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Amphiphilic copolymers of ε-caprolactone and γ-substituted ε-caprolactone. Synthesis and functionalization of poly(D,L-lactide) nanoparticles

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Abstract

Fully biodegradable and surface-functionalized poly(D,L-lactide) (PLA) nanoparticles have been prepared by a co-precipitation technique. Novel amphiphilic random copolyesters P(CL-co-γXCL) were synthesized by controlled copolymerization of ε-caprolactone and ε-caprolactone substituted in the γ-position by a hydrophilic X group, where X is either a cationic pyridinium (γPyCL) or a non-ionic hydroxyl (γOHCL). Nanoparticles were prepared by co-precipitation of PLA with the P(CL-co-γXCL) copolyester from a DMSO solution. Small amounts of cationic P(CL-co-γPyCL) copolymers are needed to quantitatively form stable nanoparticles *(ca.* 10 mg/100 mg PLA), although larger amounts of non-ionic P(CL-co-γOHCL) copolymers are needed (≥12.5 mg/100 mg PLA). Copolymers with a low degree of polymerization *(ca.* 40) are more efficient stabilizers, probably because of faster migration towards the nanoparticle-water interface. The nanoparticle diameter decreases with the polymer concentration in DMSO, e.g. from *ca.* 160 nm (16 mg/ml) to *ca.* 100 nm (2 mg/ml) for PLA/P(CL-co-γPyCL) nanoparticles. Migration of the P(CL-co-γXCL) copolyesters to the nanoparticle surface was confirmed by measurement of the zeta potential, i.e. *ca.* +65 mV for P(CL-co-γPyCL) and -7 mV for P(CL-co-γOHCL). The polyamphiphilic copolyesters stabilize PLA nanoparticles by electrostatic or steric repulsions, depending on whether they are charged or not. They also impart functionality and reactivity to the surface, which opens up new opportunities for labelling and targeting purposes.

Key words: Nanoparticles ; controlled ring-opening polymerisation ; amphiphilic copolyesters ; surface properties.

INTRODUCTION

Aliphatic poly(α-hydroxy acid)s, such as polylactide, polyglycolide, poly(ε-caprolactone), and their copolymers, are known for their unique combination of biocompatibility, (bio)degradability, and good mechanical properties, making them extremely useful for the design of drug delivery systems, resorbable implants, and scaffolds for tissue engineering. However, the lack of reactive sites along the polymeric backbone is a severe limitation whenever specific molecules have to be attached to the chains, e.g. fluorescent probes, immuno-markers, targeting moieties, bioadhesion promoters, etc. At best, aliphatic poly(α-hydroxy acid)s are easily capped by a functional group at one or both chain end(s), depending on the polymerization mechanism [1-3]. Because the content of the end-groups is directly dependent on the molecular weight, it is usually too small for many applications. Two approaches have been proposed to tackle this problem: chemical modification of preformed polyesters and (co)polymerization of functional monomers. The first strategy is illustrated by the metallation of poly(ε-caprolactone) (PCL) in the α-position of the ester carbonyl, followed by reaction with appropriate electrophiles [4]. The control of this reaction is, however, very sensitive because of the occurrence of transesterification reactions, which affect the molecular weight and polymolecularity of the chains. The second strategy is based on the copolymerization of lactide and ε-caprolactone with functionalized cyclic monomers, e.g. *N*-(carbobenzoxy)-L-lysine *N*-carboxyanhydride [5], phenylmethyl 2-(6-methyl-2,5-dioxo-3-morphonyl) ethyl ether [6], and ε-caprolactone substituted by hydrophobic [7] or hydrophilic groups [8, 9]. Some of us have reported on the controlled synthesis of ε-caprolactone γ-substituted by a protected hydroxyl [10-12], a bromide [13, 14], and a protected carboxylic acid [15]. ε-Caprolactone with an inner double bond [16] and an intracyclic ketone [17] have also been synthesized. These functional groups can be further derivatized into other desirable groups by classical organic reactions [11-13, 16-18]. γ-Functional ε-caprolactones have been homopolymerized and copolymerized with ε-caprolactone by a controlled coordination-insertion mechanism initiated by aluminum or tin alkoxides. The molecular weight is controlled by the monomer/initiator molar ratio and the molecular weight distribution is usually narrow. These new (co)polyesters have significantly increased the range of thermal

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and hydrolytic stability of neat PCL. Their biocompatibility is currently being evaluated.

The surface functionalization of colloidal drug carriers is also a very important, although challenging, issue. Surface chemistry is one of the main parameters that influences the way that nanoparticles are taken up across barriers or intracellularly [19-22]. Their bioadhesion and targeting also rely on the availability of selected functions or molecules on the surface [23-25]. As a rule, only a few studies have been devoted to the covalent binding [26-28], rather than to the adsorption of molecules of interest, to aliphatic poly(*α*-hydroxy acid) nanoparticles.

The aim of this work was to synthesize novel amphiphilic random copolymers of *ε*CL and hydrophilic *γ*-substituted *ε*CL which are able to stabilize and functionalize the surface of poly(D,L-lactide) (PLA) nanoparticles prepared by a co-precipitation technique, i.e. rapid addition of an aqueous phase to a solution of PLA/amphiphilic copolymer in DMSO [28, 29]. The amphiphilic copolymer migrates towards the nanoparticle-water interface; the coalescence of the nanoparticles is prevented by the barrier formed by the hydrophilic units; and the hydrophobic segments contribute to the anchoring of the copolymer to the PLA nanoparticles. In a previous paper, poly(methylmethacrylate-co-methacrylic acid) copolymers were used, yielding negatively charged nanoparticles [28]. In this work, these non-degradable polyamphiphiles have been replaced by potentially biodegradable copolymers of *ε*CL. Two types of hydrophilic substituents, which are relevant to drug delivery, were investigated: cationic pyridinium bromide and non-ionic hydroxyl. Random copolymers of various degrees of polymerization and contents of hydrophilic units were synthesized by controlled ring-opening polymerization initiated by aluminum alkoxide. Their ability to promote the formation of PLA nanoparticles of different sizes and surface functionalization has been demonstrated.

MATERIALS AND METHODS

Materials

ε-Caprolactone (Aldrich) was dried over CaH_2 (Aldrich) and distilled under reduced pressure before use. Aluminum triisopropoxide (Aldrich) was purified by distillation under reduced pressure, dissolved in dry toluene, and the solution was titrated by complexometry of Al with EDTA, as reported elsewhere [30]. Toluene (Lab-Scan) was dried over CaH_2 and tetrahydrofuran (Lab-Scan) was dried over sodium in the presence of benzophenone ; both were distilled under nitrogen before use.

3-Chloroperoxybenzoic acid (mCPBA) (Aldrich), cyclohexane-l,4-diol (Aldrich), sodium dichromate ($Na_2Cr_2O_7$) (Aldrich), sulphuric acid (98%; Aldrich), chlorotriethylsilane (Et_3SiCl) (Aldrich), *N*-methylmorpholine (NMM) (Aldrich), pyridine (Sigma), 40% fluorhydric acid (Riedel de Haën), and pyridinium chlorochromate (PCC) (Janssen Chimica) were used as received.

Heptane (Lab-Scan), dimethyl formamide (Aldrich), diethyl ether (Riedel de Haën), pentane (Lab-Scan), acetonitrile (Aldrich), dichloromethane (Lab-Scan), $CDCl_3$ (Cambridge Isotope Laboratories), and dimethyl sulfoxide (Merck) were used as received. Jones reagent was prepared by careful addition of 98% sulphuric acid (33 ml) to a solution of sodium dichromate (0.15 mol; 39 g) in water (134 ml).

Poly(D,L-lactide) from Boehringer-Ingelheim (R-206) was analysed by size exclusion chromatography (SEC) in chloroform with a universal calibration curve (M_n = 48 000 and M_w/M_n = 1.8).

