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FIRST AND SECOND C.M.C. OF PHOSPHATIDYLCHOLINE IN CHLOROFORM

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Synthetic phosphatidylcholine possesses the first and second c.m.c. in chloroform. The disagreement among c.m.c. values obtained from various methods was due to the fact that different methods detect different c.m.c., *i.e.* the first and second c.m.c. and the intermediate values. The average association number of first micelle was 2 and that of second micelle was 4.

Phosphatidylcholine is known to be soluble in chloroform and to form relatively small micelle. Its critical micelle concentration, c.m.c., has been determined by several methods, but the values were different with each other. The values from interfacial tension, vapor pressure depression[1)] and NMR[2)] measurements were the order of 10^{-4}, 10^{-3} and 10^{-2} mol l^{-1}, respectively. The disagreement could be elucidated by considering the existence of both first and second c.m.c.

(1) Vapor pressure depression measurement: A molecular weight apparatus (Hitachi-Perkin-Elmer, Type 115) was used. The thermister bridge reading, ΔR, which depends only on the vapor pressure at constant temperature, is plotted against the concentrations of β,γ-dimyristoyl-L-α-phosphatidylcholine(L-C_{14}) and β,γ-dipalmitoyl-DL-α-phosphatidylcholine(DL-C_{16}) (purchased from Fluka AG) and Egg-phosphatidylcholine (prepared in our laboratory[3)]) in chloroform in Fig. 1.

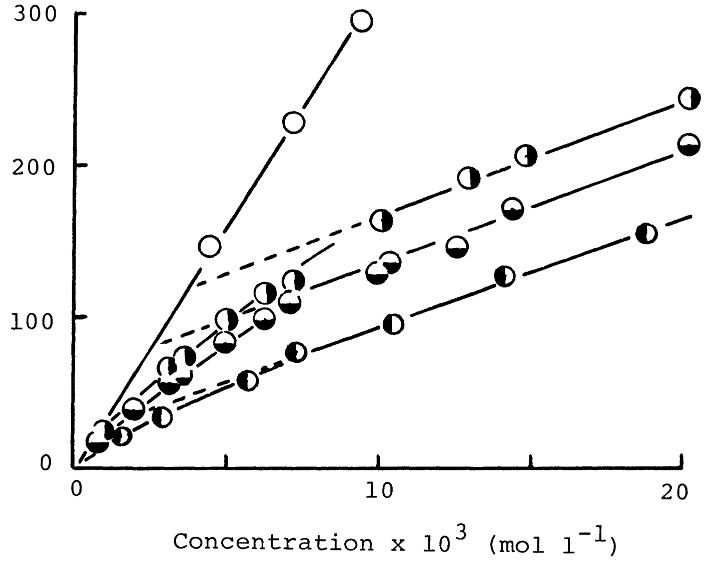

Fig. 1. Thermister bridge reading(ΔR) *vs.* solute concentration in chloroform.

◒ : β,γ-dimyristoyl-L-α-phosphatidylcholine
◑ : β,γ-dipalmitoyl-DL-α-phosphatidylcholine
◐ : Egg-phosphatidylcholine
(Average molecular weight is postulated to be 770)
○ : Benzil

For the above measurement, benzil was used as a standard substance for calibration. The relationship of ΔR *vs.* solute concentration was linear above 1 x 10^{-2} mol l^{-1}. Usually c.m.c. can be given by the intersection of the straight line with the calibration curve and the average association number by the ratio of the slope for solute to that for reference (Method I). Then at 30°C we got 2, 3 and 1 x 10^{-3} mol l^{-1} as c.m.c. of L-C_{14}, DL-C_{16} and Egg-phosphatidylcholine, respectively, and 4 as association number of each solute.

But, in this report an alternative

analysis (Method II) was adopted for the determination of c.m.c. and association number. At the first, we classified three concentration regions in equilibrium as shown in Table I. Assumptions: Below 1st-c.m.c., solute exists only as monomer. In the intermediate region between 1st- and 2nd-c.m.c., monomer and 1st-micelle, whose association number is n_1, co-exist. Above 2nd-c.m.c., monomer, 1st-micelle and 2nd-micelle, whose association number is n_2, co-exist.

Table I. Scheme of Monomer-Micelle Equilibrium.

Concentration region	Below 1st-c.m.c.		Intermediate region		Above 2nd-c.m.c.
Species	monomer		monomer $\rightleftarrows$ 1st-micelle		monomer $\rightleftarrows$ 1st-micelle $\rightleftarrows$ 2nd-micelle
Monomer concentration	C		C_m		C_m
1st-micelle concentration	0	1st-c.m.c.	$C-C_m$	2nd-c.m.c.	C_M
2nd-micelle concentration	0		0		$C-C_m-C_M$
Total concentration	C		C		C

The vapor pressure depression value caused by the existence of unit concentration of solute will be denoted by D. Then below 1st-c.m.c., observed vapor pressure value δ_{obs} can be written as follows.

