Antibacterial Activity of Polymers with Norfloxacin Moieties Against Native and Norfloxacin-Tolerance-Induced Bacteria

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ABSTRACT: Antimicrobial characteristics were investigated for norfloxacin against 18 bacteria (4 gram-positive bacteria and 14 gram-negative bacteria) with the minimum inhibition concentration (MIC) test. Among the bacteria tested, *B. cereus*, *V. fluvialis*, and *V. parahaemolyticus* formed norfloxacin-tolerant colonies. The release of ultraviolet-absorbing substances from the cells in contact with norfloxacin was examined for the native bacteria and for the norfloxacin-tolerance-induced ones. Norfloxacin was grafted to polypropylene-*graft*-maleic anhydride (MAPP) and poly(styrene-*co*-maleic anhydride) (MAPS) to synthesize polymeric antimicrobial agents. The bioactivity of MAPP–norfloxacin and MAPS–norfloxacin was evaluated and compared with that of neat norfloxacin with the shake flask test and the MIC test. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 936–943, 2005

Key words: biological application of polymers; polystyrene; polypropylene; functionalization of polymers

INTRODUCTION

Norfloxacin, a quinolone carboxylic acid derivative, is an orally absorbed fluoroquinolone antibacterial agent with a fluorine at position 6 and a piperazine ring at position 7.[1] The antibacterial spectrum of norfloxacin includes *Pseudomonas aeruginosa* and enteric pathogens. Norfloxacin is also active against both penicillin-susceptible and penicillin-resistant strains of *Nisseria gonorrhoae*, but it is less active against gram-positive cocci. Norfloxacin exerts its bactericidal effect by inhibiting the subunit of DNA gyrase, which is essential for bacterial DNA replication.[1]

Morita et al.[2] found that cells of *V. parahaemolyticus* possess an energy-dependent efflux system for norfloxacin. They cloned a gene for a putative norfloxacin efflux protein from the chromosomal DNA of *V. parahaemolyticus* with an *Escherichia coli* mutant lacking the major multidrug efflux system.

Hwangbo et al.[3] demonstrated that the addition of an excess amount of Mg^{2+} may result in the extrusion of norfloxacin from DNA. The acid pH values repress the dissociation of the carboxylic group, yielding a high proportion of the uncharged form of quinolone moieties, which is believed to penetrate the cell wall more easily than the ionized form of quinolone.[4]

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Polymeric antimicrobial agents with chemically bound bioactive molecules can significantly reduce the loss of antimicrobial activity associated with volatilization, photolytic decomposition, dissolution, and permeation.[5]

A copolymer made from pentachlorophenyl acrylate, vinyl acetate, and ethyl acrylate exhibited good antimicrobial activity against *Pseudomonas* sp.[6]

A copolymer containing phenol derivatives was synthesized by Nonaka et al.[7] The polymeric antimicrobial agent became more active against *E. coli* and *Staphylococcus aureus* as the number of phenolic hydroxyl groups increased.

Ikeda et al.[8] prepared acrylate monomers with pendant biguanide groups. Their copolymer with acrylamide exhibited high antimicrobial activity against gram-positive bacteria, although they were less active against gram-negative bacteria.

Moon and coworkers synthesized polymers with azole moieties[9] and quinolone.[10] Both polymers reduced the viable cell number significantly on contact during the shake flask test.

In this study, antimicrobial activity was investigated for norfloxacin against not only gram-positive bacteria but also gram-negative bacteria with the minimum inhibition concentration (MIC) test. The release of ultraviolet (UV)-absorbing substances from the cells was examined for the native bacteria and for the norfloxacin-tolerance-induced ones. Polypropylene-*graft*-maleic anhydride (MAPP)–norfloxacin and poly(sty-

TABLE I
Characteristics of Poly(styrene-*co*-maleic anhydride) and Polypropylene-*graft*-maleic anhydride

Sample code	[MA] (mol %)	M_n	M_w	M_w/M_n
MAPP	5	2,100	8,000	3.9
MAPS	17.5	23,000	37,100	1.62

M_n = number-average molecular weight; M_w = weight-average molecular weight.

rene-*co*-maleic anhydride) (MAPS)–norfloxacin were synthesized to prepare polymeric antimicrobial agents containing the norfloxacin moiety through the reaction between the maleic anhydride (MA) group of the polymers and the amine group of norfloxacin, and their antimicrobial activity was compared with that of neat norfloxacin. The release of norfloxacin could be either protected or at least controlled by its immobilization in the polymer matrix.

