SURFACTANTS & DETERGENTS

Syntheses of 2-(N-2-Hydroxyalkyl-N,N-Dimethylammonio)Ethyl Hydrogen Phosphates and Their Physicochemical and Antimicrobial Properties

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A series of new phosphate type of amphoterics having a 2-hydroxyalkyl group as a hydrocarbon chain, 2-(N-2-hydroxyalkyl-N,N-dimethylammonio)ethyl hydrogen phosphates (alkyl[R]:dodecyl, tetradecyl, hexadecyl), was synthesized and their physicochemical properties—isoelectric point, critical micelle concentration (CMCs), occupied area/molecule at interface and the change of free energy of micellization—and antimicrobial properties were investigated in comparison with those of sodium 2-(N-alkyl-N-methylamino)ethyl hydrogen phosphates. It was found that the CMCs and the occupied areas were observed to decrease due to a phosphobetaine moiety and a 2-hydroxyethyl group. Solution properties of the binary system of SDS/2-(N-2-hydroxytetradecyl-N,N-dimethylammonio)ethyl hydrogen phosphate in aqueous solutions were studied in terms of surface tension and pH value. On the other hand, it was observed that the Minimum inhibitory concentration (MICs) of 2-(N-2-hydroxyhexadecyl-N,N-dimethylammonio)ethyl hydrogen phosphate against fungi were smaller than those of chlorhexidine digluconate.

Lecithin is a kind of glycerophospholipid broadly distributed in natural products, such as egg, soybean, etc. This is a surface active compound consisting of hydrophobic and zwitterionic moieties. However, it is almost insoluble as it has two long chain alkyl groups as the hydrophobic moiety. If these alkyl groups could be substituted by adequate short chain alkyl groups, water-soluble amphoteric surfactants might be obtained.

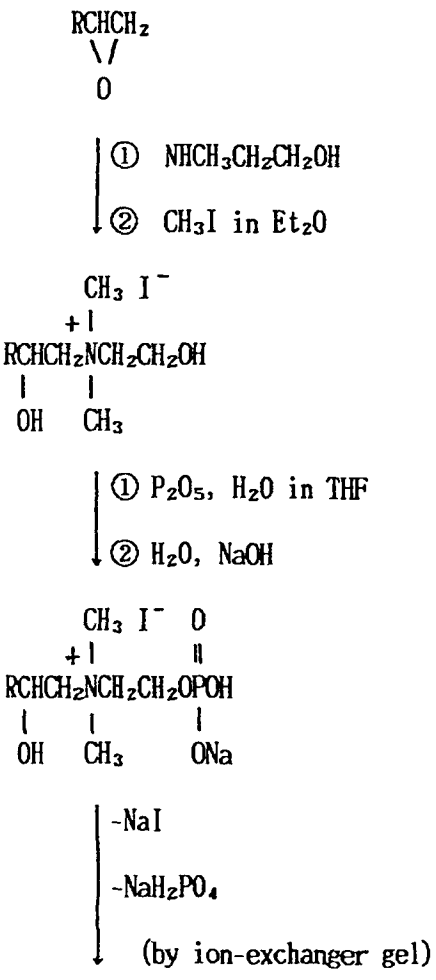
From this point of view, a series of water-soluble amphoteric surfactants, alkyl phosphorylcholines (1,2), alkyl lysophosphorylcholines (3,4) and alkyl phosphobetaines (5,6) has been reported. They have a zwitterionic moiety with a phosphoric acid group in each molecule. In addition, lysophosphorylcholines are of particular interest as potent antimetabolites of native phospholipids because they have approximately the same molecular geometry, but should be metabolized in different ways (3). Therefore, it seems important to know whether synthetic phospholipids with a particular structure are correlated with one or more biological properties.

Recently, Tsubone and his coworkers (7) have synthesized new amphoteric surfactants containing a tertiary nitrogen and a phosphoric acid group, e.g., sodium 2-(N-alkyl-N-methylamino)ethyl hydrogen phosphates (MEP) and investigated their surface active properties. In this paper, we have prepared a series of phosphobetaines, 2-(N-2-hydroxylalkyl-N,N-dimethylammonio)ethyl hydrogen phosphates (alkyl: dodecyl, tetradecyl, hexadecyl), having a 2-hydroxyalkyl, a quaternary nitrogen and a phosphoric acid group in a molecule, to investigate the physicochemical and antimicrobial properties and compare its behavior with MEP-homologues and chlorhexidine digluconate.

