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# Effects of soluble surfactants on the Langmuir monolayers compressibility: A comparative study using interfacial isotherms and fluorescence microscopy

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ABSTRACT

Langmuir monolayer isotherms and fluorescence microscopy (FM) techniques have been used to study the effect of two soluble surfactants on the methyl octadecanoate monolayer's compressibility at the air/water interface. The combination of these two techniques allows one to bridge the mechanical and morphological properties of the monolayer at different surfactant subphase concentrations. Our results show that the presence of sodium dodecyl sulfate (SDS) or dodecyltrimethylammonium bromide (DTAB) affects the monolayer elasticity differently. In addition, the outcome of this study emphasizes the role of the cationic and anionic surfactants on the monolayer compressibility. In fact, their effect was found to be primly depending on the monolayer thermodynamic situation. The isotherms of the monolayers at different surfactant concentrations underneath the monolayer preserve the characteristics behavior of the monolayer as imaged by FM. The calculated monolayer compressibility shows two different trends depending on the monolayer pressure and the surfactant type. A decreasing compressibility as a function of SDS concentration was found at pressure $\pi = 5$ mN/m, while no noticeable effect was found due to DTAB. At $\pi = 10$ mN/m both surfactants convert the monolayer from rigid to soft monolayer. Such characteristic behavior of the monolayer has been confirmed by FM.



## 1. Introduction

Interaction of organic surfactant-like molecules with lipid membrane is of great interest because of its applicability to basic science and widespread human use. In living systems, lipids are the main components of the cell membrane and exhibit different morphological structures when interacting with proteins or surfactants [1–11]. These morphological changes are not easy to monitor while the interaction is taking place between the lipids and the proteins or surfactants. However, Langmuir monolayers can provide an easy solution to have a clear insight about these interactions, since it can be easily imaged using different microscopic techniques [12–19]. Monolayers of fatty acids can provide a unique window to observe the interactions at the interface [20]. Several fatty acids undergo phase transition when compressed, heated or in interaction with other detergents, entering a state of phase coexistence. The resulting heterogeneity can be deduced from pressure-area isotherms and direct visualization of the phases associated with these transitions [20–22]. Imaging the transitions between gas (G) and liquid-expanded (LE) and liquid-condensed (LC) phases have been studied using both fluorescence microscopy (FM) [23,24] and Brewster angle microscopy (BAM) [25–27]. The penetration of insoluble monolayers by soluble amphiphiles has been extensively studied from thermodynamic and imaging perspectives providing invaluable tool to examine the interaction between the membrane perturbant and the phospholipid monolayer [28,29]. This association may affect both the morphological and mechanical properties of the monolayer [29]. Such changes can be characterized using the two dimensional compressibility modulus $\kappa$ [30,31].

In this study, we used monolayers of methyl octadecanoate (MO) at the air/water interface and two water soluble surfactants, namely sodium dodecyl sulfate (SDS) and dodecyltrimethylammonium bromide (DTAB). The selection of the fatty acid and surfactants was based on their biological significance and their electrostatic properties. MO is well characterized with respect to pressure-molecular area isotherm behavior, phase transitions and domain shapes [32–37]. The isotherms of MO/surfactant mixtures and changes in the MO phase behavior and elasticity due to the presence of soluble surfactant were presented and discussed. Fluorescence images were gathered throughout the compression or surfactant adsorption and are presented without image enhancement.

## 2. Materials and methods

### 2.1. Materials

Methyl octadecanoate consists of one 19-carbon acyl chain attached to two oxygen atoms and ester group which form together