EXPERIMENTAL

Materials

S. aureus ATCC 6538P, *S. aureus* ATCC 27735, *E. faecalis* ATCC 29212, *B. cereus* ATCC 1178, *P. aeruginosa* ATCC 11778, *P. vulgaris* ATCC 6059, *K. pneumoniae* ATCC 10031, and *E. coli* ATCC 11246 were obtained from the Culture Collection Center Institute of Microbiology at Seoul National University (Korea). *V. alginolyticus* KCTC 2928, *V. choleae* ATCC 14547, *V. fluvialis* ATCC 33809, *V. parahaemolyticus* ATCC 17802, and *V. vulnificus* KCTC 2980 were purchased from the Korean Collection for Type Cultures (Pohang, Korea). *P. fluorescens* TY1, *P. stutzeri* TY2, *A. lwoffii* TY3, *S. paucimobilis* TY4, and *B. mallei* TY5 were isolated from Tongyeong (Korea) coastal sea water.

Norfloxacin was donated by Hwail Pharmaceutical Co. (Hwasung, Korea) and was used as received.

MAPS was synthesized by the copolymerization of styrene with MA.[11] MAPP was donated by Honam Petrochemical (Yeosu, Korea). Characteristics of MAPS and MAPP are listed in Table I.

Instrumentation

Molecular weight and its distribution were measured by gel permeation chromatography (Waters 410, Milford, MA), a refractive index detector with a tetrahydrofuran eluent at 1.0 mL/min and 30°C with a column (porosity = 10 μm, Stragel HR 1, HR 2, HR 4, Linear). Narrow-molar-mass-distribution polystyrene standards (Showadenko SL-105, Oita, Japan) were used for universal calibration. Polymeric biocides were characterized by ^{1}H-NMR spectra recorded at 25°C on a Bruker AC-250 Fourier transform NMR spectrometer (Bruker Instruments, Billerica, MA). The sample (5 mg) was dissolved in 0.5 mL of dimethyl sulfoxide (DMSO)-d_6 (10 wt/vol %) and was subjected to ^{1}H-NMR measurements.

Incubation of norfloxacin-tolerant strains (NTSs)

The selected strain was initially grown in Mueller Hinton agar media with 1 μg/mL of norfloxacin. One colony was kept frozen and regrown in the medium with 2 μg/mL of norfloxacin. The procedure was repeated five times with an exponential increase of the norfloxacin concentration at each step until a concentration of 100 μg/mL norfloxacin was reached.

Shake flask method

The number of bacterial cells in the bacteria culture suspension was about 5.0×10^5 colony-forming units (cfu)/mL. The suspension in contact with the polymeric antimicrobial agents in diluted phosphate buffer saline was incubated at 37°C for 24 h, and the number of bacterial cells was calculated by multiplication of the number of colonies by the dilution factor.[12]

MIC test

MIC testing of norfloxacin was determined by a standard microdilution method with a 96-well microtiter plate. Serial dilutions of the norfloxacin in Mueller Hinton broth (50 μL) were mixed with an inoculum (10^6 cfu/mL) from a log-phage culture. Bacteria were incubated for 24 h at 37°C according to the National Committee for Clinical Laboratory Standards guidelines.[13]

Halo zone test

A halo zone test for microorganisms against the antimicrobial agents was carried out according to the method proposed by Bauer et al.,[14] under strict adherence to National Committee for Clinical Laboratory Standards. The bacteria were subcultured to Mueller Hinton broth and incubated overnight at 37°C. Then, the cells were suspended to produce a suspension of 10^6 cfu/mL. A Mueller Hinton agar plate was streaked with a sterile swab moistened with the bacterial suspension. Antimicrobial agents were dissolved in DMSO and spread on the disks made of filter paper. The disks were exposed to UV radiation for 1 h and were then aseptically applied to the surface of the agar plate. All of the test plates were incubated overnight at 37°C. The susceptibility of microorganisms to the antimicrobial agents was determined by the size of the inhibition zone.

