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SHORT COMMUNICATION

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Isotherms of phospholipid monolayers measured by a pendant drop technique

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Abstract The Axisymmetric Drop Shape Analysis (ADSA) has been used to study the surface pressure/area isotherms of insoluble surfactant monolayers. The continuous measurement of surface tension as a function of surface area by increasing and decreasing the drop volume allows to investigate the phase transitions in monolayers. The isotherms of two phospholipids, dipalmitoyl phosphatidyl choline (DPPC) and dimyristoyl phosphatidyl ethanolamine (DMPE), show good agreement with those measured by using a conventional Langmuir–Blodgett film balance, except in the coexistence region. The observed disagreements are discussed in terms of differences in compression rate, curvature of the surface and effect of impurities. Evidence of possible geometric effects on monolayer domain formation and growth is given on the basis of BAM images.

Due to the small total surface area, the ADSA technique provides advantages as regards homogeneity of temperature, surface pressure, surface concentration and the symmetry of area changes.

Key words ADSA – phospholipids – LB film balance – monolayer isotherms – BAM

Introduction

The characterization of monolayers is important for many objectives. One example is to provide basic information before transferring assembled monolayers onto solid supports for various optical and electronic devices [1]. Much work has been done on the fundamental understanding of the structure and function of a monolayer during its assembling on a molecular level and general physical features are obtained from the study of their thermodynamics, i.e., the surface pressure/area isotherm.

Since the surface tension of a surfactant solution can be determined from the shape of a liquid meniscus, and in particular from the shape of a drop, the shape analysis of a pendant drop covered with an insoluble film supplies the surface tension, and hence the surface pressure. Any change in the drop volume alters the drop surface area, the surface monolayer coverage is changed correspondingly. A controlled drop volume manipulation, coupled with the Axisymmetric Drop Shape Analysis (ADSA) is suited to determine the surface pressure isotherm of an insoluble monolayer at the surface of a pendant drop. Output of ADSA also includes the surface area and volume of the drop which makes it well-suited as a film balance for monolayer studies.

Kwok et al. [2] first used ADSA as a film balance and performed isotherm measurements of the insoluble octadecanol monolayer. Good agreement of the results with the data from common Langmuir–Blodgett (LB) film balance experiments demonstrated that ADSA is a novel tool for film balance measurements.

Phospholipids are one of the most important biosurfactants and their monolayers have widely been studied as potential model systems of biological membranes [3]. The classic experimental method of monolayer studies is the LB film balance with which the behavior of monolayers may be investigated over a wide range of area and pressure.

In this work we restudy two phospholipid substances by using the ADSA film balance and compare the experimental results with those from LB film balance measurements. The observed deviations between the two methods, especially in the coexisting region, are discussed in terms of experimental conditions, i.e., the compression rate, the curvature of the drop surface and the difference in impurity effects.

Experimental

Materials

Dipalmitoyl phosphatidyl choline (DPPC, 99 +%), and dimyristoyl phosphatidyl ethanolamine (DMPE, 99 + %), obtained from SIGMA Chemical Co., were used without further purification. GC-Chloroform (99 +%) from Aldrich Chemical Co., was used as spreading solvent. All experiments were performed with Millipore water at 20 °C.

Set-up

To measure the surface tension of a monolayer covered onto a drop surface a pendant drop apparatus was used which is shown schematically in Fig. 1. It is quite similar to that of Kwok et al. [2]. A CCD camera with an AF Nikkon 50 mm F/1.8 objective is fixed on an optical bench. The output analogue video signal is continuously transferred to a four-magnetic-head high quality video recorder which can store 25 pictures per second. A PC equipped with a "screen machine" frame grabber receives the pictures from the video tape for each pendant drop at a definite time. Each acquired image has a resolution of 640 × 480 pixels with 256 gray levels for each pixel. The screen machine software can also take the pendant drop picture directly from the CCD camera at a rate of one picture per 5 s. A syringe with a capillary tip produces a pendant drop and allows to change the drop size. A quartz cuvette isolates the drop from the environment to provide good temperature control and to reduce evaporation and air current effects. The whole set-up is mounted on a vibration-free optical platform to minimize vibrations.