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the headgroup (Fig. 1). Surfactants used in the current experiment were SDS (an anionic surfactant) and DTAB (a cationic surfactant). Both surfactants have saturated 12-carbon chains and critical micelle concentrations in excess of 8 mM. The chemical structure of these surfactants is depicted in Fig. 1. Surfactant solutions having bulk concentrations up to 500 μM have been used to examine the MO monolayer compressibility. The solutions were prepared using pure water, Millipore Milli-Q (18 MΩ, pH = 5.5). All materials (purity > 99%) used in this study were purchased from Sigma Aldrich (Germany). All experiments were conducted at temperature T = 22 °C. The surface-pressure isotherms of the Gibbs monolayers of SDS or DTAB were extensively studied in previous published reports [1,2]. The experiments described below focus on the ability of these soluble surfactants to promote or inhibit the compressibility of MO monolayers at the air/water interface. In particular we used solutions having six different bulk concentrations. These concentrations have different surface pressure or surface excess coverage which varies from 0.1 mN/m (at 50 μM) to 4.7 mN/m (at 500 μM) for SDS and from 0.6 mN/m (at 50 μM) to 10.1 mN/m (at 500 μM) for DTAB. Therefore, π-A isotherms for the lipid monolayer obtained in this study has taken this fact into account by subtracting this excess pressure from each isotherm. BAM enables both visualization of the film texture and estimation of the monolayer thickness at different stages of compression. The film thickness inferred from the relative intensity measurements of BAM was estimated to be between 1.73 ± 0.02 nm and 1.80 ± 0.02 nm for LE and LC phases respectively. With the help of this method, the monolayer thickness can be estimated with a precision of ca. 20% [4]. The molecular area (area per molecule) was calculated as the total film area, A, in $cm^2$, divided by the number of molecules in the film. Such calculations were automatically performed by Nima software [Model 312D, England].

### 2.2. Surface pressure measurements

A Nima Langmuir–Blodgett trough (Model 312D, England) was used to measure isotherms of fatty ester films adsorbed at the air/water interface. The Teflon trough has a working area of 30 × 10 $cm^2$ and was placed on a vibration isolation table and sealed in an environmental chamber. The lipid was prepared by dissolving MO in chloroform at a concentration of 2 mg/mL. The lipid films were prepared by adding 10 μL of the solution on the surface of an aqueous subphase inside of the two barriers. Experiments were begun 30 min after deposition to allow the chloroform to evaporate and the MO molecules to spread across the surface. Surface-area per molecule (π-A) isotherms were obtained by a continuous compression of the monolayer at a constant rate of 0.2 $nm^2$ $molecule^{-1}$ $min^{-1}$. This compression proved to be reversible as evidenced by a lack of hysteresis in the subsequent expansions and re-compressions of the pure monolayer up to surface pressures of 40 mN/m. Experiments were performed either with a pure subphase or after adding the SDS or DTAB into the subphase. Both surfactants were injected to the subphase by Hamilton syringe without disruption of the lipid monolayer during the injection process. Subphase concentrations were calculated under the assumption of homogeneous equilibration in the entire subphase. Experiments were carried out at constant temperature of 22 °C controlled by water circulation using Lauda circulation bath (Model RK20).

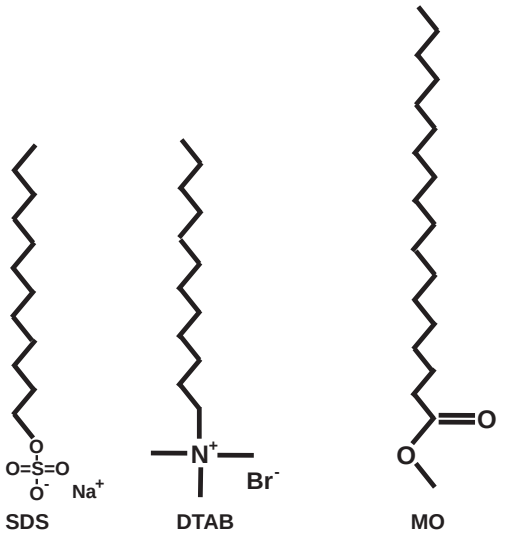


**Fig. 1.** Chemical structure of MO, SDS, and DTAB.

### 2.3. Fluorescence microscopy

To observe the morphological characteristics of the monolayer at the air/water interface, a home-made mini trough was constructed. The trough has a working area of 7 × 5 $cm^2$ which can be mounted directly under the microscope. Fluorescence from a monomolecular film doped with 2% Tex red fluorescent probe at the air/water interface was observed using a Nikon 20× long working distance objective on a Leica microscope. Excitation of the fluorescent probes was achieved using a 100-W mercury lamp with a Leica blue light pass filter. The fluorescent images were captured by a Leica video camera (Model DFC 360 FX) attached to the microscope. The Nima software was then used for further analysis of the fluorescence images.