TABLE II
MIC Values of Norfloxacin for Different Strains

	Strain	MIC of the parent strains (μg/mL)	MIC of NTS (μg/mL)[a]
Gram positive	*S. aureus* ATCC 6538p	2.57	—[b]
	S. aureus ATCC 27735	1.25	—
	E. faecalis ATCC 29212	1.25	—
	B. cereus ATCC 11778	1.25	20.7
Gram negative	*V. alginolyticus* ATCC 11778	2.57	—
	V. cholerae ATCC 14547	2.57	—
	V. fluvialis ATCC 33809	2.57	165
	V. parahaemolyticus ATCC 17802	1.25	330
	V. vulnificus ATCC 2980	1.25	—
	P. aeruginosa ATCC 11778	2.57	—
	P. vulgaris ATCC 6059	2.57	—
	K. pneumoniae ATCC 10031	1.25	—
	E. coli ATCC 11246	1.25	—
	P. fluorescens TY1	2.57	—
	P. stutzeri TY2	2.57	—
	A. lwoffii TY3	2.57	—
	S. paucimobilis TY4	2.57	—
	B. mallei TY5	2.57	—

[a] Norfloxacin-tolerance induced strains.
[b] A halo zone was not formed.

Release of uv-absorbing substances from the microorganisms

The bacterial cells were treated with 2.57 μg/mL of norfloxacin, and the amount of the UV-absorbing substances released from the cells was determined by comparison of the absorbance of UV light at 260 nm and that of the buffer blank with the UV spectrophotometer (Shimadzu, UV1201, Tokyo, Japan).

Isolation and characterization of protein

Outer membrane fractions were prepared as described previously.[15] Protein was collected separately and kept frozen at −70°C. The protein concentration was determined by the Bradford protein assay. Protein (4 μg) was suspended in a buffer solution. The samples were heated to 100°C for 3 min, eletrophoresed in discontinuous sodium dodecylsulphate (SDS)–polyacrylamide gels (12%), and then stained with 1% coomassie blue.

Synthesis of MAPS–norfloxacin

Norfloxacin (6 g) and MAPS (30 g) were first dissolved in DMSO (100 mL) at 150°C and kept under a N_2 blanket to prevent oxidation. After the mixture was stirred for 30 min, tetra *n*-butyltitanate (TNBT) was quickly added to the hot solution, and this was stirred for 24 h. The product was precipitated in acetone and dried *in vacuo* followed by Soxhlet extraction with boiling acetone for 24 h to remove the unreacted norfloxacin.

Synthesis of MAPP–norfloxacin

Norfloxacin (6 g) and MAPP (30 g) were first dissolved in 1,2,4-trichlorobenzene (100 mL) at 150°C, and MAPP–norfloxacin was synthesized following the same procedure for the synthesis of MAPS–norfloxacin.

RESULTS AND DISCUSSION

Antibacterial activity of norfloxacin

Tolerance against norfloxacin of the strains was enhanced by their cultivation in the medium with increasing concentration of norfloxacin with a modified Joaquim et al. method.[16] Among the tested strains (4 strains of gram-positive bacteria and 14 strains of gram-negative bacteria), *B. cereus*, *V. fluvialis*, and *V. parahaemolyticus* formed norfloxacin-tolerant colonies on culture medium containing 1 μ/mL of norfloxacin. *B. cereus*, having norfloxacin tolerance, was cultivated in a medium with 0.1 wt % of norfloxacin, and *V. fluvialis* and *V. parahaemolyticus*, having norfloxacin tolerance, were grown in the same medium but with 1.5 wt % norfloxacin. The NTSs thus cultivated were represented as *B. cereus*-R, *V. fluvialis*-R, and *V. parahaemolyticus*-R, respectively.

Table II summaries the MIC test results of the native strains and the NTSs. Gram-positive bacteria showed MIC values for norfloxacin in the range 1.25–2.57 μg/mL, whereas gram-negative bacteria exhibited corresponding values, also in the range 1.25–2.57 μg/mL, indicating that the MIC values of the gram-positive bacteria were not significantly different from those of

