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SOLUBILITY OF PHOSPHATIDYLCHOLINE IN CHLOROFORM. FORMATION OF HYDROGEN BONDING BETWEEN PHOSPHATIDYLCHOLINE AND CHLOROFORM

Mitsuyo OKAZAKI and Ichiro HARA
Laboratory of Chemistry, Dept. of General Education, Tokyo Medical and Dental University, Kohnodai, Ichikawashi, Japan

and

Tsunetake FUJIYAMA
Dept. of Chemistry, Faculty of Science, Tokyo Metropolitan University, Setagayaku, Tokyo

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The solubility of phosphatidylcholine (PC) was studied by the spectroscopic analysis and the measurement of the solubility. The qualitative analysis of infrared absorption spectra confirmed the existence of two types of hydrogen bondings between chloroform and PC, one between chloroform and the C=O group of PC and the other between chloroform and the phosphorylcholine group of PC. The quantitative analysis of the C–D stretching vibration bands of the chloroform-d solution of PC showed that the latter hydrogen bonding mainly contributes to the solubility and that PC dissolves in chloroform to form a complex consisting of a few or more molecules of chloroform and one molecule of PC. We discussed in this report about the molecular organization of PC in chloroform solution.

I. Introduction

It is well known that phosphatidylcholine (PC) has high solubility in chloroform, but few studies on its solubility and on the factor contributing to that have been reported. Phospholipids are generally soluble in chloroform. Comparing the solubility of phosphatidylethanolamine (PE) in chloroform to that of PC, PE containing saturated fatty acids does not dissolve in chloroform [1]. On the other hand, PC is easily soluble in chloroform independently of the chain length and the degree of saturation of fatty acids. The difference of the solubilities is attributed to the chemical structure of the head groups, phosphorylcholine and phosphorylethanolamine.

In this report, we verified the formation of hydrogen bonding between PC and chloroform by the analysis of infrared spectra and determined the number of chloro-

form molecules bonded to PC by using the method described by the authors [2]. We discussed the factor contributing to the solubility of PC in chloroform by the results of spectroscopic analysis and the measurement of solubility.

II. Experimental

A. Materials

PC (DL-α-dipalmitoylglycerylphosphorylcholine) was a commercial product (Nutritional Biochemical Co. USA) and was used after the purification by column chromatography to eliminate free fatty acids, lysophosphatidylcholine and other contaminants and the recrystallization from chloroform–acetone in checking its purity by thin layer chromatography. Palmitic acid (Kao Soap Co. LTD., Tokyo) was used after the recrystallization from acetone three times in checking its purity by gas chromatography. Chloroform and carbon tetrachloride of special grade were purchased from Wako Pure Chemical Industries, LTD., Tokyo. Chloroform was filtered through a column of activated alumina just before use. Chloroform-d was purchased from Merk and Co. LTD., and was used without further purification.

B. Absorption measurements of infrared spectra

The absorption spectra were recorded with the JASCO IR-G grating spectrometer under resolution of 1 cm^{-1}. The spectra of the solution of PC were measured with a KBr cell having a thickness of 0.1 mm. The thickness of the sample cell was checked by the interfringe method.

The chloroform-d solutions containing PC in the concentration range from zero to 0.371 moles per litre were prepared just before measurements by weighing PC and chloroform in the sample flask. Since PC is very hygroscopic, it was dried over phosphorous pentoxide in vacuo at 0.03 mm of Hg at 60°C for a few hours. Chloroform-d was thoroughly dried with Molecular Sieves 3A (Nishio Industry Co. LTD., Tokyo) and the elimination of water from the chloroform-d solution was confirmed by observing the infrared spectra with no band due to water near 3000 cm^{-1} –4000 cm^{-1}.