## 3. Results and discussion

### 3.1. Pressure-area isotherms

Fig. 2 shows π-A isotherms of MO adsorbs at the air/water interface. Different subphase concentrations of SDS or DTAB were prepared by dissolving an appropriate amount of the surfactants. The isotherm was acquired by compressing an expanded film of MO molecules. Previous studies of MO isotherm on pure water showed a surface pressure lift-off at ~18.0 $Å^2$/molecule, a liquid expanded/liquid-condensed coexistence region between 17.5 and 17 $Å^2$/molecule, and a condensed, incompressible monolayer above 17 $Å^2$/molecule [33,34]. Isotherms of MO at concentrations more than 200 μM solutions of SDS and DTAB are similar but not identical to that of MO on pure water indicating a fully condensed phase. A noticeable difference between the isotherms of MO on pure water and on the 50 and 100 μM SDS solutions is that the lift-off occurs at lower lipid molecular area on SDS containing solutions. Hence, the length of the liquid expanded/liquid condensed coexistence region increased by ~1.5 Å. This result could signify lipid-surfactant association leading to larger effective area per molecule. Similar but less pronounced behavior was observed for isotherms acquired at 50 and 100 μM DTAB solutions. For these two surfactant concentrations, the lift-off occurs at MO molecular area > 20 $Å^2$/molecule in the absence of SDS. With 50 μM SDS, the MO liquid expanded phase extends over a 2 $Å^2$ window (18–16 $Å^2$) and the liquid expanded/liquid-condensed coexistence region occurs at higher surface pressure. The pressure increase associated with the liquid condensed to solid phase transition is virtually equivalent for MO on pure water, and all SDS solutions implying that SDS is reversibly squeezed out of the monolayer at high pressures [38]. In the case of DTAB subphase solutions, coexistence occurs at approximately the same surface pressure and lower concentrations but at considerably expanded MO molecular areas. The overall shift to larger areas of the MO isotherm on the DTAB solutions implies that the cationic surfactant is integrated into the MO monolayer irreversibly. Here, the molecular area values are given in terms of area per MO molecule. The surface pressures of MO isotherms acquired from the surface of surfactant containing solutions are reported relative to the

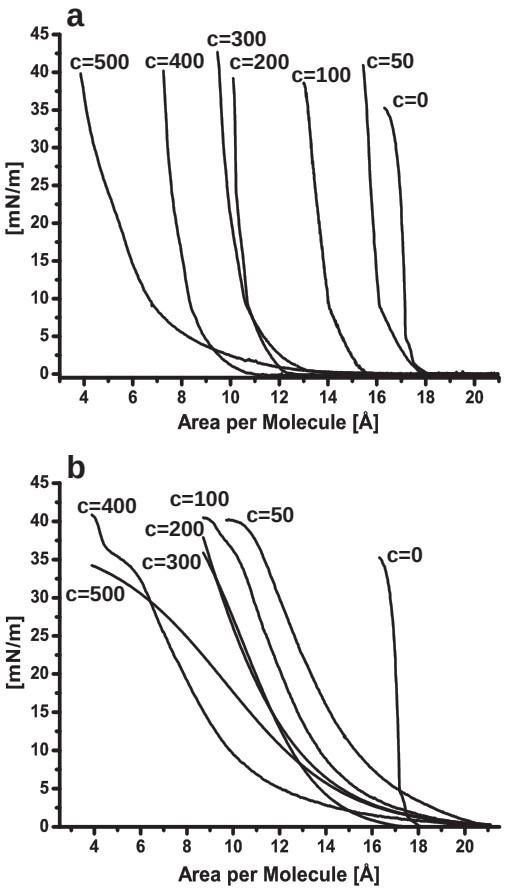


**Fig. 2.** Surface pressure (π) — area (A) isotherms of MO monolayer at temperature T = 22 °C at different subphase surfactant concentrations for (a) SDS and (b) DTAB.

equilibrated surface pressures of each surfactant solution in the absence of MO.

It is worth mentioning here that if the surfactants were not interacting with the amphiphilic molecules, MO isotherms on surfactant containing solutions would reflect the isotherms of that on pure water. The fact that lift-off occurs at larger area/molecule indicates strong, non-specific interactions between the DTAB surfactant and the amphiphilic molecules. It is likely that the increase in the surface pressure observed in the isotherm plots is mainly due to the surface active hydrophobic head group of DTAB surfactant compared with SDS molecules but not to electrostatic interactions.

