Polysaccharides Grafted with Polyesters: Novel Amphiphilic Copolymers for Biomedical Applications

ARTICLE *in* MACROMOLECULES · DECEMBER 2002

Impact Factor: 5.8 · DOI: 10.1021/ma021132a

3 AUTHORS, INCLUDING:

Ruxandra Gref
Université Paris-Sud 11
148 PUBLICATIONS **9,763** CITATIONS
SEE PROFILE

Patrick Couvreur
Université Paris-Sud 11
585 PUBLICATIONS **25,807** CITATIONS
SEE PROFILE

Articles

Polysaccharides Grafted with Polyesters: Novel Amphiphilic Copolymers for Biomedical Applications

Ruxandra Gref,*,† Jaqueline Rodrigues,‡ and Patrick Couvreur†

School of Pharmacy, UMR 8612 CNRS, Châtenay Malabry, France, and Doutorado Ciencias Biologicas, Universidade Federal de Pernambuco, Recife/PE, Brazil

Received July 16, 2002; Revised Manuscript Received November 1, 2002

ABSTRACT: New amphiphilic polysaccharides with controlled structure were synthesized by coupling between a carboxylic function present on preformed polyester chains and a hydroxyl group naturally present on polysaccharides. First, the synthesis of poly(ϵ-caprolactone) monocarboxylic acid (R−PCL−CO_2H) was carried out by ring-opening uncatalyzed polymerization of monomer in the presence of a carboxylic acid (R−CO_2H). R−PCL−CO_2H was then reacted with carbonyl diimidazole, and the resulting activated intermediate (imidazolide) was further reacted with dextran (Dex) at different molar ratios to obtain amphiphilic copolymers with various hydrophilic−lipophilic balance. The coupling reaction was followed by GPC, indicating a total conversion. The copolymers were further characterized by GPC, ^1H NMR, and FTIR. Nanoparticles of less than 200 nm, with potential interest for controlled release of bioactive compounds, were successfully prepared by using these new materials.

Introduction

One remarkable feature in drug delivery technology is the central role that polymer plays in the control of drug administration, transport, and delivery at the desired site of drug action. Encapsulation of active compounds needs to conceive new biocompatible polymers for the design of particulate carriers (nanoparticles) with engineered surface properties for targeting purpose. The concept of nanoparticle surface modification to control the specific interaction with target cells as well as the nonspecific interaction with blood components and phagocytic cells raises a question about the optimal polymer/copolymer composition. Even if polyesters such as poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA), and poly(ϵ caprolactone) (PCL) are now recognized as safe materials, none of the actual available polymers/copolymers are satisfactory. Indeed, either they are hydrophobic, thus activating the complement and leading to unwanted liver accumulation, or, when coated with hydrophilic chains such as poly(ethylene glycol) (PEG),[1,2] they do not possess reactive groups at their surface allowing ligand coupling. To address this issue, polysaccharide-based polymers represent an interesting alternative to PEG since poly- and/or oligosaccharides may have per se a lot of recognition functions allowing specific mucoadhesion or receptor recognition. For instance, fucosylated oligosaccharide ligands mediate cell−cell adhesion through binding to cell-surface selectins[3] and galactose containing oligosaccharides have affinity for asialoglycoprotein receptors in liver tumor cells.[4] Other polysaccharides like dextrans have molecular characteristics (hydrophilicity and mobility) able to prevent protein opsonization and complement activation, thus avoiding liver recognition. Thus, biocompatible polyesters with the functionality of polysaccharides should allow the design of ideal material for drug targeting.

However, there are only few examples of covalently linked polysaccharide−polyester copolymers in the literature. One strategy involved a catalytic ring-opening polymerization of monomers (lactide or caprolactone) in the presence of polysaccharides.[5−9] The major inconvenient of this approach is the difficulty to obtain controlled structures since all the hydroxyl groups on the polysaccharides, except the ones sterically hindered, initiate the polymerization.[7−9] Alternatively, it was recently suggested[10−12] that hydrophobization of polysaccharides is possible by bulk polymerization of monomers in the presence of partially protected (silylation) hydroxyl groups of polysaccharides, followed by deprotection. However, bulk polymerization lead to inhomogeneous substitution ratio and high polydispersities.[7−9] The resulting large number of grafted chains with variable length may mask the polysaccharidic backbone and modify its physicochemical properties. This is a major disadvantage for biomedical applications, where interactions of the biological environment with polymeric surfaces are of significant importance for bioadhesion, steric repulsion of seric proteins, or targeting.

In addition, ring-opening polymerizations were carried out using stannous octanoate as catalyst.[5−9] Although stannous octanoate is the most popularly used catalyst for the synthesis of biodegradable polyesters,

* Corresponding author. Present address: Faculté de Pharmacie, Université Paris Sud, UMR CNRS 8612, Tour D5, 1er étage, 5, Rue Jean-Baptiste Clément,92296 Châtenay Malabry, France. E-mail: ruxandra.gref@cep.u-psud.fr. Telephone: (33) 1 46 83 59 09. Fax: (33) 1 46 61 93 34.

† UMR 8612 CNRS.

‡ Universidade Federal de Pernambuco.

10.1021/ma021132a CCC: $22.00
Published on Web 12/05/2002

it is always chemically polluted by 2-ethylhexanoic acid so that uncontrolled secondary polymerization may occur.[13] It is worth mentioning that the degree of polymerization is often low, in particular when using stannous octanoate as catalyst.[6] Moreover, the rather high content in tin residues cannot be removed efficiently,[13] which makes the presence of this toxic compound in the resulting polymers another inconvenience for biomedical applications.

