Synthesis of Graft Copolymers by the Combination of ATRP and Enzymatic ROP in $scCO_2$

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ABSTRACT: A simple strategy is reported for the synthesis of well-defined graft copolymers of poly(methyl methacrylate-*co*-2-hydroxyethyl methacrylate) P(MMA-*co*-HEMA) with poly(ϵ-caprolactone) (PCL) grafted chains. Using $scCO_2$ as the only solvent, a one-step synthetic approach is adopted to prepare copolymer backbones via atom transfer radical polymerization (ATRP), and grafted chains are added via enzymatic ring-opening polymerization (eROP). Exhaustive study of the enzymatic grafting efficiency showed that only the hydroxyl groups in the backbone initiated the polymerization of ϵ-CL, resulting in an exceptional polymer architecture which is not accessible by conventional chemical polymerization methodology. The lower grafting density obtained (ca. 30−40%) with the enzymatic polymerization of ϵ-CL indicates that the system is likely limited by steric hindrance.

Introduction

Achieving well-defined macromolecular architectures via controlled synthesis is a major challenge in polymer science. A controlled synthetic approach allows important properties such as polymer size, composition, and topology to be defined. Considerable progress has been made in the design and synthesis of new graft copolymers. Recent advances in the area of controlled radical polymerization include atom transfer radical polymerization (ATRP)[1] and reversible addition−fragmentation chain transfer polymerization (RAFT).[2] Such methods allow the preparation of copolymers with defined structure which can then be grafted via ring-opening polymerization (ROP).[3] The compatibility of these controlled reactions allows such polymerizations to be performed either sequentially or concurrently.

Graft copolymerization from functional groups residing on an existing polymer chain offers an easy and effective approach to the incorporation of new properties into the parent polymer. This strategy is referred to as the "grafting from" technique and has the advantage not only of a limited number of reaction steps but also of a simultaneous one-step approach.[4]

Enzymatic catalysis has become an important process in industrial synthesis, since reactions can be carried out under mild conditions and often result in high enantio-, regio-, and chemoselectivity. Enzymes are also often able to replace heavy metal catalysts and in some cases reduce the use of organic solvents.[5] Major progress has been achieved over the past few years in applying enzyme catalysis in polymer science. The application of a biocatalytic approach to polymer chemistry is attractive since the polymerizations can be conducted under mild conditions, the immobilized enzyme can easily be removed from the reaction mixture, and, most importantly, enzymes are biocompatible catalysts.[6] However, to take advantage of biocatalysis in the field of polymer chemistry, the development of compatible chemo- and biocatalytic methods is required. Recently, the combination of ATRP and lipase-catalyzed ROP has been successfully demonstrated for synthesis of block copolymers in conventional solvents for sequential two-step syntheses and also for one-pot approaches.[7]

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The use of $scCO_2$ as a polymerization medium has attracted considerable interest. In addition to being an environmentally "green" solvent, with the capability to replace volatile organic and aqueous solvents, $scCO_2$ offers several advantages as a reaction medium; it has a low viscosity and is inert to free radicals. Moreover, there is no detectable chain transfer to $scCO_2$, and its solvent strength can be finely tuned through changes in temperature and pressure.[8]

Enzymes have been used as catalysts for organic reactions in $scCO_2$, thus demonstrating that they can function effectively under supercritical conditions.[9] A number of studies have been carried out in this field. The combination of $scCO_2$ and enzyme-catalyzed polymerization opens up a promising new area of research. Only a few publications report the combination of the environmentally friendly nature of enzymes and $scCO_2$ for use in polymerizations.[10−12]

Previously, we reported a simple strategy for the synthesis of block copolymers by combining simultaneously the enzymatic ring-opening polymerization (eROP) of ϵ-caprolactone (ϵ-CL) with atom transfer radical polymerization (ATRP) of methyl methacrylate (MMA) using a bifunctional initiator in $scCO_2$. It was demonstrated that enzymatic polymerization and ATRP proceeded concurrently. Moreover, the use of $scCO_2$ allows synthesis of block copolymers that might be difficult or impossible to achieve in conventional solvents.[13−15]

The purpose of this paper is to extend this methodology to the synthesis of well-defined polymethacrylate-*graft*-polycaprolactone copolymers by the combination of these two versatile polymerization methods in $scCO_2$. Two routes to the target copolymers are investigated: a one-pot, concurrent dual polymerization route and a sequential two-step route including a "grafting from" approach. The goal of our study is the preparation of a model linear polymer backbone with a defined number of hydroxyl groups that will be used as the initiator sites for the enzymatic ring-opening polymerization of caprolactone. Our aim was to then establish the grafting efficiency of the enzymatic polymerization of these systems. This approach results in core−shell amphiphilic polymeric material by combining Poly(MMA-*co*-HEMA) hydrophilic backbone core with PCL hydrophobic shell. Unreacted hydroxyl groups in the polymer backbone are then available for further chemical

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Table 1. Conditions and Results for the Synthesis of Poly(MMA-*co*-HEMA) Copolymers and for the Polymethacrylate-*g*-PCL Graft Copolymers

								after grafting	
entry	feed ratio[a] MMA/HEMA	conv (%)[b] MMA/HEMA	yield (%)[c]	ratio[d] MMA/HEMA	$M_{n,GPC}$ DRI (kDa)	PDI	N_{OH}[e]	$M_{n,GPC}$ DRI (kDa)[f]	PDI
1	10 mL/2.3 mL	45/54	56	70/30	13	1.24	33	32	1.88
2	10 mL/1.1 mL	40/70	49	87/13	8	1.37	12	24	2.02

[a] Polymerization carried out at 40 °C and 3500 psi (24.1 MPa). [b] Conversion calculated from NMR independently for each monomer. The remainder is unreacted monomer in the reaction mixture. [c] Yield was obtained gravimetrically after purification from the dried polymer. [d] Ratio MMA:HEMA in the copolymer calculated by ^{1}H NMR. [e] Average number of hydroxyl groups per backbone Poly(MMA-*co*-HEMA) chain obtained from the equation $M_n = N_{OH}(M_{HEMA} + [(1 - X_{HEMA})/X_{HEMA}]M_{MMA}$, where M_{HEMA} and M_{MMA} are the molar masses of HEMA and MMA, respectively, and X_{HEMA} is the molar fraction of HEMA units in the copolymer.[17] [f] GPC values for the graft copolymers.

