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Interaction between Polymer Capsules with Hydrophobic Cores and a Model Cellular Membrane at an Air–Water Interface

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Abstract—Submicrocapsules have been prepared from diethylaminoethyl dextran and xanthan gum on oil cores by ultrasonic treatment. These capsules have been modified with poly-L-lysine via electrostatic adsorption. The behavior of the capsules has been investigated at an air–water interface after their introduction into the aqueous subphase. The interaction of the capsules with a 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine monolayer formed on the water surface (model cellular membrane) has been studied both upon their introduction under the condensed monolayer and with the use of a dilute colloidal solution of the capsules as a subphase.

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INTRODUCTION

One of the leading trends in modern biomedicine and pharmaceutics is the development of novel nano- and microsystems of prolonged action for the targeted transport of drugs to desired areas of an organism, organs, or cells. Such carriers as nanoparticles [1, 2], liposomes [3, 4], and micelles of block copolymers [5, 6], which have different surface compositions and physicochemical properties, are being intensely investigated due to the wide range of their applications in various fields of biomedicine.

Biocompatible nano- and microcapsules are attracting the increasing attention of researchers all over the world as drug-delivery systems. The problem of the encapsulation of hydrophobic bioactive compounds is of special significance, because these compounds cannot be transferred per se with the blood stream. The use of capsules makes it possible to solve this problem, protect the contents from environmental effects, and offer opportunities for drug controlled release. Hydrophobic compounds are encapsulated mainly by methods based on the preparation of emulsions stabilized with various surfactants, including surface-active polymers and mixtures thereof [7]. These systems are widely used in the production of foodstuffs, cosmetics, fertilizers, and pharmacological preparations.

One-pot synthesis of polymer capsules with the use of ultrasound is a promising method, which induces the interaction between polymer molecules under the action of high energy released upon the collapse of cavitation bubbles at interfaces in an ultrasound field. Suslick et al. [8] were the first to use this method when preparing aqueous suspensions of albumin microcapsules loaded with liposoluble liquids. Then, this method was used to form capsules from poly (glutamic acid), avidin, and streptavidin [9–12].

Previously, we have employed this approach to obtain capsules based on natural polysaccharides—chitosan and xanthan gum [13]. In this work, chitosan has been replaced by diethylaminoethyl dextran (DEAE-dextran), which is a polymer used in pharmacology.

Modification of the surface of the capsules, which were prepared using ultrasonic treatment from chitosan and xanthan gum on hydrophobic cores, with a polycation (poly-L-lysine) promotes efficient accumulation of the capsules in the cells of mouse melanoma M3 [14]. However, some cationic polymers may destroy the molecular structure of cellular membranes with the formation of pores through which cytoplasm contents may leak [15–17]. It seems of interest to carry out experiments with model cellular membranes to gain deeper insight into these phenomena.

Langmuir monolayers of lipids have proven to be good model cellular membranes [16–18]. In such model systems, the lipid composition of the layer and the composition and temperature of the subphase may be varied to more exactly simulate the biological conditions in such a manner that the data would be useful for the prediction of the interaction between carrier particles and real cellular membranes.

The interaction of drugs or their carriers with membrane lipids is usually investigated by two methods. In one of them, lipid Langmuir monolayers on water or buffer solution surfaces are compressed to a surface pressure of 30 mN/m using movable barriers. At this surface pressure, the packing density of lipid molecules is the same as that in a cellular membrane. While maintaining a constant area of the film, variations in the surface pressure are recorded upon the addition of drugs or carrier particles to the subphase. An alternative approach involves the application of a lipid together with a drug or carrier particles onto the subphase surface in a Langmuir trough. This "hybrid" monolayer is then compressed and the compression isotherm is recorded, which is, then, compared with the isotherm for the monolayer of the pure lipid.

Variations in the morphology of the lipid layer on the subphase surface may be studied using Brewster angle microscopy [19, 20]. This method allows one to in situ investigate monolayers at air–water interfaces.

