RAFT Synthesis of Acrylic Copolymers Containing Poly(ethylene glycol) and Dioxolane Functional Groups: Toward Well-Defined Aldehyde Containing Copolymers for Bioconjugation

Nicholas A. A. Rossi,[†,‡] Yuquan Zou,[†] Mark D. Scott,[†,‡] and Jayachandran N. Kizhakkedathu*[,†]

Centre for Blood Research and the Department of Pathology and Laboratory Medicine, and, Canadian Blood Services, Life Sciences Centre, University of British Columbia, Vancouver, BC V6T 1Z3, Canada.

Received March 18, 2008; Revised Manuscript Received May 8, 2008

ABSTRACT: Copolymers of poly(ethylene glycol) methyl ether methacrylate (PEGMA) and one of two dioxolane-containing monomers, (2,2-dimethyl-1,3-dioxolane)methyl acrylate (DDMA) and (2,2-dimethyl-1,3-dioxolane)methyl acrylamide (DDMAA), were successfully synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization. RAFT copolymerization was performed in dimethylformamide (DMF) at 70 °C for 24 h using 4,4′-azobis(4-cyanovaleric acid) as initiator and *N*-(sodium ethane sulfonic acid)-2-((thiobenzyl)sulfanyl)proprionamide (CTA 1), 4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid (CTA 2), or *S*,*S*′-bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (CTA 3) as the chain transfer agent (CTA). Control over molecular weight and composition was achieved by altering the CTA concentration and the monomer feed ratio respectively. The resulting copolymers had narrow molecular weight distributions (polydispersity indices typically between 1.2 and 1.3), while monomer conversions were typically 60%. Kinetic studies revealed that PEGMA was consumed at a higher rate than the comonomers over a given time. The molecular weight of the copolymer increased linearly with conversion, while a low polydispersity was maintained throughout. The copolymerization reactivity ratios were determined using the Mayo−Lewis method. After copolymerization, the dioxolane functional groups were deprotected to form 1,2-diol groups and subsequently oxidized with HIO_4 to form reactive aldehyde groups. Subsequent chemical modification of the dioxolane moieties to aldehyde groups showed no adverse effects in terms of degradation of the copolymer (specifically ester linkages). The advantage of the current synthesis over direct copolymerization of aldehyde-based monomers is the stability of the 1,2-diol moiety compared to the corresponding aldehyde copolymer. The availability of the aldehyde groups along the polymer backbone to form stable conjugates with amine containing molecules was confirmed via a reaction with the iron chelating drug desferrioxamine (DFO). Conjugation was achieved via an aldamine reaction, followed by a reduction of the resulting Schiff base to a secondary amine. Full characterization of the copolymers was performed using NMR spectroscopy and GPC−MALLS, while UV−vis absorption spectroscopy was used to determine the efficiency of DFO conjugation.

Introduction

The synthesis of functional, biocompatible polymers for use as bioconjugates in the field of polymer therapeutics has received increasing attention in recent years.[1–9] Interest in the use of polymers to facilitate the delivery of drugs has stemmed from their ability to solubilize, camouflage, or stabilize compounds in order to improve properties such as bioavailability, biocompatibility, immunogenicity, and circulation times.[1] One of the most effective methods for overcoming the drawbacks of parent drugs is its incorporation into drug delivery vehicles. In particular, the attachment of poly(ethylene glycol) (PEG) to compounds such as polypeptides has been used to increase solubility and decrease the toxicity and immunogenicity of a wide range of drugs.[2,3] In effect, the incorporation of PEG has enabled the drugs to be administered at lower dosages and with less frequency.

Conjugation of the relevant compounds can be achieved through the use of reactive functional groups situated either along the backbone or at the terminals of the polymer.[4] The type of compound and intended application of the resulting bioconjugate determines the type of the functional group required.[5] Polymers containing aldehyde groups are particularly useful since they can be exploited to covalently attach proteins,[6] enzymes,[7] drugs, and other biologically important molecules containing reactive primary amines.[8,9] Aldehyde groups react under mild conditions with primary amines and hydrazines to form Schiff bases and hydrazone linkages respectively. In this respect, aldehyde groups have been shown to be particularly versatile and convenient. However, the synthesis of well-defined aldehyde-containing copolymers with controlled architecture and narrow polydispersity has proven to be quite challenging.

Relatively recent advances in "living"/controlled polymerization techniques have led to the successful polymerization of a wide range of novel and functional monomers. Reversible addition-fragmentation chain transfer (RAFT) polymerization[10–14] is one such technique that has received considerable attention due to both the wide range of monomers that can be polymerized and the control over molecular weights (with polydispersities often <1.1). For many biomedical purposes, it is essential to prepare polymers with narrow polydispersities, since the biocompatibility of the polymer can be influenced by molecular weight.[15] In addition, polymers with functional groups that are available for further modification, such as the covalent attachment of biologically relevant molecules, are especially useful for drug delivery applications. RAFT polymerization is based on the initiation of a standard free radical and the

* Corresponding author. E-mail: jay@pathology.ubc.ca. Telephone: 1-604-822-7085. Fax: 1-604-822-7742.
† Centre for Blood Research and the Department of Pathology and Laboratory Medicine, University of British Columbia.
‡ Canadian Blood Services, Life Sciences Centre, University of British Columbia.

10.1021/ma800606k CCC: $40.75
Published on Web 06/25/2008

reversible chain transfer of dithiocarbonyl compounds.[10] RAFT is also characterized by a linear molecular weight conversion profile and an inherent method for predicting molecular weight based on the ratio of chain transfer agent (CTA) to monomer feed. Monomers with functional groups such as acid, hydroxyl, and amine moieties and more recently activated esters,[16–19] disulfides,[20] aldehydes,[21–23] and phosphonates[24] are compatible with RAFT. A range of acrylamide-based monomers such *N*-isopropyl acrylamide (NIPAM),[25,26] acrylamide,[27] *N*,*N*′-dimethylacrylamide,[27] and *N*-*tert*-butyl acrylamide[28] have also been synthesized.

Too date, there are very few examples of aldehyde functionalized polymers synthesized via RAFT polymerization in the literature.[21–23] In addition, such compounds are difficult to store since aldehyde groups are highly reactive and relatively unstable. A more common approach involves the postpolymerization modification of polymers with protecting groups to form aldehydes. Here, we report the first RAFT copolymerization of poly(ethylene glycol) methyl ether methacrylate (PEGMA) with dioxolane-based monomers; (2,2-dimethyl-1,3-dioxolane)methyl acrylate (DDMA) and (2,2-dimethyl-1,3-dioxolane)methyl acrylamide (DDMAA). The dioxolane functional groups are subsequently deprotected and then oxidized to form reactive aldehyde groups. To the best of our knowledge, these are the first example of a copolymer containing both PEG and aldehyde functional side groups. The two dioxolane monomers were chosen due to their contrasting stabilities: the ester containing DDMA unit can be used to form a cleavable bond to a conjugate, while the DDMAA is hydrolytically more stable since it contains an amide linkage. The molecular weight characteristics, composition, and stability of the copolymers before and after modification are discussed in detail. Kinetic studies and the determination of the copolymer reactivity ratios suggest that the monomers form random copolymers. The conjugation of the iron chelating compound desferrioxamine (DFO) was demonstrated as an example of how the aldehyde groups can be utilized to conjugate bioactive molecules such as peptides, enzymes, and drugs. The copolymerization, chemical modification, and efficacy with which DFO is conjugated to the copolymer backbone are measured using NMR, GPC, and UV−vis spectroscopy.

Experimental Section

Materials and Methods. All reagents were purchased from Aldrich and used as received, unless otherwise stated. Poly(ethylene glycol) (M_n 400) methyl ether methacrylate (PEGMA) was purchased from Polysciences, Inc. (99.99%), and free radical inhibitor was removed prior to polymerization by passing through a basic alumina column. The chain transfer agents *N*-(sodium ethane sulfonic acid)-2-((thiobenzyl)sulfanyl)proprionamide (CTA 1), 4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid (CTA 2), and *S*,*S*′-bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (CTA 3) were synthesized following reported procedures.[28,29] Deferoxamine mesylate salt (∼95%) was purchased from Aldrich and used as received. Dialysis was carried out using a Spectra/Por dialysis membrane (MWCO 1000). The molecular weights of the polymers were determined by gel permeation chromatography (GPC) using a DAWN-EOS multiangle laser light scattering (MALLS) (Wyatt Technology Inc.) and Optilab RI detectors in aqueous 0.1N $NaNO_3$ solution; the details have been described previously.[30] A d*n*/d*c* value of 0.132 was used for the calculation of the molecular weight of the copolymers in 0.1 N $NaNO_3$ soution. ^{1}H NMR spectra were recorded on a Bruker Avance 300 MHz NMR spectrometer; ^{13}C NMR spectra were recorder on a Bruker Avance 400 MHz NMR spectrometer; DMSO-d_6 and $CDCl_3$ (Cambridge Isotope Laboratories) were used as solvents, with the relevant solvent peak as reference. Absorbance spectra were recorded on a Thermo Spectronic UV−vis spectrometer.

Synthesis of (2,2-Dimethyl-1,3-dioxolane)methyl Acrylate (DDMA). The synthesis of DDMA was based on a procedure described by Oguchi et al.[31] Under a dry, inert argon atmosphere, acryloyl chloride (1.44 mL, 17.7 mmol) was added slowly to a mixture of triethylamine (2.46 mL, 17.7 mmol) and solketal (2.0 g, 15.2 mmol) in dichloromethane (30 mL) at 0 °C. The reaction was allowed to reach room temperature and left to stir for 6 h. The precipitated triethylamine hydrochloride was filtered and the solution washed consecutively with $NaHCO_3$ solution (10 mL) and water (10 mL), before the organic layer was dried over anhydrous Na_2SO_4. Remaining solvent was removed under reduced pressure and the crude product (∼95% yield) was subsequently purified by flash column chromatography using hexane and ethyl acetate as the eluent. The resulting purified product was colorless, and the yield was 72%.

^{1}H NMR (DMSO-d_6): δ 1.35−1.43 (d, 6H), 3.74−3.77 (t, 1 H), 4.06−4.36 (m, 4 H), 5.95 (d, 1H), 6.1−6.2 (q, 1H), 6.3−6.37 (d, 1H).