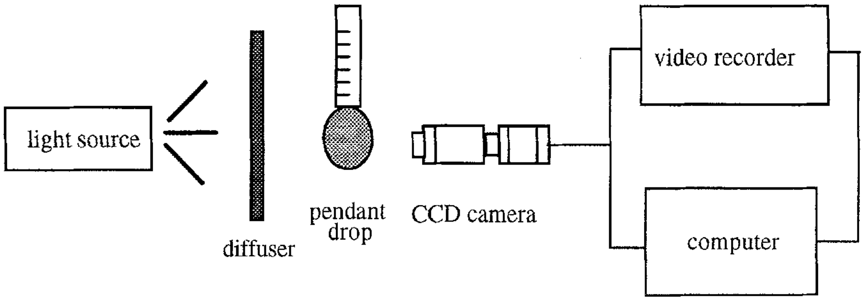
The ADSA software has been developed to determine surface or interfacial tensions from the analysis of the shape of an axisymmetric drop by fitting the Gauss–Laplace equation of capillarity to experimental drop shape coordinates [4–6]. An objective function which describes the deviation of the experimental profile from the theoretical profile is minimized by a non-linear regression procedure yielding the surface tension, drop volume and area [7–9].

Measurement procedures

To perform experiments with insoluble monolayers a micro-syringe is used to deposit a known amount of the DPPC or DMPE solution of known concentration on the surface of a small pendant water drop. Immediately after contact the drops coalesce and the surfactant molecules spread along the drop surface. The actual experiment is started about 5 min later to ensure complete evaporation of the spreading solvent chloroform. It was shown experimentally by Kwok et al. [2] that this time is sufficient. At the beginning of each measurement, the drop is made as large as possible in order to expand the phospholipid monolayer. Then the drop volume is decreased slowly and continuously in order to compress the monolayer, and the video recorder records the whole process. Once the drop becomes very small, and is close to detachment, the syringe is run in the opposite direction to perform the expansion part of the measurement cycle.

LB balance measurements (Riegler and Kirstein trough) were performed under similar experimental conditions

Fig. 1 Schematic picture of the pendant drop set-up



using the same monolayer sample. The range of compression rates on the trough was 0.063 to 0.53 Å^2/s-molecule. This compression rate is much slower compared to the pendant drop method which was operated in a range of 1.3 to 6.1 Å^2/s-molecule.

BAM experiments have been performed with an instrument described elsewhere [10].

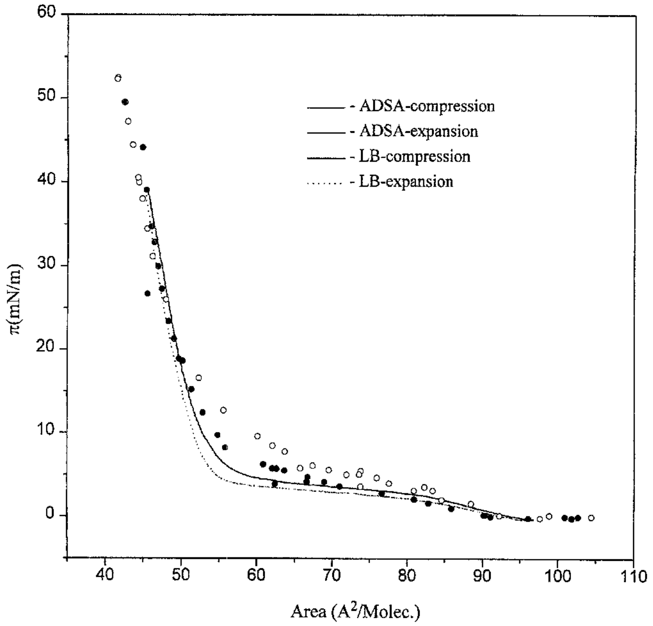
Results and discussion

When running the experiments under a successive change of the drop volume, we obtain the surface tensions of an insoluble monolayer of DPPC on a pendant water drop and consequently the complete isotherm. The results show that the surface area of the pendant drop has a variation from about 0.23 cm^2 to 0.58 cm^2 during compression, which corresponds to a surface tension change from 72.4 mN/m to 21.0 mN/m.

