The Two-step Collapse of Mixed Monolayers of Cholesterol and Its Esters

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(Received August 28, 1975)

Mixed monolayers of two insoluble components out of cholesterol and its acetate and propionate were spread on a dilute aqueous solution of disodium hexadecyl phosphate. The curves of the surface pressure against the molecular area were found to have two kink points, collapse points, although the curves of each component, and also that of the "concollapse" mixture, had only one collapse point. The diagram showing the relation between these two collapse pressures and the composition was entirely analogous to the phase diagrams of bulk mixtures which have an eutectic point. It has, therefore, been concluded that the mixed monolayer of two mutually immisible substances undergoes a two-step collapse. At the lower collapse point, one of the two components, *i*, starts separating from the monolayer. The lower collapse pressure, F_{Ci}' varies with the mole fraction, X_{i}, according to $F_{\mathrm{Ci}}'=F_{0\mathrm{i}}'-(kT/A_{0\mathrm{i}})\ln X_{\mathrm{i}}$. At the higher collapse point, the other component starts separating as well. Upon further compression, the surface pressure remains unchanged at the higher collapse pressure, which is equal to the equilibrium-spreading pressure and is independent of the composition.

In studies of insoluble monolayers it has sometimes been noticed that there is a disagreement between the collapse pressure, F_{c}, and the equilibrium-spreading pressure, F_{s}. Here, F_{c} is obtained as the kink point on a curve of the surface pressure, F, against the molecular area, A, when an insoluble monolayer is compressed, while F_{s} is observed when small particles or droplets of an insoluble substance are placed on a clean aqueous surface. Two reasons can be pointed out for this disagreement: (1) When the insoluble components have large hydrophobic groups, the molecules are oriented completely at the aqueous surface and the cohesion between the hydrophobic groups resists collapse, so that the monolayer is readily supercompressed. (2) For multicomponent monolayers, the disagreement can be explained thermodynamically. It must be emphasized here, however, that the thermodynamic discussion is significant only when the effect of the supercompression has been eliminated completely.

In the case of one-component monolayers, F_{c}, is greater than or equal to F_{s}. For example,[1)] the monolayers of tristearin, tripalmitin, and trimyristin were supercompressed so that $F_{\mathrm{c}}>F_{\mathrm{s}}$, while the monolayer of trilaurin was in equilibrium with the bulk phase so that $F_{\mathrm{c}}=F_{\mathrm{s}}$.

In the case of a mixed monolayer, however, F_{c} may be either greater or smaller than F_{s}. The mixed monolayer of tristealin and trilaurin was supercompressed so that $F_{\mathrm{c}}>F_{\mathrm{s}}$, while that of cholesterol and trilaurin was in thermodynamic equilibrium with the bulk phase so that $F_{\mathrm{c}}<F_{\mathrm{s}}$.[2)] For a mixed monolayer of triolein and tricaprylin, which belongs to the latter case with $F_{\mathrm{c}}<F_{\mathrm{s}}$, it has been verified experimentally that at $A \rightarrow 0$ agrees with F_{s}, and that on the phase diagram expressing F against the mole fraction, X, at a fixed temperature, the curve of F_{s} against X reveals an arc and that of F_{s} against X reveals another arc, indicating that these two components mix with each other at all proportions.[3)]

If the components, on the contrary, do not mix at all in the bulk phase, a phase diagram analogous to that which has an eutectic point would be expected. In this paper, mixed monolayer of two components out of cholesterol(CH) and its acetate (CA) and propionate (CP) will be discussed. For this effect, the following two experimental devices were needed:

1: As the substrate, an aqueous solution of disodium hexadecyl phosphate (SHP) was used at 0.5 mM instead of water because the mixed monolayers studied here were leaky on the water surface; the addition of as much SHP as this concentration made the monolayers stable enough for us to measure the collapse pressures without changing the essential features of the phase diagram. 2: The F—A relation is usually studied by the compression of a monolayer. In order to avoid the supercompression, however, the method of insoluble-component addition to a constant surface area was used, particularly for the monolayers containing CP.