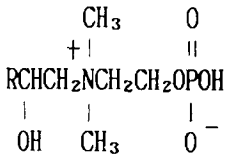
EXPERIMENTAL

In this study, the following abbreviations are used: HDMEP for 2-(N-2-hydroxyalkyl-N,N-dimethylammonio)ethyl hydrogen phosphates; the notations C_{16}-, C_{14}- and C_{12}- in front of the abbreviations denote the carbon numbers 16, 14 and 12 in 2-hydroxyalkyl chain, respectively. For example, C_{16}-HDMEP represents 2-(N-2-hydroxyalkyl-N,N-dimethylammonio)ethyl hydrogen phosphate.

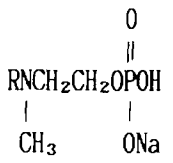
Materials. 2-(N-2-hydroxyalkyl-N,N-dimethylammonio)-1-hydroxyethyliodides (HDME) were prepared according to the general methods by reacting 1,2-epoxyalkanes and N-methylamonoethanol at 80°C for 8 hr, followed by the addition of methyliodide in diethyl ether at room temperature for 10 hr in a excellent yield (Scheme 1). 2-(N-2-



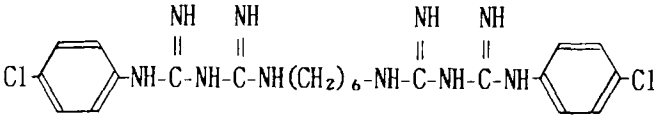
SCHEME 1



(HDMEP)



(MEP)



(Chlorhexidine)

CHART 1

hydroxyalkyl-N,N-dimethylammonio)ethyl hydrogen phosphates (HDMEP) (Chart 1) were prepared from 2-(N-2-hydroxyalkyl-N,N-dimethylammonio)-1-hydroxyethyliodides, as shown in Scheme 1.

Synthesis of 2-(N-2-hydroxyhexadecyl-N,N-dimethylammonio)ethyl hydrogen phosphate (C_{16}-HDMEP). C_{16}-HDMEP was synthesized from HDME (m.p. 67 ~ 68°C) as follows: HDME (22.9 g, 0.05 mole), distilled water (0.9 g, 0.05 mole) and diphosphorous pentaoxide (9.94 g, 0.07 mole) were mixed in tetrahydrofuran (300 mL) and stirred till clear solution obtained. After that the solution was refluxed for 8 hr with stirring. The reaction mixture was cooled to room temperature, water (3.4 g, 0.19 mole) was added and the mixture was stirred for 30 min. NaOH (5.6 g, 0.14 mole) was then added, followed by stirring for 20 min. After the evaporation of the solvent from the reaction mixture, the residue was dissolved in 150 g of water. The crude product was obtained by ion-exchanger gel (AG 501-X[8]D, Bio-Rad Laboratories, Richmond, CA) column chromatography with a water eluent as a white crystal. This was purified by silica gel column chromatography using ethanol-water (1:1, v/v) as an eluent, resulting in 8.2 g of C_{16}-HDMEP. The compound showed one spot on thin-layer chromatography (TLC), which yielded 40% : IR: 3380, 2928, 2856, 1466, 1376, 1220, 1084, 974, 752 cm^{-1}; ^{13}C-NMR (CD_3OD, δ): 14.40 ($\underline{C}\underline{C}H_3$), 23.66 ($\underline{C}H_2$), 26.09 ($\underline{C}H_2$), 30.39 ~ 30.73 ($\underline{C}H_2$), 33.01 ($\underline{C}H_2$), 37.02 ($\underline{C}H_2$), 53.44 ($NCH_3$), 59.89 ($\underline{C}H_2CH_2OPO$), 67.07 ($\underline{C}HOH$), 72.27 ($CH_2\underline{C}H_2OPO$); analysis calculated for $C_{20}H_{43}N_1O_4P_1$: C, 58.66; H, 10.83; N, 3.42. Found: C, 58.51; H, 10.43; N, 3.58.

