New Amphoteric Surfactants Containing a Phosphoric Acid Group: II. Binary System of Amphoteric/Anionic Surfactant of Sodium 2-(N-2-hydroxytetradecyl-N-methylamino)Ethyl Hydrogen Phosphate

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We studied behaviors of the binary system of 2-(N-2-hydroxytetradecyl-N-methylamino)ethyl hydrogen phosphate (C_{14}-HMP) and sodium dodecyl sulfate (SDS) in aqueous solution. We found that C_{14}-HMP reduced the ability of SDS to denature protein, to inhibit enzyme activity and to decrease transepidermal water loss. These phenomena were considered to be due to the interaction between the cationic group in the zwitterionic structure of C_{14}-HMP and the anionic group of sulfate in SDS molecule.

These behaviors suggest that C_{14}-HMP has a potential to reduce skin irritation when applied to personal care products such as cleaners and shampoos having anionic surfactants.

It has been reported that the effect on surface activity of a mixed surfactant system is superior to that of a surfactant alone (1). Consequently, it is very important to know not only the basic properties of individual surfactants but also the behaviors of the aqueous solution of such combinations. Thus, a number of solution properties for mixed surfactant systems have been studied (2–4).

Miyazawa *et al.* (5) have reported that the decreasing effects of protein-denaturation by anionic surfactant were observed in a mixed system of amphoteric/anionic surfactants. On the other hand, except for the effect of protein-denaturation, it is thought that there are many factors affecting skin-irritation, for example, the inhibitory-action of enzyme activity (6), the increasing effect of transepidermal water loss (7,8) and so on. However, concerning the mixed system of amphoteric/anionic surfactants few studies on skin-irritation have been carried out.

Our previous paper has described the preparation and basic physicochemical properties of the amphoteric surfactants, sodium 2-(N-alkyl-N-methylamino)ethyl hydrogen phosphates (9) and sodium 2-(N-2-hydroxyalkyl-N-methylamino)ethyl hydrogen phosphate (C_{14}-HMP).

In this paper, using sodium dodecyl sulfate (SDS) as a typical anionic surfactant, in order to investigate the behavior of the binary system of C_{14}-HMP/SDS in aqueous solution, we describe the reducing ability of C_{14}-HMP to denature protein, to inhibit enzyme activity, and to decrease transepidermal water loss, thus, evaluating the physicochemical properties of this mixed system.

EXPERIMENTAL METHODS

Materials. The sample of C_{14}-HMP used in this paper is the same as described in paper I of this series. SDS was reagent quality from Wako Pure Chemical Industries, Ltd., Japan. Protein and acid phosphatase are ovalbumin (Grade v, Sigma Chem. Co.) and Wheat Germ (P-L Biochemicals, Inc. USA), respectively.

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Measurements of physicochemical properties. With respect to the aqueous solutions of mixed surfactant systems, surface tension was measured at 25°C with a du Nöuy autotensiometer (Model 6801ES, RIGOSHA & CO. LTD, Japan) at concentrations from 10^{-5} to 10^{-2} mole/L. The surface tension-concentration plots were used to determine critical micelle concentration (CMC, break points).

Determination of protein denaturation potential. The protein denaturation potential was assayed according to the method of Miyazawa *et al.* (5). The protein-denaturation potency was determined from the decreasing of elution volumes of protein after 3 min mixing 1% protein aqueous solution with 0.4% surfactant aqueous solution or distilled water, by aqueous gel-permeation chromatography (Shodex OH pack B-803, Showadenko, Japan, 8 mm $\phi \times 500$ mm, as a stationary phase, 50 mM NaCl aqueous solution, 1 mL/min as a mobile phase when monitored at 240 nm). The magnitude of protein denaturation potential was calculated using the following equation:

$$\text{protein-denaturation potency (\%)} = (H_0 - Ht)/H_0 \times 100$$

where, H_0 and Ht are the peak heights of protein in the absence and presence of surfactants, respectively.

Determination of inhibitory-effect of acid phosphatase activity. Acid phosphatase (AcPase) was assayed according to the method of Imokawa and Katsumi (6). A total of 0.5 mg/mL of acid phosphatase aqueous solution and 37.1 mg/mL of sodium p-nitrophenylphosphate aqueous solution were adjusted, respectively. Mixtures of 50 μL each of the test solution and 250 μL acid phosphatase aqueous solution (pH 5.0) were warmed for 20 min at 37°C. To this, 50 μL of sodium p-nitrophenylphosphate aqueous solution was added, and the mixture was warmed for 40 min at 37°C. Subsequently, 1.6 mL of 8% trichloroacetic acid aqueous solution was added to the mixture to stop the reaction. A total of 0.5 mL of this reacted solution and 2.5 mL of 0.5 N sodium hydroxide aqueous solution were mixed to develop color, and absorbance at 410 nm was measured with spectrometer. The rate of inhibitory-effect of acid phosphatase activity in each sample was calculated using the following equation:

$$\text{inhibitory action (\%)} = (1 - ODs/ODc) \times 100$$

where ODs and ODc are the absorbance when test sample solution was added and the same when distilled water was added as a control, respectively.

