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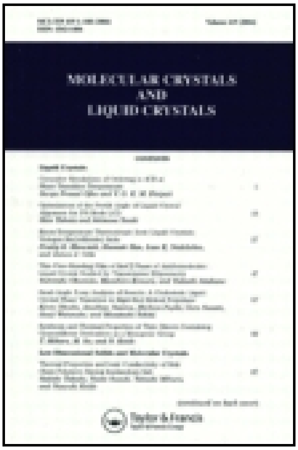

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Langmuir and Langmuir-Blodgett Films of Bilirubin

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Langmuir and Langmuir-Blodgett Films of Bilirubin

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The surface pressure-area isotherms of bilirubin (H_2BR) monolayers at an air-water interface on subphases with different pH values and on subphases containing metal ions such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Pb^{2+} ion were investigated. H_2BR can form expanded and stable monolayer on neutral and acidic subphases, while it can hardly form monolayer on basic subphases. The acid-base equilibrium of H_2BR was discussed at the air-water interface. The association-dissociation of H_2BR with H^+ ions in the interfacial region was very different from that in the bulk solution. Some information regarding the packing density and degree of ionization of the head group under different experimental conditions were obtained. The formation of H_2BR-metal complexes leads to changes in the shape of isotherms of H_2BR and changes in UV-visible absorption spectra of the monolayer assemblies. In XPS spectra new XPS peaks assigned to the metal ions containing subphases appeared. Low-angle X-ray diffraction indicates that a Y-type LB films were formed with bilayer spacing of ca. 2.50 nm.

Keywords: Bilirubin; monolayer; metal ion complexation; interface

1. INTRODUCTION

In the recent years, biomimetic strategies have focused on the application of bioinorganic nanocomposites and shaped hybrid materials with microscale organization[1,2]. Because of the importance of bile pigments in the physiological system such as calcium bilirubinates, the main component of black-pigment gallstones, an abnormal product of biomimeralization in mammal bodies and copper

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bilirubinate, a free-radical scavenger in bile and protecting phospholipids from peroxidation[3], a great deal of attention has already been given to the study of bilirubin and other bile pigments[4,5]. Excellent reviews of these studies have been made many years ago[6,7]. But many studies indicate that much is still uncertain concerning the chemistry of bilirubin. Investigations have been severely limited by the lack of stability and solubility of bilirubin in aqueous solutions including physiological solutions. This has led a number of investigators to the utilization of non-aqueous solvents for the study of the chemistry of bilirubin and its analogues. But so far there is little attempt at organized molecular films of bilirubin. Since the structural organization, coordination with metal ions and biophysical properties of bilirubin molecules in ordered molecular assemblies might be different from those in solution, therefore, if bilirubin or its derivatives can be incorporated in ordered molecular films, these ordered system may have potential applications in mimicking the mineralization processes, such as the prevention and cure of black pigment gallstones in human body and the promotion in animal body, especially in cattle and monkey bodies, as well as to investigate the structure of bilirubin, which is believed to play a very important role in the properties of bilirubin and its salts. With this in mind, the air-water monolayer of bilirubin and its amphiphilic derivative on subphases with different pH values and on subphases containing metal ions were studied. Some information regarding the packing density, phase transition and degree of ionization of the head group under different experimental conditions were obtained.

2. METHODS AND EXPERIMENTS

The H_2BR used was obtained from Sigma Chemical Co. and was of 99% purity. All the other chemicals were of A.R. grade. Formation of air-water monolayers and the deposition of the LB films have been outlined in another paper[8]. Electronic spectra were measured with a Shimadzu Model 3100 UV-VIS-NIR recording spectrophotometer. The polarized UV-visible spectra of the monolayer were measured by differential spectroscopy after M. Yoneyama et al.[9]. Low-angle X-ray diffraction results were recorded on a D/max-γ A X-ray diffractometer (Japan), using Ni-filtered Cu-K_α radiation and a scanning rate of 2° min^{-1}. The divergence and scattering slit was at 1° for $1° < 2\theta < 10°$. The analysis of X-ray photoelectron spectroscopy (XPS) was performed using an ESCALAB mark II (VG) photoelectron spectrometer, with an Al K_α X-ray radiation sources (1486.6 eV photos) under a vacuum of 10^{-7} Pa. The charging shift was corrected with the C1s line emitted from neutral hydrocarbon. Take off angle was 90°.

