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Physicochemical Properties and Antimicrobial Performance of Benzyldimethylalkylammonium Bis(2-ethylhexyl) Phosphate

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Benzyldimethylalkylammonium salts with bis(2-ethylhexyl) phosphate maintain their liquid state at room temperature. These are immiscible but can be dispersed stably in water. The benzyldimethyltetradecylammonium salt emulsion exhibited the highest antimicrobial activity among them and some advantages as an additive for aqueous media compared with conventional cationic-surfactant-type antimicrobial agents.

1. Introduction

Benzyldimethylalkylammonium [BAn^+, Fig. 1(a)] halides have been considered to be one of the safest antimicrobial agents and have a long history of efficacious use.[1,2] The conventional BAn^+ salts are solid at room temperature and are soluble in water. In a previous paper,[3] we reported that the $BA14^+$ salt with a bis(2-ethylhexyl) phosphate [$BEHP^-$, Fig. 1(b)] anion maintains its liquid state even at room temperature and is immiscible in water. BA14BEHP can be dispersed stably, and the BA14BEHP emulsion exhibited an antimicrobial performance.

In this study, BA*n*BEHPs with different *n* values (= 10, 12, 14, 16, and 18) were prepared, and their physicochemical properties and antimicrobial performance were mutually compared. The physicochemical properties of BA*n*BEHPs, such as the glass transition temperature, viscosity, conductivity, density, and solubility of water, did not vary significantly with *n*. On the other hand, the minimum inhibitory concentrations (MICs) of BA*n*BEHP against a gram-positive bacterium, *Staphylococcus aureus* IFO012732, and a gram-negative bacterium, *Escherichia coli* CK111, were lowest with $n = 12$ and 14. The MICs of BA12BEHP and BA14BEHP were the same as that of BA14Cl.

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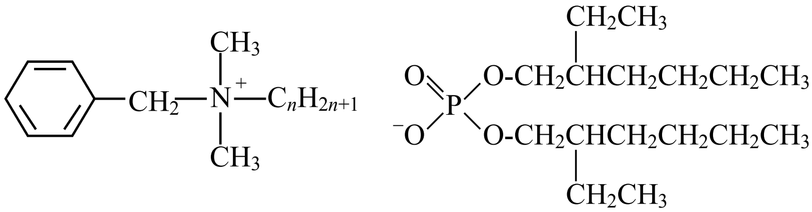

(a) (b)

Fig. 1. Chemical structures of (a) BAn^+ cation and (b) $BEHP^-$ anion.

Various types of antimicrobial materials have been the subject of research interest by some analysts.(4–7) In contrast to conventional cationic-surfactant-type antimicrobial agents such as BA14Cl, BA*n*BEHPs exhibited a low foaming property. Such a low-foaming cationic-surfactant-based antimicrobial agent seems to be promising for aqueous media such as cooling water or water-based cutting fluid.(8) The aqueous media usually contain calcium carbonate scale control agents such as polyacrylic acid sodium salt (PAAS)(9) and copper corrosion inhibitors such as 1*H*-benzotriazole (BTA). Thus, the influence of the additives on the antimicrobial performance of BA14BEHP has been studied. Also, the influence of BA14BEHP on the scale control by PAAS has been studied. Although conventional cationic-surfactant-type agents associate with anionic polymers to form colloidal particles,(10) BA14BEHP did not affect the scale control of PAAS.

