Well-Defined Biocompatible Block Copolymers via Atom Transfer Radical Polymerization of 2-Methacryloyloxyethyl Phosphorylcholine in Protic Media

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ABSTRACT: 2-Methacryloyloxyethyl phosphorylcholine (MPC) is commonly used to prepare biocompatible copolymers that have delivered clinically proven benefits in various biomedical applications. Recently, we reported that MPC could be homopolymerized to high conversions with good control via atom transfer radical polymerization (ATRP) in protic media. In the present study we describe the synthesis of a wide range of well-defined MPC-based block copolymers using either near-monodisperse macroinitiators or sequential monomer addition. With the former approach, the macroinitiators were based on either poly(alkylene oxides) or poly(dimethylsiloxane). With the latter approach, suitable comonomers included a wide range of methacrylic and other monomers, including 2-(dimethylamino)ethyl methacrylate (DMA) and its quaternized derivatives, 2-(diethylamino)ethyl methacrylate (DEA), 2-(diisopropylamino)ethyl methacrylate (DPA), methyl methacrylate, 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, and glycerol monomethacrylate. Polymerization of MPC using the three macroinitiators yielded novel PEO−MPC, PPO−MPC, and PDMS−MPC diblock copolymers. The PPO−MPC diblock copolymer proved to be thermoresponsive: molecular dissolution occurred in cold water, with colloidal aggregates being formed reversibly at elevated temperatures due to the inverse temperature solubility behavior of the PPO block. For the sequential monomer addition syntheses, the MPC monomer was generally polymerized first under optimized conditions, followed by the second monomer. High conversions were obtained for both stages of polymerization, and where applicable, aqueous GPC analyses indicated reasonably low polydispersities and good blocking efficiencies. Above pH 8, the MPC−DMA diblock copolymers also exhibited thermoresponsive behavior, forming DMA-core aggregates at elevated temperature. Spontaneous dissociation occurred on cooling to ambient temperature as the hydrophobic DMA block became hydrophilic again. The MPC−DMA, MPC−DEA, and MPC−DPA diblock copolymers proved to be pH-responsive polymeric surfactants at ambient temperature: molecular dissolution occurred in dilute acidic solution with well-defined, near-monodisperse micelles being formed at around neutral pH. In each case, the MPC block formed the biocompatible micelle coronas and the tertiary amine methacrylate block formed the hydrophobic micelle cores. In the case of the MPC−DPA diblock copolymer, the pyrene partition constant for the DPA-core micelles at pH 9 was similar to that reported previously for polystyrene-core micelles. These new MPC-based diblock copolymers are being evaluated as new nonviral vectors for DNA condensation and "stealthy" nanocapsules for the delivery of hydrophobic drugs and also for the synthesis of biocompatible shell cross-linked micelles.

Introduction

The phosphorylcholine motif is an important component of cell membranes, and it is well-known that synthetic phosphorylcholine-based polymers can be used to produce surface coatings that are remarkably resistant to protein adsorption and bacterial/cellular adhesion.[1−8] On the basis of this 'bioinspired' approach, various biomedical devices and implants with clinically proven enhanced biocompatibility have been developed over the past decade, including high-performance coronary stents and "low irritation" soft contact lenses.[3] Usually such coatings incorporate phosphorylcholine-containing vinyl monomers such as 2-methacryloyloxyethyl phosphorylcholine (MPC) and rely on conventional free radical polymerization chemistry to produce either statistical copolymers or macromonomers.[3−6] Leading players in this field include Ishihara's group,[4−6] who have suggested on the basis of differential scanning calorimetry studies that the biocompatibility performance of MPC-based coatings is related to the highly hydrophilic nature of the MPC monomer.[5b]

Although conceptually simple, this approach allows only very limited control over the target molecular weight and the molecular weight distribution, and the synthesis of well-defined diblock copolymer architectures is not possible. As far as we are aware, there have been no reports of the *controlled* polymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC). One

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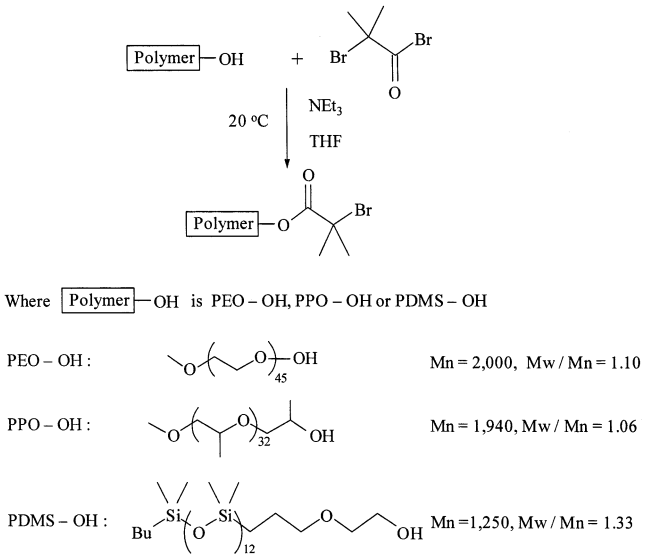

Figure 1. Reaction scheme for the block copolymerization of MPC via ATRP in protic media at 20 °C using the macroinitiator route.

problem is that such betaine monomers are normally insoluble in the organic solvents (e.g., toluene, THF) typically used for conventional ionic living polymerizations, which is the traditional method for producing controlled-structure, near-monodisperse copolymers. In 1995, atom transfer radical polymerization (ATRP) was developed independently by Wang and Matyjaszewski and Sawamoto's group.[9−12] Reversible chain-end capping using halogen atoms ensures that the instantaneous polymer radical concentration is lower than in conventional radical polymerization, which leads to the suppression of termination relative to propagation and hence enables relatively narrow molecular weight distributions to be achieved. Efficient block copolymer syntheses can be achieved under optimized conditions, and this pseudo-living free radical chemistry has gained a deserved reputation for its excellent tolerance of both monomer functionality and protic sources.[13,14]

We recently reported that ATRP is particularly effective for a wide range of hydrophilic monomers in protic media such as water and/or lower alcohols under mild conditions.[15−19] In view of these results, and given that MPC is insoluble in many organic solvents, we investigated the feasibility of polymerizing MPC via aqueous or methanolic ATRP. Our initial results regarding the controlled homopolymerization of MPC were reported in a recent communication,[20] and a follow-up paper has been recently published;[21] herein we describe the block copolymerization of MPC. Two approaches have been investigated: the macroinitiator route (see Figure 1) and the sequential monomer addition route (see Figure 2). By use of the latter route, the successful block copolymerization of MPC with a wide range of methacrylic monomers has been achieved.