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3. RESULTS AND DISCUSSION

3.1. Formation of Monolayers of H_2BR on Aqueous Subphases with Different pH Values

Fig. 1 shows the surface pressure-area isotherms (π-A) of monolayers of H_2BR on subphases of different pH values. H_2BR can form a stable monolayer with a sufficiently high collapse pressure (about 38 mN m^{-1}) on pure water subphase (pH 5.6). A limiting area of about 0.74 nm^2 (A_0) per H_2BR molecule was obtained by extrapolating the surface pressure of condensed zone to zero. As shown in Fig. 2, H_2BR exhibited three conformation isomerisms[10,11] due to its eight hydrophilic groups: two carboxylic groups (COOH), two lactam carbonyl (=O), two pyrrole N and two lactam N groups. In solid state or in $CHCl_3$ solution H_2BR exists as six H-bondings (Isomer C in Fig. 2)[10]. The hydrophilic groups were surrounded inside H_2BR molecule, so H_2BR is insoluble in water.

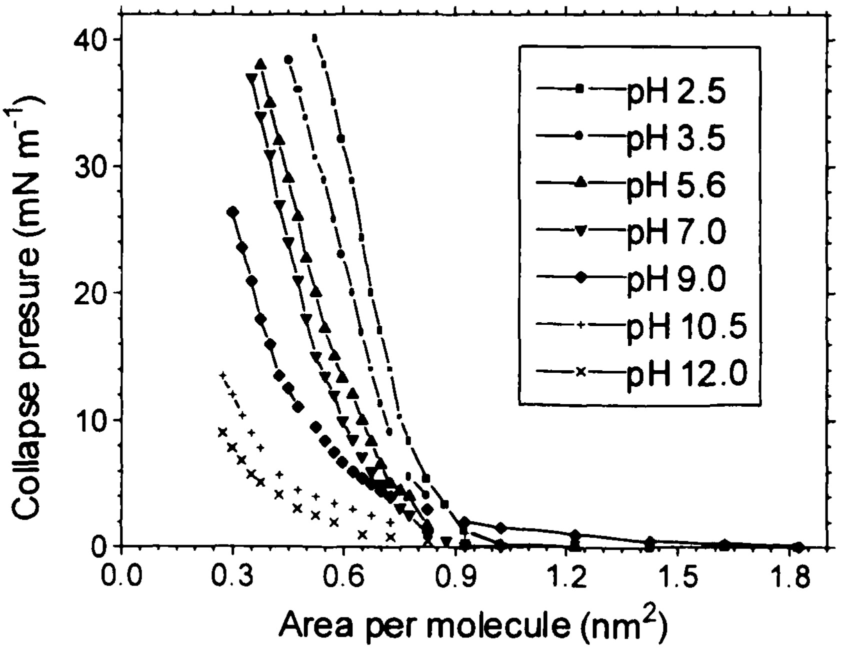

FIGURE 1 Surface pressure-area isotherms of H_2BR on aqueous subphases of different pH

According to a space-filling molecular (CPK) Model[11-13], H_2BR can be approximated as rectangular blocks with dimensions of about 1.95 × 1.20 × 0.36