Table 2. Conditions and Results for the Simultaneous One-pot Synthesis of Polymethacrylate-*g*-PCL Graft Copolymers

entry	feed ratio[a] MMA/HEMA/CL	conv (%)[b] MMA/HEMA/CL	yield (%)[c]	ratio[d] MMA/ng-HEMA/g-HEMA/CL	%[e] HEMA grafted	$M_{n,GPC}$ DRI (kDa)	PDI
1	1.0/1.1/4.0	50/50/20	47	19/32/13/36	28	13.0	1.40
2	1.0/0.5/4.0	40/65/30	57	28/26/18/28	40	11.0	1.37
3[f]	1.0/1.1/4.0	21/33/90	76	4/8/4/84	33	30.0	1.74

[a] Polymerization carried out at 35 °C at 1500 psi (10.3 MPa). [b] Conversion calculated from NMR independently for each monomer. The remainder is unreacted monomer in the reaction mixture. [c] Yield was obtained gravimetrically after purification from the dried polymer. [d] Ratio MMA/ng-HEMA/g-HEMA/CL in the copolymer calculated by NMR, where ng-HEMA is nongrafted HEMA and g-HEMA is grafted HEMA. [e] % of grafted HEMA units compared to the total amount of HEMA units in the backbone. [f] Double the amount of enzyme was used.

modification. In addition, polymethacrylate-*graft*-polycaprolactone copolymers have potential for use in biomedial applications combining properties such as biodegradability of the polyester chains and biocompatibility of the polymethacrylate backbone.

Experimental Section

Materials. ϵ-Caprolactone (ϵ-CL, 99%) and methyl methacrylate (MMA) were purchased from Aldrich, dried over CaH_2 for 24 h under nitrogen, distilled under reduced pressure with three freeze, pump, thaw cycles, and stored under nitrogen until use. 2-Hydroxyethyl methacrylate (HEMA) was dried over CaH_2 for 24 h under nitrogen and kept under molecular sieves 4 Å.

2,2′-Bipyridine (bpy, 99+ %) was purchased from Lancaster, copper(I) bromide (98%) was purchased from Aldrich, and 1,4-dioxane was purchased from Fisher and used as received. Ethyl-2-bromoisobutyrate (98%+) was purchased from Lancaster. Novozym-435 (10 wt % Lipase B from *Candida antarctica* on a macroporous acrylic resin) was purchased from Novozymes. SFC grade carbon dioxide (99.99%) was purchased from BOC gases. The high-pressure reactions were conducted in a 12.5 mL volume clamp seal autoclave, equipped with a magnetic stirrer bar. The autoclave was placed on a hot plate stirrer to allow the reaction to be stirred.[10]

General Procedure for ATRP of HEMA and MMA Using ϵ-CL as a Cosolvent in scCO$_2$ (Entry 1, Table 1). A 60 mL reactor was charged with copper(I) bromide (88 mg, 0.613 mmol) and bpy (192 mg, 1.23 mmol). It was then purged with a flow of CO_2 for 5 min to exclude air and moisture. HEMA (2.27 mL, 1.87 mmol), MMA (10 mL, 9.35 mmol), ϵ-CL (20 mL), and ethyl-2-bromoisobutyrate (90 μL, 0.613 mmol) were added to the autoclave using a syringe under a flow of CO_2 to prevent the moisture from entering the system. The autoclave was sealed and heated to 40 °C, and the pressure of CO_2 was increased to the desired level (3500 psi (24.1 MPa)). Agitation was achieved via a mechanical stirrer. Heating was stopped after 21 h, and the autoclave was placed into a dry ice/acetone bath to freeze the contents. Once the pressure inside the autoclave had fallen to atmospheric pressure, the autoclave was opened and the solid CO_2 allowed to sublime, leaving the solvent free polymer product. The polymeric material in the autoclave was dissolved in chloroform and passed through an alumina column to remove the Cu catalyst. The polymer was separated from the resulting mixture by precipitation in cold pentane (yield = 56% by gravimetric analysis).

General Procedure for the Graft Enzymatic ROP of ϵ-CL from Poly(MMA-*co*-HEMA) (Entry 1, Table 1). The 12.5 mL reactor was charged with Novozym-435 (200 mg) and poly(HEMA-*co*-MMA) (0.500 g), after which the autoclave was sealed and heated to 35 °C while under vacuum for 2 h in order to dry the enzyme beads. After 2 h, the vacuum was released by filling the autoclave with a low pressure of CO_2. ϵ-CL (4 mL, 36 mmol) was then added to the autoclave by syringe, under a flow of CO_2, to prevent the moisture from entering the system. The autoclave was sealed, the stirring started, and the pressure of CO_2 increased to 1500 psi (10.3 MPa). After a reaction time of 16 h, the autoclave was placed into a dry ice/acetone bath to freeze the contents. Once the pressure had fallen to atmospheric, the autoclave was opened and the product dissolved in chloroform. Novozym-435 was removed by filtration. The polymer was precipitated from cold pentane and dried overnight under vacuum at 50 °C (yield = 35% by gravimetric analysis).

