Determination of pH in Reversed Micelles

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A new method for the determination of pH in reversed micellar systems was proposed. The degree of dissociation (α) of Phenol Red in the systems was observed spectrophotometrically. The value of pH can be obtained by use of the Henderson-Hasselbach equation with the aid of pK_a of Phenol Red measured by means of ^{31}P-NMR in the systems containing phosphate buffer solutions. The method was applied to systems consisting of sodium octanoate, 1-hexanol, and water and some buffer solutions other than phosphate buffer solutions, and found to be effective within a wide pH range. Analysis of the fluorescence spectra of 8-anilino-1-naphthalenesulfonic acid indicates that Phenol Red molecules are present at the interface between the water core and the 1-hexanol phase in reversed micelles. The hydrolytic reaction of *p*-nitrophenyl acetate was also studied kinetically and found to change slightly with pH.

The rates of the chemical reactions are drastically accelerated in the reversed micelle.[1-5] Recently, an effective energy transfer was observed in the reversed micellar system.[6]

Hydrolytic reactions of *p*-nitrophenyl esters were studied in the reversed micelle consisting of sodium octanoate, 1-hexanol, and water. It was found that the rate of the reaction is much more enhanced as compared with that in the aqueous solution. Favorable partitioning of the substrate into the water core, the orientation of the catalyst at the interface between the water core and the 1-hexanol phase and the polarity of the water molecules are important factors to accelerate the reaction. NMR studies on the behavior of water molecules and the polar headgroup of sodium octanoate in the reversed micelle showed that the mobility and polarity of water molecules change with hydration of sodium ions.

The purpose of this study is to measure the pH in the reversed micelle in order to know the effect of pH on the hydrolytic reaction. This will also be relevant to elucidating the activity of the water molecule in the reversed micelle. The pH in the reversed micelle cannot be measured with a glass electrode. The pH value in the interior of cell membranes also cannot be measured with a glass electrode, but have been determined by several alternate methods.[7-9] ^{31}P-NMR technique has been used to measure the pH values in the interior or exterior of cell membranes.[10,11] On the other hand, the acid-base indicator is useful for measuring pH of the aqueous solution. In this case, pH is determined by the Henderson-Hasselbach equation

$$\mathrm{pH} = \mathrm{p}K_a + \log \frac{\alpha}{1-\alpha} \quad (1)$$

where K_a is the dissociation constant of the indicator, and α the degree of dissociation of the indicator. Since K_a of the indicator in the reversed micelle is not the same as that in the aqueous solution, the pH value in the reversed micelle cannot be measured only by means of indicator. The pH value in the reversed micelle consisting of the buffer solution other than the phosphate buffer solution cannot be measured by ^{31}P-NMR technique. In this study, α of Phenol Red and the pH value in the reversed micelle consisting of the phosphate buffer solution were determined by the spectrophotometry method and ^{31}P-NMR, K_a of Phenol Red being obtained by substituting α and pH in Eq. 1. Thus, the use of Phenol Red makes the pH measurement in the reversed micelle not only easier but the evaluating of the pH value in the reversed micelle consisting of any buffer solution in a wide pH range possible.

A hydrophobic probe,[12] 8-anilino-1-naphthalenesulfonic acid (ANS), is an amphiphilic compound with a chemical structure similar to that of Phenol Red. The position of Phenol Red can thus be estimated from the measurement of the fluorescence of the system.