Determination of transepidermal water loss. Transepidermal water loss (TWL) was measured according to the method of Nilson (7). Six healthy adult males were used as subjects. The test solution (40 μL) was applied to the adhesive plaster for a patch test (20 × 20 mm, Ribbon Aid, River Tape Seiyaku, Japan), and the plaster was placed on each of the 2 lateral flex regions of the subjects'

bilateral inner forearms for 24 hr. In 1 hr after removal of the patch, transepidermal water loss (TWL value) at the test site was measured with Evaporimeter (EPI type, Servomed Co., Sweden) under the environmental conditions of 24°C and 55% RH. As a control experiment, TWL value at nontreated site was measured, and relative TWL value was calculated according to the following equation:

$$\text{relative TWL (\%)} = \frac{\text{TWL value at test site}}{\text{TWL value at nontreated site}} \times 100$$

RESULTS AND DISCUSSION

Physicochemical properties of aqueous solutions of mixed systems of C_{14}-HMP/SDS. The physicochemical properties of the mixed system of C_{14}-HMP/SDS were investigated. First of all, we measured the surface tension-log concentration plots (Fig. 1). In the case of each mole ratio, the surface tension decreases with the increase of concentration, and the formation of mixed micelles in water was demonstrated by the clear evidence of a break point. Therefore, as the CMC values of mixed micelles in the C_{14}-HMP/SDS system are regarded as the concentration showing the break points, it is found that the CMC values are within the range of ca. 1 mM∼ca. 10 mM, whose values are below the CMC value of pure SDS (5.9 mM). Next, the log(CMC)-mole fraction of C_{14}-HMP plots in C_{14}-HMP/SDS system is shown in Figure 2. The CMC values decrease by adding C_{14}-HMP into SDS aqueous solution, passing the minimum level at the mole fraction of 0.75 of C_{14}-HMP. Similarly, the surface tension-molar fraction of C_{14}-HMP plots is shown in Figure 3. The surface tension of the aqueous solutions of C_{14}-HMP/SDS

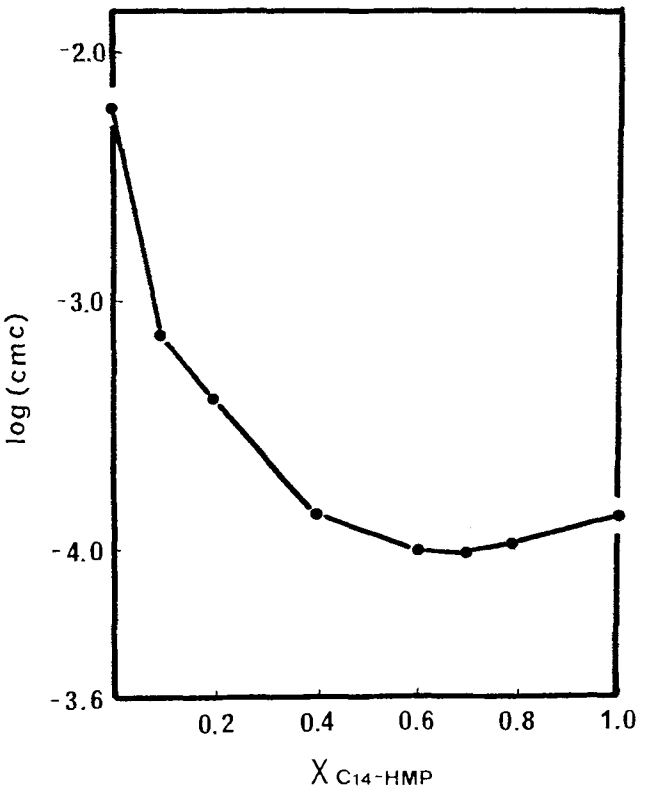

FIG. 2. Plots of log (CMC)-mole fraction of C_{14}-HMP in C_{14}-HMP/SDS system (25°C).