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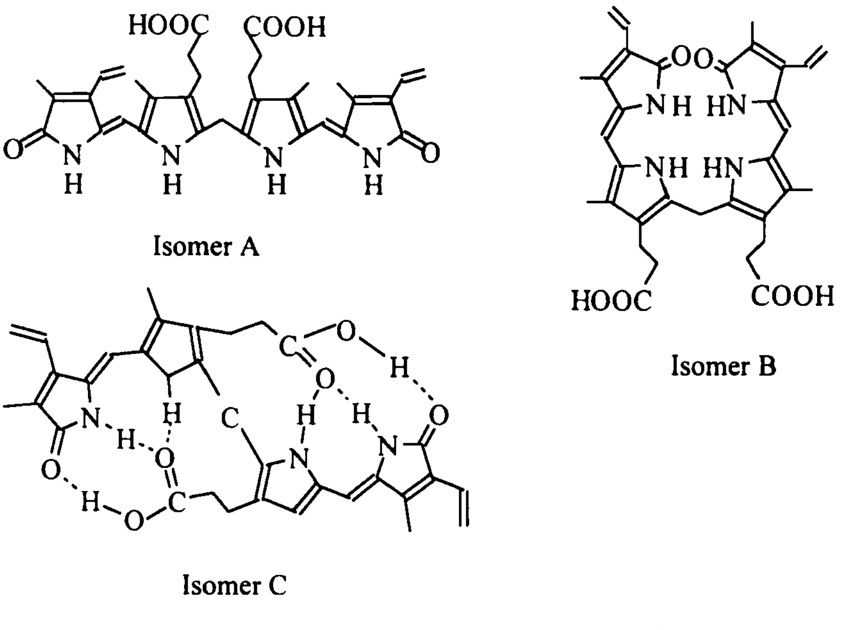
nm^3 in Isomer A, 1.20 × 1.55 × 0.36 nm^3 in Isomer B (ref. Fig. 3) and of about 1.95 × 1.05 × 0.36 nm^3 in Isomer C. The area value of 0.74 nm^2 per H_2BR molecule from Fig. 1 is much less than the face area of H_2BR in Isomer A (1.95 × 1.20 nm^2), Isomer B (1.20 × 1.55 nm^2) and Isomer C (1.95 × 1.05 nm^2) and is approximately consistent with the long side area of H_2BR in Isomer A. This suggests that the H_2BR plane orients either perpendicular to the air-water face in Isomer A or tilting at an angle (θ) with respect to the normal to the interface in Isomer B. According to the thickness measurement of H_2BR monolayer assemblies obtained from low angle X-ray diffraction, which will discussed in details later, H_2BR LB films has a double layers height of ca. 2.50 nm. So H_2BR orients in the latter fashion. In this fashion the height of H_2BR is equal to $1.55 \cos \theta$ nm, as shown in Fig. 3 (in Isomer B).



Isomer A

Isomer B

Isomer C

FIGURE 2 Stacking Isomers of H_2BR in the air-water monolayer

As shown in Fig. 1, the π -A isotherms of H_2BR depend markedly on pH values of the aqueous subphases because of the weakly acidic character of the carboxyl group and the weakly basic character of the pyrrole N atoms. On weak basic subsolution of pH 7~8 (<6.0 × 10^{-5} mol dm^{-3} NaOH), there is little change of the shape of H_2BR isotherm. For example, at pH 7.0 (2.0 × 10^{-5} mol dm^{-3} NaOH), the isotherm of the H_2BR monolayer is similar to that at pH 5.6 and the

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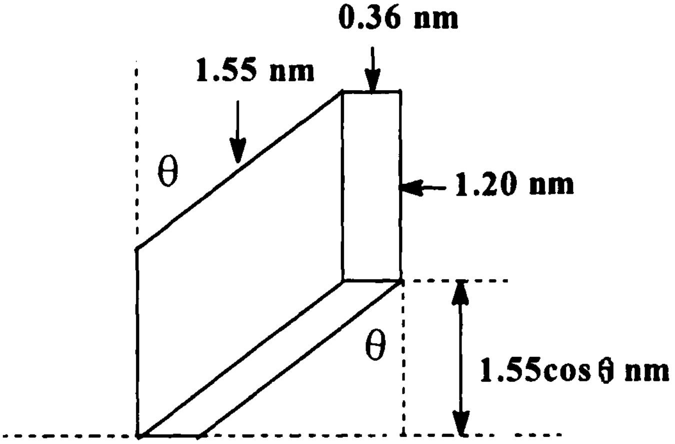

FIGURE 3 The orientation of H_2BR at the air-water interface. The digits show the molecular dimensions of H_2BR in Isomer B by C.P.K. model

collapse pressure is 37 mN m^{-1}. Further increase of NaOH will result in a great alteration of the π -A isotherm. The higher the pH in subphase, the less the molecular area of H_2BR (ref. Fig. 4). For example, at pH 10.5, although the expanded phase of the H_2BR monolayer is similar to that at pH 5.6, the condensed region is reduced and the collapse pressure is decreased to 13 mN m^{-1}. The expansion of monolayer of H_2BR and the decrease of collapse pressure with increasing basicity in the subphase may, among other factors, be due to the chemical reaction of the carboxyl groups of H_2BR with NaOH in subphase. It results a soluble salt sodium bilirubinate (Na_2BR) and prevents H_2BR from forming a condensed film. In contrast, the isotherms of H_2BR have a tendency to increase the molecular area in acidic subphases. The collapse pressure changed little.

