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The Conventional Langmuir Trough Technique as a Convenient Means to Determine the Solubility of Sparingly Soluble Surface Active Molecules: Case Study Cholesterol

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Abstract

The feasibility of a method based on mass preservation [G. Schwarz, J. Zhang, Chem. Phys. Lipids, 110 (2001) 35–45] to determine the solubility of Cholesterol in water from monomolecular films on air/water interface was investigated. Using a mass balance equation, it was found that Cholesterol undergoes an exponential desorption at very low surface pressures followed by an almost linear desorption into the subphase at higher surface pressures until monolayer collapse. Processing of the surface pressure measurements as a function of trough area in accord with the theory, enabled the accurate determination of the molecular dimensions of Cholesterol as a function of surface pressure. Slight modification of the theory enabled accurate quantification of the surface pressure-independent apparent solubility of Cholesterol and the amount of Cholesterol desorbed into the subphase as a function of surface pressure, in the nanomolar range.

Keywords

monolayer; surface pressure; surface activity; solubility; subphase concentration; molecular dimensions; compression factor; compressibility modulus

1. Introduction

Unlike other small molecules, determination of the solubility of surfactants in aqueous media by thermodynamic or other traditional methods such as suction filtration coupled with a detector of some kind is extremely difficult due to low solubility of surfactants in water, lack of chromophors on surfactant molecules and formation of micelles that could easily pass through the filter. A very attractive technique that appears to be able to determine subphase concentrations of phospholipids as low as a few nanomolar as a function of surface pressure was recently published [1]. This novel approach uses a conventional Langmuir trough to measure surface pressure of spread lipid films on air/water interface and takes advantage of the mass balance equation shown below to calculate the concentration of surfactant desorbed into the subphase,

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$$n_o = n_i + n_s = \Gamma \cdot A + n_s \quad (1)$$

where n_o = total number of moles spread; n_i = number of moles partitioned on the interface, n_s = number of moles partitioned in the subphase, Γ = surface activity expressed in moles/cm² and A = trough area in cm².

In this report, the solubility as well as other structural and thermodynamic properties of Cholesterol determined by this novel approach are presented. The advantages, limitations and potential of the method in determining solubility of sparingly soluble molecules are also discussed in some detail.

2. Materials and methods

Stock solutions of Cholesterol (99+ %, Sigma-Aldrich Inc., St. Louis, MO), were prepared by dissolving precisely weighed amounts on a CP225D Sartorius® analytical balance of readability 10 µg (Sartorius, Goettingen, Germany) in chloroform. Aliquots from the stock solution were transferred to borosilicate glass tubes and diluted further with chloroform to prepare the working solution (0.678 mM). Alternatively the organic solvent of the working solution was evaporated and the glass tubes were sealed and stored temporarily at room temperature for future use. New stock solution was prepared daily while new working solutions were prepared every 2 hr. Experiments were conducted on a Langmuir mini trough film balance (KSV Instruments Ltd., Helsinki, Finland) equipped with two polyacetal-made barriers for symmetric compression and a platinum Wilhelmy plate connected to a micro-electronic feedback for surface pressure measurement (dynamic measuring range of 0–150 mN/m). For a typical experiment, 10–40 µL of Cholesterol solution in chloroform were gently applied with precision micro volume-syringes (Hamilton Company, Reno, NV) to the surface of 150 mL deionized water contained within a thermostated trough (242.25 cm²) at 22°C. After an initial delay period of 5–10 min to ensure chloroform evaporation, the barriers were closed at a constant rate of 9.99 mm/min. Monolayer collapse areas and pressures reported herein were determined from the derivative Π - A isotherm and are onset parameters. Analysis of the constructed Force-Area isotherms for collapse pressure and monolayer compressibility modulus was carried out as described elsewhere [2].

