Polymeric Phosphonium Salts as a Novel Class of Cationic Biocides. VIII. Synergistic Effect on Antibacterial Activity of Polymeric Phosphonium and Ammonium Salts

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SYNOPSIS

Two types of homopolymers {poly[tributyl(4-vinylbenzyl)phosphonium chloride] and poly[tributyl(4-vinylbenzyl)ammonium chloride]} and copolymers, in which the compositional ratio of the phosphonium monomer to the ammonium monomer was altered, were prepared. The antibacterial activities of both the mixtures of the homopolymers in which the mixing ratio was altered and the copolymers containing positively charged phosphorus and nitrogen atoms in a single polymer chain were explored by the viable cell counting method in sterile distilled water. In the mixed system, a significant finding was the presence of an optimal mixing ratio for the antibacterial activity. The mixtures of polymeric phosphonium salt and polymeric ammonium salt clearly showed the synergistic effect on the antibacterial activity. On the other hand, the antibacterial activity of the copolymers with different cationic parts increased monotonously with increasing the phosphonium monomer units in the polymers, namely, the incorporation of two types of active moieties into a single molecule seems to be unfavorable for synergism on the antibacterial activity. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Currently, cationic biocides are extensively used in a variety of fields. For improved functionality of disinfectants, three approaches may be possible: (1) preparation of novel compounds, (2) polymerization of the disinfectants, and (3) mixing of the existing cationic disinfectants. Item 1 requires much endeavor. With respect to item 2, many studies have been performed so far, and it is found that the polymeric disinfectants are advantageous for the antibacterial activity over the low molecular weight cationic analogs.[1-3] On the other hand, few studies have been reported so far of the synergistic effects on the antibacterial activity of binary mixtures of disinfectants.[4,5] The synergism by mixing of different cationic biocides has never been reported except for a German patent in which no details for the synergism were given (item 3).[6] If the mixing of two or more kinds of disinfectants is effective to enhance the antibacterial activity, the procedure would be very favorable in view of simplicity.

We have reported that the antibacterial activity of the polymeric phosphonium salt is much higher than that of the polymeric quaternary ammonium salt with the same structure except for a cationic part.[7] In this article, we describe the preparation of homopolymers and copolymers of tributyl(4-vinylbenzyl)phosphonium chloride and tributyl(4-vinylbenzyl)ammonium chloride and the synergistic effect on the antibacterial activity by the use of both the mixtures of polymeric onium salts in which the mixing ratio was altered and copolymers with different cationic parts.

EXPERIMENTAL

The structures of onium salts used in this study are shown in Figure 1.

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Journal of Applied Polymer Science, Vol. 53, 1245–1249 (1994)
 CCC 0021-8995/94/091245-05

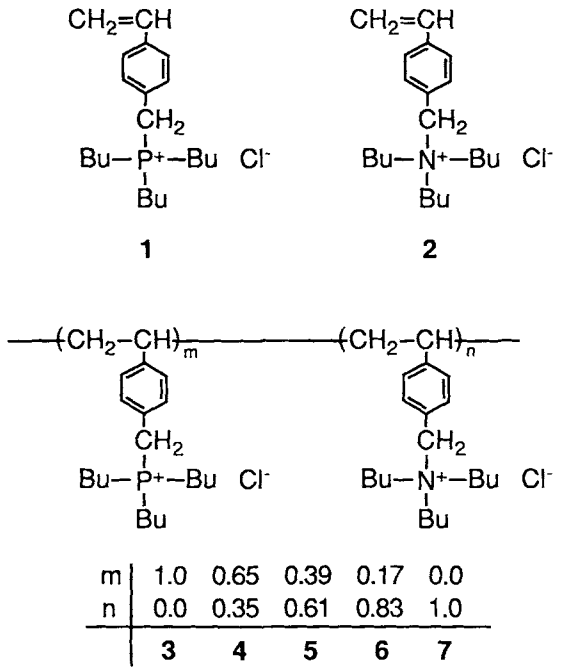

Figure 1 Structure of cations used in this study.

