Equilibrium Penetration of Monolayers VI: Cholesterol–Cetrimonium Bromide System

MARYLYN A. McGREGOR and G. T. BARNES ×

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Abstract □ Equilibrium surface pressure–area isotherms for the penetration of cholesterol monolayers by the surfactant cetrimonium bromide are presented. From these isotherms, the saturation adsorptions of surfactant for various surface concentrations of cholesterol were calculated. Plots of adsorption against the inverse of the area per cholesterol molecule revealed two linear regions, corresponding to penetration at high and low monolayer areas. At high monolayer areas, surfactant adsorption into accessible areas of the surface was similar to adsorption at a monolayer-free surface. In this region, packing of cholesterol and surfactant molecules on the surface lowered the effective cross-sectional area of the cholesterol molecule. However, at low monolayer areas, adsorption was determined by the size of the surfactant ion and the effective area of the cholesterol molecule was equal to the collapse area of a pure cholesterol monolayer.

Keyphrases □ Cholesterol monolayers—penetration by cetrimonium bromide, equilibrium surface pressure–area isotherms obtained □ Monolayers, cholesterol—penetration by cetrimonium bromide, equilibrium surface pressure–area isotherms obtained □ Cetrimonium bromide—penetration of cholesterol monolayers, equilibrium surface pressure–area isotherms obtained □ Sterols—cholesterol, monolayers penetrated by cetrimonium bromide, equilibrium surface pressure–area isotherms obtained □ Surfactants—cetrimonium bromide, penetration of cholesterol monolayers, equilibrium surface pressure–area isotherms obtained

If a surfactant is injected into the aqueous phase beneath an insoluble monolayer, it usually tends to penetrate the monolayer, forming a mixed film of monolayer substance and surfactant. This tendency is manifest as a rise in surface pressure at a given monolayer area or as an increase in area at constant surface pressure. Because of the significance of monolayer behavior in the Davson–Danielli (1) model for biological membranes, there has been considerable interest in penetration studies with monolayers of substances found in natural membranes and with surfactants of biological significance.

Cholesterol has featured prominently in these studies, as has the surfactant cetrimonium (hexadecyltrimethylammonium) bromide (I), but these two substances were used together only once previously (2). There are significant differences between these earlier results and the data presented here.

The "accessible area" theory, developed to explain the permeation of a monolayer by water evaporating from the subphase (3), was modified to describe the equilibrium penetration of a monolayer by a surfactant (4). It leads to an equation for the adsorption or penetration of surfactant into a monolayer-covered surface, Γ_f:

$$\Gamma_f = \Gamma_w - a_M\Gamma_w(1/\hat{A}_M) \qquad \text{(Eq. 1)}$$

where Γ_w is the adsorption into the accessible area of the surface, a_M is the apparent cross-sectional area of a monolayer molecule, and $\hat{A}_M$ is the area per monolayer molecule. A plot of Γ_f against $(1/\hat{A}_M)$ should be a straight line, and physically reasonable values should be obtained for Γ_w and a_M. In this paper, the measurements reported for the equilibrium penetration of cholesterol monolayers by cetrimonium bromide are analyzed in terms of the accessible area theory.

Table I—Analysis of Penetration Data in Terms of Eq. 1

Region	Γ_w, molecules/nm^2	a_M, nm^2/molecule
Low area	2.36 ± 0.19	0.40 ± 0.04
High area	1.42 ± 0.12	0.27 ± 0.03
Predicted values	1.71 ± 0.20	0.39 ± 0.02

EXPERIMENTAL

The monolayer-forming substance, cholesterol[1], was dissolved in *n*-hexane[2] for spreading. The surfactant, I[3], was found, by surface tension measurements, to have a critical micelle concentration of 0.96 mM (5). Triple-distilled water was used for all substrates and solutions.

The apparatus and techniques were described previously (5). Briefly, a monolayer was spread on a water surface, the surfactant was injected into the subphase and mixed, and the surface was then compressed in steps, with ample time for equilibrium to be established between each step. An equilibrium surface pressure (π)–area per monolayer molecule