Other HDMEP homologues, C_{14}-HDMEP and C_{12}-HDMEP, were prepared by a similar method and showed one spot on TLC. Yields and analytical data are as follows: C_{14}-HDMEP: yield 45%; IR: 3256, 2920, 2852, 1458, 1376, 1212, 1096, 924, 752 cm^{-1};^{13}C-NMR (CD_3OD, δ): 14.38 ($C\underline{C}H_3$), 23.61 ($\underline{C}H_2$), 26.05 ($\underline{C}H_2$), 30.35 ~ 30.69 ($\underline{C}H_2$), 32.96 ($\underline{C}H_2$), 36.96 ($\underline{C}H_2$), 53.46 ($N\underline{C}H_3$), 59.92 ($\underline{C}H_2CH_2OPO$), 66.95 ($\underline{C}HOH$), 71.17 ($\underline{C}H_2CH_2OPO$); analysis calculated for $C_{18}H_{40}N_1O_5O_1$: C, 56.67; H, 10.57; N, 3.67. Found: C, 56.70; H, 10.62; N, 3.56. C_{12}-HDMEP: yield 30%; IR: 3248, 2928, 2852, 1466, 1378, 1214, 1074, 940, 752 cm^{-1};^{13}C-NMR ($CD_{3O}D$, δ): 14.39 ($C\underline{C}H_3$), 23.63 ($\underline{C}H_2$), 26.05 ($\underline{C}H_2$), 30.37 ~ 30.71 ($\underline{C}H_2$), 32.98 ($\underline{C}H_2$), 36.98 ($\underline{C}H_2$), 53.37 ($N\underline{C}H_3$), 60.21 ($\underline{C}H_2CH_2OPO$), 66.98 ($\underline{C}HOH$), 71.23 ($CH_2\underline{C}H_2OPO$); analysis calculated for $C_{16}H_{36}N_1O_5P_1$: C, 57.12; H, 10.49; N, 4.16. Found: C, 56.99; H, 10.40; N, 4.15.

The physicochemical properties of their aqueous solution were measured by the standard method (7).

Assay of antimicrobial activities: Media and culture condition. Six kinds of microorganisms were used to determine the MICs of PDMEP-homologues. The media and the culture conditions of each strain are shown in Table 1.

After the cultivation of fungi, spore suspensions were prepared for the antimicrobial tests. Mycelia and the cultivated broth were transferred into mortar and pestled. The pestled solution was filtrated and the supernatant was used as a spore suspension. For the other yeasts and bacteria, the cultivated broth was used without the above procedure.

Procedure of antimicrobial test. Each of the PDMEP-homologues was dissolved in water at 0.1% (w/v) concen-

TABLE 1

Microorganisms and Their Culture Conditions

Microorganism		Culture conditions			
		Media	Temp.	Time	
Aspregillus niger	IAM 3001	GP broth (Daigo)	25°C	72 hr	Standing
Penicillium citrinum	IAM 7316	GP broth (Daigo)	25°C	72 hr	Standing
Candida albicans	IFO 0597	SCD broth (Eiken)	32°C	48 hr	Standing
Escherichia coli	IAM 1239	SCD broth (Eiken)	32°C	24 hr	Shaking
Stapyrococcus aureus	IAM 12082	SCD broth (Eiken)	32°C	24 hr	Shaking
Butillus subtilis	IAM 1069	SCD broth (Eiken)	32°C	24 hr	Shaking

tration, and then 2-fold dilutions were made and used for the tests. The mixture of 100 μL of the test solution, which consisted of 90 μL broth media (as shown in Table 1) and 10 μL of cell (spore) suspension, was added into each well of the 96 well plate (Corning Co., Ltd.). The control test, which substituted sterilized pure water for test chemical solution, was done for each strain. For fungi, the plates were incubated for 72 hr at 25°C, and for 24 hr at 32°C for yeast and bacteria. After the incubation, the MICs of PDMEP-homologues in this study were determined for each strain.

Determination of MIC. The MICs against fungi were determined as minimum concentration, at which we did not observe the mycelia growing in the incubation well.