The relative intensity of the absorption band is defined as

$$I = f_d \frac{1}{l} \int_{\text{band}} \ln\left(\frac{I_o}{I}\right) d(\ln\nu)$$

in the units of cm^{-1}, where f_d is a correction factor of the local field effect [3], l is a thickness of sample, I_o energy of incident light, I energy of transmitted light

and v frequency of light. Here, f_d is

$$f_d = \frac{9n_D}{(n_D^2 + 2)^2}$$

where n_D is the refractive index of the solution.

The refractive index n_D and the specific gravity of the solution were measured by Abbe's refractometer and a picnometer, respectively, at the same time as the absorption measurements.

All the measurements were done at 20 ± 2°C and the absorption spectra of the C–D stretching vibration for the frequency region from 2500 cm^{-1} to 2000 cm^{-1} were measured with the resolution of 1 cm^{-1} and the scanning speed of 33.3 cm^{-1} per min.

III. Results and discussions

A. Formation of hydrogen bonding between chloroform and PC

Fig. 1 shows the spectra of PC in solutions of carbon tetrachloride, chloroform and chloroform-d. It is seen from these spectra that the bands of 1207 cm^{-1}, 812 cm^{-1} and 656 cm^{-1} in the chloroform solution and the bands of 2220 cm^{-1}, 780 cm^{-1} and 637 cm^{-1} in the chloroform-d solution are never observed in the carbon tetrachloride solution. Since carbon tetrachloride has no hydrogen atom to form hydrogen bonding and all these six bands exist near the fundamental bands of chloroform or chloroform-d, it is clear that these six bands are attributed to chloroform and chloroform-d in the bonded state showing remarkable changes of frequency and intensity. The bands of 1207 cm^{-1}, 812 cm^{-1} and 656 cm^{-1} in the chloroform solution are the C–H bending, the $C–Cl_3$ degenerated stretching and the $C–Cl_3$ symmetric stretching vibrations and the bands of 2220 cm^{-1}, 780 cm^{-1} and 637 cm^{-1} in the chloroform-d solution are the C–D stretching, the $C–Cl_3$ degenerated stretching and the $C–Cl_3$ symmetric stretching vibrations, respectively. The band of the C–H stretching vibration in the bonded state can not be observed due to overlapping with that of PC. These observations suggest the existence of hydrogen bonding between chloroform and PC. In comparison of the C=O stretching vibration bands of PC in fig. 1, the band of 1738 cm^{-1} in the carbon tetrachloride solution appears at 1732 cm^{-1} in the chloroform or chloroform-d solution. The shift of 6 cm^{-1} to low frequency side suggests the existence of some molecular interaction between the C=O group of PC and chloroform or chloroform-d.

B. Properties of hydrogen bonding (qualitative analysis of infrared spectra)

The formation of hydrogen bonding with PC molecule changes many fundamental bands of chloroform or chloroform-d. Of these bands, the C–D stretch-

