Antimicrobial Activity of a Monomer and Its Polymer Based on Quinolone

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ABSTRACT: An acryl monomer containing the quinolone moiety was synthesized and then polymerized with azobisisobutyronitrile in a dimethylformamide solution. The resulting polymer as well as the corresponding monomer exhibited an excellent antibacterial activity. The poly(acrylated quinolone) (PQ) was compounded with other ordinary synthetic polymers such as low-density polyethylene (LDPE), poly(butylene succinate) (PBS), poly(methyl methacrylate) (PMMA), maleated polypropylene (PPMA), and polycaprolactone (PCL). The polymer blends reduced the viable cell number significantly on contact during the shake flask test even when the PQ content was as low as 1 wt %. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 1797–1801, 2003

Key words: biological applications of polymers; blending; functionalization of polymers

INTRODUCTION

Quinolone antibacterial agents are clinically important drugs that possess a broad spectrum of activity. The first clinically important molecule in this class was nalidixic acid, which is limited to urinary tract infections and has a limited spectrum of antibacterial activity. Nalidixic acid is reported to be more active against Gram-negative bacteria than against Gram-positive bacteria. Quinolone has not been isolated from molds or fungi, but synthesized from organic chemicals.[1]

Modification of the groups at the N1, C6, C7, and C8 positions in quinolone has been successful in the improvement of antibacterial activity. Almost all of the clinically important quinolones bear a fluorine atom at C6. Fluoroquinolones such as norfloxacin and ciprofloxacin are significantly more potent than is nalidixic acid and have broadened the spectrum of the activity.[1]

Clinically relevant quinolones bear a piperazine moiety at C7, especially when a fluorine atom is attached to the C6 position. In addition to the excellent antibacterial activity, the piperaizine ring also conferred on the quinolones an excellent combination of oral efficacy, good phamacokinetics, and favorable toxicology.[1]

Quinolones that have a cyclopropyl appendage at N1 are generally the most potent inhibitors against bacterial DNA gyrase and cell growth among the important antibacterial agents of this class. Prior to this discovery, an ethyl group was the most common functionality at this position. Excellent reviews which describe the relationship between the structure and the antimicrobial activity have been published.[1]

Polymers with antimicrobial activity are often required for food packaging, sanitary, or medical application. They can also be used as a coating material for common objects such as doorknobs, children's toys, and computer keyboards to prevent transmission of microbial infections.[2] They have usually been prepared by compounding antimicrobial agents into ordinary synthetic polymers. However, many of the antimicrobial agents steadily permeate out from the polymer matrices, giving rise to poisonous influences on the human body. Covalent bonding of antimicrobial pharmacophores to polymer matrices would reduce or eliminate the permeation problems, which can be done either by polymerization of monomers possessing antimicrobial activity or by direct chemical anchoring of pharmacophores onto functional groups of the ordinary synthetic polymers.

The reaction of pentachlorophenol with acryloyl chloride gave pentachlorophenyl acrylate, which was copolymerized with both vinyl acetate and ethyl acrylate. The resulting copolymer retarded or prevented the growth of *Aspergillus* sp., *Pseudomonas* sp., *Alternaria* sp., and *Aureobasidium pullulans*.[3] 2-Benzimidazole

