Effects of Surface Activity on Aquatic Toxicity of Binary Surfactant Mixtures

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Abstract: The purpose of this study was to discuss the effects of surface activity on the aquatic toxicity of binary surfactant mixtures comprising anionic, nonionic, and cationic surfactants. Surface tension was measured to determine the cmc (critical micelle concentration), and acute aquatic toxicity tests were conducted on *Daphnia magna* to obtain 24h-EC₅₀ (24h 50% effective concentration). TU (toxic unit) was calculated to evaluate the toxicity of the mixture. Most of the surfactant mixtures showed no synergistic increase in the aquatic toxicity. The mixture of anionic/nonionic surfactants showed synergistic interfacial activity with decreasing cmc, but the toxicity did not increase. The surface tension of the mixture at 24h-EC₅₀ (γ_{tox}), which was used as an indicator of the toxic concentration, decreased considerably and TU was >1, indicating decreased toxicity. γ_{tox} of the anionic/anionic surfactant mixture decreased when tested with hard water (hardness of 625 ppm). γ_{tox} could not be used as a toxic indicator for the anionic/cationic surfactant mixtures because they showed aquatic toxicity before their surface tension began to decrease.

Key words: surface tension, EC₅₀, water hardness, anionic surfactant, nonionic surfactant, cationic surfactant

1 INTRODUCTION

It is widely known that the toxicity of surfactants increases when the cellular permeability of aquatic species increases in response to decreased surface tension. This causes the surfactants and other chemical substances to enter the environment¹. Therefore, environmental modifying factors such as water hardness, temperature, and chemical substances and adsorbents that affects the surface tension can influence the aquatic toxicity of surfactants²⁻⁴. In our previous study, we focused on water hardness and the presence of adsorbents to discuss the effects of water properties on the aquatic toxicity of anionic and nonionic surfactants using surface tension as an indicator of the interfacial activity of the surfactants⁵. The surface tension value at 24h 50% effective concentration (24h-EC₅₀) was defined as γ_{tox} . When the same type of surfactant and test organism was used, γ_{tox} remained constant, even when EC₅₀ was varied by changing the water hardness and/or the amount of adsorbent. Therefore, we confirmed that surface tension can be a good indicator of the aquatic toxicity of a surfactant.

Several researchers have reported that the interfacial

activity of surfactant mixtures increases depending on their ionic characteristics, the type of surfactant used, the mixing ratio, and the test conditions in the mixture⁶. In particular, the interfacial activity of anionic/nonionic mixed surfactant systems has often been studied owing to their wide usage in industrial and household applications^{7,8}. Abe and Ogino⁸ found that the critical micelle concentration (cmc) of a mixture of sodium dodecyl sulfate (SDS) and alkyl polyoxyethylene (alkyl chain length: 18, number of POE units: 20) was much lower than that of SDS alone. Anionic/anionic surfactant mixtures are also widely used because their physical properties such as solubility can be efficiently controlled in the mixture⁹. Although the physical properties of surfactant mixtures have been well studied, less focus has been placed on their aquatic toxicity.

Lewis *et al.*¹⁰ studied the aquatic toxicity of binary and ternary surfactant mixtures of anionic, nonionic, and cationic surfactants using *Daphnia magna* and *Lepomis macrochirus*. They measured the toxicity of surfactant mixtures in terms of toxic units and the additive index and found that it was generally less than (decrease in toxicity) or equal to (no synergistic increase or decrease in toxicity)

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Table 1 Combination of ionicity and kind of surfactant used in each test

Ionicity	water hardness 25ppm	water hardness 625ppm
Anionic/Anionic	LAS/AS, LAS/SOAP	LAS/AS
Anionic/Nonionic (general)	LAS/AE9, AS/AE9 SOAP/AE9	LAS/AE9
Anionic/Nonionic (effect of EO units length)	LAS/AE6, LAS/AE12 LAS/AE18	
Anionic/Cationic	LAS/HTAC	LAS/HTAC

the additive toxicity of the individual surfactants. However, researchers including Lewis *et al.* have not discussed toxicity from the viewpoint of interfacial activity, which is the main cause of aquatic toxicity of surfactants. If the increased interfacial activity of a surfactant mixture can directly affect the aquatic toxicity, the toxicity of surfactant mixtures may increase. Therefore, the interfacial activity of surfactants and factors that can increase the interfacial activity such as mixing ratio and water properties should also be discussed.