Synthesis of P(CL-co-γPyCL)

γ-Bromo-*ε*-caprolactone (*γ*BrCL) was synthesized from 7-oxabicyclo[2.2.1]heptane (Aldrich, 48% aqueous), as reported elsewhere [14] (Scheme 1). It was copolymerized with *ε*-caprolactone (*ε*CL) (Scheme 2a) in a previously flamed glass reactor under nitrogen. In a typical copolymerization (copolymer A in Table 1), 1 g of *γ*BrCL (5.2×10^{-3} mol) was dried by repeated azeotropic distillation of toluene and finally 5.2 ml of *ε*CL (4.7×10^{-2} mol) was added and the solution was thermostated at 0°C. 4.3 ml of an aluminum triisopropoxide ($Al(O^iPr)_3$ solution in anhydrous toluene (0.3 mol/l) was added. After 2 h, the polymerization was stopped by the addition of an excess of 1 N HCl and the P(CL-co-*γ*BrCL) copolymer was recovered by precipitation in cold heptane and dried *in vacuo* (5.95 g; conversion: 99%). Four copolymers were synthesized, with a monomer/initiator molar ratio of 40 and 150, and a molar fraction of *γ*BrCL of 0.1 and 0.3, respectively (Table 1).

The *γ*BrCL repeating units were further modified by quaternization of an excess of pyridine at 50°C for 48 h [13] (Scheme 2a). For instance, 1 g of P(CL-co-*γ*BrCL) (copolymer A) was dissolved in 10 ml of pyridine and thermostated at 50°C for 48 h. Pyridine was eliminated under vacuum and the P(CL-co-*γ*PyCL) copolymer was purified by repeated precipitation from tetrahydrofuran (THF) into heptane. Residual pyridine was eliminated by dialysis of a copolymer solution in DMF against dimethylformamide/water mixtures of increasing content of water. After lyophilization, the P(CL-co-*γ*PyCL) copolymer was dried under vacuum until a constant weight was obtained (1.045 g; yield: 100%).

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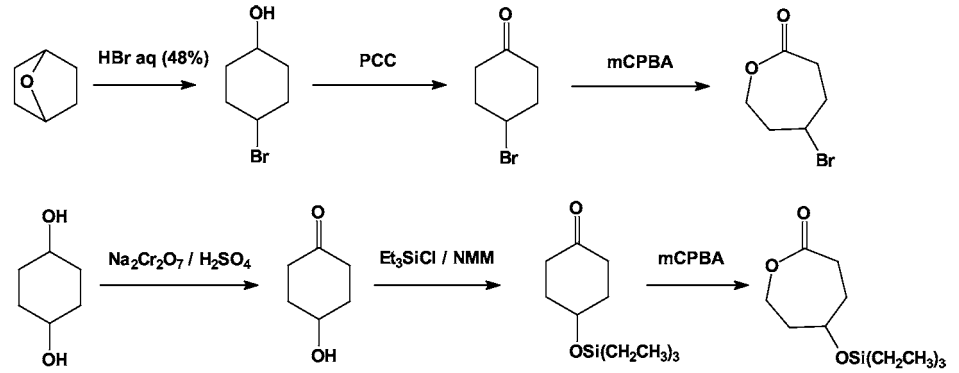
Scheme 1. *Synthesis of γ-bromo ε-caprolactone (γBrCL) and γ-triethylsilyloxy ε-caprolactone (γEt_3SiOCL), where PCC, mCPBA, Et_3SiCl and NMM stand for pyridinium cholorochromate, 3-chloroperoxybenzoic acid, chlorotriethylsilane and N-methylmorpholine, respectively.*



Scheme 2. *Synthesis of P(CL-co-γPyCL) and P(CL-co-γOHCL) copolymers. Al(O^{i}Pr)$_3$ stands for aluminum triisopropoxide.*

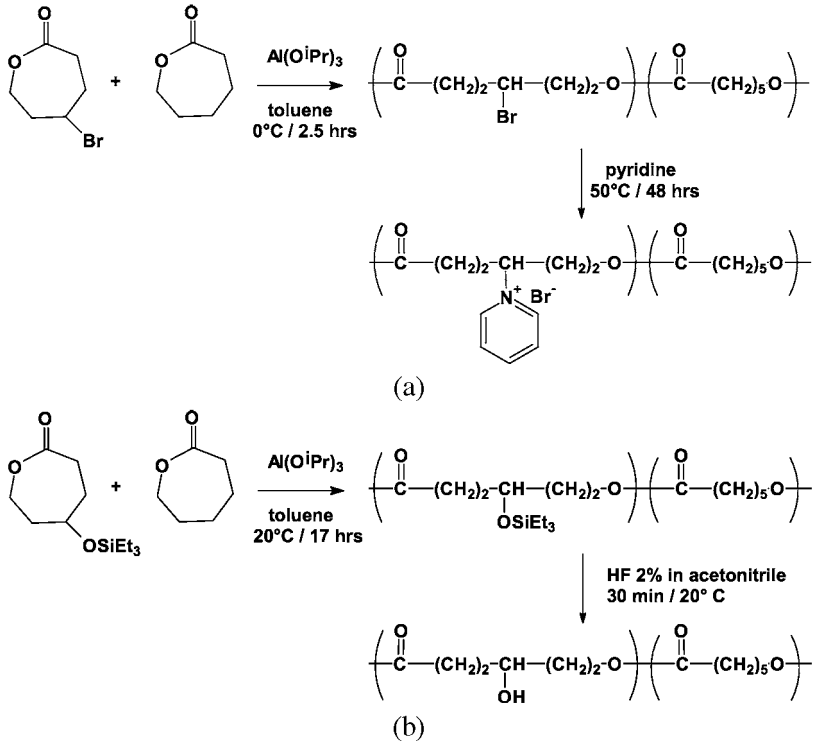


Table 1. *Molecular characteristics of the P(CL-co-γBrCL) and P(CL-co-γPyCL) copolymers*

Ref.	DP_{th} [a]	$F_{Br,th}$ [b] (mol%)	$M_{n,th}$ (× 10^{-3})	P(CL-co-γBrCL)					P(CL-co-γPyCL)		
				$F_{Br,NMR}$ (mol%)	DP_{NMR}	$M_{n,NMR}$ (× 10^{-3})	$M_{n,SEC}$ (× 10^{-3})	$M_w/M_{n,SEC}$	$F_{Py,NMR}$ [c] (mol%)	$F_{Br,NMR}$ (mol%)	DP_{NMR}
A	40	10	4.8	10	51	6.2	11.9	1.12	7	3	53
B	40	30	5.4	28	46	6.0	11.0	1.17	18	10	50
C	150	10	18.1	10	167	20.2	38.0	1.11	10	0	180
D	150	30	20.2	28	140	18.6	31.1	1.11	28	0	168

[a] Theoretical degree of polymerization = $[M]_0/[I]_0$.
[b] Molar content of γ BrCL in the feed. [c] Molar content of γ PyCL units in the copolymer.

Synthesis of P(CL-co-γOHCL)

γ-Triethylsilyloxy-ε-caprolactone (γEt_3SiOCL) was synthesized from cyclohexane-l,4-diol, as detailed elsewhere [12] (Scheme 1). Briefly, 50 g of 4-hydroxy-cyclohexanone (0.43 mol) was prepared by oxidation of cyclohexane-l,4-diol by the Jones reagent (39 g of $Na_2Cr_2O_7$ in 20% H_2SO_4) in acetone. 72 g of chloro-triethylsilane (0.48 mol) was added dropwise to a solution of 36.5 g of 4-hydroxy-cyclohexanone (0.32 mol) and 49 g of *N*-methylmorpholine (0.48 mol) in 400 ml of anhydrous THF at room temperature. After reaction for 17 h, the solution was diluted by diethyl ether, filtered, washed with a saturated NaCl solution followed by water, and finally dried over sodium sulphate, filtered, and evaporated under vacuum. 35.4 g of pure γ-triethylsilyloxy-cyclohexanone was recovered by distillation under vacuum (boiling point: 68°C at 10^{-2} mmHg; yield: 50%). 172.6 g of 3-chloroperoxybenzoic acid (0.32 mol) was added to a solution of 35.4 g of γ-triethylsilyloxycyclohexanone (0.16 mol) in 350 ml of dichloromethane. After reflux for 18 h, the mixture was cooled down to -20°C in order to precipitate the excess of *m*-chlorobenzoic acid, which was filtered away. After evaporation under vacuum, the mixture was dissolved in pentane and filtered again. The organic phase was washed several times with a saturated solution of sodium carbonate then with a saturated solution of NaCl, and finally dried over magnesium sulphate, filtered, and dried under vacuum. 25 g of pure γEt_3SiOCL was recovered after distillation under vacuum (boiling point 120°C at 10^{-2} mmHg; 0.10 mol; yield: 64%).