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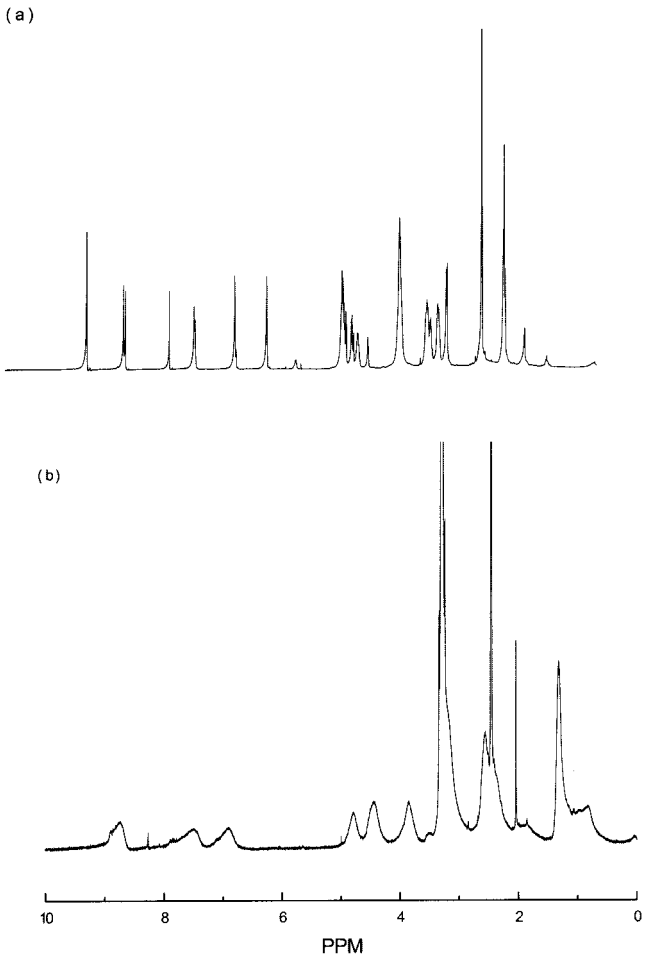


Figure 1 ^{1}H-NMR spectra of (a) MQ and (b) PQ.

carbamoyl groups were chemically linked to poly(ethylene-*co*-vinyl alcohol) (EVOH) by the transesterification of methyl 2-benzimidazole carbamate with EVOH. The EVOH with 2-benzimidazole carbamoyl groups showed a strong antimicrobial activity against *Aspergillus fumigatus* and *Penicillium pinophilum*.[4]

4-Vinylpyridine was polymerized onto a glass surface modified with acryloyl chloride followed by quaternization with various alkyl bromides.[2] Propylated, butylated, hexylated, and octylated immobilized poly(4-vinylpyridine)s (PVPs) were lethal on contact to Gram-negative bacteria as well as Gram-positive ones. However, PVP *N*-alkylated by decyl through hexadecyl bromide was totally ineffective.[2]

We prepared polymeric antimicrobial agents by polymerizing an acryl monomer with the norfloxacin moiety. Quinolone is thermally stable so that the polymeric antimicrobial agents could be processed safely at high temperatures.

Low-density polyethylene (LDPE), polycaprolactone (PCL), maleated polypropylene (PPMA), and poly(butylene succinate) (PBS) were compounded with the polymer containing quinolone groups. The antimicrobial activity and mechanical properties of the polymer blends were explored.

EXPERIMENTAL

Materials

Norfloxacin (Hwail Pharmaceutical Co., Hwasung, South Korea) and other chemicals were reagent grade; glycidyl methacrylate (GMA; Aldrich, Milwaukee, WI) was used as received. LDPE (MI = 5, M_w = 482,000, Hanhwa, South Korea), PCL (TONE Polymer P-787, M_w = 117,000, Union Carbide Co., Danbury, CT), PBS (G4500, M_w = 105,600, PDI = 2.55, Ire Chemicals, Munmak, South Korea), poly(methyl methacrylate) (PMMA; IH830H, M_w = 85,000, Hanhwa, Daejeon, South Korea), and maleated polypropylene (PPMA; M_w = 120,000, Honam Petrochemical Co., Daejeon, South Korea) were used as received.

Instrumentation

^{1}H-NMR spectra were recorded on a Bruker AM-300 (Varian Jemini 2000) spectrometer using DMSO-d_6 as a solvent. Molecular weights were determined by gel permeation chromatography (GPC; Waters 150C; μ-Styragel HT columns of 10^6, 10^5, 10^4, and 10^3 pore sizes in series; 1,2,4-trichlorobenzene at 0.5 mL/min; 140°C).

Differential scanning calorimetry (DSC) was performed with a Perkin–Elmer DSC 7 with a heating rate of 20°C/min from room temperature to 250°C. Tensile properties were tested on an Instron (Model 4462; 10 kN load cell; crosshead speed 20 mm/min) using specimens prepared according to ASTM D 638.

