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Fully degradable antibacterial poly(ester-phosphoester)s by ring-opening polymerization, "click" chemistry, and quaternization

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ABSTRACT: Fully degradable cationic poly(ester-phosphoester)s with antibacterial properties were prepared by a combination of ring-opening polymerization (ROP) and "click" reaction. First, poly(ester-phosphoester)s-bearing alkynyl groups were synthesized by the ring-opening copolymerization of 2-(2-propynyloxy)−2-oxo-1,3,2-dioxaphospholane (propynyl ethylene phosphate, PEP) and ε-caprolactone (CL) using lanthanum tris(2,6-di-tert-butyl-4-methylphenolate)s ($La(DBMP)_3$) as the catalyst. 2-Azido-N,N-dimethylethanamine ($DMEAN_3$) was then attached to the copolymers by "click" reaction, resulting in poly(ester-phosphoester)s with pendant tertiary amines. After the quaternization reactions between the copolymer and various alkyl bromides, cationic poly(ester-phosphoester)s containing ammonium groups were obtained. Optical density (OD) measurement shows that the cationic copolymers have excellent antibacterial activity, which makes them potential candidates as biomaterials. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42647.

KEYWORDS: antibacterial; catalysts; degradation; polyesters; ring-opening polymerization

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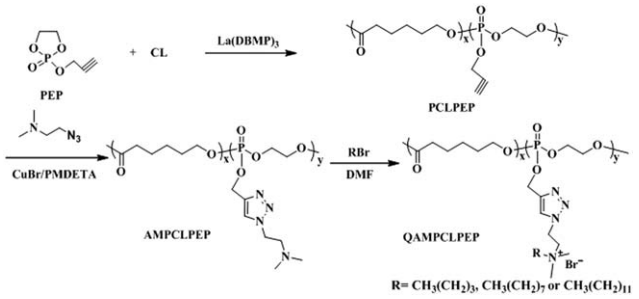
INTRODUCTION

Increasing attentions have been paid on the bacterial infection for its importance in a variety of areas, such as food packaging, biomaterials, and purification systems.[1] Many researchers have focused on the preparation of antibacterial materials.[2–4] Among these antibacterial agents, polymers containing quaternary ammonium salts (QAS) have been widely investigated for their potent antimicrobial activities.[5–10] However, many of these cationic polymers, such as poly (2-(dimethyl amino) ethyl methacrylate) (PDMAEMA)[11–13] polyethylenimine (PEI)[14,15] and poly (propylene-imine) dendrimers[16,17] are nondegradable, causing accumulation in the environment and harmful side effects. More efforts still need to be made to study degradable antibacterial polymers.

Poly(ε-caprolactone) (PCL), which could be prepared by the ring-opening polymerization (ROP) of ε-caprolactone (CL), is a U. S. Food and Drug Administration (FDA)-approved aliphatic polyester with low cost and excellent properties, including biodegradability, biocompatibility, and nontoxicity.[18–21] But the lack of functional groups in PCL backbone restrains its potential applications.[22] Copolymerizing CL with other functional monomers is one method to extend its applications.[23–33] Polyphosphoesters have been extensively studied for biomedical applications due to their biodegradability, biocompatibility, and the structural similarity to nucleic and teichoic acids.[34–36] Polyphosphoesters can be synthesized by different routes of ring-opening, polycondensation, and polyaddition.[37,38] ROP of cyclic phosphates is a more common approach with catalysts, such as $Al(OiPr)_3$[39] and $Sn(Oct)_2$.[40] Our research group has been working on the study of rare-earth catalysts, and developed a series of effective catalysts for the ROP of lactones,[41–45] lactides,[46,47] and cyclic carbonates.[48–50] Recently, we reported the ring-opening copolymerization of 2-methoxy-2-oxo-1,3,2-dioxaphospholane (methyl ethylene phosphate, MEP)[51] or 2-(2-chloroethoxy)-2-oxo-1,3,2-dioxaphospholane (CEP)[52] with CL catalyzed by lanthanum tris(2,6-di-tert-butyl-4-methylphenolate)s ($La(DBMP)_3$), to give poly(ester-phosphoester) random copolymers with high molecular weight and moderate molecular weight distribution.