In a typical copolymerization (Scheme 2b) (copolymer E in Table 2), 50 ml of anhydrous toluene was added to 1 ml of γEt_3SiOCL (4.3×10^{-3} mol) and 4 ml of εCL (36.1×10^{-3} mol). 5.8 ml of $Al(O^iPr)_3$ in anhydrous toluene (1.0×10^{-3} mol) was added. After polymerization for 17 h at 25 °C, an excess of 1 M HCl was added and the P(CL-co-γEt_3SiOCL) copolymer was precipitated in cold heptane and dried under vacuum. Eight copolymers were synthesized; the initial molar fraction of γEt_3SiOCL was 0.1, 0.3, 0.5, 0.7, and 1; and the monomer/initiator molar ratio was 40 and 150, respectively (Table 2).

The triethylsilanolate groups were hydrolysed by hydrofluoric acid (HF) in a water/acetonitrile solution (Scheme 2b). Typically, all the copolymers (Table 3) were dissolved in acetonitrile (1 wt%) and an aqueous HF solution (50%, 3.5 eq) was added dropwise. After stirring at room temperature for 15-30 min, the mixture was neutralized by sodium bicarbonate, filtered, and dried under vacuum. The P(CL-co-γOHCL) copolymer was dissolved in toluene, dried over $MgSO_4$, precipitated in heptane, and dried under vacuum. In the case of the copolymer G* (Table 4), in order to prevent degradation from occurring, the acidic hydrolysis was followed by fast neutralization and dialysis of the THF polymer solution against THF/water mixtures of increasing content of water (SpectraPor 6-8000). It was finally recovered by lyophilization.

Table 2. *Molecular characteristics of the P(CL-co-γEt_3SiOCL) copolymers*

Ref.	DP[a] theor	F_{SCL}[b] (mol%)	M_n^{thc} ($\times 10^{-3}$)	$F_{SCL,NMR}$[d] (mol%)	DP	$M_{n,NMR}$ ($\times 10^{-3}$)	$M_{n,SEC}$ ($\times 10^{-3}$)	$M_w/M_{n,SEC}$
E	40	10	5.1	9	49	6.1	12.0	1.18
F	40	30	6.2	21	45	6.5	11.3	1.15
G	40	50	7.2	44	40	6.8	12.4	1.14
H	40	70	8.2	72	36	7.6	11.5	1.10
I	40	100	9.8	100	44	10.7	11.4	1.12
J	150	10	19.1	9	158	19.8	33.5	1.09
K	150	30	23.0	28	140	21.0	31.0	1.12
L	150	50	26.9	46	133	23.1	26.1	1.13

[a] $[M]_0/[I]_0$.
[b] Molar content of γEt_3SiOCL in the comonomer feed.
[c] Theoretical M_n.
[d] Molar content of γEt_3SiOCL in the copolymer.

Table 3. *Molar content of the γEt_3SiOCL repeating units (F_{SCL}) and average length of the εCL (L_{CL}) and γEt_3SiOCL (L_{SCL}) sequences in the P(CL-co-γEt_3SiOCL) copolymers*

Ref.	^{1}H-NMR		^{13}C-NMR[a]		
	DP	F_{SCL} (mol%)	L_{CL}	L_{SCL}	F_{SCL} (mol%)
E	49	9	9.7	1.0	9
F	45	21	4.5	1.4	24
G	40	44	2.5	1.7	40
H	36	72	1.6	2.6	62

[a] δ (ppm) for the oxymethylene carbon atom: 64.05 (CL-CL), 64.15 (CL-SCL), 61.15 (SCL-SCL), and 61.05 (SCL-CL).

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Table 4. *Cleavage of the triethylsilyl protecting groups of the P(CL-co-γEt$_3$SiOCL) copolymers by acidic hydrolysis (3.5 eq HF in acetonitrile)*

Ref.	$F_{SCL,NMR}$[a] (mol%)	DP_{NMR}[a]	$M_w/M_{n,SEC}$[a] (SEC)	After acidic hydrolysis			
				Reaction time (min)	$M_w/M_{n,SEC}$[b]	$F_{OHCL,NMR}$[b] (mol%)	DP_{NMR}[b]
E	9	49	1.18	30	1.47	N/D	N/D
F[c]	21	45	1.15	30	1.19	19	43
G	44	40	1.14	20	2.30	N/D	N/D
G*[c]	44	40	1.14	15	1.28	49	35
J[c]	9	158	1.09	30	1.15	7	123
K[c]	28	140	1.12	30	1.29	23	132
L	46	133	1.13	30	2.30	N/D	N/D

[a] Before hydrolysis.
[b] After hydrolysis.
[c] Copolymers used for the preparation of nanoparticles. N/D = not determined (degrading conditions).

Poly(D,L-lactide)/P(CL-co-γXCL) nanoparticles

Typically, 1 ml of a poly(D,L -lactide) solution in DMSO (16 mg/ml) and 3.2 mg of P(CL-co-γXCL) dissolved in different volumes of DMSO were mixed. Eight millilitres of a phosphate buffer (0.13 M, pH 7.4) was rapidly added to the PLA/P(CL-co-γPyCL) solution, followed by 8 ml of water. The suspension of PLA/P(CL-co-γXCL) nanoparticles was dialysed against water for 2 h, to eliminate DMSO, and centrifuged at 3500 rpm, to remove any trace of residual solids including unstable particles and precipitated polymer. The relative amount of copolymer was changed from 50 mg down to 2.5 mg/100 mg PLA in order to determine the minimum amount required for the polymer precipitation to be quantitative. The total polymer concentration (C_P^{ORG}) in DMSO was 17.6 mg/ml for the PLA/P(CL-co-γPyCL) pair and 16 mg/ml for the PLA/P(CL-co-γOHCL) one. The influence of C_P^{ORG} on the nanoparticle size was investigated by decreasing C_P^{ORG} from 16 to 2 mg/ml, with 20 mg of copolymer per 100 mg of PLA.

Characterization of the P(CL-co-γXCL) copolymers

The actual composition of the P(CL-co-γBrCL) copolymers was calculated by ^{1}H-NMR in $CDCl_3$, from the intensity of the signal for the methylene protons in the α-position of the carbonyl ester of the γBrCL units (2.6 ppm) and the signal for the methylene protons of the εCL units in the γ-position (1.4 ppm), as explained elsewhere [13]. After quaternization of pyridine, the conversion of the γBrCL units into γPyCL was determined by ^{1}H-NMR in DMF, from comparison of the signals for the methyl protons in the α-position of the pyridinium bromide at 5.2 ppm and the methyl protons of the isopropyl ester end-group at 4.9 ppm. The degree of polymerization before and after quaternization was calculated from the signals for (i) the methyne proton of the isopropyl ester end-group at 4.9 ppm, (ii) the methylene protons in the γ-position at 1.4 ppm for the εCL units, (iii) the methylene protons in the α-position at 2.6 ppm for the γBrCL units, and (iv) the methyne proton in the α-position of the pyridinium substituent at 5.2 ppm for the γPyCL units.