Experimental

Materials. The specimen of CH used was from the Sigma Chemical Co., the CP was from The Aldrich Chemical Co., and the CA, from the Nakarai Chemical Co. was recrystallized from acetone. The specimen of SHP was the same as used in the previous paper,[4)] which had been synthesized and dissolved in water as has been reported elsewhere.[5)]

Monolayer Spreading. The insoluble components to be spread as a monolayer were dissolved in benzene and supplied from an Agla Micrometer Syringe onto the surface of water or an aqueous SHP solution in a trough (60×20×1 cm) made of methacryl-resin, coated with paraffin, and sectioned by paraffinized glass barriers.[6)]

The occupied area per molecule of insoluble components, A(Å^2/molec.), can be calculated by the following equation:

$$A = S/nN_{\mathrm{A}} \tag{1}$$

where S is the area of the aqueous surface between barriers, where n is the sum of the moles of insoluble components spread in this area, and where N_{A} is the Avogadro number. Therefore, A can be decreased either by decreasing S (SAD method) or by increasing n (ICA method). In the latter method, n was increased by dropping additional benzene solution without changing the S.

Surface Pressure. The surface pressure, F (dyn/cm), was measured by Wilhelmy's plate method. A ground plate of quartz was used after it had been cleaned by immersion in fuming nitric acid for more than one night and washing well with distilled water. The torsion balance used was Shimadzu T-NR, modified and equipped with mirror in order to magnify the movement of the plate by a lump and scale. The temperature was kept at 25±0.2 °C by circulating thermostated water through glass tubing immersed in the trough.

The surface pressure-area curve of the cholesterol monolayer thus obtained was in good agreement with that reported in the literature,[7] and the collapse pressure of the monolayer of cholesterol and its ester on the SHP solution were reproducible and stable for a few hours. Therefore, the possiblity of air oxidation[8] will not be considered here, following many papers which have been published without referring to air oxidation.[7]

Equilibrium-spreading Pressure. The equilibrium-spreading pressure, F_s (dyn/cm), was measured by Wilhelmy's plate method, taking the reading at the equilibrium reached when powder of CH, CA, or CP, or of their mixture was sprinkled on the surface of water or a SHP solution contained in a Petri dish 10 cm in diameter. In case of a mixture, its components were dissolved in ethyl ether at a given composition, the solvent was evaporated, and the residue dried in a vacuum was mixed well with a spatula before use.

Results

Monolayer of CA. Prior to the study of mixed monolayers of CH and CA, the monolayers of each component were studied. The results on the CH monolayer have been reported already.[2] On the other hand, the CA monolayer was found to be difficult to study on water, but easier on an aqueous SHP solution.

Figure 1 shows the F—A curves of CA at various values of the SHP concentration, c_2 (mM), the solution of the latter containing $c'=10$ mM of NaOH in order to prevent the binding of the proton to SHP. Figure 1 indicates the following: 1: The CA monolayer is not supercompressed, since the agreement between the data obtained by the SAD method and by the ICA method is good. 2: Each curve has one kink point, to be called the collapse point, with a collapse pressure, F_c, and a collapse area, A_c. 3: The value of F remains constant at F_c when the monolayer is compressed further beyond the collapse point. 4: The F–A curves move toward higher F values when c_2 is larger. Results similar to these have been obtained for CH as well.

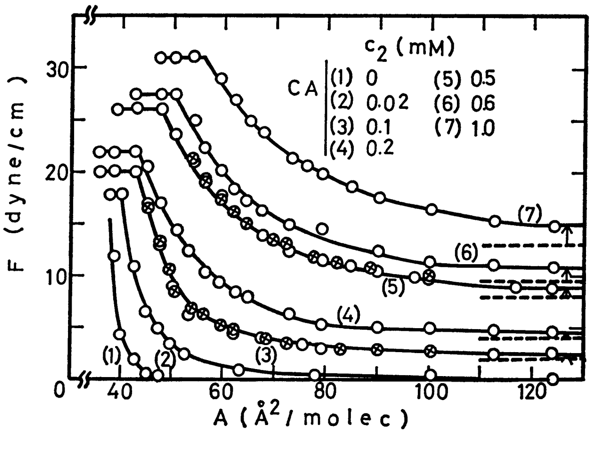

Fig. 1. Relation between the surface pressure, F, and molecular area, A, of CA monolayer at various SHP concentrations, c_2, and at fixed concentration of added NaOH, $c'=10$ mM, at 25 °C, by SAD-method (○) and ICA-method (⊗).