EXPERIMENTAL

Preparation and Characterization of Capsules

Equivolume portions of solutions of xanthan gum (Sigma-Aldrich) (0.25%, 2.5 mL, pH 2) and DEAE-dextran (Serva) (0.25%, 2.5 mL, pH 2) were mixed at room temperature. Soybean oil (25 μL) was added to the prepared solution. A titanium sonotrode 7 mm in diameter was placed at the oil–water interface, and the system was subjected to ultrasonic treatment with a Hielscher UP 400S setup (Germany) for 5 min. The sonication was carried out in a cooled cell to avoid heating of the reaction mixture. The capsules were separated by centrifugation for 5–7 min at 3000 rpm (a Sigma 2-16K centrifuge, Germany) and washed with water three times to remove excess polymers.

The capsule surface was modified via electrostatic adsorption of a polyelectrolyte. For this purpose, the capsules were placed into a poly-L-lysine solution (2 mg/mL, 0.15 M NaCl) and incubated under stirring on a shaker (IKA MS3 basic, Germany) for 15 min. Then, the capsules were washed with water three times by centrifugation/resuspension.

As a result, capsules composed of oil cores and shells formed from xanthan gum and DEAE-dextran (sample 1) and xanthan gum–DEAE-dextran capsules, the surface of which was modified with the positively charged polymer, poly-L-lysine (sample 2), were prepared.

The electrokinetic potential (ζ potential) and size distribution of the capsules were determined by dynamic light scattering with a Zetasizer Nano instrument (Malvern Instruments, United Kingdom). Images of the capsules were taken using a TCS SPE confocal laser scanning fluorescence microscope (Leica Microsystems, Germany) in the transmission mode.

Preparation and Methods of Studying Langmuir Monolayers

A Langmuir monolayer of a lipid—1,2-dimiristoyl-*sn*-glycero-3-phosphocholine (DMPC) sodium salt—was used as a model of biological membranes.

DMPC monolayers were obtained at the air–water interface and investigated in a KSV NIMA mini Langmuir trough by recording the "surface pressure–area per molecule" (π–A) and "surface pressure–monolayer relaxation time" (π–t) isotherms. The surface pressure was measured with a Wilhelmy balance at an accuracy of ±0.01 mN/m. Water with a specific resistance of 18.2 MΩ cm purified in a Millipore Direct-Q 3 UV system was used as a subphase. A 0.85 mg/mL DMPC solution (25 μL) in chloroform was carefully applied onto the air–liquid interface with a Hamilton syringe in a manner such that oscillations of π did not exceed 0.5 mN/m. The monolayer was left to relax for 15 min for complete evaporation of chloroform. Then, the monolayer was compressed at a velocity of 5 mm/min using movable Teflon barriers. All experiments were carried out at a temperature of 19 ± 2°C. Each measurement was repeated at least three times to confirm reproducibility.

A KSV NIMA BAM Langmuir trough equipped with a Brewster microscope was employed for in situ studies of the interaction between the capsules and a lipid monolayer. The microscope was equipped with a He–Ne-laser (5 mW, 658 nm) and a CDD camera. Incidence and reflection angles were preset in accordance with the Brewster angle for water in order to minimize reflection at the air–water interface. A black nonreflecting plate was placed onto the bottom of the trough to minimize the reflection of the refracted beam.

In the first series of the experiments, a suspension (2 mL) of the capsules was introduced with a mechanical pipette under the surface of a pure aqueous subphase between the barriers at a fixed area of the working surface of the trough. In this case, the distance between barriers was chosen to be as small as possible with taking into account that the microscope beam under the Brewster angle must not be overlapped. The phospholipid was not applied onto the subphase surface in these experiments.

In the second series of the experiments, a DMPC monolayer was compressed to π = 30 mN/m (until a condensed monolayer was formed). After the exposure (relaxation) of the monolayer for 30 min at its constant area, an aqueous suspension of the capsules (2 mL) was introduced into the subphase under the monolayer.

In the third series of the experiments, a dilute suspension of the capsules was used as a subphase. In this case, the suspension (2 mL) was added to 400 mL of water to prepare the subphase.