Materials

Tributyl(4-vinylbenzyl)phosphonium chloride (**1**) and tributyl(4-vinylbenzyl)ammonium chloride (**2**) were prepared as reported previously.[7]

Polymerization

Homopolymers of 1 and 2

Polymerizations of **1** and **2** were carried out at 60°C in distilled water with 2,2′-azobis-2-amidinopropane·2HCl as an initiator. Each polymerization tube was charged with the monomer (100 g/L), the initiator (2.0 g/L), and water (20 mL). It was then degassed by three freeze–pump–thaw cycles under high vacuum, sealed off, and placed in a constant temperature bath at 60°C. After 6 h, the polymerization tube was opened, and the content was poured into an excess of tetrahydrofuran (THF). The precipitated polymer was filtered off, washed with THF, and dried under vacuum. The conversion for each polymer is shown in Table I. Each polymer was purified by reprecipitation of the methanol solution into a large excess of THF. The weight-average molecular weight (M_w) was determined in methanol and is listed in the last column of the table.

Table I Conditions for Polymerization[a]

Polymer	Mol Ratio of 1 : 2 in Monomers	Mol Ratio of 1 : 2 in Polymers	Conversion (%)	M_w[b]
3	1.0 : 0	1.0 : 0	91	32,500
4	0.74 : 0.26	0.65 : 0.35	94	45,300
5	0.49 : 0.51	0.39 : 0.61	95	53,600
6	0.25 : 0.76	0.17 : 0.83	94	54,200
7	0 : 1.0	0 : 1.0	95	42,500

[a] Initiator, 2,2′-azobis-2-amidinopropane·2HCl; temperature, 60°C; time, 6 h.

[b] Determined with a low-angle, laser light-scattering photometer (KMX-6) in methanol.

Table II Conditions for Mixing of Homopolymers 3 and 7

Polymer	Mixing Ratio 3 : 7[a]
8	0.8 : 0.2
9	0.5 : 0.5
10	0.2 : 0.8

[a] In mixed polymers, based on the monomer units.

Copolymers of 1 and 2

The copolymerization procedure was the same as that of the homopolymerization. Each polymerization vessel was charged with predetermined amounts of **1** and **2**, whereas the total weight of the monomers was kept constant (100 g/L). The concentra-

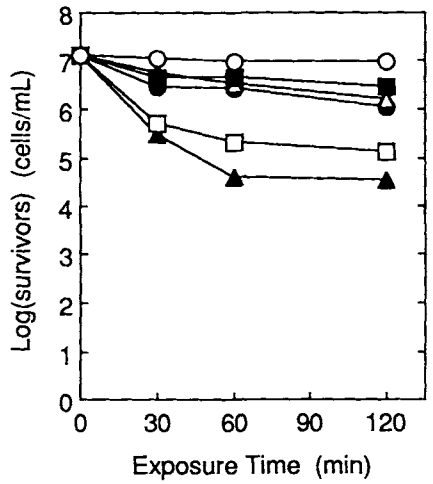

Figure 2 Plots of log(survivors) vs. exposure time for the polymers with various compositional ratios of **1** to **2** against *S. aureus:* (○) blank; (▲) **3**; (□) **4**; (●) **5**; (△) **6**; (■) **7**. Concentration, 2.8 μM, based on the total cationic part (1 μg/mL for polymer **3**).

tion of the initiator was 2.0 g/L. Composition of the copolymers was determined by elemental analysis. The conversion, molecular weight, and composition of each copolymer are listed in Table I.

Mixture of Polymers 3 and 7

Three samples were prepared in which the mixing ratio of homopolymers **3** and **7** was altered, and the binary mixtures were used in the antibacterial assessment. Characterization of these samples is shown in Table II.

Antibacterial Assessment

The antibacterial activity against *Staphylococcus aureus* (IFO 12732) was evaluated by the viable cell counting method in sterile distilled water, the details of which have been already reported.[7]

Apparatus

The M_w of the obtained polymers was determined with a KMX-6 low-angle, laser light-scattering photometer in methanol.

RESULTS

Antibacterial activity of the homopolymers (**3** and **7**) and the copolymers (**4–6**), in which the compositional ratio of tributyl(4-vinylbenzyl)phosphonium chloride to tributyl(4-vinylbenzyl)ammonium chloride was altered, was explored by the viable cell counting method. Figure 2 shows plots of log(survivors) vs. exposure time for homopolymers (**3** and **7**) and copolymers (**4–6**) against *S. aureus*. About 10^7 cells/mL of *S. aureus* were exposed to 2.8 μM, based on the total cationic units (1 μg/mL for homopolymer **3**) of the polymers in saline. When the bacterial cells were exposed to homopolymer **3**, > 99% of *S. aureus* were killed within 120 min of contact. Homopolymer **3** showed the highest activity among the polymers examined, whereas homopolymer **7** showed the lowest activity. For copolymers of **4**, **5**, and **6**, which contain 65, 39, and 17 mol % of phosphonium salt units in the copolymer, respectively, 99, > 90, and ca. 90% of bacterial cells were killed within 120 min of contact. Figure 3 shows plots of log(% survivors) vs. mol fraction of P^+ in the copolymers at 2.8 μM, based on the total cationic units in the polymers (**3–7**). This figure demonstrates a correlation between the mol fraction of P^+ in the polymers and the logarithm

