Studies on the Area-Temperature Isobars of Insoluble Monolayers at the Air/Water Interface. I. Development of a Microcomputer-Controlled Instrument for Measuring Precise Area-Temperature Isobars[1)]

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A microcomputer-controlled instrument for measuring precise area-temperature (*A–T*) isobars of insoluble monolayers at the air/water interface was developed. To obtain precise *A–T* isobars, leakage or dissolution of film material must be checked. Before starting *A–T* isobar measurements, area or structural relaxations must be completed. *A–T* isobars of monolayers of cadmium eicosanoate, L-α-dipalmitoyl phosphatidylcholine (DPPC), trioctadecanoyl glyceride (TOG), and *cis*-9-octadecen-1-ol were measured. Precise *A–T* isobars can afford two-dimensional isobaric thermal expansivities of insoluble monolayers by differentiation with respect to temperature. The thermal expansivities were evaluated as around 9.0×10^{-4} K^{-1} for condensed monolayers and around 30×10^{-4} K^{-1} for liquid-expanded monolayers.

According to Crisp or Gaines,[2)] the number of degrees of freedom of a system of an insoluble monolayer on the water surface is given by the following equation,

$$F=C^{B}+C^{S}-P^{B}-q+3, \tag{1}$$

where F=number of degrees of freedom, C^{B}=number of components in bulk and equilibrated throughout the system, C^{S}=number of components confined to the surface, P^{B}=number of bulk phases, and q=number of surface phases in equilibrium with one another. In the derivation of the phase rule, it was assumed that film material should be confined completely to the surface phase and neither dissolution nor vaporization of film material should occur.

In the case of a single component monolayer, as $C^{S}=1$, $C^{B}=2$ (water and gas) and $P^{B}=2$ (a liquid and a gas phase), the number of degrees of freedom of the system is given as follows:

$$F=2+1-2-q+3=4-q. \tag{2}$$

There are three degrees of freedom when the monolayer consists of one surface phase. They are external pressure (three-dimensional), temperature and surface pressure (two-dimensional). Since experiments on insoluble monolayers are usually performed under atmospheric pressure, external pressure is dealt with not as a variable but rather as a constant (fixed to the atmospheric pressure). There is another experimental variable used usually, i.e., molecular area which can be changed by moving barriers confining the insoluble monolayer. There are three ways to take two variables from three, and there are three types of experiments on insoluble monolayers. The first is the surface pressure-area (π–A) isotherm where surface pressure is measured as a function of the molecular area, keeping temperature constant. The second is the area-temperature (A–T) isobar where the molecular area is measured as a function of temperature, keeping surface pressure constant. The third is the surface pressure-temperature (π–T) isochore or isoarea where surface pressure is measured as a function of temperature, keeping molecular area constant. π–A isotherms are the most popular and the most widely used thermodynamic measurements of insoluble monolayers. But it is very difficult to measure precise A–T isobars or π–T isochores because dissolution of film material into the subphase water or leakage of the film material through corners of barriers, even in a very small amount, will introduce fatal errors to the results. There are only a few papers reporting A–T isobars,[3,4)] and no reports on π–T isochores.

This paper reports development of a micrompuster-controlled instrument for measuring A–T isobars of insoluble monolayers at the air/water interface and discusses procedures to obtain precise A–T isobars using this instrument. A–T isobars of some amphiphilic materials were measured with this instrument and two-dimensional isobaric thermal expansivities calculated from the isobars are also reported.

Experimental

Materials. L-α-Dipalmitoylphosphatidylcholine (DPPC, synthetic, 99+%), trioctadecanoyl glyceride (TOG, 99%) eicosanoic acid (99%), and *cis*-9-octadecen-1-ol (99%) were purchased from Sigma Chemicals and were used as film materials without further purification. Spectro-grade benzene, hexane, chloroform, ethanol, and methanol were used as spreading solvents. They were purchased from Wako Chemicals and were used without further rectification. Water for subphase was distilled using an all Pyrex distillation apparatus. Guaranteed grade sulfuric acid (Wako Chemicals) was used to adjust pH of the subphase water. Guaranteed grade cadmium acetate (Wako Chemicals) was used to prepare 10^{-3} M (1M=1 mol dm^{-3}) subphase for monolayers of eicosanoic acid.