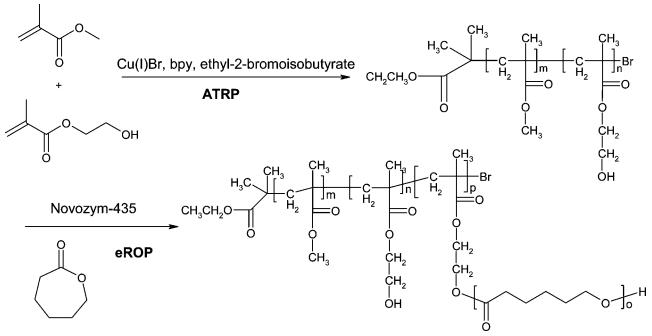
Simultaneous ATRP/eROP One-Pot Reaction (Entry 1, Table 2). The reactor was charged with Novozym-435 (0.264 mg), copper(I) bromide (15 mg, 0.102 mmol), and bpy (32 mg, 0.204 mmol), and the autoclave was sealed and heated to 35 °C while under vacuum for 2 h. After 2 h, the vacuum was released by filling the autoclave with a low pressure of CO_2. ϵ-CL (4.0 mL, 36.0 mmol), HEMA (1.1 mL, 9.07 mmol), MMA (1.0 mL, 9.36 mmol), and ethyl 2-bromoisobutyrate (15 μL, 0.102 mmol) were then added to the autoclave by syringe, under a flow of CO_2, to prevent moisture from entering the system. After all the liquid reagents had been added, the autoclave was sealed and heated at 35 °C. Stirring was started, and the pressure of CO_2 was increased to 1500 psi (10.3 MPa). After 20 h of reaction, the autoclave was placed into a dry ice/acetone bath to freeze the contents. Once the pressure inside the autoclave had fallen to atmospheric, the autoclave was opened and the contents of the vessel dissolved in chloroform. The resulting solution was passed through an alumina column to remove the catalyst, and the polymer was precipitated into hexane (yield = 47% by gravimetric analysis).

Hydrolysis. The graft copolymer (0.30 g) was dissolved in 1,4-dioxane (15 mL) at 85 °C in a 100 mL flask. 1.0 mL of concentrated hydrochloric acid was added to the solution, which was left stirring for 20 h. The hydrolyzed polymer was obtained by precipitating the solution into cold hexane.

Exhaustive studies were carried out to ensure that PHEMA was not damaged under the hydrolyzing conditions used.

Characterization. ^{1}H NMR spectra of the HEMA homopolymers, Poly(MMA-*co*-HEMA) copolymers, and also the poly(methacrylate)-*graft*-poly(caprolactone) copolymers were obtained in $CDCl_3$. ^{1}H NMR spectra were recorded using a Bruker DPX-300 spectrometer (300.14 MHz) referenced to chloroform at 7.28 ppm. Analysis of the spectra was carried out using Mestre-C software.

Scheme 1. Synthesis of Graft Copolymers by the "Grafting From" Technique[a]



[a] The first step shows the synthesis of the methacrylic backbone by ATRP, and the subsequent graft polymerization by eROP of ϵ-CL is shown in the second step.

GPC was performed on a PL-GPC-120 apparatus using THF as the eluent at 40 °C. The molecular weight was calibrated using poly(styrene) standards with toluene as the flow rate marker at a flow rate of 1 mL min^{-1}. The instrument was fitted with an RI detector for molecular weight analysis.

DSC: A TA-2920 modulated DSC (TA Instruments), calibrated with an indium standard, was used to analyze the polymers. The crystallization temperature of polymers was also determined with each run typically ranging from −50 to 160 °C for two cycles. The heating program starts with equilibration at −50 °C, then a temperature ramp of 10 °C/min to 160 °C, an isotherm for 5 min, a ramp of 10 °C/min to −50 °C, and the same steps for the second cycle.

Results

A Two-Step Approach to eROP Graft Copolymerization along the Polymethacrylate Copolymer Backbone. The synthesis of graft copolymers was performed by the "grafting from" technique, which requires the preliminary synthesis of a copolymer containing the initiator sites for graft formation. The initiator sites are the hydroxyl groups attached along the methacrylic backbone synthesized by ATRP (Scheme 1). For the synthesis of the parent copolymer, we performed the ATRP of HEMA and MMA using ϵ-caprolactone as a solvent in $scCO_2$. The use of ϵ-CL as the solvent for ATRP reactions has been reported previously in conventional solvents.[16] In addition, we have demonstrated that ϵ-CL exhibits interesting characteristics as a cosolvent for ATRP in $scCO_2$. The presence of ϵ-CL leads to better controlled living polymerization because the ϵ-CL/$scCO_2$ solubilizes the propagating chain. Initial experiments focused upon the ATRP of pure HEMA but yields were low (ca. <40%). To better understand this low yield, polymerizations were carried out in a high-pressure view cell equipped with sapphire windows. This afforded visual observation of the phase behavior of the polymerization mixture and allowed the solubility of the reaction components to be observed in $scCO_2$. The monomer HEMA and ϵ-CL (as a cosolvent), copper bromide, and bipyridine were added in the same ratios as for the polymerizations and pressurized with CO_2. Initially the system was observed to be a homogeneous brown solution. However, for the ATRP homopolymerization of HEMA, the polymeric product precipitated out of solution, preventing the polymerization from continuing. Therefore, the subsequent grafting of HEMA homopolymer with CL could not be performed. To overcome this problem, and to improve the solubility of the methacrylate polymer backbone in the $scCO_2$/ϵ-CL system, a series of HEMA−MMA copolymers were prepared.