In this study, we discussed the aquatic toxicity of binary surfactant mixtures comprising anionic, nonionic, and cationic surfactants and used surface tension as an indicator of the surface activity. *D. magna* was used as the test organism to determine the aquatic toxicity at 24h-EC₅₀. To investigate the relationship between the surface activity and aquatic toxicity, γ_{tox} was used as an indicator of the point at which toxicity emerges (24h-EC₅₀). Each ionic/ionic and ionic/nonionic surfactant mixture was evaluated at three different mixing ratios. Further, we investigated the effects of the length of the hydrophilic group of nonionic surfactants and the change in hardness of the test water on toxicity.

2 EXPERIMENTAL PROCEDURE

2.1 Surfactants and mixed surfactants

Three anionic surfactants, four nonionic surfactants, and one cationic surfactant were used in this study. The three anionic surfactants were LAS(sodium linear alkylbenzene sulfonate, Wako Chemical Co., used for a laundry detergent test in JIS, Assay>95%), AS(sodium dodecyl sulfate, Wako Chemical Co., Assay(GC)>95%), and SOAP(sodium oleate, Wako Chemical Co., Assay(GC)>60%). The carbon number distribution of LAS obtained by HPLC analysis was as follows: C₁₀: 15.9 %, C₁₁: 33.2 %, C₁₂: 30.3 %, and C₁₃: 20.54 %. The four nonionic surfactants used were AE9(alcohol ethoxylates, C:12, EO: 9, Wako Chemical Co.), AE6(alcohol ethoxylates, C:13, EO: 6, Sigma-Aldrich Co.), AE12(alcohol ethoxylates, C:13, EO:12, Sigma-Aldrich Co.), AE18(alcohol ethoxylates, C:13, EO:18, Sigma-Al-

drich Co.). Additionally, the cationic surfactant used was HTAC(hexadecyltrimethylammonium chloride, Wako Chemical Co.).

The combination of ionicity, types of surfactant used in the mixtures, and water hardness are shown in **Table 1**. Surface tension measurements and acute aquatic toxicity tests were conducted on each surfactant mixtures at a mixing ratio of 0:10, 3:7, 5:5, 7:3, and 10:0 by weight.

2.2 Test water

Standard test water with hardness of 25 ppm as CaCO₃ was prepared by dissolving 26.1 mg of CaCl₂ · 2H₂O, 17.7 mg of MgSO₄ · 7H₂O, 1.1 mg of K₂SO₄, and 25 mg of NaHCO₃ in 1 L of distilled water. In addition, test water at a water hardness of 625 ppm based on the CaCO₃ concentration was prepared using twenty-five times more of each of the water hardness components used to prepare the test water with a water hardness of 25 ppm.

2.3 Surface tension test

EZ-Pi(Kibron Inc.) with a platinum wire probe was used to measure the surface tension of the test solution by the Wilhelmy balance method. The temperature of the test water was controlled between 20°C and 22°C, and it was allowed to stand for more than 20 min before the measurement to stabilize its surface. The surface tension value of the surfactant at 24h-EC₅₀ was then determined and reported as γ_{tox} .