Synthesis of (2,2-Dimethyl-1,3-dioxolane)methyl Acrylamide (DDMAA). Under a dry, inert argon atmosphere, acryloyl chloride (3.2 mL, 39.0 mmol) was added dropwise to a stirred solution of 2,2-dimethyl-1,3-dioxolane-4-methanamine (5 mL, 38.5 mmol) and triethylamine (5.6 mL, 40 mmol) in dichloromethane (30 mL) at 0 °C. The reaction was allowed to reach room temperature and stirred for 4 h. The reaction mixture was filtered and washed with $NaHCO_3$ solution (5 mL) and water (5 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product (∼95% yield) was subsequently purified by flash column chromatography using hexane and ethyl acetate as the eluent. The resulting purified product was colorless and the yield was 67%.

^{1}H NMR (400 M Hz, $CDCl_3$): δ 1.16−1.24 (d, 6H), 3.20 (tt, 1H) 3.38 (tt, 1H), 3.50 (t, 1H), 3.87 (t, 1H), 4.08 (p, 1H), 5.47 (dd, 1H), 6.0−6.14 (m, 2H), 6.9 (s, 1H).

Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-*N*-(2,2-dimethyl-1,3-dioxolane-4-methyl)acrylamide) [P(PEGMA-*co*-DDMAA)]. In a typical RAFT copolymerization experiment, 2,2-dimethyl-1,3-dioxolane-4-methylacrylamide (0.091 g, 0.49 mmol), PEGMA (2.00 g, 4.21 mmol), *S*,*S*′-bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (CTA 3) (0.025 g, 0.090 mmol), 4,4′-azobis(4-cyanovaleric acid) (ACVA, 0.0040 g), and dimethylformamide (DMF, 10 mL) were added to a small round-bottom flask and degassed with argon. In an argon filled glovebox, the flask was stirred at 70 °C for 24 h. After the solution was allowed to cool to room temperature, water (10 mL) was added before dialyzing against water for 5 days with frequent changes (three times daily) in water. The purified polymer was recovered by lyophilization and characterized by GPC and NMR spectroscopy. The resulting product was a viscous yellow oil, and the yield was 61%.

^{1}H NMR (DMSO-d_6): δ 0.65−1.1 (b, 3 H); 1.24−1.32 (d, 6 H); 1.5−2.0 (b 2H); 3.23 (s, 3H); 3.4−3.6 (b, 32H); 4.0 (b, 2H); 7.4−7.9 (b, 1H).

^{13}C NMR (DMSO-d_6): δ 16.1, 18.0, 25.2, 26.8, 44.1, 54.0, 58.0, 63.7, 67.8, 69.6, 71.3, 73.8, 108.2, 176.0, 177.0.

Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-*N*-(2,2-dimethyl-1,3-dioxolane-4-methyl)acrylamide) [P(PEGMA-*co*-DDMA)]. The RAFT copolymerization of 2,2-dimethyl-1,3-dioxolane-4-methylacrylate and PEGMA was carried out using similar methodology described above. Subsequent chemistry performed on the dioxolane-containing units of P(PEGMA-*co*-DDMA) followed the same procedures as outlined below for P(PEGMA-*co*-DDMAA).

^{1}H NMR (DMSO-d_6): δ 0.65−1.1 (b, 3 H); 1.24−1.32 (d, 6 H); 1.5−2.0 (b 2H); 3.23 (s, 3H); 3.4−3.6 (b, 32H); 4.0 (b, 2H).

Deprotection of Ketal Groups: Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-2,3-dihydroxypropyl acrylamide) [P(PEGMA-*co*-DHPAA)]. In a typical reaction P(PEGMA-*co*-DDMAA) (1.50 g) was dissolved in water (5 mL). Glacial acetic acid (3 mL) was added dropwise to make up a 40% solution and refluxed for 3 h at 100 °C. The product was dialyzed

for 2 days against water with frequent changes (three times daily) in water. The solution was lyophilized and characterized by GPC and NMR spectroscopy.

^{1}H NMR (DMSO-d_6): δ 0.65−1.1 (b, 3 H); 1.5−2.0 (b 2H); 3.23 (s, 3H); 3.4−3.6 (b, 32H); 4.0 (b, 2H); 4.4 and 4.6 (d, 2H); 7.4−7.9 (b, 1H).

^{13}C NMR (DMSO-d_6): δ 16.2, 18.1, 44.1, 54.0, 58.0, 63.7, 67.8, 69.6, 71.3, 176.1, 176.7.

Deprotection of Ketal Groups: Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-2,3-dihydroxypropyl acrylate) [P(PEGMA-*co*-DHPA)]. A similar procedure as outlined for the synthesis of P(PEGMA-*co*-DHPAA) was used for the deprotection of P(PEGMA-*co*-DDMA).

^{1}H NMR (DMSO-d_6): δ 0.65−1.1 (b, 3 H); 1.5−2.0 (b 2H); 3.23 (s, 3H); 3.4 − 3.6 (b, 32H); 4.0 (b, 2H); 4.4 and 4.6 (d, 2H).

Formation of Aldehyde Groups: Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-*N*-(2-oxoethyl)acrylamide) [P(PEGMA-co-OEAA)]. P(PEGMA-*co*-DHPAA) (0.360 g) was dissolved in 5 mL of water and HIO_4 (0.060 g) was added. The solution was stirred for 2 h at room temperature. The product was dialyzed for 24 h against water with frequent changes in water (every 3 h). The product was not lyophilized, as removal of water tended to cause the product to become insoluble through cross-linking. However, an aliquot of the solution was lyophilized and characterized by ^{1}H NMR spectroscopy.

^{1}H NMR (DMSO-d_6): δ 0.65−1.1 (b, 3 H); 1.5−2.0 (b 2H); 3.23 (s, 3H); 3.4−3.6 (b, 32H); 4.0 (b, 2H); 4.4 and 4.6 (d, 2H); 7.4−7.9 (b, 1H), 9.45 (s, 1H).

Formation of Aldehyde Groups: Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-2-oxoethyl acrylate) [P(PEGMA-*co*-OEA)]. A similar procedure as outlined for the synthesis of P(PEGMA-*co*-OEAA) was used for the oxidation of P(PEGMA-*co*-DDMA).

^{1}H NMR (DMSO-d_6): δ 0.65−1.1 (b, 3 H); 1.5−2.0 (b 2H); 3.23 (s, 3H); 3.4−3.6 (b, 32H); 4.0 (b, 2H); 4.4 and 4.6 (d, 2H); 9.45 (s, 1H).

Synthesis of the Polymer−Desferrioxamine Conjugate (P−DFO). Desferrioxamine mesylate salt (DFO, 0.100 g) was dissolved in a solution of P(PEGMA-*co*-OEAA) (0.310 g) in water (6 mL) prior to the addition of $NaCNBH_3$ (0.1 g). The reaction was continued for 2 days at room temperature and then dialyzed for 4 days against water and lyophilized. The product was characterized by GPC and NMR spectroscopy. The amount of DFO bound to the copolymer was determined by UV−vis spectroscopy after dissolving the copolymer (2.5 mg) in ferrous sulfate solution (2.5 mL, ∼10.0 mM) and leaving to stand overnight at room temperature. On the basis of a molar absorptivity of 2300 M^{-1} cm^{-1}, the DFO content was determined by measuring the absorbance at 429 nm.[32] A similar procedure for conjugation of DFO to P(PEGMA-*co*-OEA) was also carried out.

^{1}H NMR (DMSO-d_6): δ 0.6−1.1 (b, 3 H); 1.21 (m, 6 H); 1.38 (m, 4 H); 1.51 (m, 8 H); 1.6 − 2.0 (b, 2H); 1.95 (s, 3 H); 2.27 (t, 4 H); 2.57 (t, 4 H); 2.99 (q, 4 H); 3.23 (s, 3H); 3.4 − 3.6 (b, 32H); 4.0 (b, 2H); 4.4 and 4.6 (d, 2H); 7.4 − 7.9 (b, 1H), 7.75 (s, 1H); 9.6 (s, 1H).

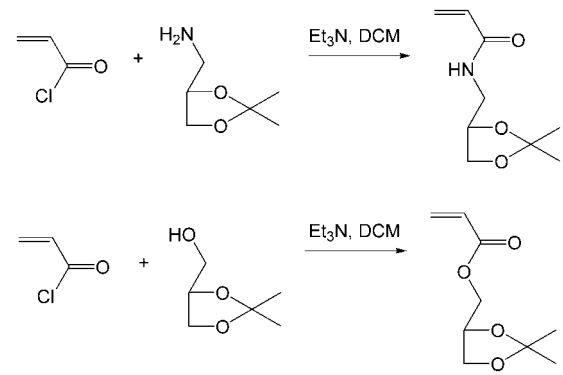
^{13}C NMR (DMSO-d_6): δ 16.2, 18.0, 20.3, 23.4, 26.0, 27.6, 28.8, 29.9, 38.7, 44.1, 54.0, 58.0, 63.7, 67.8, 69.6, 71.3, 90.5, 170.1, 171.2, 171.9, 176.1, 177.0.

RAFT Polymerization of Poly(dimethyl-1,3-dioxolane-4-methyl)acrylamide) (PDDMAA) Homopolymer. DDMAA (0.205 g, 1.10 mmol), 4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid (CTA 2) (0.0061 g, 0.022 mmol), 4,4′- azobis(4-cyanovaleric acid) (0.0010 g), and DMF (2 mL) were added to a small round-bottom flask and degassed with argon. In an argon filled glovebox, the flask was stirred at 70 °C for 24 h. After the solution was allowed to cool to room temperature, an aliquot was removed and analyzed using ^{1}H NMR. Presence of nearly 100% of the monomer indicated polymerization had failed.

RAFT Polymerization of Poly(dimethyl-1,3-dioxolane-4-methyl)acrylate) (PDDMA) Homopolymer. DDMA (0.205 g, 1.10 mmol), 4-cyano-4-((thiobenzoyl)sulfanyl)pentanoic acid (CTA 2) (0.0061 g, 0.022 mmol), 4,4′- azobis(4-cyanovaleric acid) (0.0010 g), and DMF (2 mL) were added to a small round-bottom flask and degassed with argon. In an argon filled glovebox, the flask was stirred at 70 °C for 24 h. After the solution was allowed to cool to room temperature, an aliquot was removed and analyzed using ^{1}H NMR. The polymer was recovered by precipitating into water and dried under reduced pressure (yield 45%).

^{1}H NMR (DMSO-d_6): 1.24−1.30 (d, 6 H); 1.3−2.0 (b 2H); 2.0−2.5 (b, 1H); 3.9−4.3 (b, 5H).