Fig. 4 shows the pronounced dependence of the limiting area of H_2BR (A_0) on pH values in the subphases. With increasing pH values, a steep decrease of A_0 is observed at pH 6.0–9.0. This change of limiting area relates to the conformational change of H_2BR in monolayer induced by altered ionization of H_2BR.

In order to further prove the conformational change of H_2BR in monolayer on subphases with different pH values, the polarized UV-visible spectra of H_2BR monolayer were measured[9]. Fig. 5 shows the results of H_2BR monolayer on subphase of pH 9.0. The results of H_2BR monolayers on subphases with different pH values are shown in Table I, where θ is the tilting angle of the H_2BR plane with respect to the normal to the air-water face. It can be seen that the tilting angle increases with the basicity of subphase increasing.

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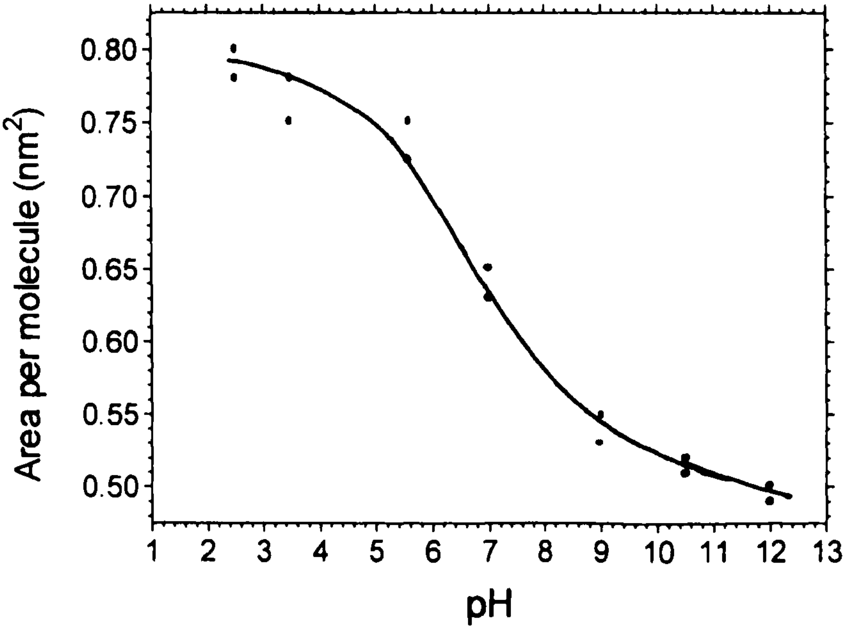

FIGURE 4 Plot of the limiting area of H_2BR monolayer as a function of pH values of subphases

TABLE I Polarized UV-visible spectra data of H_2BR monolayers

pH	*6.0*	*7.0*	*8.0*	*9.0*
$\cos^2\theta$	0.594	0.577	0.518	0.444
θ (degree)	39.6	40.5	43.9	48.2

3.2. Acid-Base Equilibria of H_2BR at Air-Water Interface

When the pH value of subphase is larger than 10.0 or less than 6.0 the π -A isotherms of H_2BR barely change, respectively. But in the pH range 6.0–9.0, a small change of the pH values of the subphase can cause a notable modification of the properties of the H_2BR monolayer film. It can thus be deduced that the association-dissociation of the carboxyl groups of H_2BR with H^+ ion is complete in the subphase pH range 6.0–9.0. When pH<6.0, H_2BR exists mainly as a protonated species ($H_{2+n}BR^{n+}$). When pH>9.0, H_2BR exists mainly as deprotonated species (BR^{2-}). The association-dissociation of H_2BR is shown in Scheme 1. H_2BR acts as a weak acid as well as a weak base at the air-water interface.