RESULTS AND DISCUSSION

According to the dynamic light scattering data, the size of capsules formed from xanthan gum and DEAE-dextran was 720 ± 40 nm, and the ζ potential of their surface was −40 mV. The image of the capsules, which was taken with the use of a confocal microscope, is given in Fig. 1. As a result of the adsorption of the positively charged polymer (poly-L-lysine), the ζ potential of the capsules changed to +25 mV, thereby indicating a successful modification of the capsule shell.

Introduction of Capsules into Subphase Free of Lipid Monolayer

Immediately after the addition of the capsules to the aqueous subphase, the surface pressure increases dramatically (Fig. 2a). In 8 and 12 min for samples 1 and 2, respectively, the surface pressure almost ceases to grow, having reached average values of 14.2 and 5.3 mN/m, respectively. Seemingly, when the oil-containing capsules are introduced into the aqueous subphase by the used method, they, because of the different densities of water and oil, rather rapidly float upward to emerge on the subphase surface, thereby causing the observed effect. Moreover, there is a mutual repulsion between the similarly charged capsules on the subphase surface, this repulsion additionally increasing the surface pressure. In the case of negatively charged capsules, a more drastic growth of the surface pressure and its higher final value are observed because of the higher ζ potential of these capsules as compared with the positively charged ones. At the same time, the additional layer of poly-L-lysine increases the mass of the polymer shell, thus hindering the emergence of the capsules.

To visualize the capsules and judge the processes occurring at the air–water interface, images of this surface were recorded using the Brewster microscope (Figs. 2b, 2c). The capsules scatter light and appear in the images as bright spots or Newtonian rings. Immediately after the addition of the capsules to the subphase, these objects are observed in a small amount. The spots and rings are absent in the experiments with the pure subphase, thereby indicating that they are relevant to the capsules indeed. Their number increases with time; i.e., the capsules emerge gradually at the air–water interface. Then, the observed pattern actually ceases to vary, and equilibrium is established in the system. Figure 2 shows the images taken from the surface of the aqueous subphase 30 min after the introduction of the capsules. The subphase surface containing positively charged capsules appears to be less "saturated" with capsules (Fig. 2c), thus confirming the suggestion that the additional poly-L-lysine layer hinders the emergence of these capsules.

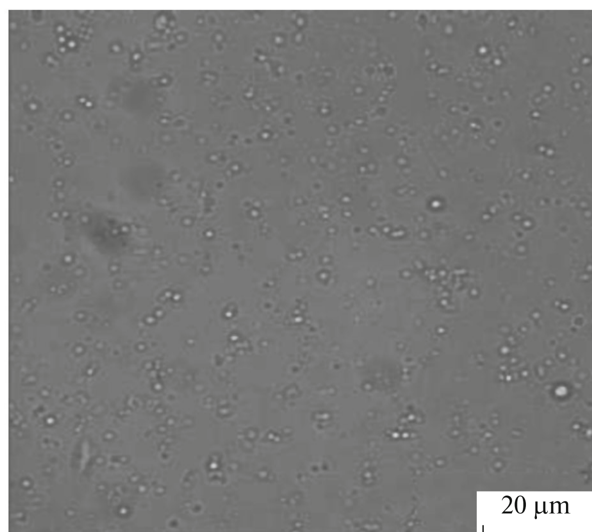

Fig. 1. Image of polysaccharide capsules taken with a confocal microscope in the transmission mode.

Introduction of Capsules under a Condensed Lipid Monolayer

After the capsules are introduced under the condensed phospholipid monolayer, the surface pressure begins to grow gradually, and, in 50 min, it increases by 2.3 and 0.4 mN/m for samples 1 and 2, respectively (Fig. 3a). In this case, π grows nonmonotonically especially in the case of the capsules with the positively charged shells. Slight variations in π indicate a weak interaction of the capsules with the condensed DMPC monolayer.

