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Contribution of "click chemistry" to the synthesis of antimicrobial aliphatic copolyester

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ABSTRACT

A straightforward strategy is proposed to impart antimicrobial properties to biodegradable poly(oxepan-2-one) (poly(ε-caprolactone) or PCL), which is based on the grafting of pendant ammonium salts by "click" chemistry. First, statistical copolymerization of 3-chlorooxepan-2-one (α-chloro-ε-caprolactone or αClεCL) with oxepan-2-one (ε-caprolactone or εCL) was initiated by 2,2-dibutyl-2-stanna-l,3-dioxepane (DSDOP). In a second step, pendant chlorides were converted into azides by reaction with sodium azide (NaN_3). Finally, quaternary ammonium containing alkynes were quantitatively added to the pendant azide groups of PCL by the copper-catalyzed Huisgen's 1,3-dipolar cycloaddition, which is a typical "click" reaction. An alternative two-step strategy based on the cycloaddition of the amine containing alkyne onto the pendant azides, followed by quaternization turned out to be less efficient. The antimicrobial activity was analyzed by the "shaking flask method" in the presence of *Escherichia coli* (*E coli).*

Keywords: Click chemistry ; aliphatic polyester ; antimicrobial polymer

1. Introduction

Infection by bacteria is quite problematic in many fields, including food packaging and hospital furniture. These micro-organisms are indeed pathogen and thus responsible for many diseases [1]. In order to get rid of them, biocides, i.e., chemicals that inhibit their growth of micro-organisms, have been made available on the market place, as alcohols [2], biguanides [3] and halogen-releasing agents [4]. Recently, cationic agents of the quaternary ammonium type proved to be potential antiseptics and disinfectants [5] for a variety of clinical purposes (e.g., preoperative disinfections and disinfection of non critical surfaces). Salton et al. proposed a five-step mechanism for the antimicrobial action of these agents [6]; (i) adsorption and penetration of the agent into the cell wall; (ii) reaction with the cytoplasmic membrane followed by its disorganization; (iii) leaking of intracellular low molecular weight material; (iv) degradation of the proteins and nucleic acids; and (v) wall lysis caused by autolytic enzymes. However, the activity of all these compounds is temporary and thus requires repeated applications for a long time biocide effect. Therefore, materials, including plastics, endowed with a permanent antimicrobial activity, are a growing sector of the specialty biocides industry. Two types of materials have to be distinguished depending on whether the additive is temporarily trapped within the polymer [7-9] or permanently attached to the chains [10,11]. Dispersion of a low molecular weight biocide, e.g., a heavy metal [7] or silver [8], within a polymer matrix is an example of the first type of materials whose major limitation is the possible migration and release of the antimicrobial. The only way of preventing this undesired effect consists in chemically bonding the active molecule to the matrix. Then, the antimicrobial action relies on the contact between the biocide and the micro-organisms. The permanency of the effect depends of course on the stability of the bonding between the biocide and the polymer. Typical examples are polymers substituted by quaternary ammonium salts [12-15], phosphonium salts [16-18] and pyridinium cations [19,20]. As a rule, these cationic biocides interact with the negatively charged membrane of the bacteria, which is accordingly disrupted and disintegrated. Based on the second strategy, Lenoir et al. prepared quaternized poly(dimethylaminoethyl methacrylate) that exhibited an antimicrobial activity [21]. After use, this material is, however, not degradable, which may be a concern. Therefore, this work aims at reporting on the development of a polymer with a biocide activity during use and elimination by degradation afterwards. This kind of material could find applications in hospital environment, e.g., as fibers in wound dressing.

Poly(oxepan-2-one) (poly(ε-caprolactone) or PCL) is an aliphatic polyester very well known for (bio)degradation. Nevertheless, a major limitation to PCL application is the lack of pendant reactive/functional groups along the chains. The last decade witnessed the synthesis and ring-opening (co)polymerization of ε-caprolactone substituted by a variety of reaction groups, mainly in α- or γ-position [22,23]. In a second step, these reactive groups were involved in derivatization reactions [22], as illustrated by the Michael addition of thiols onto pendant acrylates [24], atom transfer radical addition of terminal alkenes onto pendant chlorides [25], the addition of amines onto ketones [26], and the esterification of pendant hydroxyls by carboxylic acids [27].