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$$\underset{pH < 6.0}{H_{2+n}BR^{n+}} \quad \text{———} \quad \underset{6.0 < pH < 9.0}{H_2BR} \quad \text{———} \quad \underset{pH > 9.0}{BR^{2-}}$$

SCHEME 1 The prototype equilibrium of H_2BR at the air-water interface

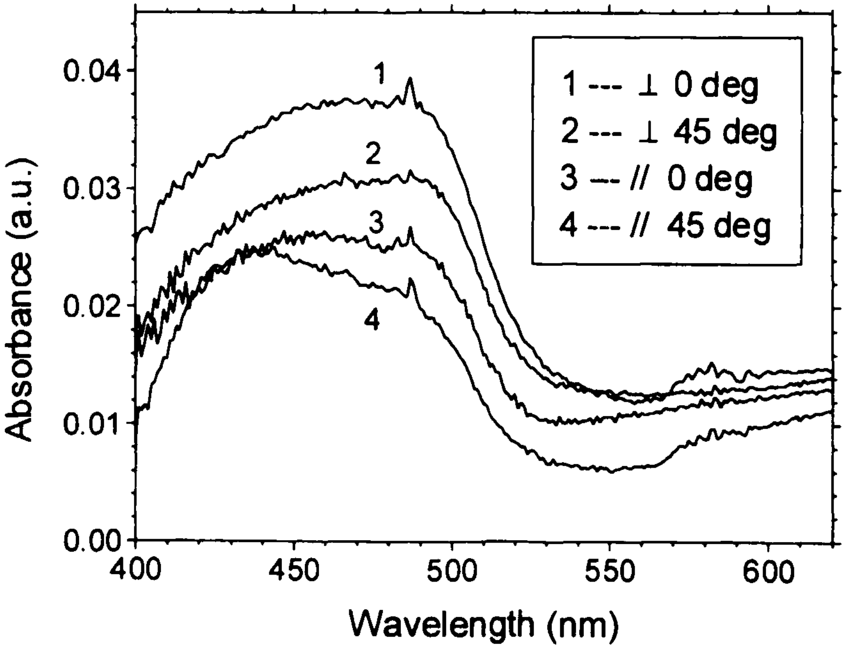

FIGURE 5 Polarized absorption spectra of a H_2BR monolayer on subphase of pH 9.0 with electro-vectors perpendicular (⊥) and parallel (//) to the dipping direction for $\beta = 0$ and 45 degrees

This association-dissociation of H_2BR at the air-water interface was much different from that of H_2BR in bulk solution. The pK_a values for H_2BR have been determined and found to be 4.4 for the carboxyl groups and 13 for the lactams[14]. That is, the association-dissociation of H_2BR in solution is complete in the pH range of ca. 3–14. It is very different from that at an interface (pH 6.0–9.0). This difference can be ascribed as the difference of pH values between the heterogeneous air-water interfacial environment and the bulk solution[15]. It can be reasonably inferred from the data obtained that the interfacial pH value is higher than that in the bulk solution by ca. 2 unit in basic subphase or lower than that by ca. 2 unit in acidic subphase[16]. H^+ or OH^- ion in the bulk solution can be accumulated in the interfacial region. So the concentration of electrolytes in the interfacial region is much higher than that in bulk solution. This is dependent on the polarity and the surface charge density of the monolayer-water interface and other factors. Similar results were observed by other researchers and us previously[17–19].

3.3 Metal Ion Complexation of H_2BR in Monolayers at Air-Water Interface

Fig. 6 shows the π -A isotherms of H_2BR monolayers in subphases containing Ca^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, and Pb^{2+} ions at pH 5.6. These isotherms are very similar. Compared with pure water subphase, the π -A isotherms of H_2BR monolayers in metal ions containing subphases are more condensed and the area per H_2BR molecule is 0.63~0.72 nm^2.