In this paper, we describe the synthesis of new comblike materials composed of a polysaccharidic backbone on which preformed polyester (PCL) chains were grafted by means of ester bridges. These polysaccharide−polyester copolymers with controlled structures were obtained by coupling between a carboxylic function present on the polyester chains and a hydroxyl group naturally existent on the polysaccharidic backbone. It was therefore necessary to first synthesize a PCL monocarboxylic acid (R−PCL−COOH) of predetermined molecular weight. Further activation of the carboxylic function allowed the direct coupling with dextran (Dex), which has been employed as a model of polysaccharide owing to its ability to prevent protein adsorption.[14] The resulting amphiphilic Dex−PCL$_n$ were characterized by various techniques. Moreover, a method has been developed to prepare nanoparticles with a mean diameter lower than 200 nm by using these new copolymers.

Experimental Section

Materials. ϵ-Caprolactone (>99%), capric acid (>99.9%), Dex from *Leuconostoc mesenteroides* with molecular weights 5×10^3 and 40×10^3 (as determined by GPC), named Dex 5K and Dex 40K were obtained from Fluka. DMSO and THF (HPLC quality) and carbonyldiimidazole (CDI) were purchased from Sigma-Aldrich. Regenerated cellulose membranes with a cutoff of 6000−8000 Da (Spectra/Por, Spectrum, Breda, The Netherlands) were used for dialysis.

Synthesis of R—PCL—COOH (R = C_9H_{19}−). Synthesis of low molecular weight [$(2-4) \times 10^3$] PCL monocarboxylic acid (R−PCL−COOH) was carried out by bulk polymerization of ϵ-caprolactone in the presence of capric acid at various molar ratios. Caprolactone was freshly distilled under CaH_2 in a vacuum before polymerization and capric acid was dried by azeotropic distillation.

For example, 20 g of ϵ-caprolactone and 1.88 g of capric acid (molar ratio 16) were weighed and put into a 50 mL round-bottomed flask which was fitted with a reflux condenserand connected to the vacuum/Ar line. The flask contents were thoroughly degassed under high vacuum using three freeze−pump−thaw cycles. Polymerizations were carried out under Ar, at 230 °C in a silicone oil bath. After 7 h, the reaction was stopped by dipping the flasks in ice. The obtained polymers were purified by four successive precipitations using THF as solvent and methanol as nonsolvent. The polymers were finally dried under vacuum. Polymer recovery yield was 75 wt %.

Synthesis of Dex—PCL$_n$. Dex and R−PCL−COOH were anhydrized by azeotropic distillation before the coupling reaction. To synthesize a polymer named Dex−PCL$_n$ where n is the number of grafted PCL chains per Dex macromolecule, a three-step reaction was carried out.

For example, 3.5 g of anhydrous R−PCL−COOH (M_n = 2100) and 290 mg of CDI (5% molar excess with regard to the COOH functions) were dissolved in 4 mL of anhydrous THF in a 50 mL round-bottomed reaction flask equipped with a reflux condenser and connected to the Ar/vacuum line. The flask was heated at THF reflux under Ar, upon which CO_2 formation was observed. After 3 h, when CO_2 was no longer observed, the reaction was stopped and THF was evaporated off. The so-obtained unisolated activated polymer (imidazolide) was dissolved in a minimal amount (~2 mL) of anhydrous DMSO, and 1.05 g of Dex 5K (23 wt %) solubilized by heating in a minimal amount (~6 mL) of anhydrous DMSO was added. The coupling reaction was carried on for minimum of 3 h at 130 °C under Ar. The reaction mixture was recovered and dialyzed against demineralized water. Precipitated polymer was isolated by centrifugation, the supernatant was discarded, and the polymer was washed again with water and THF to remove, respectively, traces of unreacted Dex and PCL. It was finally lyophilized and dried over phosphorus pentoxide. The copolymer recovery yield was higher than 80%.

To synthesize other Dex−PCL$_n$ copolymers, the weight ratio of Dex in the reaction mixture was varied from 5 to 33 wt %.

Polymer Characterization. Determination of Molecular Weight of R—PCL—COOH by Titration. An exact mass (m) of dried polymer (about 100 mg) was dissolved in 7 mL of pure acetone, and the COOH end groups were dosed with a 10^{-2} M solution of KOH in ethanol, in the presence of phenolphthalein. Average number molecular weights (M_n) were determined by the relationship

$$M_n = \frac{10^3 m}{cV}$$

where c (mol/L) is the concentration of the KOH solution (verified using a HCl solution of known concentration) and V (mL) is the added volume of KOH solution. M_n values were averaged on at least three independent measurements. The precision of this analysis was 5%.

Gel Permeation Chromatography (GPC). Dex−PCL$_n$ copolymers were analyzed by GPC using a triple detection system (Viscotek, Houston, TX). The GPC system is composed of a VE 7510 degasser (Viscotek), a VE 1121 pump (Viscotek), a Waters 712 WISP injector, a Waters 410 differential refractometer mounted in parallel with a Viscotek T60A dual (viscosity and light scattering) detector, and a Waters TCM heating column system. Polystyrene standards (Polymer Laboratories, Shropshire, U.K.) were used to determine molecular weights from universal calibration.

For the analysis of R−PCL−COOH polymers, the eluent was HPLC-grade THF at a flow rate of 1 mL/min. A GMH-HR M (Viscotek) column heated at 40 °C was used. The injected volumes were 100 μL and the polymer concentration was 5 mg/mL.

For the analysis of Dex and Dex−PCL$_n$ samples, the mobile phase was *N*,*N*-dimethylacetamide (DMAC) containing 0.4% LiBr at a flow rate of 0.5 mL/min. The injected volumes were 100 μL. Sample concentrations ranged from 5 to 10 mg/mL. To achieve the best resolutions, two GMH-HR H columns were mounted in series for samples containing Dex 5K and a GMH-HR N column was used for samples containing Dex 40K. The columns were heated at 60 °C.