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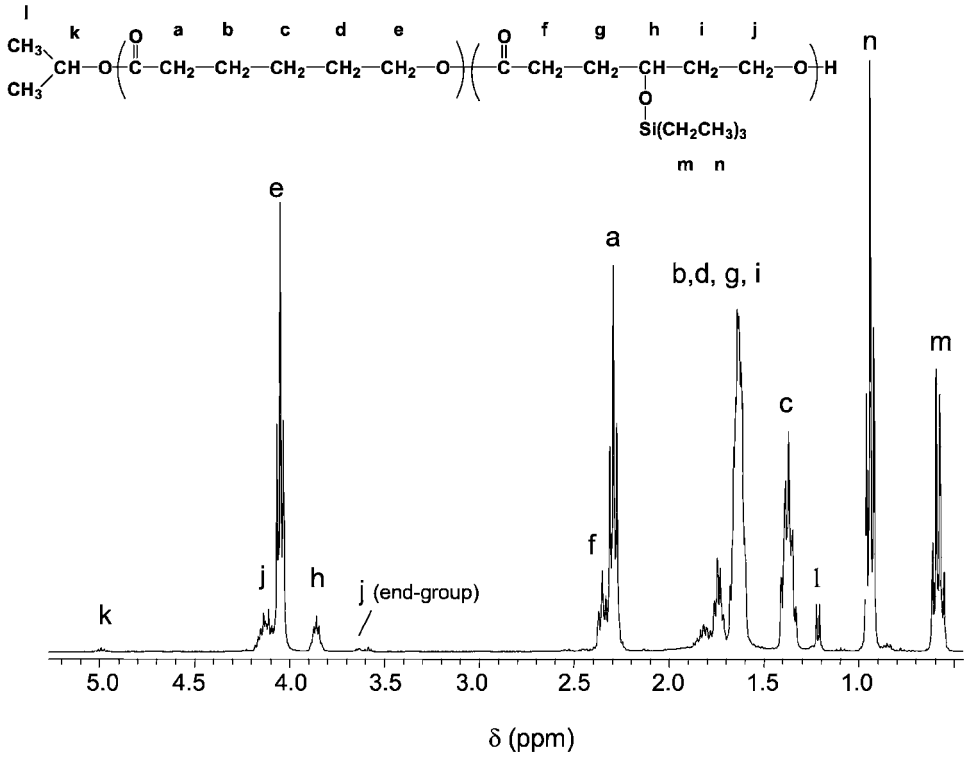
Figure 1. *^{1}H-NMR spectrum of P(CL-co-γEt$_3$SiOCL) (copolymer G in Table 2, with F_{SCL} = 44 mol% and DP = 40) recorded in CDCl$_3$ at 400 MHz.*



The molecular weight and composition of P(CL-co-γEt$_3$SiOCL) copolymers were determined by ^{1}H-NMR in CDCl$_3$ (Fig. 1). M_n was calculated from the relative intensity of the signals of the methyne proton of the isopropyl ester end-group (k, δ = 5.0 ppm), the methylene protons in the γ-position of the ester carbonyl in the εCL repeating units (c, δ = 1.4 ppm), and the methyl protons of the ethyl group in γEt$_3$SiOCL repeating units (n, δ = 0.95 ppm), according to the following equation :

$$M_n = \left(\frac{I_n}{9} \times 224.4 + \frac{I_c}{2} \times 114.0 \right) \times \frac{1}{I_k}. \qquad (1)$$

The molar fraction of γEt$_3$SiOCL (F_{SCL}) was calculated from the relative intensity of the methyl protons of the ethyl group in γEt$_3$SiOCL (n, δ = 0.95 ppm) and that of the methylene protons in the γ-position of the ester carbonyl in εCL (c, δ = 1.4 ppm).

Size exclusion chromatography (SEC) was performed in THF at 40°C using a Hewlett-Packard 1090 liquid chromatograph fitted with a Hewlett-Packard 1037A refractive index detector and four Hewlett-Packard PL gel 5μ columns (10^5, 10^4, 10^3, and 10^2 Å). ^{1}H-NMR and ^{13}C-NMR spectra were recorded in CDCl$_3$ or DMF with a Bruker 400 MHz spectrometer. Differential scanning calorimetry was carried out with a TA Instrument, at a heating rate of 10°C/min.

Characterization of the P(CL-co-γXCL)/PLA nanoparticles

Dynamic light scattering (DLS) was carried out with a Brookhaven instrument (Ar laser, 488 nm) fitted with a photon correlation spectrometer. The concentration of the nanoparticle suspension in filtered deionized water was 200 μg/ml. The size distribution was calculated by the CONTIN method and data from at least five measurements were averaged for each suspension. The zeta potential of the nanoparticles suspended in non-buffered saline (0.09% NaCl) at a polymer concentration of *ca.* 1 mg/ml was measured by Doppler electrophoretic light scattering (Coulter Delsa 440-SX) at angles of 25.6° and 35.2°.

RESULTS AND DISCUSSION

P(CL-co-γPyCL) copolymers

The synthesis of γBrCL (Scheme 1), the living copolymerization of γBrCL and εCL (Scheme 2a), and quaternization of the bromide units (Scheme 2a) have been detailed elsewhere [13, 14]. P(CL-co-γPyCL)

copolymers that contain two molar fractions of cationic γPyCL units (0.1 and 0.3) were prepared with two degrees of polymerization (i.e. monomer/initiator molar ratios of 40 and 150) (Table 1). It was previously reported that the copolymerization of εCL with γ-substituted ε-caprolactone is controlled when initiated by $Al(O^iPr)_3$ at 25 °C or lower. The theoretical degree of polymerization (DP) is then determined by the monomer to initiator ($[M]_0/[I]_0$) molar ratio corrected for the conversion of the comonomers.

Table 1 shows that $M_{n,SEC}$ is *ca.* two times higher than $M_{n,NMR}$which is not surprising because the SEC columns were calibrated by polystyrene standards. In accordance with previous data, the molecular weight distribution is narrow, in agreement with a controlled copolymerization. The random distribution of the comonomer units was previously established by ^{13}C-NMR. Finally, P(CL-co-γBrCL) was quaternized at room temperature with pyridine, without any significant change in the degree of polymerization as measured by ^{1}H-NMR. When quaternized copolyesters of higher molecular weight (C and D) are repeatedly precipitated, loss of the shortest chains could account for a higher apparent degree of polymerization.

P(CL-co-γOHCL) copolymers

Non-ionic block polyamphiphiles, such as PLA-b-poly(ethylene oxide), have also been found to stabilize PLA nanoparticles prepared by co-precipitation, although a higher copolymer content was required compared with charged polyamphiphile s [31]. In this work, novel non-ionic polyamphiphiles were synthesized, which are random copolymers of εCL and γ-hydroxyl ε-caprolactone (γOHCL).

The ring-opening copolymerization of εCL with protected γOHCL has been previously investigated in this laboratory, the hydroxyl group being protected by either an acetal group (γ-ethylene ketal ε-caprolactone, TOSUO) or a triethylsilyloxy group (γEt_3SiOCL) (Scheme 2b) [12]. Only copolymers with a low molar fraction of γEt_3SiOCL and TOSUO (0.05) were prepared, however. In this work, copolymers with higher γEt_3SiOCL contents (from 10 to 70 mol%) and a higher degree of polymerization (40 and 150) were synthesized by controlled ring-opening polymerization initiated by $Al(O^iPr)_3$ at 20°C (Table 2). The theoretical molecular weight was calculated by the equation

$$M_n^{th} = \frac{[\varepsilon CL]_0}{[Al(O^iPr)_3]_0} \times 114 + \frac{[\gamma Et_3SiO \cdot CL]_0}{[Al(O^iPr)_3]_0} \times 244, \qquad (2)$$

on the basis that the comonomer conversion is systematically close to 100%.

Consistent with controlled polymerization mediated by aluminum isopropoxide, the experimental molecular weight and the γEt_3SiOCL molar fraction are in good agreement with the expected values (Table 2) and the molecular weight distribution was narrow ($M_w/M_n < 1.2$). The resonances of the proton atoms of the isopropyl ester end-group (1.4 and 5 ppm) and the CH_2-OH end-group (j, 3.65 ppm) by ^{1}H-NMR (Fig. 1) are consistent with the accepted coordination-insertion mechanism and selective cleavage of the acyl-oxygen bond of the cyclic ester.

The sequences formed by the comonomers were analysed by ^{13}C-NMR, particularly in the 172-174 ppm range for the carbonyl carbon atom and in the 61-64 ppm range for the oxymethylene carbon atom. One single peak was observed for the homopolymers, which corresponds to homodiads of εCL (CL-CL) and γEt_3SiOCL (SCL-SCL) (Fig. 2a and 2c for the oxymethylene carbon atoms). In contrast, double resonance peaks were observed for the copolymers in the two regions. The additional peak results from a shift of the resonance when the carbon atom is part of CL-SCL and SCL-CL heterodiads (Fig. 2b). The average length of the εCL and γEt_3SiOCL sequences (L_{CL} and L_{SCL}, respectively) was calculated in the oxymethylene region [equations (3) and (4)], as described elsewhere for the P(CL-co-TOSUO) copolymers [10].

