Research Article

Effect of Fe$_3$O$_4$ Nanoparticles on Mixed POPC/DPPC Monolayers at Air-Water Interface

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Fe$_3$O$_4$ nanoparticles (NPs) as a commonly used carrier in targeted drug delivery are widely used to carry drugs for the treatment of diseases. However, the mechanism of action of between Fe$_3$O$_4$ NPs and biological membranes is still unclear. Therefore, this article reports the influence of hydrophilic and hydrophobic Fe$_3$O$_4$ NPs on mixed 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) that were studied using the Langmuir-Blodgett (LB) film technique and an atomic force microscope (AFM). From surface pressure-area (π-A) isotherms, we have calculated the compression modulus. The results showed that hydrophobic Fe$_3$O$_4$ NPs enlarged the liquid-expanded (LE) and liquid-condensed (LC) phase of the mixed POPC/DPPC monolayers. The compressibility modulus of the mixed POPC/DPPC monolayer increases for hydrophilic Fe$_3$O$_4$ NPs, but the opposite happens for the hydrophobic Fe$_3$O$_4$ NPs. The adsorption of hydrophobic Fe$_3$O$_4$ NPs in mixed POPC/DPPC monolayers was much more than the hydrophilic Fe$_3$O$_4$ NPs. The interaction of hydrophilic Fe$_3$O$_4$ NPs with the head polar group of the mixed lipids increased the attraction force among the molecules, while the interaction of hydrophobic Fe$_3$O$_4$ NPs with the tail chain of the mixed lipids enhanced the repulsive force. The morphology of the monolayers was observed by AFM for validating the inferred results. This study is of great help for the application of Fe$_3$O$_4$ NPs in biological systems.

1. Introduction

Nowadays, nanoparticles (NPs) have been used in the research of new materials, biological imaging, biosensors, drug delivery, and other biotechnologies or biologically related systems [1–5]. Fe$_3$O$_4$ NPs, which have the characteristics of low toxicity and high biocompatibility, especially play a unique role in the study of drug delivery systems [6–9]. Based on the magnetic properties of Fe$_3$O$_4$ NPs, it can be used as a carrier to carry targeted drugs to be accurately transported to cancer cell areas. However, in order for nanoparticles to enter the cell, it is necessary to understand the interaction of the nanoparticles with the biofilm. Hence, it is urgent to study the effects of Fe$_3$O$_4$ NPs and biofilms.

A biological membrane mainly contains all kinds of lipids, cholesterol, and proteins [10, 11]. Owing to the complexity of its composition, researches have used the model of lipids to study its structural characteristics. In the past years, people mainly studied the effects of Fe$_3$O$_4$ on the single lipid layer [1]. There were few researches who committed to Fe$_3$O$_4$ NPs and multilipids, especially to compare the different properties of Fe$_3$O$_4$ NPs with those of biofilm models. Therefore, it is necessary to study the effects of hydrophilic and hydrophobic Fe$_3$O$_4$ NPs on mixed lipid monolayers.

The Langmuir-Blodgett (LB) method is one of the most favorable tools for the in vitro study of the interaction at the air-water interface [12–14]. The importance of electron microscopy to modern technology is self-evident, and the atomic force microscope (AFM) is a scanning probe microscope known for its unique measurement conditions (room temperature and no vacuum) and high-resolution surface topography. AFM is widely used in many fields, such as in materials science, nanotechnology, biology, and the semiconductor industry [15]. Due to the nanoscale of the AFM tip, it can better observe the surface topography and structure of the nanoparticles and biofilm simulation. In this study, we used 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)
and 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) lipids as binary biological membrane models to explore the interaction of hydrophilic and hydrophobic Fe$_3$O$_4$ NPs with biological membrane models. We use LB and AFM techniques to study the stability, fluidity, and adsorption of monolayers.