The number n of polyester chains grafted per Dex macromolecules was calculated by subtracting the determined M_w of Dex (5000) from the M_w of Dex−PCL$_n$ copolymers and dividing by M_w of polyester grafts (2100).

^{1}H NMR and FTIR Analysis. ^{1}H NMR spectra were recorded with a 200 MHz Bruker B-ACS 60 spectrometer.

R−PCL−CO_2H samples were analyzed in chloroform-*d* (>99.8%, SDS, Peypin, France) and DMSO-d_6 (99.9 atom % deuterium (D), Sigma-Aldrich, Steinheim, Germany), whereas Dex−PCL$_n$ samples were dissolved in DMSO-d_6. Infrared spectra of dried polymer powders were recorded using a Bruker Vector 22 spectrometer. A total of 16 scans were averaged for each sample.

Nanoparticle Preparation. First, 5 mg of Dex−PCL$_n$ copolymer, 1 mL of dichloromethane, and 5 mL of demineralized water containing 0.1% (w/v) sodium cholate were stirred together for 5 min at room temperature. The emulsions thus formed were sonicated (60 s, 20 W, pulses of 1 s each, Vibra Cell, Sonics & Materials Inc., Danbury, CT). After solvent evaporation (rotary evaporator, 30 min, room temperature), the resulting nanoparticle volume diameter was determined using a particle size distribution analyzer (PL-PSDA, Polymer Laboratories, Shropshire, U.K.). The eluent was demineralized

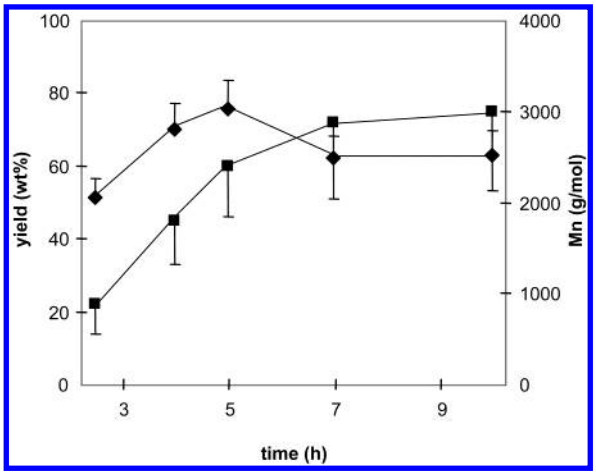

Figure 1. Yield (■) of R−PCL−COOH (R = C_9H_{19}) recovery and average number molecular weight (◆) of R−PCL−COOH polymers as a function of reaction time. The molar ratio caprolactone:capric acid in the reaction mixture was 16:1.

water containing 0.1% (w/v) Pluronic F68, at a flow rate of 2.1 mL/min. A "cartridge type 2" column (Polymer Laboratories), with a separation domain of 20−1500 nm, was used to separate the nanoparticles on the basis of their hydrodynamic radius. To detect the nanoparticles, absorbance was monitored at 254 nm.

Results and Discussion

PCL monocarboxylic acids (R−PCL−CO_2H) obtained by polymerization of ϵ-caprolactone in the presence of monocarboxylic acids are key compounds in the synthesis of amphiphilic polysaccharide−polyester copolymers with controlled structures. The molecular weight of these polymers should be a compromise. Indeed, for coupling reactions with polysaccharides, short PCL chains are required to increase the reaction rate. On the other hand, long PCL chains are needed to achieve the desired hydrophilic lipophilic balance (HLB) for nanoparticle production following an emulsification process, without increasing too much the number of grafted side chains. Under the polymerization conditions used in this work, the molecular weights M_n of the isolated polymers were in the range $(2-3) \times 10^3$ (Figure 1), suitable for grafting to Dex. The obtained M_n were practically independent of the ratio monomer/acid. Similar findings were reported in the case of ϵ-caprolactone polymerization in the presence of succinic acid.[11] According to the results in Figure 1, a 7 h reaction time was chosen because it enables one to obtain a satisfying polymer recovery yield (∼70 wt %) and appropriate molecular weights (2100−2500). A typical ^{1}H NMR spectrum and peak assignments for the R−PCL−COOH (R = C_9H_{19}) polymers are shown in Figure 2. The proton of the carboxylic function was not visible at ppm higher than 7 (very weak signal).

Average number and weight molecular weights (M_n, M_w) of these polymers were determined both by GPC and by dosage of the acidic end groups. After four successive purifications by precipitation, M_n values were constant (<5% variation) indicating that the R−PCL−COOH polymers were exempt of traces of unreacted acid. Less than 10% differences were found between the M_n determined by dosage of carboxylic end groups and by GPC (results not shown). According to GPC data, polydispersity indexes were low (<1.5).

