

Effects of different concentrations of sodium ions on the self-assembly of amphotericin B and DPPC at the air-water interface

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Received March 28, 2018

Amphotericin B is a widely used polyene antifungal drug for the treatment of deep fungal infections. This drug could cause pores on the cell membrane. In this work, we used the dipalmitoylphosphatidylcholine monolayer as the model of cell membrane in half. The influence of Na⁺ ions on the interaction between amphotericin B and biomembrane were studied by analysis of phase transition and thermodynamic properties of monolayers. The Na⁺ ions may affect the molecular orientation of amphotericin B, and it depends on the concentration of sodium ions. Low concentration of Na⁺ ions has an opposite effect to high concentration of that. The results are helpful for obtaining some information on the influence mechanism in the level of sodium ions on the interaction between amphotericin B and biomembrane in the angle of physics.

Keywords: Na⁺ ions, amphotericin B, phase transition, intermolecular interaction

Амфотерицин В является широко используемым полиеновым противогрибковым препаратом для лечения глубоких грибковых инфекций. Этот препарат может вызвать поры на клеточной мембране. Использовали двухслойный дипальмитоилфосфатидилхолин в качестве модели клеточной мембраны. Влияние ионов Na⁺ на взаимодействие между амфотерицином В и биомембраной изучалось путем анализа фазового перехода и термодинамических свойств монослоев. Ионы Na⁺ могут влиять на молекулярную ориентацию амфотерицина В в зависимости от их концентрации. Низкая концентрация ионов Na⁺ оказывает противоположное влияние чем их высокая концентрация. Результаты полезны для получения информации о механизме влияния ионов натрия разной концентрации на взаимодействие между амфотерицином В и биомембраной с точки зрения физики.

Вплив різних концентрацій іонів натрію на самоосадження амфотерицину В і DPPC на кордоні розділу повітря-вода. *Juan Wang, Tuo Li, Yahong Ma, Zongcheng Miao*

Амфотерицин В є широко використовуваним поліеновим протигрибковим препаратом для лікування глибоких грибкових інфекцій. Цей препарат може викликати пори на клітинній мембрані. У роботі використовували двошаровий дипальмітоїлфосфатиділхолін (DPPC) в якості моделі клітинної мембрани. Вплив іонів Na⁺ на взаємодію між амфотерицином В і біомембраною вивчено шляхом аналізу фазового переходу і термодинамічних властивостей моносарів. Іони Na⁺ можуть впливати на молекулярну орієнтацію амфотерицину В в залежності від їх концентрації. Низька концентрація іонів Na⁺ надає протилежний вплив ніж їх висока концентрація. Результати корисні для отримання інформації про механізм впливу іонів натрію різної концентрації на взаємодію між амфотерицином В і біомембраною з точки зору фізики.

1. Introduction

Amphotericin B (AmB) is a widely used polyene antifungal drug for the treatment of deep fungal infections [1]. Its pharmacodynamic mechanism is that amphotericin B can make pores formed on the membrane of fungi, which causes to the dead of the fungus [2]. However, when amphotericin B exerts antifungal effects, it also could influence the property of the host cell membrane [3, 4] and destroy the structure of the cell membrane, which affect the transmembrane transport of normal living ions. So, this drug has very serious toxic and side effects. It is particularly easy to cause nephrotoxicity in patients with proteinuria, azotemia, hypokalemia, anemia and other symptoms, which greatly limits its clinical application. Therefore, the study of antifungal molecular mechanism of amphotericin B and its mechanism of cell membrane toxicity has become an important scientific problem.

Many scholars have found that this membrane pores by amphotericin B formed on the membrane affects transmembrane transport of metal cations, especially of potassium [5]. At the same time, there is an interplay between metal cations and amphotericin B on the membrane. Gagos et al. [6] found that potassium and sodium ions can induce changes in the localization of amphotericin B molecules on the membrane. L.Becuuci [7] found that calcium ions could significantly promote the aggregation of amphotericin B. In addition, R. Turcu et al. [8] found that the intake of high sodium salts can, to some extent, reduce the effect of amphotericin B on mammalian cell membrane permeability. It can be seen that potassium, sodium and calcium ions may have special effects on the aggregation of amphotericin B on the membrane. However, Most scholars have pay more attention to the effects of potassium and calcium ions on amphotericin B. The studies about the effects of sodium ions are limited. The special role of sodium ions on the interaction between this drug and biomembrane can not be ignored and is worth in-depth study.

