Poly(styrene-co-4-vinylbenzyl chloride) Conjugated with 3-(Dimethylamino)phenol: Synthesis and Antibacterial Activity

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Certain phenolic compounds are bactericidal.\(^1,2\) Resins with phenol derivatives containing one, two, or three hydroxy groups were also reported to exhibit antibacterial activities due to phenolic hydroxy groups in the resins.\(^3\) Recently, we synthesized poly(styrene-alt-maleic anhydride) conjugated with 4-aminophenol (AP),\(^4\) 4-hydroxyphenol, and 4-amino-benzoic acid.\(^5\) The AP-linked polymer exhibited a bactericidal activity toward \(E.\ coli\) and \(S.\ aureus\), but it was less effective than free AP, probably due to its higher molecular weight as compared to AP.

Polymers containing quaternary ammonium salt (QAS) with at least a long alkyl chain (number of carbon atom ≥ 8) are usually very effective against a large spectrum of microorganism such as bacteria, algae, fungi, etc.\(^6,7\) The polymers are believed to be adsorbed onto the negatively charged cell surfaces by electrostatic interaction, followed by the diffusion of the long alkyl chain through the cell wall. This results in a weakening of the cytoplasmic membrane, leading to a leakage of cytoplasmic contents and eventual death of the cell.

If AP is substituted with N,N-dimethyl-3-aminophenol (DMAP) and linked to a polymer backbone by a coupling reaction between the dimethylamino group of DMAP and alkyl halide group of the polymer, the resulting polymer will contain QAS. This polymer seems to be of interest since due to its QAS in addition to the phenol group it may exhibit a stronger antibacterial activity than free DMAP.

In this study, poly(styrene-co-4-vinylbenzyl chloride) was prepared and then conjugated with DMAP to synthesize poly(ST-co-VBC)-DMAP, as shown in Scheme 1. The QAS in this system does not have a long alkyl chain, but an additive or synergistic effect was expected because the QAS is linked to the biologically active phenyl moiety. This paper briefly describes the synthesis and antibacterial activity of the polymer.

Experimental Section

Synthesis of poly(ST-co-VBC)-DMAP. A solution of styrene (3.65 g; 15.0 mmol) and 4-vinylbenzyl chloride (2.29 g; 35.0 mmol) in a 7 : 3 molar ratio in chlorobenzene (5 mL) was polymerized at 60 °C for 12 h in the presence of 2,2'-azobisisobutynitrile (0.041 g; 0.25 mmol) under a nitrogen atmosphere. The polymer was precipitated into methanol. The precipitated polymer was dissolved in tetrahydrofuran and reprecipitated into methanol. The purification procedure was repeated twice, and the precipitate was dried under vacuum to yield poly(ST-co-VBC) (75%). DMAP (0.23 g; mmol) in methanol (8.0 mL) was added to a 10 mL solution of poly(ST-co-VBC) (0.50 g) in N,N-dimethylformamide (DMF), and the mixture was stirred at room temperature for 1 h. The mixture was then heated at 100 °C for 18 h, followed by the addition of methanol (5 mL). The mixture was further heated for 6 h at the same temperature, cooled to room temperature, and finally purified by precipitation into diethyl ether to obtain poly(ST-co-VBC)-DMAP (77%).

Antibacterial test. The antibacterial activity of poly(ST-co-VBC)-DMAP was tested by the contact method,\(^9,10\) using two different types of bacteria species such as \(E.\ coli\).
(KCCM 11234) and S. aureus (KCCM 12103). Pure DMF for a blank (control) sample or a solution (0.50 mL) of either DMAP or poly(ST-co-VBC)-DMAP in DMF (2 wt%) was transferred into each test tube containing 10 mL of a bacteria species (1.2 or 1.8 $\times$ 10$^5$ cell/mL) and Luria Broth. After the test tubes were incubated at 37 °C for 24 h under shaking (130 rpm), 1.0 mL of each suspension was diluted with 9.0 mL of a Bacto peptone water. The dilution procedure was repeated five times, and 0.1 mL of the final suspension was plated on LB agar. After another incubation at 37 °C for 24 h, the number of the bacteria colony was counted. The percent reduction of the bacteria cells was calculated from the following formula:

$$\% \text{ reduction} = \left( \frac{N_b - N_t}{N_b} \right) \times 100$$

where $N_t$ is the number of the bacteria cells recovered from the inoculated suspension which contained either DMAP or poly(ST-co-VBC)-DMAP in the test tube, and $N_b$ is the number of bacteria recovered from the inoculated suspension which contained only DMF without the biocides.