TABLE III
Antibacterial Activity of Norfloxacin Measured by the Halo Zone Test

Strain	Concentration of norfloxacin (wt/vol % in DMSO, mm)						
	0.001	0.01	0.05	0.1	1	2.5	5
S. aureus ATCC 6538p	—[a]	15.0 ± 0.0	30.0 ± 0.0	35.0 ± 0.0	43.0 ± 0.0	47.0 ± 0.0	Maximum[b]
S. aureus ATCC 27735	17.0 ± 0.0	20.0 ± 0.0	25.0 ± 0.0	30.0 ± 0.0	35.0 ± 0.0	39.5 ± 0.7	Maximum
E. faecalis ATCC 29212	19.0 ± 0.0	23.5 ± 0.7	27.0 ± 0.0	30.0 ± 0.0	37.0 ± 0.0	37.5 ± 0.7	Maximum
B. cereus ATCC 11778	12.5 ± 0.7	19.0 ± 0.0	25.5 ± 0.0	29.0 ± 0.0	33.5 ± 0.7	34.0 ± 0.0	Maximum
V. alginolyticus ATCC 11778	17.0 ± 0.0	22.5 ± 0.7	28.0 ± 0.0	30.0 ± 0.0	35.0 ± 0.0	38.5 ± 0.7	Maximum
V. cholerae ATCC 14547	—	—	28.0 ± 0.0	34.5 ± 0.7	45.0 ± 0.0	51.5 ± 0.7	Maximum
V. fluvialis ATCC 33809	—	—	27.5 ± 0.7	30.5 ± 0.7	35.0 ± 0.0	40.0 ± 0.0	Maximum
V. paranaemolyticus ATCC 17802	—	—	32.0 ± 0.0	38.0 ± 0.0	45.0 ± 0.0	49.5 ± 0.7	Maximum
V. vulntficus ATCC 2980	—	—	34.0 ± 0.0	39.5 ± 0.7	47.0 ± 0.0	50.0 ± 0.0	Maximum
P. aeruginosa ATCC 11778	—	12.0 ± 0.0	28.0 ± 0.0	30.0 ± 0.0	33.0 ± 0.0	36.5 ± 0.7	Maximum
P. vulgaris ATCC 6059	25.5 ± 0.7	27.0 ± 0.0	30.0 ± 0.0	32.5 ± 0.7	37.0 ± 0.0	38.5 ± 0.7	Maximum
K. pneumoniae ATCC 10031	21.5 ± 0.7	26.5 ± 0.7	30.5 ± 0.7	35.0 ± 0.0	38.5 ± 0.7	40.0 ± 0.0	Maximum
E. coli ATCC 11246	0.0 ± 0.0	34.5 ± 0.7	40.0 ± 0.0	40.5 ± 0.7	45.0 ± 0.0	45.5 ± 0.7	Maximum
P. fluorescens TY1	18.5 ± 0.7	25.0 ± 0.0	35.0 ± 0.0	35.0 ± 0.0	47.5 ± 0.7	50.5 ± 0.7	Maximum
P. stutzeri TY2	16.5 ± 0.7	25.0 ± 0.0	34.0 ± 0.0	34.0 ± 0.0	50.0 ± 0.0	57.5 ± 0.7	Maximum
A. lwoffii TY3	30.0 ± 0.0	41.5 ± 0.7	50.0 ± 0.0	50.0 ± 0.0	65.0 ± 0.0	70.0 ± 0.0	Maximum
S. paucimobilis TY4	20.0 ± 0.0	27.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0	55.0 ± 0.0	58.0 ± 0.0	Maximum
B. mallei TY5	—	—	21.5 ± 0.7	21.5 ± 0.7	46.5 ± 0.7	52.0 ± 0.0	Maximum
B. cereus-R[c]	—	—	—	—	—	—	—
V. fluvialis-R	—	—	—	—	—	—	—
V. parahaemolyticus-R	—	—	—	—	—	—	—

[a] The halo zone was not formed.
[b] The halo zone covered all of the agar medium on the Petri dish
[c] Norfloxacin tolerant strain

the gram-negative bacteria before the norfloxacin-tolerance induction. However, *B. cereus*-R exhibited an MIC value of norfloxacin 20 times higher than that of the native *B. cereus*. The increase in MIC value as a result of the norfloxacin-tolerance induction was much more significant for both *V. fluvialis* and *V. parahaemolyticus*, gram-negative bacteria, than for *B. cereus*, a gram-positive bacterium.