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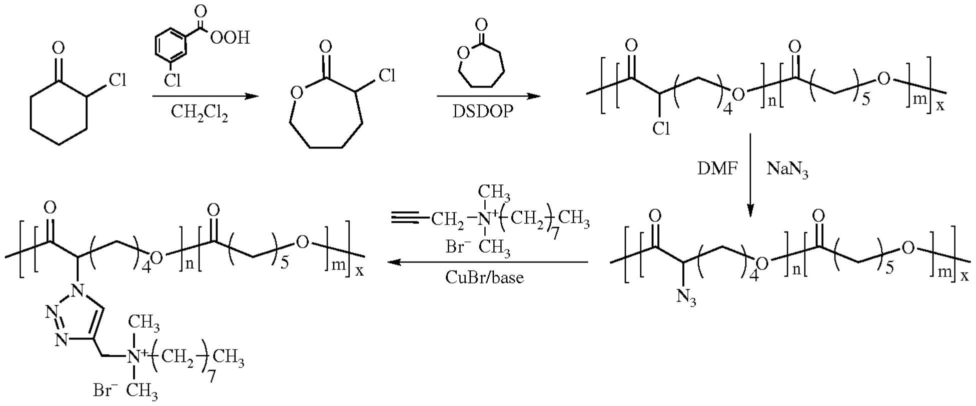
Recently, "click" chemistry, particularly the copper-mediated Huisgen's cycloaddition of azides and alkynes, proved to be a very promising functionalization method of PCL [22,28-30]. The reaction conditions were so mild (low temperature (35 °C) and short reaction time) that the grafting was quantitative, while the polyester chains were not degraded at all. For instance, hydrosoluble polyca-tionic PCL was prepared by the reaction of azide containing PCL with *N,N,N*-triethylprop-2-yn-1-ammonium bromide in the presence of CuI and triethylamine as a catalyst [29,30]. This strategy was used in this work, in order to impart an antimicrobial activity to PCL (Fig. 1). In a preliminary step, oxepan-2-one (ε-caprolactone or εCL) was copolymerized with 3-chlorooxepan-2-one (α-chloro-ε-caprolactone or αClεCL), followed by the reaction of the pendant chlorides with sodium azide. Finally, the pendant azides were involved in the Huisgen's cycloaddition of *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide in a THF/DMF mixture at 35 °C. This covalently modified PCL was actually endowed with a biocidal effect by contact, as demonstrated towards *Escherichia coli* bacteria.

Fig. 1. *General scheme for the preparation of antimicrobial PCL.*



2. Experimental section

2.1. Materials

Toluene (Chem-lab), tetrahydrofuran (THF; Chem-lab), dichloro-methane (CH_2Cl_2; Chem-lab), *N,N*-dimethylformamide (DMF; Aldrich), sodium azide (Aldrich), *N,N*-dimethylprop-2-yn-1-amine (Aldrich), 1-bromooctane (Aldrich), copper(I) bromide (Aldrich), triethylamine (Aldrich) were used as received. Poly(αClεCL-*co*-εCL) copolymers were prepared as reported elsewhere [31]. Copper bromide (Aldrich) was recrystallized in glacial acetic acid for one night. 2,2-Dibutyl-2-stanna-l,3-dioxepane (DSDOP) was prepared as reported by Kricheldorf et al. [32]. The synthesis of αClεCL was also reported elsewhere [31]. Toluene was dried by refluxing over a ben-zophenone-sodium mixture and distilled under nitrogen.

2.2. Typical preparation of a poly(αN_3εCL-co-*εCL)*

Poly(αClεCL-*co*-εCL) (5 g) with 30 mol% of αClεCL (12 mmol of αClεCL) was dissolved in 15 ml of DMF in a glass reactor, followed by the addition of 0.78 g (12 mmol) of NaN_3. The reaction mixture was stirred at room temperature overnight. DMF was evaporated in vacuo, the residual solid was dissolved in 15 ml of toluene, and the insoluble salt (NaCl) was removed by centrifugation (5000 rpm at 25 °C for 15 min). The copolymer was collected by evaporation of the solvent in vacuo.

2.3. Preparation of N,N-*dimethyl*-N-*prop-2-yn-1-yloctan-1-ammonium bromide*

1-Bromooctane (7 g, 36.2 mmol) was added in a glass reactor containing 20 ml of THF, followed by 3.6 g (43.4 mmol) of *N,N*-dimethylprop-2-yn-1-amine. After 2 days at 50 °C, the solvent was evaporated in vacuo at room temperature. The ammonium salt was dissolved in THF and purified by two repeating precipitation in cyclohexane. The final yield was 81%.