Meanwhile, cyclic phosphate monomers exhibit good flexibility of their pendant groups based on the pentavalent phosphorus.[53–57] In this work, we designed and synthesized a novel "clickable" cyclic phosphate monomer, 2-(2-propynyloxy)−2-oxo-1,3,2-dioxaphospholane (propynyl ethylene phosphate, PEP) and used it as a comonomer for the ring-opening copolymerization with CL, preparing poly(ester-phosphoester)s with pendant alkyne groups. Then tertiary amines were introduced to the copolymer backbone by "click" chemistry,[58] which was followed by quaternization with various bromoalkanes, resulting



Scheme 1. Synthesis of degradable cationic poly(ester-phosphoester)s via ROP, "click" reaction, and quaternization.

in a series of fully degradable cationic poly(ester-phosphoester)s (Scheme 1). The antibacterial properties of these cationic copolymers have been evaluated by OD600 method.

EXPERIMENTAL

Materials

Toluene, dichloromethane, ethyl ether, triethylamine (TEA), tetrahydrofuran (THF), and *N,N*-dimethylformamide (DMF) were dried over CaH_2 and distilled before use. Propynol (99%; Aladdin, China), ε-Caprolactone (CL, 99%; Acros), and ethylene glycol (99%; Aladdin, China) were distilled under reduced pressure prior to use. Lanthanum tris(2,6-di-tert-butyl-4-methylphenolate)s ($La(DBMP)_3$) was synthesized as we reported formerly.[59] Copper (I) bromide (98%; Aladdin, China) was washed with glacial acetic acid, followed by washing with methanol and ethyl ether, then dried under vacuum and kept under argon atmosphere. Poly(ε-caprolactone) homopolymer (PCL, $M_n = 69{,}700$, PDI = 1.91) was obtained from our previous work.[52] Other reagents were bought from Aladdin and used as received.

Synthesis of 2-(2-Propynyloxy)−2-oxo-1,3,2-dioxaphospholane (PEP)

PEP was prepared according to the similar method described previously.[51] Ethylene glycol (88.7 g, 1.43 mol) was added dropwise slowly to the mixture of PCl_3 (205.9 g, 1.5 mol) and dry CH_2Cl_2 (150 mL) with fiercely magnetic stirring at room temperature. HCl was removed by nitrogen flow and absorbed by a NaOH solution. The mixture was kept overnight and the solvent was evaporated under reduced pressure to get crude 2-chloro-1,3,2-dioxaphosphlane. Without any purification, the crude 2-chloro-1,3,2-dioxaphosphlane was directly dissolved in dry toluene and oxidized by bubbling O_2 at 50°C for 72 h. Then toluene was evaporated, and pure 2-chloro-2-oxo-1,3,2-dioxaphosphlane was prepared by distillation under reduced pressure (84∼88°C/40 Pa, 121.4 g, 57%). To the mixture of propynol (5.6 g, 0.1 mol), dry triethylamine (12.1 g, 0.12 mol), and dry THF (100 mL), 2-chloro-2-oxo-1,3,2-dioxaphosphlane (21.3 g, 0.15 mol) was added dropwise at 0°C. The mixture was stirred at room temperature overnight. The triethylamine hydrochloride was filtered off, and the filtrate was distilled under vacuum to remove the solvent. Then the crude product was washed three times with dry ethyl ether, and 2-(2-propynyloxy)−2-oxo-1,3,2-dioxaphospholane (PEP) was finally recovered by distillation under reduced pressure (100–104°C/40 Pa, 13.3 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.65 (s, 1H, CH≡C), 4.33 (t, 4H, OCH2CH2O), 4.74 (s, 2H, OCH2C≡CH).