### 2. Materials and Methods

**2.1. Materials.** 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (POPC) were purchased as powders from Avanti Polar Lipids (AL, USA). Hydrophilic Fe$_3$O$_4$ NP solution (10 nm avg. part. size, 5 mg/mL in H$_2$O) and hydrophobic Fe$_3$O$_4$ NP solution (10 nm avg. part. size, 5 mg/mL in toluene) were purchased from Sigma-Aldrich. In these experiments, the water was the Milli-Q water (18.2 MΩ·cm) obtained from a Millipore purification system.

**2.2. Methods.** Monolayer experiments were carried out with Langmuir-Blodgett (LB) films (KSV Minitrough, Finland). These are made up of two barriers and a Wilhelmy plate. In the experiments, a Langmuir trough and two barriers were cleaned at least three times with anhydrous ethanol and ultrapure water alternately. The temperature of the subphase was maintained at 22 ± 0.5°C by circulating water equipment. The exact volume of the lipid solution was added to the air-water interface by a Hamilton microsyringe [16].

The surface pressure-area (π-A) isotherm curve of the monolayer can be autoobtained by computer. In order to increase the reliability of the experimental data, the experimental data were repeatedly computed at least three times. The monolayer was deposited onto freshly cleaved mica at the surface pressures of 5 mN/m and 20 mN/m by a pulling device with a speed of 1 mm/min. The transfer ratio is close to a unit, indicating that mica is almost completely covered with single layer.

The microstructures of LB monolayers were observed using the SPM-9500-J3 atomic force microscope (AFM) (Shimadzu Corp., Japan) in tapping mode at room temperature. The AFM images of the maximum scanning area of 125 × 125 μm and a Z range of about 8 μm was collected using a micro-V-shaped cantilever probe (Olympus Optical Co. Ltd., Japan). The probe was made of Si$_3$N$_4$ with a spring constant of 0.06 N/m and a tip radius of 10 nm. The images were collected simultaneously with 512 × 512 points and a scanning rate of 1.0 Hz per line.

### 3. Results and Discussion

#### 3.1. π-A Isotherms and $C_s^{-1}$ of Monolayers with Hydrophilic and Hydrophobic Fe$_3$O$_4$ NPs.

The surface pressure-area (π-A) isotherms can be used to reflect the phase behavior and thermodynamic properties of the lipid monolayers. The π-A isotherms of POPC/DPPC monolayers with different molar ratios at the air-water interface are shown in Figure 1(a). For a pure DPPC monolayer, the coexistence region of the liquid-expanded and liquid-condensed (LE-LC) phase was observed at the surface pressure of 4.5 mN/m, which is consistent with literature [17]. When $X_{\text{POPC}} = 0.25$, the phase transition point of the LE-LC phase was still observed. However, when $X_{\text{POPC}}$ was increased to 0.5 and 0.75, the plateau-like LE-LC phase disappeared. The phase transition temperatures of DPPC and POPC are 41°C and -2°C, respectively [18]. At room temperature, the DPPC phase is in the gel phase and the POPC phase is in the liquid phase. The mixture of POPC/DPPC showed a different phase behavior from pure lipids.

Figures 1(b) and 1(c) show the π-A isotherms of mixed POPC/DPPC monolayers with the different subphase, respectively. In the subphase, the concentration of hydrophilic and hydrophobic Fe$_3$O$_4$ NPs was 0.016 mM, which was consistent in all experiments. We found that the isotherms moved to the direction of a larger area for the hydrophobic Fe$_3$O$_4$ NP case than for the hydrophilic Fe$_3$O$_4$ NP case.

In order to more easily understand the effect of hydrophilic/hydrophobic Fe$_3$O$_4$ NPs on the isotherms of the lipid monolayers, the relevant information is summarized in Figure 2. The lipid monolayers have three characteristic parameters: limiting area $A_{\text{max}}$ (an empirical parameter approximating the area occupied by the molecules at zero pressure), collapse pressure $\pi_c$, and lift-off area $A_L$ (the molecular occupation area where the isotherm rising just emerges related to the baseline) [19].