Copolymerization of HEMA and MMA by ATRP was initiated by ethyl 2-bromoisobutyrate and catalyzed by Cu(I)-Br using ϵ-caprolactone as a cosolvent at 40 °C in $scCO_2$. Table 1 lists the methacrylate copolymers that have been prepared. The OH content has been calculated by ^{1}H NMR from the relative intensity of the methylene protons of HEMA at 4.11 and 3.84 ppm and the methyl ester protons of MMA at 3.60 ppm. HEMA contents have been deliberately kept low in order to prepare graft copolymers with a low branching density. To confirm the presence of the hydroxyl groups in the polymer chain, the product was derivatized with oxalyl chloride. This reagent quantitatively reacted with all hydroxyl groups causing a downfield shift of ~0.45 ppm for the α-hydroxymethylene protons (H_c at 3.86 ppm and H_b at 4.13 ppm were shifted to $H_{c'}$ at 4.28 ppm and $H_{b'}$ at 4.60 ppm, respectively) (Figure 1).

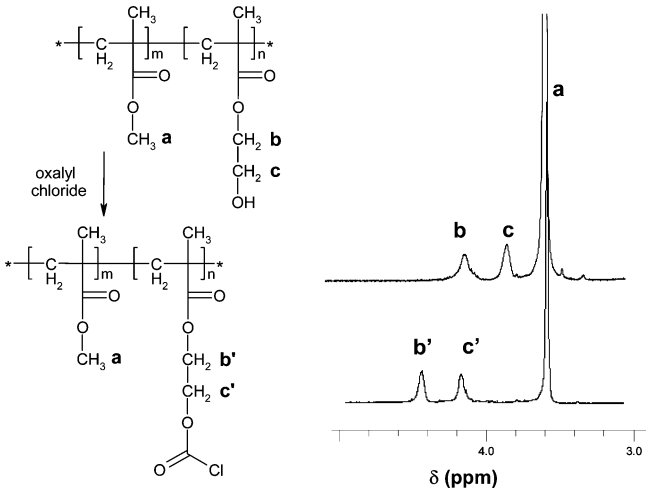


Figure 1. ^{1}H NMR spectra of the poly(MMA-*co*-HEMA) copolymer before (top) and after (bottom) addition of oxalyl chloride. A downfield shift of 0.45 ppm for the α-hydroxymethylene protons (H_b and H_c) was observed upon addition of this reagent.

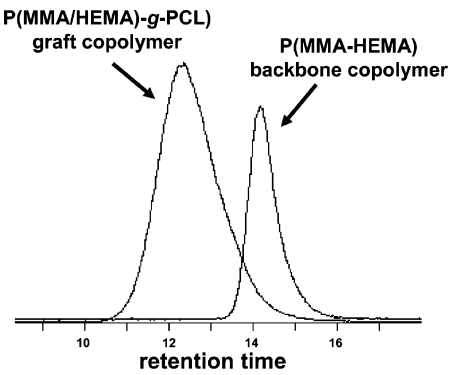
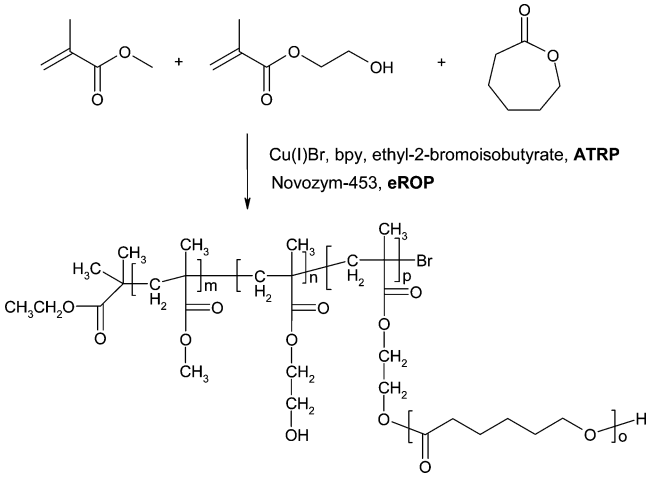


Figure 2. Comparison of the GPC traces for the poly(MMA-*co*-HEMA) copolymer and the final poly(MMA-*co*-HEMA)-*g*-PCL graft copolymer.

Using these methacrylate copolymers, graft polymerization was then carried out by eROP of ϵ-CL initiated by the hydroxyl groups of the HEMA units in the parent copolymers (Scheme 1). The content of HEMA in the copolymer was kept rather low (30% for entry 1 and 13% for entry 2 in Table 1).

GPC analysis of the graft copolymers compared to the poly(MMA-*co*-HEMA) starting material confirms the grafting reaction has occurred (Figure 2). The molar mass of the product was clearly shifted toward a lower retention time. This shows an increase in the molecular weight of the graft copolymers compared with those of the hydroxylated co(polymethacrylate) starting materials. It is worth noting that no significant trace of unreacted macroinitiator in the graft copolymer is observed. To verify the successful grafting reaction, the PCL block of the

Scheme 2. One-Pot Synthesis of P(MMA-*co*-HEMA)-*g*-PCL Graft Copolymers by the Combination of ATRP and eROP



product was hydrolyzed. GPC analysis shows a decrease in the molecular weight back to the original polymer backbone; i.e., the shift in Figure 2 was reversed. ^{1}H NMR shows the disappearance of the PCL from the product after hydrolysis. Further discussion detailing the analysis of the grafting is given later in the paper.

Simultaneous One-Step Approach for the eROP Graft Copolymerization along the Polymethacrylate Copolymer Backbone. A one-step approach means simultaneous radical copolymerization of MMA and HEMA, initiated by ethyl 2-bromoisobutyrate and catalyzed by copper(I) bromide, and the eROP of ϵ-caprolactone initiated from the hydroxyl groups from the HEMA units. Essentially, all of the reactive components were added to the autoclave at the same time. Polymerization of a 1:1.1:4 mixture of MMA, HEMA, and ϵ-CL was performed in a high-pressure vessel at 35 °C and 1500 psi CO_2 pressure. Structures of the resulting graft copolymers are shown schematically in Scheme 2, and data are summarized in Table 2.