### 3.2. Monolayer compressibility

Monolayer mechanical properties were assessed by determining the compressibility of the molecular film [20]. The compressibility modulus of a monomolecular film was computed from the surface pressure-area data isotherms according to the following equation:

$$\kappa = -\frac{1}{A(dA/d\pi)}.$$

The κ values of different monolayers subphase concentrations provide information about the elasticity and compressibility of the membrane in the presence of different surfactants in the subphase under the monolayers [20,21]. In general, the compressibility coefficient shows discontinuities during first and second order phase transitions. The presence of such discontinuities gives a direct evidence for the existence of phase transitions as well as the miscibility of the monolayer components. A lower value of κ indicates that the molecular film has a lower elasticity (rigid monolayer) and vice versa [31]. The compressibility data are shown in Fig. 3 as a function of subphase concentrations for both surfactants at two pressure values. Interestingly at π = 5 mN/m, the cationic surfactant DTAB shows almost no effect on the monolayer compressibility. The linear fit of the experimental data shows that the compressibility values fluctuate around the compressibility of the monolayer at pure water subphase. However, at the same pressure, the SDS surfactant reduces the monolayer compressibility with increasing the subphase concentrations. The reduction in the monolayer compressibility amounts to 65%. This clearly indicates that the SDS is predominately integrated into the monolayer. At higher pressure (i.e. π = 10 mN/m), both surfactants increase the values of the compressibility by the same way as a function of surfactant concentrations. These data clearly indicate the fluidizing effect of both surfactants on the monolayer at higher pressures.

The compression modulus is defined as the ability of a material to change its physical dimensions when a force is applied to it and to restore its original size and shape when the force is removed. It describes the differential change in the surface tension with respect to the differential change in the area. The modulus is large for clean air/water interface and decreases with the amount of surface-active materials present at the air/water interface. In general, κ depends on the state of the monolayer being greater for more fluid monolayers. From Fig. 3a it can be seen that κ is sensitive to changes in the monolayer. It is worth nothing that compared with MO monolayer, the MO/SDS monolayer has much lower compressibility. The low compressibility and the steep slope of its isotherm suggest that MO/SDS monolayer is rather rigid, while DTAB shows no effect on the monolayer compressibility. As SDS concentration increases in a monolayer of lipids, the monolayer becomes less compressible because the surfactant restricts the motion of the lipid hydrocarbon chains. The lower the compression modulus the more organized the physical state of the monolayer. Upon addition of SDS into the subphase, the κ value is reduced which indicates that the monolayer becomes more condensed. Therefore, SDS penetration into monolayers affects the packing of lipids. SDS separates lipid molecules from

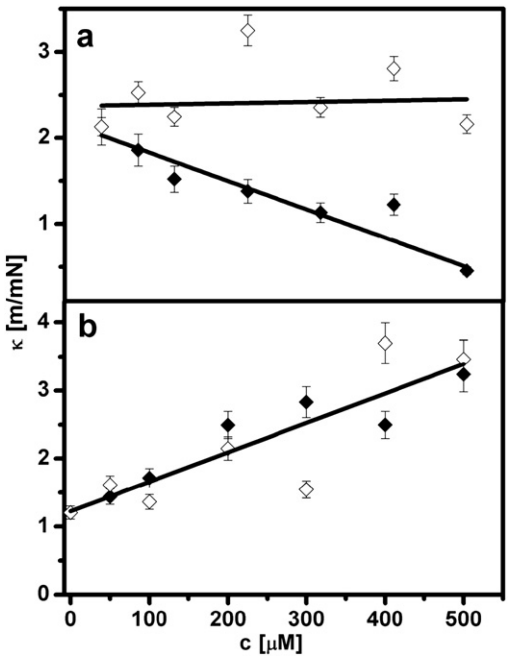


**Fig. 3.** Variation of surface compressibility modulus of MO monolayer at constant temperature as a function of subphase surfactant concentrations at pressure (a) π = 5 mN/m, and (b) π = 10 mN/m. The symbols in the figure are ◆ for SDS and ◇ for DTAB.