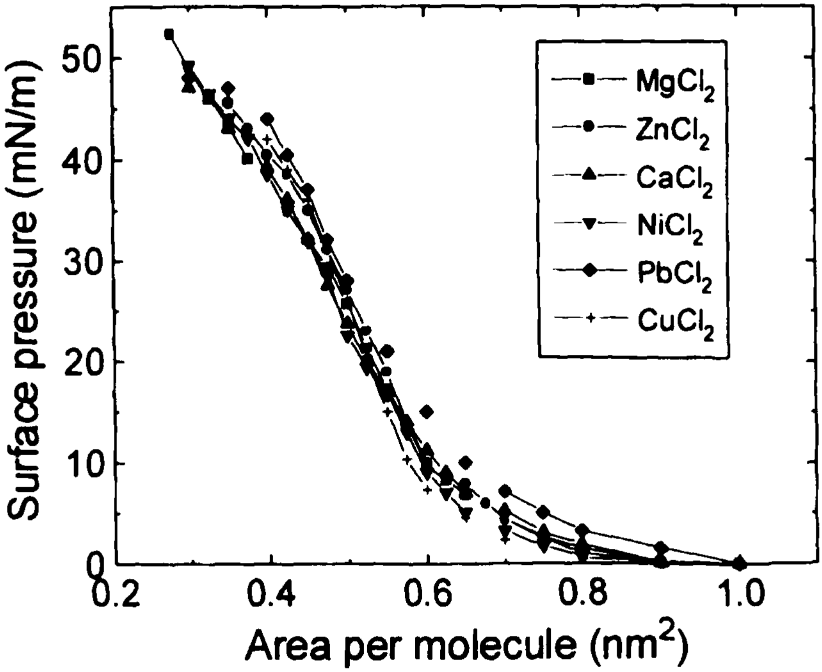

FIGURE 6 π -A isotherms of H_2BR monolayers in subphases containing metal ions

Fig. 7 shows the absorption spectra of an 11-layer LB film on a quartz substrate on pure water subphase and on subphases containing Ni^{2+} or Cu^{2+} ion

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respectively. The appearance of two new absorption bands centered at about 610 and 660 nm (602 and 660 nm for Ni^{2+}; 608 and 658 nm for Cu^{2+}) in the LB films deposited from Ni^{2+}, or Cu^{2+} ion containing subphases respectively, indicate that interfacial coordination occurs immediately after spreading the H_2BR molecules. These new bands are assigned to an electron transfer transition from the pyrrole rings of bilirubin to the hole in the 3d shell of metallic ion[17] or the oxidation of bilirubin to biliverdin catalyzed by Ni^{2+} or Cu^{2+} ion[20].

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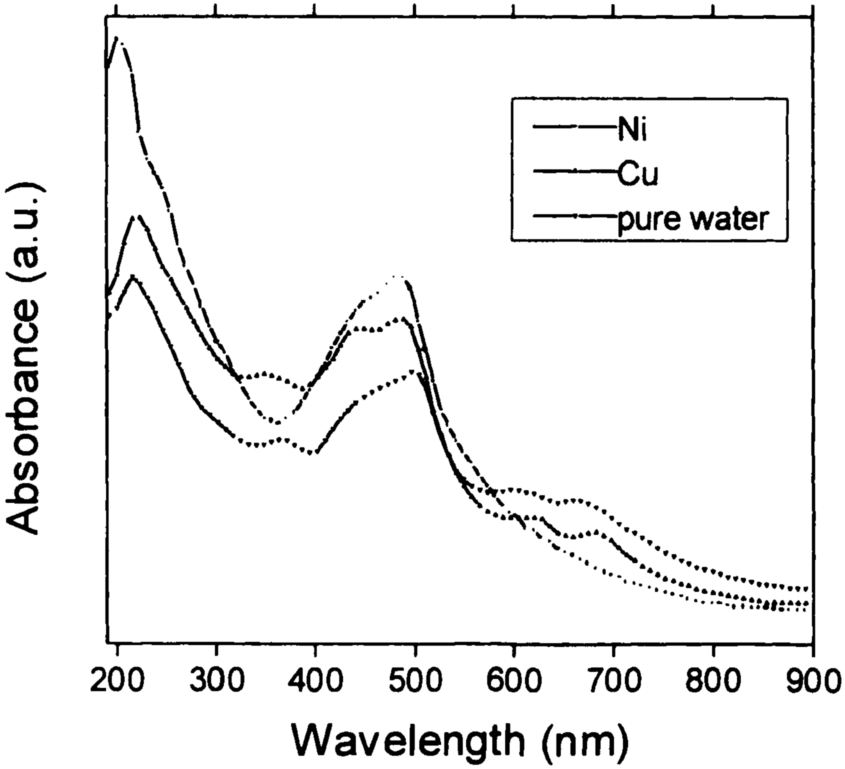