The limiting area of pure DPPC monolayers is only 38.8 Å, while the limiting area of pure POPC is 65.76 Å. After the hydrophilic/hydrophobic Fe$_3$O$_4$ NPs are added to the subphase, the limiting area of the monolayers increases and the hydrophobic Fe$_3$O$_4$ NPs are larger than the hydrophilic Fe$_3$O$_4$ NPs. The collapse pressure decreases with the increase of $X_{\text{POPC}}$. In the presence of POPC molecules, hydrophobic Fe$_3$O$_4$ NPs significantly affect the collapse pressure of the monolayers, resulting in a significant dropping of the collapse pressure. Compared with pure DPPC monolayers, the lift-off area of DPPC monolayers is higher in the presence of hydrophilic Fe$_3$O$_4$ NPs, but lower in comparison with hydrophobic Fe$_3$O$_4$ NPs.

The compression modulus $C_s^{-1}$ can be calculated from the π-A isotherms [20, 21] to study the compression or elastic properties of the Langmuir monolayers. It is calculated as follows:

$$C_s^{-1} = A \left( \frac{\partial \pi}{\partial A} \right)_T, \quad (1)$$

where $A$ and $\pi$ are the mean molecular area and surface pressure, respectively.

The maximum value of compression modulus $C_s^{-1}$ indicates the rigid state of Langmuir monolayers. Figure 3 represents the compression modulus versus area ($C_s^{-1}$ vs. A) of certain molar ratios of mixed POPC/DPPC (pure POPC black, DPPC olive, X = 0.75 red, X = 0.5 blue, X = 0.25 magenta) at the air-water interface. For convenience, the maximum value of compression modulus $C_s^{-1}$ is shown in Figure 4. In Figure 4, the $C_s^{-1}$ values of pure DPPC and POPC monolayers are 202.89 mN/m and 86.78 mN/m, respectively, indicating that...
Figure 1: The surface pressure-area ($\pi$-$A$) isotherms of a mixed POPC/DPPC monolayer. (a) Pure lipids; (b) hydrophilic Fe$_3$O$_4$ NPs; (c) hydrophobic Fe$_3$O$_4$ NPs.

Figure 2: (a) Limiting area $A_{\infty}$. (b) Collapse pressure $\pi_c$. (c) Lift-off area $A_L$ of mixed POPC/DPPC monolayers with different subphases.

Figure 3: The compression modulus versus area ($C_s^{-1}$ vs. $A$) of mixed POPC/DPPC (pure POPC black, DPPC olive, $X_{\text{POPC}} = 0.75$ red, $X_{\text{POPC}} = 0.50$ blue, and $X_{\text{POPC}} = 0.25$ magenta) with different subphases. (a) Water; (b) hydrophilic Fe$_3$O$_4$ NPs; (c) hydrophobic Fe$_3$O$_4$ NPs. Inset: plot of $C_s^{-1}$ vs. $\pi$ dependencies.
the addition of hydrophobic Fe$_3$O$_4$ NPs can increase the pure and mixed POPC/DPPC at an initial surface pressure of 5 mN/m. The change in the adsorption curves of both lipids (Figures 5(b) and 5(c)). The surface pressure of mixed lipids/hydrophobic Fe$_3$O$_4$ NPs were different from the pure lipids. However, at the initial surface pressure of 20 mN/m, the Δπ decreases with the increase of $X_{\text{POPC}}$. This may be due to the unsaturated tail chain of POPC molecules, which increases the fluidity of the monolayer and makes it easier for it to adsorb to the interface. It is also observed that hydrophobic Fe$_3$O$_4$ NPs have a greater influence on the interfacial adsorption capacity than hydrophilic Fe$_3$O$_4$ NPs. Due to the hydrophobic nature of nanoparticles, part of the lipid molecules are extruded from the interface or form NP-lipid complexes into the subphase. At the initial surface pressure of 20 mN/m, hydrophobic Fe$_3$O$_4$ NPs have a significant effect on the adsorption of lipid monolayers. The surface pressure of the adsorption curve have larger increases with the increase of $X_{\text{POPC}}$, compared with that of DPPC monolayers. The change of high surface pressure is much more than that of low surface pressure. Because of the tight intermolecular arrangement of lipid monolayers at high surface pressures, the electrostatic repulsion of lipid tail chains is enhanced.