^{1}H NMR ($CDCl_3$): δ = 4.8 (m, 2H, C$H_2$$N^+$, B), 3.6 (m, 2H, N^+CH_2, D), 3.4 (m, 6H, 2CH_3, C), 2.9 (m, 1H, C≡C*H*, A), 1.7-1.2 (m, 12H, 6CH_2, E, F), 0.8 (m, 3H, CH_3, G) ppm.

^{13}C NMR ($CDCl_3$): δ = 85 (HC≡C), 82 (HC≡C-CH_2N^+), 77 (HC≡C), 65 (N^+CH_2), 55 (CH_2-CH_2-N^+), 51 ($2CH_3N^+$), 32, 30, 27, 24 and 23 ($5CH_2$), 14 (CH_3) ppm.

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2.4. *Typical cycloaddition reaction of* N,N-*dimethyl*-N-*prop-2-yn-1-yloctan-1-ammonium bromide onto poly(αN_3εCL*-co-*εCL)*

Poly(αN_3εCL-*co*-εCL) (2 g) with 30 mol% of αN_3εCL (4.75 mmol of αN_3εCL) and 1.4 g (5.22 mmol) of *N*,*N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide were added in a glass reactor containing 5 ml of a 50/50 (v/v) THF/DMF mixture. Triethylamine (48 mg, 0.475 mmol) and 68 mg (0.475 mmol) of CuBr were then added, and the reaction mixture was stirred at 35 °C for 2 h. The solution was concentrated in vacuo, and the copolyester was precipitated in distilled water, in order to get rid of the unreacted alkyne, and dried in vacuo.

2.5. *Click cycloaddition of* N,N-*dimethylprop-2-yn-1-amine onto poly(aN_3εCL*-co-*εCL)*

Poly(αN_3εCL-*co*-εCL) (2 g) with 30 mol% of αN_3εCL (4.75 mmol of αN_3εCL) was dissolved in 5 ml of THF in a glass reactor. *N*,*N*-Dime-thylprop-2-yn-1-amine (0.430 g, 5.2 mmol) and 0.09 g of CuBr (0.475 mmol) were added and the solution was stirred at 35 °C for 2 h. The copolyester was precipitated in heptane, filtrated and dried in vacuo.

2.6. *Quaternization of the pendant tertiary amino groups of PCL*

PCL (1 g) with 30 mol% of pendant amine (2.38 mmol) was dissolved in 3 ml of THF in a glass reactor. 1-Bromooctane (0.55 g, 2.86 mmol) was added and the solution was stirred at 50 °C overnight. The copolyester was collected by precipitation in heptane.

2.7. *Antimicrobial assessment (shake flask method)*

A freeze-dried ampoule of *E. coli* (DH5α) was opened, the culture was picked up with a micropipette and added in 2 ml of a nutrient broth (Luria Bertani) (composition for 1 Lof nutrient broth: l0 g bac-totryptone, 5 g of extract of yeast, and sodium chloride), followed by incubation (Incubator shaker model G25; New Bruswick; scientific Co. INC; Edison, New Jersey, USA) at 37 °C overnight. Then 200 µL of the culture was added in 100 ml of the nutrient broth and the bacterial culture was incubated at 37 °C for 4 h. At this stage, the culture of *E. coli* contained approximately 10^8 cells/ml (absorbance at 600 nm = 0.6) and was used for the anti-bacterial test. In a 25 ml flask, a sample of UV sterilized copolyester was dispersed in 9 ml of sterile saline water mixture (8.5 g of sodium chloride in 1 Lof water milliQ in a "Schott" bottle followed by sterilization at 121 °C for 20 min). Bacterial culture (1.0 ml) that contained $\pm 10^8$ cells/ml was then added to this solution to reach the desired concentration ($\pm 10^7$ cells/ml) and the temperature of the flask was balanced at 37 °C. A blank solution was prepared without copolyester by adding 1.0 ml of the same culture to 9 ml of sterile saline water. At regular time intervals, 100 µL samples were picked out, decimal serial dilution (until 10^5) was carried out by mixing 100 µL with 900 µL of sterile saline water. Then, the surviving bacteria were counted by the spread plate method. Decimal dilutions (100 µL) were spread on a Petri dish that contained LB agar. The Petri dishes were incubated at 37 °C overnight. After incubation, the colonies were counted.

