



HAL
open science

**Novel Amphiphilic Degradable
Poly(-caprolactone)-graft-poly(4-vinyl pyridine),
Poly(-caprolactone)-graft-poly(dimethylaminoethyl
methacrylate) and Water-Soluble Derivatives**

Benjamin Nottelet, Michel Vert, Jean Coudane

► **To cite this version:**

Benjamin Nottelet, Michel Vert, Jean Coudane. Novel Amphiphilic Degradable Poly(-caprolactone)-graft-poly(4-vinyl pyridine), Poly(-caprolactone)-graft-poly(dimethylaminoethyl methacrylate) and Water-Soluble Derivatives. *Macromolecular Rapid Communications*, 2008, 29 (9), pp.743-750. 10.1002/marc.200800037 . hal-00416815

HAL Id: hal-00416815

<https://hal.science/hal-00416815v1>

Submitted on 12 Jan 2024

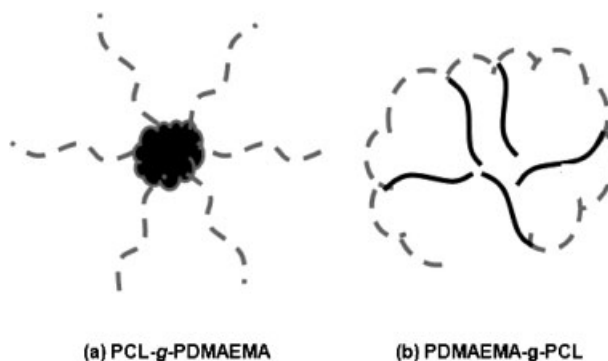
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Novel Amphiphilic Degradable Poly(ϵ -caprolactone)-*graft*-poly(4-vinyl pyridine), Poly(ϵ -caprolactone)-*graft*-poly(dimethylaminoethyl methacrylate) and Water-Soluble Derivatives

Benjamin Nottelet, Michel Vert, Jean Coudane*

New amphiphilic graft copolymers that have a poly(ϵ -caprolactone) (PCL) biodegradable hydrophobic backbone and poly(4-vinylpyridine) (P4VP) or poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA) hydrophilic side chains have been prepared by anionic polymerization of the corresponding 4VP and DMAEMA monomers using a PCL-based macropolycarbanion as initiator. The water solubility of these amphiphilic copolymers is improved by quaternization, which leads to fully water-soluble cationic copolymers that give micellar aggregates in deionized water with diameters ranging from 65 to 125 nm. In addition, to improve the hydrophilicity of PCL-*g*-P4VP, grafting of poly(ethylene glycol) (PEG) segments has been carried out to give a water-soluble double grafted PCL-*g*-(P4VP;PEG) terpolymer.



Introduction

Amphiphilic water-soluble polymers are extensively studied for their applications in numerous industrial fields, mainly because of their ability to self-assemble in

water to form macromolecular micelles.^[1-3] However, when used as a detergent or dispersant, non-biodegradable compounds become pollutants in the environment, therefore, it is necessary to propose new amphiphilic structures able to degrade under natural enzymatic or hydrolytic conditions. In addition to these industrial applications, amphiphilic copolymers have also raised increasing interest in the biomedical field, especially for gene transfection or drug-delivery applications.^[4] For these applications it is of interest to develop synthetic cationic polymers that are bioresorbable or at least degradable into smaller segments to decrease the well-known cytotoxicity of the polycations typically used to complex DNA, such as

B. Nottelet, M. Vert, J. Coudane
Max Mousseron Institute on Biomolecules, UMR CNRS 5247,
University of Montpellier 1, University of Montpellier 2,
Faculty of Pharmacy, 15 Avenue Charles Flahault, BP 14491, 34093
Montpellier cedex 5, France
Fax: +33 (0)4 67 52 08 98; E-mail: jcoudane@univ-montp1.fr

poly(ethylenimine) (PEI) and poly(L-Lysine) (PLL).^[5] To maintain the ability of amphiphilic copolymers to self-assemble while gaining degradability, the use of aliphatic polyesters as hydrophobic degradable segments has been proposed. As a result, the combination of poly(lactic acid) (PLA) and poly[lactic-co-(glycolic acid)] (PLGA) with water-soluble polymers has been extensively studied, poly(lactic acid)-*block*-poly(ethylene glycol) (PLA-*b*-PEG) copolymers being a classical structure. However, few other polyester-based amphiphiles have been synthesized. Poly(ϵ -caprolactone) (PCL), a biocompatible slow degrading polyester of interest for environmental and biomedical applications, is rarely found in amphiphilic structures, especially in graft or comb-like structures that bear cationic groups. Until now, PCL has been mainly associated with PEG segments to form di-^[6,7] or triblocks,^[8] star,^[9,10] or graft^[11,12] copolymers. Among the rare PCL-based cationic copolymers described in the literature, one can distinguish fully degradable PCL-based structures and partly degradable PCL-based structures. Fully degradable structures are composed either of natural polymers like dextran-*g*-PCL^[13] and chitosan-*g*-PCL,^[14] or poly(amino acids) like poly(asparagine)-*g*-PCL^[15] or PCL-*g*-PLL recently described by our group.^[16] It is worth noting that PCL-*g*-PLL is the only copolymer that exhibits an inverse structure composed of a hydrophobic backbone substituted by hydrophilic side chains. Partly degradable PCL-based cationic amphiphilic copolymers such as PCL-*block*-poly(2-ethyl-2-oxazoline) block and multiblock copolymers^[17,18] or PEI-*g*-(PCL-*b*-PEG)^[19] copolymers aimed at gene delivery have been described. In this article we focus on the synthesis of new graft copolymers that have a PCL main chain and poly(4-vinylpyridine) (P4VP) or poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA) hydrophilic side chains. Some PCL-based structures that exhibit vinylic hydrophilic comonomers have mainly been obtained, vinyl pyrrolidone being the comonomer in diblock,^[20] triblock,^[21] and three-arm block copolymers.^[22] As far as we know, only one example of a diblock copolymer using P4VP, which is a strong polybase ($pK_a \approx 4-5$), has been described.^[23] Compared to this unique P4VP-containing example, many copolymers of PCL and PDMAEMA have been synthesized. PDMAEMA is a weak polybase ($pK_a \approx 7.4$). It is a water-soluble polymer at room temperature over a wide range of pH. However, it exhibits inverse solubility behavior and precipitates from neutral or basic solutions between 32 and 58 °C. Some diblock,^[24] triblock,^[25] and miktoarm star copolymers^[26] have been described. In water, they form nanometric micelles with diameters in the range 10 to 50 nm, depending on PCL content and copolymer structure. PDMAEMA-*g*-PCL and brush-like copolymers have also been synthesized by Mespouille et al.^[27] and Xu et al.^[28], respectively, according to a strategy based on

