Synthesis and Properties of Polymeric Biocides Based on Poly(Ethylene-*co*-Vinyl Alcohol)

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Abstract: Poly(ethylene-*co*-vinyl acetate) with 55 wt % vinyl acetate units (EVA55) was cryogenically ground and saponified in KOH/ethanol solution to obtain poly(ethylene-*co*-vinyl alcohol) (EVOH55). Polymeric antimicrobial agents were synthesized by reacting three antimicrobial agents, 4-aminobenzoic acid (ABA), salicylic acid (SA), and 4-hydroxy benzoic acid (HBA) with EVOH55. The polymers became more flexible and exhibited lower melting peak temperature and heat of fusion as the content of the chemically bound ABA, SA, and HBA units increased. These phenomena appeared more significant in the order of ABA < HBA < SA. *S. aureus,* Gram-positive bacterium, was more susceptible to the polymeric antimicrobial agents than *P. aeruginesa,* Gram-positive bacterium. The antimicrobial activity increased in the order of EVOH55-HBA < EVOH55-ABA < EVOH-SA. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 765–770, 2004

Key words: biological application of polymers; esterification; functionalization of polymers

INTRODUCTION

Contamination of polymeric materials by microorganisms provokes serious problems in biomedical applications. One possible way to overcome this problem is to develop polymeric materials that have antimicrobial activities.[1–6,10–14]

Polymeric biocides can significantly reduce loss of antimicrobial activity associated with volatilization, photolytic decomposition, dissolution, and permeation. Moreover, increased efficiency, selectivity, and handling safety are additional benefits which may be realized.[7]

Pittman[8] synthesized a copolymer, made from penta chlorophenyl acrylate, vinyl acetate, and ethyl acrylate, which exhibited a good antibacterial activity against *Pseudomonas* sp.

Nonaka et al.[9] produced copolymer beads containing phenol derivatives. The polymeric biocides were highly active against *Escherichia coli* and *Streptococcus aureus.* The antimicrobial activity increased with increasing number of phenolic hydroxy groups in the beads.

A bactericidal monomer, 2,4,4′-trichloro-2′-acryloyloxydiphenylether was either homopolymerized or copolymerized with styrene. The weight-average molecular weight of the resulting polymers was in the range between 4100 and 11,600. The bactericidal activity against *Pseudomonos aeruginosa* decreased as a result of the polymerization.[7]

Ikeda et al.[10] prepared acrylate monomers with pendant biguanide groups. Their homopolymer with acrylamide exhibited high antibacterial activity against Gram-positive bacteria, whereas they were less active against Gram-negative bacteria.

Ikeda and Tazuke[11] synthesized various poly(trialkyl-3-(and 4-) vinyl benzylammonium chlorides). The polymeric compound with dodecyl chain showed particularly high activity. They also found that the polymers were more active than the corresponding monomers, which was ascribed to the fact that the polymers were much more favored over the monomers in terms of absorption onto the cytoplasmic membrane and of interaction with the membrane.

In this study, poly(ethylene-*co*-vinyl alcohol) (EVOH55) was prepared by saponification of poly(ethylene-*co*-vinyl acetate) containing 55 wt % of vinyl acetate units. Salicylic acid (SA), 4- aminobenzoic acid (ABA), and 4-hydroxy benzoic acid (HBA) were chemically anchored to EVOH55. EVOH55 was employed as polymer matrix, not only because it possessed hydroxyl groups to which the bioactive molecules could be chemically linked, but also because EVOH itself exhibited some antimicrobial activity. Antimicrobial activity of the resulting polymers were studied by the shake flask test against *S. aureus* and *P. aeruginesa.*

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EXPERIMENTAL

Materials

ABA (Aldrich, Milwaukee, WI), SA (Aldrich), HBA (Aldrich), triethyl amine (TEA; Aldrich), and TNBT (Aldrich) were used as received. Poly(ethylene-*co*-vinyl acetate) with 55 wt % vinyl acetate units (EVA55, Mw = 110,000, polydispersity index (PDI) = 2.51) was donated by Hanhwa (Korea). Other chemicals were used as received without further purification.