2.8. *Characterization techniques*

Size exclusion chromatography (SEC) was carried out in THF at 45 °C at a flow rate of 1 ml/min using a SFD S5200 autosampler liquid chromatograph equipped with a SFD refractometer index detector 2000. Columns of PL gel of 5 µm (10^5, 10^4, 10^3 and 100 Å) were calibrated with polystyrene or PEO standards. Size exclusion chromatography (SEC) was carried out in DMF at 40 °C at a flow rate of 1 ml/min using a Water 600 autosampler liquid chromatograph equipped with a differential refractometer index detector. Columns of Waters gel of 5 µm (10^5,10^4, 500 and 100 Å) were calibrated with polystyrene standards. ^{1}H NMR spectra were recorded in $CDCl_3$ at 400 MHz in the FT mode with a Brucker AN 400 apparatus at 25 °C. Infrared spectra were recorded with a Perkin-Elmer FT-IR 1720X. The samples were prepared by slow evaporation of a copolymer solution in THF, onto NaCl windows. Thermal gravimetric analysis (TGA) was carried out with a TA TGA Q500 apparatus. Differential scanning calorimetry (DSC) was carried out with a TA DSC Q100 thermal analyzer calibrated with indium. Glass transition and melting temperatures were measured, after a first cooling (-80 °C) and heating (100 °C) cycle. Thermograms were recorded during the second heating cycle at 10 °C/min.

3. Results

3.1. *Synthesis of poly(αN_3εCL*-co-*εCL) copolymers*

Two poly(αN_3εCL-*co*-εCL) copolymers with 30 and 50 mol% of αN_3εCL were prepared as schematized in Fig. 1 and detailed in Section 2. The molecular parameters are reported in Table 1.

Table 1 *Molecular characteristic features of poly(αN_3εCL*-co-*εCL)*

$F_{\alpha N3\varepsilon CL}$ (mol%)	M_n (g/mol) (^{1}H NMR)	M_n (g/mol) (SEC)	M_w/M_n (SEC)
51	23,000	22,000	1.5
26	17,000	19,000	1.5

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*3.2. Derivatization of the pendant azide groups of poly(αN_3εCL-*co*-εCL) into ammonium groups*

A first route towards quaternary ammonium containing PCL consists in grafting an alkyne substituted by a tertiary amine onto the pendant azide groups of poly(αN_3εCL-*co*-εCL) by the Huisgen's cycloaddition, followed by the quaternization of the amine. Previously, *N,N*-dimethylprop-2-yn-1-amine was successfully grafted onto poly(αN_3εCL-*co*-εCL) without any degradation of the polyester chain [29,30]. In this work, 1-bromooctane was used as the quaternization agent, because ammonium cations with one chain of eight carbon atoms and with a chloride or a bromide counterion are known for the higher anti-bacterial activity [33,34]. Accordingly, *N,N*-dimethylprop-2-yn-1-amine was cycloadded onto 30 mol% of αN_3εCL containing poly(αN_3εCL-*co*-εCL), in the presence of 10 mol% of CuBr in THF at 35 °C, as reported elsewhere [29]. The experimental results are reported in Table 2.

Tertiary amine containing PCL was then reacted with an excess of 1-bromooctane in THF at 50 °C overnight. After precipitation in heptane, the copolyester was analyzed by ^{1}H NMR. The coexistence of resonances at 7.7 and 8.6 ppm, assigned to the proton of the triazole ring substituted by the non-quaternized and quaternized tert-amine, respectively, was the evidence of partial quaternization, the yield being actually 76%.