Scheme 1. Synthesis of (2,2-Dimethyl-1,3-dioxolane)methyl Acrylamide (DDMAA) and (2,2-Dimethyl-1,3-dioxolane)methyl Acrylate (DDMA)



Results and Discussion

Monomer Synthesis. The primary motivation of this study is to develop a well-controlled RAFT copolymerization method for the synthesis of PEG-containing functional copolymers. In order to synthesize acrylic copolymers containing both PEG side chains and aldehyde functional groups, well-defined prepolymers were produced initially. Since it is difficult to copolymerize aldehyde containing monomers (especially aliphatic aldehydes) without side reactions using RAFT polymerization in aqueous solution, precursor monomers containing dioxolane groups were used instead. Two separate dioxolane-containing monomers were synthesized: an ester-containing dioxolane monomer, (2,2-dimethyl-1,3-dioxolane)methyl acrylate (DDMA), and an amide-containing dioxolane monomer, (2,2-dimethyl-1,3-dioxolane)-methyl acrylamide (DDMAA) (Scheme 1). In general, ester compounds tend to degrade over time under physiological conditions, while amide groups are more stable. As a result, the two classes of monomer and the corresponding copolymers can be described as either "cleavable" (DDMA) or "non-cleavable" (DDMAA). The resulting copolymers containing dioxolane functional groups are modified to form aldehyde-functionalized copolymers, which can then be used to conjugate other molecules to the polymer backbone. The advantage is that a cleavable or noncleavable linkage between the drug molecule and the polymer can be generated after attaching it through the aldehyde group. The potential benefit of having a) a cleavable ester group between the PEG chain and the polymer backbone (PEGMA unit) and b) a noncleavable amide group between the dioxolane moiety and the polymer backbone (DDMAA unit) will be addressed later in this manuscript.

Synthesis and Characterization of P(PEGMA-*co*-DDMAA) and P(PEGMA-*co*-DDMA) Using RAFT Polymerization. The first step in the formation of aldehyde-containing copolymers involved the preparation of P(PEGMA-*co*-DDMAA) and P(PEGMA-*co*-DDMA) via RAFT polymerization (Scheme 2). A range of copolymers with different molecular weight and compositions were synthesized using chain transfer agents *N*-(sodium ethane sulfonic acid)-2-((thiobenzyl)sulfanyl)proprionamide (CTA 1), 4-cyano-4-((thiobenzoyl)sulfanyl)-

Scheme 2. RAFT Copolymerization of P(PEGMA-*co*-DDMAA) and P(PEGMA-*co*-DDMA) Using 4,4′-Azobis(4-cyanovaleric acid) as Initiator and Chain Transfer Agents *N*-(Sodium ethanesulfonic acid)-2-((thiobenzyl)sulfanyl)proprionamide (CTA 1), 4-Cyano-4-((thiobenzoyl)sulfanyl)pentanoic Acid (CTA 2), and *S*,*S*′-Bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (CTA 3)

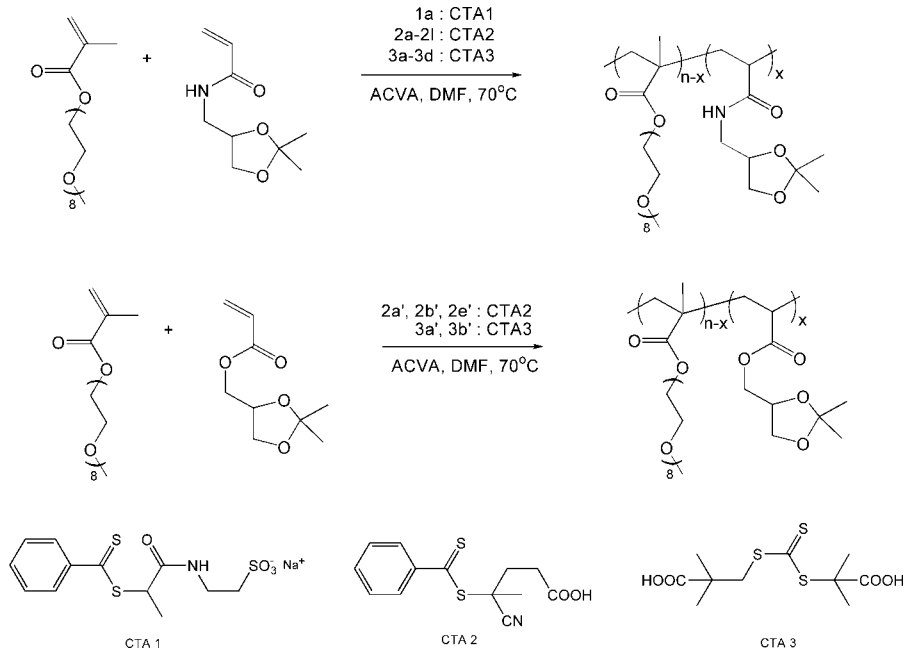


Table 1. Synthesis of Poly{poly(ethylene glycol) methyl ether methacrylate-*co*-(2,2-dimethyl-1,3-dioxolane)methyl acrylamide}, P(PEGMA-*co*-DDMAA), with Different Molecular Weights and DDMAA Composition

sample	CTA	feed ratios DDMAA: PEGMA:CTA:init[a]	M_n theory[b]	M_n GPC[c]	PDI GPC[c]	% DDMAA theory[d]	% DDMAA NMR[e]
P-1a	**1**	12:105:1:0.39	52 100	80 130	1.32	10	10
P-2a	**2**	8:72:1:0.23	35 700	68 170	1.28	10	10
P-2b	**2**	18:72:1:0.23	37 500	44 870	1.18	20	14
P-2c	**2**	31:72:1:0.23	40 000	36 910	1.23	30	18
P-2d	**2**	4:36:1:0.12	17 800	28 920	1.22	10	9.5
P-2e	**2**	5.5:49:1:0.15	24 300	44 200	1.25	10	9.5
P-2f	**2**	4.5:40:1:0.15	19 800	33 270	1.24	10	9
P-2g	**2**	12.5:49:1:0.15	25 600	21 500	1.27	20	15
P-2h	**2**	21:49:1:0.15	27 200	77 170	1.63	30	22
P-2i	**2**	15:58:1:0.2	30 300	25 360	1.35	20	15
P-3a	**3**	8:72:1:0.23	35 700	58 180	2.2	10	9
P-3b	**3**	18:72:1:0.23	37 500	71 200	1.66	20	13.5
P-3c	**3**	31:72:1:0.23	40 000	58 740	1.71	30	24
P-3d	**3**	5.5:48:1:0.16	23 800	26 600	1.65	10	11

[a] Relative concentrations of DDMAA monomer, PEGMA monomer, charge transfer agent, and initiator (4,4′-azobis(4-cyanovaleric acid)) at the start of the reaction. [b] Theoretical molecular weight determined from the ratio of CTA to total monomer concentration assuming 100% conversion. [c] Molecular weight (M_n) and polydispersity index (PDI) determined via gel permeation chromatography (GPC). [d] Molar percentage of DDMAA compared to total monomer available at the start of the reaction. [e] Molar percentage of DDMAA compared to total units in copolymer as determined by ^{1}H NMR.

Table 2. Synthesis of Poly{poly(ethylene glycol) methyl ether methacrylate-*co*-(2,2-dimethyl-1,3-dioxolane)methyl acrylate}, P(PEGMA-*co*-DDMA), with Different Molecular Weights and DDMA Composition

sample	CTA	feed ratios DDMA: PEGMA:CTA:init[a]	M_n theory[b]	M_n GPC[c]	PDI GPC[c]	% DDMA theory[d]	% DDMA NMR[e]
P-2a′	**2**	8:72:1:0.23	35 700	43 120	1.33	10	12
P-2b′	**2**	18:72:1:0.23	37 500	33 360	1.25	20	18
P-2e′	**2**	5.5:49:1:0.14	24 300	23 490	1.22	10	12
P-3a′	**3**	8:72:1:0.23	35 700	86 700	1.45	10	11
P-3b′	**3**	18:72:1:0.23	37 500	55 500	1.46	20	18

[a] Relative concentrations of DDMA monomer, PEGMA monomer, charge transfer agent, and initiator (4,4′-azobis(4-cyanovaleric acid)) at the start of the reaction. [b] Theoretical molecular weight determined from the ratio of CTA to total monomer concentration assuming 100% conversion. [c] Molecular weight (M_n) and polydispersity index (PDI) determined via gel permeation chromatography (GPC). [d] Molar percentage of DDMA compared to total monomer available at the start of the reaction. [e] Molar percentage of DDMA compared to total units in copolymer as determined by ^{1}H NMR.

pentanoic acid (CTA 2), or S,S'-bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (CTA 3). 4,4′-Azobis(4-cyanovaleric acid) was used to initiate the reaction.[33] The use of either DDMAA (Table 1) or DDMA (Table 2) as comonomer in the RAFT copolymerization with PEGMA yielded copolymers with a variety of molecular weights and compositions. Copolymers were purified by dialysis against water to remove the CTA, any remaining monomer, and DMF before recovering pure copolymer product via lyophilization. In general, a range of 10−30 mol % ketal functionality was incorporated using suitable monomer feed ratios, while molecular weights of the copolymers were varied by altering the CTA to monomer ratio.