This process can also produce free PCL homopolymer. This is initiated by adventitious water in the system and results in PCL homopolymer terminated with a carboxylic acid end group. This end group was characterized and quantified using oxalyl chloride treatment;[18] the proton NMR signal of the methylene group adjacent to the carboxylic acid groups is shifted to an uncrowded region of the spectrum ($H_{g'}$ at 2.9 ppm; Figure 5) and can be quantified by integration. Our analysis confirms that graft copolymers were formed, and the products consist predominantly of poly(methacrylate copolymer)-*g*-polycaprolactone, with water-initiated PCL typically at less than 10%. In these experiments, the water concentration in the reaction medium has been reduced to a minimum by drying the enzyme under vacuum and at 35 °C for a period of time. This drying method effectively removes most of the free and loosely bound water reducing the amount of water initiated polymerization. The water content of the predried enzyme was measured using Karl Fisher titration and results in an average value of 2 μL of water per gram of Novozym 435. This "free" water is available in the enzyme beads after drying and leads to the low level of water-initiated PCL homopolymer. Our data demonstrate that initiation takes place predominantly from the hydroxyl groups in the polyHEMA segment.

The GPC trace of the graft copolymer product was unimodal (Figure 3). To demonstrate graft copolymer formation, the PCL block of the product was hydrolyzed. ^{1}H NMR shows definitively the disappearance of the PCL from the product, leaving just the poly(methacrylate) block of the copolymer (see Supporting Information). GPC analysis of the product shows a shift to lower molecular weight, demonstrating the removal of the PCL from the graft copolymer (Figure 3).

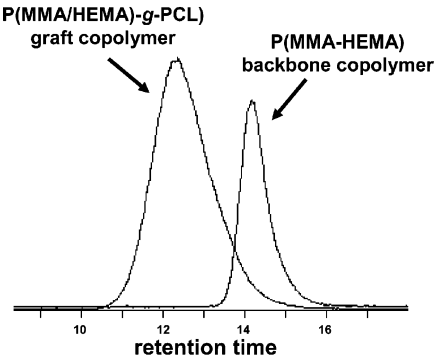


Figure 3. Comparison of the GPC traces for the final poly(MMA-*co*-HEMA)-*g*-PCL graft copolymer and the poly(MMA-*co*-HEMA) backbone copolymer after hydrolysis of the PCL block.

Discussion

A comparison of the grafting for both strategies was made: the two-step "grafting from" (Scheme 1) and the simultaneous approach (Scheme 2). The presence of MMA, HEMA, and ϵ-CL units was confirmed by the observation of characteristic signals in the ^{1}H NMR spectra (Figure 4). The appearance of a proton signal at 4.29 ppm ($H_d + H_e$) for the graft copolymer indicates esterification of the hydroxyl functionality, demonstrating that the grafting reaction has taken place. Evidence of grafting can be seen by the downfield shift of the methylene protons of polyHEMA which appear at 3.86 ppm (H_c) in the original copolymethacrylate ^{1}H NMR spectrum and is shifted to 4.29 ppm ($H_d + H_e$) in the graft copolymer spectrum. As expected, the eROP propagates from the hydroxyl groups of the HEMA units along the copolymer backbone. However, it can be seen from the NMR spectrum that the peaks at 3.86 and 4.13 ppm (H_c and H_b, respectively) do not disappear completely, indicating that not all hydroxyl groups took part in the initiation and that the efficiency of grafting was lower than 100%. Thus, some hydroxyl groups in the HEMA segment are less reactive or inaccessible toward the PCL grafting.

Unfortunately, it was very difficult to quantify the number of hydroxyl groups that take part in the ROP initiation. The addition of few drops of oxalyl chloride directly in the NMR tube facilitates the assignment of the graft copolymer and the calculation of the grafting efficiency (Figure 5). Oxalyl chloride reacts rapidly and quantitatively with both carboxyl and hydroxyl chain ends to form derivatives that can be readily identified by ^{1}H NMR spectroscopy. The addition of this reagent causes a downfield shift of ~0.45 ppm for the proton signals of the nongrafted HEMA in the copolymer (from 3.86 (H_c) to 4.28 ($H_{c'}$) in CH_2−OH and from 4.13 (H_b) to 4.60 for ($H_{b'}$) in CO−CH_2). Exactly the same shift was observed when Poly(MMA-*co*-HEMA) copolymer was treated with oxalyl chloride (Figure 1). This shift demonstrates the presence of nongrafted HEMA units along the backbone after the enzymatic reaction. At the same time, the proton signal for the grafted HEMA stays in the same position around 4.29 and 4.17 ppm (H_d and H_e); it is not modified by the addition of oxalyl chloride, as would be expected, again strengthening the evidence for grafting. The signal at 3.65 ppm (H_f) shifted to 4.37 ppm ($H_{f'}$) can be assigned to the methylene carbon next to the oxalyl chloride derivatized chain-end hydroxyl group of the PCL graft. This provides