Table 2 *Cycloaddition of* N,N-*dimethylprop-2-yn-1-amine onto poly(αN_3εCL*-co-*εCL)*

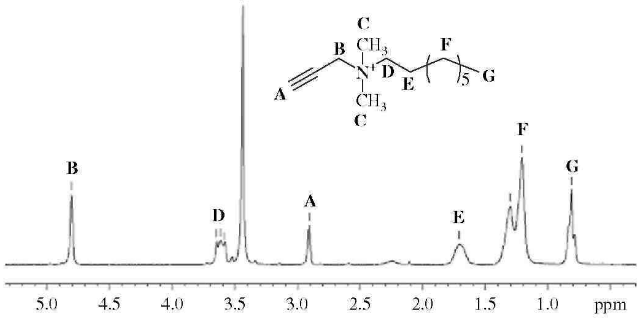
	$F_{\alpha N3\varepsilon CL}$ or F_{amine} (^{1}H NMR) (mol%)	M_n (g/mol) (SEC in DMF)	M_w/M_n (SEC in DMF)
Poly(αN_3εCL-*co*-εCL)	26	60,000	1.3
PCL-tertiary amine	26	64,000	1.3

3.3. Cycloaddition of N,N-*dimethyl*-N-*prop-2-yn-1-yloctan-1-ammonium bromide onto poly(αN_3εCL*-co-*εCL)*

The cycloaddition of an ammonium containing alkyne onto the pendant azide groups of poly(αN_3εCL-*co*-εCL) is a more straightforward route towards the targeted cationic PCL. *N,N*-Dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide was first synthesized by quaternization of *N,N*-dimethylprop-2-yn-1-amine by 1-bromooctane in THF at 50 °C for 2 days. ^{1}H NMR analysis of the crude reaction product showed that the quaternization yield was 89%. The expected *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide was purified by precipitation in cyclohexane, in which the unreacted 1-bromooctane was soluble. The structure of this ammonium salt was confirmed by ^{1}H NMR (Fig. 2).

N,N-Dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide was then reacted with the pendant azides of poly(αN_3εCL-*co*-εCL) containing 30 and 50 mol% of αN_3εCL. This 1,3-dipolar Huisgen's cycloaddition is commonly catalyzed by CuI, which is known as a bio-cide and could accordingly distort the antimicrobial testing [35]. The "click" reaction was therefore catalyzed by CuBr instead of CuI. Accordingly, poly(αN_3εCL-*co*-εCL) was reacted with 110 mol% of *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide, 10 mol% of triethylamine and 10 mol% of CuBr in THF at 35 °C. After few minutes, the copolyester started to precipitate as result of the grafting of cationic groups that decreased the solubility in THF. Upon the addition of 2 ml of DMF, the homogeneity of the reaction medium was restored. The progress of the reaction was monitored by IR spectroscopy. Indeed, the adsorption intensity by the azide at 2106 cm^{-1} decreased, whereas that one by the triazole ring at 1660 cm^{-1} increased in parallel. After 2 h, the IR analysis showed that the reaction was complete. The copolymer was then precipitated in water, in order to get rid of the small excess of the ammonium salt, and analyzed by ^{1}H NMR (Fig. 3).

Fig. 2. *^{1}H NMR spectrum of* N,N-*dimethyl*-N-*prop-2-yn-1-yloctan-1-ammonium bromide.*



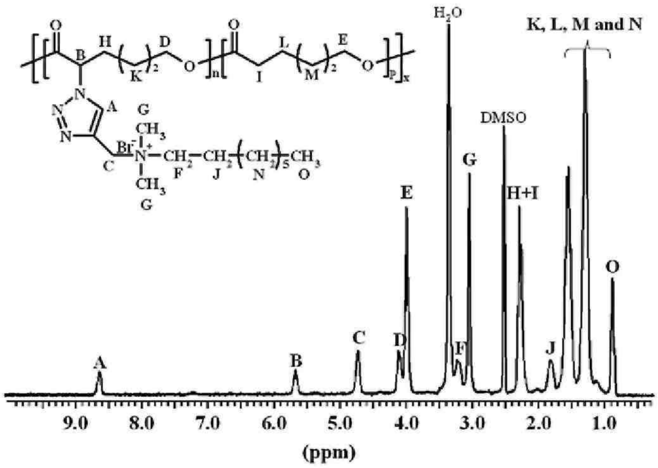
The ^{1}H NMR spectrum confirmed that the cycloaddition was quantitative. Indeed, the resonance at 3.8 ppm typical of CH-N_3 disappeared completely and was replaced by a new resonance at 8.6 ppm characteristic of the proton of the triazole ring. The assignment of the other resonances is shown in Fig. 3. The molar fraction of the pendant ammonium cations, $F_{ammonium}$, was 27 mol% as calculated by Eq. (1), which is in very close agreement with the molar fraction of the precursor αN_3εCL groups (26 mol%). The same conclusion is held for the copolyester with 50 mol% of αN_3εCL

$$F_{\text{ammonium}} = \frac{I_C}{I_D + I_E} \times 100 \qquad (1)$$

The cycloaddition of an alkyne substituted by an ammonium cation onto poly(αN_3εCL-*co*-εCL) is not only a straightforward but also a very effective strategy to cationic PCL.