Experimental

Materials. Analytical grade reagents of sodium octanoate, 1-hexanol, Phenol Red, adenosine-5′-triphosphate (ATP), 8-anilino-1-naphthalenesulfonic acid (ANS), and L-tryptophan were used without further purification. 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES, $pK_a = 7.5$), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS, $pK_a = 10.4$) and 3-[2-hydroxy-1,1-bis(hydroxymethyl)-ethylamino]-1-propanesulfonic acid (TAPS, $pK_a = 8.4$, DOJINDO Laboratories) were used. Reversed micellar solution was prepared by mixing three components, the buffer solution (phosphate or HEPES–TAPS–CAPS buffer solution), 1-hexanol and sodium octanoate, and allowing the mixture to stand at 30 °C for 24 h. In the measurements of the absorption spectra, ^{31}P-NMR spectra and the fluorescence spectra, the buffer solution containing Phenol Red (the concentration of Phenol Red is 10^{-4} M), adenosine-5′-triphosphate (10^{-2} M) and the fluorescence probe (10^{-4} M) was cosolubilized into the reversed micelle. The quencher, carbon tetrachloride, was added directly to the reversed micellar solution containing ANS molecules.

Measurements. ^{31}P-NMR was measured with JNM-PS type spectrometer at 40 MHz, the following setting of the pulse unit being employed; spectral width 4000 Hz, repeat time 2.5 s, pulse width 18.0 μs, 100 times accumulation. The absorption spectra were recorded on an EPS-3T Hitachi spectrophotometer at 30 °C. The fluorescence spectra were measured with a Shimadzu RF-502 type spectrofluorimeter at 30 °C.

Results and Discussion

Determination of pH *Value.* Figure 1 shows the dependence of the chemical shift of phosphorus in

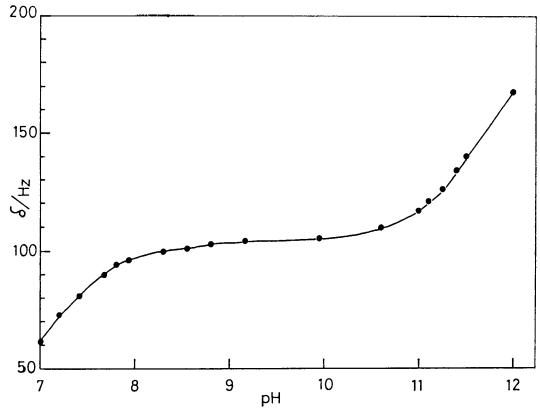

Fig. 1. The chemical shifts of phosphorus in the phosphate buffer solutions plotted as a function of pH of the buffer solution.

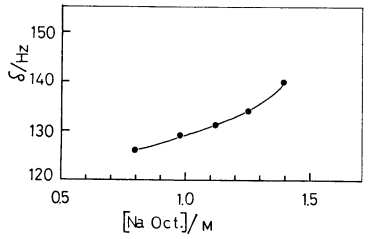

Fig. 2. Plots of the chemical shifts of phosphorus in the phosphate buffer solutions solubilized into reversed micelles against the concentration of sodium octanoate. The pH value of the phosphate buffer solution used is 7.9.

the phosphate buffer solution on the pH of the buffer solution. The steps in the chemical shift plots correspond to the processes

$$H_2PO_4^- \rightleftharpoons HPO_4^{2-} \rightleftharpoons PO_4^{3-}.$$

The chemical shifts of phosphorus were measured as a function of concentration of sodium octanoate for reversed micelles prepared by the use of phosphate buffer solutions of pH 7.9. The results are shown in Fig. 2. The chemical shift increases with increase in the concentration of sodium octanoate. This indicates that the pH value in the reversed micelle is higher than the initial pH value of the phosphate buffer solution used for the preparation of reversed micellar solutions. The activity of water molecules in the reversed micelle should decrease with increase in sodium octanoate content. However, the pH value obtained from ^{31}P-NMR method is much higher than that calculated by $[H^+]=\sqrt{K_wK_a/C}$, where K_w is the ion-product constant of water, K_a the dissociation constant of octanoic acid, and C the concentration of sodium octanoate. Such large chemical shifts of phosphorus as observed in the reversed micelle may be due to the ionic effect of the polar headgroup of sodium octanoate. Since the chemical shift of α-phosphorus of adenosine-5′-triphosphate (ATP) does not change with pH,[13] the chemical shift induced by sodium octanoate was evaluated by the following method. By means of this property of α-phosphorus of ATP, the difference between the α-P chemical shift of ATP in the aqueous solution and in the reversed micelle