3. Results

Surface pressure-trough area isotherms of Cholesterol discussed herein were performed in triplicate. As shown in Figure 1A, for a given amount of spread lipid n_o , there is a significant variability in the surface area populated by Cholesterol which could be attributed predominantly to errors in the actual volume of working solution transferred on the air/water interface. Nonetheless, the isotherms are clearly shifted to larger trough areas (in groups of three) with increasing amounts of spread lipid n_o , reflective of the tendency of Cholesterol to preferentially populate the interface. However, assuming that all Cholesterol molecules stay at the interface, we would expect all isotherms to overlap when the surface pressure Π is plotted as function of Mean molecular Area (MmA). It is important to recognize that trough areas are automatically converted into MmA (Win LB software, version 1.81) by dividing the trough area with the amount of spread lipid multiplied with the Avogadro number and assuming that the monolayer is completely insoluble ($n_s = 0$). The results depicted in Figure 1B, bottom isotherms, imply that either Cholesterol dissolves to some extent in water or the molecular packing of Cholesterol is dependent on the total number of moles sprayed on the surface. Thus, subphase partitioning or concentration-dependent packing of Cholesterol profoundly affects the molecular dimensions measured from isotherms constructed from lesser amount of spread

lipid. More specifically, the mean molecular area of Cholesterol at monolayer collapse was estimated to be $28 \text{ \AA}^2$ and $36 \text{ \AA}^2$ from isotherms of 10.163 nmoles and 27.101 nmoles, respectively.

The average values of all interfacial properties of Cholesterol monolayers are shown in Table 1. An interesting observation is the increase in the value of compressibility modulus with increasing amounts n_o of spread Cholesterol contrary to the decreasing rates of surface pressure as a function of trough area. This is surprising because, in principle, the compressibility modulus should remain invariant for a given material irregardless of unit length or unit area. The strain should be proportionally smaller for the smaller unit area under constant stress conditions or, as it is in our case under conditions of constant area compression per unit time, a proportionally bigger stress is required to compress the smaller area monolayer unit. Either way the rate of surface pressure with surface area should be proportionally higher for the smaller monolayer area than for the larger one. Therefore, despite the paradoxical increase of the compressibility modulus of Cholesterol monolayer with the total amount n_o of spread Cholesterol, our results of the rate of surface pressure with area are at least in a qualitative agreement with theory.

Another interesting peculiarity apparent from Figure 1A and Figure 1B is that at low n_o the

slope $\left(\frac{\partial \Pi}{\partial A}\right)_{n_o}$ is much steeper than $\left(\frac{\partial \Pi}{\partial (MmA)}\right)_{n_o}$. In order to quantify this discrepancy both slopes were calculated at low and high n_o . As shown in Table 2, when compression is carried out at a constant rate $\Delta A/\Delta t = 10 \text{ mm/min}$ and low n_o ,

$$\left(\frac{\partial \Pi}{\partial A}\right)_{n_o=9.775} = 1.37 > \left(\frac{\partial \Pi}{\partial (MmA)}\right)_{n_o=9.775} = 0.810, \text{ while at high } n_o,$$

$\left(\frac{\partial \Pi}{\partial A}\right)_{n_o=29.325} = 4.74 < \left(\frac{\partial \Pi}{\partial (MmA)}\right)_{n_o=29.325} = 8.00$. At this point a word about the way the experiment is carried out on the Langmuir film balance is appropriate. The operator first fills in information of the experimental conditions, such as volume, concentration and MW of the compound prior to initiating a variable or constant barrier compression. All experiments described herein were conducted at a constant symmetric barrier compression rate. It is now apparent that the origin of this discrepancy lies in the formula

$$MmA \left(\text{\AA}^2 / \text{molecule} \right) = \frac{\text{Trough Area, } A \left(\text{cm}^2 \right) \cdot 10^{16} \text{ \AA}^2 / \text{cm}^2}{n_o \left(\text{moles} \right) \cdot N_A \left(\text{molecules / mole} \right)}$$
 which is used to convert the raw data (Π -Trough Area) into a Π -Mean molecular Area plot. Contrary to the constant value of the numerator, the value of the denominator increases with n_o , thus, the value ΔMmA and of

the slope $\left(\frac{\partial \Pi}{\partial (MmA)}\right)_{n_o}$ decreases and increases, respectively, with increasing n_o . The choice of trough areas that correspond to higher surface pressures (Table 2) as a result of higher interfacial Cholesterol density is the reason for the bigger magnitude of both slopes at higher n_o .