3.3. The Thermodynamic Analysis of Mixed POPC/DPPC Monolayers. For the miscibility and stability of mixed lipid monolayers, we can clarify their thermodynamic properties with the excess mean molecular area ($A_{\text{exc}}$) and excess Gibbs free energy ($\Delta G_{\text{ex}}$). For binary mixtures, we give the surface pressure $\pi$, ideal area per molecule $A_{\text{ideal}}$, excess mean molecular area $A_{\text{exc}}$, and excess Gibbs free energy $\Delta G_{\text{ex}}$ [16, 23, 24]; they are defined as follows:

$$A_{\text{ideal}} (POPC/DPPC) = X_{\text{POPC}} A_{\text{POPC}} + X_{\text{DPPC}} A_{\text{DPPC}},$$

$$A_{\text{exc}} (POPC/DPPC) = A_{12} - A_{\text{ideal}} (POPC/DPPC),$$

$$\Delta G_{\text{ex}} = N_A \int_{0}^{\pi} A_{\text{exc}} d\pi,$$

where $A_{\text{POPC}}$ and $A_{\text{DPPC}}$ are the areas per molecule of POPC and DPPC in pure monolayers at the considered $\pi$, and $X_{\text{POPC}}$ and $X_{\text{DPPC}}$ are the molar fractions of POPC and DPPC in the binary mixtures, respectively; $A_{12}$ is the experimental evaluation of the area per molecule of the binary mixtures, $\pi$ is surface pressure, and $N_A$ is the Avogadro number. The $A_{\text{exc}}$ values of the mixed lipids in the case of ideal miscibility or complete immiscibility is zero [25]. According to previous reports, $A_{\text{exc}} > 0$ indicates that the lipid molecules are repulsive interactions, and $A_{\text{exc}} < 0$ indicates that the lipid molecules are attractive interactions [26]. Figure 6 shows the excess mean molecular area and excess Gibbs free energy of lipid monolayers. A negative value of $A_{\text{exc}}$ ($X_{\text{POPC}} = 0.25$) indicates that there is an attractive interaction. A positive value of $A_{\text{exc}}$ ($X_{\text{POPC}} = 0.5$) indicates that the equimolar
POPC and DPPC have a repulsive interaction. In the experiments, hydrophilic Fe$_3$O$_4$ NPs enhanced the attraction interaction of mixed POPC/DPPC ($X_{POPC} = 0.25$) monolayers. The content of POPC molecules in the monolayers was further increased, and the molecular interaction in the mixed monolayers were transformed from repulsive to attractive interactions (Figure 6(b)). This is because the interaction between hydrophilic Fe$_3$O$_4$ NPs and the polar head of mixed lipids weakens the interaction of lipid molecules. In the presence of hydrophobic Fe$_3$O$_4$ NPs (Figure 6(c)), the value of $A_{ex}$ decreases to the negative value as the surface pressure increases, indicating that the interaction between the force changes from repulsive force to attractive force. At $X_{POPC} = 0.5$ and $X_{POPC} = 0.75$, the values of $A_{ex}$ are positive and negative, respectively. In contrast to hydrophilic Fe$_3$O$_4$ NPs, the repulsion interaction between lipid molecules is enhanced or the attraction interaction is attenuated in the presence of hydrophobic Fe$_3$O$_4$ NPs. In Figure 6(d), the value of $\Delta G_{ex}$ has a minimum at $X_{POPC} = 0.25$, indicating that the monolayers are most stable at POPC:DPPC (1:3), and the case of hydrophilic Fe$_3$O$_4$ NPs is similar to this condition. However, for the hydrophobic Fe$_3$O$_4$ NP subphase, the most stable lipid monolayers are POPC:DPPC (3:1).

3.4. The AFM Images of Pure POPC and DPPC and Different Molar Ratios of Mixed POPC/DPPC Monolayers with Hydrophilic and Hydrophobic Fe$_3$O$_4$ NPs at the Air-Water Interface. The AFM images of mixed POPC/DPPC monolayers at the initial pressures of 5 mN/m and 20 mN/m are shown in Figures 7 and 8, respectively.