GPC-MALLS analysis revealed the copolymers varied in molecular weight from 21 500 to 80 000. In general, the

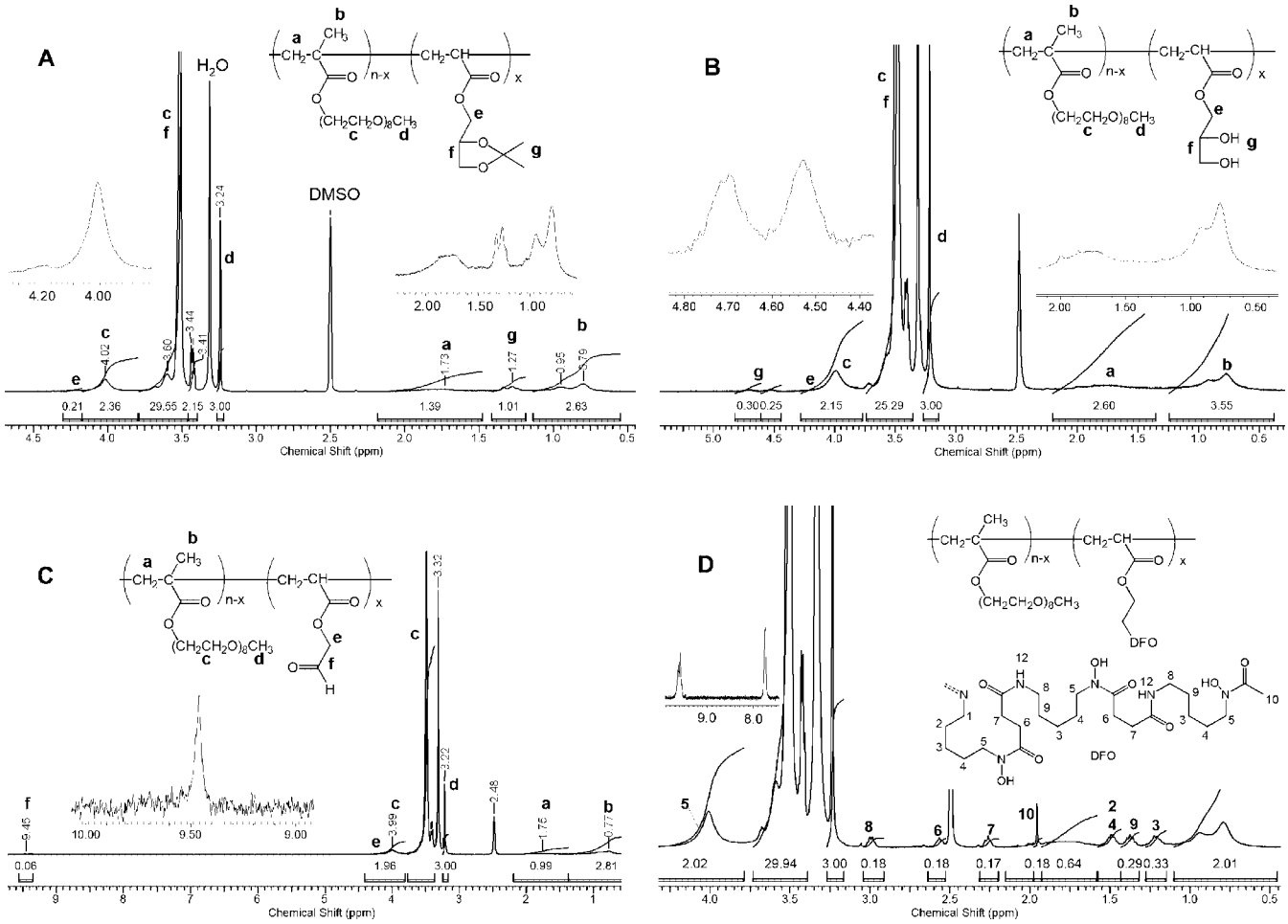


Figure 1. Following the modification of the copolymer via ^{1}H NMR spectroscopy; copolymer **P-3a′** (Table 2) is used as an example: (A) copolymer synthesized via RAFT; (B) formation of hydroxyl groups; (C) formation of aldehyde; (D) coupling of DFO to copolymer (P−DFO **3a′**).

molecular weight of the copolymers were in good agreement with theoretical values calculated from the monomer to CTA feed ratio. However, in some cases the experimental molecular weights were slightly higher compared to the theoretical values (Table 1). An explanation for this trend can be attributed to a loss of CTA efficiency. CTA concentration was altered compared to the total monomer and initiator concentration to obtain copolymers of various lengths with similar compositions. For example, **P-2a**, **P-2d**, and **P-2e** were synthesized with varying CTA 2 concentrations: at high CTA 2 concentration (a 1:40 molar ratio of CTA:total monomer), **P-2d** has a relatively low molecular weight (M_n 28 920); conversely, with half as much CTA 2 (1:80 molar ratio), **P-2a** is over twice the length of **P-2d** (M_n 68 170). The same relationship is true for copolymers synthesized using CTA 3, where a 1:80 molar ratio of CTA: total monomer yielded a copolymer (**P-3a**, M_n 58 180) of higher molecular weight compared to **P-3d** (1:53.5 molar ratio, M_n 26 600).

In addition, the choice of either the amide or ester monomer seems to have an effect on the molecular weight characteristics and composition of the copolymers. A comparison of copolymers synthesized with different monomers using similar reaction conditions (e.g., comparison of **P-2a** with **P-2a′**, **P-2b** with **P-2b′**, **P-2e** with **P-2e′**, etc., Tables 1 and 2) reveals that the acrylate P(PEGMA-*co*-DDMA) samples have molecular weights that are closer to the theoretical values. For example, the amide containing copolymer **P-2a** has a $M_n \sim 68\,000$, while the analogous ester containing copolymer **P-2a′** has a $M_n \sim 43\,000$, which is much closer to the theoretical value of M_n 35 700 calculated from the CTA:monomer ratio. As will be described later, one explanation for this trend is the higher reactivity ratio of DDMA compared to DDMAA in the copolymerization. However, control over polymerization is indicated for most copolymers synthesized using CTA 2 since a similarly low polydispersity (1.2−1.3) is observed for both types of monomer.

Control over the molecular weight distributions of the copolymers ($M_w/M_n \sim 1.2-2.2$) seems to be dependent on the type of CTA, with CTA 2 giving the best results. In addition to CTA 2, two other CTA were also investigated (scheme 2). CTA 3, a trithio compound, yielded copolymers (**P-3a** to **P-3d**, **P-3a′**, and **P-3b′**) with higher than expected molecular weights. For example, the M_n of **P-3a** is 58 180, whereas the theoretical M_n is 35 700. CTA 3 also produced copolymers with relatively high polydispersities (1.4−2.2) for both types of copolymers. For example, for analogous copolymers synthesized using the same reaction conditions, CTA 2 gave a much lower polydispersity (e.g., M_w/M_n of **P-2b** = 1.18) compared to CTA 3 (e.g., M_w/M_n of **P-3b** = 1.66). CTA 1 was also used in one case, and it gave good control over polydispersity (**P-1a**). However, a higher than expected molecular weight (80 000) for **P-1a** was observed. Since CTA 2 seemed to give the most consistent molecular weights and lowest polydispersities, efforts were focused on using CTA 2 to synthesize a range of copolymers with varying compositions (e.g., DDMAA:PEGMA monomer ratios of 10: 90, 20:80, and 30:70) and molecular weights.

The structure and composition of the copolymers were confirmed using ^{1}H and ^{13}C NMR spectroscopy. As an example, the ^{1}H NMR of copolymer **P-3a′** is shown in Figure 1A. It was difficult to deduce exact compositions of the copolymer using the integrals of the NMR peaks, since there is considerable

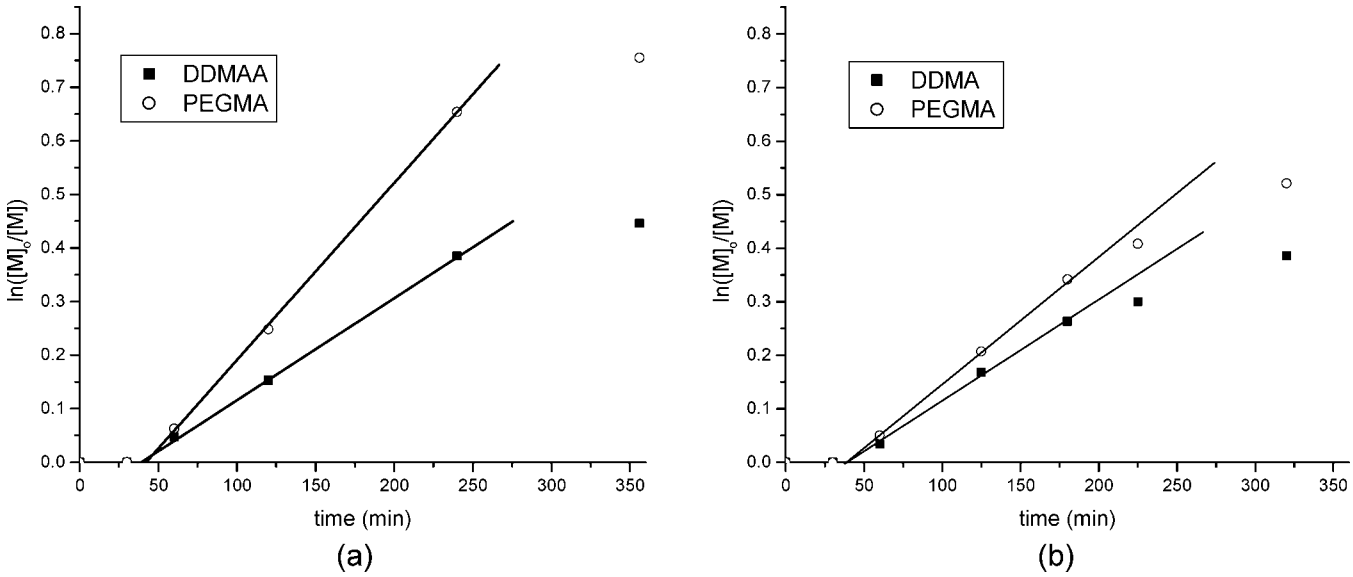


Figure 2. Monomer conversion vs time for synthesis of (a) P(PEGMA-*co*-DDMAA) **P-2b** and (b) P(PEGMA-*co*-DDMA) **P-2b′** at 70 °C in DMF at a relative molar ratio of [DDMA(A)]:[PEGMA]:[CTA]:[init] = 18: 72: 1: 0.23.

overlap between the $-CH_3$ and $-CH_2$ peaks associated with polymeric PEGMA and the CH_3 peaks associated with the dioxolane monomers between 0.5 and 2.0 ppm. Nevertheless, it is possible to deduce that at low concentrations (~10% DDMA or DDMAA composition) copolymers had similar compositions to the feed ratios. Conversely, at higher DDMA or DDMAA feed ratios (~30%), experimentally derived compositions were lower than expected (~22% DDMA or DDMAA composition). This can be attributed to the difference in the reactivity ratios of the monomers compared to PEGMA.

Attempts to synthesize homopolymers of DDMA and DDMAA were also made using similar reaction conditions described for the RAFT copolymerization. In the case of the amide-containing DDMAA monomer, no polymerization occurred, while the ester-based DDMA monomer underwent polymerization. This suggests the amide monomer has a very low homopolymer reactivity under the RAFT polymerization conditions described here. Hence, the presence of PEGMA monomer is essential to the propagation of DDMAA. In addition, the PDDMA homopolymer was found to be insoluble in water, while the PEG-containing copolymers were soluble in both organic solvents (e.g., DMSO, DMF and DCM) and water, highlighting one of the reasons for using PEGMA as comonomer. Interestingly, both monomers have been shown to be polymerizable using atom transfer radical polymerization (ATRP) in concurrent studies performed by our group.[34] There are also many examples in the literature of the copolymerization of PEGMA using techniques such as ATRP and RAFT to improve biocompatibility and solubility.[35–40] Although ATRP may also be used to copolymerize the monomers described here, RAFT polymerization was chosen as the preferred method. Compared to ATRP, RAFT tends to form polymers of lower polydispersity, especially for low molecular weight chains. In addition, the removal of copper catalyst in ATRP is a concern, especially for polymers used for biological applications.