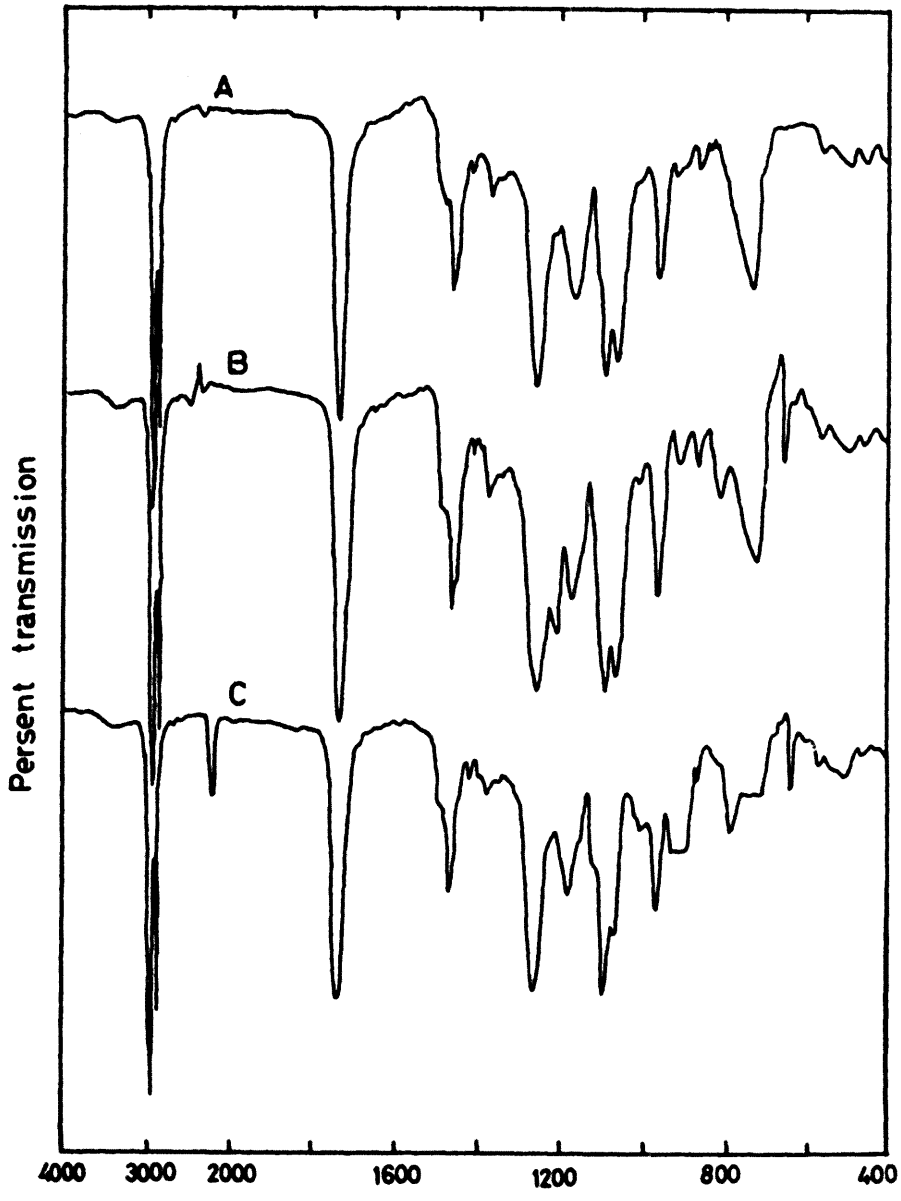

Fig. 1. Infrared spectra of PC in solutions of carbon tetrachloride (A), chloroform (B) and chloroform-d (C). PC concentrations are fixed about 5 weight percents and absorption from solvents is completely cancelled out by putting solvents in the reference side.

ing vibration band of chloroform-d is the most appropriate for quantitative study of hydrogen bonding, because it shows remarkable change due to hydrogen bond formation and is well isolated from other absorption bands. The absorption spectra of the chloroform-d solution of PC in the frequency region of 2500–2000 cm^{-1} are shown in fig. 2. The C–D stretching vibration band of pure chloroform-d is observed with a sharp band at 2254 cm^{-1} (fig. 2B). In the case of the chloroform-d solution of PC, two bands at 2254 cm^{-1} and 2214.5 cm^{-1} are observed (fig. 2A). The former corresponds to that of pure chloroform-d and the latter showing increased intensity and band width and the large shift of 39.5 cm^{-1} to low frequency