In this work, we used the dipalmitoylphosphatidylcholine (DPPC) monolayer as the model of cell membrane in half. The influence of Na^+ ions on the interaction between amphotericin B and biomembrane were studied by analysis of phase transition and thermodynamic properties of monolayers. The results are helpful for obtaining some information on the influence mechanism in the molecular level of sodium ions

on the interaction between amphotericin B and biomembrane in the angle of physics.

2. Experimental

2.1 Materials

Dipalmitoylphosphatidylcholine (DPPC: purity \geq 99%), power amphotericin B (purity $>$ 75%) and Tris (hydroxymethyl) aminomethane (Tris: purity \geq 99.9%) were purchased from Sigma, USA. The other chemicals were of analytical grade and were used without further purification. DPPC were dissolved in a chloroform/methanol mixture (9:1, v/v) to give a final concentration of 0.57 μM lipid membrane-forming solution. AmB (0.70 μM) was dissolved to a concentration of approximately $6 \cdot 10^{-4}$ M in a 3:1 (v/v) mixture of dimethylformamide and 1 M HCl (aq). A subphase buffer composed of 10 mM Tris-HCl buffer, pH 7.4, was prepared for the Langmuir experiment. High purity water obtained from a Milli-Q plus water purification system (18.2 M Ω /cm, Millipore, USA) was used in all experiments.

2.2 Langmuir technique

A Langmuir trough (KSV-Minitrough, Finland) was used to obtain the surface pressure-area per molecule curves of the monolayer at the air-water interface. A Wilhelmy-type tensiometer was used as the pressure sensor. The accuracy of the sensor was 0.1 $\mu\text{N/m}$. A monolayer at the air-water interface of the trough subphase could be symmetrically compressed or expanded with two Teflon barriers at a desired rate. For all the experiments, the temperature was maintained at 35 ± 0.1 °C by an external circulator, and the trough was filled with buffer as the subphase.

Before each run, the Teflon trough (trough size 323 \times 75 \times 5 mm) was washed with ethanol and rinsed with purified water. The appropriate volume of sample solution was deposited at the air-water interface with a Hamilton microsyringe. Spreading solvent evaporation over 15 min and then the monolayers were compressed with a barrier speed of 20mm/min. The surface pressure was recorded during the interface compression process. Repeated experiments were performed independently to confirm the reproducibility of the isotherm measurements.