each other which may hinder lipid chain motion and packing resulting in more ordered phases [20,21]. The above experiment has been carried out at another pressure π = 10 mN/m (see Fig. 3b) to assess the qualitative description of the effects of adding soluble surfactants to the subphase. This experiment has been performed to measure how well the soluble surfactants are maintained within the monolayer upon compression to high surface pressures. In a pretreatment experiment, the MO monolayer was spread out at a high area per molecule (π ≈ 0 mN/m). Without compressing the lipid film, SDS or DTAB was injected into the subphase to allow maximal insertion and adsorption at the air/water interface. The presence of the surfactants at the interface gave rise to an instantaneous increase in the surface pressure at the given temperature. The lipid–surfactant system was then maintained at π = 10 mN/m for more than 30 min. As the surfactant concentration increases, the monolayer reverts slowly to LE-phase, suggesting that the surfactant has been eliminated from the film. At successively higher concentrations, the squeezing-out pressure of the surfactant is increased. This is due to the interplay of the two effects that change the properties of the lipid–surfactant system at different pressure and constant temperature, namely, the phase behavior of the lipid monolayer structure and the physical properties of the surfactants. Using vibrational sum-frequency spectroscopy, Can et al. [11,39] demonstrated how SDS and DTAB architecture affected its propensity to be incorporated into the lipid monolayer. In their case, all spectroscopic features revert back to those characteristic of pure lipid monolayer when the pressure has changed to higher values. They inferred this observation as a result of less amount of surfactant insertion due to enhanced solubility of the surfactant at higher pressures. Our results show that the ability of these two surfactants to be maintained within the monolayer at higher pressure is decreased in support of their assertion [16,38–41].

### 3.3. Morphology analysis using fluorescence microscopy

To demonstrate the mechanism of two dimensional phase transitions at different surface pressures and surfactant concentrations, FM images has been used to confirm these effects directly. The FM images in Fig. 4 show the morphological information on the MO monolayer at SDS concentration of 200 μM which reveals the phase structure as discussed above. For example, Fig. 4a shows a typical MO monolayer taken at pure water (π = 5 mN/m, T = 22 °C). The figure demonstrates the coexistence of liquid/condensed phases consisting of small dark LC-domains evolving in a large area of bright LE-phase. Waiting for 30 min after injecting the SDS into the subphase to allow homogeneous equilibrium between them, the SDS surfactant has changed the monolayer from LE-rich phase to more condensed phase. The new observed morphology exhibited by the monolayer after the SDS adsorption presented in Fig. 4b which clearly indicates that SDS is integrated well into the monolayer. This effect has been also observed when SDS or DTAB interacts with DPPC monolayers [11,12,16]. To further investigate the monolayer morphological properties at higher pressure, a monolayer of MO was further compressed to π = 10 mN/m on the air/water interface. At this pressure a large area of LC region where semicircular to bola-shaped LE-domains are evolving in the LC-matrix (Fig. 4c). The FM images taken 30 min after dissolving the SDS into the subphase has changed the monolayer morphology to small dark LC-domains evolving in a large LE-rich phase region (Fig. 4d). In fact, we have also investigated the effect of DTAB (data not shown) and the results confirm the behavior seen in Fig. 3. The data show that at pressure π = 10 mN/m both surfactants are squeezed out from the monolayer.

### 3.4. Implications for cell membrane

The elastic parameters describing the capacity of the membrane for bending or compressing laterally under the action of an external stimulus have been measured from a variety of biophysical studies carried out on native membranes as well as model membranes built up as artificial bilayers and monolayers [41]. In fact, among them SDS and DTAB have been demonstrated to cause dissolution of the plasma membrane [42]. This alteration is observed in many experiments which related to insertion and release of these macromolecules from the cell membrane [43,44]. In this section, we briefly comment on the effects of these two particular surfactants on phase behavior of monolayer system as compared with cell membrane bilayer.