Table 1. The observed chemical shift (δ), the chemical shift induced by sodium octanoate (δ'), $\delta-\delta'$ and pH obtained from ($\delta-\delta'$) for reversed micellar samples

[Sodium octanoate][a]	δ/Hz	δ'/Hz	$(\delta-\delta')$/Hz	pH
1.38	140.0	39.0	101.0	8.6
1.24	133.7	33.0	100.7	8.5
1.10	131.7	31.3	100.4	8.4
0.97	129.9	29.5	100.4	8.4
0.69	125.5	25.4	100.1	8.3

a) The molar concentration (M) of sodium octanoate in 1-hexanol.

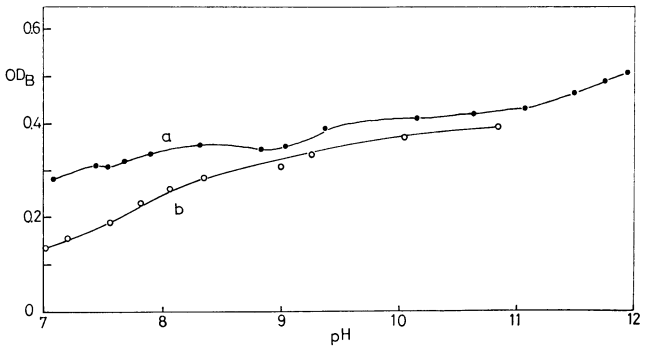

Fig. 3. Plots of the absorbance of Phenol Red due to the ionized form (OD_B) against the initial pH value of the phosphate buffer solution (a) and HEPES–TAPS–CAPS buffer solution (b) solubilized into the reversed micelle.

was measured, and used as the chemical shift induced by sodium octanoate. The real chemical shift of phosphorus in the reversed micelle should be obtained by subtracting the shift thus obtained from the chemical shift observed for the reversed micelle. From the result, the real pH value (pH′) in the reversed micelle can be obtained by means of Fig. 1. The results are given in Table 1.

The absorption spectra of Phenol Red in reversed micelles show a peak at 450 nm due to unionized form and a peak at 571 nm due to ionized form of Phenol Red and the isosbestic point at 490 nm. The absorbance at 571 nm (OD_B) due to the ionized form of Phenol Red in the reversed micelle against the initial pH value of the buffer solution (phosphate buffer or HEPES–TAPS–CAPS buffer) solubilized into the reversed micelle is plotted in Fig. 3. Although the pH values of the phosphate buffer solution containing Phenol Red vary in the range 7—12, the absorbance due to the ionized form of Phenol Red does not appreciably change with pH. This indicates that pH at the position where Phenol Red exists does not change much with pH of the phosphate buffer solution. Thus, pH in the reversed micelle is determined by the buffer capacity of sodium octanoate rather than that of the phosphate buffer solution. A similar experiment was carried out in the reversed micelle with use of another buffer solution, HEPES–TAPS–CAPS. The results are shown in Fig. 3. The transformation of Phenol Red from the acid form (unionized form) to the basic form

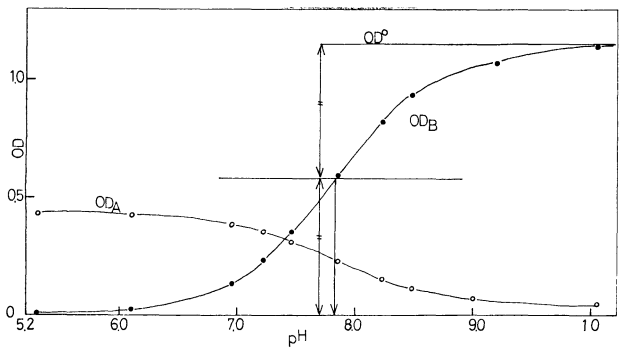

Fig. 4. Plots of the absorbance of Phenol Red due to the ionized (OD_B) and the unionized (OD_A) forms against pH of the phosphate buffer solution. This figure shows pK_a of Phenol Red in the phosphate buffer solution is 7.82.