Instrumentation. Figure 1 shows a block diagram of the instrument developed. Signals from two Wilhelmy-type surface balances and sheath platinum wire resistance thermometers were A/D converted and inputed into a 16-bit microcomputer (PC-286V, Epson). The microcomputer

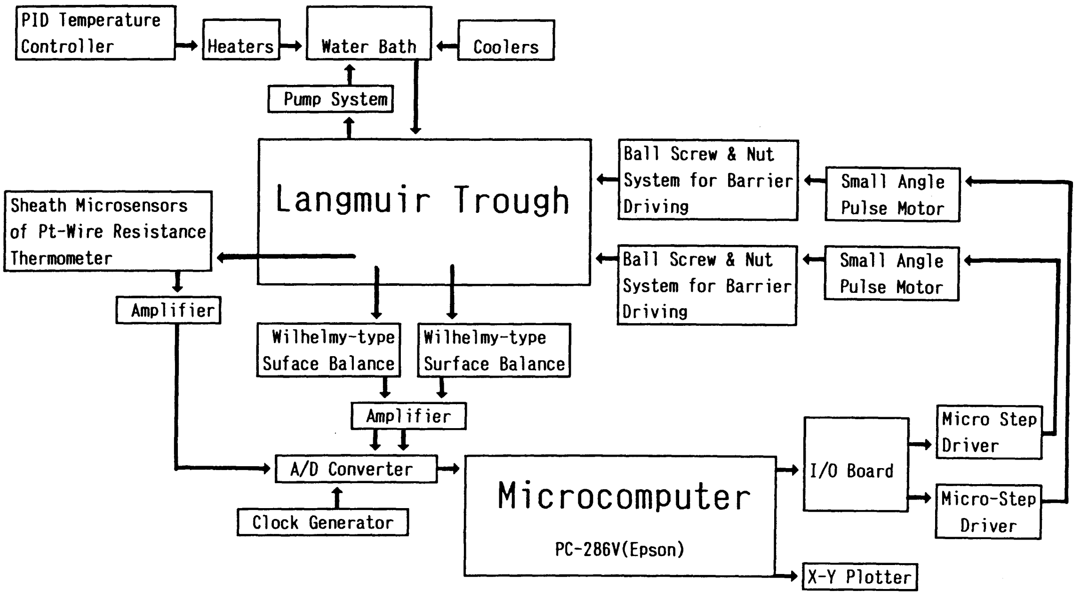

Fig. 1. Block diagram of the instrument.

feedback-controlled the monolayer surface pressure and maintained it strictly constant, based on the signals from the two surface balances by the method stated below. Temperature of subphase water was changed by circulating temperature controlled water from a water bath, which was equipped with control-heaters and coolers, through in a double-bottomed Langmuir trough. The monolayer temperature was measured with thin sheath microsensors of platinum wire resistance thermometers (sheath diameter=0.5 mm, element wire diameter=50 μm, 100 ohm), whose vertical position could be finely adjusted using a rack-and-pinion device.