the use of an atom transfer radical polymerization (ATRP)-polymerizable PCL derivative.

These examples illustrate the interest in PCL-based amphiphilic cationic copolymers. However, except for the mentioned PDMAEMA-*g*-PCL,^[27] none of these copolymers are graft-type compounds, although it is remarkable that amphiphilic graft copolymers are known to generate much more stable objects in water with lower critical aggregation concentrations, in comparison to diblock copolymers. In this paper, we describe the synthesis of PCL-*g*-P4VP and PCL-*g*-PDMAEMA copolymers, their quaternized cationic derivatives, and the characterization of their self-assembly behavior in water. To the best of our knowledge, amphiphilic graft copolymers with a PCL backbone and pH sensitive water-soluble side chains of P4VP or PDMAEMA have never been reported.

Experimental Part

Materials

Poly(ϵ -caprolactone) ($\bar{M}_n = 42\,500 \text{ g} \cdot \text{mol}^{-1}$, $\bar{M}_w = 80\,000 \text{ g} \cdot \text{mol}^{-1}$), lithium diisopropyl amide (LDA, 2 M in tetrahydrofuran (THF)/heptane/ethylbenzene), 2-(*N,N*-dimethylamino)ethyl methacrylate (DMAEMA, 98%), 4-vinylpyridine (4-VP, 95%), monomethoxy-poly(ethylene glycol) (MeO-PEG, $\bar{M}_n = 2\,000 \text{ g} \cdot \text{mol}^{-1}$), and iodomethane (CH_3I , >99%) were obtained from Aldrich (St. Quentin Fallavier, France). Ammonium chloride (NH_4Cl) was obtained from Fluka (St. Quentin Fallavier, France). MgSO_4 was obtained from Prolabo (Paris, France). Diethyl ether, dichloromethane, dimethylformamide, ethanol, and methanol were obtained from Riedel de Haën (St. Quentin Fallavier, France). All these chemicals were used as received. THF was obtained from Acros Organics (Noisy-le-Grand, France) and distilled over benzophenone/sodium until the formation of a deep blue color. Spectra/Por 6 dialysis membranes (MWCO 1000) were obtained from SpectrumLaboratories Inc. (Breda, Netherlands).

Synthesis of Poly(ϵ -caprolactone)-graft-Poly(4-vinylpyridine) (PCL-*g*-P4VP)

Typically, PCL (2 g, 17.5 mmol) was dissolved in anhydrous THF (200 mL) in a reactor equipped with mechanical stirring, and kept at $-78 \text{ }^\circ\text{C}$ under an argon atmosphere. A solution of LDA (2.9 cm³, 5.8 mmol) was injected with a syringe through a septum to form a macropolycarbanion (PCL^-) and the mixture was kept at $-78 \text{ }^\circ\text{C}$ for 30 min. 4VP (3.8 mL, 35 mmol) was then injected with a syringe through a septum into the reaction medium. The reaction was carried out for 2 h between -70 and $-50 \text{ }^\circ\text{C}$ with stirring. The mixture was then directly precipitated in an excess of cold deionized water. The precipitate was washed with cold ethanol and dried under vacuum (10^{-3} mbar) for several hours (Table 1, R1F1). The ethanol was further evaporated and the resulting oil was precipitated in diethyl ether. The precipitate was dried under vacuum (10^{-3} mbar) for several hours (Table 1, R2F2). Overall yield was 87% for PCL and 74% for P4VP.

Table 1. Characteristics of the synthesized PCL-*g*-P4VP and PCL-*g*-DMAEMA copolymers.

Run	Copolymer	Monomer/ ϵ -CL units	T °C	Fraction and wt.-%	ϵ -CL/4VP or ϵ -CL/ DMAEMA ^{a)}	Global yield for ϵ -CL	Global yield for 4VP or DMAEMA	$\overline{M}_{n,SEC}$ ^{b)} g · mol ⁻¹	PDI	$T_c/T_m/T_g$ °C
Run 1	PCL- <i>g</i> -P4VP	2	-70, -50	R1F1-64	50/50	87	74	9 100	2.0	51/17/-
				R1F2-36	15/85			25 500	1.8	-/-/106
Run 2	PCL- <i>g</i> -P4VP	2	-40, -20	R2F1-100	12/88	23	67	-	-	-
Run 3	PCL- <i>g</i> -DMAEMA	1.5	-60, -50	R3F1-100	80/20	-	-	27 400	2.0	58/28/-
Run 4	PCL- <i>g</i> -DMAEMA	2.5	-70, -60	R4F1-50	80/20	62	82	15 400	2.8	51/7/-
				R4F2-50	70/30			-	-	-

^{a)}Molar ratio; ^{b)}SEC with THF as the eluent.