Preparation of 1-ethyl-6-fluoro-7-{4-[2-hydroxy-3-(2-methylacryloyloxy) propyl] piperazin-1-*yl*}-4-oxo-1,4-dihyroquinolin-3-carboxylic acid (MQ)

A suspension of norfloxacin (1.00 g, 3.13 mmol) and GMA (0.445 g, 3.13 mmol) in dimethylformamide (5

TABLE I
Shake Flask Test Results for MQ

Sample code	Cell number reduction (%)							
	E. coli (−)		*S. aureus* (+)		*B. subtilis* (+)		*M. luteus* (+)	
Blank	2.0×10^7	—	2.05×10^7	—	7.65×10^5	—	1.42×10^6	—
MQ	n.d.	100	n.d.	100	n.d.	100	n.d.	100

n.d.: not detected.

TABLE II
Shake Flask Test Results for PQ Annealed at Different Temperatures

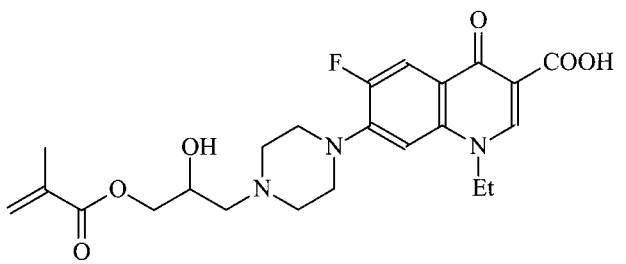
Sample code	Cell number reduction (%)							
	E. coli (−)		*S. aureus* (+)		*B. subtilis* (+)		*M. luteus* (+)	
Blank	3.54×10^8	—	2.89×10^8	—	9.1×10^8	—	7.54×10^8	—
PQ-100°C	n.d.	100	n.d.	100	n.d.	100	n.d.	100
PQ-120°C	n.d.	100	n.d.	100	n.d.	99.99	n.d.	99.99
PQ-140°C	n.d.	100	n.d.	100	n.d.	100	n.d.	100
PQ-180°C	n.d.	100	n.d.	100	n.d.	100	n.d.	99.99

n.d.: not detected.

mL) was stirred for 24 h at 40°C. The reaction mixture was cooled, poured into water, and extracted with dichloromethane (MC). The organic extract was concentrated *in vacuo* to give the crude product (1.33 g, 92%). The analytical sample was obtained by recrystallization from MC–hexane (mp 144–148°C).

^{1}H-NMR ($CDCl_3$, 200 MHz): 1.48 (t, 3H), 1.86 (s, 3H), 2.46–2.87 (m, 6H), 3.23-3.40 (m, 4H), 6.77(d, 1H), 7.89 (d, 1H), 3.45–3.76 (m, 1H), 3.80–4.30 (m, 4H), 5.00 (s, 1H), 6.05 (s, 1H). IR (film, cm^{-1}): 3429, 1722, 1632, 1480, 1256.

ANAL. Calcd for $C_{23}H_{28}FN_3O_6$: C, 59.86%; H, 6.12%; N, 9.11%. Found: C, 59.80%; H, 6.19%; N, 9.26%.



Structure of MQ

Polymerization of MQ

A suspension of MQ (4.00 g, 8.67 mmol) and AIBN (0.1 g, 0.609 mmol) into dimethylformamide (16 mL) was stirred for 24 h at 40°C. The reaction mixture was cooled and poured into water–acetone. The resulting solid was filtered and washed with acetone to give a yellow solid (3.44 g, 97%).

Polymer blending

The LDPE/PQ mixture was fed into the cam-type mixer (Brabender: Germany, type 810602) set at 180°C. The rotor speed was maintained at 50 rpm and the blending was continued for 10 min in the closed mixer. Blend sheets were made by hot pressing at 200°C for 5 min under 1.55 atm and quickly immersed into ice water. The film thus formed was free from any distortion problems.