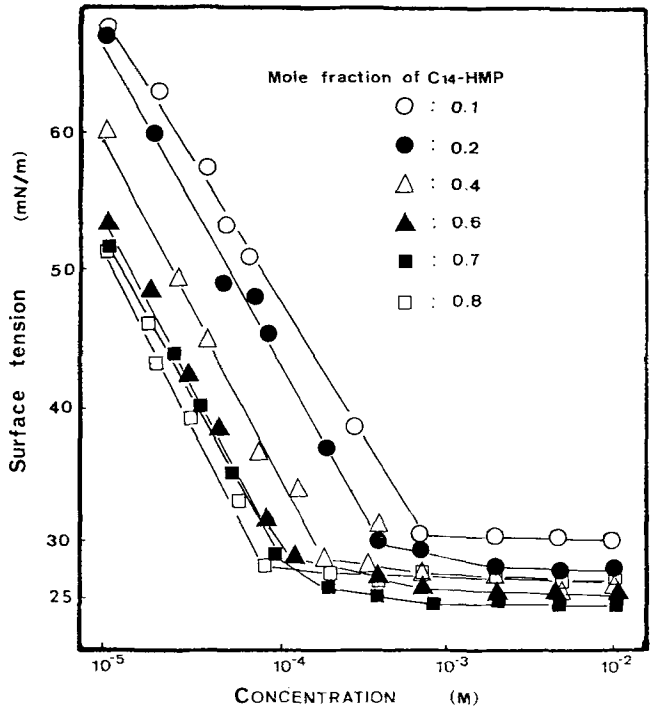

FIG. 1. Plots of surface tension-concentration in C_{14}-HMP/SDS system (25°C).

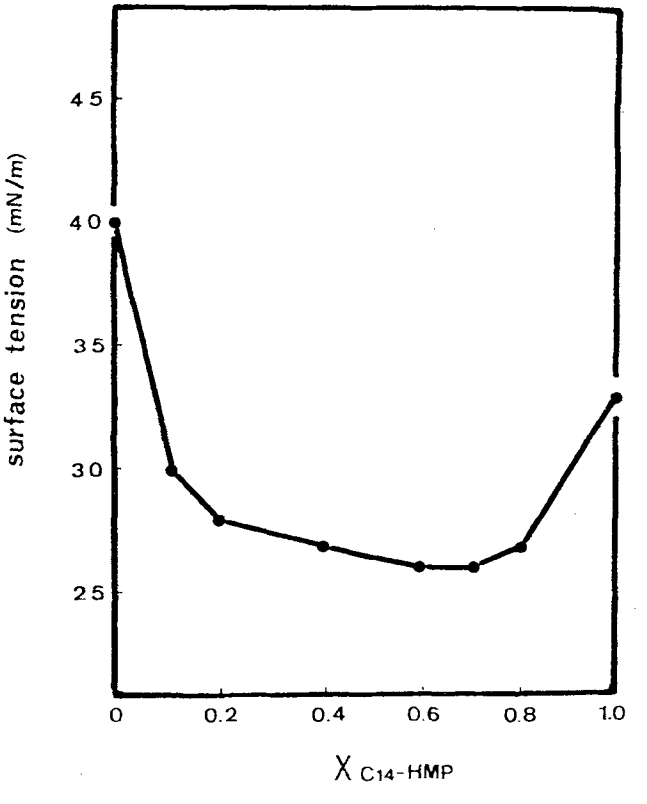

FIG. 3. Plots of surface tention-mole fraction of C_{14}-HMP in C_{14}-HMP/SDS system (concentration: 10 mM, 25°C).

mixture at 10 mM (above the CMC) is plotted against the mole fraction of C_{14}-HMP. The surface tension decreases with the mole fraction of C_{14}-HMP and then increases through a minimum. The surface tension has a minimum at a mole fraction of 0.7 for C_{14}-HMP. These phenomena might be attributed to the formation of mixed micelles by interaction between the cationic group in the zwitterionic structure of C_{14}-HMP and the anionic group of sulfate in SDS molecule. The interaction is affected by the component ratio, as shown in Figure 2 and Figure 3.

Skin-irritating properties of aqueous solutions of mixed systems of C_{14}-HMP/SDS. Firstly, the percent protein-denaturation by C_{14}-HMP/SDS mixtures was investigated with a variant concentration of C_{14}-HMP in constant concentration of SDS (Table 1). The percent protein-denaturation decreases with increasing of C_{14}-HMP. The percent protein-denaturation was very low value when the weight ratio of C_{14}-HMP/SDS was 10. Thus, we found that C_{14}-HMP reduced the ability of SDS to denature protein.

TABLE 1

Protein-Denaturation Potency of C_{11}-HMP/SDS System

Surfactant	Protein-denaturation (%)
0.2 % SDS	49.1 ± 1.9
0.2% SDS + 0.1% C_{14}-HMP	40.6 ± 1.5
0.2% SDS + 0.2% C_{14}-HMP	31.3 ± 2.5
0.2% SDS + 0.2% C_{14}-HMP	31.3 ± 2.5
0.2% SDS + 2.0% C_{14}-HMP	−6.0 ± 3.7

Mean ±SD (n = 3).