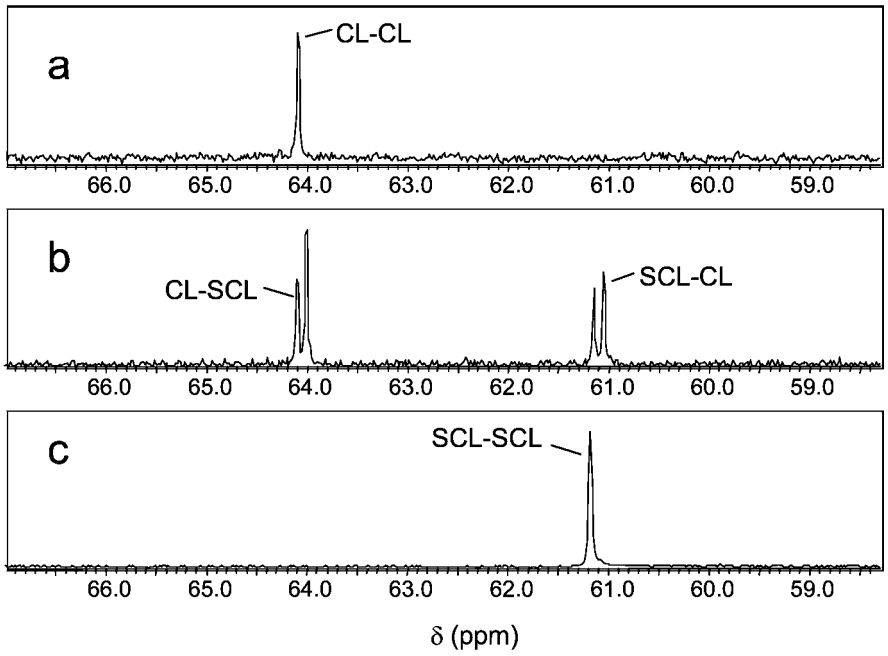
$$L_{CL} = \frac{I_{CL-CL}}{I_{CL-SCL}} + 1, \qquad (3)$$

$$L_{SCL} = \frac{I_{SCL-SCL}}{I_{SCL-CL}} + 1, \qquad (4)$$

where I_{I-J} is the intensity of the signal for the oxymethylene carbon in the *I-J* diad. L_{CL} and L_{SCL} for the copolymers E to G of lower DP are reported in Table 3. The molar content of the γEt_3SiOCL units was calculated from L_{CL} and L_{SCL}, according to the equation:

$$F_{SCL} = \frac{L_{SCL}}{L_{SCL} + L_{CL}}. \qquad (5)$$

Figure 2. *^{13}C-NMR spectra expanded in the resonance region of the oxymethylene groups of PCL (a), P(CL-co-γEt$_3$SiOCL) (copolymer G in Table 2) (b) and P(γEt$_3$SiOCL) (c), recorded in CDCl$_3$.*



Good agreement was found with the data calculated from the ^{1}H-NMR spectra, which supports the randomness of the copolymers.

The thermal behaviour of PCL, P(CL-co-γEt$_3$SiOCL), and P(γEt$_3$SiOCL) was analysed by scanning differential analysis (Fig. 3a). As expected, homo-PCL showed a glass transition temperature (T_g) at -60°C and a melting temperature (T_m) at 60°C. Homopolymer P(γEt$_3$SiOCL) was completely amorphous, with a T_g at -50°C, a value slightly higher than the T_g of PCL. The copolymers that contained less than *ca.* 25 mol% of γEt$_3$SiOCL units remained semi-crystalline (E and F), with T_m decreasing with increasing F_{SCL}(Fig. 3b). Copolymers of higher F_{SCL} were amorphous and T_g increased slightly with increasing F_{SCL}(Fig. 3b).

The protecting triethylsilyl group of the hydroxyl functions was cleaved by acidic hydrolysis (Scheme 2b). In our previous study, this hydrolysis reaction was carried out quantitatively with trifluoroacetic acid (1.5 eq TFA with respect to the triethylsilyl groups, added to a THF/water polymer solution) without chain degradation [12]. However, when the copolymer J (X = 9 mol%, DP = 150) was treated under the same conditions, the cleavage of the triethylsilyl groups was quantitative, but the copolymer was degraded after 15 min. Hydrolysis with HCl (10 eq in THF) and n-butyl ammonium fluoride (2 eq in THF) was also accompanied by rapid degradation, whereas acetic acid (successfully used for the hydrolysis of the t-butyldimethylsilyl protecting group [9]) prevented the copolymer from degrading but failed to cleave the triethylsilyl groups after 20 h (10 eq in THF). Only fluorhydric acid (HF, 3 eq in acetonitrile) was able to deprotect the copolymer without degradation after 30 min. As reported in Table 4, this method is effective as long as the γEt$_3$SiOCL content is low (i.e. copolymers F, J, and K, with F_{SCL} = 21, 9 and 28 mol%, respectively), except for copolymer E (F_{SCL} = 9 mol%), which is degraded. The ^{1}H-NMR spectrum of a completely hydrolysed copolymer (F) is consistent with the total cleavage of the Et$_3$Si protecting groups (no signal at 0.59 and 0.94 ppm). The signal at 4.34 ppm is characteristic of the methyne proton (h) in the α-position of the released hydroxyl group (Fig. 4a). The DP and polymolecularity of copolymer F after hydrolysis (DP = 43 and M_w/M_n = 1.15) are close to the values for the silylated precursor (DP = 45 and M_w/M_n = 1.19), which confirms limited chain degradation, if any.

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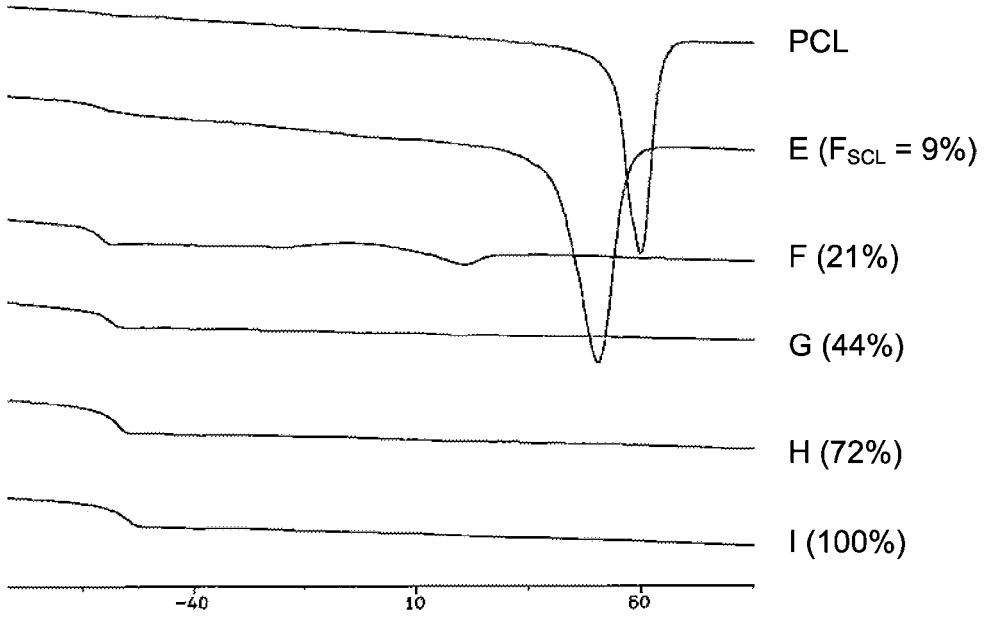
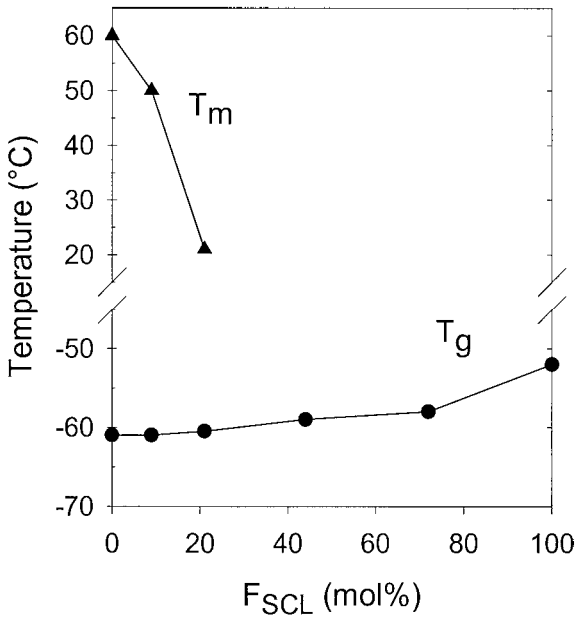
Figure 3. *(a) Differential scanning calorimetry of PCL and P(CL-co-γEt$_3$SiOCL) (copolymers E-I in Table 2), recorded at a heating rate of 20°C/min (first run). (b) Melting and glass transition temperatures for P(CL-co-γEt$_3$SiOCL) copolymers of various contents in γEt$_3$SiOCL units (F_{SCL}).*