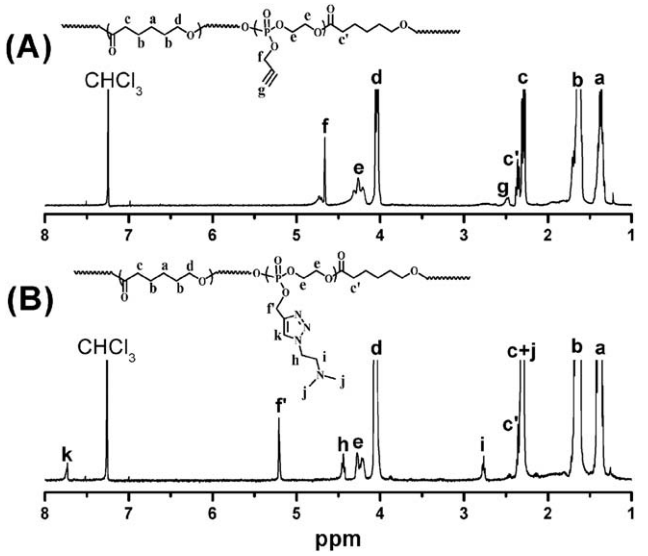


Figure 1. ^{1}H NMR spectra of (A) PCLPEP2 and (B) AMPCLPEP2.

Synthesis of 2-Azido-*N,N*-dimethylethanamine ($DMEAN_3$)

2-Chloro-*N,N*-dimethylethanamine hydrochloride (7.2 g, 0.05 mol) and NaN_3 (3.25 g, 0.05 mol) were dissolved in 50 mL deionized water and then KOH (4.2 g, 0.075 mol) was added. The mixture was stirred for 5 days at room temperature. The resulting mixture was extracted by CH_2Cl_2 and then 2-azido-N,N-dimethylethanamine ($DMEAN_3$) was obtained by evaporating CH_2Cl_2 under reduced pressure. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.29 (s, 6H, $(CH_3)_2N$), 2.52 (t, 2H, CH_2N), 3.36 (t, 2H, N_3CH_2).

Table I. Synthesis of PCLPEPs with Various Chemical Compositions

Sample	PEP : CL (in feed)	PEP : CL[a] (in polymer)	Yield (%)	M_n[b] (kg/mol)	PDI[b]
PCLPEP1	5 : 95	5.5 : 94.5	87.1	29.9	3.03
PCLPEP2	10 : 90	12.7 : 87.3	81.2	21.5	2.54
PCLPEP3	20 : 80	23.6 : 76.4	77.5	9.9	1.90

Polymerization conditions: [PEP + CL]/[La] = 800 (molar ratio), 30°C, 5 h.
[a] Molar fraction of PEP in copolymers, calculated from ^{1}H NMR spectra.
[b] Measured by GPC calibrated with polystyrene standards.

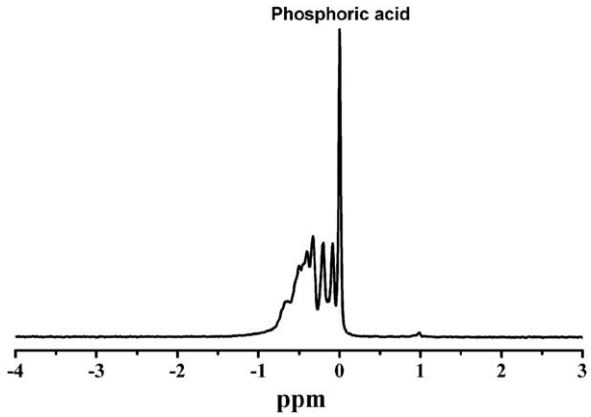


Figure 2. ^{31}P NMR spectrum of PCLPEP2.