Table III shows the antibacterial activity of norfloxacin determined from the halo zone tests. Some native strains began to form an inhibition zone at norfloxacin contents as low as 0.001 wt %, whereas four out of the five *Vibrio* species and *B. mallei* TY5 did not form the inhibition zone until the norfloxacin content was 0.05 wt % or more. When the norfloxacin content was 5 wt %, all of the native strains were completely inhibited. In marked contrast, *B. cereus*-R, *V. fluvialis*-R, and *V. parahaemolyticus*-R did not form the inhibition zone at norfloxacin contents up to 5 wt %.

Figure 1 represents the concentration of UV-absorbing substances released from the microbial cells as a result of contact with 2.57 μg/mL norfloxacin. In the absence of norfloxacin, release of UV-absorbing substances was not detected. However, after contact with 2.57 μg/mL of norfloxacin, the amount of UV-absorbing substances from the native *B. cereus* increased sharply and then reached a plateau value within 10 min. In contrast, the release of UV-absorbing substances from *B. cereus*-R proceeded much more slowly,

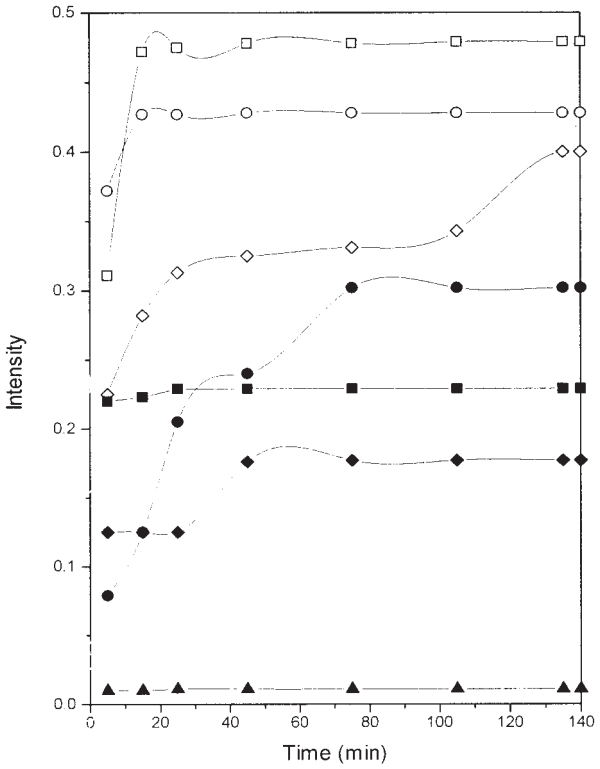


Figure 1 UV absorption intensity of the substances released from the microbial cells treated with 2.57 μ/mL of norfloxacin: (▲) control, (●) *B. cereus*-R, (○) *B. cereus* ATCC 11778, (◆) *V. fluvialis*-R, (◇) *V. fluvialis* ATCC 33809, (■) *V. parahaemolyticus*-R, and (□) *V. parahaemolyticus* ATCC 17802.

TABLE IV
Effect of Metal Cations on the MIC Values of Norfloxacin

Strain	Minimal inhibitory concentration (μg/mL)				
			Concentration of the cation (mM)		
	Control	Cation	1	30	100
B. cereus ATCC 11778	1.25	K^+	1.25	1.25	2.57
		Na^+	1.25	1.25	2.57
		Ca^{2+}	20.62	41.25	41.25
		Mg^{2+}	41.25	82.5	165.00
V. fluvialis ATCC 33809	2.57	K^+	2.57	2.57	5.15
		Na^+	2.57	2.57	5.15
		Ca^{2+}	80.00	160.00	330.00
		Mg^{2+}	160.00	330.00	330.00
V. paraheamolyticus ATCC 17802	1.25	K^+	2.57	2.57	5.15
		Na^+	2.57	2.57	5.15
		Ca^{2+}	80.00	160.00	330.00
		Mg^{2+}	160.00	330.00	330.00

and release continued for 70 min. The same tendency was observed for V. *fluvialis* and *V. parahaemolyticus*, in that the amount of UV-absorbing substances was much higher and that the release rate was much faster in comparison to those from the corresponding NTS.