The images taken from these systems using the Brewster microscope (Figs. 3b, 3c) differ significantly from the corresponding images of the subphase free of the lipid monolayer (Figs. 2b, 2c). While only faint Newtonian rings indicating the presence of capsules in the near-surface layer are visualized at the interface of sample 1 (Fig. 3b), large bright spots corresponding to aggregates of the capsules can be seen in the images taken from the subphase containing positively charged capsules (Fig. 3c). Hence, in spite of the weak effect of the capsules with positively charged shells on the structure of the condensed DMPC monolayer, which is evident from almost unchanged values of π, the electrostatic interaction with oppositely charged phospholipid molecules causes concentrating of these capsules under the monolayer, thus leading to their aggregation. The aggregation of the capsules coated with poly-L-lysine is facilitated by their low surface charge ($|\zeta| < 30$ mV), which decreases still more due to the partial compensation upon the interaction of the capsules with the negatively charged phospholipid monolayer.

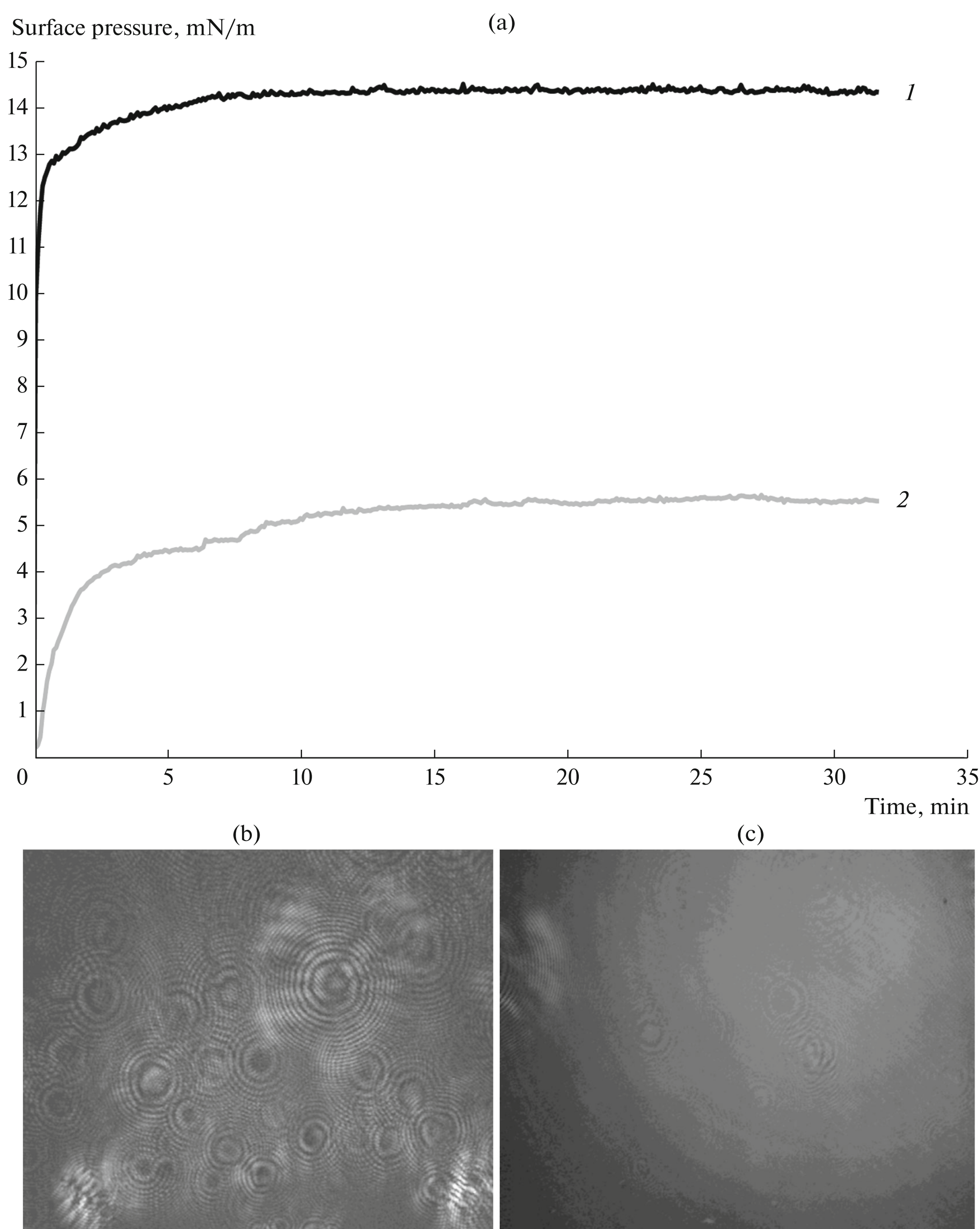

Fig. 2. (a) Time variations in the surface pressure and (b, c) images taken with a Brewster microscope from the surface of the aqueous subphase after the addition of capsules to the subphase. Curve *1* and image (b) correspond to the capsules of sample 1, while curve *2* and image (c) correspond to the capsules of sample 2. Images were obtained at $t = 30$ min; their sizes are 311 μm × 418 μm.

Spreading of a DMPC Monolayer onto a Capsule-Containing Subphase

A large number of bright spots, which correspond to capsules that have emerged, are observed in the images of the surfaces of both subphases, which initially contained the capsules (Fig. 4a). Immediately after the spreading of the lipid monolayer, the number of bright spots decreases significantly (Fig. 4b). This may be explained by the fact that the monolayer is formed by the application of droplets of a DMPC solution in a volatile hydrophobic solvent onto the surface of an aqueous subphase. The spreading hydrophobic layer, on the one hand, shields the surface that existed before its formation from observation with the