Synthesis of Poly(ϵ -caprolactone)-graft-poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PCL-*g*-PDMAEMA)

The reaction was carried out under the same conditions as those used for the synthesis of PCL-*g*-P4VP and was carried out for 2 h between -70 and -50 °C with stirring. The mixture was then hydrolyzed by 200 mL of a 2 M aqueous solution of NH₄Cl. The pH was adjusted to ≈ 7 by addition of 37% HCl. The reaction medium was then extracted with 2 \times 200 mL of dichloromethane. The combined organic phases were washed with 2 \times 200 mL of deionized water, dried over anhydrous MgSO₄, and filtered. The solvent was distilled off under reduced pressure until a thick oil was obtained and poured into a large volume of cold methanol that contained HCl. The precipitate was filtered out and dried under vacuum (10⁻³ mbar) for several hours (Table 1, R3F1 and R4F1). The milky filtrate was further centrifuged. The obtained solid was dried under vacuum (10⁻³ mbar) for several hours (Table 1, R4F2). The overall yield was 62% for PCL and 82% for PDMAEMA.

Quaternization of PCL-*g*-P4VP and PCL-*g*-PDMAEMA

Typically, the copolymer was dissolved in DMF (10 mmol in 100 mL) and a large excess of CH₃I (50 equivalents) was added. The reaction was carried out for 24 h at 65 °C. After completion, solvent, iodomethane, and part of the DMF were evaporated. The residue was diluted with deionized water and dialyzed against deionized water using dialysis tubes (MWCO \approx 1 000 Da). Quaternized copolymers were obtained by lyophilization of the final aqueous solution.

Synthesis of PCL-*g*-(P4VP;PEG) Brush Copolymers

Typically, PCL-*g*-P4VP (Table 1, R1F1, 2.15 mmol) was dissolved in an anhydrous mixture of THF and dimethyl sulfoxide (DMSO) (5:1, 200 mL) in a reactor equipped with mechanical stirring, and kept at -78 °C under argon atmosphere. A solution of LDA (3 mmol, 1.4 equivalents per ϵ -CL unit) was injected with a syringe through a

septum to form a macropolycarbanion (PCL⁻-*g*-P4VP) and the mixture was kept at -78 °C for 30 min. In parallel, monomethoxy-poly(ethylene glycol) (MeO-PEG, 2.5 mmol) was activated by bromoacetylation (bromoacetyl bromide, 0.25 mmol) following a method already described.^[11] One equivalent of the activated MeO-PEG with regard to the ϵ -CL units was dissolved in anhydrous THF (100 mL). The reaction was carried out for 1 h between -70 and -50 °C with stirring. The solution was concentrated under reduced pressure, and precipitated into diethyl ether. The precipitate was dissolved in deionized water and dialyzed against deionized water (MWCO \approx 1 000 Da). Finally, the terpolymer was precipitated from the aqueous phase by adjusting the pH to \approx 10–11, filtered off, and dried under vacuum (10⁻³ mbar) for several hours. Overall yields in PCL and P4VP were 82 and 40%, respectively.

Hydrolytic Degradation of PCL-*g*-P4VP and PCL-*g*-PDMAEMA

Typically, the copolymer (0.01 mole) was dissolved in THF (50 mL) and the solution was poured into a round-bottomed flask equipped with a septum. An aqueous solution (2 mL) of NaOH 20% (w/v) was then added with stirring. Degradation was carried out at room temperature with stirring, and aliquots were taken at fixed intervals and analyzed by size-exclusion chromatography (SEC). After total degradation of the PCL chains, residual non-hydrolyzable polymer was precipitated in MeOH, dried under vacuum (10⁻³ mbar), and analyzed by SEC and ¹H NMR spectroscopy.

Characterization

Molecular weights were measured by SEC using a Waters chromatograph fitted with a 60 cm long 5 μ m mixed C PLgel column as the stationary phase, THF at a flow rate of 1 cm³ · min⁻¹ was used as the mobile phase, and a Waters 410 refractometer as detector. For SEC analysis of water-soluble copolymers, a mixture of water/methanol (90:10; v/v) was used as the mobile phase in association with a Waters chromatograph fitted with two 30 cm

PlaquagelOH 40 8 μm columns as the stationary phase. Typically, polymer (10 mg) was dissolved in the eluent (2 mL) and the resulting solution was filtered on a 0.45 μm Millipore filter prior to injection in a 20 μL sample loop. Molecular weights \overline{M}_n and \overline{M}_w were expressed with respect to polystyrene or poly(ethylene glycol) standards.

^1H and ^{13}C NMR spectra were recorded at room temperature on a Bruker spectrometer operating at 300 or 50 MHz, respectively. Deuterated chloroform or dimethyl sulfoxide was used as solvent, and chemical shifts were expressed in ppm from the signal of tetramethylsilane (TMS).

FT-IR spectra were recorded on a Perkin-Elmer 1760 spectrometer, polymers being cast on NaCl plates from organic solution or ZnS plates for aqueous solution.

The mean diameter of micelle-like objects was determined by dynamic light scattering at 90° using a Spectra-Physics Stablite 2017 LASER (514 nm, 200 mW, 30 A) and a Brookhaven 9863 collimator. Samples were filtered using a 0.45 μm filter, kept at 25°C in a decaline bath, and data were treated by the default CONTIN method.

Thermal transitions were measured under a nitrogen flow by differential scanning calorimetry using a DSC6 Perkin Elmer system. Scanning conditions were the following: heating from 20 to 150°C at $10^\circ\text{C}\cdot\text{min}^{-1}$, cooling to -70°C at $10^\circ\text{C}\cdot\text{min}^{-1}$ and a second heating scan to 150°C at $10^\circ\text{C}\cdot\text{min}^{-1}$. Thermal transitions were measured on the second heating scan.