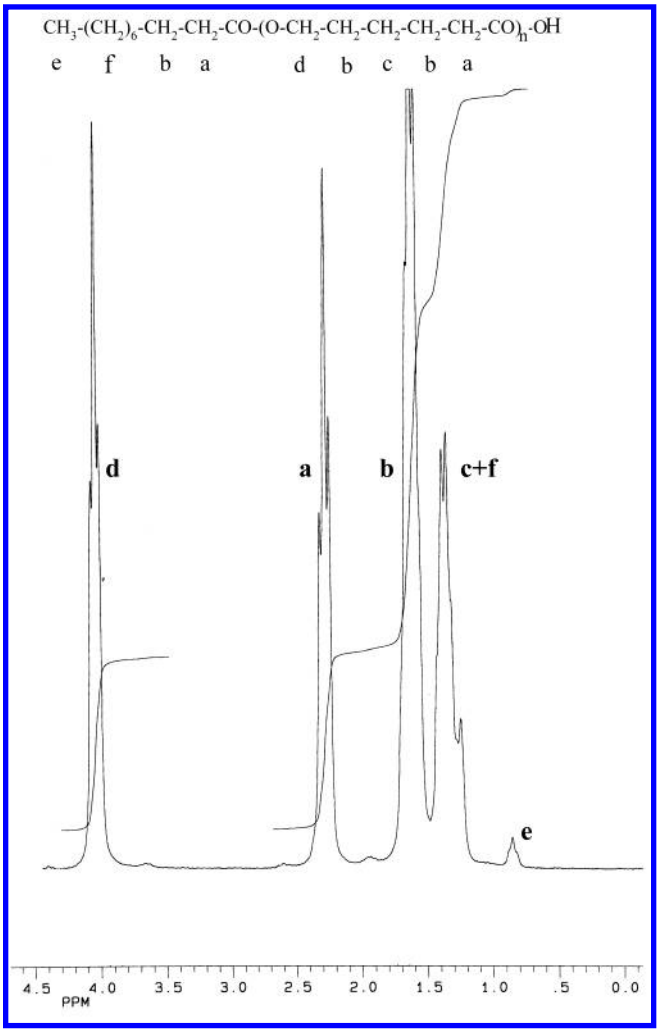

Figure 2. ^{1}H NMR (200 MHz) of R−PCL−COOH (R = C_9H_{19}, M_n 2100) in chloroform-*d*.

The theoretical molecular weights (M_t) (at total monomer conversion) are defined as

$$M_t = \frac{n_{\epsilon CL}}{n_{acid}} 114 + 172$$

where $n_{\epsilon CL}$ and n_{acid} are, respectively, the number of moles of monomer and capric acid in the reaction mixture and 114 and 172 are, respectively, the molecular weights of monomer and capric acid. A good agreement was found between M_t and M_n determined by titration, after 7 h reaction time. For example, for a molar ratio monomer:capric acid of 16, M_n was 2100 and M_t was 2000.

It was found that trace water accelerates the polymerization of ϵ-caprolactone, but it might lead to the formation of undesired HO−PCL−COOH polymers. Studies concerning the influence of initiator structure were reported by Bixler et al.[16] Because water is a competitive initiator of polymerization, drastic conditions (high purity reagents and anhydrous conditions) were necessary to obtain polyester carboxylic acids.

For the coupling reaction between R−PCL−COOH and Dex, different strategies were envisaged. For example, the carboxylic function of R−PCL−COOH was activated with *N*-hydroxysuccinimide (NHSI), previous to coupling to polysaccharides. The conversion of COOH in corresponding ester of NHSI in the presence of

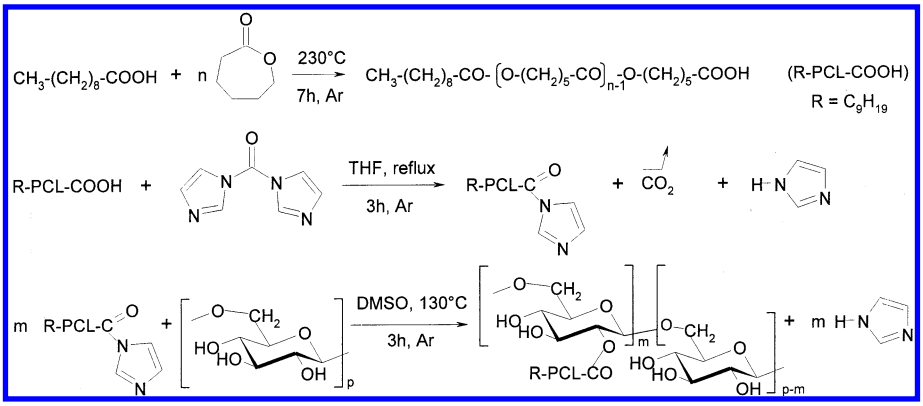

Figure 3. Chemical reactions involved in the synthesis of Dex−PCL$_n$ copolymers.

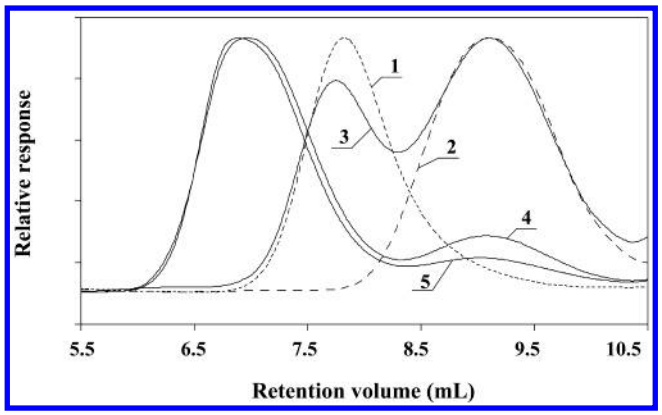

Figure 4. Coupling reaction time course as followed by GPC: Dex (40000) (1), R−PCL−COOH (2100) (2), starting mixture (Dex and R−PCL−COOH) (3), and reaction mixture after 1 (4) and 3 h (5).

dicyclohexylcarbodiimide (DCC) was quantitative, but coupling with polysaccharide needed several days. The reaction yield did not exceed 50%. DCC was used to couple polyesters and PEG.[17] However, in our case, direct coupling using DCC required prolonged reaction times and led to low conversion yields.

The best results were obtained using CDI as coupling agent (short reaction times, good conversion yields). The selectivity, reactivity, and efficiency of this acylating agent under various conditions were studied.[18] Examples of applications in polymer chemistry were reported particularly in the case of polysaccharides.[19,20] Coupling with CDI can be carried on in various usual solvents such as THF, DMF or DMSO.[18] In this work, DMSO was chosen because it is the best solvent for the polysaccharides used. The coupling reaction with CDI was carefully inspected and the reaction conditions were optimized using Dex 5K and 40K as starting materials. The different steps of the synthesis of Dex−PCL$_n$ copolymers are schematized in Figure 3. First, CDI in excess was used for the conversion of carboxylic acid of R−PCL−COOH in corresponding imidazolide intermediate. In a second stage, Dex was added to the non-isolated mixture containing imidazole, and the reaction mixture was heated at 130 °C.