Instrumentation

Molecular weight and its distribution were measured by using GPC [Waters model 150C plus; Waters Instruments, Rochester, MN; 1,2,4-trichlorobenzene eluant, 1.0 ml/min, 135°C; column [porosity: 10 μm, Stragel® HT6E (effective molecular-weight range: 5100–1 $\times$ 10^7), HT5 (5100–4 $\times$ 10^6), HT3 (500–30,000)] employing polystyrene (Showadenko SL-105, Japan) as a standard.

Polymers were characterized by ^{1}H-NMR spectra recorded at 110°C on a FT-NMR (Bruker AC-250 FT-NMR spectrometer, Bruker Instruments, Billerica, MA). Ten milligrams of the copolymer was dissolved in 0.5 ml of 1,2-dichlorobenzene-*d* (20 wt/vol %) and was subjected to the ^{1}H-NMR measurements.

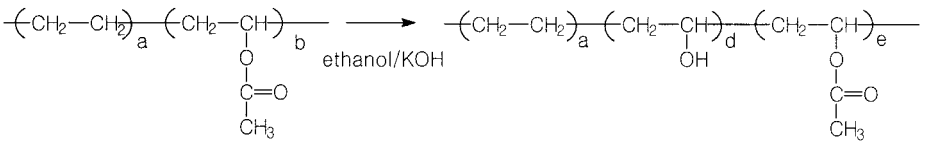
Thermal properties of the polymers were determined by using DSC (Perkin–Elmer DSC 7, Norwalk, CT). Thermal history of the products was removed by scanning to 200°C at the heating rate of 10°C/min (first scan). After cooling down at the rate of −200°C/min to room temperature, the sample was reheated at 10°C/min to 200°C and the second-scan DSC thermograms were obtained.

Mechanical properties of the films were determined with a universal test machine (Instron model 4200; Canton, MA) at a cross-head speed of 20 mm/min according to ASTM D 638 at 20 ± 1°C, and relative humidity of 65%. Sheets were made by hot pressing (Lab Press, Carver) at 180°C for 1 min under 4.0 atm and by quickly immersing into ice water. The film thus formed was free from any distortion problems.

Preparation of EVOH55

Poly(ethylene-*co*-55 wt %-vinyl acetate) (EVA55) pellets were ground into powder (ca. 250 μm). Ground EVA55 (6 g) was saponified in 200 ml of 0.5*M* KOH in ethanol solution (1000 ml ethanol/28.05 g KOH solution). The saponified EVA55 was not soluble in the reaction system. The heterogeneous solution was refluxed with stirring for 72 h, precipitated by excess distilled water, filtrated, washed with distilled water and methanol, and dried under vacuum at 60°C for 1 week.[12]

The content of the copolymers were determined from ^{1}H-NMR peaks corresponding to methine protons (4.9 ppm) and methyl protons of vinyl acetate units (2.1 ppm) by using eqs. (1) and (2)



$$\frac{3b}{4a + 6b} = \frac{\text{peak at 4.9 ppm and 2.1 ppm}}{\text{total peak area}} \quad (1)$$

$$\frac{3e}{4a + 4d + 6e} = \frac{\text{peak at 4.9 ppm and 2.1 ppm}}{\text{total peak area}} \quad (2)$$

$$\text{degree of saponification} = \frac{b - e}{b} \times 100(\%) \quad (3)$$

Synthesis of polymeric biocides using TNBT catalyst

The saponified EVOH55 (20g) was first dissolved in 1,4-dioxane (300 ml) and HBA (10g) was added to the above solution. The solution was refluxed at 110°C and then stirred for 15 h in the presence of titanium(IV) butoxide (TNBT)(0.1 ml) as a catalyst for the esterification. The product was precipitated into acetone and dried *in vacuo*, followed by Soxhlet extraction with boiling ethanol for 1 day to remove the unreacted antimicrobial agents.