In Figure 7(a), the pure DPPC monolayers at an initial surface pressure of 5 mN/m were in the LE-LC phase, and a polygonal irregular sheet-like structure was observed in the AFM image. With the increase of $X_{POPC}$, it can be observed that the DPPC domains are gradually reduced.

Figure 5: Surface pressure-time ($\pi$-t) adsorption curve of different molar ratios of mixed POPC/DPPC at the initial surface pressure of 5 mN/m and 20 mN/m. (a and d) Lipid, (b and e) hydrophilic Fe$_3$O$_4$ NPs, and (c and f) hydrophobic Fe$_3$O$_4$ NPs.
and the POPC liquid phase appears. The AFM pattern of the DPPC monolayers with hydrophilic Fe₃O₄ NPs shows a partially dispersed platelet-like structure. The mixed POPC/DPPC (X_{POPC} = 0.25) monolayers with hydrophilic Fe₃O₄ NPs showed a porous, irregular sheet-like structure, which was more compact than pure X_{POPC} = 0.25. Due to

Figure 6: Excess mean molecular area (A_{exc}) and excess Gibbs free energy (ΔG_{ex}). (a and d) Lipid, (b and e) hydrophilic Fe₃O₄ NPs, and (c and f) hydrophobic Fe₃O₄ NPs.

Figure 7: AFM images of mixed POPC/DPPC at the initial pressure of 5 mN/m.

and the POPC liquid phase appears. The AFM pattern of the DPPC monolayers with hydrophilic Fe₃O₄ NPs shows a partially dispersed platelet-like structure. The mixed POPC/DPPC (X_{POPC} = 0.25) monolayers with hydrophilic Fe₃O₄ NPs showed a porous, irregular sheet-like structure, which was more compact than pure X_{POPC} = 0.25. Due to
the interaction between the hydrophilic Fe₃O₄NPs and the head of the DPPC molecules, the DPPC molecules or NP-DPPC complexes enter the subphase, which affects the DPPC monolayers in the interface arrangement. For the hydrophobic Fe₃O₄ NP condition, it showed many large patches of uniform structure. With the increase of XPOPC, the large platform structure collapses into many small domains. This may be because of the repulsive interaction of hydrophobic Fe₃O₄ NPs with the tail chain of the mixed lipid molecules resulting in the lipid-NP complexes entering the subphase.

When the surface pressure is raised to 20 mN/m, the DPPC monolayers show a more uniform layered structure. With the increase of XPOPC, the monolayer forms more small-area structures. The monolayers have the phase separation structure. At high surface pressure (20 mN/m), the lamellar structure of DPPC monolayers is more compact in the presence of hydrophilic Fe₃O₄ NPs. When the subphase is Fe₃O₄ NPs, the DPPC monolayers become more condensed. However, for the hydrophobic Fe₃O₄ NPs, the monolayer structure becomes more obvious. The interaction of hydrophilic Fe₃O₄ NPs with the lipid head enhances the attraction interaction between lipid molecules.

Figures 9(a) and 9(b) represent the surface roughness for the AFM images of mixed POPC/DPPC monolayers at the initial pressures of (a) 5 mN/m and (b) 20 mN/m.
when the initial surface pressure is 20 mN/m, the effect of Fe$_3$O$_4$ NPs on the roughness of mixed POPC/DPPC is not obvious, and the variation range is only 0.15 nm. This may be due to the disordered arrangement of lipid molecules at low surface pressure. When the surface pressure increases, the order between the lipid molecules increases and leads to the dense distribution of lipid molecules, and the effect of Fe$_3$O$_4$ NPs on the roughness of the mixed POPC/DPPC monolayer is not significant.