It must be noted that the SEC analysis of this positively charged PCL is quite a problem because of disturbing adsorption during elution in an organic solvent and insolubility in water.

Fig. 3. *^{1}H NMR spectrum of PCL containing 30 mol% of ammonium bromide.*



3.4. Thermal properties of functional PCL

PCL containing 30 mol% of chlorine, azide and tertiary amine is semi-crystalline, with quasi the same melting temperature (32±1 °C; Table 3). T_g is also comparable, with a slightly higher value when the tertiary amine is the substituent (Table 3). In contrast, PCL containing 30 mol% of quaternary ammonium is amorphous. Fig. 4 compares the TGA profiles for the different copolyesters. Clearly, the thermal stability and the degradation mechanism depend on the functional group, the lower stability being imparted by the *tert*-amine substituent.

Fig. 4. *TGA curves of the PCL substituted by 30 mol% of different functional groups.*

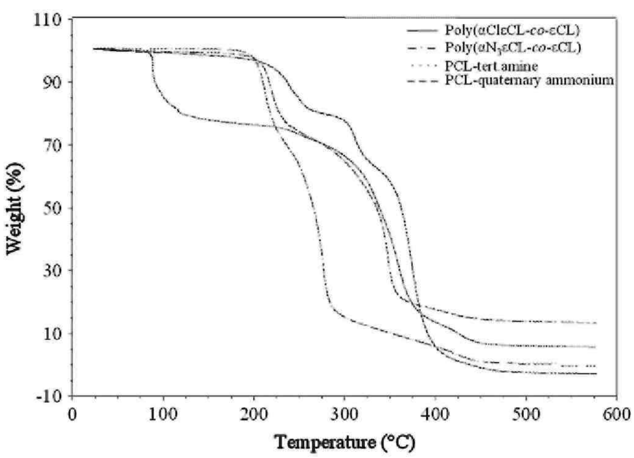


***Table 3** T_g and T_m of the PCL substituted by 30 mol% of different functional groups*

	T_g (°C)	T_m (°C)
Poly(αClεCL-*co*-εCL)	-61	33
Poly(αN_3εCL-*co*-εCL)	-59	31
PCL-tertiary amine	-53	32
PCL-quaternary ammonium	-28	-

3.5. Antimicrobial activity

The dynamic shake flask method was used to assess the ability of the herein reported copolyesters to kill bacteria. In these experiments, a sample of *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide and each of the functional copolyesters, respectively, were shaken with 10 ml of a bacterial suspension (10^8 cells/ml), for 20, 60 and 120 min at 37 °C. The viable cells in the suspension were counted after dilution, and kept for overnight incubation on agar plates. Bacterial suspension (10 ml) without copolyester was also analyzed as a reference.

The antimicrobial activity of *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide was first determined. According to Kanazawa et al., a quaternary ammonium containing polymer showed a biocidal activity in water, when the biocide concentration was 3.5 x 10^{-4} M [36]. Therefore, 1 mg (0.035 mmol) of *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1 -ammonium bromide was added to 10 ml of the bacterial solution. However, 2 h later, no biocide effect was detected, consistent with the observation by Kawabata et al. that ammonium cations were less active in the monomeric form than in the polymeric one, at the same concentration [37]. Expectedly, high biocidal activity was reported when the concentration was increased up to 17.5 x 10^{-3} M (Fig. 5).