Synthesis of polymeric biocides using thionyl chloride

To a solution of HBA (10 g) in THF (100 ml) at 20°C, thionyl chloride (5 ml) was added dropwise and was then refluxed at 70°C. After stirring for 2 h, EVOH (20 g) was added to the hot solution and stirred for another 15 h in the presence of TEA. The product was precipitated into acetone and dried *in vacuo*, followed by Soxhlet extraction with boiling ethanol for 1 day to remove the unreacted antimicrobial agents.

Antimicrobial activity test (Shake flask method[13])

The number of bacterial cells in the culture suspension was ~4.0–6.0 $\times$ 10^5 CFU/mL. After their contact with

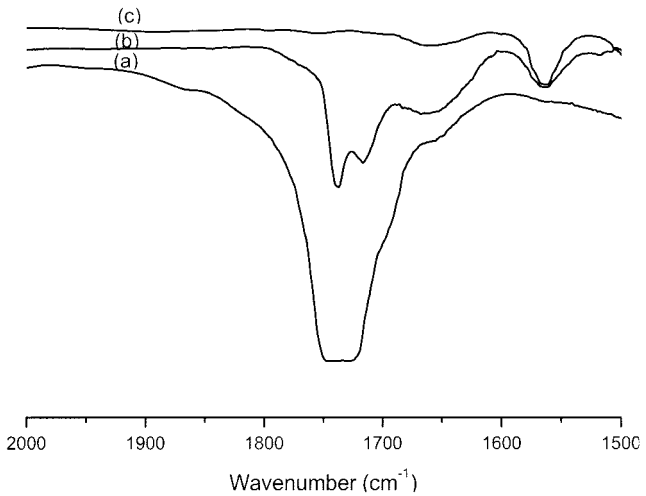


Figure 1 FTIR spectra of (a) EVA55 as received, (b) EVA55 after 6 h of the saponification, and (c) EVA55 after 48 h of the saponification.

the antibacterial agents in diluted PBS for 24 h at 37°C, the suspension was incubated at 37°C for 24 h, and the number of the bacterial cells was calculated by multiplying the number of colonies by the dilution factor.

RESULTS AND DISCUSSION

Preparation of the polymeric biocides

EVA55 containing 55 wt % of vinyl acetate units was ground cryogenically and saponified in ethanol/KOH solution in a heterogeneous manner.

Figure 1 shows FTIR spectra of EVA55 before and after the saponification. The sharp peak, appearing at 1650–1755 cm^{-1} and corresponding to C═O stretching band of the vinyl acetate units, disappeared almost completely after 48 h of the saponification.

HBA, ABA, and SA were reacted with the saponified EVA55 (EVOH55) in the presence of titanium(IV) butoxide (TNBT).

Amount of ABA grafted to EVOH55 was much larger than that of HBA and SA. Carboxylic acid groups of HBA and SA were converted to acid chloride groups by treating them with thionyl chloride. When the resulting compounds were reacted with EVOH55, the amount of grafted HBA and SA increased considerably.

Figure 2 shows ^{1}H-NMR spectra of EVA55, EVOH55, and EVOH55 reacted with HBA (EVOH55-HBA). The methylene protons exhibit their peaks at 1.26 ppm. The peaks appearing at 3.6 ppm correspond to methine protons of vinyl alcohol units (VOH). EVOH55-HBA shows new peaks at 6.8 and 7.8 ppm, which are ascribed to the phenyl ring protons of HBA units.