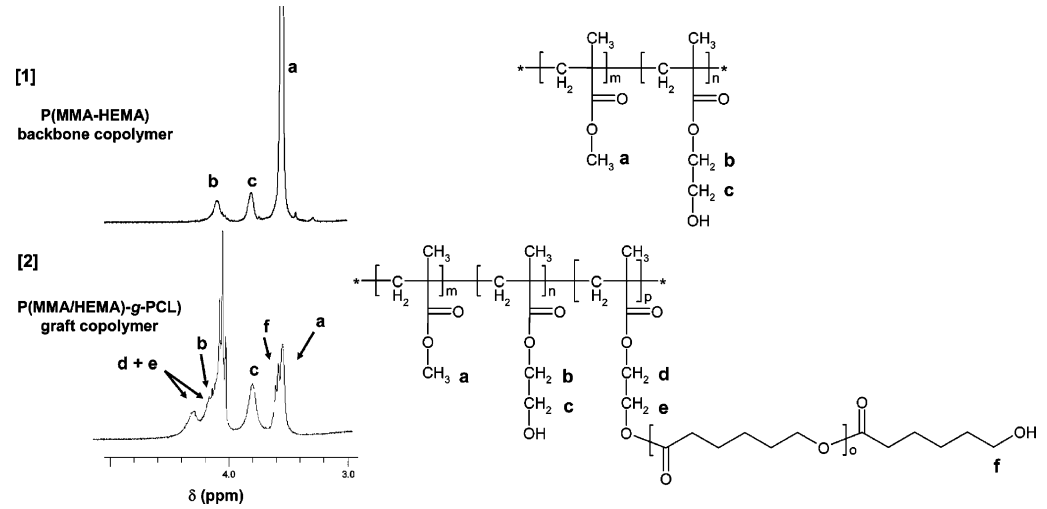


Figure 4. Comparison between the ^{1}H NMR spectra of Poly(MMA-*co*-HEMA) copolymer backbone [1] and the ^{1}H NMR spectra of Poly(MMA-*co*-HEMA)-*g*-PCL graft copolymer [2]. Note that the occurrence of grafting is clearly demonstrated by the appearance of a proton signal at 4.29 ppm ($H_d + H_e$).

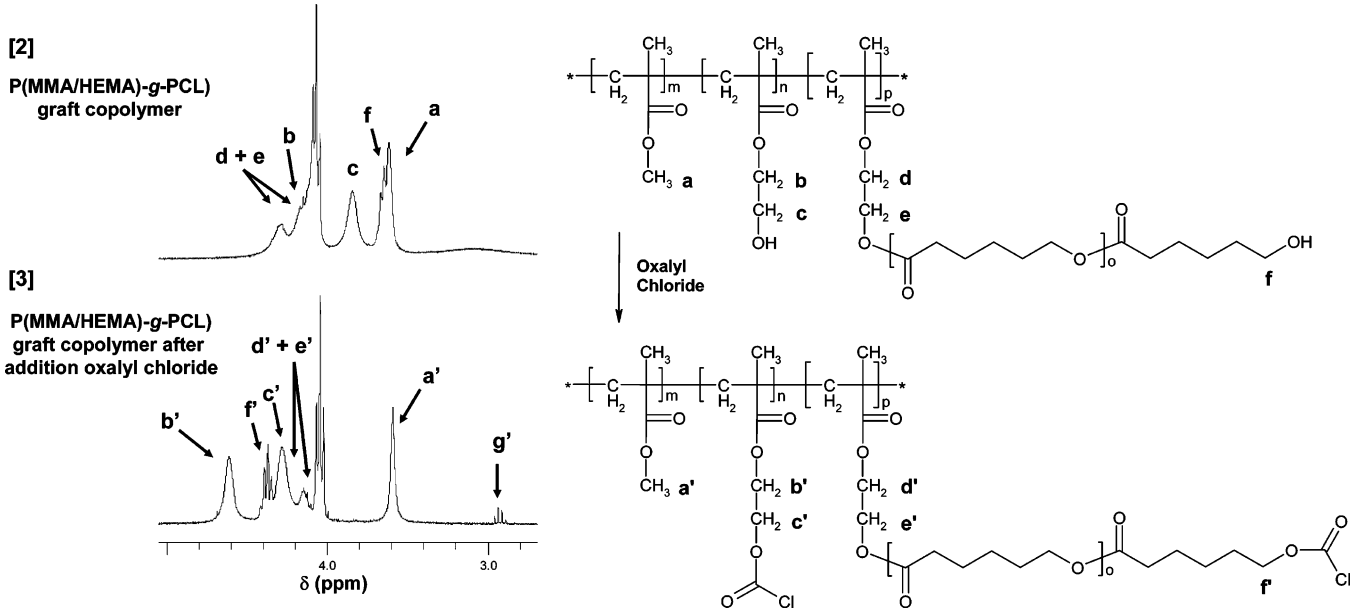


Figure 5. ^{1}H NMR spectra of graft copolymer before [2] and after [3] addition of oxalyl chloride. Few downfield shifts were observed upon addition of oxalyl chloride, demonstrating that only some of the hydroxyl functionalities are modified. Note that $H_{g'}$ clearly demonstrated the presence of a low concentration of PCL homopolymer from adventitious water initiation.

evidence that the polyester grafts are end-capped by a hydroxyl group, as one would expect for eROP.

These observations confirm that the grafting reaction has taken place from some of the hydroxyl groups along the polymethacrylate backbone and also that there are some unreacted hydroxyl groups in the backbone which have not been grafted with CL.

The approximate composition of the graft copolymer can be calculated from the ^{1}H NMR spectrum. On the basis of the ratio of the areas under the different peaks, the chemical composition in the product (entry 1, Table 2) was calculated to give a ratio MMA:nongrafted HEMA:grafted HEMA:CL of 19:32:13:36. It can be seen from this ratio that 30% of the HEMA is grafted with caprolactone chains, leaving ca. 70% of the OH groups in the poly(MMA-*co*-HEMA) unreacted. To assess the reproducibility of the experiments, each was repeated twice, yielding consistent results with grafting of ±2%.

It has been shown previously that the molecular weight of PCL increases with higher enzyme-to-monomer ratio. [10] Therefore, the PCL block length could, in principle, be controlled. Under the initial conditions, it can be seen that only short lengths of the grafted PCL blocks in the final copolymer were produced (entries 1 and 2, Table 2). To obtain longer PCL blocks, the amount of enzyme to monomer was doubled (entry 3, Table 2), leading to a higher PCL content in the copolymer together with higher conversion of the CL monomer. The increase of the amount of enzyme in the reaction resulted in longer graft lengths, but no further increase of the grafting density was observed. Therefore, the efficiency of grafting remained the same.