The surface pressure of a monolayer must be strictly constant during an *A*–*T* isobar measurement. But it is difficult to keep it constant, based on signals from a single Wilhelmy-type surface balance because the zero-point of the balance itself changes with changing temperature of the subphase water. There are three reasons for drift of the zero point of the surface balance with changing temperature, i.e., changes of surface tension and density of subphase water, change of position of water surface by evaporation, and change of the dimensions of the Langmuir trough itself by thermal expansion. To overcome these difficulties, this instrument was equipped with two Wilhelmy-type surface balances; one was located in the monolayer-covered water area and another was in the clean water area. The microcomputer feedback-controlled positions of two barriers confining the monolayer through an I/O and a control boards (SMBMP, San-ei Electric Co., Ltd.), micro-step drivers (FD-5500WS, San-ei Electric Co., Ltd.), small-angle pulse motors (Vexta, Oriental Motors Co., Ltd.) and ball-screw and nut systems, to maintain the difference of outputs of the two surface balances constant. By this means, all causes of the zero-point drift of the surface balances with changing temperature could be cancelled completely and the pressure could be kept strictly constant during *A*–*T* isobar measurements. Four control parameters (the trigger interval, the real-time smoothing number, the surface compressibility of a monolayer and the feedback gain) were also used to control the surface pressure precisely.[5] Pressure fluctuation during the *A*–*T* isobar measurements was controlled within ±0.15 $\mathrm{mN\,m^{-1}}$ in general. The spreading technique of monolayers restricted reproducibility in the molecular area of the *A*–*T* isobars and it was ±1.0% but the fluctuation in area during measurements was ±0.001 $\mathrm{nm^2\,molecule^{-1}}$ at most. These fluctuations were much less than those reported in previous *A*–*T* isobar measurements.[3]

A very shallow-type Langmuir trough was constructed using long rectangular rods of Teflon on a double-bottomed brass base which was coated with a thin pressure-sensitive Teflon adhesive sheet (inside dimensions of the trough, 80×16×0.4 cm). Only 600 ml of subphase water was required for the reason that the heat capacity of the system should be as small as possible to effect temperature change of the system rapidly.

Leakage and/or dissolution of the monolayers introduces serious errors into the *A*–*T* isobar. It is difficult to prevent leakage of monolayers at higher surface pressures and at higher temperatures by conventional rectangular barriers. The barriers used for this instrument were made from Teflon and have recesses of 1.5 mm depth in both ends to fit the inside width of the rims of the Langmuir trough (Fig. 2 (a)). Moreover, the barriers were equipped with flexible Teflon sheet-made skirts at the four corners of the recesses which slided smoothly without leakage of film molecules even when the temperature of the system changed widely (Fig. 2 (b) and (c)).

Dissolution of film material must be also avoided. Since film dissolution is a rate process and is accelerated by increasing temperature, the time that the film material is at high temperature must be short. Thus, the rates of temperature rising and lowering should be as high as possible to minimize the effect of dissolution. In this instrument, the maximum rate of temperature change was about 2 °C $\mathrm{min^{-1}}$ on average. Dissolution of film material can be checked by comparing an *A*–*T* isobar obtained at temperature rising with one at temperature lowering. If the two isobars coincide well with one other, we can confirm that there was no leakage or dissolution of the film material during measurement.

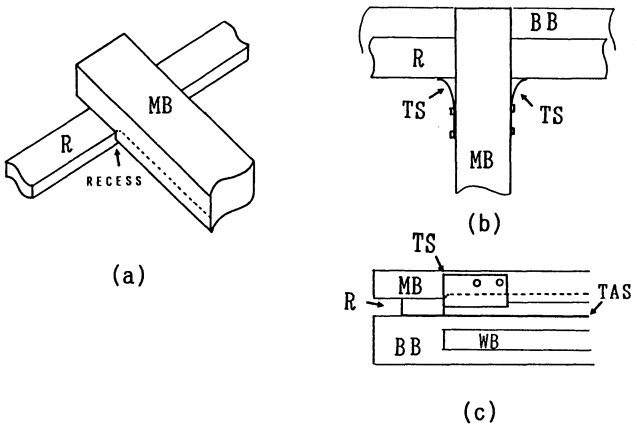

Fig. 2. Structure of the barriers used. (a) Fitness of the recesses of the barriers with rims of the Langmuir trough. (b) Top view of the barrier with flexible Teflon sheet-made skirts which slide smoothly against rims of the Langmuir trough. (c) Side view of the barrier and the trough. MB: moving barrier, R: rim of the Langmuir trough, TS: Teflon-made skirt, TAS: Teflon adhesive sheet, BB: brass base, WB: water bath, ----: water level.