The surface pressure, F, is defined ,as usual, on the basis of the surface tension of pure water, γ_w, by means of the following equation:

$$F=\gamma_w-\gamma \quad (2)$$

where γ is the surface tension of the surface covered with a monolayer. If there is no insoluble monolayer, the surface pressure of the adsorption monolayer of SHP, F_{SHP}, is given by:

$$F_{SHP}=\gamma_w-\gamma_{SHP} \quad (3)$$

where γ_{SHP} is the surface tension of the SHP solution. Each F–A curve on a SHP solution shown in Fig. 1 approaches, when A becomes sufficiently large, a horizontal broken line which represents the F_{SHP}-value. Therefore, the shift of the F–A curves with c_2 toward higher F-values is mainly due to the increase in F_{SHP}. To eliminate this, the practical surface pressure, F', is introduced; it is defined on the basis of the surface tension of the substrate instead of that of pure water:

$$F'=\gamma_{SHP}-\gamma=F-F_{SHP} \quad (4)$$

According to this equation, Fig. 1 is redrawn as Fig. 2, in which the practical collapse pressure, F_c', has turned out to be independent of c_2, at least within the range examined here. On the other hand, the molecular area increases with c_2. This will be discussed in detail in a separate paper.

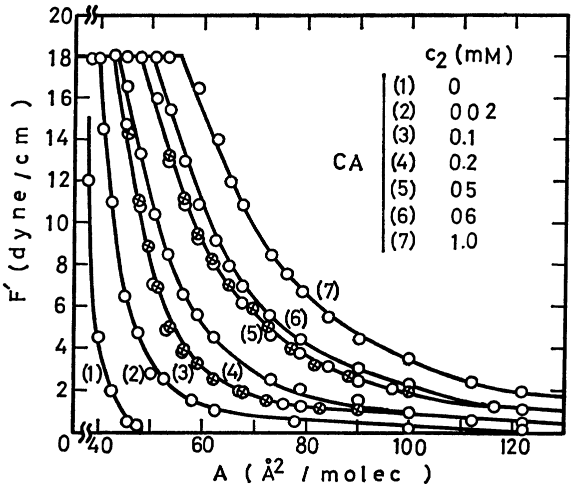

Fig. 2. Relation between the practical surface pressure, F', and molecular area, A, of CA obtained from the data shown in Fig. 1.

Mixed Monolayer of CH and CA. In this case of mixed monolayers, too, the practical surface pressure, F', is adopted: the results obtained for the mixed monolayer of CH and CA on the SHP solution of $c_2=$ 0.5 mM are shown in Fig. 3. It may be seen in this figure that each F'–A curve of the CH–CA mixed monolayer has, in general, two kind points, in contradistinction to the CH and CA monolayers which had only one kind point. Both of the two kink points of each F'–A curve are collapse points, as will be discussed in detail later on. The mixed monolayer, therefore, undergoes a two-step collapse.

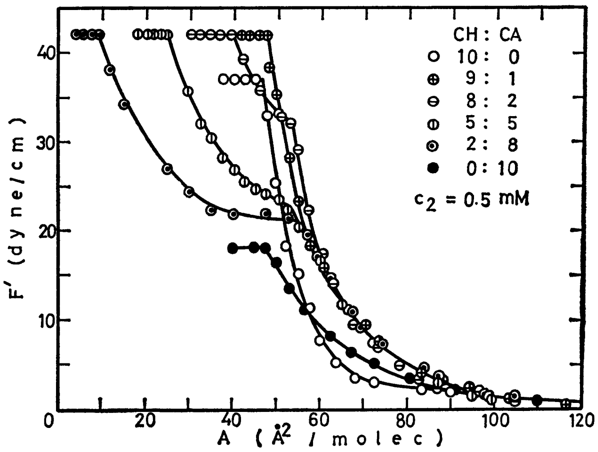

Fig. 3. Variation of F'-A curves of CH–CA monolayers with the composition, at $c_2=0.5$ mM, $c'=10$ mM and at 25 °C.

The collapse pressure and the collapse area at the lower collapse point, which is one located at the lower surface pressure, are designated by $F'_{c\,l}$ and $A'_{c\,l}$, while those at the higher collapse point are designated by $F'_{c\,h}$ and A_{ch}. Therefore, as may be seen from Fig. 3, $F'_{c\,l}$ depends on the composition of the mixed monolayer, whereas $F'_{c\,h}$ is independent of the composition of the mixed monolayer and F' remains unchanged at $F'_{c\,h}$ when the monolayer is compressed further. Two other points particularly noteworthy in Fig. 3 are that the mixed monolayer at the mole ratio of CH : CA $=9:1$ has only one collapse point, while the F'_c of this monolayer coincides with $F'_{c\,h}$ at other compositions.