The surface tension as a function of surface area transformed into the corresponding pressure/area per molecule isotherm $\pi(A)$ of DPPC is shown in Fig. 2. The surface pressure varies from 0 mN/m to about 55 mN/m as the film is densely compressed.

The DPPC isotherm measured with a classical LB trough is also shown in Fig. 2. It is well-known from literature [11] that DPPC undergoes several phase transitions on compressing the film. At 20 °C an obvious increase of surface pressure occurs at $A < 90$ Å^2/molecule. ADSA reveals that this pressure increase appears at about 91 Å^2/molecule. The succeeding coexistence region of liquid expanded (LE) and liquid condensed (LC) states starts at 79 Å^2/molecule and undergoes an area variation of about 22 Å^2/molecule. The results of the pendant drop measurements exhibit that the coexistence region covers a molecular area interval from 82 Å^2 to 58 Å^2 with a surface pressure of about 6 mN/m. Comparing the whole process of phase transitions, ADSA shows consistent results with the conventional film balance measurements, only the absolute values in the coexistence region show significant differences.

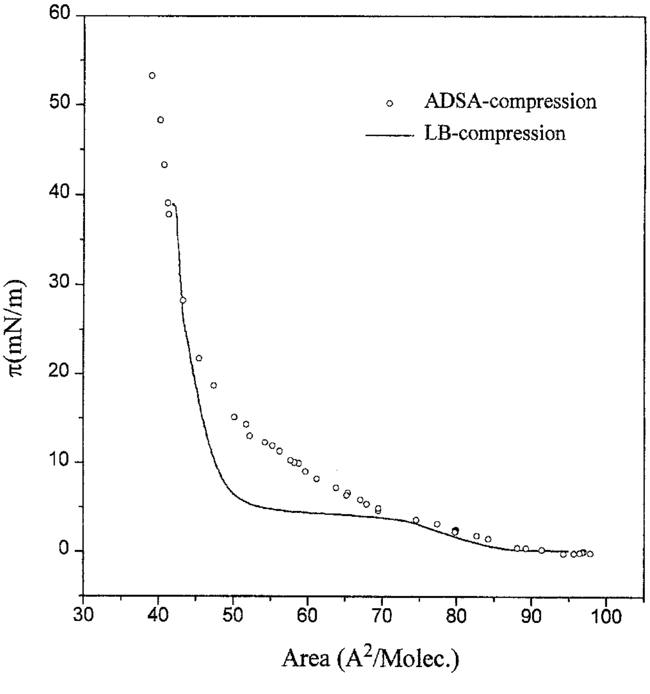
Fig. 2 Pressure/area isotherm for compression-expansion experiments of a DPPC film using ADSA and a conventional film balance (continuous lines)



In addition to the DPPC monolayer studies, a DMPE monolayer has been investigated by both methods. DMPE has a smaller head group than DPPC. Owing to this difference the monolayers show a different phase behavior [12]. For example, the DMPE monolayer exhibits a vertical transition of the isotherm at a surface pressure of about 30 mN/m. The results in Fig. 3 demonstrate that the ADSA and the LB trough measurements exhibit the same shape of $\pi(A)$ isotherm. Both isotherms display the pronounced break in the slope corresponding to the expected phase transitions.

Above a pressure of 32 mN/m, the slope of the isotherm from LB balance measurements become steeper as the phase behavior reaches the solid-like state. Although

Fig. 3 Compression isotherm from pendant drop measurements of DMPE monolayer and a conventional film balance (continuous lines)