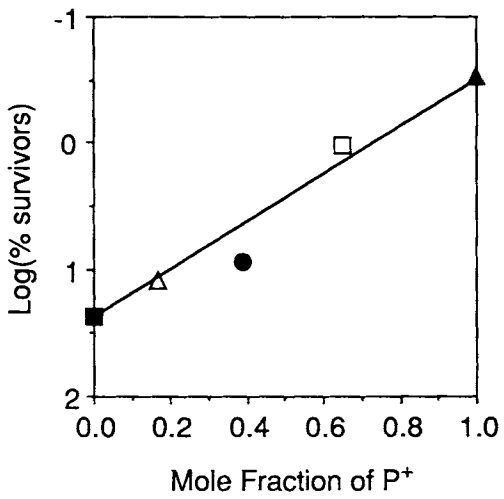

Figure 3 Plots of log(% survivors) vs. mol fraction of P^+ in the polymers with various compositional ratios of **1** to **2** at 2.8 μM, based on the total cationic units in the polymers, against *S. aureus:* (○) blank; (▲) **3**; (□) **4**; (●) **5**; (△) **6**; (■) **7**.

of % survivors. Antibacterial activity of the polymers in the unit concentration increased in the order of **3** > **4** > **5** > **6** > **7**. These results clearly indicate that the antibacterial activity of the copolymers against *S. aureus* increased with increasing the phosphonium monomer units in the copolymers. However, a synergistic effect on the antibacterial activity was not observed for the copolymers containing positively charged phosphorus and nitrogen atoms in a single polymer chain. Incorporation of two types of active moieties into a single molecule seems to be unfavorable for synergism on the antibacterial activity.

The synergistic effect on antibacterial activity by mixing of homopolymers (**3** and **7**) was investigated for mixtures (**8–10**). Figure 4 shows plots of log(survivors) vs. exposure time for homopolymers (**3** and **7**) and mixtures (**8–10**) against *S. aureus*. The concentration of mixtures used to obtain these data was 2.8 μM (based on the total cationic units, 1 μg/mL for homopolymer **3**). When ca. 10^7 cells/mL of *S. aureus* were exposed to **9**, which contained 50 mol % of phosphonium salt in the mixture, ca. 99.99% of *S. aureus* were killed within 120 min of contact, and this mixture exhibited the highest activity among the samples. For **8** and **10**, 99.9 and ca. 90% of the *S. aureus* cells were killed after 120 min exposure, respectively. Figure 5 shows plots of log(% survivors) vs. the mol fraction of P^+ in the mixtures at 2.8 μM (based on the total cationic units) of the samples (**3** and **7–10**). A significant

finding is the presence of an optimal mixing ratio for the antibacterial activity. At low mol fraction of P^+ below 50 mol %, the antibacterial activity increased with the mol fraction of P^+, whereas at a high mol fraction, the activity decreased as the mol fraction of P^+ increased. Sample **9**, possessing the intermediate mixing ratio, exhibited the highest activity against *S. aureus* among the samples examined. The mixtures of polymeric phosphonium salt and polymeric quaternary ammonium salt clearly showed the synergistic effect on the antibacterial activity.

DISCUSSION

Many studies on the interaction of cationic biocides with the cytoplasmic membranes of bacteria that are composed mainly of phospholipids and membrane proteins have been performed so far.[3,8,9] The mode of interaction is assumed in the following terms: (1) adsorption onto the membrane surface; (2) inducing fluidization and phase separation of the membrane; and (3) solubilization of the components of the membrane: phospholipid molecules and membrane proteins. However, it is still ambiguous how the cationic biocides interact with the cytoplasmic membrane with subsequent disruption. Many physiological events have been observed that are related to events 1 and 2 described above,[10-12]