Precise A–T isobars can afford two-dimensional isobaric thermal expansivities by differentiation with respect to temperature. If there is no phase transition of the film material in the temperature range measured, the isobar will appear as a straight line as a whole, and hence it is easy to calculate the thermal expansivity by numerical differentiation with respect to temperature using linear regression analysis. If there are some phase transitions, the A–T isobar has some kinks. Correlation factors of the linear regression analysis are also calculated. All results are finally sent to an X-Y plotter for drawing normalized figures.

Results and Discussion

Dissolution. Figure 3 shows A–T isobars of DPPC at 40 mN m^{-1} exhibiting the effect of partial dissolution at higher temperature. The monolayer of DPPC was spread from a mixed solvent solution of hexane and ethanol (9:1 in volume) on pH 2 water. A Cloehn microsyringe of a gas-tight type which was connected to a synchronous motor-driven micrometer was used to administer the desired quantity of the spreading solution. The temperature was raised up to 45 °C at a rate of 2 °C min^{-1} and was kept constant there for 40 min, then lowered to 10 °C at the same rate. A phase transition begins at 42 °C. Dissolution of DPPC molecules occurred at the higher temperature and the A–T isobar measured under temperature lowering shifted downward about 4% in molecular area. The two isobars, however, were almost parallel below 42 °C, and this means that dissolution may be neglected in this temperature span.

Many of the A–T isobars of film materials forming stable insoluble monolayers could be measured up to about 45 °C without taking into account the dissolution under the conditions of this high rate of temperature change.

Area or Structural Relaxation. Figure 4 shows A–T isobars of an eicosanoic acid monolayer at 10 mN m^{-1} on water containing 10^{-3} M cadmium acetate. The monolayer was spread from a benzene solution. There are two isobars shown in the figure. Curves (a) and (b) are isobars measured with the temperature rising and lowering, respectively. The discrepancy between isobars (a) and (b) shows an area-relaxation effect of the monolayer.

An insoluble monolayer after just being compressed to a certain high pressure at low temperatures is in some unstable state and exhibits a tendency to relax to a more stable closed-packed state with time. The relaxation time depends on many factors, such as temperature, surface pressure, pH or ion types in the subphase etc. In general, the relaxation time is very long at low temperatures and at high pressures. Moreover, the relaxation takes longer time in a solid state than in a liquid state. Cadmium eicosanoate

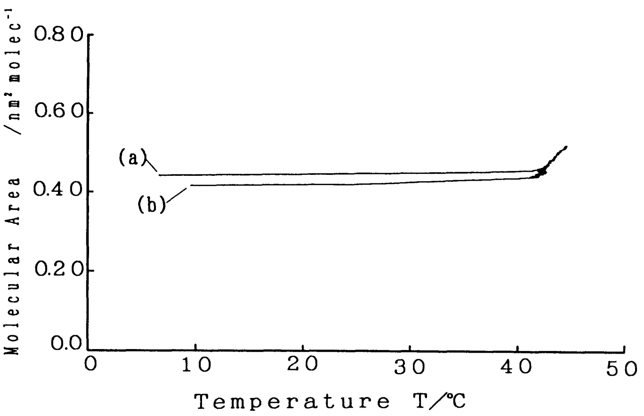

Fig. 3. A–T isobars of DPPC at 40 mN m^{-1} showing partial dissolution of the film material. (a) A–T isobar of DPPC measured at temperature rising. (b) A–T isobar of DPPC measured at temperature lowering, after keeping at 45 °C for 40 min.