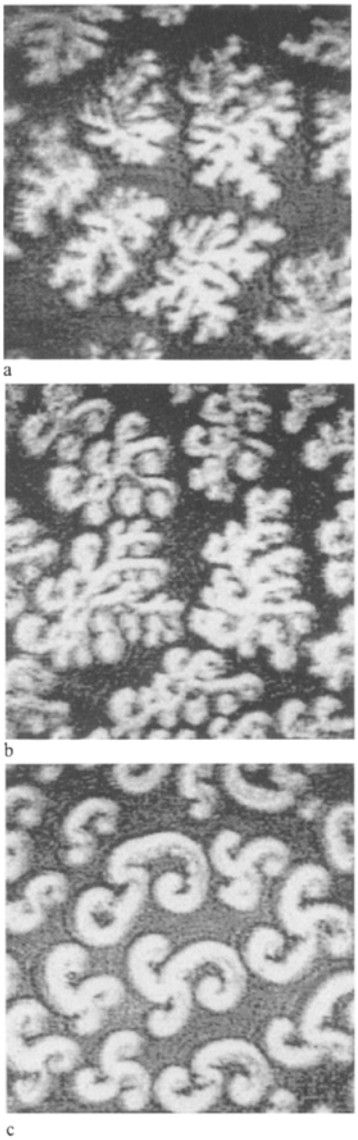


Fig. 4 BAM images of a DPPC monolayer in the coexisting region of LC and LE states at different compression rates; a) $A = 60$ Å^2/molecule, compression rate = 0.083 Å^2/s molecule, b) $A = 60$ Å^2/molecule, compression rate = 0.012 Å^2/s molecule, c) $A = 60$ Å^2/molecule, compression rate = 0.0033 Å^2/s molecule

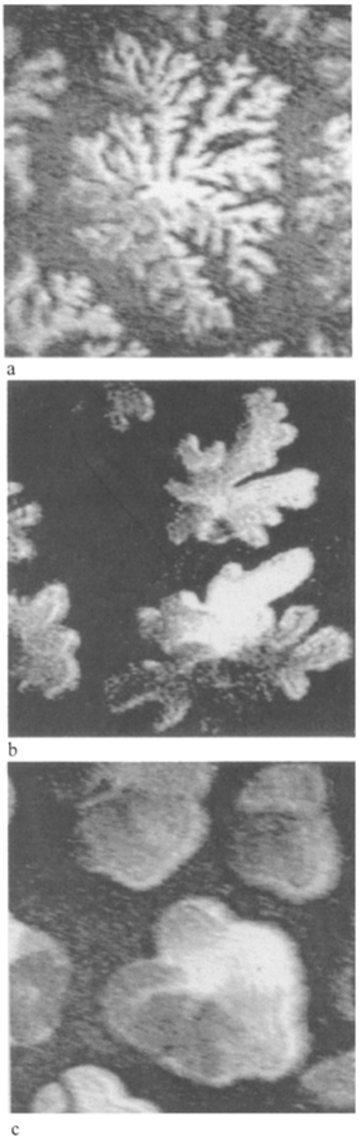


Fig. 5 BAM images of a DMPE monolayer in the coexisting region of LC and LE states at different compression rates; a) $A = 60$ Å^2/molecule, compression rate = 0.083 Å^2/s molecule, b) $A = 60$ Å^2/molecule, compression rate = 0.012 Å^2/s molecule, c) $A = 60$ Å^2/molecule, compression rate = 0.0033 Å^2/s molecule

this kink is not so clear from ADSA measurements, the surface pressure/area slope also becomes steeper.

With ADSA, the coexistence region for both substances, especially tending to the high density order phase (LC phase), is less pronounced and shows clearly higher surface pressures. The reason for this surface pressure shift could be explained in different ways.

First, there could be an effect of the curved drop surface on the monolayer structure. The change of the domain shape and number along the curved surface during compression or expansion may be different compared to a flat surface in LB balance experiments. It has been confirmed by fluorescence microscopy that the number of domains is a non-equilibrium property [13] and depends on the nucleation process. Compressing the curved surface the exchange between the domains and/or the change of the domain number of LE and LC may need a longer period of time and hence causes the pressure upshift. On the other hand, the domains also grow far from equilibrium on a curved surface [12]. This could be understood as a diffusion limited aggregation process on a bent surface and may lead to fractal structures where domain growth is limited [14].

This idea is supported by rate dependence experiments of compression and expansion performed by Kwok [15]. With increasing compression rates a shift of the part of the isotherm in the coexisting region upwards and a change of the isotherm shape can be observed. Studies of the morphology of DPPC and DMPE monolayers with Brewster Angle Microscopy (BAM) show significant changes in the domain structure when the compression rate is changed [10]. As an example, this effect is demonstrated in Figs. 5 and 6 for DMPE and DPPC monolayers obtained at different compression rates. It is easy visible that the domain structure changes significantly with the compression rate. The even higher compression rates in ADSA experiments and in addition the curved surface of the drop could be reasons for a remarkable difference in the mechanism of the transition process from one phase into the other leading finally to a shift and change of shape of the $\pi(A)$ isotherm.