4. Conclusion

In this paper, the influence of the subphase of mixed POPC/DPPC monolayers was studied using the LB technique. The different content has great influence on the structure of monolayers. The subphase especially contains Fe$_3$O$_4$ NPs with different properties. To further illustrate the interaction of lipids with Fe$_3$O$_4$ NPs, we used AFM to study the surface morphology between them. The results show that the repulsive interaction of hydrophobic Fe$_3$O$_4$ NPs with the tail chain of the mixed lipid molecules results in lipid-NP complexes entering the subphase. The interaction of hydrophilic Fe$_3$O$_4$ NPs with the lipid head enhances the attraction interaction between lipid molecules. Meanwhile, Fe$_3$O$_4$ NPs can increase the roughness of the mixed POPC/DPPC monolayer at low surface pressure and the effect of hydrophobicity is stronger than that of hydrophilicity; however, the effect is not obvious under high surface pressure. This study helps us gain new insights for the interaction between nanoparticles and molecules. This could have a potential application in designing the targeted drug liposomes.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] C. Hao, J. Li, W. Mu et al., “Adsorption behavior of magnetite nanoparticles into the DPPC model membranes,” *Applied Surface Science*, vol. 362, no. 30, pp. 121–125, 2016.

[2] M. Liong, J. Lu, M. Kovochich et al., “Multifunctional inorganic nanoparticles for imaging, targeting, and drug delivery,” *ACS Nano*, vol. 2, no. 5, pp. 889–896, 2008.

[3] A. Roychoudhury, S. Basu, and S. K. Jha, “Dopamine biosensor based on surface functionalized nanostructured nickel oxide platform,” *Biosensors and Bioelectronics*, vol. 84, pp. 72–81, 2016.

[4] R. Sarkar, P. Pal, M. Mahato, T. Kamilya, A. Chaudhuri, and G. B. Talapatra, “On the origin of iron-oxide nanoparticle formation using phospholipid membrane template,” *Colloids and Surfaces B: Biointerfaces*, vol. 79, no. 2, pp. 384–389, 2010.

[5] D. P. Singh, C. E. Herrera, B. Singh, S. Singh, R. K. Singh, and R. Kumar, “Graphene oxide: an efficient material and recent approach for biotechnological and biomedical applications,” *Materials Science and Engineering: C*, vol. 86, pp. 173–197, 2018.

[6] M. Cao, Z. Li, J. Wang et al., “Food related applications of magnetic iron oxide nanoparticles: enzyme immobilization, protein purification, and food analysis,” *Trends in Food Science & Technology*, vol. 27, no. 1, pp. 47–56, 2012.

[7] C. Z. Ye and P. A. Ariya, “Co-adsorption of gaseous benzene, toluene, ethylbenzene, m-xylene (BTEx) and SO$_2$ on recyclable Fe$_3$O$_4$ nanoparticles at 0–101% relative humidities,” *Journal of Environmental Sciences*, vol. 31, pp. 164–174, 2015.

[8] L. Wu, F. Zhang, Z. Wei et al., “Magnetic delivery of Fe$_3$O$_4$-polydopamine nanoparticle-loaded natural killer cells suggest a promising anticancer treatment,” *Biomaterials Science*, vol. 6, no. 10, pp. 2714–2725, 2018.

[9] H.-L. Liu, M. Y. Hua, H. W. Yang et al., “Magnetic resonance monitoring of focused ultrasound/magnetic nanoparticle targeting of delivery therapeutic agents to the brain,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 34, pp. 15205–15210, 2010.

[10] D. Rose, J. Rendell, D. Lee, K. Nag, and V. Booth, “Molecular dynamics simulations of lung surfactant lipid monolayers,” *Biophysical Chemistry*, vol. 138, no. 3, pp. 67–77, 2008.

[11] A. Olżyńska, M. Zubek, M. Roeselova, J. Korchowiec, and L. Ciwiklik, “Mixed DPPC/POPC monolayers: all-atom molecular dynamics simulations and Langmuir monolayer experiments,” *Biochimica et Biophysica Acta (BBA) - Biomembranes*, vol. 1858, no. 12, pp. 3120–3130, 2016.

[12] A. D. Roy, D. Dey, J. Saha, P. Debnath, D. Bhattacharjee, and S. A. Hussain, “Study of cholesterol derivative and phospholipid (DPPC) mixed film using LB technique and FRET: design of cholesterol sensor,” *Sensors and Actuators B*, vol. 255, pp. 519–528, 2018.