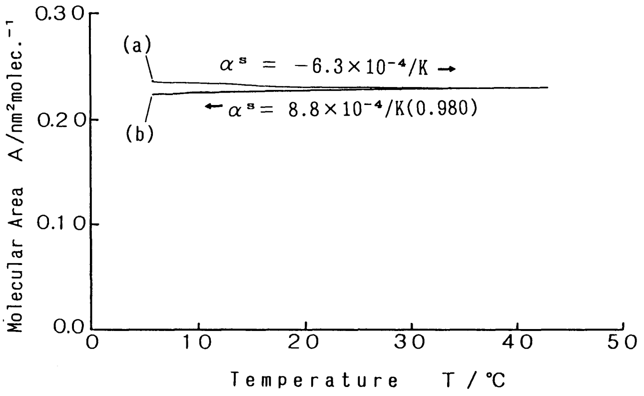

Fig. 4. A–T isobars of an eicosanoic acid monolayer at 10 mN m^{-1} on 10^{-3} M cadmium acetate showing the "thermally stimulated area relaxation" effect. (a) A–T isobar measured with the first rising of temperature without "isobaric thermal treatment." (b) A–T isobar measured with the temperature lowering. (See text.)

(see Fig. 4) is in a solid state in monolayers at 10 mN m^{-1}, and it takes very long time to relax especially at the starting low temperature of the *A–T* isobar measurement. The relaxation time becomes shorter as the temperature increases, and curve (a) gradually approaches curve (b) which exhibits no relaxation effect. It can be considered that curve (b) shows the true isobar of a cadmium eicosanoate monolayer under these conditions. The thermal expansivity calculated from isobar (a) was negative as shown in Fig 4. Curve (a) shows a "temperature accelerated" or "thermally stimulated" area relaxation process of the insoluble monolayer.

It is very interesting to point out that the discrepancy of the isobars (a) and (b) at 6 °C, about 5% in molecular area, corresponds well to the "hole" area detected by electron microscopic observation of monolayers of long-chain acids and their barium salts.[6] From the results shown in Fig. 4, it can be inferred that such "holes" in monolayers will disappear by "isobaric thermal treatment" up to a certain high temperature.

To eliminate the relaxation effect from *A–T* isobar measurements, we adopted the following experimental procedures: A monolayer is spread and is compressed to some constant pressure at room temperature. Then, the temperature of the system is raised to a certain high value where the relaxation time is very short, followed by lowering of the temperature to the starting point of the *A–T* isobar measurement, keeping pressure constant through out the whole time. The maximum temperature of the "isobaric thermal treatment" depends on the film material, the constant pressure and other experimental conditions. *A–T* isobars shown hereafter were measured with the procedures stated above, and it was checked that there was no significant leakage or dissolution of film material during measurement.

***A–T* Isobars of Some Typical Insoluble Monolayers.** Figure 5 shows *A–T* isobars of a cadmium eicosanoate monolayer under the same conditions as those shown in Fig. 4. The relaxation effect disappeared and the two isobars, (a) and (b), coincided well with each other, except the part above 20 °C where isobar (b) showed a small undulation. The minimum surface compressibility of the monolayer at 9.8 °C was 1.0×10^{-3} m mN^{-1} which was a very small value corresponding to that of a solid state. The two-dimensional isobaric thermal expansivities and the correlation factors for the two isobars were calculated as 9.0×10^{-4} K^{-1} (0.998) and 9.9×10^{-4} K^{-1} (0.974) respectively.