Mixed Monolayer of CA and CP. Before studying the mixed monolayer, the monolayer of CP was examined; it is shown in Fig. 4. The data obtained by the SAD method show that the F'_c of CP increased with c_2 even when the value is expressed as the practical surface pressure, in contradistinction to the cases of CH and CA mentioned above. This is verified to be due to the supercompression on the basis of the observation of the change with time and measurements by the ICA method as follows: 1: The values of F'_c decrease with the time as is shown by the vertical arrows in Fig. 4, and stop decreasing at $F'_c=7.5$ dyn/cm, the value at $c_2=0$ (pure water). 2: The F'—A curves obtained by means of the ICA method showed no change with time, agreed well with the curves obtained by means of the SAD method in the region of low F' values, and gave the value of $F'_c=7.2$ dyn/cm, which is practically equal to the above-mentioned value of the "equilibrium" collapse pressure obtained by means of the SAD method. In Fig. 4, an example obtained by means of the ICA method is shown for $c_2=0.5$ mM.

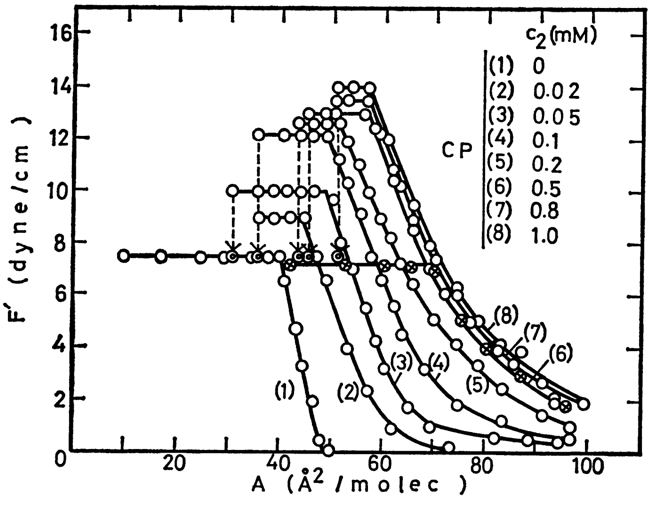

Fig. 4. The F'-A curves of CP monolayer at various c_2 values ($c'=10$ mM) at 25 °C. SAD-method (○), its equilibrium values (◎), and ICA-method (⊗).

The mixed monolayer of CA and CP at $c_2=0.5$ mM gave the F'–A curves shown in Fig. 5, obtained by means of the SAD method. The general features of the CA–CP system are identical with those of the CH–CA system described in the preceding section, since each F'–A curve has, in general, two collapse points except

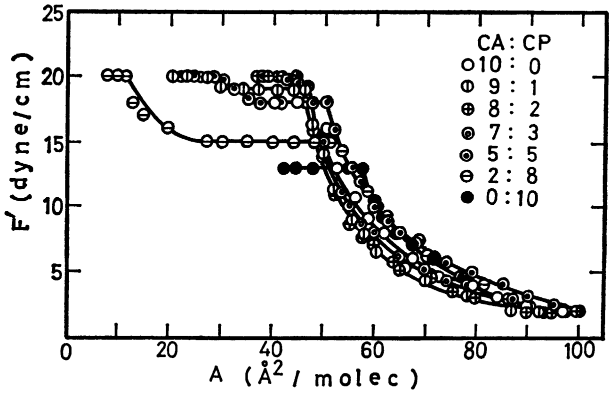

Fig. 5. Variation of F'-A curves of CA–CP monolayers with the composition, at $c_2=0.5$ mM, $c'=10$ mM and at 25 °C.