To better understand the reasons behind the discrepancy in the molecular dimensions of Cholesterol, we further studied how the compression pressure varies with the trough Area when the total number of spread molecules n_o is held constant. As shown in Figure 2A and 2B, significant differences in the instantaneous rate of change of pressure with area as a function of surface pressure and the average rate of surface pressure with surface area, respectively, are obvious with n_o . More specifically, monolayers formed with lowest amounts of Cholesterol exhibited on average a 54% higher rate of surface pressure change at a constant rate of compression than the monolayers formed with almost three times the amount of Cholesterol

spread on the interface. On the other hand, the similarity in the shape of the slopes $\left(\frac{\partial \Pi}{\partial A}\right)_{n_o}$ as a function of surface pressure, the very dilute range of concentrations of Cholesterol solutions applied on the interface (67.80 – 180.7 nM), the relatively constant surface pressure at monolayer collapse and the characteristic pre transition observed in all isotherms at a surface pressure of 12 – 15 mN/m (Table 1 and Figure 3), provide strong evidence that the character of the Cholesterol monomolecular layer on the air/water interface remains largely invariable at all n_o used in this study.

Having ascertained that the surface pressure Π varies with the trough area similarly with all different n_o , we proceeded with the analysis of the isotherms in accord with the mass balance equation. Trough areas were therefore plotted as a function of total spread Cholesterol n_o at different constant surface pressures and the results were fitted using a least squares analysis into a line represented by equation 1, where surface density Γ is the slope and n_s is the intercept (as $A \rightarrow 0, n_i \rightarrow 0 \Leftrightarrow n_o = n_s$). As demonstrated in Figure 4 the amount of Cholesterol partitioning in the subphase increases with increasing surface pressure suggesting that increased compression causes Cholesterol desorption from the monolayer film into the subphase. A question at this point would be about the physical significance of extrapolating the lines to the ordinate for assumption of a zero trough area can not be realistic. An equivalent to extrapolating the linear fits to the ordinate would be the use of trough areas A and the slope Γ to calculate corresponding n_i for every n_o . The amount of Cholesterol present in the subphase n_s could then be calculated using equation 1 ($n_s = n_o - n_i$). The result would be the calculation of many different n_s at a particular surface pressure for different n_o and the average value would be a value similar to that found from the y-intercepts of the linear fits of the mass conservation plot of Figure 4. As demonstrated in Figure 5A (solid diamonds) the Cholesterol present in the subphase n_s has increased exponentially at low surface pressures followed by an almost linear desorption extended up to monolayer collapse. We hypothesized that since Cholesterol undergoes desorption into the subphase with compression, extrapolation of the measured amount of Cholesterol into the subphase, n_s , as a function of surface pressure at infinite dilution of the interface ($\Pi \rightarrow 0$) should yield the true solubility of Cholesterol in water at 22°C. Unfortunately, due to the exponential nature of the desorption at low surface pressures, linear regression analysis of the data points in the linear region would be a very unreliable measure of the true solubility. We therefore analyzed surface pressures as low as 0.15 mN/m using the mass balance equation. As shown in the inset of Figure 5A, desorption practically stops at surface pressures below 0.25 mN/m. The uncertainty in the measurement from the average value increased from 5% at 0.50 mN/m to 8%, 12%, 23% and 52% at 0.30, 0.25, 0.20 and 0.15 mN/m, respectively. The solubility of Cholesterol ($n_{s(\Pi \rightarrow 0)} = n_{sol}$) was estimated from linear regression analysis of the lowest four points of surface pressure to be equal to 0.978 nmoles ($C_s = 6.52$ nM). Subtracting the amount dissolved in the subphase (n_{sol}) at zero Π from the total amount spread on the interface, n_o , not only has improved the molecular dimension estimate but it also reduced the variation in the measurement. Specifically the dimensions of Cholesterol were shifted by $3.0 \text{ \AA}^2$, $1.3 \text{ \AA}^2$ and $1.9 \text{ \AA}^2$, for the low n_o , high n_o and on average, respectively (Figure 1B-top isotherms and Table 1). The apparent partition coefficient of