Experimental Section

Materials. The MPC monomer (99.5% purity) was obtained from Biocompatibles UK Ltd. The Cu(I)Br and 2,2′-bipyridine (bpy) were both purchased from Aldrich and used as received. The silica used for removal of the ATRP copper catalyst was column chromatography grade silica gel 60 (0.063−0.200 mm) purchased from E. Merck (Darmstadt, Germany). Methanol and 2-propanol were purchased from Aldrich. The water used in all experiments was deionized and doubly distilled prior to use. The OEGBr initiator was synthesized and purified according to a literature procedure.[15−17,22] The two monohydroxy-capped poly(alkylene oxide) precursors were donated by Laporte Performance Chemicals (Hythe, UK). Poly(ethylene oxide) (PEO-OH) had an M_n of 2000 (Dp = 45) and an M_w/M_n of 1.10. Poly(propylene oxide) (PPO-OH) had an M_n of 1940 (Dp = 33) and an M_w/M_n of 1.06. These two precursors were each converted into the corresponding ATRP macroinitiators (PEO-Br and PPO-Br) by reaction with 2-bromoisobutyryl bromide using previously reported protocols.[22] Monocarbinol-terminated and dicarbinol-terminated poly(dimethylsiloxane) (PDMS), each with a nominal molecular weight of 1000, were purchased from Gelest Inc., Germany. ^{1}H NMR studies in $CDCl_3$ indicated a number-average molecular weight of 1250 for the monocarbinol PDMS (corresponding to a Dp of 13) and a number-average molecular weight of 1330 for the dicarbinol PDMS (also corresponding to a Dp of 13). GPC analyses gave polydispersities of 1.16 and 1.25 for the monocarbinol- and dicarbinol-terminated PDMS, respectively.

[2-(Methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)ammonium hydroxide (SBMA), methyl methacrylate (MMA), 2-(dimethylamino)ethyl methacrylate (DMA), and 2-(diethylamino)-

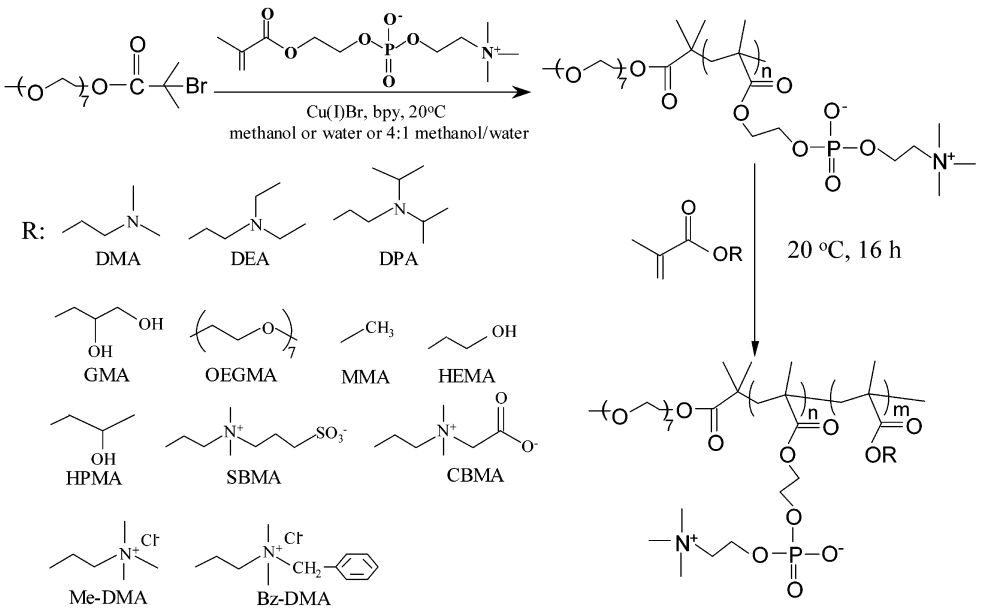

Figure 2. Reaction scheme for the block copolymerization of MPC via ATRP in protic media at 20 °C using the sequential monomer addition route.

ethyl methacrylate (DEA) were purchased from Aldrich. Glycerol monomethacrylate (GMA) was a gift from Röhm (Germany). The methyl chloride and benzyl chloride quaternized derivatives of DMA (designated MeDMA and BzDMA, respectively) were kindly provided as 75 wt % aqueous solutions by Elf Atochem, Germany. 2-Hydroxyethyl methacrylate (HEMA) and monomethoxy-capped oligo(ethylene glycol) methacrylate (OEGMA) were donated by Laporte Performance Chemicals (Hythe, UK). 2-(Diisopropylamino)ethyl methacrylate (DPA) was purchased from Scientific Polymer Products. The carboxybetaine methacrylate (CBMA) was synthesized by reacting DMA with sodium iodoacetate in water at 20 °C for several days at pH 8, followed by precipitation into either THF or acetone to remove the sodium iodide byproduct.