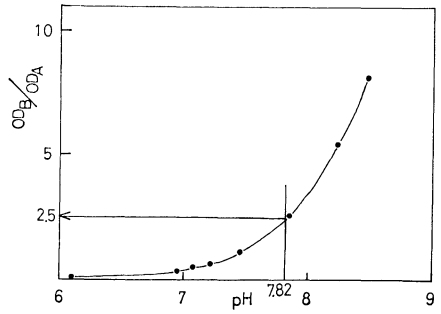

Fig. 5. Plots of OD_B/OD_A against pH of the phosphate buffer solution.

(ionized form) takes place to a greater extent than in the phosphate buffer solution, especially in the pH range 7—9. In order to estimate the degree of dissociation (α) of Phenol Red, it is necessary to measure the absorbance due to the completely ionized form of Phenol Red ($OD_B°$). However, the value of OD_B at the highest pH (pH=12) showed no constant value (Fig. 3). The following experiment was made in aqueous solutions. The absorbances of Phenol Red at 571 nm and at 450 nm were measured as a function of the pH of the buffer solution (Fig. 4). The dissociation constant *K*a of Phenol Red in this buffer solution (phosphate buffer solution) was found to be 7.82 by means of Eq. 1. The ratio of the absorbance at 571 nm (OD_B) to that at 450 nm (OD_A) is plotted against pH of the buffer solution (Fig. 5). We see that OD_B/OD_A is 2.5 for pH 7.82. The values of OD_B/OD_A were obtained for the four buffer solutions; phosphate buffer solution, HEPES–TAPS–CAPS, 90% phosphate buffer solution +10% ethanol, and 90% phosphate buffer solution +10% dioxane; the results are summarized in Table 2. The OD_B/OD_A value becomes 2.5 at the point pH=pK_a where OD_B is equal to a half of $OD_B°$. Thus, $OD_B°$ can be obtained from the value of OD_B at the pH where the value of OD_B/OD_A becomes 2.5. In the four buffer solutions, the value of $OD_B°$ thus obtained was equal to that estimated from Fig. 4. In the reversed micelle, $OD_B°$ was evaluated with the assumption that OD_B at pH of OD_B/OD_A=2.5 is also equal to a half of $OD_B°$. The following procedure was taken to determine pH

Table 2. The dissociation constant pK_a and the ratio OD_B/DA_A for four buffer solutions

	pK_a	OD_B/DA_A
Phosphate buffer	7.82	2.50±0.02
90% phosphate buffer+10% ethanol	7.90	2.50±0.02
90% phosphate buffer+10% dioxane	7.88	2.51±0.02
HEPES-TAPS-CAPS	7.82	2.48±0.02

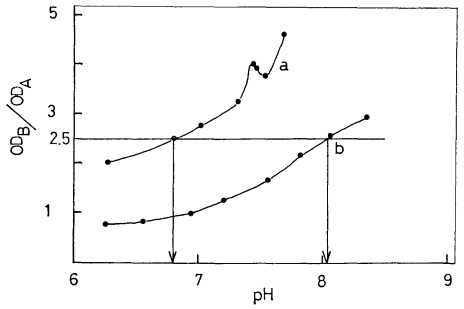

Fig. 6. Plots of OD_B/OD_A of Phenol Red against the original pH value of the buffer solution solubilized into the reversed micelle. a) Phosphate buffer, b) HEPES–TAPS–CAPS.

in the reversed micelle.

1. The values of OD_B/OD_A were determined as a function of the pH of the original buffer solution solubilized into the reversed micelle. The results are shown in Fig. 6.