Cholesterol was also calculated as a function of Π using $K_p = \frac{\Gamma \cdot A}{n_s}$ where A is the maximum trough area (242.25 cm^2), and the results are plotted in Figure 5B. Although the surface density of Cholesterol Γ , increases with surface pressure (see Figure 6), the total number of molecules of Cholesterol on the interface n_i , decrease with increasing Π due to desorption into the subphase. As a result the apparent partition coefficient of Cholesterol at the air/water interface decreases with surface pressure.

The relationship of Cholesterol interfacial density Γ as a function of surface pressure is shown in Figure 6. The isotherm comprises five distinct regions. The first region depicts an initial exponential increase of the surface pressure Π with the surface density of Cholesterol up to 0.60 mN/m followed by a region of linear increase of surface pressure with surface density. The third region is characterized by a concavity change at surface pressure 14 mN/m (monolayer pretransition, Table 1) and extends up to 24 mN/m. Subsequently, another inflection point signals the onset of the forth region that ends at 34–38 mN/m (onset of monolayer collapse). As depicted in the last region the isotherm bends abruptly at surface pressure 45–46 mN due to monolayer collapse (Figure 6A–B).

In Figure 6B the compression factor Z of the Cholesterol monolayer was calculated using

$Z = \frac{\Pi}{\Pi_{ideal}}$, where $\Pi_{ideal} = R \cdot T \cdot \Gamma$, and plotted as a function of surface pressure and interfacial density Γ . Substituting the ideal value of unity for the compression factor in the corresponding 2D equations of state, derived from regression analysis of the data points, yielded values of ideal surface density Γ_{ideal} and ideal surface pressure Π_{ideal} of 0.3910 nmol/cm² and 9.72 mN/m, respectively (see Figure 6B, point of intersection of the two curves with vertical dashed-line). At surface pressures below 9.72 mN/m the monolayer is more compressible than ideal films, presumably because intermolecular attractive forces dominate. Above the Γ_{ideal} intermolecular distances are very small, thus, repulsive forces render the monolayer less compressible than an ideal film. Finally at Π_{ideal} , the repulsion forces exactly counteract the intermolecular attraction forces and the Cholesterol monolayer behaves like an ideal film.

4. Discussion

The work described in this report investigated the potential of a novel approach of processing surface pressure measurements of monolayers spread on air/water interface to determine the water solubility of Cholesterol [3]. In this method, the amount of spread Cholesterol, n_o , and the compression (trough) area, A , are known and are varied systematically, the surface pressure Π is measured (as a function of A) and the surface activity Γ , the amount of Cholesterol in water and on the interface n_s and n_i , respectively, the partition coefficient K_p , and the mean molecular dimensions of Cholesterol, MmA , are calculated.

Quality control of the method was achieved with inspection and regression analysis of the

collected raw data (Π - A graph) to verify the shape similarity of the slopes $\left(\frac{\partial \Pi}{\partial A}\right)_{n_o}$ at various n_o . In addition, pending the different values of the Cholesterol two-dimensional compressibility modulus, the similarity of all other parameters collected in Table 1 argue in favor of a fairly uniform monomolecular packing of Cholesterol on air/water interface at the various n_o used in this study.