General Polymerization Protocols. 1. Polymerization of MPC Using Macroinitiators. Typical protocols for the controlled polymerization of MPC using the PEO-Br, PPO-Br, and PDMS-Br macroinitiators in methanol, 2-propanol, or a 4:1 methanol/2-propanol mixture were as follows. PEO-Br (1.01 g, 0.505 mmol, 1 equiv) was dissolved in methanol (10 mL). After purging with nitrogen for 30 min, the Cu(I)Br catalyst (72.0 mg, 0.505 mmol, 1 equiv) and bpy ligand (158 mg, 1.01 mmol, 2 equiv) were added to the stirred solution under nitrogen. MPC (6.00 g, 20.2 mmol) was then added as a solid to the reaction mixture under nitrogen. The reaction mixture immediately became dark brown and progressively more viscous. In aqueous-based reaction solutions, exotherms of 2−4 °C were typically observed, indicating the onset of polymerization. After approximately 24 h, ^{1}H NMR analysis indicated that more than 99% of the MPC had been polymerized (disappearance of vinyl signals at δ 5.5−6.0). On exposure to air, the reaction solution turned blue, indicating aerial oxidation of the Cu(I) catalyst. The resulting PEO−MPC diblock copolymer was precipitated into THF, then redissolved in water, and passed through a silica column to remove the spent ATRP catalyst. The diblock copolymer was dried by freeze-drying for 24 h. Aqueous GPC analysis indicated an M_n of 12 000 (vs poly(ethylene oxide) standards) and an M_w/M_n of 1.19.

MPC (6.24 g, 2.1×10^{-2} mol) was block copolymerized in 10 mL of methanol using the PPO-Br macroinitiator (0.45 g, 2.1×10^{-4} mol). The relative molar ratio of [MPC]:[PPO-Br]:[CuBr]:[bipy] was 100:1:1:2. After 24 h, an MPC−PPO diblock copolymer was obtained with an MPC conversion of almost 100%, as indicated by ^{1}H NMR spectroscopy. The reaction solution was then passed through a silica gel column to remove the spent ATRP catalyst. After solvent evaporation, the remaining copolymer was dried in a vacuum oven at room temperature to yield a colorless solid.

Finally, block copolymerization of MPC using PDMS-Br was conducted in 10 mL of a 4:1 methanol/2-propanol solvent mixture under the following conditions: MPC (4.00 g, 1.35×10^{-2} mol); [MPC]:[PDMS-Br]:[CuBr]:[bipy] = 100:1:1:2. After 25 h, ^{1}H NMR spectroscopy indicated no residual vinyl double bonds at δ 5.5−6.0. The blue alcoholic solution of the MPC−PDMS diblock copolymer was then passed through a silica gel column to remove the spent catalyst. After solvent evaporation, the remaining copolymer was dried in a vacuum oven at 20 °C for at least 16 h to yield a colorless solid. A similar protocol was employed for the bifunctional Br-PDMS-Br macroinitiator.

2. Block Copolymerization of MPC with Other Methacrylic Monomers. The following four examples are illustrative of the general synthetic protocols employed.

MPC−HEMA Diblock Copolymer. MPC was polymerized first (6.00 g, 1.35×10^{-2} mol) in 10 mL of methanol, using [MPC]:[OEGBr]:[CuBr]:[bpy] = 30:1:1:2. After 2.5 h, the monomer conversion was greater than 99%, and the MPC homopolymer obtained had a low polydispersity (M_w/M_n = 1.19) with M_n = 9000 (vs poly(ethylene oxide) standards). HEMA monomer (4.39 g, 3.37×10^{-2} mol, target Dp = 50) was then added to this reaction solution. The reaction mixture was maintained under a dry nitrogen purge for the duration of the polymerization. On exposure to air after 24 h, the reaction solution turned blue, indicating aerial oxidation of the ATRP catalyst. ^{1}H NMR studies indicated a HEMA monomer conversion of 99%. The reaction solution was passed through a silica gel column to remove the spent catalyst. After solvent evaporation, the copolymer was washed with excess THF to remove residual HEMA monomer and then dried in a vacuum oven at room temperature to yield a colorless solid.

MPC−SBMA Diblock Copolymer. MPC (4.00 g, 1.35×10^{-2} mol) was polymerized first in water (10 mL) using [MPC]:[OEGBr]:[CuBr]:[bpy} = 20:1:1:2. After 2 h, the MPC conversion was greater than 99%, and the homopolymer obtained had an M_n of 6200 and an M_w/M_n of 1.20. SBMA (3.80 g, 1.35×10^{-2} mol, Dp = 20) was then added to this reaction solution as a solid. After 21 h, ^{1}H NMR studies indicated that both monomers had been consumed. The MPC−SBMA diblock copolymer had an M_n of 12 000 and an M_w/M_n of 1.27, as determined by aqueous GPC. The reaction solution was then passed through a silica gel column to remove the spent catalyst. After solvent evaporation, the purified copolymer was dried in a vacuum oven at 20 °C for at least 24 h to yield a colorless solid. This purification protocol resulted in the loss of up to 15% MPC−SBMA diblock copolymer due to adsorption onto the silica gel.

MPC−MeDMA Diblock Copolymer. MPC (6.00 g, 2.02×10^{-2} mol) was homopolymerized first in a 4:1 methanol/water solvent mixture using [MPC]:[OEG-Br]:[CuBr]:[bipy] = 30:1:1:2. After 1 h, over 99% conversion was achieved and the isolated homopolymer had an M_w/M_n of 1.19. A 75 wt % aqueous solution of MeDMA monomer (5.50 g, 2.02×10^{-2} mol, target Dp = 30) was then added to the polymerizing solution. After 46 h, an MPC−MeDMA diblock copolymer was obtained at an overall conversion of more than 99%, as indicated by ^{1}H NMR spectroscopy (no residual vinyl double bonds at δ 5.5−6.0). The reaction solution was then passed through a silica gel column to remove the copper catalyst, and the diblock copolymer was freeze-dried from water overnight. This purification protocol resulted in the loss of up to 20% diblock copolymer due to adsorption. The diblock copolymer had an M_n of 16 000 and an M_w/M_n of 1.27 as determined by aqueous GPC using poly(2-vinylpyridine) standards.