2.4 Acute aquatic toxicity test

A toxicity test was performed according to JIS K 0229 (1992), and *D. magna* was used as the test organism. To use larval organisms born within 24 h of the test, the adult organisms were transferred to breeding vessels 24 h prior to the test⁶⁾. A total of 9.5 mL of test water and test surfactant solution were poured in a 20 mL volumetric glass tube. Five test organisms were then transferred into each test tube and distilled water was added to the 10 mL line. Four test tubes were prepared for each concentration of the sample, and two test tubes without surfactant were prepared for the blank test. The tubes were then incubated under a 16 h/8 h light/dark cycle at 20°C to 22°C for 24 h,

after which the dead and immobilized individuals were counted. Next, the immobilization rate was calculated by dividing the number of dead and immobilized individuals by the number of total individuals. Next, 24h-EC₅₀ was determined by graphical plotting of the immobilization rates against the surfactant concentration.

2.5 Toxic unit concept

The toxic unit (TU) is an indicator of toxicity that is defined as the actual concentration in solution divided by the lethal threshold concentration¹¹⁾. To determine the toxicity of a mixture, the toxic units of each surfactant were added together using the following equation¹²⁾:

$$(Am/Ai) + (Bm/Bi) = TU \quad [1]$$

where Ai is the EC₅₀ value for surfactant A alone, Bi is the EC₅₀ value for surfactant B alone, Am is the concentration of surfactant A in the surfactant mixture, and Bm is the concentration of surfactant B in the surfactant mixture. The TU of surfactant A (= Am/Ai) and surfactant B (= Bm/Bi) were added together to express the TU of the surfactant mixture. If the TU is equal to 1, the toxicity is simply additive (no synergistic increase or decrease in toxicity), while the toxicity is less-than-additive (synergistic decrease in toxicity) if the TU is greater than 1 and it is greater-than-additive (synergistic increase in toxicity) if it is less than 1.

3 RESULTS AND DISCUSSION

3.1 Surface activity and aquatic toxicity of individual surfactants

The cmc was used to evaluate the surface activity and 24h-EC₅₀ among groups was compared to evaluate the aquatic toxicity of individual surfactants (Table 2). LAS showed the highest surface activity of the three anionic surfactants, with the lowest cmc (100 mg/L). The cmc val-

Table 2 Critical micelle concentration (cmc) and 24h-EC₅₀ of test surfactants

Surfactant	cmc (mg/L)	EC ₅₀ (mg/L)
LAS	100	10
AS	500	34
SOAP	560	4
AE9 (C=12)	56	3
AE6 (C=13)	50	4.5
AE12 (C=13)	180	27
AE18 (C=13)	320	50
HTAC	180	0.01

ues of the other two anionic surfactants, AS (500 mg/L) and SOAP (560 mg/L), were relatively close and much higher than that of LAS. Despite the high surface activity, the toxicity of LAS was lower than that of SOAP. With a water hardness of 25 ppm, SOAP showed the strongest toxicity among the three anionic surfactants, while AS showed the weakest toxicity. The nonionic surfactants, AE9 and AE6, showed higher surface activity and stronger aquatic toxicity than AE12 and AE18, which had longer EO chain lengths. These findings are similar to those reported by Kikuchi¹³⁾, who found that the toxicity of C12-AE to *Oryzias latipes* increased as the EO unit increased from 3 to 16. Based on the results obtained with AE9 and AE6 in the present study, the difference in toxicity caused by the difference in EO chain length was not apparent because their alkyl chain lengths were different (AE9, C = 12; AE6, C = 13). The cmc of the cationic HTAC was about 180 mg/L, which was similar to that of AE12, while 24h-EC₅₀ was significantly low as expected because HTAC is often contained in pesticides.