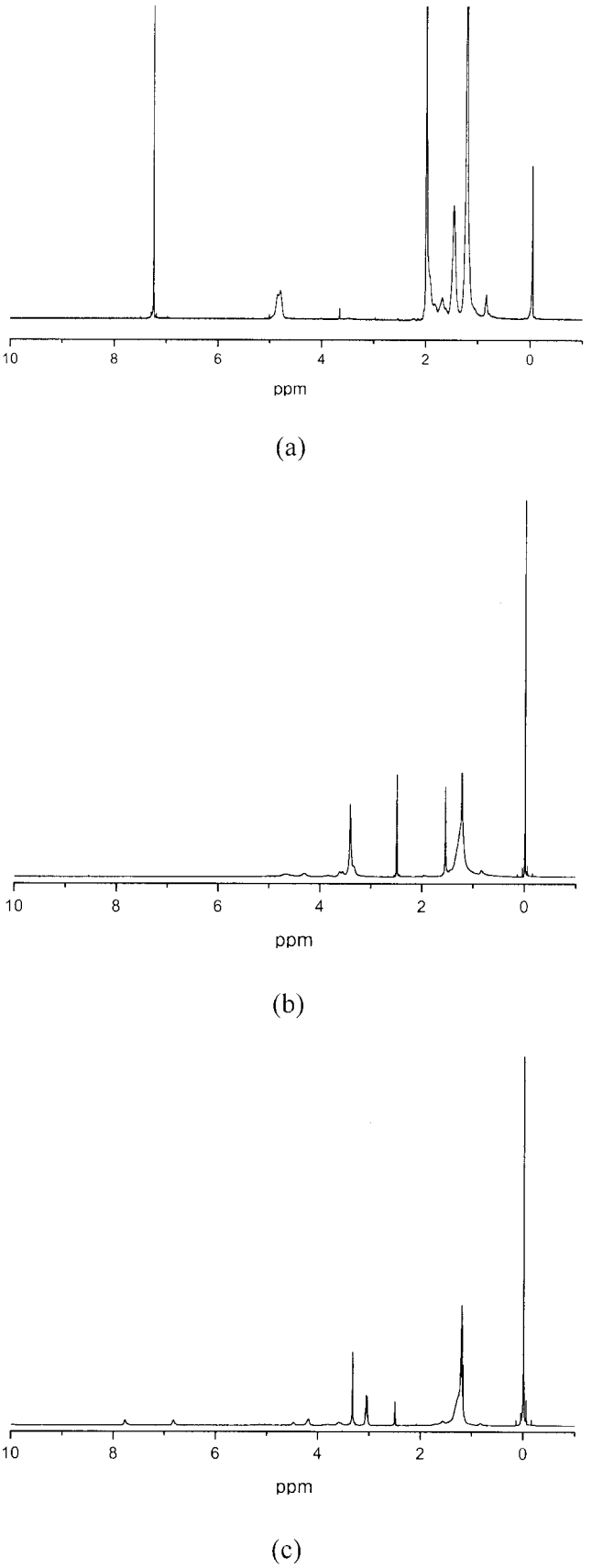


Figure 2 ^{1}H-NMR spectra of (a) EVA55, (b) EVOH55 produced by the saponification of EVA55 for 48 h, and (c) EVOH55 reacted with 4HBA.

TABLE I
Thermal and Tensile Properties EVOH55 with Different Amount of the Grafted Biocides

Sample code	Biocide content[a] (mol %)	Thermal properties[b]		Tensile properties		
		T_m (°C)	ΔH_f (cal/g)	E Modulus (MPa)	Stress at max. load (MPa)	Elongation at break (%)
EVOH55	—	134.0	21.6	141.2 ± 26.3	3.81 ± 0.8	5.0 ± 1.0
EVOH55-HBA0.5	0.5	133.1	19.9	615.8 ± 58.0	16.7 ± 3.3	5.1 ± 0.75
EVOH55-HBA2.1	2.1	126.3	18.4	403.8 ± 36.8	20.1 ± 1.4	11.0 ± 2.9
EVOH55-HBA5.4[c]	5.4	84.9	10.1	29.6 ± 2.8	9.2 ± 0.5	304.6 ± 24.3
EVOH55-HBA16[c]	16.3	80.5	5.24	7.66 ± 3.3	5.7 ± 0.05	340.5 ± 54.5
EVOH55-SA2.3[d]	2.3	63.4	2.09	4.10 ± 0.5	0.97 ± 0.13	125.1 ± 45.2
EVOH55-SA5.5[d]	5.5	60.5	1.92	1.35 ± 0.08	2.8 ± 0.5	722.7 ± 79.2
EVOH55-ABA9.0[e]	9.0	113.5	19.37	254.7 ± 16.9	8.8 ± 2.0	6.1 ± 0.8