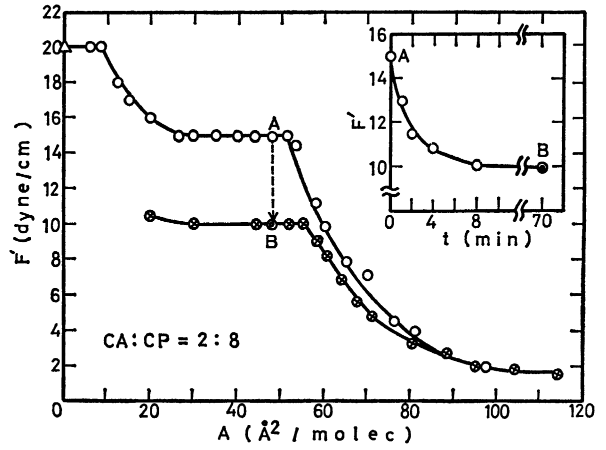

Fig. 6. Comparison of the practical surface pressure, F', obtained by SAD- and ICA-method and the variation with time, t, of the mixed monolayer at the mole ratio of CA : CP=2 : 8, at $c_2=0.5$ mM, $c'=10$ mM and at 25 °C.
SAD-method (○), its equilibrium values (◎), ICA-method (⊗), and the equilibrium spreading pressure, F'_s (△).

for CA : CP=8 : 2, the F'_{ch} is independent of the composition and is in agreement with F'_{s}, and F'_{cl} depends on the monolayer composition. In contradistinction to the CH–CP system, however, the value of F'_{cl} of this CA–CP system was not steady, but decreased with the time.

As example, the case of a mixed monolayer with the mole ratio of CA : CP=2 : 8 is shown in Fig. 6. The data were obtained by means of the SAD method. Moreover, the vertical arrow in Fig. 6 shows that F' decreased, while the surface area was kept constant, from A to B with the time, t (min), as is shown in the insert; the "equilibrium" collapse pressure at B coincides with the F'_{cl} obtained by means of the ICA method. The same result was also obtained from the experiments at other compositions of the CA–CP system.

Mixed Monolayer of CP and CH. The F'–A curves of CP–CH systems at c_2=0.5 mM are given in Fig. 7. As for the CH monolayer, the agreement between the data obtained by means of the SAD method and ICA method is shown. All the others were obtained by means of the ICA method, becase the time dependence of F' was expected for the monolayers containing CP. The broken lines were drawn according to the calculated value of F'_{ch} and by extrapolation from lower F'-values in order to estimate the A_{ch} values, which will be discussed later on.

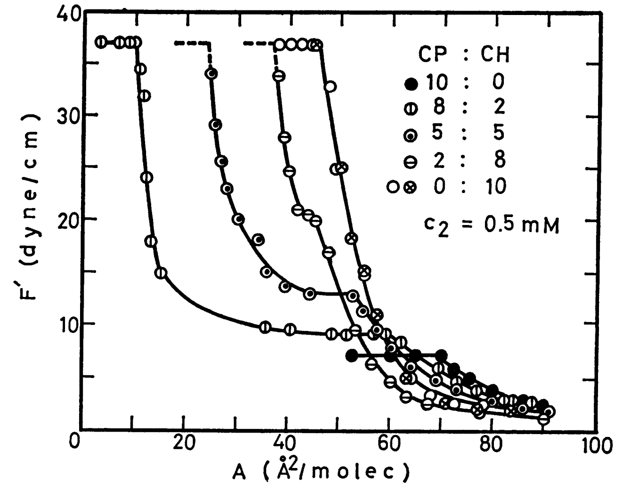

Fig. 7. Variation of F'–A curves of CP–CH monolayers with the composition, at c_2=0.5 mM, c'=10 mM and at 25 °C, by DAD-method (○) and ICA-method (all others).

Discussion

Two-step Collapse. According to the experimental results shown in Figs. 3, 5, and 7, each F'–A curve of the mixed monolayer consisting of two components out of CH, CA and CP has, in general, two collapse points, indicating that the mixed monolayers undergo a two-step collapse when they are compressed.

The dependence of F_{c} of the monolayer composition is shown in Fig. 8, taking ,on the abscissa, the mole fraction in the insoluble components, X_4, as defined by:

$$X_4 = n_4/(n_3+n_4) \tag{5}$$

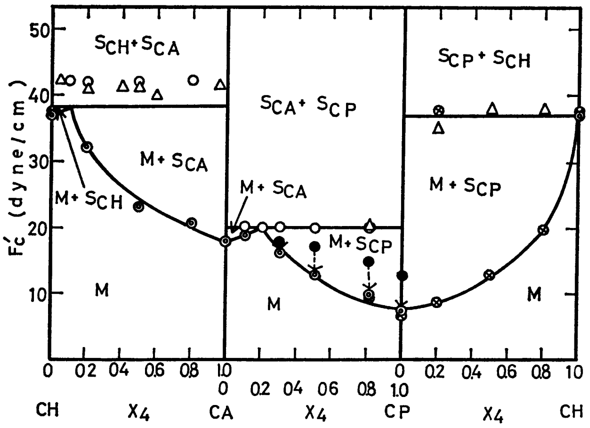

Fig. 8. Relation between the collapse pressure, F'_{c}, and mole fraction X_4, for CH–CA, CA–CP, and CP–CH systems at c_2=0.5 mM, c'=10 mM and at 25 °C. Solid lines are theoretical by Eq. (15). F'_{ch} (○), F'_{cl} by SAD-method (●), and its equilibrium values (◎), F'_{c} by ICA-method (⊗), and F'_{s} (△).

Here, n is the moles and the 3 and 4 suffixes indicate the respective insoluble components, reserving the 1 and 2 suffixes for water and SHP respectively. It has been confirmed here as well as experimentally that the shift of curves with c_2 is mostly cancelled by taking F' instead of F. In Fig. 8, the values of the equilibrium-spreading pressure, which is measured on the same SHP solution and expressed in terms of the practical surface pressure, F'_{s}, are also plotted to show that the values are independent of X_4 and agree well with F'_{ch}.

Figure 8 shows the dependence of the practical surface pressure at these two collapse points, F_{cl} and F'_{ch}, on the mole fraction in the insoluble components, X_4, indicating that F'_{ch} is independent of X_4 and agrees with F'_{s}, while F'_{cl} varies with X_4 and its curves merge with F'_{ch} at one point, which can be called a "concollapse point". As a result, the two mixed monolayers of CH : CA=9 : 1 (Fig. 3) and CA : CP= 8 : 2 (Fig. 5) have only one collapse point, whose collapse pressure agrees with the F'_{ch} values of the monolayers at other X_4 values. Thus, Fig. 8 is completely analogous to the ordinary-phase diagram representing the pressure-composition relation, at a given temperature, of a solid-liquid equilibrium of mixtures which have an eutectic point[9] and may be considered to be a phase diagram representing the equilibrium between the monolayer and the bulk phase separated from the monolayer due to the collapse.

Therefore, the following conclusions may be drawn: When a mixed monolayer is compressed, one of the components, which is presented in more than the concollapse composition, starts separating at F'_{cl}. This separation continues during the further compression, accompanying the increase in F' and the approach of the value of X_4 to that of the concollapse monolayer. When the F' reaches F'_{ch}, the other component starts separating simultaneously, but this second deposit does not mix with the first one. Under a given pressure, temperature, and SHP concentration, the freedom of the system become zero when the two bulk phases have been separated.[2,3,10)] Therefore, further compression beyond the higher collapse point does not change F' from F'_{ch}, the latter being independent of the composition of the starting monolayer, X_4, and being equal to F'_{s}. Thus, in the regions designated by M, M+S and S_3+S_4 in Fig. 8, there exist the monolayer, the monolayer and a bulk phase, and two bulk phases respectively.

In the CP–CH system, both the F'_{ch} and F'_{s} of the mixed monolayers agree with those of pure CH. This is because the F'_{c} of CP is much lower than that of CH, that means CP is far easier to collapse than CH, so that the component remaining in the concollapse monolayer is mostly CH.

Collapse Pressure and Composition. The monolayer to be studied here has three components, because the mixed monolayer of two insoluble components, 3 and 4, contains also the soluble component 2 (SHP in our case), which has penetrated from the substrate: The chemical potential of the j component in the monolayer, μ_j^{m}, is written:[10)]

$$\mathrm{d}\mu_j^{\mathrm{m}} = kT\,\mathrm{d}\ln\xi_j + \omega_j\mathrm{d}F \qquad (j=2, 3 \text{ or } 4) \qquad (6)$$

or, if the SHP concentration c_2 is constant so that F_{SHP} is also constant:

$$\mathrm{d}\mu_j^{\mathrm{m}} = kT\,\mathrm{d}\ln\xi_j + \omega_j\mathrm{d}F' \qquad (j=2, 3 \text{ or } 4) \qquad (6a)$$