MPC−DPA Diblock Copolymer. MPC was polymerized first (6.00 g, 2.02×10^{-2} mol) in 10 mL of methanol, using [MPC]:[OEGBr]:[CuBr]:[bpy] = 30:1:1:2 under a nitrogen atmosphere at 20 °C. After 2.0 h, the MPC conversion was greater than 99%, and the MPC homopolymer obtained had a low polydispersity (M_w/M_n = 1.09 with M_n = 10 000 vs poly(ethylene oxide) standards). Then DPA monomer (8.65 g, 4.04×10^{-2} mol, target Dp = 60) was added to this reaction solution. After 40 h, ^{1}H NMR studies indicated that both monomers had been consumed. The reaction solution was passed through a silica gel column to remove the spent ATRP catalyst, which resulted in the loss of around 10% copolymer due to adsorption onto the silica. After solvent evaporation, the solid copolymer was washed with excess *n*-hexane to remove any traces of residual DPA monomer, redissolved in water, and then freeze-dried overnight. The resulting colorless MPC_{30}−DPA_{60} diblock copolymer had an M_n of 21 000 and an M_w/M_n of 1.27, as determined by aqueous GPC using poly(2-vinylpyridine) standards.

Copolymer Characterization. GPC Protocols. The molecular weights and molecular weight distributions of selected MPC homopolymers and diblock copolymers were determined by aqueous gel permeation chromatography (GPC). The standard GPC protocol involved using a Pharmacia Biotech "Superose 6" column connected to a Polymer Labs ERC-7517A refractive index detector. The eluent was 0.20 M $NaNO_3$ solution with 50 mM Trizma buffer at pH 8−10, and poly(ethylene oxide) standards were used for calibration. For MPC-based diblocks based on cationic monomers (DMA, DEA, DPA, MeDMA, BzDMA) an alternative protocol was employed. This involved using two ViscoGEL columns (G5000 PWXL and G2500 PWXL) connected to a Polymer Labs ERC-7517A refractive index detector. The eluent was an aqueous solution comprising 0.50 M acetic acid and 0.30 M Na_2SO_4 at pH 2; poly(2-vinylpyridine) standards (PSS, Germany) were used for calibration.

^{1}H NMR Spectroscopy. All ^{1}H NMR spectra were recorded using a 300 MHz Bruker Avance DPX300 spectrometer. The kinetics of polymerization were determined for reactions carried out in either D_2O or CD_3OD by comparing the peak integrals due to the monomer vinyl signals at δ 5.5 and 5.9 to those of the methacrylate backbone at δ 0.5−1.1. If appropriate, the macroinitiator was used as an "end group" to determine the degree of polymerization of the MPC block. In these calculations it was assumed that the macroinitiator efficiency was 100%, that chain transfer was negligible, and that every polymer chain contained a macroinitiator end group.

Dynamic Light Scattering Studies. The MPC−DEA and MPC−DPA diblock copolymers were molecularly dissolved in water at pH 2 at different concentrations, and the solution pH was adjusted to pH 8−9 to induce micelle formation. Dynamic light scattering (DLS) studies were performed at 25 °C on a Brookhaven Instruments Corp. BI-200SM goniometer equipped with a BI-9000AT digital correlator using a solid-state laser (125 mW, $\lambda = 532$ nm) at a fixed scattering angle of 90°. The intensity-average hydrodynamic diameter, $\langle D_h \rangle$, and polydispersity, μ_2/Γ^2, of the micelles were obtained by cumulants analysis of the experimental correlation function.[18,19] The polydispersity, μ_2/Γ^2, is a model-independent estimate of the width of the size distribution, which is equal to the normalized second moment from a cumulant analysis of the scattered light autocorrelation function. For relatively narrow size distributions the polydispersity may be equated to the variance of the distribution.

Fluorescence Studies of Micellization. *Critical Micellization pH Studies.* The MPC_{30}−DMA_{60}, MPC_{30}−DEA_{60}, and MPC_{30}−DPA_{60} diblocks were molecularly dissolved in turn in doubly distilled water at pH 2. Pyrene/copolymer solutions were prepared by adding acetone solutions of pyrene into dry 10.0 mL volumetric flasks. After evaporation of the acetone, diluted copolymer stock solutions were added so as to obtain a final copolymer concentration of 0.50 g L^{-1} in each case; the final concentration of pyrene was fixed at 6.0×10^{-7} mol dm^{-3}. NaOH solution was added to each copolymer solution to increase the solution pH; this pH adjustment caused micelles to be formed in situ. Each copolymer solution was left for 30 min at room temperature to ensure equilibration between the pyrene in the micelles and that in the aqueous solution. Pyrene emission spectra were recorded at 25 °C on a Cary Eclipse fluorescence spectrometer using an excitation wavelength of 333 nm. The emission and excitation slit widths were set at 2.5 and 10 nm, respectively. Spectra were accumulated with a signal-to-noise ratio of 1000.

Pyrene Partition Experiments. The MPC_{30}−DMA_{60}, MPC_{30}−DEA_{60}, and MPC_{30}−DPA_{60} diblocks were molecularly dissolved in turn in doubly distilled water at pH 2. Pyrene/copolymer solutions were prepared by adding acetone solutions of pyrene into dry 10.0 mL volumetric flasks. After evaporation of the acetone, diluted copolymer stock solutions were added so as to obtain final copolymer concentrations ranging from 1×10^{-4} to 5.0 g L^{-1}; the final concentration of pyrene was fixed at 6.0×10^{-7} mol dm^{-3}. A drop of 2 M NaOH solution was added to each copolymer solution to obtain pH 9; this pH adjustment caused micelles to be formed in situ. Each copolymer solution was left for 3 days at room temperature to ensure equilibration between the pyrene in the micelles and that in the aqueous solution. Pyrene excitation spectra were recorded at 25 °C on the same fluorescence spectrometer as that described above using an emission wavelength of 373 nm. The emission and excitation slit widths were set at 10 and 2.5 nm, respectively. Spectra were accumulated with a signal-to-noise ratio of 1000.