2. Experimental Methods

2.1 *Chemicals*

Benzyldimethyltetradecylammonium chloride (BA14Cl, Tokyo Chemical Industry) and bis(2-ethylhexyl) hydrogenphosphate (HBEHP) (Wako Pure Chemical Industries) were used to prepare BA14BEHP as follows: 20 mmol of BA14Cl and 20 mmol HBEHP were mixed in 200 ml of water. 20 ml of 2 mol l^{-1} Na_2CO_3 aqueous solution was added slowly to the mixture. The mixture was heated while stirring until the solid substance was dissolved. The stirring was stopped, and then the mixture separated into an oily substance and an aqueous phase. The mixture was transferred to a separating funnel, and the aqueous phase was removed. The resultant crude BA14BEHP was purified by shaking with water several times. BA14BEHP was dried at 80 °C under 50 mmHg until the water content became 1% or less. BA14BEHP was confirmed by IR and UV spectra. The BA*n*BEHPs (n = 10, 12, 16, and 18) were obtained in the same manner as BA14BEHP with benzyldimethyldecylammonium chloride (Fluka), benzyldimethyldodecylammonium bromide, benzyldimethylcethylammonium chloride, and benzyldimethylstearylammonium chloride. The last three BAn^+-salts were obtained from Tokyo Chemical Industry. Polyacrylic acid sodium salt (molecular weight, 2100) and 1*H*-benzotriazole were obtained from Sigma and Wako, respectively. Other chemicals were reagent-grade materials. They were used without further purification.

2.2 *Apparatus*

The DSC analysis (DSC6220, SII Nanotechnology, Japan) was performed at a heating rate of 10 °C min^{-1}. Thermogravimetric analysis (TG-50, Shimadzu, Japan) was performed at a heating rate of 10 °C min^{-1} under nitrogen flow of 50 ml min^{-1}. A vibro viscometer (A&D SV-10), a conductivity meter (CM-40V, DKK-TOA, Japan), and a Karl Fischer titrator (AQV-5, Hiranuma Sangyo, Japan) were used for the determination of the viscosity, conductivity, and water content, respectively. The experiments were performed at 25 ± 1 °C.

2.3 *Antimicrobial test*

The antimicrobial performance of the BA*n*BEHP emulsion was examined according to the Japanese Pharmacopoeia with *S. aureus* IFO012732 and *E. coli* CK111. The bacterial cells were grown at 37 °C with shaking in LB medium and diluted at a density of 2.5–10 × 10^5 cells ml^{-1}. 50 µl of the bacterial suspension was mixed with 1 ml of test BA*n*BEHP emulsion, and then incubated at 25 °C for 1 h. 50 µl of the mixture was added to 3 ml of super broth, and then the bacterial cells were grown at 37 °C for 20 h with shaking. The bacterial growth was examined by visual observation of the degree of turbidity of each culture medium. The antimicrobial test was performed with the BA*n*BEHP emulsion at different weight concentrations to determine the MIC.

3. Results and Discussion

3.1 *Physicochemical properties*

The physicochemical properties of the present BA*n*BEHP are listed in Table 1. The values are in conjunction with those for BA14BEHP prepared with a standard material of benzyldimethyltetradecylammonium chloride (Zephiramine, Dojindo).[3] BA14BEHP did not display a solid-liquid phase transition, but displayed a glass transition in the DSC curve, and the glass transition temperature was determined to be −8 °C. In thermogravimetric analysis, BA14BEHP exhibited thermal stability up to *ca*. 210 °C. Thus, BA14BEHP exists in liquid state over a wide temperature range. The viscosity of BA14BEHP was determined to be 4500 mPa s at 25 °C, which is much higher than those of conventional water-immiscible ionic liquids, such as imidazolium-based ionic liquids and quaternary ammonium salts with bis(perfluoroalkylsulfonyl)imides.[11,12] Also, the

Table 1
Physicochemical properties of BA*n*BEHP (at 25 °C).

n	10	12	14	16	18
Glass transition temperature (°C)	−3	−6	−8	−10	−11
Viscosity (mPa s)	6800	4700	4500	5800	3300
Conductivity (mS cm^{-1})	0.003	0.002	0.002	0.002	0.003
Density (g ml^{-1})	0.947	0.948	0.971	0.954	0.947
Water (wt%)	25.4	19.6	18.4	12.8	12.2