Here, ξ_j is the mole fraction of the j component in the monolayer:

$$\xi_j = n_j/(n_2+n_3+n_4) \qquad (7)$$

which is different from the mole fraction to X_j, defined by Eq. (5), and ω_j is the partial molecular area of the j component and is related to the (average) molecule area of the insoluble components, A, by:

$$A = \nu_2\omega_2 + X_3\omega_3 + X_4\omega_4 \qquad (8)$$

where ν_2 is the mole of the penetrated component, 2. per mole of insoluble components:

$$\nu_2 = n_2/({}_3 + n_4) \qquad (9)$$

The mole fraction which can be assigned experimentally is not ξ_i, but X_i. Therefore, by using the relation:

$$\mathrm{d}\ln\xi_i = \mathrm{d}\ln X_i - [\xi_i/(1-\xi_i)]\,\mathrm{d}\ln\xi_i \qquad (i=3 \text{ or } 4) \qquad (10)$$

Equation (6a) can be rewritten when c_2 is constant and $\mathrm{d}\,\mu_2^{\mathrm{m}}=0$:

$$\mathrm{d}\,\mu_i^{\mathrm{m}} = kT\,\mathrm{d}\ln X_i + A_{0i}\mathrm{d}F' \qquad (i=3 \text{ or } 4) \qquad (11)$$

where;

$$A_{0i} = \omega_i + \nu_2\omega_2 \qquad (i=3 \text{ or } 4) \qquad (12)$$

This equation may then be used to determine the penetration amount of the 2 component, as will be discussed in a separate paper to be published later. The quantity, A_{0i}, defined by Eq. (12) is a kind of partial molecular area of the i component on the surface of the solution of the 2 component; it is related to the average molecular area of the insoluble components, A, by:

$$A = X_3A_{03} + X_4A_{04} \qquad (13)$$

When a mixed monolayer is in equilibrium with the bulk phase, i, separated from the monolayer at the lower collapse point, the chemical potential of the i component in the monolayer, μ_i^{m}, is equal to that in the bulk phase, μ_i^{b}, and the latter is independent of the monolayer composition, X_i, because the other insoluble component is not separated at the lower collapse point. Integrating Eq. (11) with $\mathrm{d}\,\mu_i^{\mathrm{m}} = \mathrm{d}\,\mu_i^{\mathrm{b}} = 0$, the following is obtained for the lower collapse pressure:

$$F'_{\mathrm{c\,l}} = F'_{0\,i} - (kT/A_{0i})\ln X_i \qquad (i=3 \text{ or } 4) \qquad (14)$$

Here, $F'_{0\,i}$ is introduced to determine the integration constant on condition that $F'_{\mathrm{c\,l}} = F'_{0\,i}$ at $X_i=1$; it is the collapse pressure of the monolayer of the i component on the aqueous SHP solution. To obtain Eq. (14), it has been assumed that A_{0i} is a constant. This is acceptable because the molecular area at the lower collapse point, A_{cl}, does not change much with the composition, X_i, at a given c_2.

According to Eq. (14), the theoretical values of $F'_{\mathrm{c\,l}}$ shown in Fig. 8 by solid curves as a function of X_4 are calculated by using the parameter values, $F'_{0\,i}$ and A_{0i}, obtained experimentally from the collapse point of the one-component monolayer on the aqueous SHP solution; these values are given in Table 1. The agreement between the theoretical curves and the experimental plots in Fig. 8 is good. The intersection of two curves is the concollapse point. For this point, the practical surface pressure, F'_{e}, and the mole fraction, X_{e}, are calculated; they are given in Table II. The concollapse pressure, F'_{e}, is, as has been stated before, equal to the higher collapse pressure, F'_{ch}, shown by the horizontal solid line in Fig. 8. The agreement between the theoretical value and the experimental plot is, again, fairly good.

TABLE 1. EXPERIMENTAL VALUES OF THE PARACTICAL COLLAPSE PRESSURE, $F'_{0\,i}$, AND COLLAPSE AREA, A_{0i}, AT c_2=0.5 mM AND c'=10 mM AT 25 °C

Component i	F_{0i} (dyn/cm)	A_{0i} (Å^2/molec.)
CH	36.9	46.5
CA	18.0	47.0
CP	7.5	55.0

TABLE 2. CALCULATED VALUES OF CONCOLLAPSE PRESSURE, F'_{e}, AND CONCOLLAPSE MOLE FRACTION, X_{e}, AT c_2=0.5 mM AND c'=10 mM AT 25 °C