The optimal reaction time corresponding to more than 90% conversion was determined by GPC. A typical GPC followup of the coupling reaction is shown in Figure 4. The reactants were Dex 40K (curve 1) and R−PCL−COOH 2100 (curve 2). The amount of Dex 40K in the reaction mixture was 10 wt %. On the chromatogram of the initial reaction mixture (curve 3), both peaks of Dex and R−PCL−COOH can be observed. After 1 h of reaction at 130 °C, the area of the peak corresponding to R−PCL−COOH (curve 2) was drastically reduced. It became negligible after 3 h (curve 5), showing a practically total conversion of R−PCL−COOH. Therefore, reaction times of 3 h were chosen for the coupling reactions.

After 1 and 3 h (curves 4 and 5), a new peak corresponding to higher molecular weights appears at shorter retention volumes. This, together with the disappearance of the peaks corresponding to Dex and R−PCL−COOH, clearly indicates that R−PCL−COOH was grafted to the Dex backbone. Moreover, no residual acidity from R−PCL−COOH could be detected by titrimetry in the isolated Dex−PCL$_n$ copolymers, showing once more that the grafting was efficient.

The copolymers were then analyzed by GPC, ^{1}H NMR, and FTIR. These techniques allowed one to determine their average molecular weights, polydispersity indexes, gyration radiuses, and viscosities, as well as their global compositions. In particular, it was possible to determine the amount of Dex effectively incorporated into the Dex−PCL$_n$ copolymers and the substitution degree.

Dex−PCL$_n$ copolymers obtained using Dex 40K and more than 15 wt % Dex in the reaction mixture were poorly soluble in DMAC, and therefore, their analysis by GPC was not possible. This was not the case with a family of Dex−PCL$_n$ copolymers prepared using Dex with lower M_w (Dex 5K), even in amounts up to 33 wt % in the reaction mixture.

All these isolated Dex−PCL$_n$ copolymers showed an unique peak in GPC. Table 1 gathers the GPC characteristics of Dex−PCL$_n$ copolymers obtained using 5, 20, and 33 wt % Dex 5K in the reaction mixture and a R−PCL−COOH molecular weight of 2100. Average number and weight molecular weights (M_n and M_w), gyration radius, and intrinsic viscosity increased with

Table 1. GPC Characteristics of Dextran (Dex5K) and Three Dex−PCL$_n$ Copolymers with 3, 5.5, or 7.1 Grafted Polyester Side Chains: Average Weight (M_w) and Number (M_n) Molecular Weights, Polydispersity (Pd), Average Weight Intrinsic Viscosity (IV$_w$), Average Weight Gyration Radius (R_{g_w}), and Variation of the Specific Refraction Index with Concentration (dn/dc)[a]

copolymer	Dex	Dex−PCL$_3$	Dex−PCL$_{5.5}$	Dex−PCL$_{7.1}$
M_w (g/mol)	5000	10 900	16 000	19 100
M_n (g/mol)	4700	9900	11 700	13 500
Pd	1.06	1.10	1.37	1.41
IV$_w$ (dL/g)	0.087	0.12	0.12	0.098
R_{g_w} (nm)	2.47	3.57	4.07	3.92
dn/dc (mL/g)	0.147	0.088	0.084	0.052

[a] For GPC conditions see the Experimental Section. These data were obtained from the triple detection Viscotek unit.