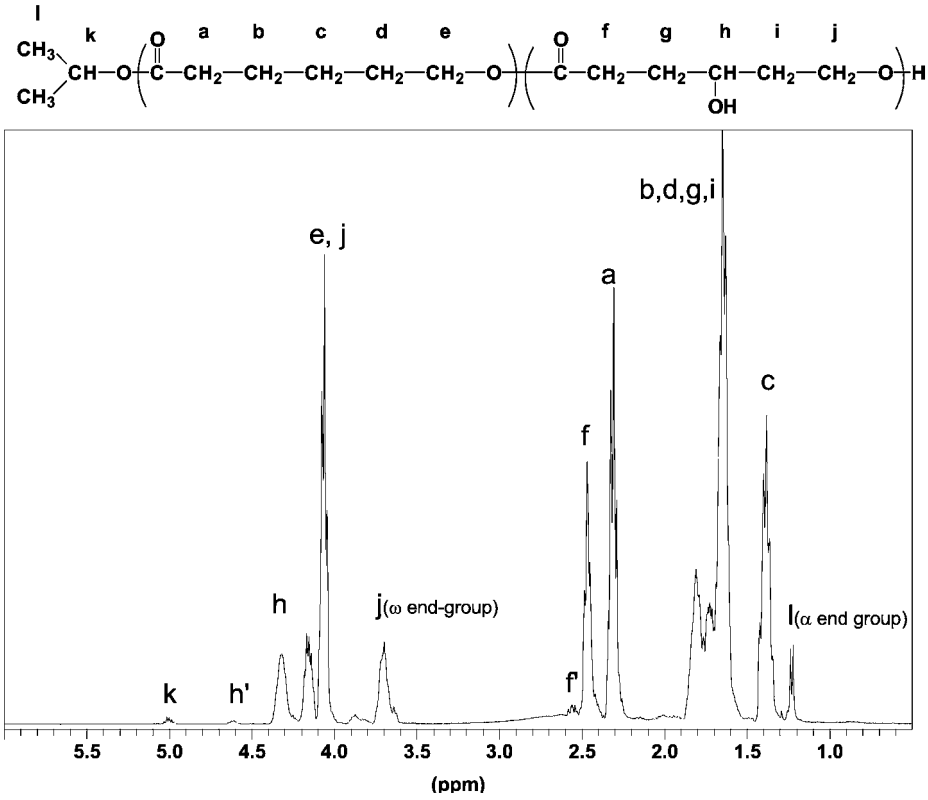


(a)



(b)

Figure 4. *^{1}H-NMR spectra of the P(CL-co-γOHCL) copolymer G* (Table 4) before (a) and after (b) acidification, recorded in $CDCl_3$ at 400 MHz.*



In contrast, copolymers with a higher γEt$_3$SiOCL content (copolymers G and L with F_{SCL} = 50 and 46 mol%) are rapidly degraded, more likely as a result of intramolecular transesterification of an internal ester group by the hydroxyl released in the γ-position and the formation of a five-membered butyrolactone (Scheme 3). The same rearrangement was previously reported for monomeric γ-hydroxy ε-caprolactone, with the formation of 3-(2-

hydroxyethyl)-γ-butyrolactone [9]. When only a few γOH groups are released, this transesterification reaction is slow enough for the unaltered polyester to be recovered. However, at a high γOH content, the probability that chain scissions occur is high enough for chain degradation to be detected by ^{1}H-NMR and FTIR. Indeed, the ^{1}H-NMR spectrum of these desilylated copolymers shows additional signals at 4.54 and 2.49 ppm, compared with the deprotected copolymers of a lower γEt_3SiOCL content. The multiplet at 4.54 ppm can be attributed to the methyne proton (h') of the butyrolactone ring and the triplet at 2.49 ppm can be assigned to two methylene protons in the α-position of the ester carbonyl (f) (Fig. 4b). Consistently, the intensity of these signals, which is low for the desilylated copolymer G* (Table 4), increases upon further acidification (Fig. 4a and 4b). The FTIR spectrum of the acidified copolymer G* shows an additional absorption at 1772 cm^{-1}, which is characteristic of the butyrolactone carbonyl group, in agreement with the intramolecular rearrangement (Fig. 5a). In contrast, vibration of the carbonyl ester of the chains is observed at 1724 cm^{-1} before the acidification of G* and no absorption is visible at 1772 cm^{-1} (Fig. 5b).

Scheme 3. *Scission of P(CL-co-γOHCL) chains by intramolecular rearrangement of the γOHCL monomeric unit.*

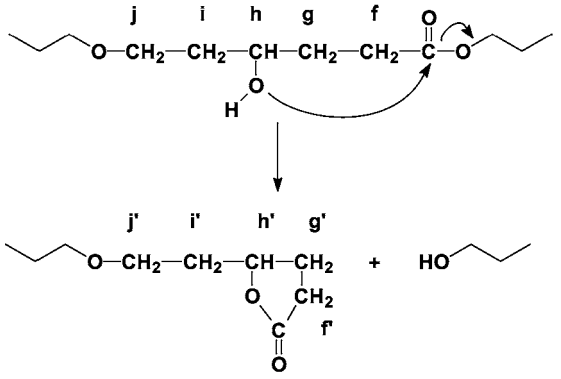
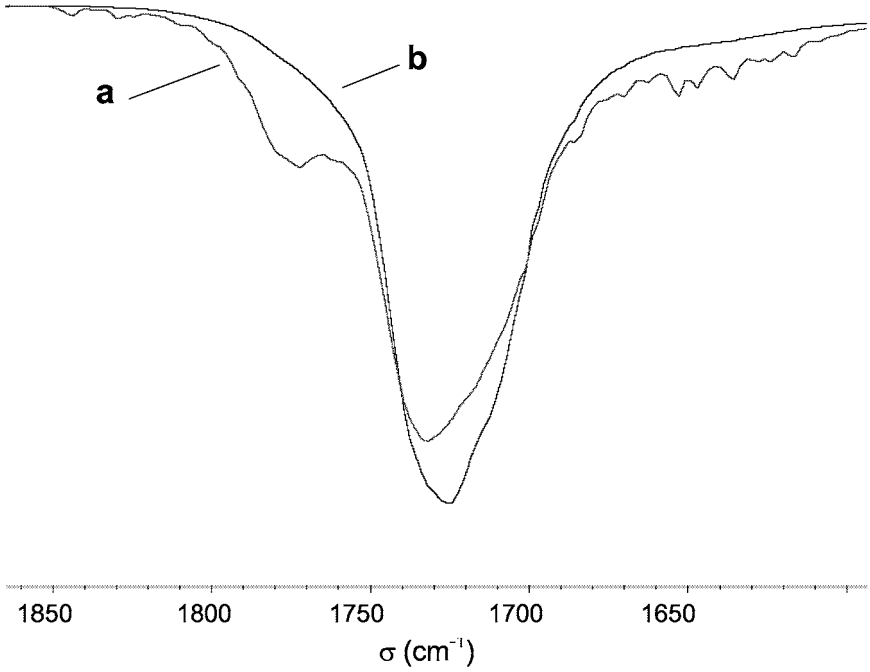