The MICs for the other yeast and bacteria were determined by the following procedure. One volume of inoculating loop of each strain was inoculated from each well on the agar medium plate, which was the same medium as shown in Table 1, and the agar plates were incubated for 48 hr at 32°C. After the incubation, the MICs were determined. In this case, each MIC was defined as minimum concentration at which no growth colony was observed or where there were significantly fewer colonies than control on the agar plate.

RESULTS AND DISCUSSION

Physicochemical properties of aqueous solution of HDMEP. These phosphobetaines are soluble in water at room temperature. The values of pK_1 and pK_2 of C_{14}-HDMEP were obtained by the potentiometric titration method as 4.9 and 9.8, respectively. This result shows that the cationic structure is formed below pH 4.8, the zwitterion exist in the range of pH 4.9 ~ 9.8, whereas the anionic form is present above pH 9.8.

The surface tension-concentration plots of HDMEP-homologues by the Donuy method are shown in Figure 1. The CMCs, the lowering ability of surface tension (γ CMC), e.g., the surface tensions measured close to CMC and the occupied area/molecule at the surface (A) are summarized in Table 2 with the data of other amphoteric surfactants (7). It can be seen that all compounds reduce the surface tension of water, and the formation of micelle in water was shown by the clear break points and that, as expected, the break in the curve shifted to a lower concentration owing to the increase of the hydrophobicity as the increase of the carbon number of the alkyl chain. It is clear that the effects of the length of the alkyl chain are evident upon the surface properties. The CMC values were 0.16 mmole/L for C_{12}-HDMEP, 0.10 mmole/L for C_{14}-HDMEP and 0.06 mmole/L for C_{16}-HDMEP, whose values were found to be lower than those of homologues of 2-(N-alkyl-N-methylamino)ethyl hydrogen phosphate (Chart 1, MEP) (7) because of the introduction of a 2-hydroxyethyl group and a phosphobetaine moiety. The plots of logarithm of the CMC values gainst the carbon number of alkyl chain (N) (Fig. 2) showed the following relation:

$$\text{Log (CMC)} = 2.78 - 0.1025\ N$$

The γ CMC values of HDMEP-homologues were between 37 ~ 41 mN/m like another phosphate type of amphoterics (7). Figure 3 shows that surface tension at CMC decreases as the carbon number of alkyl chain increases. On the other hand, the occupied areas per molecule at the air/water interface (A) were 43.8×10^{-2} nm^2 for C_{12}-HDMEP, 45.3×10^{-2} nm^2 for C_{14}-HDMEP and 47.5×10^{-2} nm^2 for C_{16}-HDMEP. These values increase linearly with the increase of the number of carbon atoms in alkyl chain (Fig. 3). A value of C_{12}-HDMEP is smaller than that of C_{12}-MEP because of methyl substitution, and is comparable to that of sodium dodecyl sulfate (8).

The effect of temperature on CMC value of C_{14}-HDMEP was investigated in order to understand the thermodynamics of the micellization process in water