Table 3 Critical micelle concentration (cmc), 24h-EC₅₀, and TU of anionic/anionic surfactant mixtures at water hardness of 25 ppm

	Mixed ratio	cmc (mg/L)	EC ₅₀ (mg/L)	TU
LAS/AS	10:0	100	10	1.00
	7:3	180	12	0.91
	5:5	180	12	0.74
	3:7	320	12	0.58
	0:10	500	34	1.00
LAS/SOAP	10:0	100	10	1.00
	7:3	100	9	1.31
	5:5	320	7.5	1.31
	3:7	560	11	2.26
	0:10	560	4	1.00

3.2 Relationship between surface tension and aquatic toxicity of binary surfactant mixture for varying ionicity and mixing ratio

3.2.1 Anionic/anionic surfactant mixture

The cmc was used to evaluate the surface activity and the TU values were compared to evaluate the aquatic toxicity. Additionally, the γ_{tox} was used to discuss the relationship between surface activity and aquatic toxicity.

The change in cmc and TU of LAS/AS and LAS/SOAP with different mixing ratios is shown in Table 3. The cmc values of LAS/AS increased as the ratio of AS in LAS/AS increased. The aquatic toxicity of LAS/AS was slightly greater than additive. The 3:7 mixture showed the greatest toxicity, as indicated by a TU value was 0.58, while that of the 7:3 mixture was 0.91 and that of the 5:5 mixture was 0.74. On the other hand, the toxicity of LAS/SOAP was less than additive and the decrease in toxicity was greater for the 3:7 mixture, as indicated by a TU value of 2.26. These findings indicate that small amounts of LAS have a relatively strong influence upon the toxicity of the LAS/anionic mixture.

Figures 1 and 2 show the γ_{tox} of LAS/AS and LAS/SOAP, which is the surface tension value at the concentration corresponding to 24h-EC₅₀. The γ_{tox} of the mixtures changed depending on the mixing ratio of the LAS/AS, but the γ_{tox} of LAS/SOAP was almost the same. Therefore, the γ_{tox} change in the anionic/anionic surfactant mixtures varies depending on the type of surfactant used. However, a characteristic tendency was observed in both mixtures in which the γ_{tox} values were lower than that of AS or SOAP and higher than

Fig. 1 γ vs concentration curves for LAS/AS mixtures with different mixing ratios.

Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

Fig. 2 γ vs concentration curves for LAS/SOAP mixtures with different mixing ratios.

Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

that of LAS. For example, the γ_{tox} values of LAS/AS ranged from 48 to 52 mN/m, while those of LAS and AS were 45.7 mN/m and 62 mN/m, respectively (Fig. 1).

3.2.2 Anionic/nonionic surfactant mixture

The cmc values of anionic/nonionic surfactant mixtures were lower than those of the individual surfactants and were the lowest of all the compounds evaluated (Table 4). These findings are in agreement with those of a study conducted by Okano, who showed synergistically enhanced surface activity of anionic and nonionic surfactant mixtures⁷⁾. The cmc of the 5:5 mixture of LAS/AE9 was lower than the values of LAS and AE9. Additionally, the cmc values of AS/AE9 and SOAP/AE9 decreased greatly when a small amount of AE9 was added. For example, the cmc of the 7:3 mixture of AS/AE9 was about 100 mg/L, while that of the 10:0 mixture (AS) was about 500 mg/L. Despite such synergistic increase in surface activity, the TU values of the anionic/AE9 mixtures were all greater than 1, indicating that those toxicities were less than additive. In other words, the toxicity of the anionic/AE9 mixtures decreased.

Unlike the anionic/anionic surfactant mixture, there was a distinctive trend observed in the γ_{tox} of the anionic/nonionic surfactant mixtures. Specifically, a decrease in γ_{tox} to values lower than those of both individual surfactants was seen in all anionic/AE9 mixtures (Fig. 3, 4 and 5). In the case of the anionic/anionic surfactant mixtures, the γ_{tox} values of the mixtures were between the values of the individual surfactants.