$$\delta_{obs} = CD \qquad (1)$$

or

$$\frac{\delta_{obs}}{D} = C \qquad (1')$$

or

$$\frac{\delta_{obs}}{CD} = 1 \qquad (1'')$$

where C is the total concentration. Between 1st- and 2nd-c.m.c., δ_{obs} can be written as follows.

$$\delta_{obs} = C_m D + \frac{C - C_m}{n_1} D \qquad (2)$$

where C_m is the monomer concentration above 1st-c.m.c.. Eq. 2 can be rearranged as Eq. 2' or Eq. 2".

$$\frac{\delta_{obs}}{D} = C_m\left(1-\frac{1}{n_1}\right) + \frac{1}{n_1}C \qquad (2')$$

$$\frac{\delta_{obs}}{CD} = \frac{C_m}{C}\left(1-\frac{1}{n_1}\right) + \frac{1}{n_1} \qquad (2'')$$

When C_m is constant above 1st-c.m.c. and equal to it, by plotting against C we can get $1/n_1$ or n_1 from the slope of the line, and 1st-c.m.c. from the intersection of two lines expressed as Eq. 1' and 2'. For the study of the behavior of solute in relatively high concentration region, Eq. 2" is more convenient to use than Eq. 2'. We can get $1/n_1$ as the intersection of the line expressed as Eq. 2" on ordinate. Above 2nd-c.m.c., δ_{obs} can be expressed as Eq. 3 or Eq. 3'.

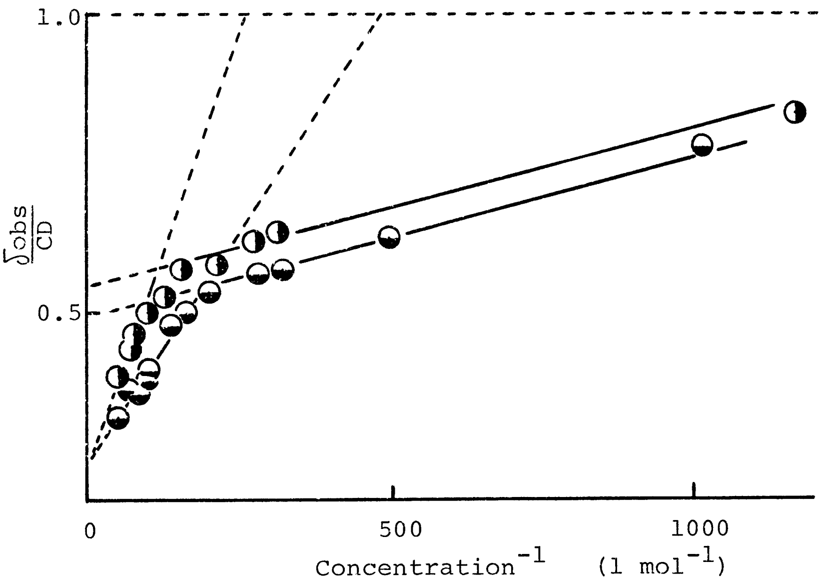

Fig. 2. $\frac{\delta_{obs}}{CD}$ *vs.* $\frac{1}{C}$ plots.

◒ : β,γ-dimyristoyl-L-α-phosphatidylcholine

◑ : β,γ-dipalmitoyl-DL-α-phosphatidylcholine

$$\delta_{obs} = C_m D + \frac{C_M}{n_1} D + \frac{C - (C_m + C_M)}{n_2} D \qquad (3)$$

$$\frac{\delta_{obs}}{CD} = \frac{1}{C}\left\{C_m\left(1-\frac{1}{n_2}\right) + C_M\left(\frac{1}{n_1}-\frac{1}{n_2}\right)\right\} + \frac{1}{n_2} \qquad (3')$$

where C_M is the concentration of 1st-micelle in monomer unit. When C_m, C_M, n_1 and n_2 are constant in the concentration region where total concentration $C >$ 2nd-c.m.c., we can expect that δ_{obs}/CD *vs.* 1/C plot represents a straight line and $1/n_2$ can be obtained from the intersection of the line on ordinate. 2nd-c.m.c. can be obtained from the intersection of two lines expressed as Eq. 2" and Eq. 3'. δ_{obs}/CD *vs.* 1/C plots of L-C_{14} and DL-C_{16} were shown in Fig. 2, where the relations expressed by Eq. 2" and Eq. 3' are experimentally confirmed. However, δ_{obs}/CD *vs.* 1/C plot of Egg-phosphatidylcholine did not show clear 2nd-c.m.c.

(2) Interfacial tension measurement: A phosphatidylcholine is not expected to have surface activity at air/organic media interface since ordinary oil soluble surfactant except fluorocarbon derivative does not. But, it was previously reported that Aerosol OT, typical oil soluble surfactant, shows interfacial activity at *polar phase/organic solution* interface by drop weight method$^{4)}$. As shown in Fig. 3, the plot of interfacial tension *vs.* Egg-phosphatidylcholine concentration in logarithmic scale shows a behavior similar to that of surface tension of aqueous solutions of surfactants near c.m.c.. We identified the breaking point in Fig. 3 as c.m.c. of phosphatidylcholine in chloroform, *i.e.* 1×10^{-4} mol l^{-1}.