FIGURE 7 The absorption spectra of an 11-layer LB film on a quartz substrate on pure water subphase and on subphases containing Ni^{2+} and Cu^{2+} ions

In contrast to the well-documented instability of H_2BR in solution, H_2BR confers an unusually high stability in the monolayers. The UV-visible spectra of H_2BR and its amphiphilic complexes MBR in organized molecular films can be stabilized for two week without apparent change, showing the apparent difference between ordered molecular films and the bulk solution.

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The coordination of H_2BR with metal ions was still confirmed by X-ray photoelectron spectroscopy (XPS) of the LB film of H_2BR deposited in subphases containing these metal ions. In the XPS survey scans of 2-layer LB films of H_2BR deposited from subphases with Ca^{2+}, Cu^{2+}, Zn^{2+} ions, in addition to the constituents of H_2BR, the O1s, N1s and C1s core level peaks common to all the spectra, the spectra of the H_2BR LB films deposited from metal ions containing subphases are marked by the presence of the corresponding metal core level peaks, Ca2p, Cu2p, or Zn2p[21], indicating the presence of metal ion in the LB films. That is, a complexation of H_2BR with metal ions was formed at the air-water interface and the H_2BR-M complex (MBR) monolayers were transferred onto quartz plates.

Compared with the binding energy of N1s (399.6 eV) and O1s (532.3 eV) in H_2BR LB films, N1s increased to 400.2~400.6 eV in the H_2BR-M LB films, while O1s nearly remains unchanged (532.2~532.4 eV). It is clear that the lone pair electrons from the nitrogen atoms are shifted into the metal atoms and thereby a complexation was formed. H_2BR is coordinated to the metal ions in subphase through the pyrrole N atoms and the lactam N atoms, but the oxygen atoms in H_2BR do not take part in coordination. The charge-transfer from the N atoms of H_2BR to metal ions leads to a decrease of charge density and an increase of binding energy of N atoms. That is, H_2BR coordinates metal ion with four N atoms, then a ring structure complex (MBR) analogue to metalloporphyrin formed. MBR orient with COOH groups in contact with the aqueous phase and the pyrrole rings plane orients in the air to form condensed monolayers. The pyrrole rings plane tilts at an angle with respect to the normal to the interface.

This 1:1 coordination ratio of H_2BR with divalent metal ions M^{2+} is different from that of bilirubin in organic and aqueous basic solution. Bilirubin can form 1:1, 1:2, sometimes and 2:1 complexes with metal ions such as Ca^{2+}, Zn^{2+}, Cd^{2+}, and Cu^{2+} ion[22-24]. This difference was recognized as the difference between the heterogeneous air-water interfacial environment and bulk solution.

3.4. Thickness Measurements H_2BR LB Films

In order to explain the suggested model of H_2BR monolayer assemblies, low-angle X-ray diffraction was used to obtain some useful structural information on the film organization. In the low-angle X-ray diffraction spectrum of 31 layers Langmuir-Blodgett (LB) films of H_2BR deposited from subphase containing $CuCl_2$, a broad Bragg diffraction peak at 3.54° (2θ) was observed. It shows that the LB films have a periodic structure. The layer spacing for CuBR LB films calculated by using Bragg diffraction formula $2d\sin\theta = n\lambda$ to be: 2.50 nm, which is larger than the double height (2 × 1.20 nm) of H_2BR in Isomer A and less than that (2 × 1.55 nm) in Isomer B. This result shows that the CuBR LB films were of Y-type and CuBR molecules are tilted in Isomer B at an angle with respect to the normal to the interface.

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