Table 4 Critical micelle concentration (cmc), 24h-EC₅₀, and TU of anionic/nonionic surfactant mixtures at water hardness of 25 ppm

	Mixed ratio	cmc (mg/L)	EC ₅₀ (mg/L)	TU
LAS/AE9	10:0	100	10	1.00
	7:3	40	9	1.75
	5:5	30	9	1.82
	3:7	40	6	1.26
	0:10	56	3	1.00
AS/AE9	10:0	500	34	1.00
	7:3	100	9	1.09
	5:5	100	8	1.45
	3:7	56	6	1.45
	0:10	56	3	1.00
SOAP/AE9	10:0	560	4	1.00
	7:3	100	6	1.65
	5:5	100	4	1.17
	3:7	56	5	1.39
	0:10	56	3	1.00

Fig. 3 γ vs concentration curves for LAS/AE9 mixtures with different mixing ratios. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀. **Fig. 4** γ vs concentration curves for AS/AE9 mixtures with different mixing ratios. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

3.2.3 Anionic/cationic surfactant mixture

The cmc decreased greatly, especially in the 5:5 mixture, showing an increase in surface activity (Table 5). The cmc value of the 5:5 mixture was approximately 10 mg/L, while the values of LAS was 100 mg/L and that of HTAC was 180

mg/L. Ogino reported that the formation of anionic-cationic complexes increases the surface activity of the anionic/cationic surfactant mixture⁹. The values of TU for LAS/HTAC were greater than or equal to 1; therefore, the aquatic toxicities of the anionic/cationic surfactant mixtures were less

Fig. 5 γ vs concentration curves for SOAP/AE9 mixtures with different mixing ratios. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

Fig. 6 γ vs concentration curves for LAS/HTAC mixtures with different mixing ratios. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

than additive, which was in agreement with Lewis' report (Table 5). Therefore, the increase in surface activity did not increase the toxicity of the anionic/cationic surfactant mixture.

An index of γ_{tox} cannot be used as a toxicity indicator of anionic/cationic surfactant mixtures because the toxicity emerged before the surface tension began to decrease (Fig. 6). The γ_{tox} values of the surfactant mixtures were equal to that of HTAC, which was about 72.8 mN/m (surface tension value of distilled water). These findings indicate that the main cause of the toxicity of the anionic/cationic surfactant mixtures is not the interfacial activity.

3.2.4 Effect of number of EO units on relationship between surface activity and aquatic toxicity of LAS/AE mixed systems

The LAS/AE9 mixture showed the greatest decrease in cmc among anionic/nonionic surfactant mixtures. AE is

widely used because its surface activity can be controlled by adjusting the length of the hydrocarbon chain and EO chain¹⁴. We focused on the EO chain length and its effect on the surface activity, aquatic toxicity, and γ_{tox} of the LAS/AE mixture.

The results revealed the same tendency in the anionic/nonionic surfactant mixtures. Specifically, the cmc of the surfactant mixtures decreased below the values of the individual surfactants (Table 6). However, the decrease in the cmc value was different when the EO chain length changed. The cmc values of LAS/AE12 showed the greatest decrease from 180 mg/L (10:0) to 56 mg/L (3:7). In the case of LAS/AE18, the cmc value decreased from 320 mg/L (10:0) to 100 mg/L (3:7). The cmc values of LAS/AE6 only decreased from 50 mg/L (10:0) to 40 mg/L (3:7), which was not significant. Therefore, it can be assumed that AE with an EO chain length of 12 (AE12) has the strongest interac-

Table 5 Critical micelle concentration (cmc), 24h-EC₅₀, and TU of anionic/cationic surfactant mixtures at water hardness of 25 ppm

	Mixed ratio	cmc (mg/L)	EC ₅₀ (mg/L)	TU
LAS/HTAC	10:0	100	10	1.00
	7:3	100	0.07	2.10
	5:5	10	0.04	2.00
	3:7	32	0.02	1.40
	0:10	180	0.01	1.00

Table 6 Critical micelle concentration (cmc), 24h-EC₅₀, and TU of anionic/AE (EO unit length=6, 12, 18) surfactant mixtures at water hardness of 25 ppm

	Mixed ratio	cmc (mg/L)	EC ₅₀ (mg/L)	TU
LAS/AE6	10:0	100	10	1.00
	7:3	40	9	1.16
	5:5	40	7	0.89
	3:7	40	5	0.68
	0:10	50	4.5	1.00
LAS/AE12	10:0	100	10	1.00
	7:3	56	18	1.42
	5:5	56	23	1.87
	3:7	56	22	1.78
	0:10	180	27	1.00
LAS/AE18	10:0	100	10	1.00
	7:3	80	14	1.06
	5:5	56	20	1.52
	3:7	100	28	2.13
	0:10	320	50	1.00

tion with LAS.