Kinetic Analysis of RAFT Copolymerization. Kinetic studies were performed to follow the copolymerization reaction and to determine whether the comonomers were randomly distributed along the chain. As an example, a kinetic study involving a 20 mol % DDMAA and 80 mol % PEGMA (**P-2b**) using CTA 2 is shown in Figure 2a. The conversion plot reveals an incubation period of approximately 30 min before polymerization was initiated. As expected for a 'living' RAFT polymerization, a semilogarithmic plot indicating a first order relationship between molecular weight and monomer consumption was observed until ~40% conversion. The rate of consumption of the PEGMA monomer was higher than that of the DDMAA monomer, which suggests that DDMAA has a lower reactivity under these conditions. After 6 h, the rate of polymerization had slowed until no further growth occurred. This may also explain the yield (60%) of copolymer, and the fact that monomer conversion does not exceed 60% (Figure 4a). Similar conversion rates have been observed for other RAFT polymerizations in which the polymerization can no longer be deemed to be living or controlled beyond 60% monomer conversion.[41,42] At this point, no significant increase in molecular weight was observed and conversion slowed significantly. The GPC-MALLS profiles of copolymers formed at different time intervals are also given in Figure 3b and indicate a unimodal distribution of molecular weights. It has been postulated that this is due to irreversible terminations and side reactions,[25,43,44] leading to the loss of the dormant RAFT species.[45,46] Typically for this type of reaction, the polydispersity of the copolymer increased only slightly during polymerization and remained quite low (~1.2). The RAFT polymerization of PEGMA using macroinitiators have shown similar pseudofirst order kinetic plots and polydispersities ranging from 1.1 to 1.3.[47]

Kinetic studies were also carried out on the copolymerization of the ester form of the monomer, DDMA (20 mol %), with PEGMA (80 mol %). As shown in Figure 2b, a similar first order, semilogarithmic plot was observed for the copolymerization of **2b′**. Although the rate of consumption of DDMA was still lower than that of PEGMA, the difference in monomer consumption was not as pronounced. Considering homopolymerization of DDMA was possible under these conditions, this observation would indicate a relatively higher reactivity ratio compared to DDMAA. Similar to the results found for the DDMAA-containing copolymers, yields of 60% and a linear relationship between molecular weight and monomer conversion until approximately 40% was observed (Figure 3). According to GPC traces taken at various time points during the reaction (Figure 4b), a unimodal distribution of molecular weights is observed, while polydispersity remains almost constant ($M_w/M_n \sim 1.2$) (Figure 4a). The narrow distribution is maintained as the copolymer continues to grow, indicating controlled polymerization.

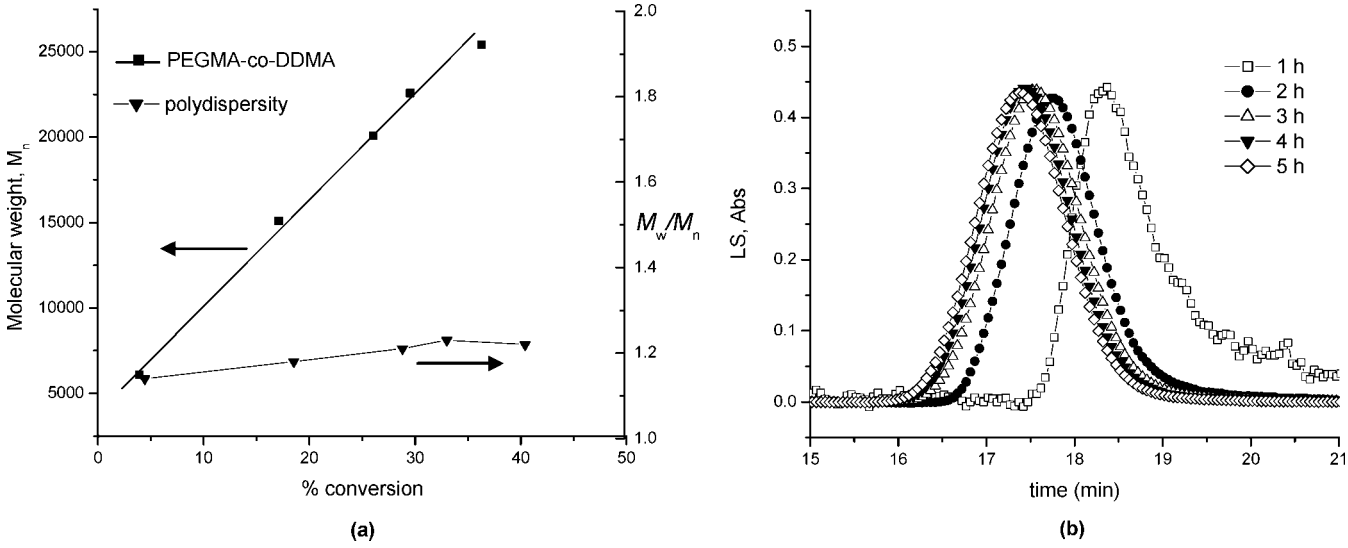


Figure 3. (a) Molecular weight vs percentage conversion of PEGMA and DDMA for synthesis of P(PEGMA-*co*-DDMA) **P-2b** at 70 °C in DMF at a relative molar ratio of [DDMA]:[PEGMA]:[CTA]:[init] = 18:72:1:0.23 and (b) corresponding GPC overlays (right).

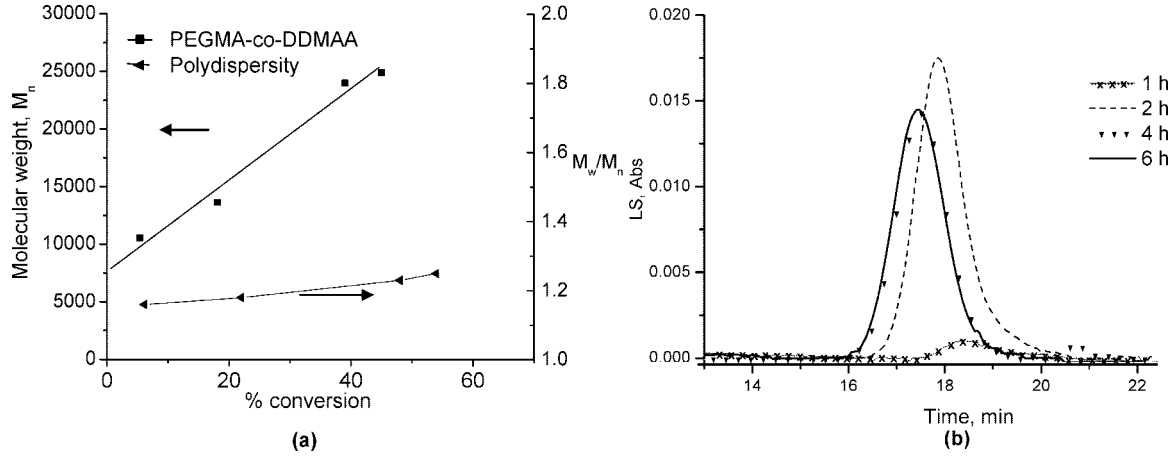


Figure 4. (a) Molecular weight vs percentage conversion of PEGMA and DDMAA for synthesis of P(PEGMA-*co*-DDMAA) **P-2b′** at 70 °C in DMF at a relative molar ratio of [DDMAA]:[PEGMA]:[CTA]:[init] = 18:72:1:0.23 and (b) corresponding GPC overlays.

Reactivity Ratios. Reactivity ratios were determined using the Mayo−Lewis method.[48] By establishing the relationship between the monomer feed ratio of the copolymers at the beginning of the reaction and the copolymer compositions at low conversion (~10%), it was possible to derive the reactivity ratios using the equation below:

$$f(F-1)/F = r_2 - (f^2/F)r_1 \quad (1)$$

$$F = F_1/F_2 f = f_1/f_2 \quad (2)$$

where r_1 and r_2 are the reactivity ratios of the respective monomers, F is the ratio of mole fractions (F_1, F_2) of monomers being added to the chain at any given time, and f is the ratio of the mole fractions (f_1, f_2) of the monomers in the feed ratio. The values for f and F can be measured experimentally using integral data taken from ^{1}H NMR spectra of the copolymerization reactions (Table 4). For the copolymers discussed here, the ratio of the polymeric CH_3 (0.5−1 ppm) group located along the backbone of the PEGMA unit was compared to the olefinic peaks (5.5−6.5 ppm). From these values, it is possible to obtain plots of $f(F-1)/F$ vs f^2/F, which contain slopes and intercepts corresponding to r_1 and r_2 respectively. Plots for the copolymerization of PEGMA with DDMA and DDMAA, with monomer feed ratios of 80% and 20% respectively and in both cases, are shown in Figure 5. According to these plots, $r_{PEGMA} = 1.06$ and $r_{DDMA} = 0.43$ for PEGMA-*co*-DDMA, and $r_{PEGMA} = 1.14$ and $r_{DDMAA} = -0.02$ for PEGMA-*co*-DDMAA. The lower reactivity ratio of DDMAA compared to DDMA is in agreement with the observation that DDMAA is not homopolymerizable under RAFT conditions and that the rate of copolymerization of DDMAA with PEGMA is slower. Indeed, it has been reported previously that the controlled RAFT polymerization of neutral acrylamides has proved to be quite difficult.[49–52] According to published free radical copolymerization reactivity ratios, acrylamide based monomers consistently show markedly lower reactivity compared to (meth)acrylate monomers.[53] For example, the reactivity ratios for *N*-methyl acrylamide and methyl methacrylate are quoted as 0.05 and 1.14 respectively in conventional free-radical polymerization.[54]