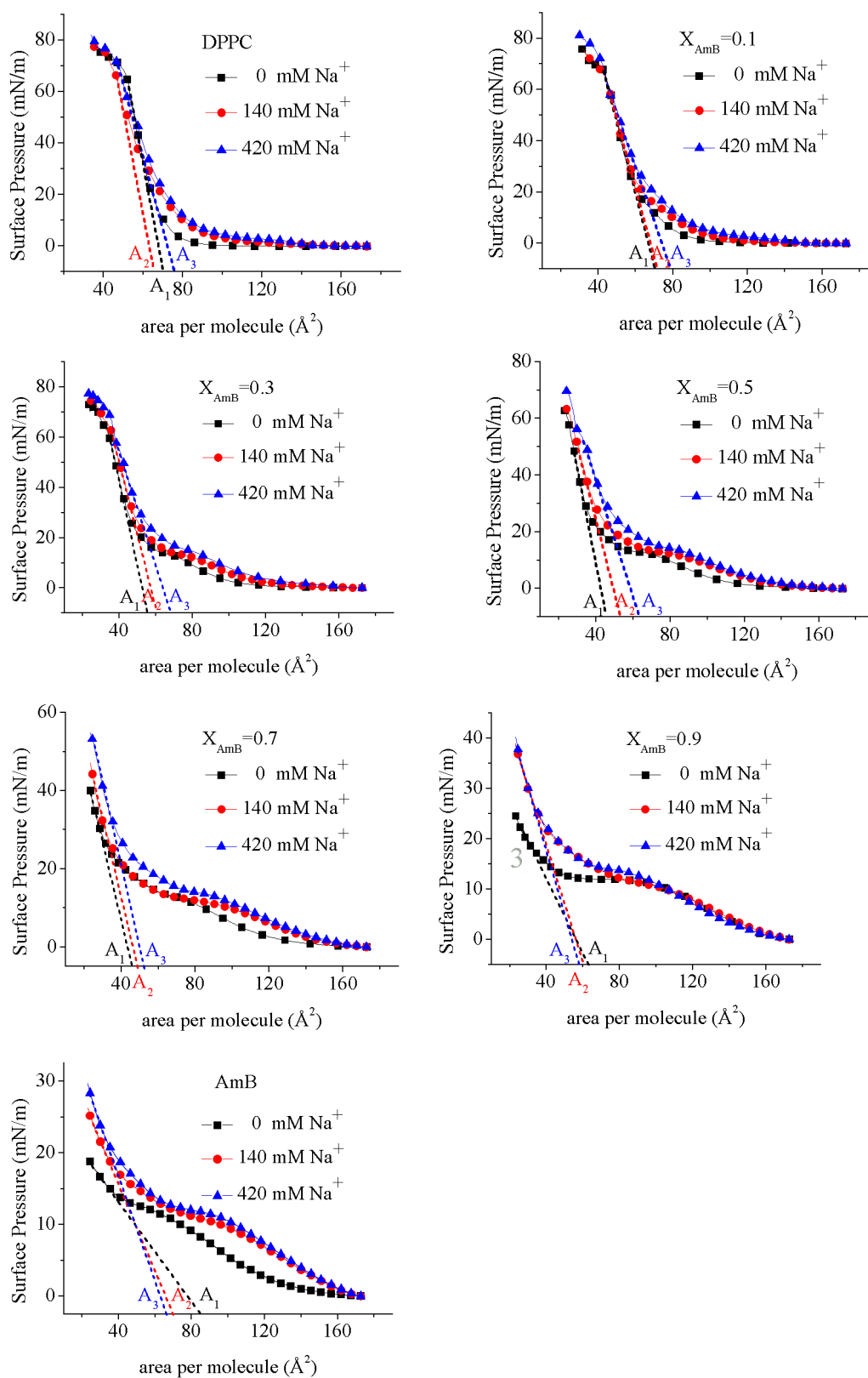


Fig. 1. Surface pressure-area per molecule curves of mixed monolayers (A_1 , A_2 , A_3 is the limiting molecular area of the mixed monolayer in 0mM, 140mM, 420mM Na^+ , respectively).