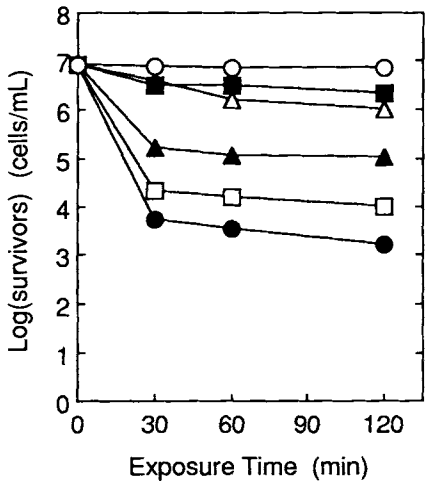

Figure 4 Plots of log(survivors) vs. exposure time for the polymers with various mixing ratios of **3** and **7** against *S. aureus:* (○) blank; (▲) **3**; (■) **7**; (□) **8**; (●) **9**; (△) **10.** Concentration, 2.8 μM, based on the total cationic part (1 μg/mL for polymer **3**).

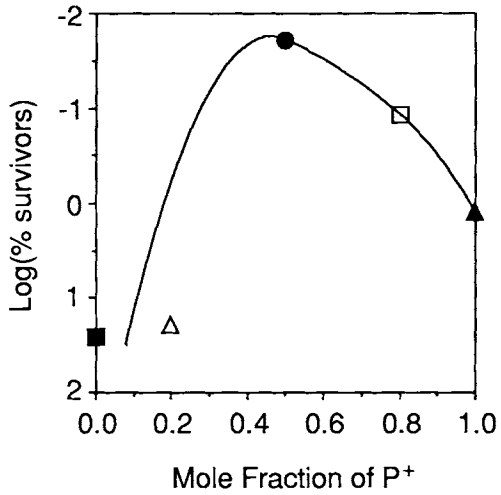

Figure 5 Plots of log(% survivors) vs. mol fraction of P^+ in the mixtures with various mixing ratios of **3** and **7** at 2.8 μM, based on the total cationic units in the samples, against *S. aureus:* (○) blank; (▲) **3**; (■) **7**; (□) **8**; (●) **9**; (△) **10.**

whereas few studies have been reported on the interaction of the cationic biocides with the cytoplasmic membranes that are related to event 3.

Surfactants, including cationic biocides with hydrophilic and hydrophobic parts in a single molecule, have a critical micelle concentration (cmc), which is very important for the ability of surfactants. At concentrations above cmc, the surfactants form aggregates in aqueous solution. A synergistic effect on the properties of mixed systems has been investigated for the binary mixtures of alkyltrimethylammonium bromides with different chain lengths and of one of these compounds and chlorhexidine digluconate.[13] Currently, antiseptic solutions are commercially available that contain mixtures of alkyltrimethylammonium bromides and an antibacterial agent: chlorhexidine digluconate. These binary mixtures give mixed micelles in aqueous solution, and the changes of solution properties in mixed micellar systems are known to affect both the stability and antiseptic properties of the solution.[13] Furthermore, it has been reported that the components of the cytoplasmic membrane are solubilized into the hydrophobic parts of the surfactant micelles that can diffuse through the cell wall of the microbes.[14] On the basis of these arguments, it is most likely, at the present stage of research, to interpret the observed synergistic effect on the antibacterial activity as a result of solubilization of membrane constituents by mixed micelles of phosphonium and am-

monium salts. Polyelectrolytes like polycations used in this study form micelles in aqueous solution even at very low concentrations. In fact, trials to determine the values of cmc for homopolymers **3** and **7** by conventional electrical conductivity measurements, however, failed. It was expected that these values for polycations would be too small to be determined reliably by conventional methods. This consideration would lead to an expectation in that the mixtures of polymeric phosphonium salts and polymeric ammonium salts form mixed micelles even at very low concentrations and the properties of the mixed micelles depend strongly on the composition of the phosphonium and ammonium salts. Solubilization of the membrane constituents (mainly phospholipids and membrane proteins) would definitely alter the membrane functions, which would be lethal and result in the death of the bacterial cells.

The effects of changing the positively charged atom in surfactants on micelle size and dye solubilization efficiency were investigated for low molecular weight phosphonium and ammonium salts with the same substituents in aqueous solution.[15] The results indicate that the micellar aggregation number of phosphonium salts was greater than that of ammonium salts and the ability of the phosphonium salts to solubilize the dye was superior to that of ammonium salts, namely, the amount of materials being solubilized into the micelles is strongly affected by the micelle size. If antibacterial activity is influenced by the solubilization, the synergistic effect observed in this study is attributable to the state of aggregation in mixed systems.

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Received February 7, 1994
Accepted March 14, 1994