Using a statistical derivation of the copolymer equation, it is possible to determine the microstructure of the copolymers as defined by the distributions of the various lengths of each monomer (M_{PEGMA} and $M_{DDMA/A}$) sequence. Both the average sequence length distribution of each monomer and the probabilities of forming sequences of various lengths can be determined. In the case of the amide containing copolymer, PEGMA-*co*-DDMAA, the probability of forming a sequence of only one DMAA unit is 100% (i.e., the formation of dyads, triads, etc. is 0%). The number average sequence length ratio (PEGMA:DDMAA) in this case is calculated to be 5.6:1 (i.e.,

Table 3. Efficacy of Coupling Copolymers with DFO and Effect on Molecular Weight Characteristics

sample	M_n (PDI)[a] before	M_n (PDI)[a] after	% DFO[c] theory	% DFO[c] NMR	% DFO[c] UV	DFO/chain NMR[d]
P−DFO 2a	68 170 (1.28)	127 400 (2.7)[b]	10	7	5	10
P−DFO 2d	28 920 (1.22)	31 650 (1.24)	9.5	5.5	4	4.5
P−DFO 2e	44 200 (1.25)	45 660 (1.26)	9.5	6	5	5.5
P−DFO 2f	33 270 (1.24)	36 400 (1.25)	9	7	4	5
P−DFO 2g	21 500 (1.27)	27 110 (1.27)	15	10	7	5.5
P−DFO 2h	77 170 (1.63)	90 600 (1.84)	22	15	10	26
P−DFO 2a′	43 120 (1.33)	43 290 (1.28)	12	6.5	5	6

[a] Molecular weight (M_n) and polydispersity index (PDI) comparison of unmodified copolymer (Before) and DFO-functionalized copolymer, P−DFO (after). [b] **2a** was modified with DFO after the aldehyde-containing copolymer, P(PEGMA-*co*-OEAA), had been isolated via lyophilization. [c] Percentage of polymer units containing DFO moieties as determined from the precursor polymer (theory), ^{1}H NMR, and UV−vis absorption (at 420 nm) of Fe(II) chelated P−DFO. [d] The number of DFO units per polymer chain was determined using the percentage of DFO determined from the NMR data and the molecular weight of the copolymer from GPC.

Table 4. Data for Copolymer Reactivity Ratio Determination[a]

	F_{PEG}	f_{PEG}	F_{DDM}	f_{DDM}	$f = f_1/f_2$	$F = F_1/F_2$
PEGMA-*co*-DDMA	0.928	0.9	0.072	0.1	9	11.8
	0.906	0.9	0.094	0.1	9	9.84
	0.847	0.85	0.153	0.15	5.67	5.54
	0.867	0.8	0.133	0.2	4	6.54
	0.752	0.75	0.248	0.25	3	3.03
	0.717	0.70	0.283	0.3	2.33	2.55
PEGMA-*co*-DDMAA	0.918	0.9	0.082	0.1	9	12.5
	0.925	0.9	0.075	0.1	9	11.2
	0.885	0.85	0.115	0.15	5.67	7.69
	0.818	0.8	0.182	0.2	4	5.32
	0.843	0.8	0.157	0.2	4	5.42
	0.829	0.75	0.171	0.25	3	4.5
	0.769	0.7	0.231	0.3	2.33	3.33

[a] F values determined from copolymer compositions using ^{1}H NMR at low conversions (typically ∼ 10%); f values determined from monomer feed ratios (at 0 min). PEGMA-*co*-DDMA: poly{poly(ethylene glycol) methyl ether methacrylate-*co*-(2,2-dimethyl-1,3-dioxolane)methyl acrylate}; PEGMA-*co*-DDMA: poly{poly(ethylene glycol) methyl ether methacrylate-*co*-(2,2-dimethyl-1,3-dioxolane)methyl acrylamide}.

there are 5.6 PEGMA units for every 1 DDMAA unit in the polymer backbone) which is higher than the monomer feed ratio of 4:1. Conversely, the number average sequence length ratio for the ester containing PEGMA-*co*-DDMA copolymer was calculated to be 4.7:1. The probability of finding dyads and triads of DDMA units in the copolymer is 8.8% and 0.85% respectively. The probability of a DDMA unit existing in a triad monomer sequence of PEGMA-DDMA-PEGMA is 90.3%. These results are consistent with the observation that DDMA is homopolymerized, whereas DDMAA is not. It also shows that the dioxolane units (and the subsequent aldehyde units) are distributed 'randomly' along the backbone.

Synthesis of P(PEGMA-*co*-DHPAA) and P(PEGMA-*co*-DHPA). The transformation of P(PEGMA-*co*-DDMAA) and P(PEGMA-*co*-DDMA) from ketal-functionalized copolymers to aldehyde-containing copolymers is described in the following section. It was important to modify the ketal groups without altering the molecular weight characteristics or compositions of the copolymers. The first step toward the formation of the aldehyde groups involved the deprotection of ketal moieties using acetic acid to produce highly water soluble copolymers containing 1,2-diols (Scheme 3). The presence of hydroxyl groups and the concomitant absence of ketal groups were determined using ^{1}H NMR spectra (DMSO-d_6; δ 4.5 ppm and δ 1.27 ppm respectively). Figure 1B shows a typical ^{1}H NMR spectrum for a deprotected form of the copolymer (**P-3a′**). According to the ^{1}H NMR spectra of all the copolymers, the ratio of peak intensities from the $-OCH_3$ groups of PEGMA and the CH_2 and CH_3 groups from the polymer backbone remained constant. Hence, deprotection using acetic acid did not have any adverse effects on the ester linkages present (DMSO-d_6; δ 4.0 ppm). In addition, GPC was also used to monitor the effects, if any, of copolymer modification. An overlay of the GPC spectra of **P-2f** before and after deprotection is shown as an example (Figure 6). Degradation caused by the acidic reaction conditions would be indicated by a change in molecular weight and polydispersity of the polymer. No changes in the molecular weights occurred for any of the copolymers.

Synthesis of P(PEGMA-*co*-OEAA) and P(PEGMA-*co*-OEA). The aldehyde groups on the copolymer were generated by the oxidation of the 1,2-diol moieties using periodic acid at room temperature (Scheme 4).[55,56] The presence of characteristic aldehyde proton peaks (δ 9.45 ppm) coupled with the disappearance of the hydroxyl peaks (δ 4.5 ppm) confirmed the formation of the desired polymer product by ^{1}H NMR (Figure 1C). It is recommended that the aldehyde form of the copolymer should not be isolated via lyophilization as cross-linking occurred on a number of occasions. For example, in the case of sample P−DFO **2a** (Table 3), the aldehyde-functionalized copolymer was isolated prior to conjugation with the amine containing iron chelator, desferrioxamine[57] (DFO). A significant increase in polydispersity is indicative of cross-linking. Indeed, other samples isolated in this manner became so cross-linked that they were either sparingly or totally insoluble in both organic and protic solvents. Therefore, conjugation with amine containing compounds such as DFO was carried out immediately after dialysis of the aldehyde-containing copolymer. In contrast, the dialyzed and lyophilized diol form of the copolymer was found to be highly soluble in water and was stable over a period of time in the refrigerator at 4 °C. The advantage of the current synthesis is that the precursor polymer containing 1,2-diols is much more stable than the corresponding aldehyde polymer. Therefore, it is possible to store the copolymers for extended periods of time, while the aldehyde groups should be generated under mild conditions immediately prior to conjugation.

Previously, different methods of forming aldehyde-containing polymers have involved the use of monomers containing either acetals or aromatic aldehydes. For example, reactive aldehyde groups were produced by hydrolysis of acetals following ATRP of 3,3-diethoxypropyl methacrylate (DEPMA) in slightly acidic conditions.[58] Although DEPMA may be a viable alternative monomer for the copolymers synthesized here, it may be that the monomer is not as stable the diol derivatives. At low pH (below ∼6), the acetal groups would be susceptible to hydrolysis and could generate reactive aldehyde groups. This could be a concern during postpolymerization modification and purification in water (dialysis). Using the method described here, it is not only necessary to generate the aldehyde polymer, but also to store the precursor polymer for extended periods of time without losing stability.

In addition, the properties of the aldehydes generated using the synthetic methodology outlined here differ to the aromatic aldehyde moieties generated previously on other polymers. First, it has been reported that polymers containing aromatic rings may be more cytoxic than aliphatic polymers. For example, Uhrich and co-workers reported that some polymers containing aromatic cores were found to be toxic.[59] In another study, Henry *et al.* also reported the cytotoxicity of styrene−maleic ahydride based polymers.[60] By incorporating aliphatic aldehydes, it may help to eliminate any possibility of long-term toxicity caused by these polymers. Since the ultimate aim of developing the polymers is to use them as circulating drugs in the blood, the presence of aromatic rings may be to the detriment of its overall biocompatibility. Another important reason for the choice of monomer concerns the relative hydrophobicity and aqueous

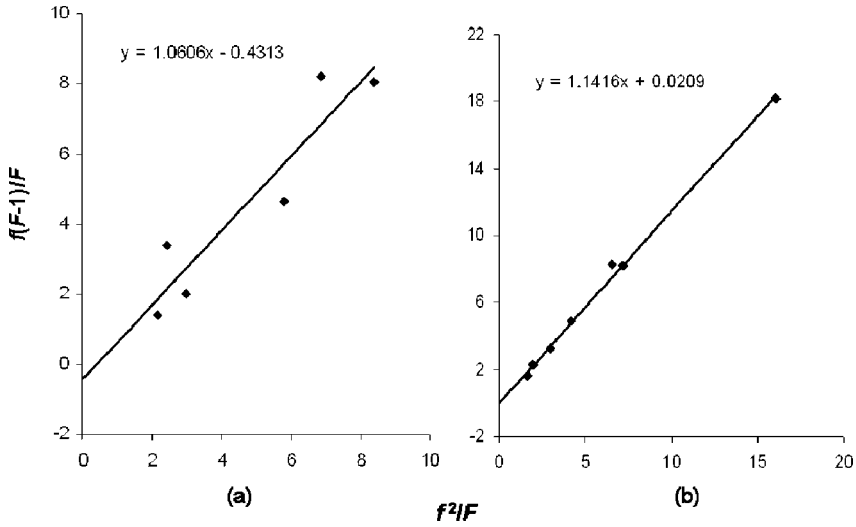
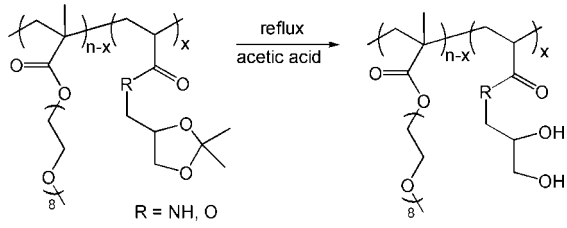


Figure 5. Determination of reactivity ratios of (a) PEGMA and DDMA, and (b) PEGMA and DDMAA via plots of $f(F-1)/F$ vs f^2/F: (a) poly(PEGMA-*co*-DDMA), r_1 = slope = 1.06, r_2 = −(intercept) = 0.43; (b) poly(PEGMA-*co*-DDMAA), r_1 = slope = 1.14, r_2 = −(intercept) = −0.02.