Synthesis of Poly(ε-caprolactone-co-propynyl ethylene Phosphate) (PCLPEP)

A typical polymerization was described as follows: 1.62 g of PEP (10 mmol) and 10.27 g of CL (90 mmol) were added to the ampoule under argon atmosphere. 0.125 mmol of La $(DBMP)_3$ was added into the ampoule. The ampoule was put into an oil bath at 30°C for 5 h. The product was dissolved in THF and precipitated into ethyl ether. The copolymer was recovered by filtration, and dried under vacuum to constant weight at room temperature (yield: 9.65 g, 81.2%).

Synthesis of PCLPEP Containing Tertiary Amines (AMPCLPEP) via "Click" Reaction

The copolymer 1.5 g (1.76 mmol of alkyne group), 0.22 g $DMEAN_3$ (1.93 mmol of azide group) were dissolved in argon-purged DMF (20 mL) in an ampoule. CuBr (25.2 mg, 0.176 mmol) and *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA) (30.5 mg, 0.176 mmol) were added in order, and the reaction mixture was degassed by three freeze–pump–thaw cycles, left in argon, and stirred at room temperature overnight. The solution was passed through an alumina column to remove the salts. The AMPCLPEP was recovered by precipitation into cold ethyl ether and dried in vacuum to constant weight (yield: 1.51 g, 88.2%).

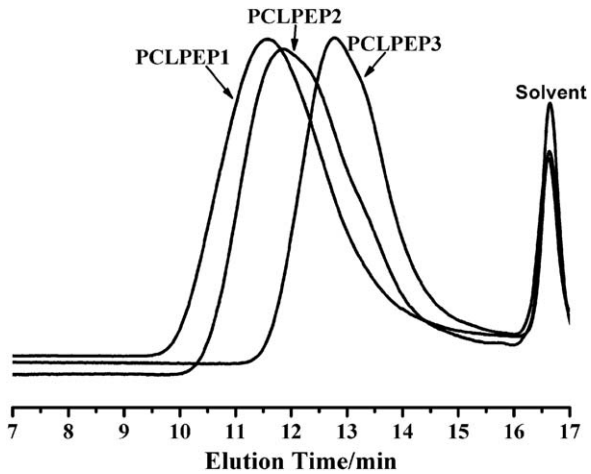


Figure 3. GPC traces of PCLPEPs.

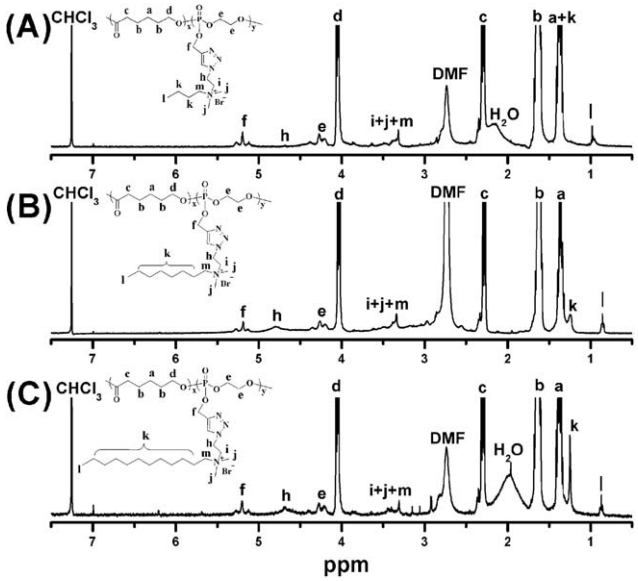


Figure 4. ^{1}H NMR spectra of quaternized AMPCLPEP2: (A) QAMPCLPEP2-B (with 1-bromobutane); (B) QAMPCLPEP2-O (with 1-bromooctane); and (C) QAMPCLPEP2-D (with 1-bromododecane).

Quaternization of AMPCLPEP

A typical quaternization was described as follows, taking 1-bromobutane as an example: 1.0 g copolymer AMPCLPEP (1.05 mmol of amine), 0.48 g 1-bromobutane (3.5 mmol), and 30 mL DMF were added to a 100-mL flask, which was put into an oil bath at 50°C for 3 days. DMF was removed by reduced pressure distillation. The resulting mixture was then dissolved in dichloromethane and precipitated into ethyl ether twice to remove the 1-bromobutane. The final product was dried in a vacuum oven to constant weight (yield: 0.98 g, 81.7%).