Table IV demonstrates the effects of metal cations on the MIC values of norfloxacin. The addition of Ca^{2+} or Mg^{2+} to the culture medium increased the MIC value of norfloxacin considerably for both *B. cereus*-R, *V. fluvialis*-R, and *V. parahaemolyticus* -R. However, the increase in the MIC values was not significant when Na^+ or Ka^+ ions were added instead of Ca^{2+} or Mg^{2+}. This was because divalent or trivalent cations such as Ca^{2+}, Mg^{2+}, and $A1^{3+}$ can alter the absorption of norfloxacin into the microbial cells[17] due to the formation of complexes.[3]

Proteins from the native *B. cereus*, *V. fluvialis*, and *V. parahaemolyticus* and the corresponding NTS were separated by SDS–polyacrylamide gel electrophoresis, as shown in Figure 2. A significant difference was not observed between *B. cereus* and *B. cereus*-R. As shown in Figure 2, the band of protein expressed by *V. fluvialis*-R and *V. parahaemolyticus*-R appearing at about 45 kDa was slightly thicker than that from the native *V. fluvialis* and *V. parahaemolyticus* (lanes 3 and 6), respectively. However, a definite conclusion on the shock protein could not be drawn because a new additional band for the shock protein due to the stress coming from the contact with norfloxacin was not observed in Figure 2 after the tolerance induction.

Antibacterial activity of MAPS–norfloxacin and MAPP–norfloxacin

MAPP–norfloxacin and MAPS–norfloxacin were synthesized to anchor norfloxacin to the polymers through a chemical reaction between the maleic group of the polymers and the amine group of norfloxacin. Figure 3 shows the ^{1}H-NMR spectrum of MAPS and MAPS–norfloxacin. The methylene and phenyl ring protons of the styrene units exhibited their peaks at 1.2–2.3 and 6.3–7.8 ppm, respectively. The peaks at 3.0–3.7 ppm corresponded to the methine protons of maleic anhydride units. Figure 3(b) shows the ^{1}H-NMR spectrum of MAPS–norfloxacin. The new peaks at 2.5–2.7, 3.0–4.0, 4.2, and 9.0 ppm confirmed that the chemical reaction between MAPS and norfloxacin took place.

Table V shows the MIC test results for MAPP–norfloxacin and MAPS–norfloxacin against the native

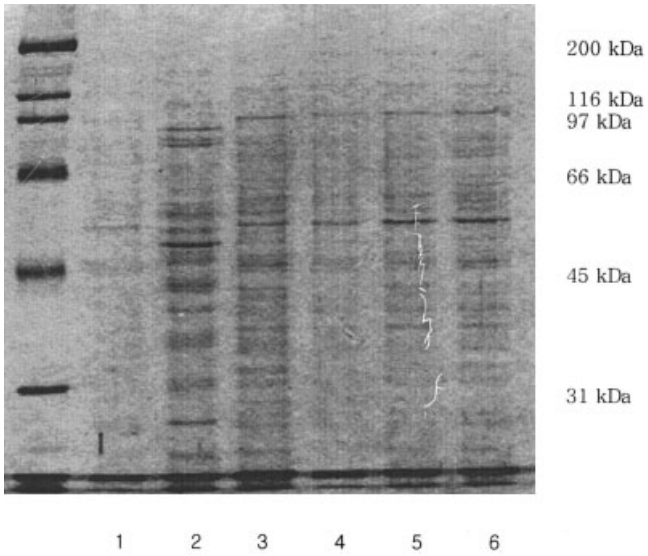


Figure 2 SDS–12% PAGE of the total protein: (1) *B. cereus* ATCC 11778, (2) *B. cereus*-R, (3) *V. fluvialis*-R, (4) *V. fluvialis* ATCC 33809, (5) *V. parahaemolyticus* ATCC 17802, and (6) *V. parahaemolyticus*-R.