(3) Discussion : In Fig. 4, δ_{obs}/CD *vs.* 1/C relation are schematically shown. (i) The c.m.c. value estimated from Fig. 1 according to Method I corresponds to Point B" in Fig. 4 and shows an intermediate value between the 1st- and 2nd-c.m.c.. (ii) The NMR method for determining c.m.c. is based on the measure of environmental difference around a particular proton or conformational difference of solute between above and below c.m.c.. R. Herz et al obtained 1.4×10^{-2} mol l^{-1} as c.m.c. value of DL-C_{16} in chloroform at 24°C from the analysis of H_2O chemical shift associated with its polar group$^{2)}$. This value coincided with the 2nd-c.m.c. from Method II and therefore may correspond to Point B' in Fig. 4. Of course, it can be expected to obtain the value of 1st-c.m.c. from NMR, by measuring the chemical shift in extreme low concentration region in more

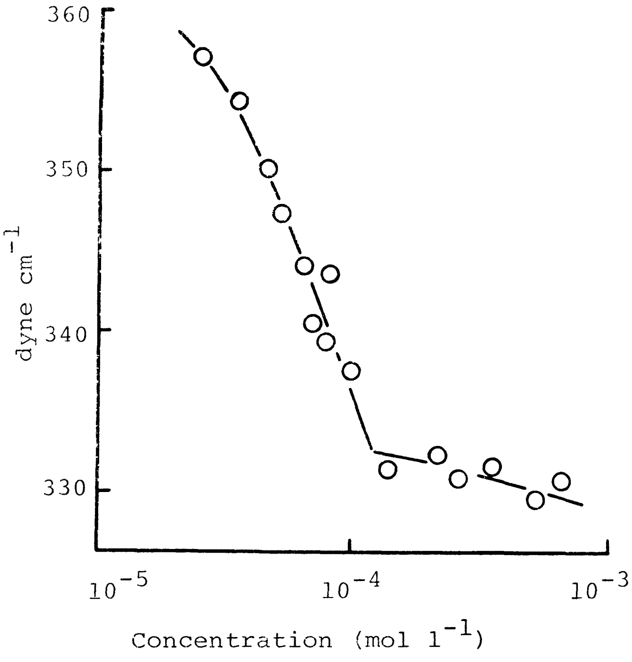

Fig. 3. Interfacial tension between mercury and chloroform solutions *vs.* Egg-phosphatidylcholine concentration in logarithmic scale.

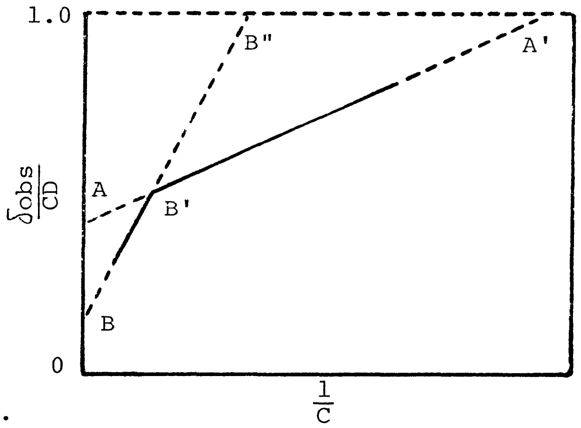

Fig. 4. Scheme of $\frac{\delta_{obs}}{CD}$ *vs.* $\frac{1}{C}$ plot

Table II. First and Second C.m.c. Estimated by the Various Methods (mol l^{-1})

	Method I	Method II	Interfacial tension	NMR*2
L-C_{14}	intermediate 2×10^{-3}	first 5×10^{-4}		
		intermediate 2×10^{-3}		
		second 6×10^{-3}		
DL-C_{16}	intermediate 3×10^{-3}	first 5×10^{-4}		second 1.5×10^{-2}
		intermediate 3×10^{-3}		
		second 1×10^{-2}		
Egg*1	intermediate 1×10^{-3}		first 1×10^{-4}	

*1 Average molecular weight is postulated to be 770.

*2 Reference 2.

detail. (iii) The breaking point of interfacial tension *vs*. solute concentration in logarithmic scale shows the concentration at which the first micelle appears. Therefore, the concentration corresponds to Point A' in Fig. 4, and shows the 1st-c.m.c.. The c.m.c. values obtained from several methods are summarized in Table II. The average association numbers of the first and second micelle can be estimated from Point A and Point B, respectively, and are summarized in Table III.

(4) Conclusion: Synthetic phosphatidylcholine possesses 1st- and 2nd-c.m.c.. The disagreement among c.m.c. values obtained from various methods of measurements was found to be due to the fact that each method does not detect the identical c.m.c., but different c.m.c., *i.e.* first and second c.m.c. and the intermediate values.

Table III. Association Number n_1(First Micelle) and n_2(Second Micelle)

	Method II		NMR*2
	n_1	n_2	
L-C_{14}	2	4	
DL-C_{16}	2	4	3
Egg*1		4(Method I)	

*1 Average molecular weight is postulated to be 770.

*2 Reference 2

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