Results and Discussion

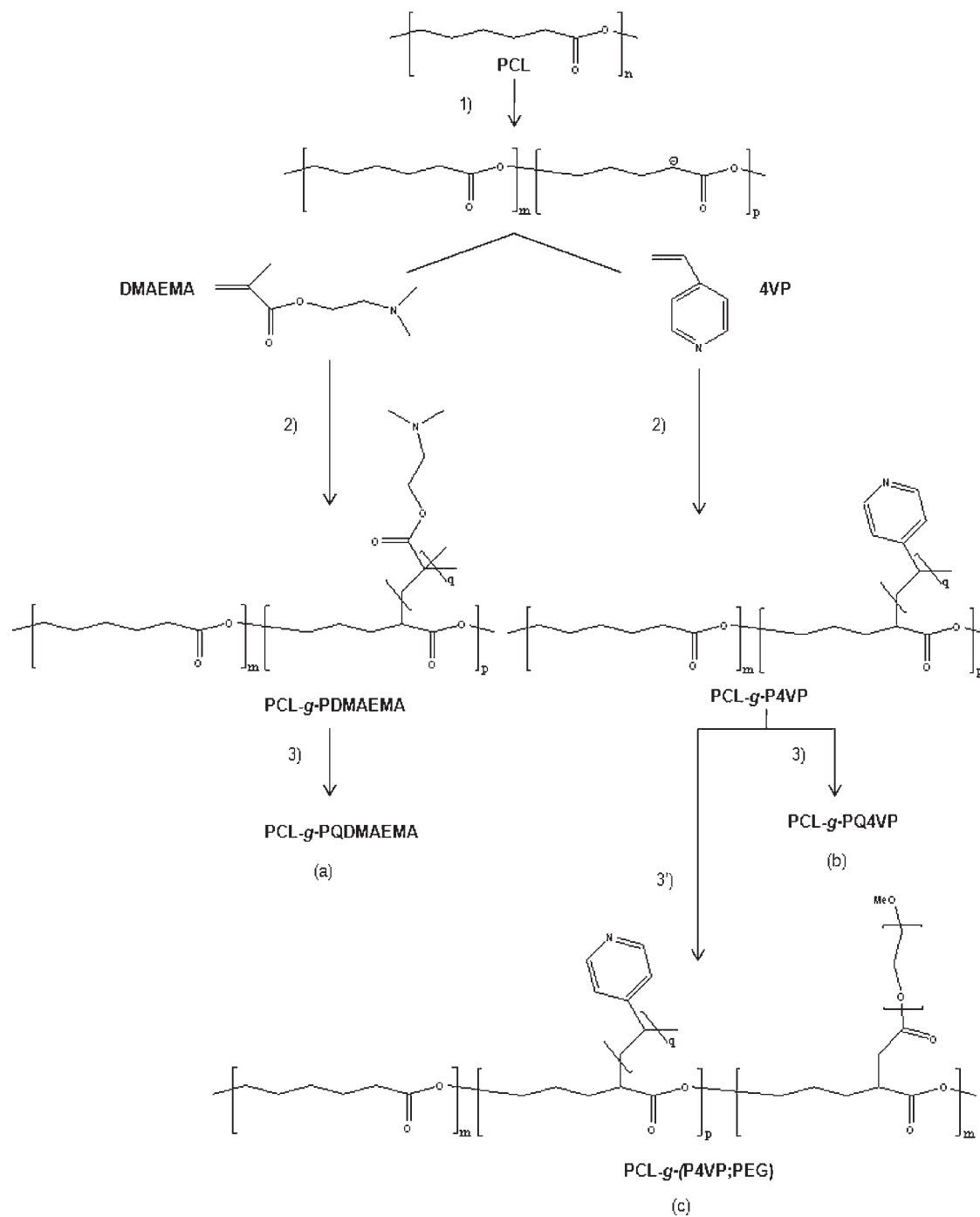
The aim of this work was to synthesize original degradable and cationic amphiphilic graft copolymers with a hydrophobic skeleton and hydrophilic side chains. We recently proposed a simple general method to chemically modify aliphatic polyesters chains, especially PCL.^[29] This method is based on the anionic activation of the PCL chain by removal of a proton of the methylene group in the α position of the ester carbonyl, using a non-nucleophilic base such as lithium diisopropyl amide (LDA). In a second step, various electrophilic reagents were added to the macropolycarbanion generated in the first step. The method was applied to the formation of PCL-based graft copolymers by a grafting 'from' or a grafting 'onto' technique.^[11,16] In this article we report the synthesis of novel PCL-*g*-P4VP and PCL-*g*-PDMAEMA copolymers by the grafting 'from' strategy (Scheme 1).

Synthesis and Quaternization of PCL-*g*-P4VP

Two copolymers have been synthesized at different temperatures (Table 1, runs 1 and 2) to obtain two compositions determined by ^1H NMR. Figure 1 shows a typical spectrum of PCL-*g*-P4VP with signals that correspond to PCL (I1, I2 and I3) and P4VP (I1 and I4) in which typical peaks of the pyridine moiety are shown at approx. 6.4 and 8.2 ppm.