Scheme 3. Deprotection of Ketal Groups: Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-2,3-dihydroxypropyl acrylamide) P(PEGMA-*co*-DHPAA) (R = NH) and Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-2,3-dihydroxypropyl acrylate) P(PEGMA-*co*-DHPA) (R = O)



solubility of aromatic aldehydes. Water solubility is an important criterion in the development of polymers for blood contacting applications (e.g., those administered intravenously).

Demonstration of Bioconjugation. DFO is an iron chelator[57] used to treat 'iron overload', a condition which leads to the presence of bioreactive iron and the iron-driven free radical oxidation of lipids, proteins, carbohydrates and nucleic acids.[61] In order to demonstrate the efficacy with which biologically

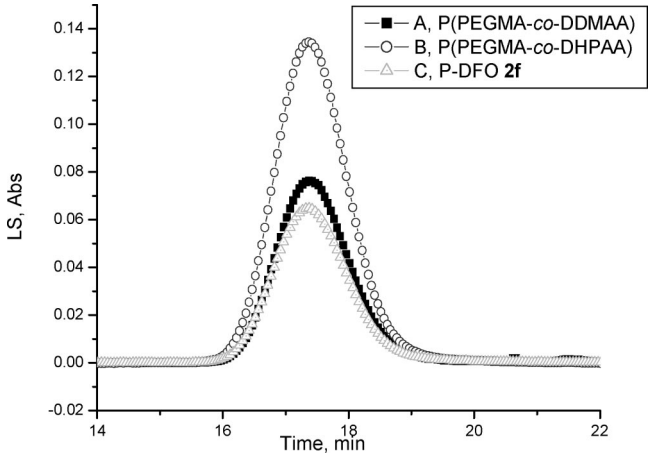
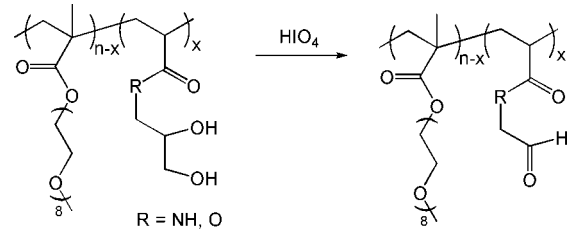
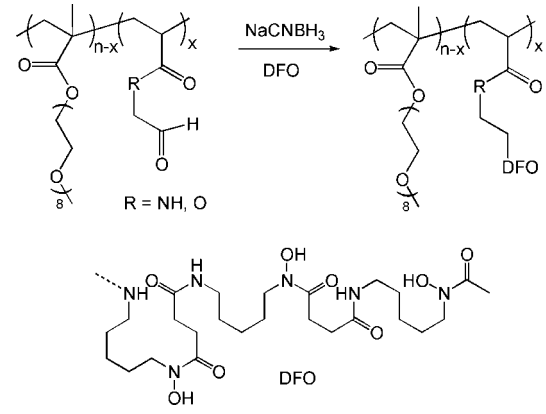


Figure 6. GPC overlay following the functional group modification of P(PEGMA-*co*-DDMAA) **P-2f**: (A) P(PEGMA-*co*-DDMAA); (B) P(PEGMA-*co*-DHPAA), diol derivative; (C) P−DFO **2f**, DFO functionalized copolymer.

Scheme 4. Formation of Aldehyde Groups: Synthesis of Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-*N*-(2-oxoethyl)acrylamide) P(PEGMA-*co*-OEAA) (R = NH) and Poly(poly(ethylene glycol) methyl ether methacrylate-*co*-2-oxoethyl acrylate) P(PEGMA-*co*-OEA) (R = O)



Scheme 5. Synthesis of the Polymer−Desferrioxamine Conjugate (P−DFO)



active compounds can be conjugated to the copolymers synthesized here, DFO was covalently coupled to the polymer backbone via reaction with the aldehyde group. The aldehyde groups on the copolymer were reacted with the primary amine of DFO through an aldamine reaction and reduction of the resulting Schiff base to a secondary amine using $NaCNBH_3$ (Scheme 5).[29] The resulting copolymer-DFO conjugate (P−DFO) was purified by dialysis and recovered via lyophilization. Dialysis of the conjugate for 5 days was carried out to ensure any unbound DFO had been completely removed. ^{1}H NMR spectroscopy revealed the presence of characteristic DFO peaks;

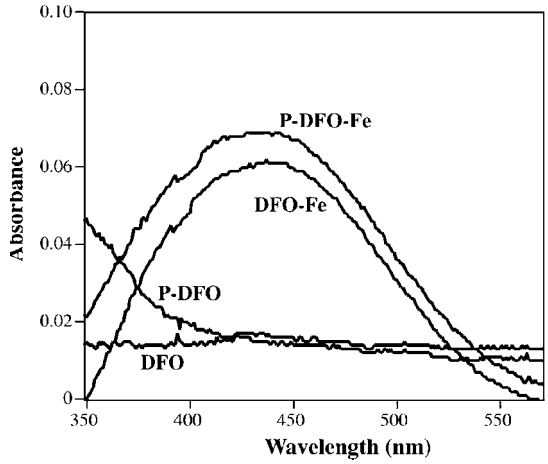


Figure 7. UV−visible spectra of DFO and P−DFO **3a′** ± bound Fe(II).

the example of P−DFO **3a′** is shown in Figure 1D. Using the integral data from the ^{1}H NMR spectra, it was possible to make an approximation of the concentration of DFO present within each copolymer conjugate (Table 3). For example, the average number of DFO units per polymer chain was varied from 4.5 (P−DFO **2d**) to 26 (P−DFO **2h**). In general, the composition of DFO present was less than the expected theoretical value. A loss of some of the functionality may be caused by the highly reactive nature of the aldehyde groups in water.

After conjugation with DFO, the molecular weight characteristics of each copolymer were analyzed; the example of **2f** is shown in Figure 6. In most cases, the molecular weights of the copolymers remained constant, as did the reasonably low polydispersity ($M_w/M_n \sim 1.2$). The similarity between the GPC traces of the synthesized RAFT copolymer **A**, the diol containing copolymer **B**, and the DFO-functionalized copolymer **D**, supports the notion that the polymer backbone remains hydrolytically stable during deprotection, oxidation, and conjugation. Table 3 lists some of the molecular weight characteristics of the copolymers before modification and after conjugation with DFO.

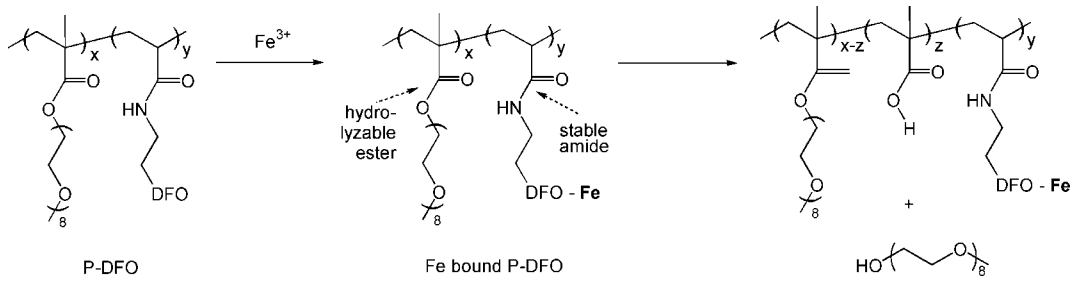
UV−vis absorption spectroscopy was used to determine the content of DFO contained within each copolymer. Upon dissolution in aqueous $FeSO_4$ solution, P−DFO copolymers showed the characteristic absorption of the DFO−Fe(II) complex (Figure 7) with a λ_{max} at 429 nm. The absorbance was used for calculating the DFO content using the Beer−Lambert equation. It was reported previously that the conjugation of molecules like DFO to a polymer will not affect the efficacy of drug *in vivo*.[32] Conjugation was highly efficient and the values obtained here were in good agreement with the values obtained from ^{1}H NMR spectroscopy. For example, P−DFO **2e** complexed with Fe(II) gave a UV−vis absorbance value corresponding to approximately 5 mol% DFO content. This value is in good agreement with the 6 mol% value extracted from the ^{1}H NMR data. Finally, by taking into account the length of the chain as determined by GPC, it is possible to predict the average number of DFO molecules per chain. In this case, P−DFO **2e** contains between 5 and 6 DFO molecules.

The ultimate aim of this research is to develop well-defined and degradable copolymers for application in polymer therapeutics. After circulating in the blood, the copolymer will ideally degrade (via the ester linkages between PEG and the copolymer backbone), enabling excretion through the kidney. Excretion through the urine is dependent on molecular weight and the kidney has a molecular weight cut off of 50 kDa − 65 kDa (above which compounds are not excreted). In this respect, the stable amide containing DDMAA monomer is of interest since the conjugated molecule will remain with the copolymer (Scheme 6). Since the PEG chains, with a molecular weight of ~400 Da, are linked to the copolymer through degradable bonds, the molecular weight of the copolymer will decrease. For example, P−DFO **2h** has an initial molecular weight of ~90 kDa. As degradation of the ester linkages occur, PEG chains will be removed from the copolymer, increasing the rate of clearance through the kidney. In effect, the clearance from circulation can be tuned by controlling the initial molecular weight properties of the copolymer drug. In cases where DFO is linked to the copolymer via an ester linkage (i.e., precursor copolymers synthesized from DDMA) degradation may also occur, forming toxic monomeric DFO in the process. The degradation and *in vitro* characteristics of the conjugated copolymers described here will be the subject of a forthcoming publication.