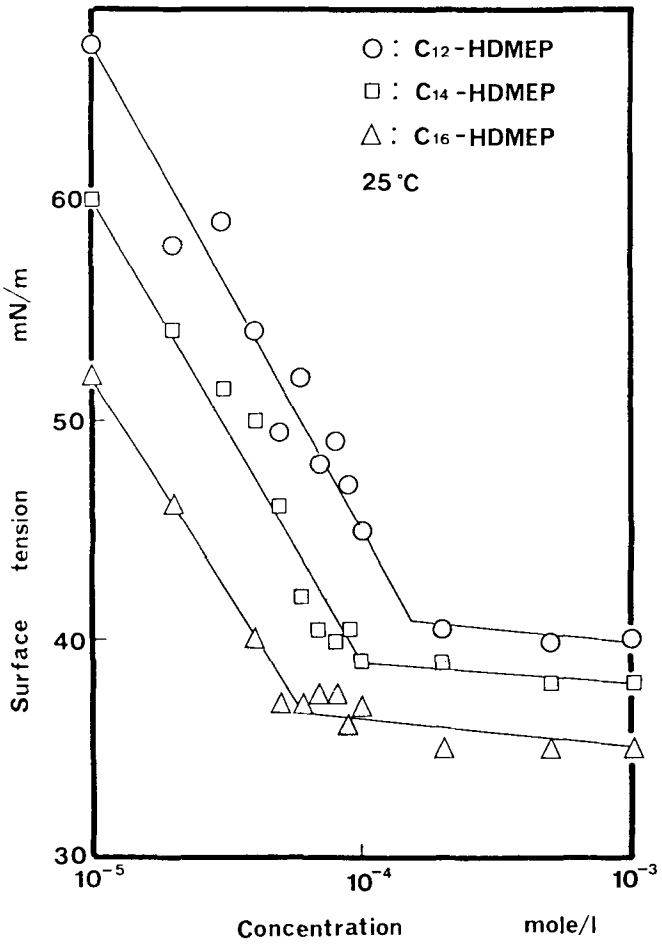


FIG. 1. Surface tension-concentration plot.

TABLE 2

Interfacial Properties of Surfactants (7)

Surfactant	CMC mmole/L (°C)	γ cmc mN/m	A × 10^2 nm^2
C_{12}-HDMEP	0.16 (25)	41.0	43.8
C_{14}-HDMEP	0.10 (25)	39.0	45.3
C_{16}-HDMEP	0.06 (25)	37.0	47.5
C_{12}-MEP	0.36 (25)	39.2	51.4
C_{14}-MEP	0.20 (25)	38.0	59.3
C_{16}-MEP	0.10 (25)	37.0	79.4

at several ionic conditions. Slopes of logarithm of CMC against 1/T for C_{16}-HDMEP are shown in Figure 4. Changes of free energy of micellization (9,10), $\Delta G°CMC$, were calculated from the following equation:

$$\Delta G°CMC = 2.303\ RT\ (\log CMC - \log w),$$

where w is the molar concentration of water.

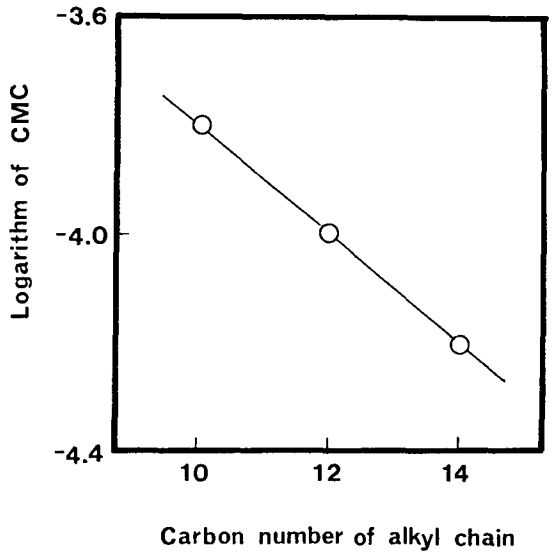


FIG. 2. Plots of logarithm of CMC against carbon number of alkyl chain.