In the next step, the inhibition of growth of *E. coli* by cationic PCL was investigated. For sake of comparison, the same concentration of ammonium used by Kanazawa et al. was also used in this work. Therefore, 2.44 mg of PCL with 30 mol% of ammonium (0.003 mmol) were added to 10 ml of the bacterial solution. Data in Fig. 6 show the absence of biocidal effect at this concentration. The reason might be due to the insolubility of the copolyester in water, such that the majority of the ammonium cations was not available to contact with bacteria. In line with this tentative explanation, the amount of copolyester was increased until a biocide effect was observed. At least 50 mg (0.065 mmol) was needed to observe a moderate biocidal effect. A further increase of up to 126 mg of the copolyester (0.164 mmol) in 10 ml of the bacterial solution allowed all the bacteria to be killed within 1 h. Remarkably, PCL with 30 mol% of quaternary ammonium was more active than *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide, at the same ammonium concentration, although the monomeric ammonium was soluble in water in contrast to the polymeric counterpart. For instance, 50 mg (0.175 mmol) of *N,N*-dimethyl-*N*-prop-2-yn-1-yloctan-1-ammonium bromide was unable to kill bacteria within 2 h in contrast to 126 mg of cationic PCL (0.163 mmol) that killed all the bacteria within 1 h. This observation is thus in qualitative agreement with the observations reported by N. Kawabata et al. about the comparative antimicrobial activity of monomeric and polymeric ammonium species [37].

***Fig. 5.** Plots of log(E. coli survivors) versus exposure time towards no (○), 1 mg (Δ), 10 mg (□), 50mg (◊) of* N,N-*dimethyl*-N-*prop-2-yn-1-yloctan-1-ammonium bromide.*

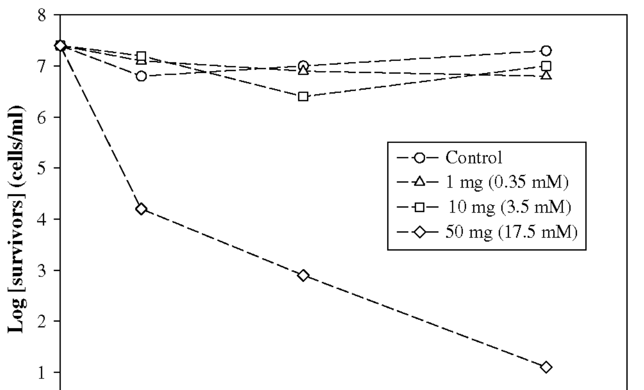


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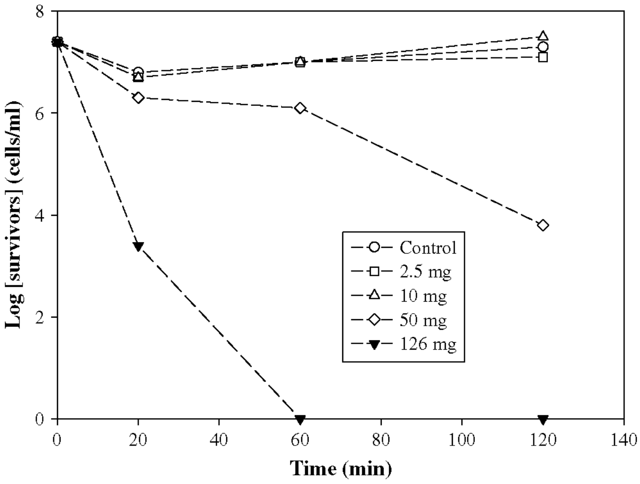
Fig. 6. *Plots of log(E. coli survivors) versus exposure time for cationic PCL (30 mol% of dimethyoctylammonium) no (○); 2.5 mg (□);10 mg (Δ); 50 mg (◊) and (▼) 126 mg.*

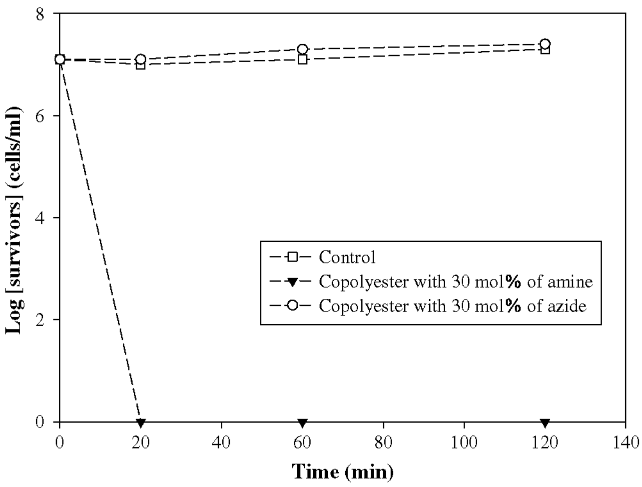


In order to confirm that the antimicrobial activity results from the contact of the bacteria with the copolyester and not from ammonium salts that would be released by hydrolysis of the polyester chains, the solution collected after the antimicrobial test, was analyzed by UV. No UV absorption characteristic of a triazole substituted ammonium was observed. The reported biocidal effect is thus a contact effect between the copolyester and bacteria.