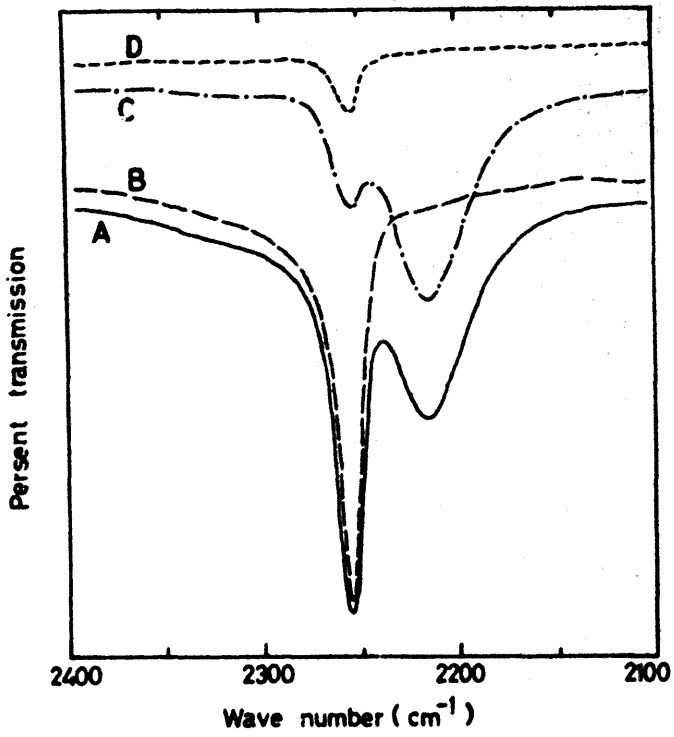

Fig. 2. Infrared spectra of chloroform-d solutions in the C–D stretching region. A: Infrared spectra of chloroform-d solution of PC. PC concentration is 0.171 mole per litre. B: Infrared spectra of pure chloroform-d. C: Infrared spectra of A. D: Infrared spectra of chloroform-d solution of palmitic acid. Palmitic acid concentration is 0.19 moles per litre. In C and D, absorption from free chloroform-d is completely cancelled out by putting chloroform-d in the reference side.

side is attributed to that in the bonded state. Comparing the band width of 2254 cm^{-1} of pure chloroform-d (fig. 2B) with that of the chloroform-d solution of PC (Fig. 2A),the latter is slightly larger than the former, from which we are able to estimate another band in the bonded state near the free band at 2254 cm^{-1}.

The difference spectra of the chloroform-d solution of PC are also shown in fig. 2 to confirm this estimation (fig. 2C). In this spectra another bonded band is observed at 2256 cm^{-1} plus 2214.5 cm^{-1}. The intensity and band width of the bonded band of 2256 cm^{-1} are not so large as that of 2214.5 cm^{-1}. Consequently, it is confirmed that there are at least two types of hydrogen bonding between chloroform and PC molecule.

As described before, it is clear from the frequency shift of the C=O stretching vibration band of PC that there is some molecular interaction between the C=O group of PC and chloroform. The difference spectra of the chloroform-d solution of palmitic acid has the same bonded band at 2256 cm^{-1} as that of the chloroform-d solution of PC (fig. 2D). Accordingly, the band of 2256 cm^{-1} is attributed to the formation of hydrogen bonding between the C=O group of PC and chloroform-d. On the other hand, the band of 2214.5 cm^{-1} is due to the formation of hydrogen bonding between the phosphorylcholine group of PC and chloroform-d.

In these two types of hydrogen bonding, the hydrogen bonding between the phosphorylcholine group of PC and chloroform mainly contributes to the solubility of PC in chloroform. Phosphatidylethanolamine (PE) containing saturated fatty acids does not dissolve in chloroform, although PE has the C=O group. Therefore, we studied the intensity and the properties of the hydrogen bonding between the phosphorylcholine group of PC and chloroform-d by using the method reported by the authors [2].

C. Quantitative analysis of spectral measurements

The absorption spectra of the chloroform-d solutions of PC in various concentrations in the frequency region of 2500–2000 cm^{-1} are shown in fig. 3. For each solution, we are able to determine the relative intensities of the C–D stretching vibration bands in the free and the bonded state, I_f and I_b, respectively.

In table 1, we summarize the specific gravity, d, the refractive index, n_D and the molar concentration of chloroform-d and PC of the solution, C_{CDCl_3} and C_{PC}, where C_{CDCl_3} and C_{PC} are calculated from the weight concentration and the density of the solution.