Also, the shapes of domains in the coexisting region of DPPC and DMPE monolayers differ [16] which was also observed by fluorescence microscopy [16]. Nevertheless, both domain types were considered as stable. Thus in the pendant drop experiments, whether or not the expansion rate and the surface curvature really have effects on the transition process of domains and consequently on the shape of the $\pi(A)$ isotherm needs further clarification.

Impurities in the substance could be another reason for the observed differences in the coexistence region of the isotherms. Impurities affect the course of an isotherm, especially when they are measured under non-equilibrium conditions. A LB trough has a much larger capacity to solve a possibly sparsely soluble impurity while a single drop has only a small volume so that most of such an impurity will remain at the drop surface. Thus, possible impurity effects would be much more pronounced in ADSA experiments.

Conclusion

ADSA is a useful novel tool for studying the thermodynamic behavior of insoluble films. The pendant drop experiments of phospholipid monolayers, DPPC and DMPE, show good agreement with the LB balance measurements, except in the coexistence region. These discrepancies are discussed in terms of the high supersaturation with the low density fluid phase and the formation of non-equilibrium condensed phase structures due to the high compression rate. Furthermore, the curvature can affect the number and structure of domains. Also, the different sensitivity of ADSA and LB trough experiments to possible impurities in the phospholipid samples must be considered.

The ADSA technique allows measurements in a very broad molecular area interval. The detection with ADSA of higher surface pressure values close to the collapse indicates that three-dimensional ordered films may be formed on the water drop surface. The uniform temperature, small amount of surfactant required, and the absence of consolidation gradients in the monolayer are further important advantages of the ADSA film balance.

In the ADSA technique, the use of a small amount of substance is particularly important for very expensive samples. However, due to the fact that very small amounts of surfactant material are spread onto the drop, the calculation of the molecular area is very sensitive and may result in a shift of the measured curve along the area-axis.

A further advantage is that ADSA is not only applicable to study insoluble films at the liquid/gas interface, but can also be applied to liquid/liquid systems [17]. In such systems, the direct measurements of surface tension from the drop shape analysis makes the experiment much easier to perform than with a LB balance.

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References

1. Ambwani DS, Fort Jr T (1979) Surface and Colloid Science. Plenum Press, New York
2. Kwok DY, Vollhardt D, Miller R, Li D, Neumann AW (1994) Colloids and Surfaces A 88:51
3. Phillips MC, Chapman D (1968) Biochim Biophys Acta 163:301
4. Rotenberg Y, Boruvka L, Neumann AW (1983) J Colloid Interface Sci 93:169
5. Cheng P, Li D, Boruvka L, Rotenberg Y, Neumann AW (1990) Colloids and Surfaces 43:151
6. Cheng P (1990) Ph.D Thesis, University of Toronto
7. Li D, Cheng P, Neumann AW (1992) J Colloid Interface Sci 39:347
8. Cheng P, Neumann AW (1992) Colloids & Surfaces 62:297
9. Miller R, Joos P, Fainerman VB (1994) Adv Colloid Interface Sci 49:249
10. Weidemann G, Vollhardt D (1995) Colloids and Surfaces A 100:187
11. Albrecht O, Gruler H, Sackmann E (1978) J Physique 39:301
12. Möhwald H (1993) Rep Progr Phys 56:653
13. Helm CA, Möhwald H (1988) J Phys Chem 92:1262
14. Miller A, Möhwald H (1987) J Chem Phys 86:4258
15. Kwok DY, Tadros B, Deol H, Cabrerizo-Vilchez MA, Vollhardt D, Miller R, Neumann AW (1995) Langmuir submitted
16. Lösche M, Möhwald H (1989) J Coll Interface Sci 131:56
17. Li JB, Miller R, Wüstneck R, Möhwald H, Neumann AW (1995) Colloids and Surfaces A 96:295