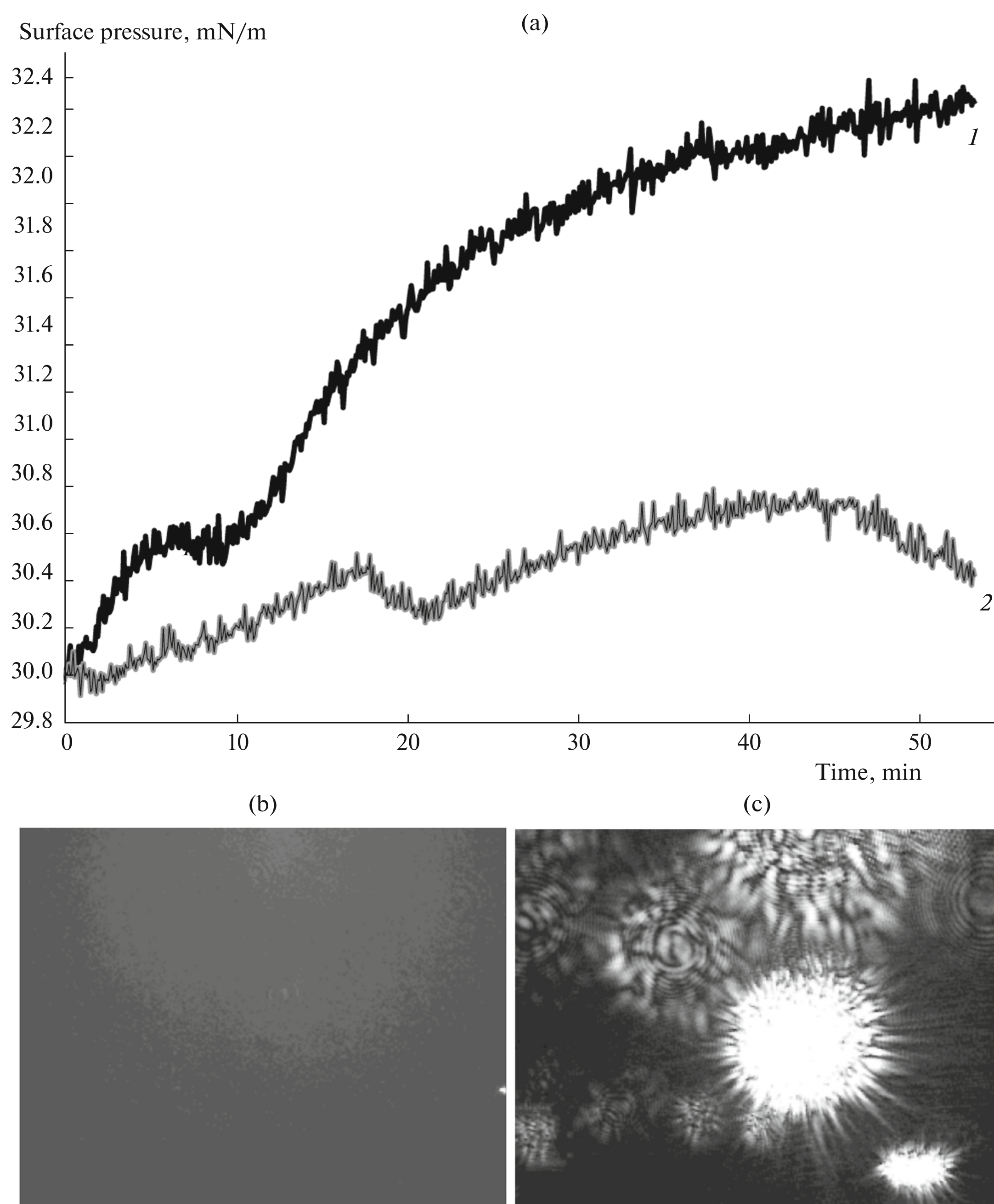

Fig. 3. (a) Time variations in the surface pressure and (b, c) images taken with a Brewster microscope from condensed DMPC monolayers after the addition of capsules to the subphase. Curve *1* and image (b) correspond to the capsules of sample 1, while curve *2* and image (c) correspond to the capsules of sample 2. Images were obtained at $t = 50$ min; their sizes are 311 μm × 418 μm.

microscope at the Brewster angle and, on the other hand, the layer being applied may mechanically repel the capsules that have emerged at the surface.

After the relaxation of DMPC monolayers, their surface pressures were 14 and 1 mN/m on the subphases containing the capsules of samples 1 and 2, respectively (Fig. 5). In the case of the capsules with negatively charged shells, a similar growth of π was