Why is the level of grafting apparently fixed at ca. 30−40%? Clearly the graft distribution along the backbone is dictated by the reactivity of the monomers MMA and HEMA. These have been accurately calculated using Fineman−Ross methods for ATRP of HEMA and MMA, showing that HEMA is about 3 times more reactive than MMA.[19] There have been several reports on the determination of reactivity ratios in different transition-metal-mediated radical copolymerizations in conven-

tional solvent, and these have shown a significant difference in reactivity of MMA and HEMA.[20,21] If we assume that the reactivity ratios remain similar in $scCO_2$, then HEMA will polymerize more quickly, leading to a tapered distribution with a higher proportion of HEMA units and hence hydroxyl functionalities bunched close to one end of the polymethacrylate backbone. Since HEMA polymerizes faster than MMA, the reaction was also performed with a lower content of HEMA in the feed to study the effect of polymerization conditions on the grafting density. However, conditions were never achieved in which 100% grafting was observed. Only a slight increase from 28% to 40% in the grafting density was observed (entries 1 and 2 in Table 2).

Additionally, in this one-pot simultaneous process, it is also possible for the HEMA monomer to be acted upon by the lipase-forming PCL-grafted HEMA monomer. Such monomer could also be incorporated into the growing HEMA/MMA chain. One might expect that the incorporation of this HEMA macromonomer into the chain and the close proximity of the HEMA functionalities would also cause some significant steric hindrance, thus preventing grafting of all the OH groups. Finally, there are steric considerations around the enzyme-active site, determined by the mode of action of Novozym-435.[22] In the first step, ϵ-caprolactone, an acyl donor, binds to the Ser-105 of the Novozym-435 to form an acyl-enzyme intermediate. When the polymethacrylate chain enters the active site, the OH group on a HEMA unit must attack the acylated enzyme. The acyl-enzyme intermediate is then deacylated, and the main polymer chain is released from the active site with one grafted CL unit attached. The chain propagation of polycaprolactone continues by this mechanism. But to allow this to happen, the poly(methacrylate) chain has to bend in order to enter into the enzyme cavity and locate the hydroxyl function close to the acyl-enzyme intermediate. If the OH groups are too close to each other along the poly(methacrylate) backbone, this could hinder the access to some of the OH groups, thus preventing ROP from occurring at all possible sites. Indeed, it will be more favorable to promote the propagation of a preexisting grafted PCL chain (on a neighboring OH functionality) than to initiate a new chain from a new OH group. All of these factors explain why the grafting efficiency is somewhat less than 100% for enzymatic polymerization: about 33% of the OH groups for the two-step approach and a maximum of 40% for the simultaneous one-pot approach.

A comparison was also made between polymerization using enzyme and using chemical catalysts. For the chemically catalyzed polymerization, a reaction was carried out using tin(II) bis(2-ethylhexanoate) [$Sn(Oct)_2$] in THF and resulted that all hydroxyl groups of the P(MMA-*co*-HEMA) were initiated, leading to 100% grafting as reported previously.[20] Once again, steric hindrance might be the reason for the low initiation efficiency in enzymatic catalysis. We assume that these differences are a consequence of different activation mechanisms.

These results are consistent with the exhaustive study recently published by Moeller comparing the enzymatic and the chemical grafting of CL from a linear and a star-shaped polyglycidol macroinitiator.[23] The authors reported quantitative grafting of a multiinitiator by chemical catalysis but only partial grafting by enzymatic catalysis. The difference in grafting density can be explain by the different polymerization mechanisms governing both polymerization techniques. Steric hindrance might be the reason for the low initiation efficiency in enzymatic catalysis.

Additionally, in the sequential two-step approach, this steric limitation and the difficulty of grafting PCL from OH along the backbone mean that any adventitious water present in the system will easily initiate formation of nongrafted PCL homopolymer. In the simultaneous one-step approach, free water is much less of a problem. The higher concentration of HEMA monomer in addition to the Poly(MMA-*co*-HEMA) copolymer formed in the system ensures that water is relatively much less important and does not compete with the OH groups of the HEMA units. Experimentally, we do indeed observe more PCL homopolymer in the sequential two-step approach (Figure 5) compared to the simultaneous one-step approach.

A comparison was made with enzymatic grafting polymerization of the poly(methacrylate) backbone in conventional solvents. The enzymatic ROP grafting polymerization of ϵ-caprolactone via a sequential two-step approach was performed using the P(MMA-*co*-HEMA) as a multiple macroinitiator in toluene at 70 °C, leading to 30% grafting density. These experiments showed that the two-step enzymatic grafting of caprolactone from a multifunctional macroinitiator backbone leads to a limited grafting density lower than 100% in either toluene or $scCO_2$ due to steric hindrace. However, the simultaneous eROP and ATRP polymerization only works in $scCO_2$ (not in conventional solvents). We believe that this is a result of the very effective plasticization of the Poly(MMA-*co*-HEMA) copolymer chain in the ϵ-CL/$scCO_2$ system. We demonstrate that ϵ-CL monomer and $scCO_2$ act as very effective cosolvents for the ATRP of MMA and HEMA. To achieve an insight into the phase behavior during the simultaneous process, the polymerization was carried out in a view cell. Under the reactions conditions, the mixture remained homogeneous throughout the period of polymerization, and thus the simultaneous one-pot approach proceeded successfully.