The TU values of LAS/AE12 and LAS/AE18 were greater than 1 (less than additive), which indicates a decrease in toxicity (Table 6). Only the toxicity of the 5:5 and 3:7 mixtures of LAS/AE6 showed a slight increase in TU values to 0.89 and 0.68, respectively. However, a great decrease in γ_{tox} was observed in all three mixtures of LAS/AE6 (Fig. 7), LAS/AE12 (Fig. 8), and LAS/AE18 (Fig. 9).

3.3 Effect of water hardness on relationship between surface activity and aquatic toxicity

3.3.1 Anionic/anionic surfactant mixture

The existence of bivalent metal ions such as calcium and magnesium increases the surface activity of anionic surfactants^{3,5}. Thus, the cmc values of LAS, AS, and LAS/AS decreased significantly when tested with water with a hardness of 625 ppm (Table 7). However, the increase in surface activity was not synergistic. Unlike the results observed when the water hardness was 25 ppm, the aquatic toxicity of LAS/AS was less than additive, showing TU values greater than 1.

Contrary to the results observed when the water hardness was 25 ppm, a decrease in the γ_{tox} of the LAS/AS mixtures was observed when tested with a water hardness of 625 ppm (Fig. 10). When the water with a hardness of 25 ppm was used, this tendency was only seen for anionic/nonionic surfactant mixtures that showed a decrease in cmc and toxicity. For hard water, the relationship between

Fig. 7 γ vs concentration curves for LAS/AE6 mixtures with different mixing ratios.

Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

surface activity and aquatic toxicity of the LAS/AS was similar to that of anionic/nonionic surfactant mixtures.

Fig. 8 γ vs concentration curves for LAS/AE12 mixtures with different mixing ratios. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

Fig. 9 γ vs concentration curves for LAS/AE18 mixtures with different mixing ratios. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

Table 7 Critical micelle concentration (cmc), 24h-EC₅₀, and TU of LAS/AS at water hardness of 625 ppm

	Mixed ratio	cmc (mg/L)	EC ₅₀ (mg/L)	TU
LAS/AS	10:0	40	5.5	1.00
	7:3	32	11	1.52
	5:5	56	14	1.52
	3:7	100	18	1.43
	0:10	100	28	1.00

Table 8 Critical micelle concentration (cmc), 24h-EC₅₀, and TU of LAS/AE9 at water hardness of 625 ppm

	Mixed ratio	cmc (mg/L)	EC ₅₀ (mg/L)	TU
LAS/AE9	10:0	40	5.5	1.00
	7:3	16	8.0	1.55
	5:5	20	8.8	1.77
	3:7	20	6.2	1.29
	0:10	45	4.5	1.00

3.3.2 Anionic/nonionic surfactant mixtures

The results obtained using the anionic/nonionic surfactant mixtures were the same as those obtained using the anionic/anionic mixtures for water with hardness of 25 ppm. Additionally, synergistic decreases in cmc and toxic-

ity were observed (Table 8). A decrease in the γ_{tox} of LAS/AE9 mixtures was also observed when tested using water with a hardness of 625 ppm (Fig. 11). The decrease in the surface tension value at γ_{tox} was greater in mixtures than individual surfactants. These findings indicated that γ_{tox} of

Fig. 10 γ vs concentration curves for LAS/AS mixtures with mixing ratios of 5:5 in test water with hardness of 25 ppm and 625 ppm. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

Fig. 11 γ vs concentration curves for LAS/AE9 mixtures with mixing ratios of 5:5 in test water with hardness of 25 ppm and 625 ppm. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h-EC₅₀.