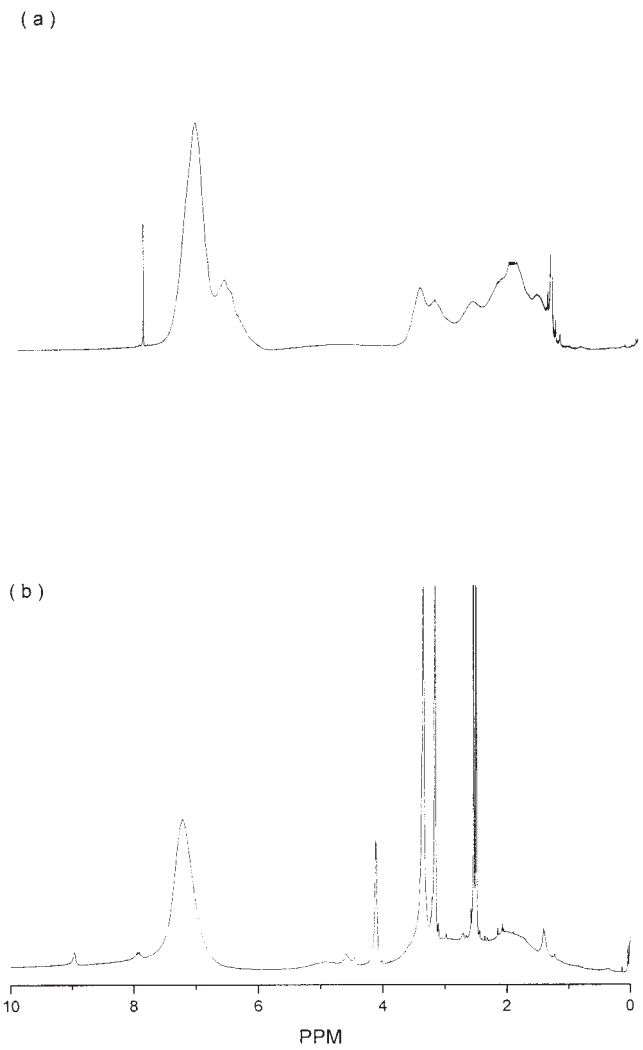


Figure 3 ^{1}H-NMR spectra of (a) MAPS and (b) MAPS–norfloxacin.

strains and the corresponding NTS. The MIC values of both MAPP–norfloxacin and MAPS–norfloxacin against the native strains on the basis of unit weight of the norfloxacin unit were 4–16 times higher than those of neat norfloxacin. *B. cereus*-R, *V. fluvialis*-R, and *V. parahaemolyticus*-R also exhibited higher MIC values of MAPP–norfloxacin and MAPS–norfloxacin than those of neat norfloxacin, indicating that the immobilization of norfloxacin on the polymers reduced the antimicrobial activity of the bioactive agent (Scheme 1).

The shake flask tests were carried out to examine the antimicrobial activity of MAPP–norfloxacin and MAPS–norfloxacin. Table VI summaries the shake flask test results. The addition of 1 wt % MAPP–norfloxacin and MAPS–norfloxacin reduced 99% of the viable cells of the native strains. In contrast, the viable cell number of *B. cereus*-R, *V. fluvialis*-R, and *V. parahaemolyticus*-R did not decrease at all, even after contact with 1 wt % MAPP–norfloxacin and MAPS–norfloxacin. However, when the content of the polymeric antimicrobial agents increased to 5 wt %, the number of the viable cells in the three NTSs was decreased by 73–98%, demonstrating that the polymeric antimicrobial agents were somewhat bioactive against the bacteria even when the bacteria attained tolerance against norfloxacin.