Conclusions

In summary, well-defined copolymers containing PEGMA and dioxolane units were synthesized via RAFT copolymerization. Two types of dioxolane monomers were chosen based on their relative hydrolytic stabilities: ester-containing DDMA and amide-based DDMAA. Polymers with a range of molecular weights, low polydispersities, and predetermined compositions were synthesized. Control over molecular weight and composition was achieved by altering the CTA concentration and the monomer feed ratio respectively. Kinetic studies revealed that PEGMA was consumed at a higher rate than the comonomers, while reactivity ratios showed that the acrylate monomer, DDMA, was more reactive than the acrylamide monomer, DDMAA. Successful postpolymerization modification of the dioxolane groups to form reactive aldehyde groups was achieved without degradation or cross-linking of the copolymer. The availability of the aldehyde groups along the polymer backbone to form stable conjugates with amine containing molecules was confirmed via a reaction with the iron chelating drug desferrioxamine (DFO). NMR, GPC, and UV−vis absorption spec-

Scheme 6. Proposed Degradation Pathway of P−DFO[a]



[a] P−DFO is circulated and chelates free iron (Fe^{3+}); During degradation, molecular weight decreases as various PEG ($M_n \sim 400$) moieties are cleaved, enabling the copolymer to be cleared at a faster rate through the kidney.

troscopy was used to determine compositions, molecular weight characteristics, and the efficiency of the DFO conjugation respectively. The biocompatibility and performance of the DFO conjugated copolymers described here will be described in a forthcoming paper. In addition, it is hoped that the copolymers described here will be used to conjugate a number of different biologically important molecules.

Acknowledgment. This research was funded by the Canadian Blood Services (CBS) and the Canadian Institutes of Health Science-CBS Research Partnership Fund. The authors thank the LMB Macromolecular Hub at the UBC Centre for Blood Research for the use of their research facilities; the infrastructure facility is supported by the Canada Foundation for Innovation (CFI) and the Michael Smith Foundation for Health Research (MSFHR). N.A.A.R. is a recipient of a CIHR/CBS postdoctoral fellowship in Transfusion Science and J.N.K. is a recipient of a CIHR/CBS new investigator in Transfusion Science.

References and Notes

(1) Harris, J. M.; Chess, R. B. *Nature Rev.* **2003**, *2*, 214–221.
(2) Merrill, E. W.; Salzman, E. W. *ASAIO J.* **1983**, *6*, 60–64.
(3) Abuchowski, A.; McCoy, J. R.; Palczuk, N. C.; Es, T. V.; Davis, F. F. *J. Biol. Chem.* **1977**, *252*, 3582–3586.
(4) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200–1205.
(5) Slomkowski, S. *Prog. Polym. Sci.* **1998**, *23*, 815–874.
(6) Christman, K. L.; Maynard, H. D. *Langmuir* **2005**, *21*, 8389–8393.
(7) Slomkowski, S.; Basinska, T.; Miksa, B. *Polym. Adv. Technol.* **2002**, *13*, 906–918.
(8) Lin, C.; Zhang, Z. P.; Zheng, J. F.; Liu, M. M.; Zhu, X. X. *Macromol. Rapid Commun.* **2004**, *25*, 1719–1723.
(9) Salo, H.; Virta, P.; Hakala, H.; Prakash, T. P.; Kawasaki, A. M.; Manoharan, M.; Lonnberg, H. *Bioconjugate Chem.* **1999**, *10*, 815–823.
(10) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559–5562.
(11) Quinn, J. F.; Rizzardo, E.; Davis, T. P. *Chem. Commun.* **2001**, 1044.
(12) Goto, A.; Sato, K.; Tsujii, Y.; Fukuda, T.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2001**, *34*, 402.
(13) Vana, P.; Quinn, J. F.; Davis, T. P.; Barner-Kowollik, C. *Aust. J. Chem.* **2002**, *55*, 425.
(14) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379–410.
(15) Godwin, A; Hartenstein, M.; Muller, A. H. E.; Brocchini, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 594–597.
(16) Schilli, C. M.; Muller, A. H. E.; Rizzardo, E.; Thang, S. H.; Chong, Y. K. *Adv. Controlled/Living Radical Polym.* **2003**, *854*, 603–618.
(17) Favier, A.; D'Agosto, F.; Charreyre, M. T.; Pichot, C. *Polymer* **2004**, *45*, 7821–7830.
(18) Savariar, E. N.; Thayumanavan, S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 6340–6345.
(19) Vosloo, J. J.; Tonge, M. P.; Fellows, C. M.; D'Agosto, F.; Sanderson, R. D.; Gilbert, R. G. *Macromolecules* **2004**, *37*, 2371–2382.
(20) Ghosh, S.; Basu, S.; Thayumanavan, S. *Macromolecules* **2006**, *39*, 5595–5597.
(21) Shi, M.; Li, A.-L.; Liang, H.; Lu, J. *Macromolecules* **2007**, *40*, 1891–1896.
(22) Hwang, J.; Li, R. C.; Maynard, H. D. *J. Controlled Release* **2007**, *122*, 279–286.
(23) Sun, G.; Cheng, C.; Wooley, K. L. *Macromolecules* **2007**, *40*, 793–795.
(24) Rixens, B.; Severac, R.; Boutevin, B.; Lacroix-Desmazes, P. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *44*, 13–24.
(25) Ganachaud, F.; Monteiro, M. J.; Gilbert, R. G.; Dourges, M. A.; Thang, S. H.; Rizzardo, E. *Macromolecules* **2000**, *33*, 6738–6745.
(26) Carter, S. R.; England, R. M.; Hunt, B. J.; Rimmer, S. *Macromol. Biosci.* **2007**, *7*, 975–986.
(27) Thomas, D. B.; Convertine, A. J.; Myrick, J. L.; Scales, C. W.; Smith, A. E.; Lowe, A. B.; Vasilieva, Y. A.; Ayres, N.; McCormick, C. L. *Macromolecules* **2004**, *37*, 8941–8950.
(28) Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754–6756.
(29) Thang, S. H.; Chong, Y. K.; Mayadunne, R. T. A.; Moad, G.; Rizzardo, E. *Tetrahedron Lett.* **1999**, *40*, 2435–2438.
(30) Kizhakkedathu, J. N.; Brooks, D. E. *Macromolecules* **2003**, *36*, 591–598.
(31) Oguchi, K.; Sanui, K.; Ogata, N.; Takahashi, Y.; Nakada, T. *Polym. Eng. Sci.* **1990**, *30*, 449–452.
(32) Hallaway, P. E.; Eaton, J. W.; Panter, S. S.; Hedlund, B. E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 10108–10112.
(33) Liu, G.; Qiu, X. *Polymer* **2004**, *45*, 7203–7211.
(34) Zou, Y.; Brooks, D.; Kizhakkedathu, J. *Macromolecules* **2008**, submitted for publication.
(35) Wang, X. S.; Armes, S. P. *Macromolecules* **2000**, *33*, 6640–6647.
(36) Neugebauer, D.; Zhang, Y.; Pakula, T.; Sheiko, S. S.; Matyjaszewski, K. *Macromolecules* **2003**, *36*, 6746–6755.
(37) Holder, S. J.; Rossi, N. A. A.; Yeoh, C. T.; Durand, G. G.; Boerakker, M. J.; Sommerdijk, N. A. J. M. *J. Mater. Chem.* **2003**, *13*, 2771–2778.
(38) Ishizone, T.; Han, S.; Okuyama, S.; Nakahama, S. *Macromolecules* **2003**, *36*, 42–49.
(39) Bes, L.; Angot, S.; Limer, A.; Haddleton, D. M. *Macromolecules* **2003**, *36*, 2493–2499.
(40) Ling, Z.; Uyen, N. T. L.; Bernard, J.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Biomacromolecules* **2007**, *8*, 2890–901.
(41) Convertine, A. J.; Ayres, N.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2004**, *5*, 1177–1180.
(42) Ren, Y.; Zhu, Z. L.; Huang, J. L. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 3828–3835.
(43) Monteiro, M. J.; de Brouwer, H. *Macromolecules* **2001**, *34*, 349–352.
(44) Kwak, Y.; Goto, A.; Tsujii, Y.; Murata, Y.; Komatsu, K.; Fukuda, T. *Macromolecules* **2002**, *35*, 3026–3029.
(45) Thomas, D. B.; Convertine, A. J.; Hester, R. D.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2004**, *37*, 1735–1741.
(46) Martina, H. S.; Thomas, P. D.; Anthony, G. F. *J. Mater. Chem.* **2003**, *13*, 2090–2097.
(47) Hu, Y. Q.; Kim, M. S.; Kim, S. K.; Lee, D. S. *Polymer* **2007**, *48*, 3437–3443.
(48) Mayo, F. R.; Lewis, F. H. *J. Am. Chem. Soc.* **1944**, *66*, 1594–1601.
(49) Donovan, M. S.; Sanford, T. A.; Lowe, A. B.; Sumerlin, B. S.; Mitsukami, Y.; McCormick, C. L. *Macromolecules* **2002**, *35*, 4570.
(50) Donovan, M. S.; Lowe, A. B.; Sumerlin, B. S.; McCormick, C. L. *Macromolecules* **2002**, *35*, 4123.
(51) Thomas, D. B.; Sumerlin, B. S.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2003**, *36*, 1436.
(52) Convertine, A. J.; Lokitz, B. S.; Vasileva, Y.; Myrick, L. J.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2006**, *39*, 1724–1730.
(53) Greenley, R. Z. In *Polymer Handbook*, 4th ed.; Brandrup, J., Immergut, E. H., Grulke, E. A., Eds.; John Wiley & Sons, Inc.: New York, 1999; Chapter 2, p 181.
(54) Orbay, M.; Laible, R.; Dulog, L. *Makromol. Chem.* **1982**, *183*, 47–63.
(55) Veh, R. W.; Corfield, A. P.; Sander, M.; Schauer, R. *Biochim. Biophys. Acta* **1977**, *486*, 145–160.
(56) March, J. In *Advanced organic chemistry: reactions, mechanisms, and structure*; Wiley: New York, 1992.
(57) Hershko, C.; Graham, G.; Bates, G. W.; Rachmilewitz, E. A. *Br. J. Hamaetol.* **1978**, *40*, 255–263.
(58) Li, R. C,; Broyer, R. M.; Maynard, H. D *J. Polym. Sci. A: Polym. Chem.* **2006**, *44*, 5004–5013.
(59) Schmalenberg, K. E.; Frauchiger, L.; Nikkhou-Albers, L.; Uhrich, K. E. *Biomacromolecules* **2001**, *2*, 851–855.
(60) Henry, S. M.; El-Sayed, M. E. H.; Pirie, C. M.; Hoffman, A. S.; Stayton, P. S. *Biomacromolecules* **2006**, *7*, 2407–2414.
(61) Hebbel, R. P. *Clin. Haematol.* **1985**, *14*, 129–140.

MA800606K