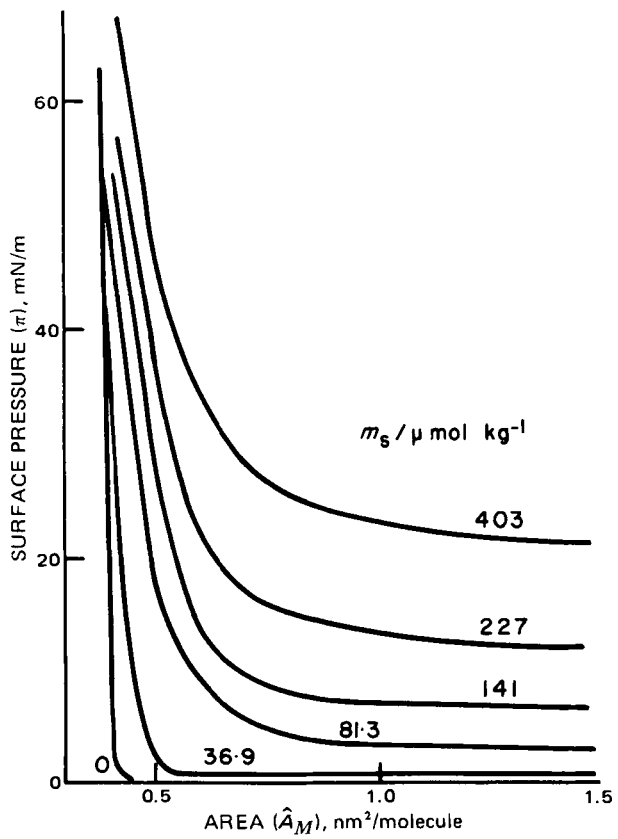

Figure 1—*Surface pressure–area isotherms of cholesterol monolayers on I solutions of various concentrations (T = 298 °K).*

[1] "For Biochemistry" grade, Merck.
[2] Spectroscopic grade, BDH.
[3] "Pro analysi" grade (>99% purity), Merck.

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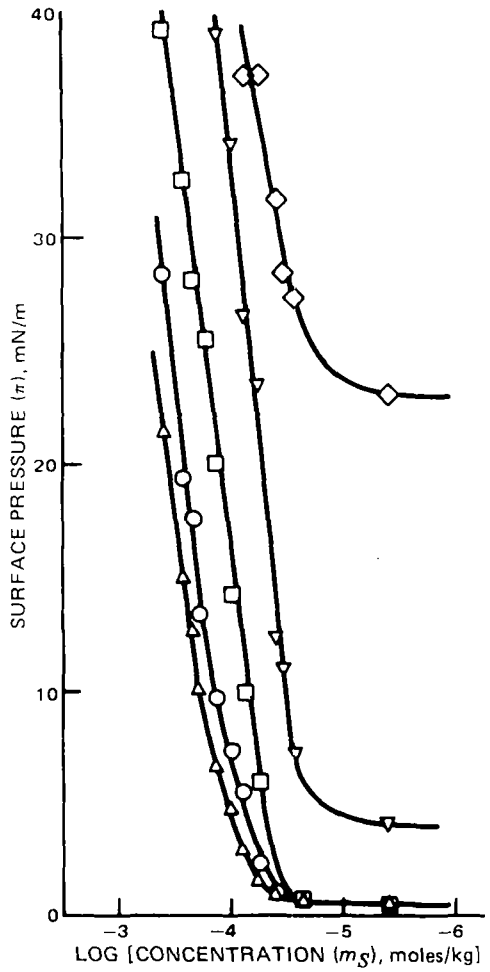

Figure 2—*Surface pressure as a function of I concentration for cholesterol monolayers at 0.4 (◊), 0.45 (▽), 0.55 (□), 0.70 (○), and 1.40 (△) nm²/monolayer molecule.*

($\hat{A}_M$) isotherm was thus obtained for one particular concentration of surfactant, m_S, in the subphase. The concentration of surfactant in such an experiment was determined at the completion of the run by sweeping the monolayer off the surface and measuring the surface tension of the subphase. The surface tension was then compared with a calibration curve (5).

RESULTS AND DISCUSSION

Equilibrium surface pressure–area isotherms for cholesterol with various concentrations of surfactant are shown in Fig. 1. From these curves, plots of surface pressure against log m_S were prepared for selected values of $\hat{A}_M$ (Fig. 2). The curves in Fig. 2 were approximately linear at surface pressures greater than 10 mN/m, indicating saturation adsorption. The slopes of the linear sections, $d\pi/d$ log m_S, were plotted against the area per monolayer molecule, $\hat{A}_M$ (Fig. 3). As expected, the values of $d\pi/d$ log m_S at high areas tended toward the slope for a monolayer-free surface.

Similar experiments with this system were reported (2) but over a narrower range of surfactant concentrations. There are significant differences between the two sets of results. The previous (2) surface pressure–area curves were appreciably more expanded than those shown in Fig. 1, and their monolayers appeared to collapse at 30–36 mN/m and about 0.7 nm²/molecule compared with the collapse pressures of 55–65 mN/m and areas less than 0.5 nm²/molecule reported here. In view of differences in the source of materials and techniques, there can be no firm explanation of these discrepancies. However, the results are consistent with the possibility that Ahmad *et al.* (2) had not allowed sufficient time in the stepwise compression of their monolayers for proper equilibria to be established.