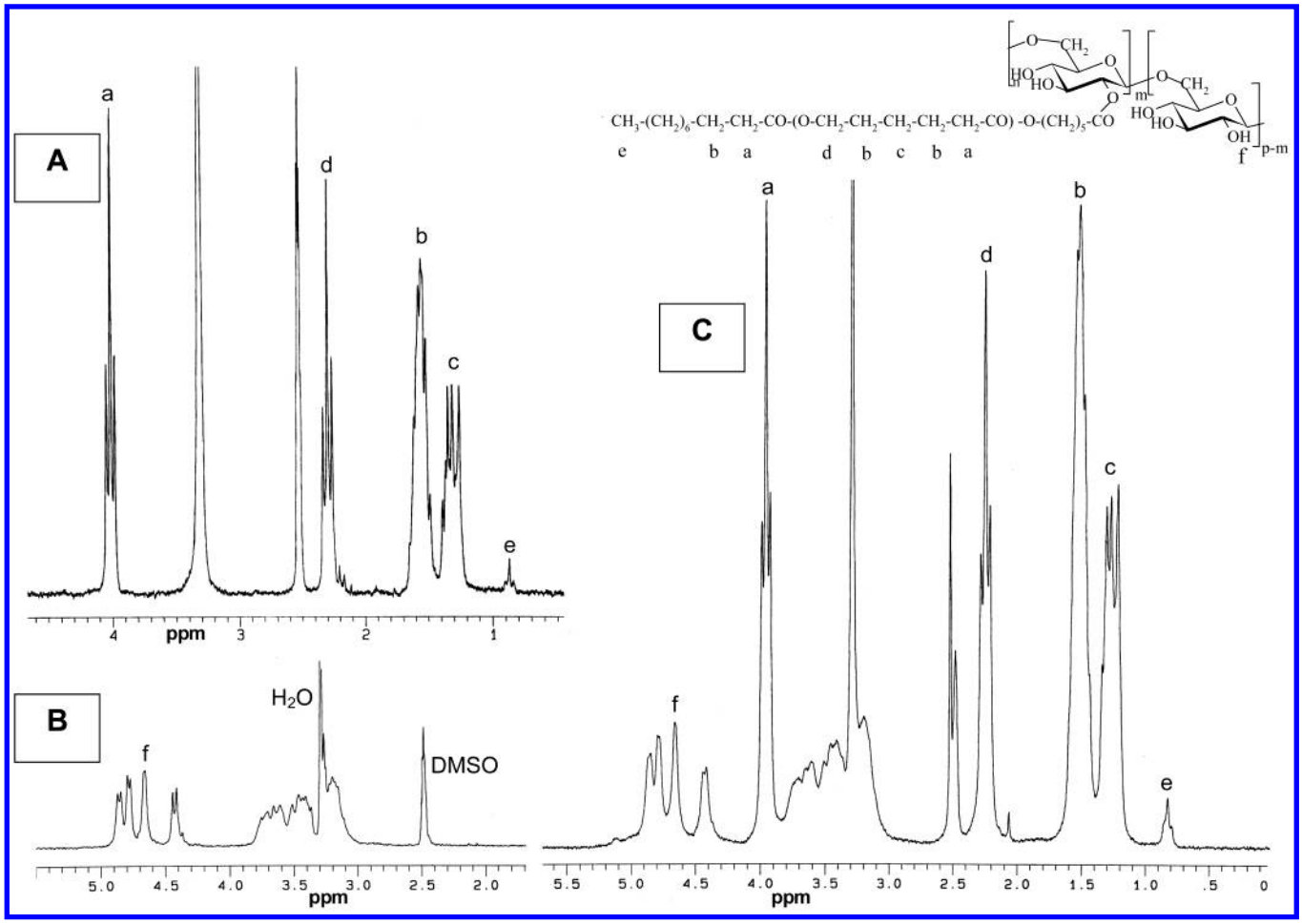

Figure 5. ^{1}H NMR (200 MHz) in DMSO-d_6: R−PCL−COOH (R = C_9H_{19}, M_n 2100) (A), Dex 5K (B), and Dex−PCL_3 copolymer (C).

the wt % R−PCL−COOH in the reaction mixture, as a consequence of the increase of the number n of grafted PCL chains. Polydispersity of the Dex−PCL_n copolymers slightly increased with their M_w but remained low (<1.5).

Data in Table 1 allowed one to calculate the number of grafted PCL chains per Dex backbone. It appears that approximately 3, 5.5, and 7.1 polyester chains were grafted to Dex in Dex−PCL_n copolymers synthesized with respectively 33%, 20%, and 5 wt % Dex in the reaction mixture.

A representative ^{1}H NMR spectrum of PCL, Dex, and Dex−PCL_3 is shown in Figure 5, together with the attribution of the various peaks. All and only the peaks corresponding to Dex and to PCL can be observed in the spectrum of Dex−PCL_3 copolymer. Because of the very low number of grafted PCL chains, about 3 in this case, no peak corresponding to the ester bridges between PCL and Dex could be observed.

In particular, it can be observed on Figure 5 that the signal from the anomeric proton (f) of the glucopyranosyl ring (4.65 ppm) of Dex is well separated from the other proton signals (3.2−3.7 ppm) and the signals from the OH groups (4.4, 4.8, 4.9 ppm). Therefore, the Dex wt % in the Dex−PCL_n copolymers can be calculated using the following relationship

$$\text{Dex wt \%} = \frac{162A_{4.65}}{162A_{4.65} + \frac{114}{2}A_4}$$

where 162 and 114 are respectively the molecular weight of α-D-glucopyranose and caprolactone units. $A_{4.65}$ and A_4 are respectively the areas of the peaks corresponding to the anomeric H of Dex and to the two H's of the CH_2 group linked to −C(O)-O in PCL chains (peak a, Figure 5). For example, the so-calculated value of Dex in the copolymer, 31 wt %, was very close to 33 wt %, initially introduced in the reaction mixture. Differences could possibly be due to the elimination of the less-substituted fractions by repetitive washing. In the case of the less-substituted polymers, the differences between the theoretical and the calculated values of Dex in the copolymer were higher (∼15%).

Figure 6 presents the superposition of FT-IR spectra of polymer powders: Dex 5K (curve 1), R−PCL−COOH (curve 5), and three Dex−PCL_n copolymers (n = 3−7). Dextran presents a broad absorption band in the region 3200−3500 cm^{-1}, assigned to the OH stretching vibrations. A large peak at about 2900 cm^{-1} attributed to the CH/CH_2 vibrations is also observed. In the case of PCL chains, the peaks corresponding to these vibrations (2944 and 2865 cm^{-1}) are sharper. The characteristic C═O stretching vibration of PCL chains can be observed at 1722 cm^{-1}. The second main IR absorption band of Dex has a maximum at 999.3 cm^{-1}. This region is dominated by ring vibrations overlapped with stretching vibrations of (C−OH) side groups and the (C−O−C) glycosidic bond vibration.[21] The intensity of all the characteristic peaks of Dex increases in the Dex−PCL_n copolymers with the Dex wt % increase. Simultaneously,