Depending on the temperature of the polymerization step, different copolymer compositions are obtained. At high temperatures (between -40 and -20°C), the copolymer contained a higher ratio of P4VP (Table 1, R2F1). This is a consequence of the occurrence of chain scissions of PCL, which are more pronounced at a higher temperature. As a result, soluble PCL oligomers were eliminated during the purification process and the final isolated copolymer contained more 4VP units. On the other hand, at low temperatures (between -70 and -50°C) balanced compositions were obtained, the main fraction being composed of 50% of each monomer (Table 1, R1F1). A minor fraction (R1F2) that contained 85% of P4VP was formed during the last polymerization stage when the temperature rose to -50°C . The polymerization rate of 4VP increased with temperature, to give longer chains of P4VP. A consequence of the faster P4VP chain growth is the absence of melting and crystallization temperatures (T_m , T_c), and the high glass transition temperature (T_g) (106°C) in copolymer R1F2, which is characteristic of P4VP ($T_g \approx 140^\circ\text{C}$) segments. Contrarily, the physico-chemical properties of copolymer R1F1 ($T_m = 51^\circ\text{C}$ and $T_c = 17^\circ\text{C}$) are close to those of pure PCL ($T_m = 57^\circ\text{C}$ and $T_c = 24^\circ\text{C}$). In all cases there is only one T_g and/or one T_m , which shows the probable compatibility of the amphiphilic structures. The structure of the copolymer was studied by the hydrolytic degradation of the PCL backbone followed by SEC analysis in water/methanol (90:10; v/v) of the residual P4VP chains. \overline{M}_n was $710\text{ g}\cdot\text{mol}^{-1}$ and PDI = 1.6. Taking into account the molar composition (50/50) given by NMR spectroscopy, copolymer R1F1 was assumed to have a PCL backbone with a degree of polymerization \overline{DP} of 40, substituted by an average of 8 short side chains ($\overline{DP} \approx 7$) of P4VP. Interestingly, it is accepted that short chains of methacrylic or methacrylamide-type polymers ($\overline{M}_n < 30\text{ kDa}$) can be cleared from the body by renal excretion.^[30,31] PCL-*g*-P4VP can thus be considered as fully bioresorbable since the PCL backbone is biodegradable and P4VP chains are short enough to be excreted.

Behaviour of the main fraction of R1F1 in aqueous solution has been evaluated. Taking into account the low degree of protonation of the P4VP chains, because of the low $\text{p}K_a$ of pyridine ($\approx 4-5$), R1F1 was not water soluble in deionized water at pH 7.0 and only water-dispersible at pH 3 despite a 50% ratio of pyridine groups. For this reason, quaternization of the pyridine moiety has been carried out using iodomethane, to yield pyridinium groups (Scheme 1b). The FT-IR spectrum showed the presence of the stretching vibration bands of ammonium groups at 2410 and 2760 cm^{-1} . ^1H NMR spectra showed a shift of the aromatic peaks of the pyridine group from 6.4 and 8.2 ppm to 7.6 and 8.5 ppm, respectively. Quaternization was found to be quantitative and did not change the overall composition of the copolymer. The quaternized copolymer



Scheme 1. Synthesis of a) PCL-*g*-PQDMAEMA, b) PCL-*g*-PQ4VP, and c) PCL-*g*-(P4VP;PEG) using a three-step reaction with 1) anionic activation of PCL, 2) anionic polymerization of monomers by a grafting from method using PCL macropolymer as a macroinitiator, and 3) quaternization of amino groups or 3') anionic activation of PCL-*g*-P4VP and grafting of activated MeO-PEG

(PCL-*g*-PQ4VP) has a lower melting temperature (46 °C) compared to PCL-*g*-P4VP (51 °C) and is only slightly crystalline ($\Delta H_f = 8 \text{ J} \cdot \text{g}^{-1}$ vs $44 \text{ J} \cdot \text{g}^{-1}$ for PCL). SEC analysis in a mixture of water and methanol (90:10) showed two peaks, a main peak for $\bar{M}_n = 2500 \text{ g} \cdot \text{mol}^{-1}$, and a minor

one for $\bar{M}_n = 45000 \text{ g} \cdot \text{mol}^{-1}$. The second peak may reflect the presence of aggregates in the solution, as a result of the amphiphilic character of PCL-*g*-PQ4VP. Accordingly, light scattering of a $10 \text{ mg} \cdot \text{mL}^{-1}$ solution of PCL-*g*-PQ4VP in deionized water showed that PCL-*g*-PQ4VP formed

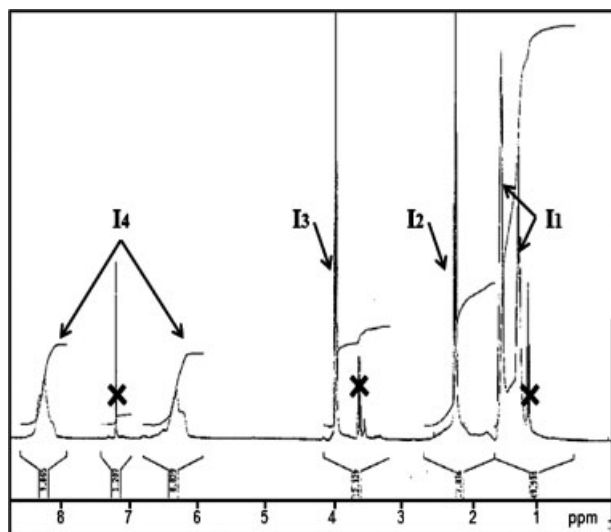


Figure 1. ^1H NMR spectrum of PCL-*g*-P4VP (fraction R1F1) in CDCl_3 (NMR solvent and traces of solvent used for purification are indicated by black cross).

micelle-like objects in water with a mean diameter of ≈ 75 nm (Figure 2a).

In addition to the quaternization of amino groups, another strategy was employed to obtain a water-soluble PCL-*g*-P4VP copolymer. This strategy is based on the grafting of an activated hydrophilic MeO-PEG segment