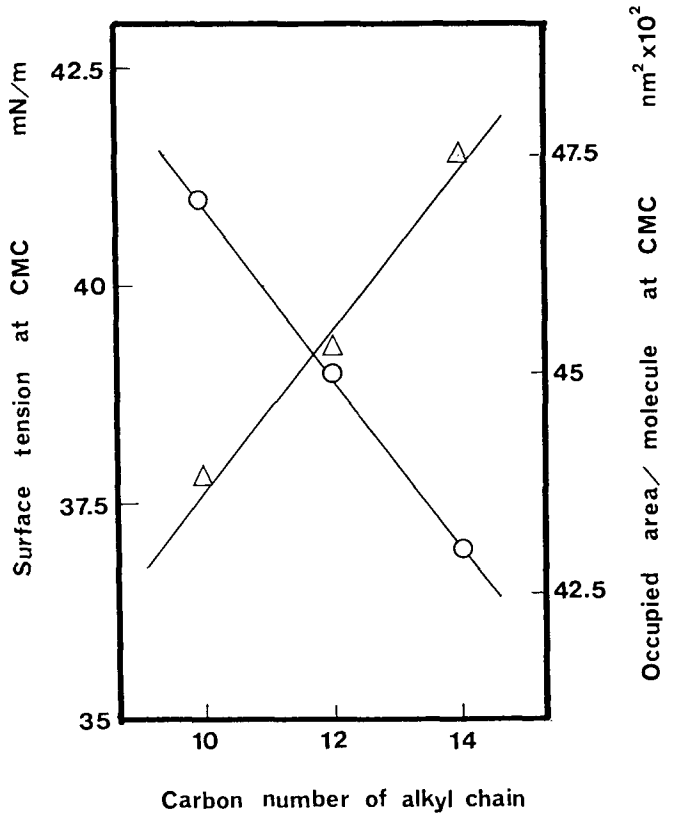


FIG. 3. Plots of surface tension (O) and occupied area per molecule at air water interface (△) against carbon number of alkyl chain.

At pH 11, the CMC increases remarkably with the increasing temperature. This tendency is consistent with other ionic surfactants. This shows that C_{16}-HDMEP behaves as an anionic surfactant by the deprotonation of a phosphate group. The free energy of micellization $\Delta G°CMC$ was found to be −5.95 kcal/mole. On the other hand, at pH 6.0, the CMC decreases with the increasing

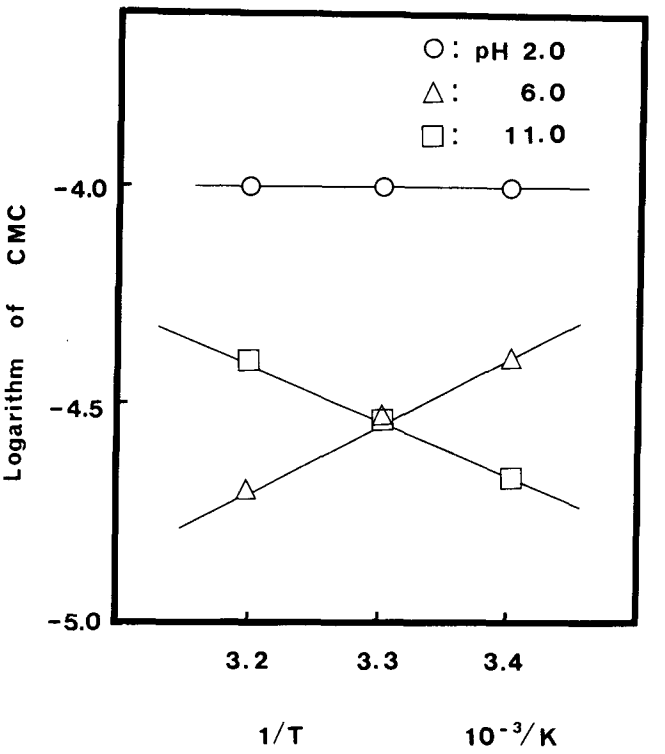


FIG. 4. Effect of temperature on CMC of C_{14}-HDMEP.

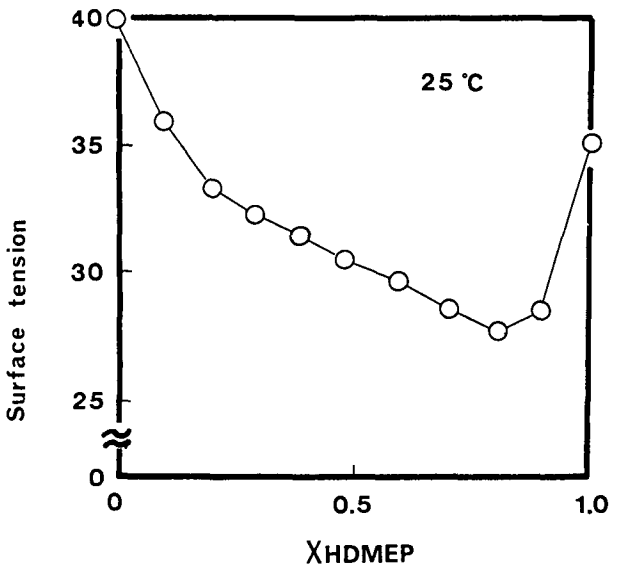


FIG. 5. Surface tension-concentratin plot (pure and mixed surfactants).