observed in the experiment performed without the spreading of the monolayer (Fig. 2a). In this case, an increase of the surface pressure was not observed before the spreading of the monolayer, probably because of the more uniform distribution of the capsules in the subphase and the larger area of the working surface area of the trough. After a 15-min relaxation of the monolayer on the subphase containing negatively charged capsules, the surface pressure could increase due to the mutual repulsion of the gaseous lipid monolayer and similarly charged capsules in the subphase.

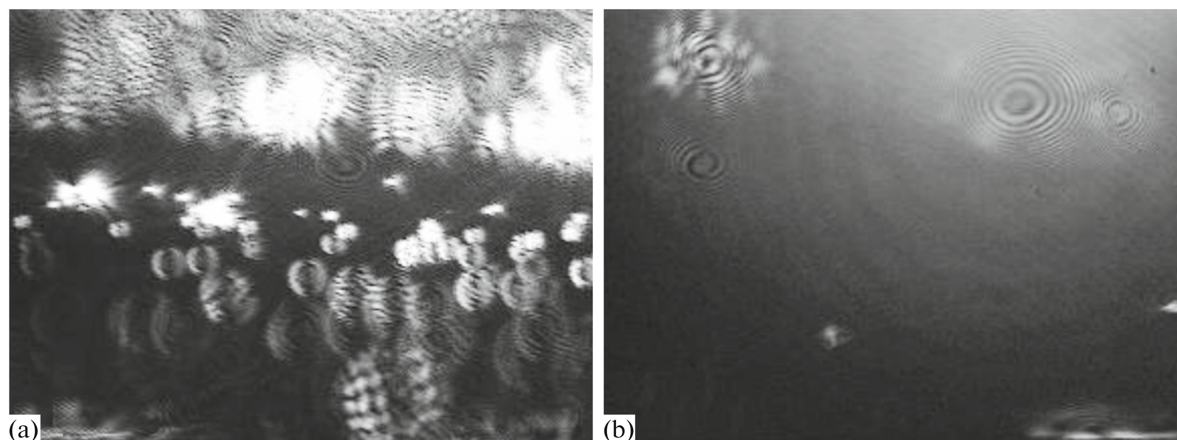

Fig. 4. Images taken with a Brewster microscope from the surface of the aqueous subphase containing capsules of sample 2 (a) before and (b) after the spreading of a DMPC monolayer (image sizes are 311 μm × 418 μm).