The thermal properties of the copolymer were investigated by DSC. Crystallization behavior was only seen in copolymers that were rich in PCL units. PCL homopolymer has a crystallization temperature (T_c) of 41.5 °C, whereas Poly(MMA-*co*-HEMA) copolymer is amorphous and does not exhibit a crystallization temperature. DSC was used to look for crystallization of the copolymer by comparison with polymer blends. We prepared a physical mixture of Poly(MMA-*co*-HEMA) copolymer and PCL homopolymer with a content of PCL that matched the graft copolymers. The samples were run for two cycles (ensuring intimate mixing and blending), and we analyzed the second run of each sample. The physical blend showed no alteration in the crystallization temperature of the PCL as one would expect for such phase-separated materials. However, the graft copolymer (entries 1 and 2, Table 2) does not exhibit a crystallization peak. The absence of crystallinity in the graft copolymer is due to the inhibiting effect of the Poly(MMA-*co*-HEMA) upon the effective packing of the PCL chain in the crystallites. The crystallization peak was not observed for contents lower than 40 mol % CL (entries 1 and 2 in Table 2). However, crystallization behavior was observed for entry 3 in Table 2 and all entries in Table 1 where the PCL content is equal to or higher than 80%.

Conclusion

Well-defined poly(alkyl methacrylate)-*graft*-polycaprolactone copolymers have been successfully synthesized in supercritical carbon dioxide by combination of two versatile polymerization techniques: atom transfer radical polymerization and enzymatic ring-opening polymerization. Both a simple one-pot simultaneous polymerization and a two-step "grafting-from" technique lead to graft copolymers. Exhaustive studies show that ca. 30−40% of the hydroxyl functionalities can be initiated to form

PCL grafts from the polymer backbone. Various experiments suggest that the limited amount of grafting is due to steric hindrance reasons. The synthesis of amphiphilic core−shell polymers with exceptional architectures was successfully achieved by the enzymatic polymerization grafting. The resulting structures contain unreacted hydroxyl groups in the polymer backbone available for further chemical modification. These novel copolymers have the unique feature of being reactive since not only is each polyester graft end-capped by a hydroxyl end group, which can be easily derivatized into other organic functionalities, but also the presence of free hydroxyl groups in the backbone which can be also labeled. These copolymers can thus be used as intermediates for the design of more complex macromolecular systems. This is again an additional benefit of the grafting route. Moreover, these polymeric materials could be used in a wide range of biomedical applications.

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Supporting Information Available: ^{1}H NMR spectra of the final poly(MMA-*co*-HEMA)-*g*-PCL graft copolymer and the remaining poly(MMA-*co*-HEMA) copolymer after hydrolysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Wang, J. S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614.
(2) Chong, B. Y. K.; Le, T. P. T.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1999**, *32*, 2071.
(3) Mecerreyes, D.; Jérôme, R.; Dubois, P. *Adv. Polym. Sci.* **1999**, *147*, 1.
(4) Mercerreyes, D.; Moineau, G.; Dubois, P.; Jérome, R.; Hedrick, J. L.; Hawker, C. J.; Malmström, E. E.; Trollsas, M. *Angew. Chem., Int. Ed.* **1998**, *37*, 1274.
(5) Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. *Nature (London)* **2001**, *409*, 258.
(6) a) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793. (b) Gross, R. A.; Kumar, A.; Kalra, B. *Chem. Rev.* **2001**, *101*, 2097.
(7) (a) Meyer, U.; Palmans, A. R. A.; Loontjes, T.; Heise, A. *Macromolecules* **2002**, *35*, 2873. (b) Peeters, J.; Palmans, A. R. A.; Veld, M.; Scheijen, F.; Heise, A.; Meijer, E. W. *Biomacromolecules* **2004**, *5*, 1862.
(8) Woods, H. M.; Silva, M. M. C. G.; Nouvel, C.; Shakesheff, K. M.; Howdle, S. M. *J. Mater. Chem.* **2004**, *14*, 1663.
(9) Mesiano, A. J.; Beckman, E. J.; Russell, A. J. *Chem. Rev.* **1999**, *99*, 623.
(10) Loeker, F. C.; Duxbury, C. J.; Kumar, R.; Gao, W.; Gross, R. A.; Howdle, S. M. *Macromolecules* **2004**, *37*, 2450.
(11) Ryu, K.; Kim, S. *Korean J. Chem. Eng.* **1996**, *13*, 415.
(12) Takamoto, T.; Uyama, H.; Kobayashi, S. *e-Polym.* **2001**, *4*, 1.
(13) Duxbury, C. J.; Wang, W.; de Geus, M.; Heise, A.; Howdle, S. M. *J. Am. Chem. Soc.* **2005**, *127*, 2384.
(14) Villarroya, S.; Zhou, J.; Duxbury, C. J.; Heise, A.; Howdle, S. M. *Macromolecules* **2006**, *39*, 633.
(15) Zhou, J.; Villarroya, S.; Wang, W.; Wyatt, M. F.; Duxbury, C. J.; Thurecht, K. J.; Howdle, S. M. *Macromolecules* **2006**, *39*, 5352.
(16) Wang, W. X.; Yin, Z. H.; Detrembleur, C.; Lecomte, P.; Lou, X. D.; Jerome, R. *Macromol. Chem. Phys.* **2002**, *203*, 968.
(17) Xu, X.; Huang, J. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5523.
(18) Mahapatro, A.; Kalra, B.; Kumar, A.; Gross, R. A. *Biomacromolecules* **2003**, *4*, 544.
(19) Yilmaz, E.; Küçükyavuz, Z. *Polymer* **1993**, *34*, 145.
(20) Matyjaszewski, K. *ACS Symp. Ser.* **2003**.
(21) Ydens, I.; Degée, P.; Dubois, P.; Libiszowski, J.; Duda, A.; Penczek, S. *Macromol. Chem. Phys.* **2003**, *204*, 171.
(22) Cordova, A.; Iversen, T.; Hult, K.; Martinelle, M. *Polymer* **1998**, *39*, 6519.
(23) Hans, M.; Gasteier, P.; Keul, H.; Moeller, M. *Macromolecules* **2006**, *39*, 3184.

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