 350 Pa) reaching a film thickness, typical for common black films (CBF) [a CBF is stabilized by the electrostatic repulsion between the identically charged film surfaces] of about 51 nm. The film is very stable against the increase in the applied pressure from 350 to 1300 Pa, reaching an equilibrium thickness of 37 nm

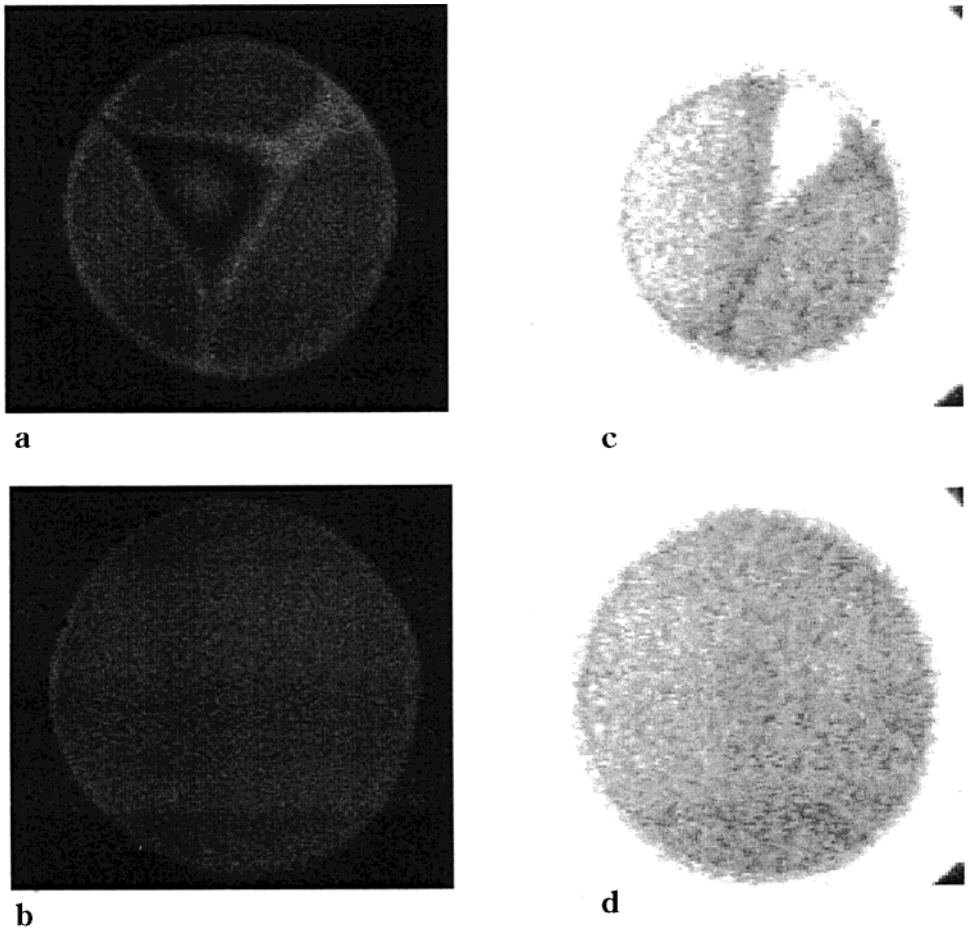


**Figure 3.** Photographs of an aqueous FITC−PAH/$C_{16}$TAB film illuminated with white light (a) during transition and (b) after the transition has finished, and illuminated by fluorescence excitation light (450−480 nm) (c) during transition and (d) after the transition has finished.

at the highest applied pressure. The electrostatic repulsion plays a central role in the stability of free-standing foam films due to the high double layer potential, which value is about 145 mV for the pure surfactant film.[4−5]

Figure 3 shows digital images taken with CCD camera at different stages of the film thinning using white light and fluorescence excitation light (450−480 nm). The photos recorded during illumination of white light (a) show black spots that indicate a transition from thick to thin common black film. After 1 min, the film reaches its homogeneous film thickness (b). The corresponding images during illumination with fluorescence excitation light show that a fluorescein rich phase is pressed out of the film during the transition (c). This means that the polyelectrolyte also leaves the film, since the dye is covalent bound to the polyelectrolyte. Once the film is in equilibrium again (d) the film is homogeneously dark.