Figure 5. *FTIR spectra of the P(CL-co-γOHCL) copolymer G* (Table 4) after (a) and before acidification (b).*

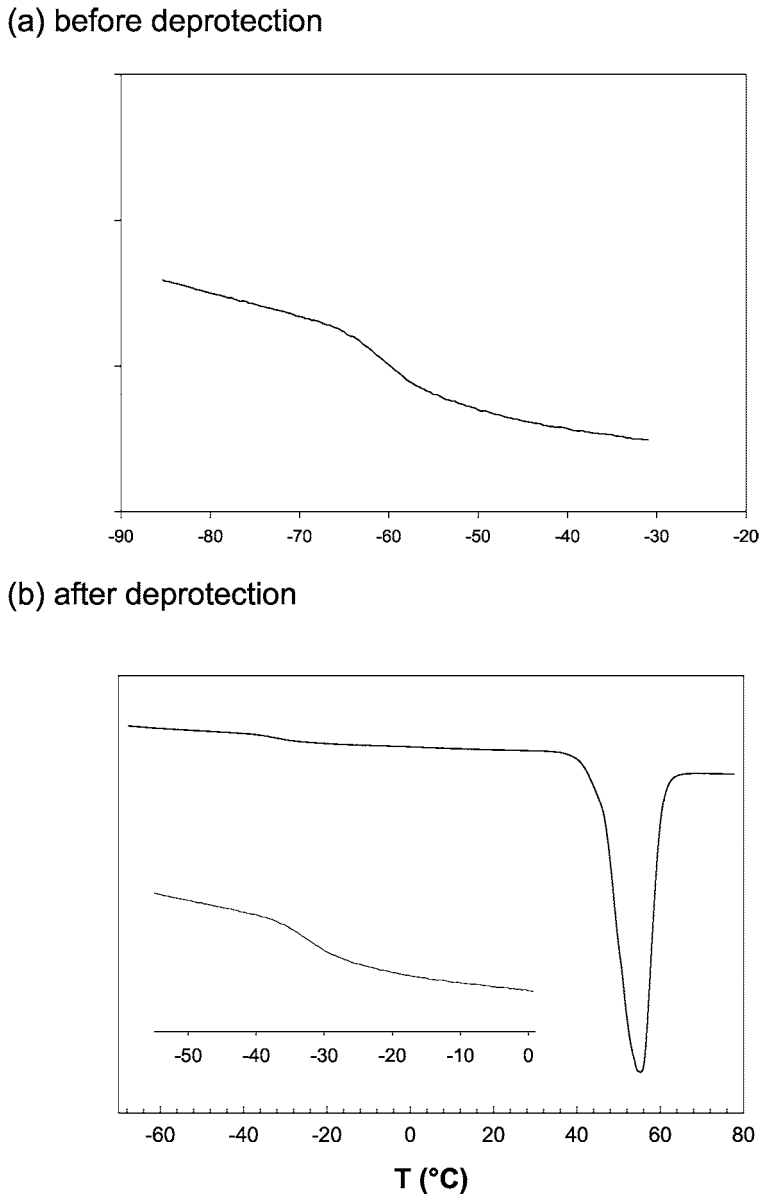


A way to restrict chain scission after deprotection of the hydroxyl groups is to eliminate rapidly the excess of acid by neutralization, or by dialysis against THF/water mixtures of increasing water content. Dialysis was successfully tested in the case of copolymer G (ref. G* in Table 4), as assessed by the limited chain degradation

(M_w/M_n = 1.28 instead of 1.14) at complete deprotection. Hydrolysis by pyridine · HF, a precursor of anhydrous HF [32], is not effective. Indeed, only 66% of the triethylsilyl groups of copolymer G were cleaved after 30 min and degradation occurred to some extent, as confirmed by a higher polymolecularity (M_w/M_n = 1.46).

After deprotection, P(CL-co-γOHCL) copolymers are semi-crystalline, whatever the γOHCL content. Compared with the amorphous silylated copolymer G (Fig. 3a), the T_g of the deprotected copolymer G* is higher (-31°C vs. -59°C, respectively) and a melting temperature is observed at +56°C, close to the T_m of PCL (Fig. 6).

Figure 6. *Differential scanning calorimetry of the P(CL-co-γOHCL) copolymer G* (Table 4) recorded at a heating rate of 20°C/min (first run).*



Preparation of PLA nanoparticles by co-precipitation with P(CL-co-γXCL)

As previously reported, co-precipitation of poly(D,L-lactide) with small amounts of random amphiphilic copolymers of methyl methacrylate (MMA) and methacrylic acid (MA), P(MMA-co-MA), yielded stable sub-200 nm nanoparticles [28]. Addition of 10 mg of P(MMA-co-MA) per 100 mg PLA was required to observe the complete conversion of PLA into stable nanoparticles (initial polymer concentration = 17.5 mg/ml in DMSO). The conversion was partial for more concentrated polymer solutions. In this work, non-degradable methacrylic copolymers were replaced by PCL partly substituted by pyridinium and hydroxyl groups, respectively, with the

purpose of making degradable colloidal carriers available.

Amount of P(CL-co-γXCL) required for the formation of stable PLA nanoparticles

The weight ratio [P(CL-co-γXCL)]/[PLA] was varied in order to determine the smallest amount of P(CL-co-γXCL) required for the formation of nanoparticles. Beyond a key value, the yield is quantitative and the particle size remains unchanged, while keeping constant the total polymer concentration in the organic phase. Cationic P(CL-co-γPyCL) copolymers are able to stabilize PLA nanoparticles, even when the γPyCL content is as low as 7 mol%. This observation is consistent with the experiments conducted with statistical copolymers of MMA and MA, P(MMA-co-MA), which were effective stabilizers whenever the content of the hydrophilic MA units was higher than 5 mol%. Table 5 shows that the smallest amount of P(CL-co-γPyCL) required to convert quantitatively PLA into stable nanoparticles depends on both the γPyCL content (*X*) and the copolymer DP. For a DP of *ca.* 50, 10 mg of copolymer A per 100 mg of PLA is needed, compared to a two-fold smaller amount (5 mg/100 mg PLA) of copolymer B that contains 2.5 times more hydrophilic units (18 mol%). It happens that the number of hydrophilic units per 100 mg PLA is quasi the same in the two experiments (*ca.* 6×10^{-3} mol). This amount is higher in the case of chains of higher DP (copolymers C and D), i.e. more than 10^{-2} mol/100 mg PLA. One possible explanation is that longer copolymer chains face more problems in migrating towards the nanoparticle surface upon fast polymer precipitation. A larger initial amount of ionic groups is therefore needed to prevent nanoparticles from coalescing. It must be noted that the mean diameter of the nanoparticles formed with the minimum amount of copolymer is independent of the copolymer DP and the pyridinium content (copolymers A to D). As a rule, the ideal copolymer DP is a compromise between two opposite tendencies, i.e. the faster migration of shorter chains to the nanoparticle surface and the more efficient anchorage of longer chains to the PLA matrix.

Table 5. *Amount of P(CL-co-γXCL) required for the complete conversion of PLA into stable nanoparticles (in mg and mol per 100 mg PLA). Average diameter and zeta potential of the nanoparticles*

γXCL	Ref.	F_X (mol%)	DP	C_P^{ORG} (mg/ml)	P(CL-co-γXCL) (mg/l00mg PLA)	γXCL (10^{-3} mol/ 100 mg PLA)	Mean diameter[a] (nm)	Zeta potential (mV)
γPyCL	A	7	53	17.6	10	6	156 ± 16	+61 (±10)[b]
	B	18	50	17.6	5	7	176 ± 9	+66 (±10)[b]
	C	10	180	17.6	10	8	172 ± 13	N/D
	D	28	168	16.8	5	10	160 ± 14	N/D
γOHCL	F	19	43	16.0	20	28	184 ± 11	-7 (±3)[c]
	G*	49	35	16.0	12.5	28	213 ± 22	-8 (±3)[c]
	J	7	123	16.0	50	29	N/D	N/D
	K	23	132	16.0	20	33	N/D	N/D

[a] Measured by DLS with the CONTIN calculation method (10 measurements).
[b] 10 mg copolymer/100 mg PLA.
[c] 20 mg copolymer/100 mg PLA.