Table 9 Critical micelle concentration (cmc), 24h-EC₅₀, and TU of LAS/HTAC at water hardness of 625 ppm

	Mixed ratio	cmc (mg/L)	EC ₅₀ (mg/L)	TU
LAS/HTAC	10:0	40	5.5	1.00
	7:3	18	0.42	3.99
	5:5	10	0.75	11.79
	3:7	56	0.11	2.41
	0:10	70	0.03	1.00

the LAS/AE9 mixtures is more easily affected by the water hardness than LAS and AE9.

3.3.3 Anionic/cationic surfactant mixture

Similar to the results obtained when water with a hardness of 25 ppm was used, cmc values of 7:3 and 5:5 mixtures synergistically decreased below the values of LAS and HTAC when the water hardness increased (Table 9). The surface activity also synergistically increased in mixtures, but the surface tension curve and cmc value of the 5:5 mixture was not affected by the water hardness when compared to the results obtained when water with a hardness of 25 ppm was used (Fig. 12). Conversely, the aquatic toxicity of the 5:5 mixture was greatly affected by the water hardness, showing a significant increase in TU value (Table 9). The TU of the 5:5 mixture was about 12, while the value of the 7:3 mixture was 4 and that of the 3:7 mixture

was 2.4. Since no significant change in the surface tension was observed, it can be assumed that molecules of the LAS/HTAC complex in the bulk phase were affected by the existence of metal ions, which caused the decrease in toxicity. Although the mechanism of the decrease in toxicity is not clear, it is a very interesting and noteworthy phenomenon associated with the toxicity of mixtures of surfactants.

4 CONCLUSION

In this study, we discussed a new perspective for assessing the aquatic toxicity of surfactants on a laboratory scale by considering the surface activity of surfactant mixtures as the main cause of toxicity. The relationship between the surface activity and aquatic toxicity of anionic/anionic, an-

Fig. 12 γ vs concentration curves for LAS/HTAC mixtures with mixing ratios of 5:5 in test water with hardness of 25 ppm and 625 ppm. Solid lines and dashed lines represent the individual surfactants and the surfactant mixtures, respectively. Circles on each curve represent the points corresponding to γ_{tox} , i.e., the surface tension at 24h- EC_{50} .

ionic/nonionic, and anionic/cationic surfactant mixtures was investigated. γ_{tox} was used as a toxic indicator to represent the surface tension at the point at which toxicity emerges (24h- EC_{50}).

The relationship changed in response to the ionic combinations of the surfactants, but similar tendencies were observed for each combination. The changes in the surface activity, aquatic toxicity, and γ_{tox} of each mixture in test

water with hardness of 25 ppm and 625 ppm are shown in Table 10. We established the relationship between the surface activity and aquatic toxicity of binary surfactant mixtures and found that an increase in the surface activity did not necessarily increase the toxicity of the surfactant mixture. Instead, the aquatic toxicity of most of the surfactants decreased in the mixtures.

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Table 10 Summary of the relationship between surface activity and aquatic toxicity of anionic/anionic, anionic/nonionic, and anionic/cationic surfactant mixtures

Ionicity	Water hardness	Surface activity	Aquatic toxicity	γ_{tox}
Anionic (-) Anionic(-)	25 ppm	→	↗ LAS/AS ↘ LAS/SOAP	→
	625 ppm (LAS/AS)	→	↘	↘
Anionic (-) Nonionic (±)	25 ppm (Include change in EO unit length)	↗	↗ LAS/AE6 ↘ LAS/AE12 LAS/AE18	↘
	625 ppm (LAS/AE9)	↗	↘	↘
Anionic (-) Cationic (+)	25 ppm	↗	↘	Not related
	625 ppm	↗	↘	Not related

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