[a] Determined from the corresponding ^{1}H-NMR spectra.
[b] Measured by DSC.
T_m: peak melting temperature; ΔH_f: heat of fusion (cal/g).
[c] HBA was treated with thionyl chloride and then reacted with EVOH55.
[d] SA was treated with thionyl chloride and then reacted with EVOH55.
[e] ABA was reacted with EVOH55 in the absence of thionyl chloride.

Molar content of the biocidal units, HBA, ABA, and SA, grafted to EVOH55 determined from the corresponding ^{1}H-NMR spectra, are demonstrated in Table I.

The abbreviation of the sample code in Table I, EVOH55-HBA5.4, for example, means that the content of HBA units in the polymer is 5.4 mol %.

As cited above, it was hard to graft more than 2.1 mol % of HBA to EVOH55 without the help of thionyl chloride. Reaction of HBA with thionyl chloride could produce oligomers of HBA as well as 4-hydroxy benzoic acid chloride. However, the ratio of intensity of the peaks at 4.1–4.8 ppm to that of the peaks at 6.8–7.8 ppm was 1 : 4 from the ^{1}H-NMR spectrum of EVOH55-HBA, indicating that each of the esterified hydroxy groups afforded only one HBA unit. This fact excludes the possibility that EVOH-HBA had some oligomeric branches composed of HBA units.

Mechanical and thermal properties of the polymeric biocides

The crystallinity of EVOH55 moiety decreased by the grafting of the biocides as evidenced by the decrease in peak melting temperature (T_m) and heat of fusion (ΔH_f). It is worth noting that the decrease of T_m and ΔH_f was much less in the case of EVOH55-ABA9.0 in comparison with that corresponding to EVOH55-HBA5.4 and EVOH55-SA5.5. Moreover, elongation modulus of EVOH55-ABA9.0 was much higher than that of EVOH55-HBA5.4 and that of EVOH55-SA5.5.

EVOH55-HBA5.4, EVOH55-HBA16.3, EVOH55-SA2.3, and EVOH55-SA5.5 showed rubberlike properties. As the vinyl alcohol units in EVOH are esterfied by the three biocidal molecules, intermolecular distance increased to decrease interactions between the molecules. Hence, glass transition temperature (T_g) should decrease, as is evidenced by a decrease in tan δ peak temperature (Fig. 3). EVOH55-SA has lower T_m and lower elongation modulus compared to EVOH55-HBA, the content of the grafted biocidal units in the respective polymers being similar, because intermolecular interaction of EVOH55-SA should be weaker than that of EVOH55-HBA because of the greater shield of hydroxy groups of SA units compared to those of HBA units.