In order to check that the copolyester and not copper catalyst residues is at the origin of the biocide effect, 1 mg (0.005 mmol) of copper iodide, the highest possible contamination, was dissolved into 10 ml of a bacteria solution. After 120 min of contact, 100 µL of this solution was spread on a Petri dish that contained LB agar. No biocide effect was detected after an overnight incubation. Consistently, the antimicrobial activity of the copolyesters cannot be accounted for by residues of the copper catalyst.

One of the last questions to be addressed is to know to which extent the quaternization of tertiary amine substituents is needed for a polymer to exhibit an antimicrobial activity. In this respect, Igna-tova et al. reported that non-quaternized pory(dimethylamino methacrylate) was active although less than the quaternized version [38]. In this work, 33 mg of PCL with 30 mol% of tertiary amine (0.065 mmol of tertiary amine) were added to 10 ml of the bacteria suspension. A biocidal activity was also observed, all the bacteria being killed within 20 min (Fig. 7). This anti-bacterial activity was surprisingly higher than that one of the cationic counterpart, which needed 60 min for all the bacteria to be killed although the amount of ammonium (0.163 mmol) was higher than the tertiary amine used in the test (0.065 mmol).

Fig. 7. *Plots of log(E. coli survivors) versus exposure time for the control (□) and the copolyester containing 30 mol% of azide (○) and tertiary amine (▼).*



This discrepancy with the previous work by Ignatova et al. is only apparent because PCL with 30 mol% of quaternary ammonium was sticking on the bottom of the flask in contrast to PCL with 30 mol% of tertiary amine that was finely dispersed in the bacterial solution and thus offered a much larger surface area of contact with bacteria and accordingly a higher biocidal activity. Although the quaternized and non-quaternized tertiary amine containing PCL were compared at the same nominal concentration of ammonium and tertiary amine, respectively, no reliable conclusion about the biocidal activity can be drawn, because of a completely different aggregation state of the copolyester chains in water.

Since sodium azide is a common disinfectant for water, it was important to test the effect of the azide containing PCL against *E. coli.* So, 28 mg (0.066 mmol) of poly($\alpha N_3 \varepsilon$CL-*co*-εCL) with 30 mol% of $\alpha N_3 \varepsilon$CL were added to 10 ml of the bacterial solution. No biocidal effect was, however, observed after 2 h of contact.

Finally, it might be argued that the positive anti-bacterial tests result from the absorption of the bacteria on the polymer surface, without being actually killed. In order to evaluate the validity of this hypothesis, the cationic PCL was separated from the bacterial solution at the end of the test and transferred into 10 ml of nutrient broth. After one night of incubation at 37 °C, the solution remained clear consistent with a negligible growth of bacteria. This observation gave credit to the non-absorption of living bacteria onto the polymer surface and thus to the biocidal activity of the cationic PCL.

4. Conclusions

"Click" chemistry is a very effective route for the grafting of ammonium cations onto PCL previously modified by pendant azides, the purpose being to impart anti-bacterial properties to this biodegradable aliphatic polyester. Remarkably, the one-step grafting of alkynes substituted by an ammonium salt was more efficient than the two-step grafting of alkynes bearing a tertiary amine followed by quaternization. Moreover, as pointed out in previous papers, the "click" Huisgen's cycloaddition has the major advantage of being carried out under very mild conditions that preserve the length of the polyester chains. The biocidal effect of PCL grafted by ammonium cations was established by the 'shake flask method'. Moreover, it was shown that quaternization of the pendant tertiary amines was not mandatory for PCL to be endowed with a substantial biocidal effect. One main objective of this work was to develop a polymer which exhibits a permanent biocide activity during use and degrade afterwards. Although PCL is very well known for its biodegradability, a detailed investigation of the biodegradability of the ammonium containing polyesters synthesized in this work is mandatory and is under current investigation in our laboratory. This work is beyond the scope of this paper and will be reported elsewhere in the near future. The thermodynamic miscibility with other (co)polymers, such as ABS and PVC, is also a remarkable property of PCL. The next step of this work will investigate whether these miscible (co)polymers can be made antimicrobial by blending with or coating by cationic PCL or PCL-*b*-cationic PCL copolymers.

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