Since the bonded band is separately observed from the free band in low frequency side, we can directly determine the relative intensity of the C–D stretching vibration band in the bonded state, I_b. As for that in the free state, I_f, we can not determine it directly, because another bonded band between the C=O group of PC and chloroform-d is completely overlapped with the free band. Accordingly, I_f^a in table 2 contains the overlapping bonded band, and so it is indispensable to estimate the

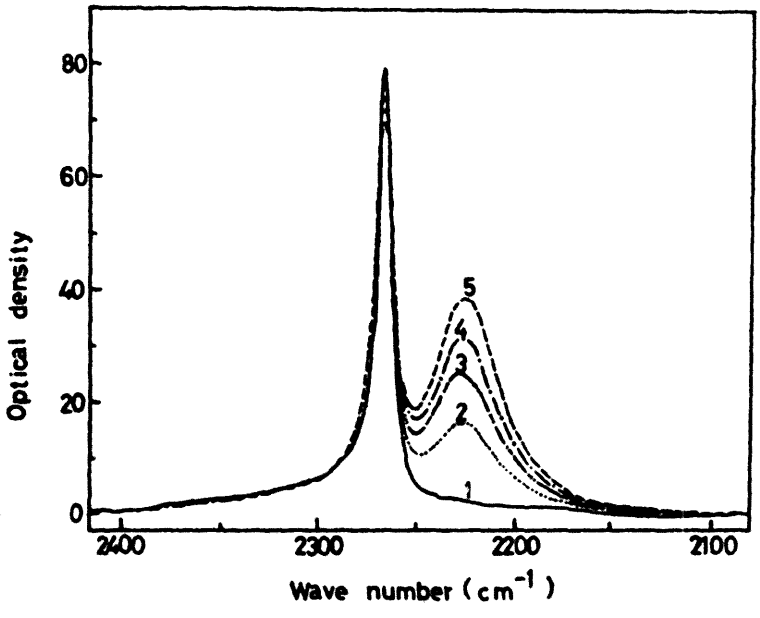

Fig. 3. Infrared spectra of chloroform-d solutions of PC in the C–D stretching region. The number on the absorption bands refers to the sample number in tables 1 and 2.

Table 1
The observed values for the chloroform-d solutions of PC and the C–D stretching vibration bands.

Sample number	Weight % of PC	d	n_D	C_{CDCl_3}	C_{PC}	$-\Delta\nu$(C–D)	$\Delta\nu_{\frac{1}{2}}$
1	0	1.48	1.4470	12.30	0	—	—
2	5.24	1.4468	1.4500	11.39	0.103	39.5	42
3	11.30	1.4251	1.4512	10.79	0.171	39.5	42
4	13.06	1.3981	1.4524	10.12	0.249	39.5	42
5	19.91	1.3675	1.4540	9.06	0.370	39.5	42

n_D is refractive index and d is specific gravity of the solution. C_{CDCl_3} and C_{PC} are the molar concentrations of chloroform-d and PC of the solution in the units of mole per litre. $-\Delta\nu$(C–D) and $\Delta\nu_{\frac{1}{2}}$ are the frequency shift and the half band width of the bonded band in the units of cm^{-1}.

molar absorption coefficient of the C–D stretching vibration band in the bonded state between the C=O group of PC and chloroform-d to calculate I_f from I_f^a. For this purpose, the absorption spectra of the chloroform-d solution of palmitic acid were measured and the molar absorption coefficient was determined with the assumption of 1 : 1 complex formation between the C=O group and chloroform-d. Using this value, we calculate the values of I_f as shown in table 2.

The values of I_f^* or I_b^* in table 2 are the products of I_f or I_b and f_c; where f_c is the correction factor to reduce the molar concentration of chloroform-d of the solution to that of pure chloroform-d. As described in our report [2], by plotting I_f^* against I_b^* at a series of concentrations, a straight line with a slope of $-(\Gamma_b/\Gamma_f)$ is generated so far as the absolute intensities in the free state, Γ_f and those in the bonded state, Γ_b are constant over the concentration range employed. And so, we can calculate Γ_f and Γ_b from the slope of a straight line and the extrapolated value of I_f^* at $I_b^* = 0$.