Hydrolytic Degradation

The polymer films were prepared by compression molding and cut into 2 × 2 cm^2 pieces (about 200 μm thickness). Each piece was then placed into a capped bottle containing 10 mL phosphate buffer solution (PBS, 0.05 M, pH = 7.4). The bottles were incubated at 37°C. The buffer solution was changed every day. At predetermined time intervals, three parallel samples were withdrawn from the degradation medium, rinsed with distilled water for five times, and then dried to constant weight in vacuum at room temperature. Weight loss was determined gravimetrically by comparing the dry weight remaining at a specific time with the initial weight.

Evaluation of Antibacterial Properties

The antibacterial properties of the copolymers were evaluated according to our previous reports.[60] Briefly, the polymer films (2 × 2 cm^2, about 200 μm thickness) were sterilized with UV light and suspended in 7 mL of bacterial broths in the 6 well plates, and incubated at 37°C with 80 rpm shaking. One milliliter of broths were withdrawn in each well at 0, 3, 6, 9, and 24 h, diluted to 2 mL with LB culture medium and measured three times by a spectrophotometer at 600 nm wavelength to obtain the optical density (OD). Bacterial broth without any polymer was taken as control.

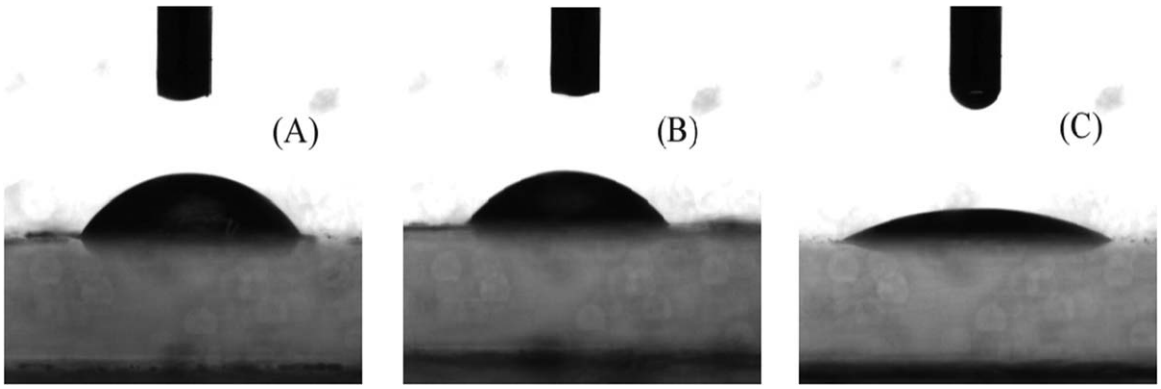


Figure 5. SWCA measurements: (A) QAMPCLPEP2-B, CA = 50.2°; (B) QAMPCLPEP2-O, CA = 43.9°; (C) QAMPCLPEP2-D, CA = 22.8°.

Characterization

^{1}H and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance DMX500 spectrometer in $CDCl_3$ with tetramethylsilane and phosphoric acid (85%) as internal standards, respectively. Gel permeation chromatography (GPC) measurements were performed on a Waters 208 apparatus equipped with Waters 2410 RI detector in THF (1.0 mL/min) at 40°C and calibrated with commercial polystyrene standards. Static water contact angle (SWCA) measurements were obtained by a contact angle system (OCA20, Dataphysics, Germany) equipped with video capture at room temperature. Samples were dissolved in THF with concentration of 10 mg/mL, and dried on the glass slide for 1 day. Each sample was determined 5 times to get the average value.