Determination of the Residual ATRP Catalyst Level. After silica or alumina treatment to remove the spent ATRP catalyst, aqueous solutions of selected, purified MPC-based diblock polymers were analyzed for their Cu contents using a Perkin-Elmer Plasma 400 inductively coupled plasma atomic emission spectrometer (ICP-AES) using the following protocol. Approximately 50 mg of polymer was accurately weighed, dissolved in distilled water containing 5.0% (v/v) "trace analysis" grade nitric acid, and made up to 5.00 mL. The unknown aqueous polymer solution was introduced into the argon plasma, and the Cu emission at 327.396 nm was measured and compared to that found for stock solutions containing 0.100, 0.050, and 0.020 ppm Cu; a blank solution containing 5% (v/v) nitric acid in distilled water was used as a fourth calibration solution. For sample solutions that exceeded 0.100 ppm of Cu, a second analysis was performed using calibration solutions containing 1.000 and 0.100 ppm together with the blank solution. The manufacturer's estimated detection limit is around 0.0097 ppm of Cu for the above emission wavelength. This ICP-AES technique indicated residual Cu levels in the range of 1−3 ppm for the purified diblock polymers analyzed.

Results and Discussion

Recently, we reported a detailed optimization study of the homopolymerization of MPC via ATRP.[20,21] Excellent yields, first-order monomer kinetics, linear M_n vs conversion plots, and relatively narrow polydispersities ($M_w/M_n = 1.15-1.35$) were obtained in both aqueous and alcoholic media at 20 °C. However, slower polymerizations and narrower polydispersities were always obtained in alcoholic solution, and chain extension experiments indicated significantly greater living character (i.e., greater self-blocking efficiencies) under these conditions.[21] ATRP kinetics were significantly slower in 2-propanol (IPA) than in methanol due to the reduced polarity of the former solvent. However, acceptable rates of polymerization and reasonable control were obtained at elevated temperature in IPA. Alternatively, the addition of a relatively small amount of water to the IPA led to a significantly faster polymerization at ambient temperature.[23] The effect of varying the ligand type and target Dp was also investigated, with optimal results being obtained for 2,2′-bipyridine and target Dp's of 20−200. Herein we have extended this initial homopolymerization study in order to prepare a wide range of controlled-structure MPC-based block copolymers. In view of the good living character obtained for the homopolymerization of MPC, we elected to polymerize this monomer first in all of the "one-pot" block copolymer syntheses involving sequential monomer addition. Alternatively, well-defined diblock and triblock copolymers were obtained using macroinitiators derived from hydroxy-capped polymers for the homopolymerization of MPC. These two approaches are discussed separately below.

Preparation of MPC-Based Diblock Copolymers Using Macroinitiators. Three types of macroinitiators based on poly(ethylene oxide) (PEO), poly(propylene oxide) (PPO), and poly(dimethylsiloxane) (PDMS) were employed in this study. Molecular weight and polydispersity data for the near-monodisperse hydroxy-functional PEO, PPO, and PDMS precursors prior to esterification using 2-bromoisobutyryl bromide are given in the Experimental Section and also summarized in Figure 1.

The evolution of conversion vs time and the corresponding semilogarithmic plot are shown together in Figure 3 for the homopolymerization of MPC using the PEO-Br macroinitiator. The linear semilogarithmic plot indicates that the polymerization is first order with respect to MPC and implies that the polymer radical concentration remains constant on the time scale of the polymerization. The corresponding aqueous GPC curves for the PEO macroinitiator and the final PEO−MPC diblock copolymer are shown in Figure 4a. There is a clear shift to higher molecular weight for the PEO−MPC diblock compared to the PEO-Br macroinitiator,

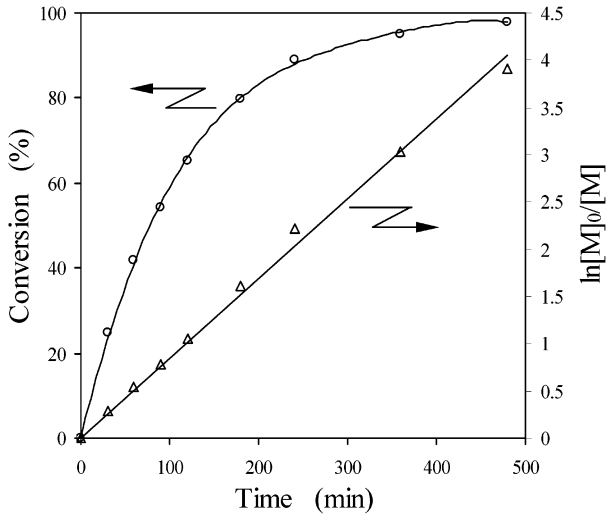

Figure 3. Conversion vs time curve and the corresponding semilogarithmic plot for the homopolymerization of MPC in methanol using a PEO_{45}-Br macroinitiator at 20 °C. Conditions: MPC (6.0 g, 2.02×10^{-2} mol), PEO_{45}-Br (1.01 g, 5.05×10^{-4} mol), the relative molar ratios of MPC:PEO_{45}-Br:Cu(I)Br:bpy were 40:1:1:2.