Shake flask method[5]

The number of bacterial cells in the bacteria culture suspension was about 5.0×10^5/mL. After their contact with the antibacterial agent in diluted PBS for 24 h at 37°C, the suspension was incubated at 37°C for 24 h,

TABLE III
Antibacterial Activity of the LDPE/PQ Blends Measured by the Shaking Flask Method

Sample code	Cell number reduction (%)							
	E. coli (−)		*S. aureus* (+)		*B. subtilis* (+)		*M. luteus* (+)	
Blank	7.37×10^7	—	7.01×10^7	—	3.27×10^7	—	5.82×10^7	—
150-1.0	4.90×10^5	99.34	5.96×10^6	91.50	5.58×10^6	82.94	9.04×10^6	84.47
150-2.5	4.87×10^5	99.34	3.60×10^6	94.86	2.41×10^6	92.63	6.58×10^6	88.69
150-5.0	6.11×10^3	99.99	6.03×10^3	99.99	5.59×10^3	99.98	8.77×10^5	98.49
170-1.0	3.70×10^5	99.50	5.73×10^4	93.84	5.35×10^6	83.64	8.90×10^6	84.71
170-2.5	3.09×10^5	99.58	9.91×10^5	98.59	2.42×10^6	92.60	6.97×10^6	88.02
170-5.0	6.71×10^3	99.99	3.74×10^3	99.99	4.97×10^3	99.98	8.31×10^5	98.57
200-1.0	5.27×10^5	99.28	4.30×10^6	93.87	5.61×10^6	82.84	8.24×10^6	85.84
200-2.5	3.54×10^5	99.52	7.95×10^5	98.87	1.64×10^6	94.98	6.87×10^6	88.20
200-5.0	4.01×10^3	99.99	4.05×10^3	99.99	4.39×10^3	99.99	8.23×10^5	98.59

The sample code 150-1.0 means that LDPE was compounded at 150°C with 1.0 wt % PQ.

TABLE IV
Tensile Properties of LDPE/PQ Blends

Compounding temperature (°C)	Sample code	Elongation modulus (MPa)	Stress at maximum load (MPa)	% Strain at maximum load (%)
170	LDPE	53.2 ± 2.60	10.4 ± 1.13	476.5 ± 35.9
150	LDPE/PQ-1.0%	46.5 ± 10.1	7.27 ± 1.37	173.4 ± 46.4
	LDPE/PQ-2.5%	48.4 ± 12.3	7.31 ± 0.39	104.0 ± 18.0
	LDPE/PQ-5.0%	64.2 ± 10.1	6.49 ± 0.44	65.8 ± 24.8
170	LDPE/PQ-1.0%	43.5 ± 0.53	7.66 ± 0.10	169.6 ± 29.1
	LDPE/PQ-2.5%	48.1 ± 3.08	7.20 ± 0.66	126.0 ± 42.3
	LDPE/PQ-5.0%	47.2 ± 6.08	2.52 ± 0.55	97.9 ± 22.0
200	LDPE/PQ-1.0%	58.6 ± 10.5	8.42 ± 1.02	142.6 ± 16.9
	LDPE/PQ-2.5%	59.7 ± 5.58	7.80 ± 0.64	102.6 ± 24.2
	LDPE/PQ-5.0%	78.5 ± 5.90	8.18 ± 0.12	68.1 ± 6.69

and the number of the bacterial cells was calculated by multiplying the number of colonies by the dilution factor.

RESULTS AND DISCUSSION

Norfloxacin was reacted with GMA to produce acrylated quinolone (MQ). ^{1}H-NMR spectra of the MQ monomer is demonstrated in Figure 1. MQ was powdery and was insoluble in water.

The antibacterial activity of MQ was assessed by using the shake flask test method. The results, summarized in Table I, indicate that MQ was a strong antibacterial agent.

The peaks of the ^{1}H-NMR spectra of MQ in Figure 1(a) were assigned as described in the Experimental section. Polymerization of MQ was carried out by using 2.5% AIBN as an initiator at 40°C for 24 h in a dimethylformamide solution. Figure 1(b) demonstrates the ^{1}H-NMR spectra of the produced polymer (PQ). The vinyl protons of MQ, which were expected to appear at 6.05–6.77 ppm, disappeared as a result of the polymerization. GPC analysis of PQ was as follows:

$$M_w : 1.42 \times 10^5$$

$$\frac{M_w}{M_n} : 2.76$$

PQ was annealed at 100–180°C for 24 h. The antimicrobial activity of PQ was determined by the shake flask test as shown in Table II. The viable cell number after 24 h of incubation at 37°C after contact with PQ (25 mg/mL) was nil, indicating that PQ was a very potent antimicrobial agent and that PQ was thermally stable enough to be processed by the existing ordinary polymer processing equipment. However, PQ was so brittle and rigid that the molding of PQ alone was practically impossible. The compounding of PQ with other easily processible polymers would provide a solution to render PQ moldable. Hence, PQ was compounded first with LDPE at different temperatures and the antimicrobial activity of the LDPE/PQ blends was tested using the shake flask test method.