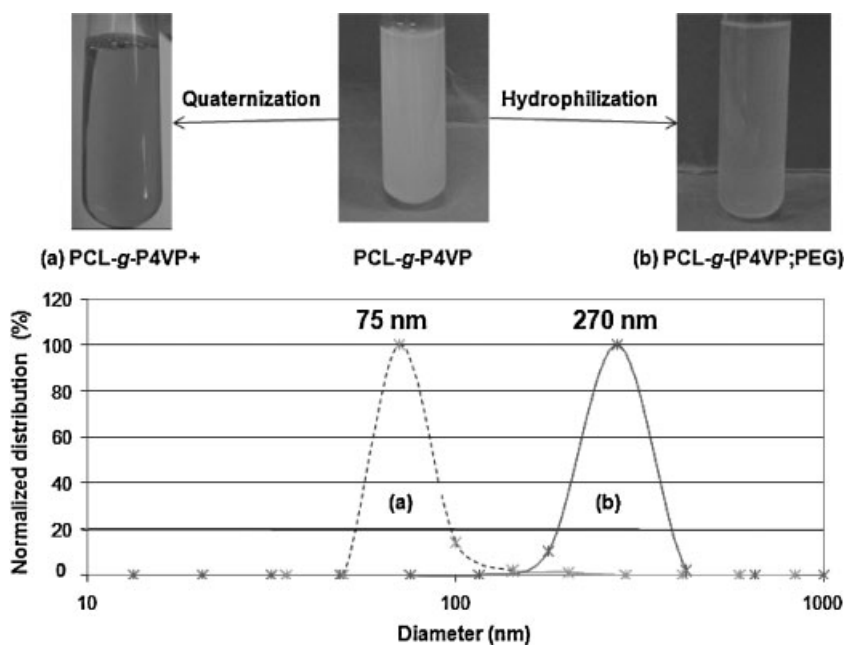


Figure 2. Pictures of aqueous solutions of PCL-*g*-P4VP (acidic conditions, pH 3), PCL-*g*-PQ4VP (neutral conditions, pH 7), and PCL-*g*-(P4VP;PEG) (acidic conditions, pH 3) at $10 \text{ mg} \cdot \text{mL}^{-1}$ and size distribution obtained by dynamic light scattering analysis for PCL-*g*-PQ4VP (a) and PCL-*g*-(P4VP;PEG) (b).

onto PCL-*g*-P4VP (Scheme 1c). Activated MeO-PEG was obtained by reaction with bromoacetyl chloride as already described in a previous article.^[11] PCL-*g*-P4VP has been re-activated by reaction with LDA to form a new macropolycarbanion, which reacts with the activated MeO-PEG. After purification by precipitation and dialysis, the terpolymer PCL-*g*-(P4VP;PEG) was characterized by ^1H NMR spectroscopy in CDCl_3 . Signals of the three units were detected, in particular a new peak at 3.65 ppm, which corresponds to the protons of PEG chains. The percentage in monomeric units, i.e., CL/4VP/EG, was 65/29/6, which corresponded to the grafting of one MeO-PEG chain on the PCL backbone. It is worth noting that the CL/4VP ratio has changed, compared to its value in copolymer R1F1 (CL/4VP = 1). The explanation was found by analysis of the basic aqueous medium used to precipitate the terpolymer. A minor fraction (5% by weight) was recovered and analyzed by ^1H NMR spectroscopy to showing a PCL/P4VP/PEG percentage of 19/75/6, respectively. The composition of this fraction is a consequence of a new cleavage of the PCL skeleton during reactivation in the presence of LDA. However, 89% of the PCL and 40% of the P4VP were recovered in the main fraction. In addition, despite the rather low PEG proportion in the terpolymer, the hydrophilic PEG chains increased the water solubility of the macromolecule. The PCL-*g*-P4VP fraction (R1F1) that contained 50% of 4VP units is water dispersible at pH 3, while PCL-*g*-(P4VP;PEG) is water soluble at the same pH,

despite a higher CL proportion (65%). A PCL-*g*-(P4VP;PEG) SEC chromatogram in THF showed a molecular weight, \bar{M}_n , of $5000 \text{ g} \cdot \text{mol}^{-1}$, instead of $\bar{M}_n = 9100 \text{ g} \cdot \text{mol}^{-1}$ in the starting PCL-*g*-P4VP. The apparent decrease of molecular weight may be a consequence of chain cleavage of the PCL backbone, and also of interactions of PEG chains on the SEC columns, which increase the elution time. Micelle-like objects (mean diameter ≈ 270 nm) were detected by dynamic light scattering of acidic solutions of PCL-*g*-(P4VP;PEG) ($10 \text{ mg} \cdot \text{mL}^{-1}$, pH 3) (Figure 2b). This diameter is larger than that of PCL-*g*-PQ4VP, because of the extension of the relatively long PEG chains ($\bar{M}_n = 2000 \text{ g} \cdot \text{mol}^{-1}$) in water and the partial protonation of 4VP residues at pH 3. Using the Henderson–Hasselbach equation and considering a $\text{p}K_a$ of 4–5 for the pyridine groups in the terpolymer, the ratio between the non-protonated (4VP) and the protonated (4VP $^+$) residues is in the range 10^{-2} to

Table 2. Characteristics of the synthesized PCL-*g*-DMAEMA copolymers and their cationic PCL-*g*-QMAEMA derivatives.

Quaternization	ϵ -CL/DMAEMA	ϵ -CL/QMAEMA	Initial $\overline{M}_{n,SEC}^{a)}$	Final $\overline{M}_{n,SEC}^{b)}$	Mean diameter
			$g \cdot mol^{-1}$	$g \cdot mol^{-1}$	
Q1	80/20	80/20	27 400	2 000	123
Q2	80/20	80/20	15 400	1 400	90
Q3	70/30	45/55	1 650	1 300	64

^{a)}SEC with THF as the eluent; ^{b)}SEC with water/MeOH (90:10; v/v) as the eluent.

10^{-1} . The presence of PEG side chains and the absence of quaternization give an amphiphilic character, which leads to larger objects than those obtained with the cationic PCL-*g*-PQ4VP.