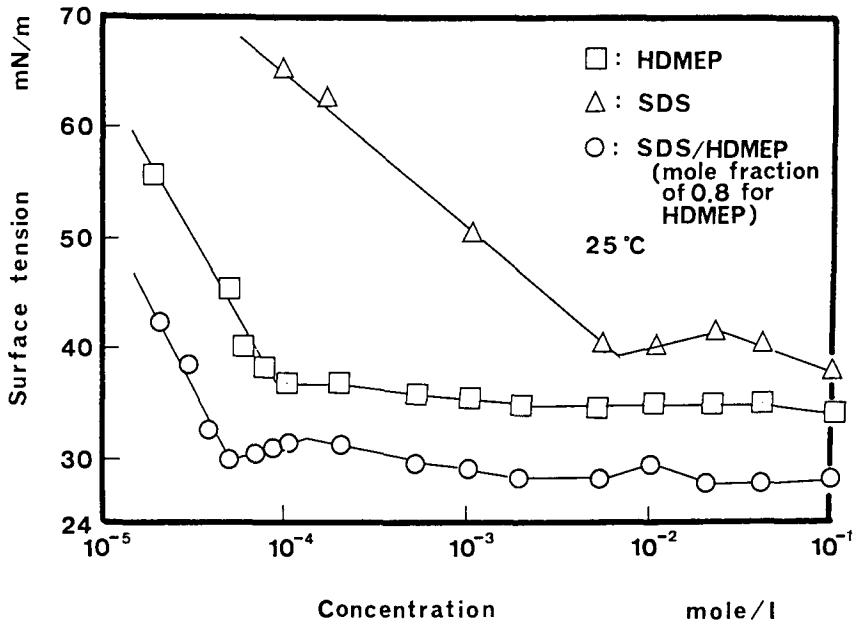


FIG. 6. Surface tension-mole fraction of C_{14}-HDMEP in SDS/C_{124}-HDMEP mixing system (concentration: 20 mM, 25°C).

temperature, as in the case of nonionic surfactants, and ΔG°CMC was 6.87 kcal/mole. This shows that C_{14}-HDMEP behaves as a zwitterion. At pH 2.0, the CMC remains unchanged with the temperature.

Properties of aqueous solutions of binary mixing system of SDS/C_{14}-HDMEP. Understanding how surfactants interact in mixed micelles is important for the many industrial applications of surfactants (11). Many papers have been previously published on solution properties of mixed surfactant systems (12-14). Therefore, solution properties of the binary mixing system of SDS/C_{14}-HDMEP in aqueous solutions were studied in terms of surface tension and pH value.

The change in surface tension with the concentration of each surfactant and the mixed surfactant system of SDS/C_{14}-HDMEP (0.8 mole fraction for C_{14}-HDMEP) system was investigated (Fig. 5). It is clear that the surface tension is lowered with the increase of the concentration, and that after going to the break point the surface tension shows almost constant value. Evidence for mixed micelle formation is obvious from the concentration vs surface tension plot. The break point shifts to a lower value than the individual surfactant components, and the effectiveness of surface tension reduction is also increased. The CMC value of this surfactant system was 5.0×10^{-5} mole/L.

The surface tension of the aqueous solutions of SDS/C_{14}-HDMEP system at 20 mM (above the CMC) is plotted against the mole fraction of C_{14}-HDMEP (Fig. 6). The surface tension decreases with the increasing the mole fraction of C_{14}-HDMEP and then increases through a minimum. The surface tension has a minimum at a mole fraction of 0.8 for C_{14}-HDMEP.