Compression isotherms of DMPC on capsule-containing subphases have a similar slope (Fig. 5, curves *1*, *2*) and differ remarkably from the compression isotherm of DMPC on pure water (Fig. 5, curve *3*). The lower slope of isotherms *1* and *2* is, presumably, caused by the interaction of monolayer molecules with the capsule surface. This effect is more pronounced for the capsules with shells that are charged oppositely to phospholipid molecules; upon reaching the minimum possible subphase surface area upon compression, the value of π in this system has not exceeded 25 mN/m (Fig. 5, curve *2*). The further compression could be accompanied by "squeezing" of the capsules from the near-surface layer into the subphase and the formation of a condensed monolayer. This assumption is based on the fact that the DMPC monolayer, which initially contains "impurity" nanoparticles, recovers its molecular structure (unit cell parameters and positions of the peaks in the diffraction pattern) at a surface pressure above 35 mN/m [21]. This conclusion is confirmed by the inflections in isotherms *1* and *2* at surface areas per molecule of 51 and 54 Å^2, respectively (Fig. 5), which can be related to the end of the "squeezing" of the capsules and the onset of monolayer condensation.

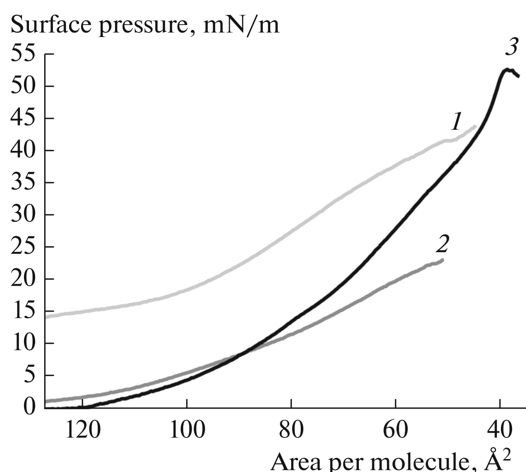

Fig. 5. Compression isotherms of DMPC monolayers on subphases containing capsules of samples 1 (curve *1*) and 2 (curve *2*) and on pure water (curve *3*).

CONCLUSIONS

Polymer capsules with hydrophobic cores emerge at the surface of an aqueous subphase because of the difference between the densities of oil and water. This leads to an increase in the surface pressure after both their introduction into the subphase under the air–water interface and the spreading and relaxation of the gaseous monolayer of the phospholipid on the subphase surface initially containing the capsules. Therewith, negatively charged capsules provide a higher surface pressure than positively charged ones do because of the significantly higher absolute value of the ζ potential of these capsules and the mutual repulsion of a similarly charged gaseous lipid monolayer and the capsules present in the subphase. Moreover, the additional layer of polylysine increases the mass of the polymer shell, which hinders the emergence of the capsules.

The compression isotherms of the DMPC monolayer spread onto the capsule-containing aqueous subphase lead us to conclude that the emergence of both positively and negatively charged capsules at the surface hinders the formation of the condensed phase of the monolayer. This presumably occurs due to the electrostatic interactions between lipid molecules and the polymer shells in combination with the mutual repulsion of the capsules under the monolayer.

The interaction between the condensed DMPC monolayer and the capsules coated with poly-L-lysine leads to the formation of capsule aggregates under the

monolayer, with these aggregates being observed using Brewster angle microscopy. This interaction is also electrostatic; however, it seems to be rather weak, because the aggregation has almost no effect on the surface pressure of the monolayer, which characterizes the structure of this ordered lipid film.

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