Table II—Determinations of the Cross-Sectional Area of the Surfactant Ion

Cross-Sectional Area of Surfactant Ion, nm²/molecule	Method of Determination	Reference
0.39	Leybold models	5
0.29	Molecular models	6
0.37	Amagat equation	6
0.40	Alexander equation	6
0.37	$1/\Gamma_w$	5
0.42	$1/\Gamma_w$	Present work

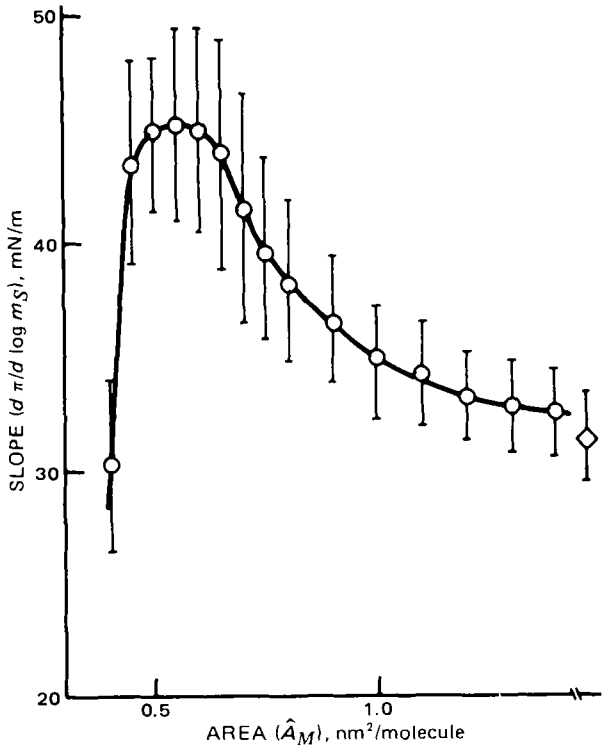

Figure 3—*Slope of the surface pressure–log (surfactant concentration) plot as a function of the area per cholesterol molecule.*

Adsorption values were calculated from the slopes of the linear sections of the π–log m_S curves by using Pethica's modification to the Gibbs ad-

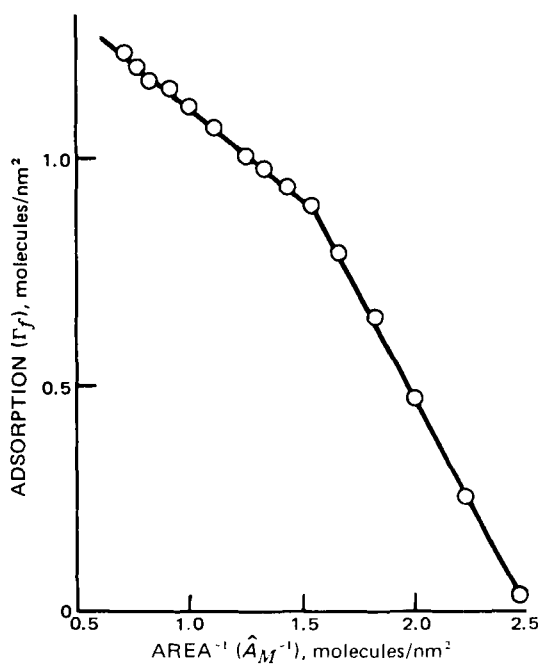

Figure 4—*Adsorption of I into cholesterol monolayers plotted according to Eq. 1.*