Non-ionic P(CL-co-γOHCL) copolymers are less effective stabilizers of PLA nanoparticles than charged P(CL-co-γPyCL) chains, consistent with the lower efficiency of a steric barrier compared with an electrostatic one (Table 5). Indeed, at least 20 mg of copolymer F is required to precipitate quantitatively 100 mg of PLA, although only 5 mg of copolymer B of similar DP and content of hydrophilic units is needed. The molar amount of γOHCL repeating units required to stabilize the PLA nanoparticles is much higher, at least 28×10^{-3} mol per 100 mg PLA, *ca.* four times more than the pyridinium units. The DP of the non-ionic chains plays a minor role, because at least 28×10^{-3} mol of hydroxyl group per 100 mg of PLA is needed for a short copolymer (copolymer F, DP = 43), compared with 33×10^{-3} mol for longer chains (copolymer K, DP = 132).

When prepared with the minimum amount of P(CL-co-γOHCL) of low γOHCL content (F, J and K) and a polymer concentration of 16 mg/ml DMSO, the PLA nanoparticles do not remain dispersed after dialysis against an electrolyte solution, even one of low concentration (e.g. 0.09% NaCl). This instability is, however, overcome by increasing the relative amount of copolymer and the content of the hydrophilic units. For instance, 20 mg instead of 12.5 mg of copolymer G* per 100 mg of PLA allows the PLA suspension to withstand an electrolyte concentration of 0.9% while keeping the size distribution unchanged.

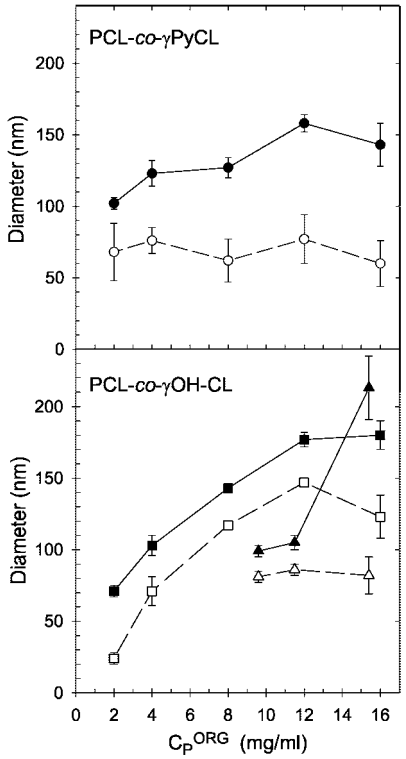
It appears that at comparable C_P^{ORG}, the nanoparticle diameter tends to increase when stabilized by P(CL-co-γOHCL) rather than by P(CL-co-γPyCL). Either copolymers bearing hydroxyl groups migrate more slowly towards the nanoparticle surface when the co-precipitation is triggered, or the steric barrier formed by the

hydroxyl groups is less effective against coalescence than the cationic electrostatic barrier. The particle size distribution is comparable whatever the copolymer used.

A difference in the solvency properties of DMSO towards PLA and the copolymers might have an effect on the nanoparticle formation and stabilization. Indeed, DMSO is a good solvent for PLA, a non-solvent for PCL, and the solubility of the copolymers increases with the content of the hydrophilic units. Nevertheless, the conversion of PLA into nanoparticles and the particle size are basically independent of the copolymer composition, as emphasized by the series of cationic copolymers.

Finally, the zeta potential of the nanoparticles was measured in 0.09% NaCl (Table 5) in order to confirm the surface functionalization. The zeta potential is positive for the nanoparticles stabilized by P(CL-co-γPyCL) (*ca.* +65 ± 10 mV) and is slightly negative (*ca.* -7.5 ± 3 mV) when P(CL-co-γOHCL) is the stabilizer. This is strong evidence for the preferential localization of the pyridinium and hydroxyl groups at the nanoparticle surface. In a previous study on the stabilization of PLA nanoparticles by anionic P(MMA-co-MA) copolymers, the zeta potential was negative (*ca.* -60 mV) and remained basically unchanged when the copolymer amount was increased, which suggested that there is a critical density of hydrophilic units at the surface of the co-precipitated nanoparticles. The functional groups available on the surface can be used for binding purposes. For example, anionic (macro)molecules can associate to nanoparticles bearing cationic pyridinium groups by ionic interactions. Hydroxyl groups or organic functions derivatized therefrom are potential binding sites for drugs, markers, and targeting moieties.

Figure 7. *Influence of the total polymer concentration (C_P) in the organic phase on the size distribution of PLA nanoparticles prepared with (a) P(CL-co-γPyCL) copolymer B (●,○) and (b) P(CL-co-γOHCL) copolymer F (■,□) and G^+ (▲,Δ). Solid symbols stand for the average diameters and empty symbols stand for the lower diameters (N = 10 measurements, CONTIN calculation method).*



Influence of the polymer concentration in the organic phase on the nanoparticle size

The influence of the total polymer concentration in DMSO on the nanoparticle size was investigated for copolymers with a low DP, i.e. the cationic copolymer B and the non-ionic copolymers F and G*. The nanoparticle diameter decreased, although not dramatically, with the polymer concentration in the organic phase, at least until a lower concentration limit, beyond which the dispersion became unstable (Fig. 7). For instance, the mean diameter of the nanoparticles stabilized by the cationic copolymer B is 158 ± 6 nm at C_P^{ORG} = 12 mg/ml and 102 ± 4 nm at C_P^{ORG} = 2 mg/ml. Nevertheless, the drop in particle size is more important when the

concentration of the non-ionic copolymer F is changed within the same limits, i.e. from 177 ± 5 nm down to 71 ± 4 nm. The size distribution of the collected nanoparticles was estimated from the difference between the mean and the lower diameter measured by the CONTIN method (Fig. 7). The polydispersity of the two series of nanoparticles is comparable whenever they are prepared at C_P^{ORG} = 16 mg/ml. Dilution of the native solution results in nanoparticles of a higher polydispersity when they are stabilized by P(CL-co-γPyCL) rather than by P(CL-co-γOHCL). This is thought to reflect the difference in the stabilization mechanism, i.e. electrostatic repulsions vs. steric barrier against coalescence.

CONCLUSIONS

Potentially biodegradable sub-200 nm nanoparticles have been prepared by the co-precipitation of PLA with novel amphiphilic copolyesters from DMSO. Two series of random copolyesters of ε-caprolactone and ε-caprolactone γ-substituted by a bromide (γBrCL) and a triethylsilyloxy group (γOHCL), respectively, were synthesized by ring-opening copolymerization initiated by aluminum triisopropoxide. These copolyesters were endowed with amphiphilicity by quaternization of pyridine by the γBrCL co-units and the acidic hydrolysis of the γEt_3SiOCL co-units, respectively.

PLA is quantitatively converted into stable nanoparticles by co-precipitation with relatively small amounts of the cationic P(CL-co-γPyCL) copolymers (≤10 mg/ 100 mg PLA) of a low content of pyridinium bromide. Much larger amounts of the non-ionic P(CL-co-γOHCL) copolymers with similar DP and composition (≥20 mg/100 mg) are needed however, which makes copolymers with higher contents of γOHCL units more attractive. For this purpose, the acid cleavage of the triethylsilyl groups has required optimization for being conducted without significant chain degradation. Indeed, the occurrence of chain scission and intramolecular rearrangement with the formation of a butyrolactone end-group is as important as the γEt_3SiOCL content is high. P(CL-co-γOHCL) copolymers with a hydroxyl content as high as 49 mol% were accordingly prepared. The zeta potential of the PLA nanoparticles agrees qualitatively with the preferential location of the pyridinium and hydroxyl groups at the surface. Indeed, this potential is positive in the case of stabilization by pyridinium-containin g copolyesters and close to zero when the hydroxyl groups are the hydrophilic moieties. This surface reactivity can be exploited further to bind molecules of interest to the particles, such as tracers and targeting molecules, while basically retaining (bio)degradability. Indeed, only a low content of polyamphiphiles has to be used to modify the surface of the particles, whose bulk properties are unmodified.

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