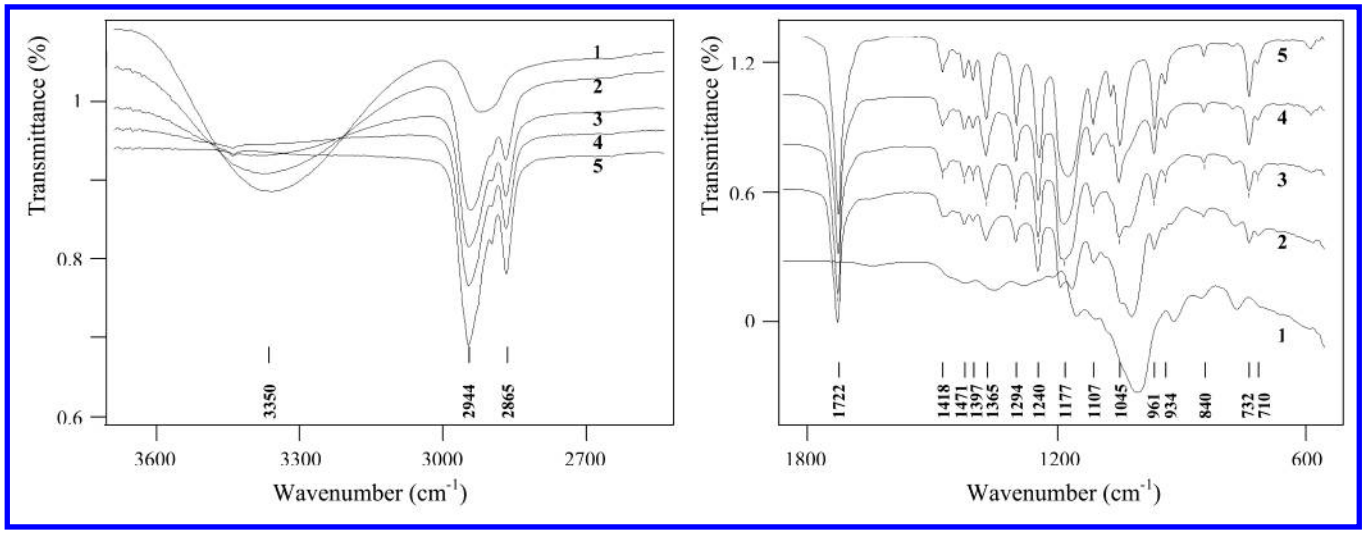

Figure 6. FTIR spectra of Dex 5K (1), Dex−PCL_3 (2), Dex−$PCL_{5.5}$ (3), Dex−$PCL_{7.1}$ (4) ,and R−PCL−COOH (R = C_9H_{19}, 2100) (5) copolymers.

Table 2. Composition (wt % Dex) of a Series of Dex−PCL_n Copolymers with an Average of 3, 5.5, and 7.1 Grafted PCL Chains, As Determined by FTIR and ^{1}H NMR Spectroscopies, Compared to the Dex wt % in the Reaction Mixture (rm)

copolymer	Dex−$PCL_{7.1}$	Dex−$PCL_{5.5}$	Dex−PCL_3
wt % Dex (rm)	5	20	33
wt % Dex (FTIR)	9 ± 4	16 ± 3	26 ± 3
wt % Dex (^{1}H NMR)	n.d.[a]	17 ± 2	30.7 ± 2.3

[a] Not determined (n.d.), because of the too low intensity of the Dex signals.

the intensity of the characteristic peaks of PCL decreases. Therefore, in a manner similar to that for NMR spectroscopy, FTIR spectroscopy showed that the Dex, Dex−PCL_n, and PCL samples were structurally related.

Moreover, the global composition of the Dex−PCL_n copolymers could be calculated from the intensity of the carbonyl group band at 1722 cm^{-1}, characteristic of R−PCL−COOH (Table 2). Table 2 allows a comparison of the two different techniques used to determine the Dex wt % in Dex−PCL_n copolymers. FTIR spectroscopy is revealed to be the most accurate one. NMR was accurate too, but could not be applied to copolymers with low amounts of Dex, because of the very low intensity of the corresponding peaks. In the case of Dex−PCL_3 and Dex−$PCL_{5.5}$ (respectively 33 and 20 wt % Dex in the reaction mixture), a good agreement was found between IR and NMR methods. Both these methods gave Dex wt % values lower than the ones in the reaction mixture. Possibly, during polymer purification by washing, the fraction of Dex−PCL lost in the aqueous phase was the less-substituted one (with the highest solubility in water and/or forming colloidal dispersions). As a consequence, the recovered polymer was impoverished in Dex.

As a conclusion, GPC is revealed to be effective to determine the selectivity and the conversion ratio of the coupling reaction, in particular to prove that Dex−PCL_n did not contain the starting material (R−PCL−COOH and Dex) and to calculate polymer characteristics such as average molecular weights and polydispersity indexes (Table 1). IR and NMR spectroscopies were effective to determine the global composition of the Dex−PCL_n copolymers, in particular their wt % of Dex and PCL.

The synthesized Dex−PCL_n copolymers revealed excellent oil-in-water emulsion stabilizing abilities, due

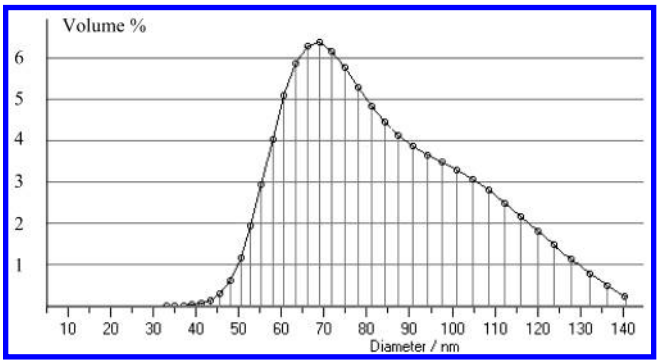

Figure 7. Volumic diameter distribution of nanospheres prepared using Dex−$PCL_{5.5}$ copolymer and following an "interfacial migration/solvent evaporation" technique.

to their amphiphilic nature. This property was further used to elaborate core−shell nanoparticles from these copolymers. The nanoparticle preparation methods generally involve the dissolution of the preformed polymers in an organic phase, but since the graft Dex−PCL_n copolymers here synthesized were insoluble in the most commonly used organic solvents for nanoparticle preparation (acetone, ethyl acetate, methylene chloride), an "interfacial migration/solvent evaporation" method has been developed. For this, the amphiphilic copolymers suspended in a mixture of water and of an immiscible organic phase (ethyl acetate, methylene chloride) were allowed to migrate at the oil/water interface, by stirring the two phases. The diameter of the oil droplets was reduced by sonication and the solvent was evaporated, leading to the formation of nanoparticles. A typical diameter distribution in the case of nanoparticles made of Dex−$PCL_{5.5}$ copolymer is shown in Figure 7. Nanoparticle diameters ranged from 45 to 140 nm, with an average volume diameter of 70 nm. This size, being lower than 200 nm, is particularly advantageous for intravenous administration.[1] The investigation of the pharmaceutical applications of this new type of particles is underway.