[13] C. Lim, S. Park, J. Park, J. Ko, D. W. Lee, and D. S. Hwang, “Probing nanomechanical interaction at the interface between biological membrane and potentially toxic chemical,” *Journal of Hazardous Materials*, vol. 353, no. 5, pp. 271–279, 2018.

[14] M. Kawaguchi, “Interfacial characteristics of binary polymer blend films spread at the air-water interface,” *Advances in Colloid and Interface Science*, vol. 247, pp. 163–171, 2017.

[15] Y. Wang, S. Wu, L. Xu, and Y. Zeng, “A new precise positioning method for piezoelectric scanner of AFM,” *Ultramicroscopy*, vol. 196, pp. 67–73, 2019.

[16] E. Guzmán, L. Ligigeri, E. Santini, M. Ferrari, and F. Ravera, “Mixed DPPC–cholesterol Langmuir monolayers in presence of hydrophilic silica nanoparticles,” *Colloids and Surfaces B: Biointerfaces*, vol. 105, pp. 284–293, 2013.

[17] A. Wnętrzak, K. Łątka, and P. Dynarowicz-Lątka, “Interactions of alkyphosphocholines with model membranes—the Langmuir monolayer study,” *The Journal of Membrane Biology*, vol. 246, no. 6, pp. 453–466, 2013.

[18] K. A. Okotrub, S. Y. Amstislavsky, and N. V. Surovtsev, “Raman spectroscopy reveals the lipid phase transition in
preimplantation mouse embryos during freezing,” Archives of Biochemistry and Biophysics, vol. 635, pp. 37–43, 2017.

[19] R. Wang, Y. Guo, H. Liu, Y. Chen, Y. Shang, and H. Liu, “The effect of chitin nanoparticles on surface behavior of DPPC/DPPG Langmuir monolayers,” Journal of Colloid and Interface Science, vol. 519, pp. 186–193, 2018.

[20] J. T. Davies and E. K. Rideal, Interfacial Phenomena, Academic Press, New York, NY, USA, 1963.

[21] A. K. Panda, F. Possmayer, N. O. Petersen, K. Nag, and S. P. Moulik, “Physico-chemical studies on mixed oppositely charged surfactants: their uses in the preparation of surfactant ion selective membrane and monolayer behavior at the air water interface,” Colloids and Surfaces A: Physicochemical and Engineering Aspects, vol. 264, no. 1-3, pp. 106–113, 2005.

[22] D. Jiang, K. L. Dinh, T. C. Ruthenburg et al., “A kinetic model for β-amyloid adsorption at the air/solution interface and its implication to the β-amyloid aggregation process,” The Journal of Physical Chemistry B, vol. 113, no. 10, pp. 3160–3168, 2009.

[23] C. Hao, Q. Liu, Q. Li, J. Zhang, and R. Sun, “Thermodynamic and structural studies of DMPC and DSPC with DOTAP mixed monolayers at the air–water interface,” Russian Journal of Physical Chemistry A, vol. 90, no. 1, pp. 214–219, 2016.

[24] M. Rojewska, M. Skrzypiec, and K. Prochaska, “Surface properties and morphology of mixed POSS-DPPC monolayers at the air/water interface,” Colloids and Surfaces B: Biointerfaces, vol. 150, pp. 334–343, 2017.

[25] E. Guzmán, M. Ferrari, E. Santini, L. Liggieri, and F. Ravera, “Effect of silica nanoparticles on the interfacial properties of a canonical lipid mixture,” Colloids and Surfaces B: Biointerfaces, vol. 136, pp. 971–980, 2015.

[26] G. Neunert, J. Makowiecki, E. Piosik, R. Hertmanowski, K. Polewski, and T. Martynski, “Miscibility of dl-α-tocopherol β-glucoside in DPPC monolayer at air/water and air/solid interfaces,” Materials Science and Engineering C, vol. 67, pp. 362–368, 2016.