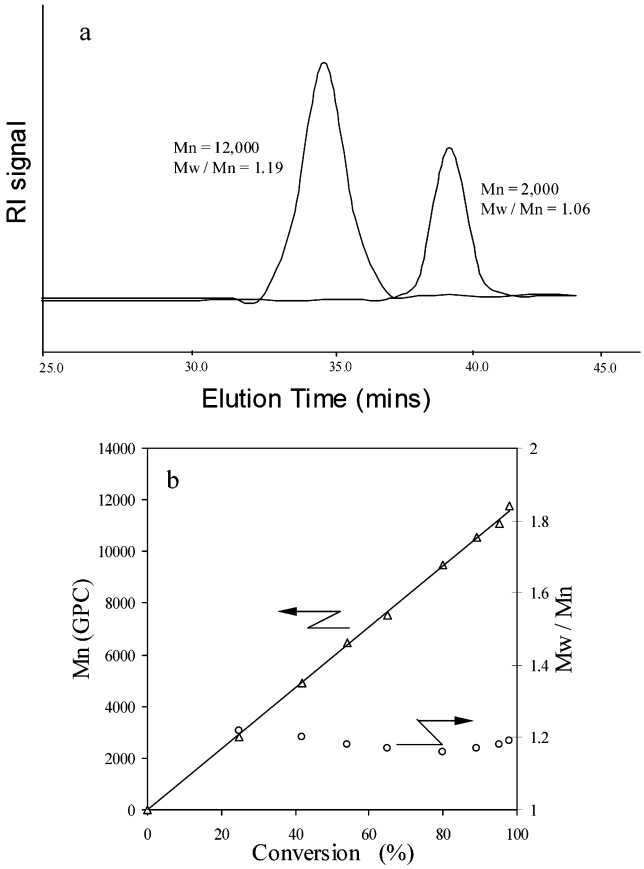

Figure 4. (a) Aqueous GPC data for the PEO_{45}-Br macroinitiator and the corresponding PEO_{45}−MPC_{40} diblock copolymer. (b) Evolution of M_n and M_w/M_n with conversion for the same homopolymerization. See Figure 3 caption for the reaction conditions.

and there is essentially zero contamination, indicating very high macroinitiator efficiency. The evolution of M_n is linear with conversion, and polydispersities remain low throughout the polymerization (see Figure 4b), as expected for MPC polymerizations conducted in methanol.[20,21]

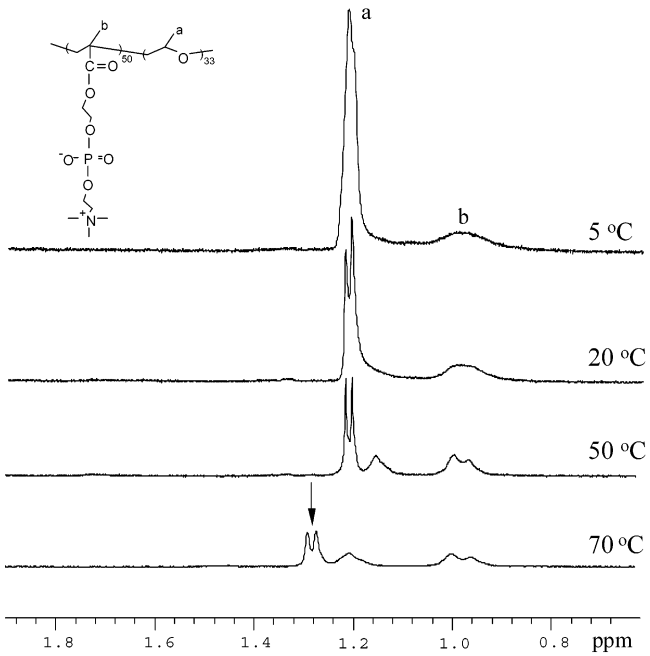

Figure 5. Variable temperature ^{1}H NMR studies of the thermoresponsive MPC_{50}−PPO_{33} diblock copolymer. Note the attenuation of the NMR signals assigned to the PPO block.

The semilogarithmic plot of monomer concentration vs time for the polymerization of MPC (target Dp = 50) using the PPO macroinitiator was linear up to 80% conversion (not shown). Unfortunately, the PPO block is not sufficiently hydrophilic to allow aqueous GPC analysis, and the MPC block is insoluble in THF and other common GPC solvents such as DMF, which precludes conventional nonaqueous GPC analysis. Attempts to use methanol/water mixtures were unsuccessful, since this led to adsorption of the PEO standards on the column. Thus, GPC analysis of the PPO−MPC diblock copolymers was not possible. However, the GPC data obtained for the PEO−MPC diblock copolymer and also our earlier results on related PPO-based diblock copolymers indicate that these PPO−MPC diblock copolymers should be near-monodisperse.[16c,17−19] Furthermore, attempts to remove residual PPO homopolymer by *n*-hexane extraction were unsuccessful: ^{1}H NMR analysis of the MPC−PPO diblock copolymer before and after *n*-hexane treatment indicated no significant change in block composition, which suggested that a high macroinitiator efficiency had been achieved during MPC homopolymerization. By varying the MPC/PPO-Br molar ratio, the target degree of polymerization of the MPC block could be varied (see Table 1). It is well-known that the PPO block is thermoresponsive, with an LCST of around 15−20 °C.[18,19] Thus, we studied the dilute aqueous solution behavior of the PPO_{33}−MPC_{50} diblock copolymer by dynamic light scattering at various temperatures. As expected, molecular dissolution was achieved at 5 °C (i.e., below the cloud point of the PPO block), but micellization occurred at elevated temperatures. The intensity-average micelle diameter was around 65 nm at 60 °C. The micelles were somewhat polydisperse, but the addition of salt reduced the width of the size distribution considerably. Variable temperature ^{1}H NMR spectra were recorded for this PPO_{33}−MPC_{50} diblock copolymer (see Figure 5). The signal labeled "a" due to the PPO backbone at δ 1.2 becomes broadened and attenuated relative to the methacrylate