2. The pH of the buffer solution giving the value OD_B/OD_A=2.5 was obtained from the OD_B/OD_A *vs.* pH curve shown in Fig. 6.

3. The OD_B value corresponding to the pH value obtained from step 2 was obtained from the OD_B *vs.* pH curve given in Fig. 3. Thus, the value of $OD_B°$ can be obtained as twice the value of OD_B.

4. The degree of dissociation (α) of Phenol Red in the reversed micelle is thus obtained as $OD_B/OD_B°$.

5. The dissociation constant K_a of Phenol Red in the reversed micelle can be calculated from the degree of dissociation of Phenol Red and the pH value obtained by ^{31}P-NMR, by means of Eq. 1. When the phosphate buffer solution of pH 7.9 was solubilized into the reversed micelle, the α and pH values were found to be 0.65 and 8.6 by spectrometric method and ^{31}P-NMR, respectively. By substituting these values into Eq. 1, the pK_a value of Phenol Red was found to be 8.3.

6. By use of α and pK_a (8.3) of Phenol Red, the real pH value (pH′) in the reversed micelle containing a buffer solution was obtained in a wide pH range. The pH′ values in the reversed micelle containing phosphate buffer solutions and HEPES buffer solutions are shown in Fig. 7.

Fluorescence Spectra. It is important to study the position of Phenol Red molecule. It may be in the water core, 1-hexanol phase or at the interface between the water core and 1-hexanol phase. Fluorescence has been used for elucidating the position. 8-Anilino-1-naphthalenesulfonic acid (ANS), which is an amphiphilic compound with a structure similar to that of Phenol Red, was used. The positions of

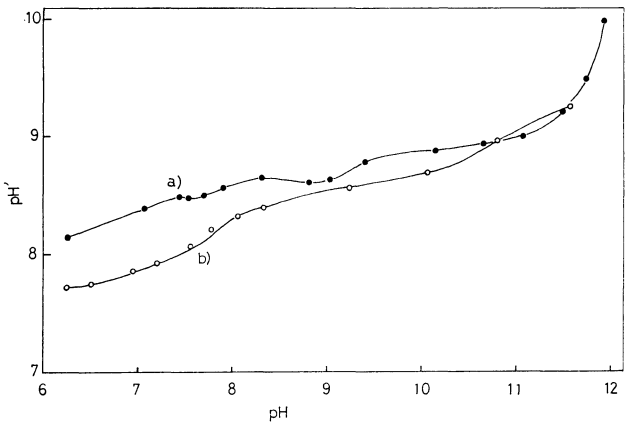

Fig. 7. Plots of the real pH value (pH') against the original pH value of the buffer solution solubilized into the reversed micelle. a) Phosphate buffer, b) HEPES–TAPS–CAPS buffer.

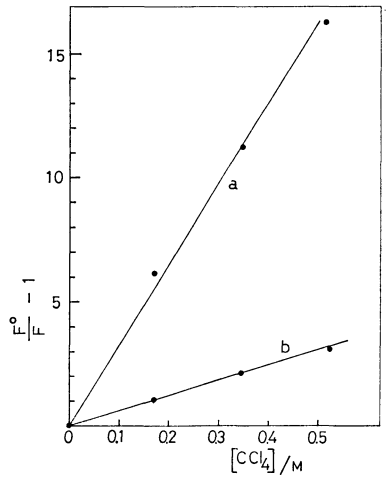

Fig. 8. The Stern-Volmer plots in 1-hexanol and the reversed micelle. The fluorescence probe used is ANS. a) 1-Hexanol, b) in the reversed micelle.

the emission maxima and the relative intensities of ANS in 1-hexanol, water, and the reversed micelle are given in Table 3. The position of the emission maximum and the very low intensity in water as compared with those in the reversed micelle indicates that ANS molecules do not exist in the water phase of the reversed micelle.