Synthesis of PCL-*g*-PDMAEMA

PCL-*g*-PDMAEMA copolymers have been synthesized under similar conditions (Scheme 1a). Depending on the run, the ratio of DMAEMA to CL units and the polymerization temperature have been changed, and various compositions were obtained leading to various solubility properties (Table 1, run 3,4). PDMAEMA ($pK_a \approx 7.4$) is almost not protonated at neutral pH and when the proportion of PCL is higher than 70%, the copolymer is not soluble in deionized water. Insoluble fractions R3F1 and R4F1 and soluble fraction R4F2 have been quantitatively quaternized with iodomethane to give water-soluble degradable cationic copolymers. The quaternized copolymers poly(ϵ -caprolactone)-*graft*-poly(2-(*N,N,N*-trimethylammonium) ethyl methacrylate) (PCL-*g*-PQDMAEMA) have been analyzed by i) SEC in water/methanol (90:10), ii) dynamic light scattering carried out on $10 \text{ mg} \cdot \text{mL}^{-1}$ neutral aqueous solutions, and iii) ^1H NMR in D_2O and $\text{DMSO}(d_6)$. Results are summarized in Table 2. Quaternization was characterized in the ^1H NMR spectra by a shift of the methyl signal of the amino groups from 2–2.5 ppm to 3–3.25 ppm (depending on the NMR solvent used). For a given composition, the size of the objects in deionized water decreased with the molecular weight (Table 2, runs Q1 and Q2; Figure 3). It is also worth noting that the diameter decreased when the proportion of QDMAEMA increased (Table 2 fractions Q2 and Q3, Figure 3). It is of interest to compare our results with the ones obtained by other groups. Xu et al.^[28] have

synthesized brushes of PCL and PDMAEMA by free radical copolymerization of PCL and PDMAEMA macromonomers. They obtained micelles with a diameter of 55 nm (PCL/PDMAEMA = 2.4) by a solvent displacement method. In the present work, a simple dissolution in deionized water of PCL-*g*-PQDMAEMA copolymer Q3 led to similar micelles (diameter ≈ 64 nm) (PCL/PQDMAEMA = 1). Because these copolymers have a similar architecture, one can expect that, like in PCL/PDMAEMA brushes, PCL-*g*-PQDMAEMA formed much more stable micelles, with a CMC 100 times lower than in the corresponding diblock copolymers. Inverse structures of PQDMAEMA-*g*-PCL synthesized by Mespouilles et al.^[27] present a poor water-solubility under neutral and even acidic conditions when the CL percentage is above 50%. When the CL ratio is below 50% large aggregates are obtained at pH = 4.8 (diameters ≈ 250 and 350 nm for concentrations of 0.1 and $1 \text{ g} \cdot \text{L}^{-1}$, respectively). It is worth noting that the water solubility of the PCL-*g*-PDMAEMA synthesized in this work was obtained even under neutral conditions as soon as the CL proportion was below 70%. This improved water solubility, compared to that of PDMAEMA-*g*-PCL, might be explained by the particular conformation of the macromolecules in water where hydrophilic segments can easily adopt an extended

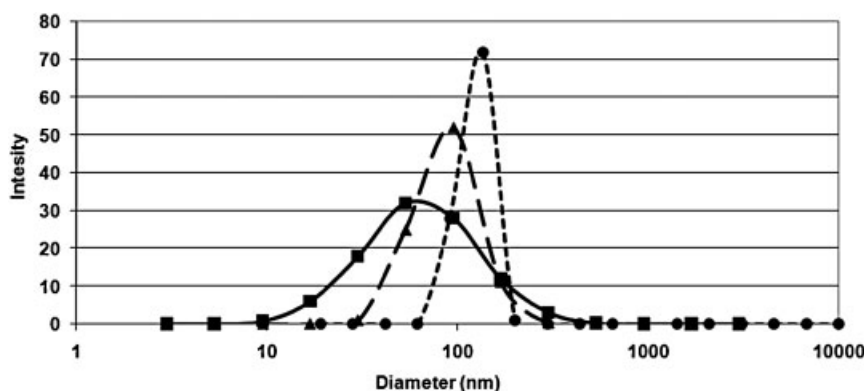
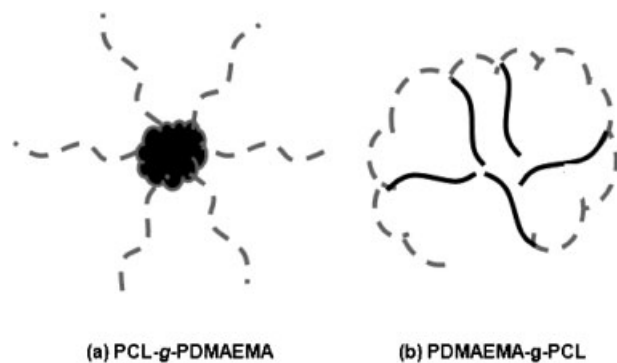


Figure 3. Size distribution of PCL-*g*-PQDMAEMA micellar aggregates in deionized water obtained by dynamic light scattering analysis of solutions at $10 \text{ mg} \cdot \text{mL}^{-1}$ for Q1 (circles), Q2 (triangles), and Q3 (squares).



Scheme 2. Schematic representation of the expected structures of the micellar objects obtained by dissolution of a) PCL-g-PDMAEMA and b) PDMAEMA-g-PCL in aqueous medium (PCL segments = black full line, PDMAEMA segments = grey dot line).

conformation in water, to give an improved stabilization of the hydrophobic PCL core (Scheme 2).

Conclusion

Novel amphiphilic graft copolymers PCL-g-P4VP and PCL-g-PDMAEMA have been synthesized. They present an original invert structure with a hydrophobic backbone and hydrophilic side chains. Because of the low pK_a of pyridine groups, the PCL-g-P4VP copolymers are not soluble in water at neutral pH and only water-dispersible at pH 3. Quantitative quaternization with iodomethane gives fully water-soluble cationic PCL-g-PQ4VP that contains 50% ϵ -CL and 50% Q4VP units, which spontaneously forms micellar objects in deionized water with a mean diameter of 75 nm. As an alternative method to increase the hydrophilicity of PCL-g-P4VP, PEG chains have been grafted on the copolymer. A non-ionic water-soluble terpolymer bearing a PCL backbone substituted by P4VP and PEG side chains, namely PCL-g-(P4VP;PEG), was obtained. In deionized water, this terpolymer formed nanometric micellar objects. All PCL-g-PDMAEMA copolymers were water-soluble provided the DMAEMA proportion was above 30%. Because of their inverted structure, improved water solubility was obtained compared to PDMAEMA-g-PCL. Quaternization of various PCL-g-PDMAEMA copolymers led to cationic PCL-g-QDMAEMA which spontaneously forms micellar objects whose diameters are in the range 65 to 125 nm.