Figure 6 shows an *A–T* isobar of a trioctadecanoyl glyceride monolayer which was spread from a benzene solution on pH 3 subphase (20 mN m^{-1}). The material has three hydrophobic long chains and forms very stable insoluble monolayers. The minimum surface compressibility at 6.4 °C was as small as 1.5×10^{-3} m mN^{-1}, corresponding to a value of a solid state. The monolayer exhibits no phase transition at 20 mN m^{-1} in the temperature span measured and the curve looks like a straight line. The two-dimensional thermal expansivity and the correlation factor calculated from the isobar were 9.0×10^{-4} K^{-1}

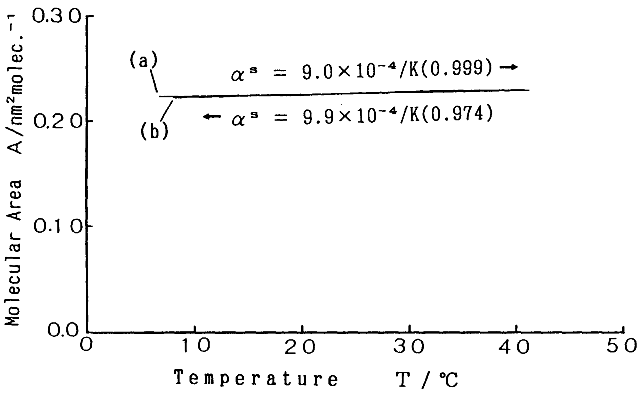

Fig. 5. *A–T* isobars of an eicosanoic acid monolayer which was measured following the procedures described in the text to eliminate the relaxation effect. Other experimental conditions are the same as those shown in Fig. 4. (a) An *A–T* isobar measured at temperature rising. (b) An *A–T* isobar measured at temperature lowering.

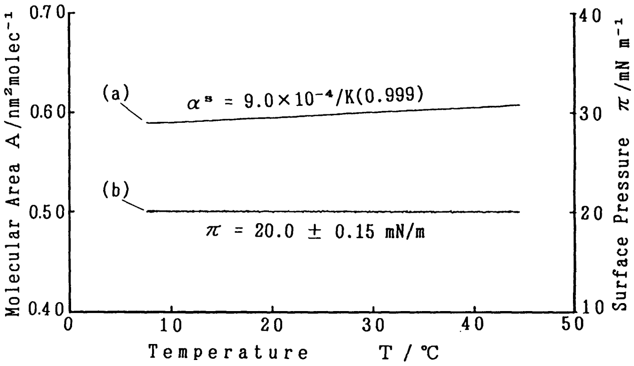

Fig. 6. (a) An *A–T* isobar of a TOG monolayer on pH 3 subphase at 20 mN m^{-1} measured at temperature rising, and (b) surface pressure fluctuation.

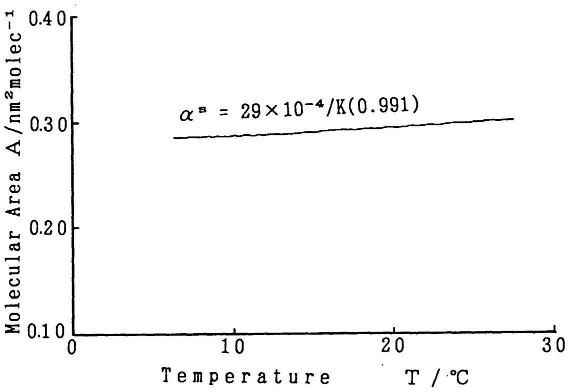

Fig. 7. An *A–T* isobar of an *cis*-9-octadecen-1-ol monolayer on distilled water at 15 mN m^{-1}.

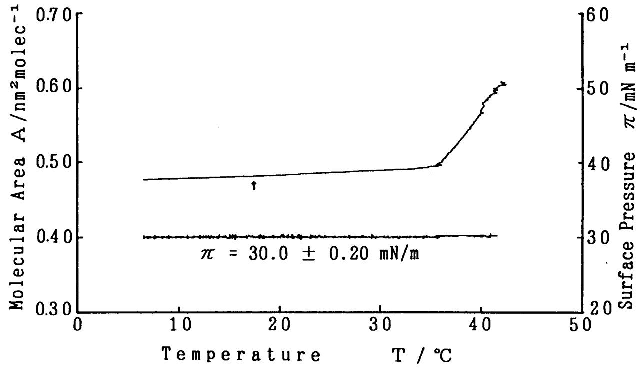

Fig. 8. An A–T isobar of a DPPC monolayer on pH 3 subphase at 30 mN m^{-1}. The isobar shows two phase transitions. The second-order one is detected at 18 °C and the "first-order" one at above 36 °C. The pressure was controlled strictly at 30.0 ± 0.20 mN m^{-1} in spite of the existence of phase transitions.