Carbon tetrachloride can quench the fluorescence of ANS in 1-hexanol. Quenching of the excited state of ANS can be quantitatively treated by the Stern-Volmer equation[14] $F^\circ/F-1=k^\circ\tau[Q]$, where F and F° are the fluorescence intensities of the probe with and without the quencher, respectively, k° the rate constant of quenching, τ° the fluorescence lifetime of the probe in the absence of the quencher, and [Q] the concentration of the quencher. Figure 8 shows the plots of the data obtained by means of this equation in 1-hexanol and in the reversed micelle. Carbon tetrachloride quenches the fluorescence of ANS in 1-hexanol to a greater extent than in the reversed micelle. The results indicate that no appreciable amount of ANS exists in the 1-hexanol phase of the reversed micelle. Thus, the probe molecules should exist at the interface between the water core and the 1-hexanol phase.

Table 3. Position of emission maximum and the relative intensity of ANS in water, 1-hexanol and the reversed micelle

	Water	1-Hexanol	Reversed micelle[a)]	
			A	B
Emission maximum/nm	515	460	465	470
Relative intensity	1	254	131	109

a) A; 1.38 M of sodium octanoate in 1-hexanol. B; 0.69 M of sodium octanoate in 1-hexanol.

The same experiment was carried out in the reversed micellar system with use of L-tryptophan, an amphiphilic compound. L-Tryptophan is not soluble in 1-hexanol, but can be quenched a little by carbon tetrachloride in the reversed micelle. The results indicate that L-tryptophan molecules also exist at the interface between the water core and the 1-hexanol phase. Thus, the amphiphilic molecules are considered to be located at the interface between the water core and the 1-hexanol phase, due to the balance of the hydrophobic and lyophilic interactions. Phenol Red exists at the interface between the water core and the 1-hexanol phase as in the case of L-tryptophan and ANS, the real pH (pH') given in Fig. 7 being pH at the interface of the reversed micelle.

Dissociation Constant of Phenol Red and the Surface Potential in the Reversed Micelle. By substituting the pH value obtained by ^{31}P-NMR and the degree of dissociation of Phenol Red by spectrometric methods in Eq. 1, we have obtained the dissociation constant of Phenol Red as 8.3, which is larger than that in the aqueous solution (7.8). The dissociation constant of Methyl Red measured by Montal and Gitler[15] in the aqueous solution of sodium dodecyl sulfate was also larger than that in the aqueous solution. By means of the gel filtration technique, they showed that Methyl Red is influenced by the surface charges of the micelle. The concentration of H^+ ion at the surface of the micelle would differ from that in the bulk phase of the system due to the difference in electrical potential between that on the micellar surface and that in the bulk phase. The dissociation constant of Methyl Red on the micellar surface, K_s, is given by the equation $pK_s=pK_b-\varepsilon\psi/2.3\,kT$, where K_b is the dissociation constant of Methyl Red in the bulk phase, ψ the surface potential, ε the electrical charge, T the absolute temperature, and k the Boltzmann constant. For Methyl Red in the aqueous solution of sodium dodecyl sulfate, $\psi=-86$ mV, and for Phenol Red, $\psi=-30$ mV in the reversed micelle. The difference in surface potential between Methyl Red and Phenol Red would be caused by the difference in the location of the indicators and in the nature of the headgroups of the surfactants, *i.e.*, the sulfate group of sodium dodecyl sulfate and the carboxylate group of sodium octanoate.

Effect of pH *on the Reaction in the Reversed Micelle.* The rate constant of the hydrolytic reaction of *p*-

nitrophenyl acetate in the reversed micelle in which the phosphate buffer solution is solubilized is as large as that in which the HEPES buffer solution is solubilized. On the other hand, pH′ in the reversed micelle in which the phosphate buffer solution is solubilized is higher than that in which the HEPES buffer solution is solubilized (Fig. 7). Thus it appears that, although the hydrolytic reaction depends slightly on the pH in this system, it is not an important factor in the enhancement of this reaction rate in the reversed micelle.

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