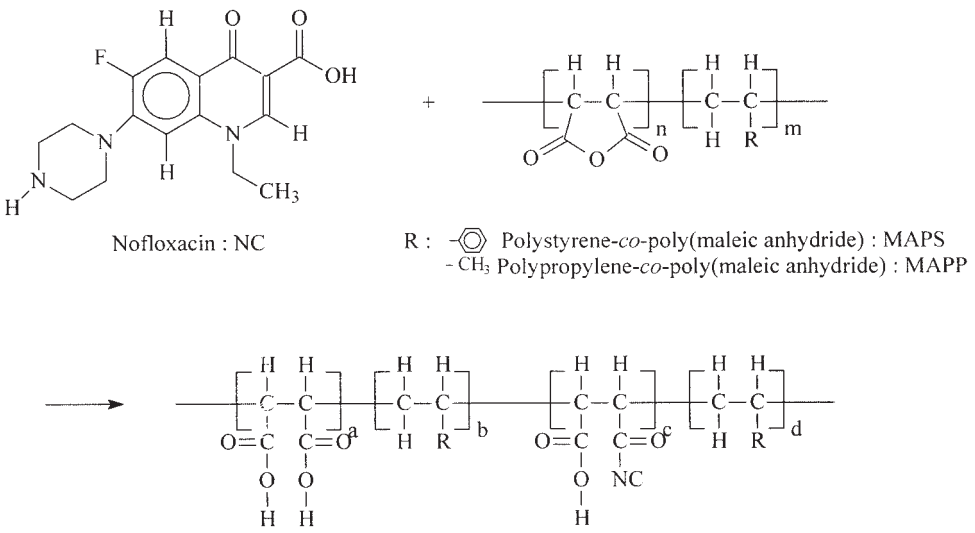
CONCLUSIONS

Among the 18 strains tested, *B. cereus*, *V. fluvialis*, and *V. parahaemolyticus* formed norfloxacin-tolerant colonies. The NTSs exhibited MIC values of norfloxacin 20 times higher than the corresponding native strains. The amount of UV-absorbing substances released from the NTS cells in contact with norfloxacin was much smaller, and the release rate was much slower than that from the native bacteria. The addition of Ca^{2+} or Mg^{2+} ions to the culture medium increased the MIC value of norfloxacin considerably, whereas monovalent metal cations such as Na^+ and K^+ exerted little effect on the MIC values because of the formation of a complex be-

TABLE V
MIC Values of the Synthesized Polymeric Antimicrobial Agents

Strain	Polymeric biocide	Norfloxacin content in the polymeric biocides (wt %)[a]	MIC of the parent strains (μg/mL)	MIC of the NTS (μg/mL)[b]
B. cereus ATCC 11778	MAPP–norfloxacin	5	825	3300
	MAPS–norfloxacin		825	3300
V. fluvialis ATCC 33809	MAPP–norfloxacin	5	825	6600
	MAPS–norfloxacin		415	6600
V. parahaemolyticus ATCC 17802	MAPP–norfloxacin	5	825	6600
	MAPS–norfloxacin		415	6600

[a] Measured by NMR.
[b] Norfloxacin tolerance-induced strains.



Scheme 1 Synthesis of the polymeric antimicrobial agents.

tween the divalent cations and norfloxacin. Polymeric antimicrobial agents with norfloxacin units were synthesized by the reaction of norfloxacin with MAPP and MAPS. The polymeric antimicrobial agents were less active than norfloxacin. However, the polymeric antimicrobial agents were still bioactive against the bacteria even when the bacteria attained tolerance against norfloxacin.

TABLE VI
Antibacterial Activity of the Synthesized Polymeric Biocides Measured by the Shake Flask Test

Strain	Polymeric antimicrobial agent[a]	Concentration of antimicrobial agent (wt/vol %)	cfu/mL	Reduction rate (%)[b]
B. cereus ATCC 11778	—	Blank	2.3×10^8	—
	MAPP–norfloxacin	1	0.7×10^1	99
	MAPS–norfloxacin	1	0.2×10^1	99
V. fluvialis ATCC 33809	—	Blank	7.3×10^7	—
	MAPP–norfloxacin	1	1.2×10^1	99
	MAPS–norfloxacin	1	0.4×10^1	99
V. parahaemolyticus ATCC 17802	—	Blank	1.3×10^7	—
	MAPP–norfloxacin	1	1.4×10^1	99
	MAPS–norfloxacin	1	1.1×10^1	99
B. cereus-R[a]	—	Blank	6.2×10^2	—
	MAPP–norfloxacin	1	7.5×10^2	—
	MAPS–norfloxacin	1	7.9×10^2	—
V. fluvialis-R	—	Blank	1.8×10^3	—
	MAPP–norfloxacin	1	2.2×10^3	—
	MAPS–norfloxacin	1	1.8×10^3	—
V. parahaemolyticus-R	—	Blank	2.8×10^3	—
	MAPP–norfloxacin	1	4.2×10^3	—
	MAPS–norfloxacin	1	5.2×10^3	—
B. cereus-R	—	Blank	6.2×10^2	—
	MAPP–norfloxacin	5	3.1×10^1	95
	MAPS–norfloxacin	5	1.2×10^1	98
V. fluvialis-R	—	Blank	1.8×10^3	—
	MAPP–norfloxacin	5	4.7×10^2	73
	MAPS–norfloxacin	5	2.2×10^2	87
V. parahaemolyticus-R	—	Blank	2.8×10^3	—
	MAPP–norfloxacin	5	3.6×10^2	87
	MAPS–norfloxacin	5	1.6×10^2	94

[a] Norfloxacin tolerant strain.

[b] Reduction rate (%) $= \dfrac{\text{Bacteria number in blank} - \text{Bacteria number in MAPP (or MAPS)–norfloxacin}}{\text{Bacteria number in blank}} \times 100.$

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