In several occasions, the relative concentrations of lipids present in the interface and subphase were used to calculate the apparent partition coefficient of lipids, as a function of surface pressure [1,4]. The partition coefficient of a molecule into two phases is considered a thermodynamic quantity (ratio) that remains constant under conditions of constant pressure and temperature. The large values of the K_p coupled with the negative value of the chemical potential difference, $\Delta\mu^0 = -R \cdot T \cdot \ln K_p < 0$, where $\Delta\mu^0 = \mu_s^0 - \mu_i^0$, would seem to denote the tendency of the Cholesterol molecules to migrate from the subphase toward the air/water interface. However, for technical reasons all experiments were performed with Cholesterol amounts that exceeded the maximum solubility of it in water. Thus, the calculated partition coefficient not only cannot offer any insight as to the spontaneous preference of the Cholesterol to partition into one phase over another but it can only at best represent an approximation of

the minimum value of the true partition coefficient of Cholesterol between water and air/water interface at zero surface pressure, strictly for the given trough area and subphase volume (Figure 5B; inset). An additional complication in this sort of calculation is the dynamic nature of the air/water interface, that is, the irrevocable capacity of Cholesterol to constantly modify the properties of the interface just by accumulating in it.

It needs to be recognized that although methods that measure surface tension can only monitor changes in surface concentration, the use of the mass balance equation herein allowed monitoring of the total amount of surfactant in the system. As mentioned in the Results section solubility of Cholesterol in water was calculated by extrapolating the linear portion of the subphase amount n_s at very low surface pressures on the ordinate (Figure 5A-inset). This was necessary due to the surface pressure-dependent variable rate of molecular desorption. The mass balance equation $n_o = n_i + n_s$ implies that n_s is a composite of two factors, that is, $n_s = n_{sol} + n_{des}$, where n_{sol} is the amount of Cholesterol dissolved in water and it has a constant value at constant Pressure, Volume and Temperature, while n_{des} is the amount of Cholesterol desorbed from the monolayer into the subphase as a function of surface pressure. Using this method, we were able to calculate an aqueous solubility of Cholesterol of 6.52 nM, a value which is very close to 10 – 40 nM reported in the literature [5,6]. The correct amount of Cholesterol desorbed as a function of surface pressure can be calculated after subtraction of the Cholesterol solubility in water from the n_s . The empty diamonds joined by the dashed-line in Figure 5A depict the true values of Cholesterol desorption as a function of surface pressure.

The method of mass preservation was described originally by Dr. Schwarz to calculate the actual partitioning of surface active molecules into the subphase as a function of surface pressure. The true dimensions of the molecules were calculated as a function of surface pressure, from the values of surface activity Γ that were in turn calculated using the mass balance equation. It is extremely important to emphasize that the slopes of the lines shown in Figure 4 are independent of the actual value of the ordinate. That is, whether the actual value of the Cholesterol solubility is subtracted or not from the total has absolutely no effect on the slope determination. The molecular dimensions of Cholesterol as a function of surface pressure are summarized in Figure 6A, empty circles. Gupta and coworkers have measured the molecular area of Cholesterol on Langmuir-Blodgett films using AFM to be 36.5 – 38.5 Å² in close agreement with our value of 41.0 Å² determined at monolayer collapse [7]. This is a tremendous difference from the molecular dimensions determined at low n_o and the average uncorrected MmA of Cholesterol of 27.7 Å² and 32.7 Å², respectively (Table 1). Given a molecular area of Cholesterol a_c at monolayer collapse equal to 41.0 Å² and a molar volume equal to 391.4 cm³/mole (ACD/Chemsketch Freeware version 8.17, Advanced Chemistry Developments Inc., Canada), the thickness of the monomolecular film was estimated to be 1.60 nm. This value is in close agreement with the 14.5 Å of Cholesterol monolayer thickness measured by grazing-incidence X-ray (GIXR) and ellipsometry [8]. Interestingly, the three-dimensional pressure across the film at Π 0.15 mN/m and at Π_{ideal} was determined to be 0.9200 atm and 59.95 atm, respectively. The essentially identical to atmospheric pressure compression pressure calculated at low surface pressure is consistent with an L₁-G phase of the Cholesterol monolayer and justify our find that no desorption occurs in those very low surface pressures.