Table 2
The results of quantitative analysis for the C–D stretching vibration bands of the chloroform-d solutions of PC

Sample number	I_f^a	I_f	I_b	f_c	I_f^*	I_b^*	C_b	n
1	1.316	1.316	0	1.00	1.316	0	0	—
2	1.218	1.115	0.632	1.100	1.227	0.695	0.69	6.7
3	1.168	0.997	1.009	1.177	1.173	1.188	1.10	6.4
4	1.132	0.883	1.327	1.278	1.128	1.696	1.44	5.8
5	1.067	0.697	1.597	1.479	1.031	2.360	1.74	4.7

As for the definition of I and I^*, see Ref. [2]. I_f^a is an apparent relative intensity and I_f is calculated from I_f^a (see the text). f_c is $12.30/C_{CDCl_3}$ and C_b is the molar concentration of chloroform-d in the bonded state (moles per litre). n is the number of chloroform-d molecules bonded to one molecule of PC, C_b/C_{PC}.

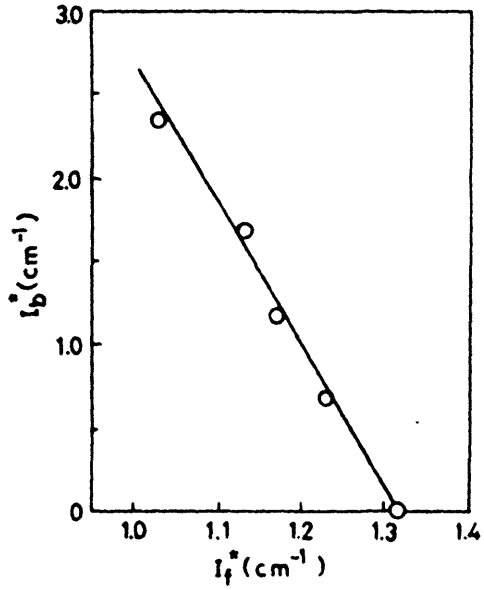

Fig. 4. $I_f^* - I_b^*$ plot for chloroform-d and PC system (see table 2).

The plot of I_f^* versus I_b^* for chloroform-d and PC system is shown in fig. 4. It can be seen from the figure that a linear relationship exists between I_f^* and I_b^* and the values of Γ_f and Γ_b are 107 and 920, respectively. We are able to determine the molar concentration of chloroform-d in the bonded state of the solution, C_b, from Γ_b and I_b. Accordingly, the number of chloroform-d molecules bonded to one molecule of PC, n, can be obtained from C_b and C_{PC}. The calculated values of C_b and n are shown in table 2. It is concluded from table 2 that about 5–7 molecules of chloroform-d and one molecule of PC form a complex in the solution.

The linear relationship between I_f^* and I_b^* shows that the absolute intensities in the free and bonded state, Γ_b and Γ_f, are constant over the concentration range employed. If the ratio of the absolute intensity, Γ_b, in the bonded state to the absolute intensity, Γ_f, in the free state, Γ_b/Γ_f, can represent the strength of hydrogen bonding between chloroform-d and the acceptor molecule, it is concluded that the strength of hydrogen bonding between chloroform-d and the phosphorylcholine group of PC is constant over the concentration range employed. This conclusion dose not contradict with the fact that the frequency shift, $-\Delta\nu$(C–D), and the half band width, $\Delta\nu\frac{1}{2}$, for the C–D stretching vibration band in the bonded state are constant over the concentration range employed as shown in table 1. Although we can not directly determine I_f, it is at least concluded that PC molecule dissolves in chloroform to form a complex consisting of a few molecules of chloroform and one molecule of PC by hydrogen bonding.

The product of the intensity ratio, Γ_b/Γ_f, and the solvation number, n, is an appropriate measure to estimate the stabilization energy of an acceptor molecule through hydrogen bonding formation in the solution. In the system of chloroform-d and PC, the stabilization energy is about 40–60 and this value is nearly the same as the values obtained for some surfactants and chloroform-d systems, 50–100 [2].