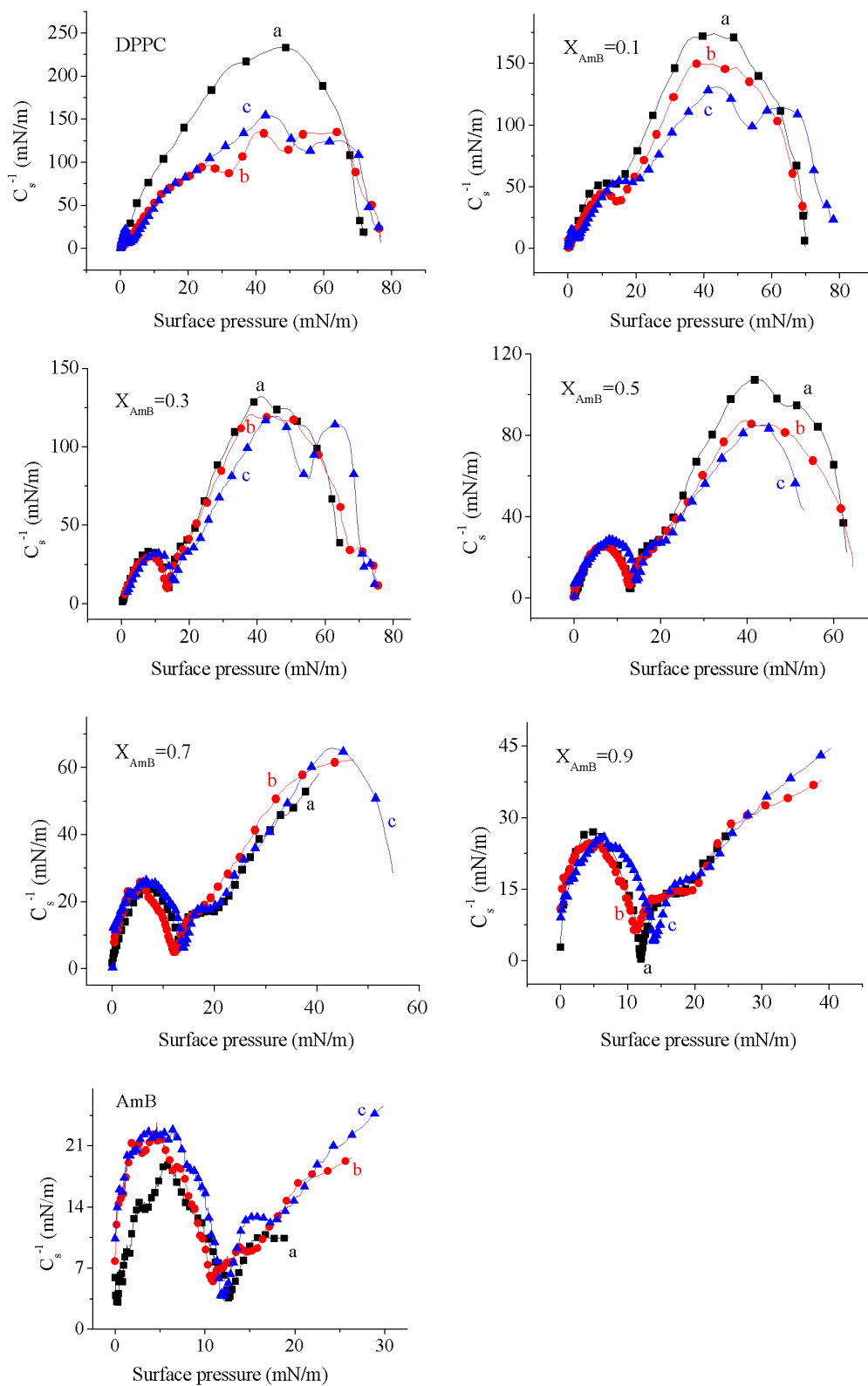


Fig. 2. Compression modulus C_s^{-1} of mixed monolayers in 0mM Na^+ (a), 140 μM Na^+ (b), 420 μM Na^+ (c).

Table. Limiting molecular area of the mixed monolayer

Limiting molecular area (\AA^2)	DPPC	$X_{AmB} = 0.1$	$X_{AmB} = 0.3$	$X_{AmB} = 0.5$	$X_{AmB} = 0.7$	$X_{AmB} = 0.9$	AmB
A_1	71.3	71.6	55.7	46.2	46.3	63.8	85.9
A_2	65.2	72.3	60.9	54.0	49.2	60.7	71.1
A_3	76.9	79.9	68.8	64.1	52.3	58.1	67.2

2.3 Thermodynamic parameters of monolayer

Compression modulus C_s^{-1} can be calculated according to the surface pressure-area per molecule curve by the formula [9-11]:

$$C_s^{-1} = -\frac{A d\pi}{dA} \quad (1)$$

where A is the area per molecule at the indicated surface pressure π . The minimum of C_s^{-1} suggests the phase transition of monolayer.

Excess Gibbs free energy of mixing ΔG^{exc} can suggest the information on the intermolecular interaction of monolayer. It was calculated for binary Langmuir monolayers at a given π value according to its definition [12,13]:

$$\Delta G^{exc} = N \int_0^\pi A^{exc} d\pi, \quad (2)$$

where N is Avogadro's number.

A^{exc} is the excess area defined as follows [14, 15]:

$$A^{exc} = A_{12} - A_1 X_1 - A_2 X_2, \quad (3)$$

where A_{12} is the mean molecular area at a given π value for the binary monolayer, and A_1 and A_2 are the molecular areas of components 1 and 2, respectively, at the same surface pressure. X_1 and X_2 are the molar fractions of single components in the mixed monolayer.

A^{exc} can be calculated to determine whether two components are immiscible or ideally miscible. A negative value of ΔG^{exc} indicates that the intermolecular interaction exhibits attraction [14].