5. Conclusions

Our interest to explore new ways to determine aqueous solubility of cationic lipids was triggered by the old and recent findings of a relationship between lipid exchange and transfection activity [9,10]. The method described in this report is certainly capable of assessing concentrations of surfactant in water in the nanomolar range. Perhaps, the most attractive feature of the method is that unlike all the other methods that aim to directly measure dissolved amounts of sparingly soluble molecules in the subphase, this method measures amounts that

are excluded from the bulk, thus, the less soluble the molecule the more sensitive is the method. To increase the sensitivity of the assay the use of deeper troughs and smaller surface areas is recommended in order to increase the amount of surfactant dissolved in the subphase. The main disadvantage of the method is the requirement to measure surface pressures of monolayers existing in the L_1 -G phase. The steady-state molecular exchange between the interface and the bulk is the primary reason for the increased noise and reduced sensitivity of the assay at very low surface pressures. Performing these experiments at reduced temperature may prove an effective way to reduce the variations in the measurement.

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Figure 1.

A Surface pressure – interfacial area isotherms of Cholesterol at 22°C. Amount of spread lipid n_o in nmol from left-to-right (triplicate runs): 10.163; 13.550; 16.938; 20.326; 23.713; 27.101. B. Representative surface pressure, Π – Mean molecular Area, MmA isotherms of Cholesterol at 22°C. Amount of spread lipid n_o represented by each isotherm is the same as in Figure 1A. Isotherms recovered by the LB software automatically assuming that all Cholesterol molecules stay in the interface are shown in the bottom. The curves directly above are the isotherms normalized to account for the solubility of Cholesterol in the subphase calculated as $\Pi \rightarrow 0$ (see text).

Figure 2.

A. Average of three experiments of the instantaneous rate of change of surface pressure, Π , with interfacial area, A , as a function of surface pressure Π , for variable amounts of spread lipid n_o . Error bars were omitted for the sake of clarity. B. Π -A isotherms were fitted using the method of least squares analysis from 5–35 mN/m and slopes were plotted as a function of the total moles of spread Cholesterol, n_o . Error bars represent the standard deviations of three experiments. The solid horizontal line is the average value of the slopes. The maximum deviation of the individual slopes from the average value was calculated using

$$\%rel \cdot Error = \frac{st \cdot deviation}{Average \ Value} \times 100 \text{ and found to be } 24 \ \%.$$

Figure 3.

Representative surface pressure Π (line) and derivative isotherm (empty symbols joined by line) plotted as a function of interfacial area, A . Notice the inflection point and the maximum in the $\Pi - A$ isotherm that reflects a reorganization of the Cholesterol packing and the collapse of the monolayer, respectively.

Figure 4.

Nanomoles of total Cholesterol n_o , versus the interfacial area, A , at fixed volume, atmospheric pressure and constant temperature 22°C. Linear fits at surface pressure Π from top-to-bottom (or left-to-right): 30 mN/m, 20 mN/m, 10 mN/m, 5 mN/m and 0.5 mN/m. Error bars are the standard deviation of the average of three experiments.

Figure 5.

A. Subphase amount of Cholesterol n_s , as a function of surface pressure, Π . The horizontal dashed-line represents the apparent solubility of Cholesterol in water at 22°C calculated from a least square analysis of the desorption of Cholesterol as a function of surface pressure as explained in the text. Solid symbols represent the total amount of Cholesterol present in the subphase n_s , whereas empty symbols joined with a line denote the amount of Cholesterol desorbed from the monolayer into the subphase with increasing pressure. B. Apparent partition coefficient plotted as a function of surface pressure.