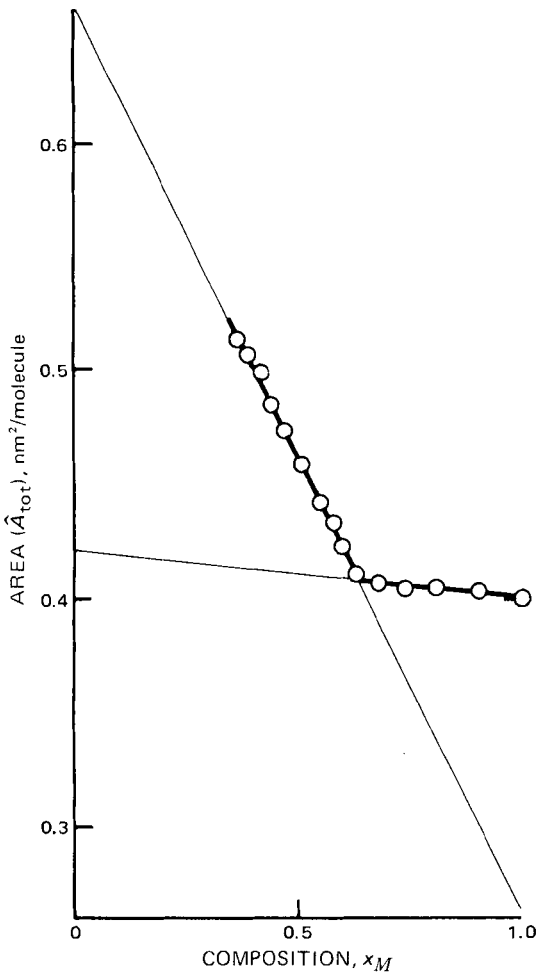

Figure 5—*Total area plotted against mole fraction of monolayer material to determine partial molecular areas.*

sorption isotherm. A plot of adsorption, Γ_S, against the inverse of the area per monolayer molecule, $1/\hat{A}_M$, as required for the accessible area theory (Eq. 1), is given in Fig. 4. Two distinct linear regions can be seen in this plot; for convenience, they will be called the high area region and the low area region. Values of the parameters Γ_w and a_M were determined for both regions using regression analysis (Table I).

The values obtained for Γ_w, the adsorption into the accessible area of the surface, in the two linear regions were similar to those for the octadecanol–I system (5).

In the high area region, Γ_w had practically the same value as Γ_S: the adsorption at a monolayer-free surface (Table I). In this region, the electrostatic forces limiting adsorption at the monolayer-free surface had a similar effect in the presence of the monolayer, even though the average surface concentration of surfactant molecules was less than at the monolayer-free surface. This result suggests that the surfactant is adsorbing or penetrating into large gaps in the monolayer and that the local surface concentration in these gaps is similar to that at a monolayer-free surface.

However, in the low area region, Γ_w was much higher than Γ_S. Here, the data indicate that the surfactant is adsorbed in small holes in the monolayer with only one molecule per hole. Thus, the size of the surfactant molecule is important and $1/\Gamma_w$ (0.42 nm²/molecule) should be a good estimate of the cross-sectional area of the surfactant ion. Table II summarizes values for the cross-sectional areas of the ion. There is reasonable agreement between all values except the second.

Table III—Partial Molecular Areas as Determined from a Plot of Total Area against Composition

Region	$\bar{A}_M$, nm²/molecule	$\bar{A}_S$, nm²/molecule	$\Gamma = 1/\bar{A}_S$, molecules/nm²
Low area	0.40	0.42	2.38
High area	0.26	0.67	1.50

The values obtained for a_M in the two regions differed substantially. At low areas, the value agreed well with the collapse area of cholesterol, 0.39 nm²/molecule. At high areas, the value for a_M was appreciably smaller. Probably the effective cross-sectional area of the monolayer molecule is reduced because the cholesterol is able to fit between the widely spaced surfactant molecules with only a moderate increase in their spacing.

A plot of total area against composition yielded values for the partial molecular areas of the two single components (Fig. 5). Table III summarizes values for the partial molecular areas obtained for both the low and the high area regions. These values agreed well with the corresponding values obtained for $1/\Gamma_w$ and a_M in the two regions. Indeed, it can be shown (7) that $1/\Gamma_w$ and a_M are theoretically identical to the partial molecular areas of surfactant and monolayer, respectively, and that the accessible area theory provides an alternative means of estimating these areas.

The equilibrium penetration of cholesterol monolayers by sodium lauryl sulfate was studied by Pethica (8) and analyzed in terms of the accessible area theory (4). The data were restricted to a region corresponding approximately to the low area region of the present system. Within this region, the two systems are very similar. The value of a_M obtained from Pethica's data was 0.374 nm²/molecule, in reasonable agreement with the value found here; the value of Γ_w again indicated that penetration was primarily determined by the size of the surfactant ion.

Neither Pethica's data nor the data presented here indicate that a complex might be formed between cholesterol and the surfactant. However, complex formation has been suggested between cholesterol and sodium hexadecyl sulfate (9, 10) and for the present system (2). It is likely that the longer chain length of the hexadecyl sulfate ion would lead to a stronger interaction with cholesterol than occurs between the lauryl sulfate ion and cholesterol. Nevertheless, Goddard and Schulman (10) suggested that the interaction is not strong enough for complex formation. Ahmad *et al.* (2), working with the cholesterol–I system, suggested that complex formation could explain the large collapse areas. However, the present data do not show this feature (Fig. 1), and analysis by the accessible area theory gives no indication of complex formation (11).

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