Acknowledgements: The authors thank RHODIA and the French Ministry of Education and Research for the CIFRE fellowship of Benjamin Nottelet, as well as Sylvie Hunger for NMR spectra.

Keywords: amphiphiles; biodegradable; cationic polymer; graft copolymers; micelles; poly(ϵ -caprolactone); poly(dimethylaminoethyl methacrylate); poly(4-vinyl pyridine); quaternization

- [1] S. Garnier, A. Laschewsky, J. Storsberg, *Tenside Surfact. Det.* **2006**, *43*, 88.
- [2] A. Halperin, *Polym. Rev.* **2006**, *46*, 173.
- [3] A. Laschewsky, *Adv. Polym. Sci.* **1995**, *124*, 1.
- [4] L. Y. Qiu, C. Zheng, Y. Jin, K. J. E. Zhu, *Expert Opin. Ther. Pat.* **2007**, *17*, 819.
- [5] D. Domurado, M. Vert, *J. Biomater. Sci., Polym. E.* **2007**, *18*, 287.
- [6] I. L. G. Shin, S. Y. Kim, Y. M. Lee, C. S. Cho, Y. K. Sung, *J. Controlled Release* **1998**, *51*, 1.
- [7] S. Y. Kim, Y. M. Lee, *Biomaterials* **2001**, *22*, 1697.
- [8] M. H. Huang, L. M. Suming, J. Coudane, M. Vert, *Macromol. Chem. Phys.* **2003**, *204*, 1994.
- [9] B. Bogdanov, A. Vidts, A. Van Den Bulcke, R. Verbeeck, E. Schacht, *Polymer* **1998**, *39*, 1631.
- [10] F. Quaglia, L. Ostacolo, G. De Rosa, M. I. La Rotonda, M. Ammendola, G. Nese, G. Maglio, R. Palumbo, C. Vauthier, *Int. J. Pharm.* **2006**, *324*, 56.
- [11] S. Ponsart, J. Coudane, J. McGrath, M. Vert, *J. Bioact. Compat. Pol.* **2002**, *17*, 417.
- [12] R. Riva, S. Schmeits, C. Jerome, R. Jerome, P. Lecomte, *Macromolecules* **2007**, *40*, 796.
- [13] C. Lemarchand, R. Gref, S. Lesieur, H. Hommel, B. Vacher, A. Besheer, K. Maeder, P. Couvreur, *J. Controlled Release* **2005**, *108*, 97.
- [14] L. Liu, Y. Li, H. Liu, Y. Fang, *Eur. Polym. J.* **2004**, *40*, 2739.
- [15] J. H. Jeong, H. S. Kang, S. R. Yang, J. D. Kim, *Polymer* **2003**, *44*, 583.
- [16] B. Nottelet, A. El Ghzaoui, J. Coudane, M. Vert, *Biomacromolecules* **2007**, *8*, 2594.
- [17] S. C. Lee, Y. K. Chang, J. S. Yoon, C. H. Kim, I. C. Kwon, Y. H. Kim, S. Y. Jeong, *Macromolecules* **1999**, *32*, 1847.
- [18] S. C. Lee, S. W. Kang, C. Kim, I. C. Kwon, S. Y. Jeong, *Polymer* **2000**, *41*, 7091.
- [19] X. T. Shuai, T. Merdan, F. Unger, M. Wittmar, T. Kissel, *Macromolecules* **2003**, *36*, 5751.
- [20] T. W. Chung, K. Y. Cho, H. C. Lee, J. W. Nah, J. H. Yeo, T. Akaike, C. S. Cho, *Polymer* **2004**, *45*, 1591.
- [21] B. S. Lele, J. C. Leroux, *Macromolecules* **2002**, *35*, 6714.
- [22] A. Leiva, F. H. Quina, E. Araneda, L. Gargallo, D. Radic, *J. Colloid Interface Sci.* **2007**, *310*, 136.
- [23] S. C. Chan, S. W. Kuo, C. H. Lu, H. F. Lee, F. C. Chang, *Polymer* **2007**, *48*, 5059.
- [24] F. Bougard, M. Jeusette, L. Mespouille, P. Dubois, R. Lazzaroni, *Langmuir* **2007**, *23*, 2339.
- [25] S. Motala-Tirrol, D. Jhurry, *Eur. Polym. J.* **2007**, *43*, 3042.
- [26] H. Liu, J. A. Xu, J. L. Jiang, J. Yin, R. Narain, Y. L. Cai, S. Y. Liu, *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 1446.
- [27] L. Mespouille, P. Degee, P. Dubois, *Eur. Polym. J.* **2005**, *41*, 1187.
- [28] P. S. Xu, H. D. Tang, S. Y. Li, J. Ren, E. Van Kirk, W. J. Murdoch, M. Radosz, Y. Q. Shen, *Biomacromolecules* **2004**, *5*, 1736.
- [29] S. Ponsart, J. Coudane, M. Vert, *Biomacromolecules* **2000**, *1*, 275.
- [30] M. Kissel, P. Peschke, V. Subr, K. Ulbrich, J. Schuhmacher, J. Debus, E. Friedrich, *PDA J. Pharm. Sci. Technol.* **2001**, *55*, 191.
- [31] X. Jiang, M. C. Lok, W. E. Hennink, *Bioconjugate* **2007**, *18*, 2077.