Table 1. Summary of the Conversion and Molecular Weight Data for MPC-Based Diblock Copolymers Prepared by Alcoholic ATRP at 20 °C Using the Macroinitiator Route[a]

target block copolymer composition	[MPC] (mol × 10^2)	[macroinitiator] (mol × 10^4)	solvent composition	reaction time (h)	MPC conversion[b] (%)	residual Cu by ICP-AES[c] (ppm)
PEO_{45}−MPC_{40}	2.02	5.05	MeOH	24	100	2.0
PEO_{45}−MPC_{10}	1.35	13.5	MeOH	3	100	1.5
PPO_{33}−MPC_{20}	2.0	10.0	MeOH	3	100	2.5
PPO_{33}−MPC_{30}	2.02	6.73	MeOH	20	100	3.1
PPO_{33}−MPC_{50}	2.10	4.20	IPA	23	100	2.9
PPO_{33}−MPC_{100}	2.10	2.10	MeOH	24	>99	3.2
$PDMS_{13}$−MPC_{100}	1.35	1.35	4:1 MeOH:IPA	25	>99	2.7
$PDMS_{13}$−MPC_{50}	2.0	4.0	4:1 MeOH:IPA	23	100	1.5
$PDMS_{13}$−MPC_{30}	2.02	6.73	4:1 MeOH:IPA	23	100	1.8
MPC_{50}−$PDMS_{16}$−MPC_{50}	2.02	2.02	MeOH	21	>99	2.4
MPC_{30}−$PDMS_{16}$−MPC_{30}	1.22	2.02	MeOH	20	>99	1.3
MPC_{10}−$PDMS_{16}$−MPC_{10}	2.70	1.35	MeOH	18	>99	2.4

[a] The relative molar ratios of macroinitiator:Cu(I)Br:bpy were 1:1:2, and the total solvent volume was 10 mL for all syntheses. [b] Conversions determined by ^{1}H NMR spectroscopy. [c] Cu levels determined by inductively coupled plasma atomic emission spectroscopy.

Table 2. Summary of the Conversion and Molecular Weight Data for MPC-Based Diblock Copolymers Prepared by ATRP in Protic Media at 20 °C Using the Sequential Monomer Addition Route[a]

expt no.	target composition	solvent	time for >95% conversion[b] homo (h)	time for >95% conversion[b] diblock (h)	Mn (GPC)[c] homo	Mn (GPC)[c] diblock	M_w/M_n [c] homo	M_w/M_n [c] diblock	residual Cu by ICP-AES/ppm[d]
1	MPC_{30}−$HEMA_{50}$	MeOH	2.5	24	9 000	19 000	1.19	1.29	2.0
2	MPC_{50}−$HEMA_{40}$	MeOH	1.6	24	13 000	21 000	1.16	1.45	1.9
3	MPC_{50}−GMA_{40}	MeOH	1.5	10	12 000	20 000	1.17	1.21	1.7
4	MPC_{50}−GMA_{100}	MeOH	1.5	24	12 000	27 000	1.17	1.28	1.9
5	MPC_{100}–MMA_{30}	MeOH	3.0	20	28 000		1.15		1.6
6	MPC_{100}−$CBMA_{100}$	MeOH	3.0	22	28 000	50 000	1.12	1.20	1.9
7	MPC_{20}−$SBMA_{20}$	H_2O	0.5	21	6 000	12 000	1.12	1.27	2.1
8	MPC_{20}−$OEGMA_{20}$	H_2O	1.0	20	6 000	16 000	1.21	1.34	1.8
9	MPC_{30}−$HPMA_{60}$	MeOH	2.0	21	9 000		1.19		1.4
10	MPC_{30}-$MeDMA_{30}$	4:1 MeOH:H_2O	1.0	48	10 000	16 000	1.19	1.27	2.0
11	MPC_{30}−$BzDMA_{30}$	4:1 MeOH:H_2O	1.0	48	11 000	16 000	1.18	1.32	1.9

[a] The relative molar ratios of OEG-Br initiator:Cu(I)Br:bpy were 1:1:2. [b] As determined by ^{1}H NMR spectroscopy. [c] As determined by aqueous GPC using poly(2-vinylpyridine) calibration standards. [d] As determined by inductively coupled plasma atomic emission spectroscopy [ICP-AES].

Table 3. Summary of the Conversion and Molecular Weight Data for MPC−Tertiary Amine Methacrylate Diblock Copolymers Prepared by Methanolic ATRP at 20 °C Using the Sequential Monomer Addition Route[a]

exp no.	target composition	time for >95% conversion[b] homo (h)	time for >95% conversion[b] diblock (h)	M_n (GPC)[c] homo	M_n (GPC)[c] diblock	M_w/M_n[c] homo	M_w/M_n[c] diblock	Cu[d]/ppm
1	MPC_{30}−DMA_{30}	2.0	15	10 000	22 000	1.19	1.27	2.0
2	MPC_{30}−DMA_{60}	2.0	24	10 000	34 000	1.15	1.29	1.5
3	MPC_{30}−DMA_{100}	2.0	48	10 000	46 000	1.17	1.32	1.7
4	MPC_{30}−DEA_{30}	2.0	20	9 000	21 000	1.15	1.30	1.6
5	MPC_{30}−DEA_{60}	2.0	24	10 000	31 000	1.19	1.29	1.6
6	MPC_{30}−DEA_{100}	2.0	48	10 000	43 000	1.17	1.28	1.9
7	MPC_{30}−DPA_{30}	2.0	20	10 000	14 000	1.08	1.20	1.6
8	MPC_{30}−DPA_{60}	2.0	24	10 000	21 000	1.09	1.27	2.3
9	MPC_{30}−DPA_{100}	2.0	40	10 000	30 000	1.12	1.27	1.9

[a] The relative molar ratios of OEG-Br initiator:Cu(I)Br:bpy were 1:1:2. [b] As determined by ^{1}H NMR spectroscopy. [c] As determined by aqueous GPC using poly(2-vinylpyridine) calibration standards. [d] As determined by inductively coupled plasma atomic emission spectroscopy [ICP-AES].

backbone signal "b" at elevated temperature, indicating reduced mobility and solvation of the PPO block as it forms the partially dehydrated micelle cores.[16c] As expected, this temperature-induced micellization is fully reversible: on cooling to room temperature the original NMR spectrum is obtained. Thus, this new PPO−MPC polymeric surfactant is an interesting biocompatible analogue of the well-known PPO−PEO "Pluronic" or "Synperonic" series.