Table III reveals that the LDPE/PQ blends possessed a good antibacterial activity even though the content of PQ in the blend was as low as 1 wt % and the compounding temperature was as high as 200°C. However, the tensile properties decreased precipitously even though a small amount of PQ was compounded with LDPE due to the incompatibility between the two polymers as shown in Table IV.

TABLE V
Tensile Properties of Polymer Blends Containing 5 wt % of PQ

Compounding temperature (°C)	Sample code	Elongation modulus (MPa)	Stress at maximum load (MPa)	% Strain at maximum load (%)
180	PCL	130.4 ± 1.0	24.5 ± 6.1	1229.0 ± 226.9
	PCL/PQ	48.4 ± 12.3	11.2 ± 11.2	919.1 ± 59.6
180	PPMA	43.5 ± 6.53	7.66 ± 0.20	169.6 ± 29.1
	PPMA/PQ	486.4 ± 66.6	8.50 ± 1.01	3.35 ± 0.37
180	PBS	266.3 ± 8.1	32.9 ± 2.0	270.3 ± 115.1
	PBS/PQ	178.3 ± 34.7	17.5 ± 2.36	28.27 ± 2.57
180	PMMA	116.8 ± 1.93	8.24 ± 1.93	4.29 ± 1.34
	PMMA/PQ	380.1 ± 73.2	10.9 ± 0.37	4.92 ± 6.69

TABLE VI
Antibacterial Activity of the PPMA/PQ, PMMA/PQ, and PCL/PQ Blends Measured by the Shaking Flask Method

Sample code	Cell number reduction (%)							
	E. coli (−)		*S. aureus* (+)		*B. subtilis* (+)		*M. luteus* (+)	
Blank	1.0×10^9	—	2.83×10^9	—	1.1×10^{10}	—	7.81×10^9	—
PPMA	5.0×10^6	99.5	1.19×10^6	99.96	4.0×10^6	99.96	8.0×10^6	99.90
PMMA	1.6×10^7	98.4	1.49×10^6	99.95	1.26×10^8	98.85	1.56×10^6	99.98
PCL	1.0×10^6	99.9	3.14×10^5	99.99	2.06×10^8	98.13	3.0×10^6	99.96

Blend composition: polymer 95 wt %/PQ 5 wt %.

PQ was compounded with other polymers which were less hydrophobic than was LDPE. However, the tensile properties of polypropylene maleated with 5 wt % of maleic anhydride units (PPMA) still decreased precipitously as a result of the compounding with 5 wt % of PQ (Table V). The decrease in the elongation properties due to compounding with 5 wt % of PQ was less significant in the PCL/PQ blend than in the PMMA/PQ, PBS/PQ, and PMMA/PQ blends, indicating that PQ is more compatible with PCL than with the other polymers.

To our knowledge, quinolone has not yet been used as a modifier for polymer antimicrobial agents.[6] Table VI demonstrates that PCL/PQ as well as PPMA/PQ and PMMA/PQ blends exhibited a good antibacterial activity.

By taking into consideration the antibacteral activity together with the mechanical properties and the thermal stability, the PCL/PQ blend is expected to find a practical application such as in food-packaging materials, which would be composted together with food garbage because they would be eventually biodegraded at a controllable rate depending on the PQ content.

CONCLUSIONS

Both the acrylated quinolone monomer (MQ) and its polymer (PQ) exhibited an excellent antibacterial activity against Gram-negative bacteria as well as Gram-positive ones. The thermal stability of PQ was good enough to be melt-processed with other ordinary synthetic polymers. The tensile properties of LDPE, PPMA, PBS, and PMMA decreased drastically as a result of compounding with PQ. The PCL/PQ blend exhibited acceptable mechanical properties and was effective in reducing the viable cell number on contact.

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