conductivity was 0.002 mS cm^{-1}, which is much lower than those of the conventional ionic liquids. This result suggests that the interaction between the $BA14^{+}$ cation and the $BEHP^{-}$ anion is strong. A BA14BEHP-water mixture (1:1 volume ratio) was shaken at 25 °C. The mixture separated into two phases, but both phases emulsified immediately after shaking. The aqueous phase continued to emulsify even several days later. On the other hand, the emulsion particles in the BA14BEHP phase disappeared within 1 d. The solubility of water in BA14BEHP was determined to be 18.4 wt%, which corresponds to the water content of the BA14BEHP phase. This solubility is much higher than that for conventional water-immiscible ionic liquids.[11,12] The high solubility suggests that the phosphate group of the $BEHP^{-}$ anion interacts with some water molecules in the BA14BEHP phase. The density of the water-saturated BA14BEHP medium was 0.976 g ml^{-1}, which is close to that of water. This may be one of the reasons that BA14BEHP can be dispersed stably in water. The median diameter of BA14BEHP particles in the emulsion was determined to be 13 μm by a dynamic light scattering particle size analyzer. The foaming property was screened by shaking ten times a 100 ml graduated cylinder containing 30 ml of sample solution. The foam volume of the 400 ppm BA14Cl solution was 50–60 ml immediately after shaking and 40–50 ml after 10 min. On the other hand, the foam volume of the 400 ppm BA14BEHP emulsion was less than 2 ml immediately after shaking, and the foam disappeared within 10 min.

The physicochemical properties of BA*n*BEHPs (n = 10, 12, 16, and 18) are also listed in Table 1. The glass transition temperature, viscosity, conductivity, density, and solubility of water are in the range of −11–−3 °C, 3300–6800 mPa s, 0.002–0.003 mS cm^{-1}, 0.94–0.96 g cm^{-3}, and 12.2–25.4 wt%, respectively. It is noted that the values did not vary much with n in the range tested. The BA*n*BEHPs can be dispersed also in water, and the emulsions were low-foaming. Therefore, the antimicrobial activity of the BA*n*BEHPs as water additives can be assayed as described below.

3.2 *Antimicrobial performance*

The antimicrobial performance of BA*n*BEHPs against *S. aureus* IFO012732 and *E. coli* CK111 was assayed at 20 h contact. The MICs are listed in Table 2. Among the BA*n*BEHP's tested, BA12BEHP and BA14BEHP yielded the lowest MICs against the bacteria. The dependence of n on the MIC is similar to that of BA*n*Cl in an aqueous solution.[1,2] The MICs of BA12BEHP and BA14BEHP against the gram-positive bacterium *S. aureus* IFO012732 are lower than those against the gram-negative bacterium *E. coli* CK111. This result is also similar to that of BA14Cl. For comparison, we also assayed tetra-*n*-hexylammonium and tetra-*n*-octylammonium salts with the $BEHP^{-}$

Table 2
Minimum inhibitory concentration (MIC in ppm) of BA*n*BEHP and BA14Cl against *S. aureus* IFO012732 and *E. coli* CK111.

	BA*n*BEHP					BA14C1
	n = 10	12	14	16	18	
S. aureus IFO012732	200	100	50	200	>1000	100
E. coli CK111	1000	200	400	1000	>1000	400

anion, which can be dispersed in water. They did not kill the bacteria even at 1000 ppm. This result suggests that the antimicrobial effect of BA*n*BEHP's is mainly attributed to the action of the BAn^+ cation. The $BA14^+$ salt with bis(2-ethylhexyl) sulfosuccinate anion also maintains its liquid state even at room temperature.[3] The BA14BEHP analogue is also water-immiscible and can be dispersed in water. However, the emulsion could not kill the bacteria even at 1000 ppm, indicating that the counter anion influences the antimicrobial action of the water-insoluble $BA14^+$ salts. The contact efficiency of the BA14BEHP emulsion particles to the bacterial cells is considered to be much lower than that of dissolved BA14Cl species; nevertheless, the MICs of BA14BEHP are the same as those of BA14Cl. Thus, although the mechanism of the antimicrobial action is not clear at present, the experimental results indicate that BA12BEHP and BA14BEHP can be used as antimicrobial agents advantageously for aqueous media. In the following, the applicability of BA14BEHP for cooling water or water-based cutting fluid is discussed.