3. Results and discussion

3.1 Phase transition of self-assembled monolayers

In the process of self-assembly of pure DPPC molecules at the air-water interface,

as the concentration of Na^+ increases, the extent of expansion for πA curves at the liquid expansion phase is gradually enhanced. When $X_{AmB} = 0.1, 0.3, 0.7$, the extent of expansion has a similar change to that of pure DPPC monolayer with the increasing of Na^+ ions' concentration. However, the difference is the extent of expansion for curves at the liquid condensed phase is also gradually enhanced in the increasing of Na^+ ions' concentration after addition of AmB.

The limit molecular area was calculated from the data points on the surface pressure-area per molecular curve, and the results are shown by dotted lines in the Fig. 1. When the Na^+ ions' concentration was 0 μM , 140 μM , and 420 μM , the limiting molecular area of the monolayer was A_1 , A_2 , and A_3 , respectively (Table).

For a pure DPPC monolayer curve, as the concentration of Na^+ ions increases, the limiting molecular area decreases firstly and then increases, and $A_2 < A_1 < A_3$. When X_{AmB} , the limiting molecular area also gradually increases with the increase of sodium ions ($A_1 < A_2 < A_3$). However, when, the area of the limiting molecule gradually decreases with the increase of the sodium ion concentration ($A_3 < A_2 < A_1$), which is similar to that for pure AmB monolayer. The difference is that the change is more obvious.

The minimum value of C_s^{-1} indicates the phase transition point of the monolayer film. From the Fig. 2, we can see that for pure DPPC, when the concentration of Na^+ is 420 mM, the curve has a phase transition point at over the surface pressure of 56 mN/m. When the concentration of Na^+ is reduced to 140 μM , two phase transition points appears at 32 mN/m and 48 mN/m. This may be due to the fact that the orientation of the DPPC molecules at the interface are affected by the Na^+ ions. At a certain range of surface pressure, the DPPC molecules may be rearranged. This phenomenon can not be observed without sodium ions.