RESULTS AND DISCUSSION

Syntheses and Characterizations of Polymers

PCLPEP with different compositions were prepared via the ring-opening copolymerization of CL and PEP catalyzed by $La(DBMP)_3$ as shown in Table I. The yields of the polymerizations are around 80%, demonstrating the high catalytic activity of $La(DBMP)_3$. Figure 1(A) shows a typical ^{1}H NMR spectrum of PCLPEP2 (Table I), in which CL and PEP units owned their corresponding signal assignment. The chemical shifts at 2.29 (Hc) and 2.36 (Hc') ppm were assigned to the appearance of CL–CL segment and CL–PEP segment, indicating the random structure of the copolymer. In addition, ^{31}P NMR was employed to characterize the copolymer (Figure 2). There are several peaks in the spectrum, which could be attributed to the different chemical environments of phosphorus atom in the random copolymers. The molecular weights of the copolymers were measured by GPC (Figure 3), in which the unimodal peaks indicates that the obtained products are copolymers rather than mixtures of PCL and PEP homopolymers.

AMPCLPEP containing tertiary amines was prepared by "click" reaction between PCLPEP and $DMEAN_3$. ^{1}H NMR spectrum of the AMPCLPEP2 sample (from PCLPEP2) is shown in Figure 1(B), in which the signals corresponding to $DMEAN_3$ moieties (H^h, H^i, and H^j) could all be observed clearly. Moreover, the characteristic peaks of triazole ring at 7.75 ppm (H^g) was detected and the signal at 4.67 ppm [Hf, in Figure 1(A)] entirely shifts to 5.21 ppm [$H^{f'}$, in Figure 1(B)], demonstrating the completed "click" reaction of alkynyl groups with $DMEAN_3$.

AMPCLPEP2 quaternized with 1-bromobutane, 1-bromooctane, and 1-bromododecane were named as QAMPCLPEP2-B, QAMPCLPEP2-O, and QAMPCLPEP2-D, respectively. Figure 4 presents the ^{1}H NMR spectra of these quaternized polymers. After quaternization, the signals of alkyl protons from bromoalkanes (H^k, H^l, and H^m) appeared. Moreover, the integral areas

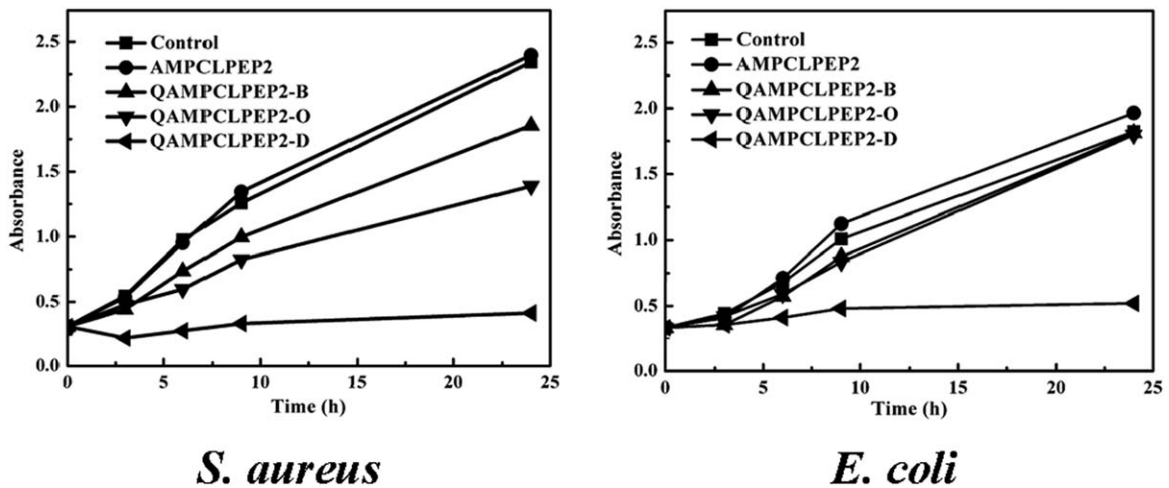


Figure 6. Antibacterial evaluations of QAMPCLPEP2s against *S. aureus* and *E. coli* by OD600 method (Conditions: 37°C, in pH 7.4 bacterial broths).