Components 3—4	F_{c} (dyn/cm)	$X_{\mathrm{e},4}$
CH-CA	38.2	0.11
CA-CP	20.0	0.20
CP-CA	37.0	0.98

Collapse Area and Composition. The relation between the collapse area, A_{c}, and X_4 at c_2=0.5 mM is shown in Fig. 9. The molecular area at the higher collapse point, A_{ch}, is, of course, smaller than that at the lower collapse point, A_{cl}. The latter does not depend much on the composition, as has been mentioned before, while the former varies almost linearly with X_4,

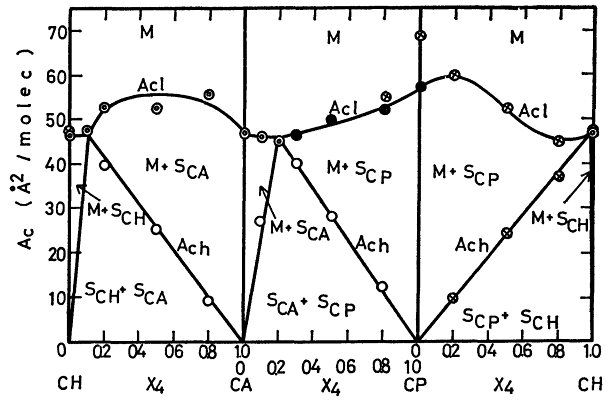

Fig. 9. Relation between the collapse area, A_c, and the mole fraction, X_4, for CH–CA, CA–CP, and CP–CH systems at c_2=0.5 mM, c'=10 mM and at 25 °C. Symbols are same as in Fig. 8.

describing a triangle, the top of which is the concollapse point with the molecular area of A_e and the mole fraction of X_i.

When a mixed monolayer, consisting of n_3 and n_4 moles of two insoluble components, 3 and 4, respectively, is compressed beyond the lower collapse point, the 4 component (if $X_4 > X_{e,4}$) is separated from the monolayer, resulting in a marked decrease in the surface area, S. Therefore, the molecular area, A, calculated on the basis of the initial moles of the insoluble components (n_3+n_4):

$$A = S/[(n_3+n_4)N_A] \tag{1a}$$

decreases remarkably at the lower collapse point.

When the monolayer reachs the higher collapse point upon further compression, the mole fraction in the monolayer becomes equal to that of the concollapse monolayer, X_e, and the total area is expressed as $S = A_e\ (n_3/X_{e,3})N_A$, because n_3 does not change in the process. Therefore, after the introduction of this into Eq. (1a),

$$A_{ch} = A_e X_3/X_{e,3} \quad (X_4 > X_{e,4}) \tag{15}$$

or:

$$A_{ch} = A_e X_4/X_{e,4} \quad (X_4 < X_{e,4}) \tag{16}$$

These equation are tested in Fig. 10 and proved to be satisfactory, since the experimental A_{ch} value is found to be proportional to $(X_i/X_{e,i})$, the proportionality coefficient being A_e=46.0 (Å^2/molec.) if the $X_{e,i}$ values given in Table II are used. The straight line in Fig. 9, drawn by using this value of A_e and the values of $X_{e,4}$ given in Table 2, agree well with the experimental plots.

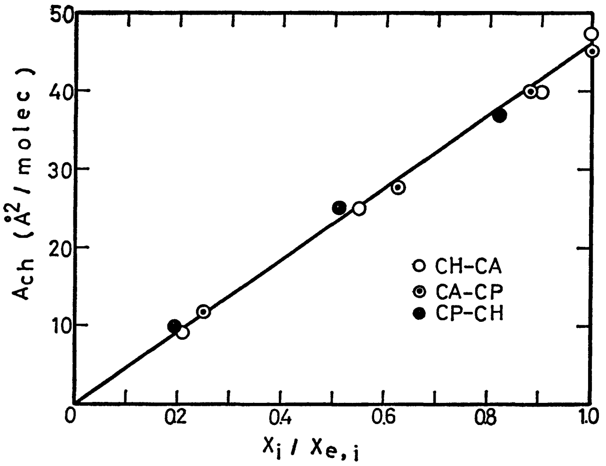

Fig. 10. The proportionality of A_{ch} to $(x_i/X_{e,i})$ expected by Eq. (15), for CH–CA (○), Ca–CP (◎), and CP–CH(●).

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