**Results and Discussion**

The FT-IR spectra (a JASCO FT-IR spectrophotometer) in Figure 1 revealed that the intensity of a characteristic C-Cl peak at 1260 cm$^{-1}$ was significantly decreased after the coupling reaction of poly(ST-co-VBC) with DMAP, but the OH and C-N peak emerged clearly at near 3360 and 1363 cm$^{-1}$, respectively. This result indicates that most Cl atoms of the benzyl groups were replaced by DMAP during the coupling reaction.

The important proton NMR peaks of poly(ST-co-VBC) and poly(ST-co-VBC)-DMAP were assigned, as shown in Figure 2 (a JEOL-JMN 400 MHz spectrometer). The molar ratio of styrene to 4-vinylbenzyl chloride units in poly(ST-co-VBC) was estimated to be about 1.8 : 1. A peak at 4.5 ppm in the poly(ST-co-VBC) spectrum was reduced and a new peak emerged at 4.30 ppm in poly(ST-co-VBC)-DMAP spectrum. The peaks at 4.50 and 4.30 ppm represent the unreacted methylene protons (-CH$_2$-Cl) and ammonium nitrogen-linked methylene protons (-CH$_2$-DMAP), respectively. The other proton peaks of the reacted DMAP moiety were overlapped with those of poly(ST-co-VBC). Based on the peak integrals at 4.50 and 4.30 ppm, about 60% Cl atoms of the benzyl groups in poly(ST-co-VBC) was replaced by DMAP, as shown in Scheme 1 ($x = 0.4, y = 0.6$).

The number- and weight-average molecular weight of poly(ST-co-VBC) were 21800 and 37800, respectively (a Waters 440 HPLC calibrated with polystyrene standard samples). According to the proton NMR data, the conversion of the benzyl chloride group to the ammonium group was about 60%. Thus the number-average molecular weight of poly(ST-co-VBC)-DMAP was estimated to be 27100. The poly(ST-co-VBC)-DMAP was not well soluble in most organic solvents, but readily soluble in methanol.

The thermal degradation of poly(ST-co-VBC) and poly(ST-co-VBC)-DMAP was studied with thermogravimetric analysis (TGA) using a DuPont 2000 differential scanning calorimeter, and the TGA thermograms are shown in Figure 3. Poly(ST-co-VBC) began to decompose at 377 °C. However, the thermal decomposition of poly(ST-co-VBC)-DMAP proceeded in two steps at 128 and 350 °C, respectively. From the beginning of the heating at below 128 °C, a slight weight loss was observed, which may be attributed to the probable evaporation of moisture and/or solvent. The true initial weight loss at 128 °C may be due to the cleavage of DMAP moiety in the polymer.

The antibacterial activity of poly(ST-co-VBC)-DMAP was investigated with the contact method toward E. coli and
S. aureus, which are the Gram negative and Gram positive bacteria, respectively. After the contact with either DMAP or poly(ST-co-VBC)-DMAP, the suspensions were incubated at 37 °C for 24 h, and the number of the bacteria colony was counted. The number of bacteria cell per mL was calculated by multiplying the number of colonies by the dilution factor and the result is listed in Table 1.

The E. coli cells in the DMAP specimens disappeared completely (100% reduction), but the number of the S. aureus cells was reduced only to 82.7% as compared to those in the control specimens. On the other hand, the E. coli and S. aureus cells in the poly(ST-co-VBC)-DMAP specimens were reduced only to 50.7% and 13.3%, respectively. However, it should be considered that the cell concentrations of E. coli and S. aureus cells were somewhat different from each other. The present experimental result indicates that the bactericidal activity of poly(ST-co-VBC)-DMAP against S. aureus and E. coli is much lower than that of DMAP. Consequently, it led us to conclude that the QAS groups in the polymer do not effectively contribute to the antibacterial activity of the polymer, and this ineffectiveness of QAS may probably be due to the lack of a long alkyl chain which is usually required for high activities against microorganisms. Furthermore, the polymer should have more problem in diffusing across the cell wall of the bacteria cells due to higher molecular weight as compared to DMAP. The concentration or dilution effect should be also considered because the DMAP moiety in poly(ST-co-VBC)-DMAP was only about 24 wt% based on the NMR data.

In conclusion, poly(ST-co-VBC)-DMAP was successfully synthesized by reacting DMAP with poly(ST-co-VBC). It was thermally decomposed in two consecutive steps at 128 °C and 350 °C. After the contact with DMAP for 24 h, the E. coli cells were completely removed (100% reduction), but the S. aureus cells were reduced only to 82.7%. The bactericidal activity of poly(ST-co-VBC)-DMAP toward E. coli and S. aureus was much lower than that of DMAP. The lower activity of the polymer as compared to that of DMAP was attributed to the lack of a long alkyl chain in the QAS moiety, higher molecular weight, and dilution effect.

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**References**