This suggests that the stabilization energy through hydrogen bonding formation between chloroform and the phosphorylcholine group of PC is the main factor contributing to the solubility of PC in chloroform.

D. Solubility and molecular organization of PC in chloroform

The solubility curve of PC in chloroform is shown in fig. 5. Two characteristic properties of the solubility of PC in chloroform are observed in fig. 5. The first property is that PC dissolves in chloroform at fairly low temperature when the molar ratio of chloroform to PC is over 10, but it dose not dissolve at 60°C when the molar ratio is below 6. The second property is that there is a critical solubility temperature (CST) near −10°. The first can be explained by the spectroscopic analysis of a complex formation consisting of a few molecules of chloroform and one molecule of PC. As for the second, the existence of a CST is experimentally confirmed in other systems by the authors [4], where we can observe a CST depending on the length of the alkyl chain using a series of alkylphosphorylcholines synthesized by the authors. Consequently, it can be concluded that the existence of a CST is a characteristic phenomenon observed in the solubilities of the compounds carrying the phosphorylcholine group in chloroform. Comparing this phenomenon with Kraft point observed in an aqueous solution of surfactants, the formation of micelles in the chloroform solution has to be studied.

There have been many studies on the formation of PC micelles in benzene and carbon tetrachloride [5–12]. In these studies, the structural organization of PC micelles is confirmed as an inverted micelle with the polar head groups in the interior of the micelle and hydrocarbon chains directed outward into the solvent and the size of the micelle is so large as to be solubilized by many water molecules in the interior of the micelle.

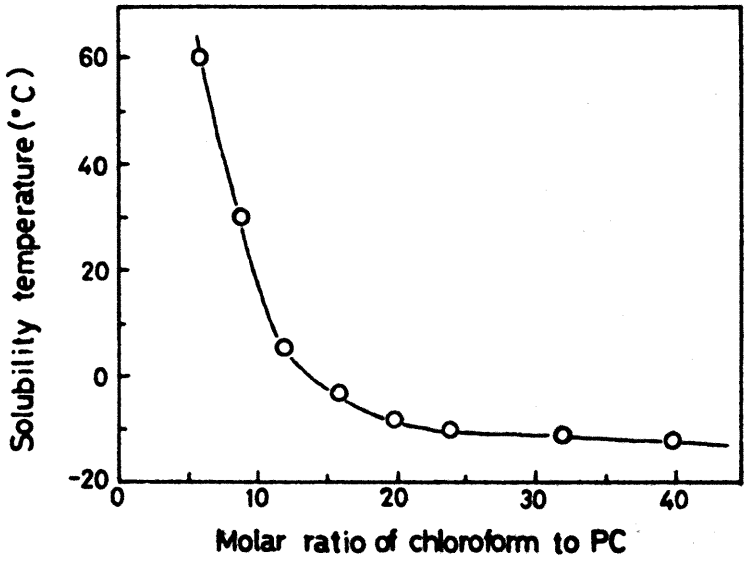

Fig. 5. Solubility of PC in chloroform.

But, as for the formation of PC micelles in chloroform, there have been few definitive studies [13–18]. The inverted micelles as those in benzene and carbon tetrachloride are reported in these studies, although their molecular organization or their size is variable. Haque et al. [17] reported that 3 molecules of PC formed a micelle in chloroform. From these studies and our results in this report, the properties of PC micelles in chloroform may be different from those in benzene and carbon tetrachloride. If the aggregation of PC molecules exists in the chloroform solution, the inverted micelles with the packed molecular organization might be unfavorable and the number of aggregation might be too small. Because a few or more molecules of chloroform are surrounding the PC molecule by two kinds of hydrogen bondings they may neutralize to some extent the polarity of the phosphorylcholine group. The experiments on these points with a series of alkylphosphorylcholines carrying a different chain length will be reported later.

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