Figure 6.

A. Surface pressure as a function of the Cholesterol surface density Γ calculated from the slopes of the lines shown in Figure 4. Cholesterol molecular dimensions, a , calculated as a function

of surface pressure using $a = \frac{1}{\Gamma_c \cdot N}$ are also shown (empty circles). Solid and broken-line segments connect the points to enhance the visual clarity of the graph. B. Compression factors for Cholesterol monolayer at 295.15 K plotted as a function of surface pressure and interfacial density (empty squares).

Table 1
Monolayer parameters of Cholesterol spread on water at 22 °C

Total nmoles (n_0)	MmAc ($\text{\AA}^2$)	*MmAc ($\text{\AA}^2$)	Π_c (mN/m)	K (mN/m)	$\Delta\Pi/dA$	Π_{pr} (mN/m)
10.163	27.7 ± 1.8	30.7 ± 2.0	35.2 ± 3.4	323 ± 12	20.0 ± 0.5	15.2 ± 2.4
13.551	30.6 ± 1.3	32.9 ± 1.6	37.4 ± 0.6	415 ± 67	17.4 ± 2.9	12.7 ± 2.5
16.938	32.7 ± 0.7	34.7 ± 0.7	35.1 ± 3.9	446 ± 71	15.0 ± 1.4	13.7 ± 1.0
20.326	33.6 ± 2.1	35.4 ± 2.2	34.7 ± 1.1	493 ± 65	13.7 ± 1.4	12.3 ± 1.1
23.713	35.1 ± 0.8	36.4 ± 0.8	36.2 ± 3.0	536 ± 27	13.3 ± 1.2	13.7 ± 4.8
27.101	36.4 ± 0.7	37.7 ± 0.8	35.1 ± 2.1	650 ± 130	12.6 ± 1.6	14.0 ± 2.6
Average $\pm$ SD	32.7 ± 3.1	34.6 ± 2.7	35.6 ± 2.4	477 ± 111	15.3 ± 3.0	13.6 ± 2.5

MmAc Π_c and Π_{pr} denote monolayer collapse and precollapse parameters, respectively. They were determined from the first derivative of the compression isotherm and they are onset parameters.

$d\Pi/dA$, expressed in units of $mN \cdot m^{-1} \cdot cm^{-2}$, is the instantaneous rate of change of surface pressure with surface area at monolayer collapse.

Average values and standard deviations (SD) were calculated from three samples.

* Collapse dimensions after taking into account the solubility of Cholesterol in the subphase

Table 2
Calculated slopes $\left(\frac{\partial \Pi}{\partial A}\right)_{no}$ and $\left(\frac{\partial \Pi}{\partial (MmA)}\right)_{no}$ at a constant ΔA for a low and high n_o .

$n_0 = 9.775$ nmol					
A (cm ²)	MmA (Å ²)	Π (mN/m)	ΔA (cm ²)	* ΔMmA (Å ²)	ΔΠ (mN/m)
21.74	36.92	1.78	-0.27	-0.46	0.37
21.47	36.46	2.15			
$n_0 = 29.325$ nmol					
A (cm ²)	MmA (Å ²)	Π (mN/m)	ΔA (cm ²)	ΔMmA (Å ²)	ΔΠ (mN/m)
72.04	40.79	15.45	-0.27	-0.16	1.28
71.77	40.63	16.73			

$$* \text{ Calculated using } \Delta MmA \left(\text{\AA}^2 / \text{molecule} \right) = \frac{\Delta A \left(\text{cm}^2 \right) \cdot 10^{16} \text{\AA}^2 / \text{cm}^2}{n_o \left(\text{moles} \right) \cdot N_a \left(\text{molecules} / \text{mole} \right)}$$