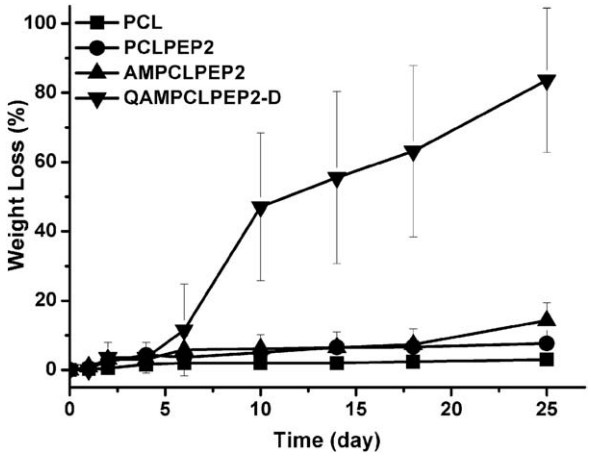


Figure 7. Weight losses of PCL, PCLPEP2 AMPCLPEP2, and QAMPCLPEP2-D as a function of hydrolytic degradation time.

of those protons agree with theoretic values. These facts indicate that the complete quaternization has been achieved and cationic poly (ester-phosphoester)s with well-defined chemical compositions were obtained.

The hydrophilicity of the quaternized polymers were evaluated by SWCA measurements (Figure 5). Due to the hydrophilic PEP units and ammonium moieties, small SWCAs were observed, which even lowered with longer alkyl chain length under the same charge density.

Antibacterial Properties

The antibacterial activity of these cationic poly(ester-phosphoester)s was explored by using optical density (OD) method, against *Staphylococcus aureus* and *Escherichia coli* bacteria, respectively. Figure 6 presents OD values at 600 nm versus exposure time for QAMPCLPEP2s. The OD values of QAMPCLPEP2-D are much less than those of other quaternized polymers as well as control experiment, which were stable, even after 24 h culturing. This phenomenon indicates that QAMPCLPEP2-D has a high rate of killing cells and high antibacterial activity against both gram-negative and gram-positive bacteria. It is well known that the antibacterial activity of QAS is affected mainly by positive charge density and the hydrophobic/hydrophobic interactions between the alkyl substituent and the hydrophobic inner part of the bacterial cell wall.[61] It has been found that QAS with longer alkyl chain exhibits higher antibacterial activity[62] due to the hydrophobic/hydrophobic interactions, which could also be concluded from our results.

Hydrolytic Degradation

Polyesters and polyphosphoesters possess degradability due to the hydrolysis of ester linkages. Figure 7 shows the weight loss profiles versus hydrolytic degradation time for the samples of PCL, PCLPEP2, AMPCLPEP2, and QAMPCLPEP2-D by using buffer solution method. The existence of hydrophilic PEP units and tertiary amines slightly promoted the degradations of PCLPEP2 and AMPCLPEP2, compared with PCL homopolymer. Nevertheless, the quaternized polymer QAMPCLPEP2-D exhibited much higher degradation rate than its unquaternized precursor. The weight loss of 47% was achieved within 10 days for QAMPCLPEP2-D. Then a nearly complete degradation of QAMPCLPEP2-D was observed after 25 days. The more hydrophilic QAS moieties in the side groups could account for the faster degradation of QAMPCLPEP2-D.

CONCLUSIONS

Fully degradable poly(ester-phosphoester)s containing QAS were prepared facilely via ROP, "click" chemistry, and quaternization. OD600 measurements reveal that the copolymer with long alkyl groups in QAS moiety exhibits excellent antibacterial activity against both gram-negative and gram-positive bacteria. Therefore, this cationic poly(ester-phosphoester) holds great potentials for biomedical applications, such as wound dressing, medical device coating, and tissue engineering scaffold.

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