After addition of AmB, when X_{AmB} , the two phase transition points of mixed

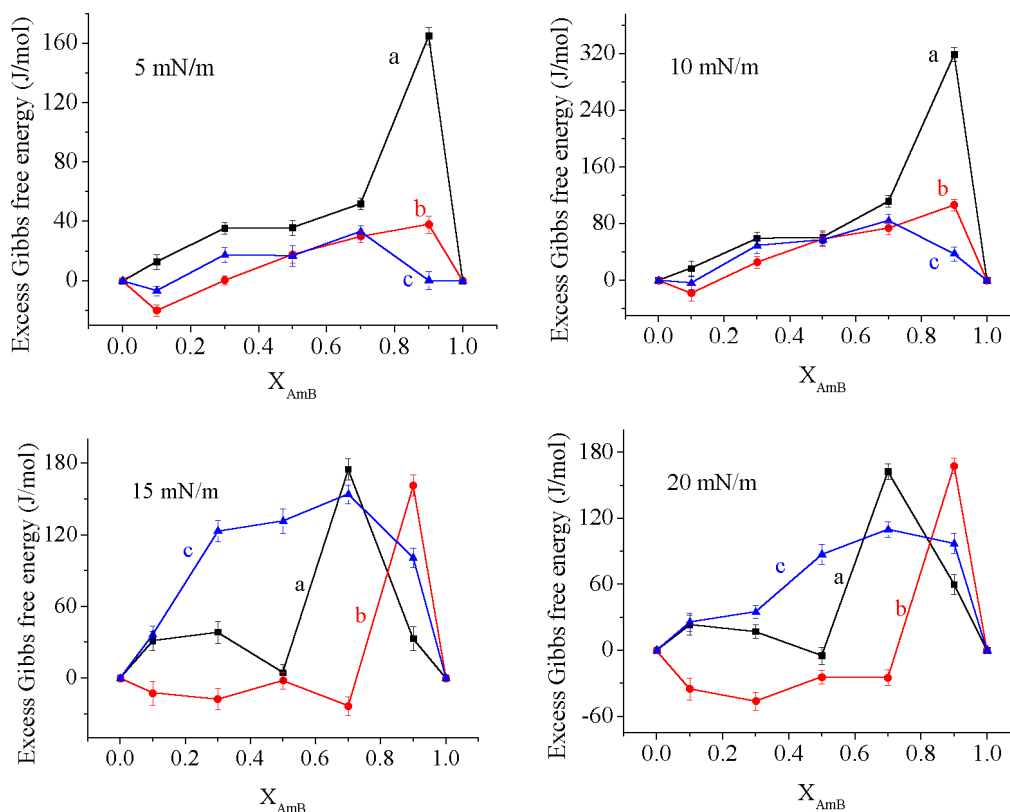


Fig. 3. Excess Gibbs free energy of mixed monolayers in 0mM Na^+ (a), 140 mM Na^+ (b), 420 mM Na^+ (c)

monolayer at the surface pressure of 32 and 48 mN/m have disappeared in 140 mM Na^+ . But when the sodium ions' concentration was 420 μM , the phase transition point at 56 mN/m is still present. As the proportion of AmB continues to increase, the phase transition point disappears.

It is shown that the concentration of sodium ion has a great influence on the phase transition of DPPC monolayer. In addition, AmB changes the phase transition of DPPC at high surface pressures in the presence of sodium ions.

3.2 Interaction between the super-molecules

When X_{AmB} , at the surface pressure of 5 or 10 mN/m, the excess gibbs free energy is positive in the absence of Na^+ ions (Fig. 3). This suggests that there is repulsion between the molecules. However, in the presence of Na^+ ions, the excess gibbs free energy is negative, which shows that the interaction between molecules is gravity. In addition, the larger the sodium concentration, the excess gibbs free energy of the monolayer is smaller, which means the interaction force between the molecules is

weaker. Differently, at the surface pressure of 15 or 20 mN/m, the excess gibbs free energy becomes positive when the concentration sodium ions goes up to 420 mM, and the intermolecular interactions become repulsive.

When X_{AmB} , at the surface pressure of 5 or 10 mN/m, the excess gibbs free energy is positive and the intermolecular interactions is repulsion. Differently, at the surface pressure of 15 or 20 mN/m, the value of excess gibbs free energy becomes negative, and the interaction between molecules is shown as gravitation when the concentration of sodium ions is 140 mM. This indicates that the sodium ions in appropriate concentration could promote the tight arrangement of molecules.

When X_{AmB} at 5 mN/m or 10 mN/m, the value of the excess gibbs free energy is positive. Moreover, with the increasing of sodium ion concentration, the value of excessive gibbs free energy decreases gradually. It is shown that the interaction force between molecules on the monolayer is repulsion, and the repulsion decreases gradually as the increasing of sodium ion concentration. When the surface pressure goes up

to 15 and 20 mN/m, the value of the excess gibbs free energy is positive, which is similar to that at low surface pressure. However, when in the presence of 140 mM Na⁺ ions, the excess gibbs free energy is maximal, and the repulsion between molecules is stronger than that in other conditions of this work. The interaction between molecules is closely related to the distribution and arrangement of molecules on the monolayer.

4. Conclusions

From the above, the effect of sodium ion on the liquid expansion phase, the limiting molecular area, phase transition and intermolecular forces of mixed monolayer films depends on the composition of amphotericin B in the membrane. The influence of sodium ions on the limiting molecular area is relative to the proportion of amphotericin B in mixed membrane. The smaller the area of the limiting molecule, the smaller the distance between the molecules on the mixed monolayer, and the closer the formation of the monolayer. The effect of sodium ions on the phase change of mixed monolayer is also related to the concentration of amphotericin B. With the increase of concentration of amphotericin B at high surface pressure, sodium ions make the phase transition points of mixed monolayer gradually disappear, which shows that sodium ions mainly interact with amphotericin B.

There is a correlation between the intermolecular forces and the monolayer membrane structure. The Na⁺ ions may affect the molecular orientation of amphotericin B, and it may mainly depend on the concentration of sodium ions. The low concentration of sodium ions could increase the attraction between molecules but the high

concentration of sodium ions increases the mutual repulsive force between molecules.

Acknowledgements

The study was supported by the National Natural Science Foundation of China (Grant: 51407184), the Doctor's Special Foundation of Xijing University (Grant: XJ17T02).

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