3.3 *Influence of scale control agent and corrosion inhibitor*

Since aqueous media usually contain calcium carbonate scale and copper corrosion inhibitors, the antimicrobial activity of BA14BEHP against *S. aureus* IFO012732 and *E. coli* CK111 was assayed also in the presence of 100 ppm BTA and 500 ppm PAAS. The MICs are listed in Table 3. Because 100 ppm BTA and/or 500 ppm PAAS solution in the absence of BA14BEHP did not kill the bacteria, the antimicrobial effect of the mixture is attributed to the action of BA14BEHP. The MICs of BA14BEHP in the presence of 100 ppm BTA are about the same as those in the absence of additives.

The MICs of BA14BEHP in the presence of 500 ppm PAAS seem to be lower than those in the absence of additives. One of the reasons for the effect of PAAS may be the decrease in the size of BA14BEHP particles to enhance the contact efficiency to the bacterial cells. Using a microscope, 10–20 μm BA14BEHP particles were mainly observed in 400 ppm BA14BEHP emulsion, and 1–5 μm BA14BEHP particles were observed in the presence of 500 ppm PAAS. The decrease in particle size was observed also by the addition of KCl at 1 mol l^{-1}, suggesting that the particle size depends on ionic atmosphere.

3.4 *Influence on calcium carbonate scale control*

A 250 ppm (as $CaCO_3$) $Ca(HCO_3)_2$ solution was prepared by mixing 250 ppm $CaCl_2$ and 250 ppm $NaHCO_3$. The pH was adjusted to 8.5 with NaOH or HCl. The solution was stored at 60 °C for 20 h to form the $CaCO_3$ scale according to the reaction:

$$Ca(HCO_3)_2 \rightarrow CaCO_3 + CO_2 + H_2O. \quad (1)$$

Table 3
MICs (in ppm) of BA14BEHP and BA14Cl against *S. aureus* IFO012732 and *E. coli* CK111 in the presence of 100 ppm BTA and 500 ppm PAAS.

	BA*n*BEHP		BA*n*Cl	
	BTA	PAAS	BTA	PAAS
S. aureus IFO012732	100	20	100	100
E. coli CK111	200	100	200	100

Then, the reaction mixture was cooled to room temperature and centrifuged. The concentration of Ca^{2+} species in the supernatant (c_{Ca}) was determined to be 80 ± 30 ppm by spectrophotometry with Arsenazo-III. Anionic polymers, such as PAAS and polyphosphoric acid, can control the $CaCO_3$ scale formation by association with the Ca^{2+} ion to form water-soluble Ca^{2+}-polyanion complexes.(8,9) The c_{Ca} obtained with the 250 ppm $Ca(HCO_3)_2$ solution containing 500 ppm PAAS was 250 ± 10 ppm, indicating that the $CaCO_3$ scale formation shown by eq. (1) is controlled successfully by PAAS. The c_{Ca} obtained with the 250 ppm $Ca(HCO_3)_2$ solution containing 500 ppm PAAS and 400 ppm BA14BEHP was determined to be 250 ± 10 ppm, indicating that BA14BEHP did not affect the scale control by PAAS. Similarly, it was shown that BA14BEHP did not affect the scale control by polyphosphoric acid. Interestingly, the c_{Ca} obtained with the 250 $Ca(HCO_3)_2$ solution containing 400 ppm BA14BEHP was 230 ± 5 ppm, suggesting that BA14BEHP can inhibit the formation of the $CaCO_3$ scale as well as organophosphorus compounds. The effect may be attributed to the adsorption of $BEHP^-$ on the fine particles of $CaCO_3$.(13)

4. Conclusion

BA14BEHP can be applied as an antimicrobial agent for aqueous media. The antimicrobial test is now being extended to other bacteria.

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