As the electronegativity of oxygen is stronger than that of nitrogen, the hydrogen bond between hydroxy groups and carbonyl groups in EVOH55-HBA is more probable than that between amine groups and carbonyl groups in EVOH55-ABA. However, both T_m and elongation modulus of EVOH55-ABA are higher than those of EVOH55-HBA, indicating that intermolecular interaction of EVOH55-ABA is stronger than that of EVOH55-HBA. This should be in part ascribed

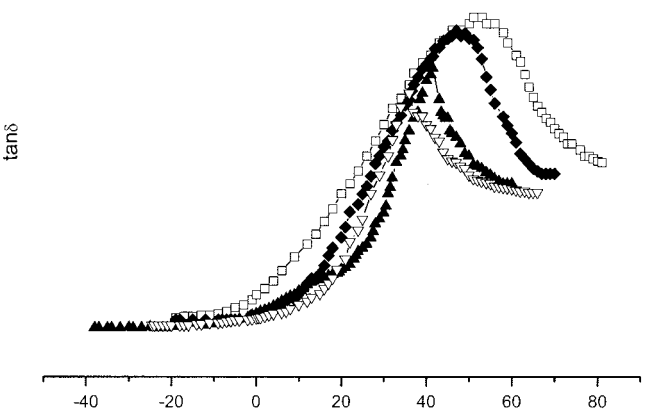


Figure 3 Tan δ curve of polymer biocides (□:EVOH55; ◆: EVOH55-HBA2.1; ▲: EVOH55-HBA5.4; ▽: EVOH55-HBA16).

TABLE II
Antibacterial Activity Measured by the Shake Flask Test

Strain	Sample code	Biocide con. (wt/vol %)	CFU/ml	Reduction rate (%)
P. aeruginosa (−)	Blank	—	4.37×10^6	—
	EVA55	1.0	4.53×10^6	—
		2.5	5.20×10^6	—
		5.0	6.00×10^5	—
	EVOH55	1.0	5.20×10^6	—
		2.5	7.30×10^6	—
		5.0	8.10×10^6	—
	EVOH-ABA9.0	1.0	3.08×10^6	29.5 + 12.8
		2.5	2.61×10^6	40.3 ± 8.23
		5.0	5.60×10^4	98.7 ± 0.25
	EVOH-SA[a]2.3	1.0	3.22×10^6	26.3 + 5.03
		2.5	2.96×10^6	32.3 ± 2.28
		5.0	2.85×10^6	34.8 ± 5.94
	EVOH-SA[a]5.5	1.0	5.17×10^6	—
		2.5	4.15×10^5	90.5 ± 0.01
		5.0	1.52×10^4	99.7 ± 0.03
	EVOH-HBA2.1	1.0	3.63×10^6	16.9 + 0.01
		2.5	1.26×10^6	71.2 ± 1.60
		5.0	1.03×10^5	76.4 ± 2.45
	EVOH-HBA[a]5.4	1.0	3.18×10^6	27.2 ± 6.80
		2.5	2.60×10^6	40.5 ± 1.70
		5.0	1.79×10^6	58.9 ± 5.28
	EVOH-HBA[a]16	1.0	6.90×10^5	84.2 + 1.60
		2.5	3.46×10^5	92.1 ± 0.54
		5.0	2.41×10^5	94.5 ± 1.28
S. aureus (+)	Blank	—	4.71×10^6	—
	EVA55	1.0	6.20×10^6	—
		2.5	5.00×10^6	—
		5.0	3.38×10^6	28.2 ± 10.8
	EVOH55	1.0	7.00×10^6	—
		2.5	2.55×10^6	45.9 ± 8.06
		5.0	1.50×10^6	68.2 ± 10.4
	EVOH-ABA9.0	1.0	7.90×10^6	—
		2.5	1.97×10^6	58.2 ± 4.03
		5.0	1.17×10^5	97.5 ± 0.50
	EVOH-SA[a]2.3	1.0	3.00×10^6	36.3 + 10.6
		2.5	1.72×10^5	96.3 ± 0.78
		5.0	6.50×10^1	100 ± 0.02
	EVOH-SA[a]5.5	1.0	4.30×10^6	90.9 ± 0.04
		2.5	2.92×10^3	99.9 ± 0.03
		5.0	8.00×10^2	100 ± 0.02
	EVOH-HBA2.1	1.0	1.79×10^6	17.6 + 0.01
		2.5	1.50×10^6	71.2 ± 1.60
		5.0	8.00×10^5	76.4 ± 2.45
	EVOH-HBA[a]5.4	1.0	1.00×10^6	78.8 ± 8.85
		2.5	5.12×10^5	89.1 ± 1.60
		5.0	3.65×10^5	92.3 ± 0.37
	EVOH-HBA[a]16	1.0	1.19×10^6	74.7 + 3.18
		2.5	3.26×10^5	93.1 ± 1.57
		5.0	2.26×10^5	95.2 ± 0.97