Figure 4a shows the variation of the fluorescence intensity during film drainage. The intensity diminishes as the film thickness becomes thinner, indicating that labeled polyelectrolyte is pressed out of the film. These spectra complete clearly the results described above, but in order to get information if the polyelectrolyte remains in the CBF or not, a baseline is needed. This can be done by recording a emission spectra after the film is broken. [Sometimes this task is not easy, since some films are very stable and need to be broken mechanically with the additional problem of recreating the same experimental conditions as before film rupturing.] Our measurements showed that this baseline is not exactly zero due to a random contribution from the filter glass filled already with the dye containing solution. Since an increase in pressure could lead to an expulsion of liquid from the fritted glass to the capillary tube [in both the TBF and the FTBF the fritted glass is connected with the reference pressure (atmospheric pressure) by a capillary tube] and, therefore, to a reduction of fluorescence intensity, the background was measured at different pressures. In the case of a broken film, no reduction of the intensity with increasing pressure was detectable. The intensity of the baseline (broken film) and fluorescence for the equilibrium CBF at lowest pressure (around 350 Pa) is shown in Figure 4b. The difference in intensity shows clearly that some FITC−PAH remains in the CBF at 350 Pa. (An increase in pressure to 1100 Pa, leading to a reduction in thickness of about 15 nm, seems to cause a very small effect in the fluorescence intensity (less than 10%), which we interpret as corresponding to a few polyelectrolyte chains being pressed out of the film, but much less than in the case of the stepwise expulsion during the jump in film thickness from a thick to a thin CBF.)

**4. Conclusion.** An experimental setup to investigate changes in the fluorescence of free-standing films (foam films) is presented. Digital image analysis and spectroscopic results show that the amount of polyelectrolyte in the film is reduced during the film thinning. That means that polyelectrolyte is pressed out of the film during the stepwise transition and it is not collapsed

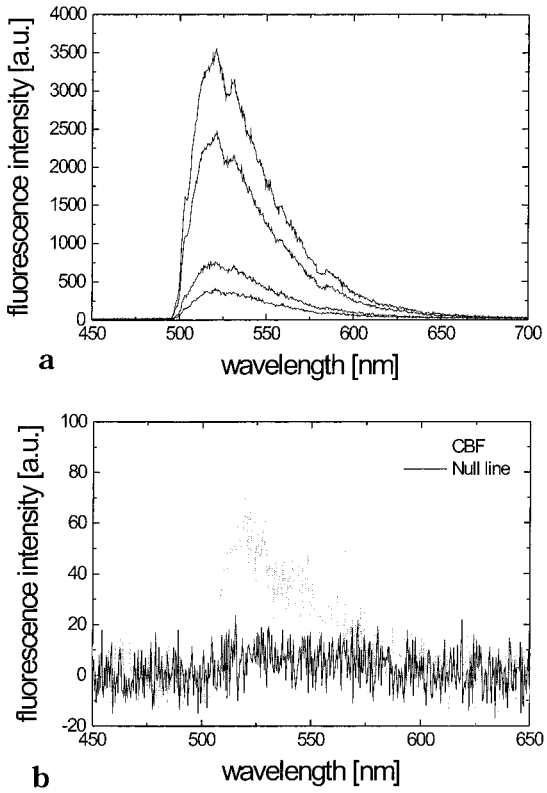


**Figure 4.** (a) Fluorescence emission spectra during drainage of a FITC−PAH/$C_{16}$TAB film. (b) Comparison between the fluorescence intensity of a common black film (thickness: ∼45 nm) and the intensity after the film was broken (both spectra are recorded at an applied pressure of 350 Pa).

in the film core. Further analysis shows that even at high-pressure some polyelectrolyte remains in the common black film (40−50 nm).

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