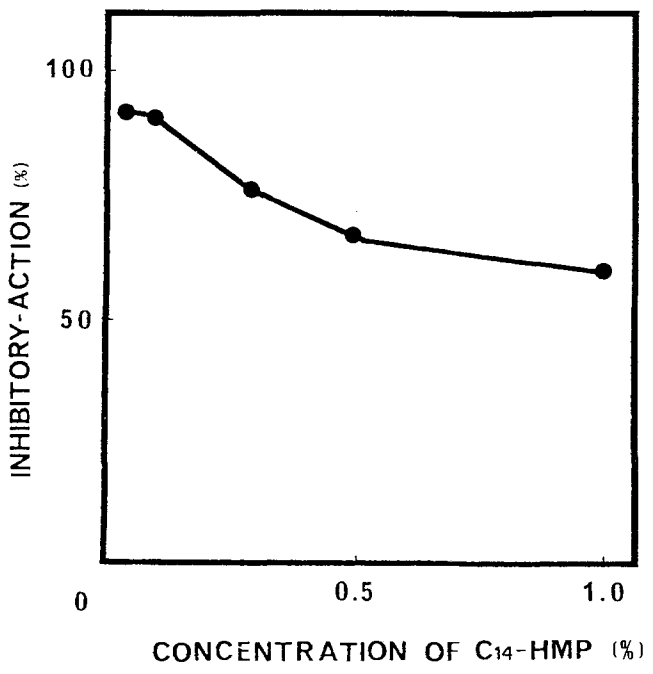

FIG. 4. Depressing effect on inhibitory-action of acid phosphatase activity by C_{14}-HMP/SDS mixtures.

TABLE 2

Transepidermal Water Loss (TWL) Values and Relative Values Against Distilled Water After Closed Patch Test for 24 hr*

	0.25% SDS	2.5% C_{14}-HMP + 0.25% SDS	2.5% C_{14}-HMP	distilled water
TWL (g/m^2hr)	1.4 ± 0.7	1.0 ± 0.5	0.7 ± 0.2	0.7 ± 0.3
Relative value	202 ± 74	142 ± 19	110 ± 23	100 ± 0

*Mean ±SD (n = 6).

One of the reasons for the development of protein-denaturation is due to adsorption of surfactant onto protein surface. The reducing ability of C_{14}-HMP/SDS mixture to denature protein might be attributed to the inhibition of adsorption of SDS molecules on the surface of protein caused by the presence of definite amounts of C_{14}-HMP, which complexed strongly with SDS by ionic-interaction.

When chemical compounds give some hindrances onto horney layer of skin, it is known that acid phosphatase activity is depressed with the evolution of free acid phosphatase from cell membranes. In other words, surfactants having a certain level of skin-irritating seem to have a marked inhibitory effect on acid phosphatase (6).

Thus, secondly, the reducing ability to inhibit enzyme activity by C_{14}-HMP/SDS mixture was investigated (Fig. 4). We found that C_{14}-HMP reduced the ability of SDS to inhibit enzyme activity as the ability increases with the increasing of C_{14}-HMP.

If the dermal corneum is damaged by physical and chemical stimulation, percutaneous evaporative water loss increases in proportion to the degree of the damage (10). In other words, it is presumed that the higher the increase in the percutaneous evaporative water loss after pasting on the sample, the dermal stimulation from the sample is higher. Thus, thirdly, transepidermal water losses were investigated for 0.25% SDS, 0.25% SDS + 2.5% C_{14}-HMP, 2.5% C_{14}-HMP and distilled water. These results are shown in Table 2. By statistical treatments, in regard to the differences between each average value of tested solutions, a significant difference was not observed between 2.5% C_{14}-HMP aqueous solution and distilled water as a control. Therefore, the dermal stimulations measured this time estimated to be as follows:

$$0.25\%\ \text{SDS} > 0.25\%\ \text{SDS} + 2.5\%\ C_{14}\text{-HMP}$$

$$> 2.5\%\ C_{14}\text{-HMP} \geqslant H_2O$$

HMP has a weak dermal barrier hypofunction (10), for which its safety when applied to the skin is presumed to be high. And from the fact that an aqueous solution containing 0.25% SDS + 2.5% C_{14}-HMP has lower tendencies to damage dermal barrier competence than the aqueous solution containing only 0.25% SDS, we found that C_{14}-HMP reduced the ability of SDS to increase transepidermal water loss.

From these *in vitro* and *in vivo* behaviors of the binary system of C_{14}-HMP/SDS in aqueous solution, we found that C_{14}-HMP has a potential to reduce some factors of dermal stimulation (11) by SDS. These reducing potentials were considered to be due to the interaction between C_{14}-HMP and SDS as described above following the formation of mixed micelles.

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