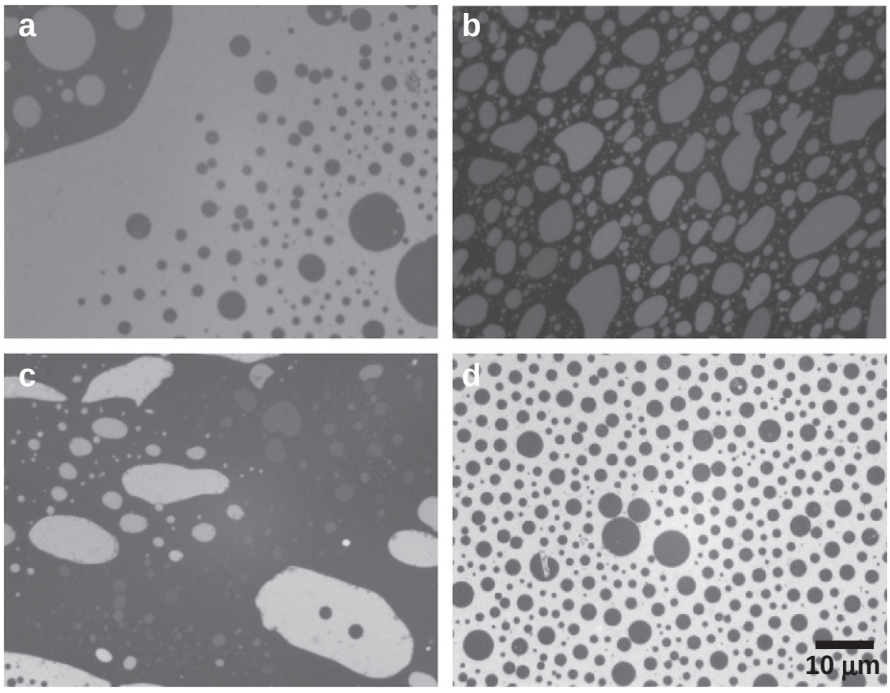


**Fig. 4.** Fluorescence microscopy images of MO monolayer at π = 5 mN/m and T = 22 °C on a pure water (a) and 200 μM SDS (b). Images (c) and (d) are the same as (a) and (b) but at pressure π = 10 mN/m.

In the current work, molecular interactions between SDS or DTAB and the lipid monolayer have been investigated by the Langmuir film balance technique, which uses a lipid monolayer at the air/water interactions as a model for the membrane lipid bilayer. The advantages of such a simpler model membrane is that the measured surface pressure versus surface area isotherms of the monolayer as well as some imaging techniques (i.e., FM) can be quantitatively analyzed for the determination of its properties, which can then be compared with the corresponding properties of the cell membrane bilayer. The morphological changes in the lipid monolayer have been monitored for two final pressures ($\pi = 5$ mN/m and $\pi = 10$ mN/m) after surfactant penetration from the subphase into the MO monolayer at low initial pressure. The chosen initial pressure of the lipid monolayer reflects the lipid density and the molecular packing of the corresponding lipid bilayer. Our observations are not exactly equivalent to that of a lipid bilayer, but serves as an excellent model of the outer leaflet of the cell membrane [45]. Since there are many ways in which the cell membrane regulates the packing density of its membrane components, insertion of peptide or other molecules is one of them. Comparison of degree of SDS or DTAB insertion between $\pi = 5$ mN/m and $\pi = 10$ mN/m clearly shows an increase in surfactant squeezing-out or release of the monolayer as more tails are packed in the film. The results obtained using monolayers with various pressures and tail packing clearly point to the effects of the final pressure on the surfactants squeezing-out of the monolayer. This observed phenomenon can be viewed as a simple biological process that occurs in mammalian cells [44,46].

### 3.5. Future study

It is known that phospholipids are differentiated from each other by their head group, chain length, and degree of chain unsaturation [47,48]. For a biophysical viewpoint, it would be important to investigate the molecular interactions between biosurfactants and lipids of various head group types, chain lengths, and degree of chain unsaturation. More importantly, because cholesterol is a very important compartment of the cell membrane, effects of cholesterol component on the molecular interactions between biosurfactants and lipids within the cell membrane could be studied [48]. Also, the penetration ability of such soluble molecules into the cell membrane would have significant meaning in biological and medical applications. This work is in progress in our laboratory and only preliminary results have been presented here.

## 4. Conclusions

The interactions of cationic and ionic surfactants with Langmuir monolayers were examined to clarify their effect on the monolayer compressibility. The results obtained at two pressure values show different interaction mechanisms between the monolayer and the surfactants: (1) at $\pi = 5$ mN/m, the SDS surfactant tends to reduce the monolayer elasticity while no effect was observed for DTAB. (2) At $\pi = 10$ mN/m both surfactants soften the monolayer almost in the same way. Fluorescence microscopy measurements of the monolayer at the air/water interface confirmed the pressure-area isotherm results.

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