and 0.999, respectively. The surface pressure fluctuation is also shown in the figure. In spite of the wide range of temperature change, it can be seen that pressure was controlled at 20.0 ± 0.15 mN m^{-1} during the A–T isobar measurements.

Figure 7 shows an A–T isobar of a *cis*-9-octadecen-1-ol monolayer at 15 mN m^{-1} spread from a benzene solution on distilled water. This material has a cis-unsaturation in the middle of the hydrophobic chain and forms a typical liquid-expanded monolayer. As the solubility of this material in water is much larger than that of the corresponding saturated material, measurement was stopped at about 27 °C to reduce dissolution. A small relaxation effect was observed in the low temperature region of the isobar. The two-dimensional thermal expansivity of the liquid-expanded monolayer was calculated from the isobar data except at the low temperature region as 29×10^{-4} K^{-1} (0.991) which is much larger than those obtained for monolayers in a condensed state.

Figure 8 shows an A–T isobar of a DPPC monolayer at 30 mN m^{-1} on pH 3 subphase as well as the pressure fluctuation during the measurement. This material also forms stable insoluble monolayers but exhibits two phase transitions, one appears as a slight inflection at 18 °C (pointed by an upward arrow) and the other appears at 35 °C as a large stepwise change in molecular area as seen from the figure. In spite of the fact that the monolayer exhibits a phase transition with accompanying large area change, it can be seen that the surface pressure was kept constant at 30.0 ± 0.20 mN m^{-1}. It can be considered that the phase transition detected at 18 °C corresponds to the second-order from phase IV (crystalline, non tilted) to phase III (crystalline, tilted) of DPPC.[3] Two-dimensional thermal expansivities of the condensed state below and above this transition were 9.0×10^{-4} K^{-1} (0.980) and 1.3×10^{-3} K^{-1} (0.995), respectively. The transition at the higher temperature corresponds to the first-order one from phase III to phase II (anisotropic fluid). Thermodynamics predicts that a first-order phase transition should appear as a vertical line in an A–T isobar. In practice, however, the A–T isobar in the transition region is not vertical but slanted. This may be because the molecular processes involved in the transition require some time to complete or the transition has a second-order character by approaching a critical point of the coexisting region of phase II and III as pointed out by Albrecht.[3]

Conclusion

A computer-controlled instrument for measuring A–T isobars of insoluble monolayers at the air/water interface was developed. By adopting a double surface balance system, the pressure could be controlled within ±0.15 mN m^{-1} using four control parameters. To obtain precise A–T isobars, it must be checked that there is no significant dissolution of film material into the subphase or no leakage of the material as shown by the agreement of two isobars measured at temperature rising and lowering. Before starting measurements, structural or area relaxations after compression must also be completed at a certain high temperature. In relation to the relaxation processes, the "isobaric thermal treatment" technique was proposed to densify insoluble monolayers, and this treatment will be a powerful tool to obtain dense and "defect-free" LB films.

Precise A–T isobars can afford two-dimensional isobaric thermal expansivities of insoluble monolayers that have never been reported. The thermal expansivities are about 30×10^{-4} K^{-1} for monolayers in the liquid-expanded state and about 10×10^{-4} K^{-1} for monolayers in the condensed states. A–T isobars will play important roles, together with the π–A isotherms, in considering physical properties or polymorphism of insoluble monolayers at the air/water interface.

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