The pH values of the aqueous solutions of SDS/C_{14}-HDMEP system are plotted against the mole fraction of C_{14}-HDMEP at the total concentrations of above and below the CMC (Fig. 7). The pH values increase

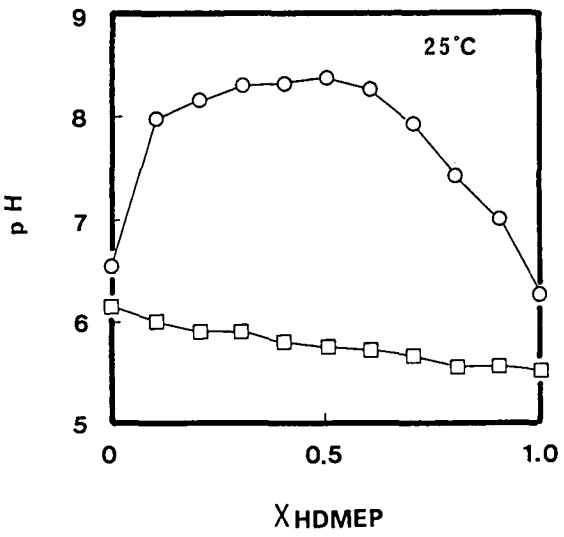


FIG. 7. pH-mole fraction of C_{14}-HDMEP mixing system (○, 20 mM; □, 0.025 mM; 25°C).

with the increasing mole fraction of C_{14}-HDMEP, and then decrease after a maximum in the total concentrations above the CMC. The pH value has a maximum in the vicinity of a mole fraction of 0.5 for C_{14}-HDMEP. On the other hand, the pH values decrease monotonously with the increasing the mole fraction of C_{14}-HDMEP in the total concentrations below the CMC. It is interesting to note that the pH values above the CMC are higher than those below the CMC in SDS/C_{14}-HDMEP system.

Antimicrobial activities. The MICs of HDMEP from dodecyl to hexadecyl are shown in Table 3 in comparison with MEP-homologues and chlorhexidine digluconate (Chart 1), which is a bactericide that is widely used

TABLE 3

Antimicrobial Activities (MIC, mg/mL)

	Aspergillus niger	*Penicillum citrinum*	*Candida albicans*	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*
C_{12}-HDMEP	625	156.3	>1250	>1250	>1250	312.5
C_{14}-HDEMP	156.3	156.3	>1250	>1250	>1250	156.3
C_{16}-HDEMP	78.1	39.1	>1250	>1250	>1250	78.1
C_{12}-MEP	625	156.3	>1250	>1250	>1250	625
C_{14}-MEP	156.3	39.1	156.3	625	312.5	19.5
C_{16}-MEP	78.1	39.1	NT[a]	>1250	NT[a]	78.1
Chlorhexidine[b]	250	62.5	15.6	7.8	7.8	3.9

[a]NT: not tested. [b]Chlorhexidine digluconate.

because of its strong antimicrobial activity and broad antimicrobial spectrum (15). It is clear that the antimicrobial activities of these surface active compounds are markedly effected by their alkyl chains as other antimicrobial compounds (1,2) are effected by hydrophobic effect, and that C_{16}-HDMEP and C_{16}-MEP have the highest activities against fungi, such as *Aspergillus niger* and *Penicillium citrinum*, and the same MICs values, whose values are smaller than the corresponding CMCs values. This shows that their micelle type of aggregates do not interact with the cell membranes of fungi, but that their monomers do. On the other hand, C_{16}-HDMEP is more hydrophobic than C_{16}-MEP, because the CMC of C_{16}-HDMEP is lower than that of C_{16}-MEP. Thus, the antimicrobial activities of C_{16}-HDMEP and C_{16}-MEP cannot be explained by hydrophobic effect alone. This shows that another effect relates with their antimicrobial activities.

The hydrophobic character, due to the fatty hydrocarbon chain and the absence of charge, is likely allow the adsorption and the transport across membranes of microorganisms (16). The phosphobetaines, in this study, have a charge of a 2-hydroxyl group in a long hydrocarbon chain, and MEP has a charge of a tertiary nitrogen in their molecules. Accordingly, it is presumed that the presence of a charge effects their antimicrobial activities. The elucidation of hydrophobic, electronic and steric factors of the mechanisms of the antimicrobial actions of these compounds, as well as the relation between the structure and the antimicrobial activities in comparison with the activities of other phosphobetaines, 2-(N,N,N-trialkylammonio)alkyl hydrogen phosphates with 2-(N-alkyl-N,N-dimethylammonio)ethyl hydrogen phosphates (17), will be discussed in a later paper.

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[Received August 14, 1989; accepted January 17, 1990]
[JS/D5774]