Conclusion

The method described in this paper allows to obtain a family of copolymers of the type Dex−PCL_n (n = 3−7) with well-defined structures. The number of grafted PCL chains is predetermined from the mass ratio Dex:

R−PCL−COOH in the reaction mixture. With the aim to further use these materials for biomedical applications, grafting was achieved through labile ester bridges to ensure a good biodegradability. The synthesis involves three steps. First, a functionalized polyester, R−PCL−COOH, was obtained by uncatalyzed ring-opening polymerization of ϵ-caprolactone in the presence of capric acid. This polymer was activated with CDI. The reactive imidazolide intermediate was effectively coupled to the Dex backbone. Although in general substitution reactions on polysaccharides are difficult, in this study it was shown that CDI was an excellent acylation agent for Dex. A followup of the coupling kinetics by GPC revealed that the reaction was fast (∼3 h) and that conversion was practically complete (>90%). However, the copolymer recovery yield was about 70 wt %, because of loss by extensive washing and colloidal particle formation. The solubility of the Dex−PCL_n copolymers depends on their substitution yield. The HLB balance varied in a large domain (1−7). The new copolymers had excellent abilities to stabilize emulsions. This property was used to elaborate nanoparticles of less than 200 nm by using Dex−PCL_n copolymers. This low diameter is compatible with intravenous administration. Studies are underway to determine the structure of these nanoparticles and to explore their biomedical applications. By taking into consideration the physicochemical properties of a given drug, it should be possible to chose the optimal Dex−PCL_n copolymer composition to achieve the best results in terms of entrapment and release.

Current research deals with coupling of mono or dicarboxylic polyesters to oligo-and polysaccharides such as cyclodextrins, amilose, chitosan, or hyaluronic acid.

Acknowledgment. This work was supported by the French National Research Center (CNRS). We acknowledge N. Marnheineke (Viscotek) for help with the GPC analysis.

References and Notes

(1) Gref, R.; Minamitake, Y.; Peracchia, M. T.; Trubetskoy, V.; Torchilin, V.; Langer, R. *Science* **1994**, *263*, 1600−1603.
(2) Bazile, D.; Prud'homme, C.; Bassoulet M. T.; Marlard, M.; Spenlehauer, G.; Veillard, M. *J. Pharm. Sci.* **1995**, *84*, 493−498.
(3) Listinski, J. J.; Siegal, G. P.; Listinski, C. *Am. J. Clin. Pathol.* **1998**, *110*, 425−440.
(4) Stahn, R.; Zeisig, R. *Tumor Biol.* **2000**, *21*, 176−186.
(5) Choi, E. J.; Kim, C. H.; Park, J. K. *Macromolecules* **1999**, *32*, 7402−7408.
(6) Donabedian, D. H.; McCarthy, S. P. *Macromolecules* **1998**, *31*, 1032−1039.
(7) Dubois, P.; Krishnan, M.; Narayan, R. *Polymer* **1999** 40, 3091−3100.
(8) Li, Y.; Volland, C.; Kissel, T. *Polymer* **1998**, *39*, 3087−3097.
(9) Breitenbach, A.; Li Y. X.; Kissel, T. *J. Controlled Release* **2000**, *64*, 167−178.
(10) Rutot, D.; Duquesne, E.; Ydens, I.; Degée, P.; Dubois, P. *Polym. Degrad. Stab.* **2001**, *73*, 561−566.
(11) Ohya, Y.; Maruhashi, S.; Ouchi, T. *Macromol. Chem. Phys.* **1998**, *199*, 2017−2022.
(12) Nouvel, C.; Ydens, I.; Degée, P.; Dubois, P.; Dellacherie, E.; Six J. L. *Polymer* **2002**, *43*, 1735−1743.
(13) Schwach, G.; Coudane, J.; Engel, R.; Vert, M. *J. Polym. Sci., Polym. Chem.* **1997**, *35*, 3431−3440.
(14) Bergström, K.; Österberg, E.; Holmberg, K.; Hoffman, A.; Schuman, T.; Kozlowski, A.; Harris, J. *J. Biomater. Sci., Polym. Ed.* **1994**, *6*, 123−132.
(15) Zhang, Q.; Wang, B.; Hong, K.; Zhu, Q. *Macromol. Chem. Phys.* **1994**, *195*, 2401−2407.
(16) Bixler, K.; Calhoun, G. C.; Scholsky, K. M.; Stackman, R. W. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.* **1990**, *31*, 494−495.
(17) Bae Y. H.; Huh K. M.; Kim, Y.; Park K. H. *J. Controlled Release* **2000**, *64*, 3−13.
(18) Staab, H. *Angew. Chem., Int. Ed.* **1962**, *1*, 351−367.
(19) van Dijk-Wolthuis W. N. E.; Tsang S. K. Y.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *Polymer* **1997**, *38*, 6235−6242.
(20) Bethell, G. S.; Ayers, J. S.; Hearn, M. T. W.; Hancock, W. S. *J. Chromatogr.* **1981**, *219*, 361−372.
(21) Kacurakova, M.; Capek, P.; Sasinkova, V.; Wellner, N.; Ebringerova, A. *Carbohydr. Polym.* **2000**, *43*, 195−203.

MA021132A