[a] Thionyl chloride was used for the synthesis of the polymeric biocides.

to the fact that the amine groups accept the hydrogen of the unesterified hydroxy groups as well as the carbonyl groups in EVOH55-ABA.

Antimicrobial activity of the polymeric biocides

Antimicrobial activity of the polymeric biocides was tested by using the shake flask test method, and the results are compared in Table II. Because of the experimental uncertainties, it is hard to draw definite conclusions from the results in Table II. However, it can be said that *S. aureus*, the Gram-positive bacterium, is more susceptible to the polymeric biocides than *P. aeruginosa*, the Gram-negative bacterium. The cell wall of Gram-positive bacteria contains peptidoglycan, to which polymers such as teichoic acids, polysaccha-

rides, and proteins are covalently linked. In Gram-negative bacteria, the inner cell wall is a thin layer of peptidoglycan, which is again covered by an outer membrane composed of lipopolysaccharide and protein. Hence, in many cases, Gram-negative bacteria are more resistant to antimicrovial agents than Gram-positive ones.[14]

It is curious to observe that EVOH55 itself had some antimicrobial activity against *S. aureus.* EVOH75 at 5 wt % concentration extirpated 67% of the viable cells of *S. aureus.* In contrast, *Vibrio cholerae* ATCC 1547 and *V. vulnificus* KCTC 2980 were not affected at all in the presence of EVOH75 (data not shown).

As *iso*-propanol was reported to be a more active biocide than methanol and ethanol, it is possible that some small molecular weight EVOH in EVOH55 and EVOH75 could contribute to the reduction of the viable cells. However, we do not have at present a clear answer for the reason *V. cholerae* ATCC 1547 and *V. vulnificus* KCTC 2980 was insenstive to EVOH75, whereas *S. aureus* was sensitively susceptible to EVOH75.

Jeong et al.[13] synthesized poly(styrene-*co*-maleic anhydride) (SMA). ABA and HBA were chemically linked to SMA through esterification reaction between the succinic anhydride units in SMA and the amine group of ABA or the hydroxy group of HBA. Antibacterial activity of the resulting polymers was lower than that of the corresponding free bioactive molecules. They attributed this to the slow release rate of the bioactive agents from the polymer backbones via hydrolysis. Hydrolysis of the amide linkage should take place more slowly than that of the ester linkage at the experimental conditions. SMA with ABA units were far less active against microorganisms than SMA with HBA.[13]

EVOH-SA5.5 reduced viable cell number of both *P. aeruginosa* and *S. aureus* more significantly than EVOH-HBA16 and EVOH-ABA9.0, indicating that EVOH-SA was a more potent polymeric antimicrobial agent than the other two polymers.

CONCLUSION

Polymers having ABA, SA, and HBA moieties were prepared and their antimicrobial activity was assessed against *S. aureus* and *P. aeruginosa.* EVOH was employed as polymer matrix, because it not only had hydroxy groups to which the bioactive molecules could be chemically linked, but also EVOH itself exhibited some antimicrobial activity. The antimicrobial activity increased in the order of EVOH-HBA $<$ EVOH-ABA $<$ EVOH-SA. Grafting of a small amount of the bioactive molecules to EVOH transformed the EVOH into an elastomer-like material. The susceptibility of bacteria to the polymeric antimicrobial agents was more pronounced for Gram-positive bacterium than for Gram- negative bacterium.

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