A series of PDMS−MPC diblocks and MPC−PDMS−MPC triblocks were also synthesized, and the results are summarized in Table 1. Unfortunately, the very different solubility behavior of the MPC and PDMS blocks again precluded GPC analysis. However, it is noteworthy that a highly hydrophilic monomer such as MPC can be polymerized efficiently using a highly hydrophobic PDMS-based macroinitiator in protic media.

Preparation of MPC-Based Diblock Copolymers Using Sequential Monomer Addition. A wide range of diblock copolymers were prepared by sequential monomer addition via ATRP at 20 °C using MPC in combination with various methacrylic comonomers (see Tables 2 and 3). In all cases the MPC was polymerized first, followed by the second monomer. In most cases the solvent used was methanol, and the conditions were selected to provide optimal living character and hence high blocking efficiencies. In Table 2, 9 of the 11 entries are so-called "double-hydrophilic" diblock copolymers. In contrast, entries 5 and 9 are examples of hydro-

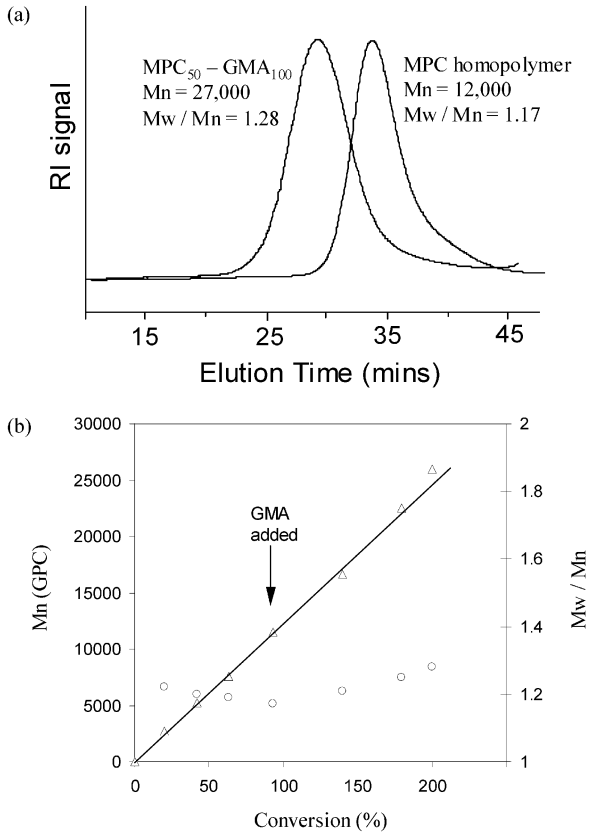

Figure 6. (a) Aqueous GPC data for the MPC_{50}−GMA_{100} diblock copolymer. Conditions: MPC (6.0 g, 2.02×10^{-2} mol), OEGBr (0.20 g, 4.04×10^{-4} mol), the relative molar ratios of MPC:OEGBr:Cu(I)Br:bpy were 50:1:1:2, with GMA (6.47 g, 4.04×10^{-2} mol) being added as the second monomer. (b) Evolution of M_n and M_w/M_n with conversion during the synthesis of the MPC−GMA diblock copolymer [same conditions as in (a) above].

philic−hydrophobic diblock copolymers, since the HPMA and MMA blocks remain permanently water-insoluble regardless of the solution pH or temperature. Unfortunately, this precluded aqueous GPC analysis of these MPC−HPMA and MPC−MMA diblock copolymers; only the extracted MPC homopolymers were suitable for analysis by aqueous GPC (see Table 2). In contrast, aqueous GPC could be used to assess the living character (blocking efficiency) of selected "double-hydrophilic" diblock copolymers. For example, in Figure 6a the aqueous GPC curves for the initial MPC homopolymer and the final MPC−GMA diblock copolymer are shown. There is a shift of the whole distribution curve to higher molecular weight as expected for the diblock copolymer, with minimal MPC homopolymer contamination. Similar results were obtained with the MPC−HEMA, MPC−SBMA, and MPC−CBMA diblock copolymers (not shown). The evolution of M_n and M_w/M_n with conversion for the MPC−GMA diblock is shown in Figure 6b. The GMA was added after approximately 95% conversion of the MPC, and the M_n vs conversion plots were linear for both stages of the polymerization. It is noteworthy that the M_w/M_n decreases with conversion for the MPC polymerization but increases gradually for the GMA polymerization. This suggests that the GMA polymerization has less living character than the MPC polymerization, although further work is required to confirm this hypothesis. The final polydispersity of the MPC−GMA diblock copolymer was 1.28.

Like the MPC−PPO diblocks described above, the MPC−HEMA diblock copolymers also proved to be thermoresponsive: as the solution temperature was increased above the cloud point of the HEMA block, near-monodisperse micelles of 63 nm were formed at 50 °C, and ^{1}H NMR studies (not shown) indicated that these micelle cores were relatively hydrated.

A summary of the various MPC-based diblock copolymers prepared by sequential monomer addition of the three tertiary amine methacrylates is provided in Table 3. The MPC−DMA diblock copolymers exhibited thermoresponsive micellization behavior at neutral pH and elevated temperature. Variable temperature ^{1}H NMR spectra of a MPC_{30}−DMA_{60} diblock copolymer recorded at 20, 40, and 60 °C are shown in Figure 7. All the signals due to the DMA residues are visible at 20 °C, with signals "c" and "d" being particularly prominent. At elevated temperature signal "d" is almost completely attenuated, and signal "c" is strongly attenuated compared to signals "h" and "i" due to the MPC residues. This is consistent with the DMA blocks forming dehydrated micelle cores, with the hydrophilic MPC blocks forming the micelle coronas. DLS studies of the same diblock copolymer at 50 °C indicated an intensity-average micelle diameter of approximately 26 nm.

The pH-induced micellization behavior of an MPC−DEA diblock copolymer was briefly reported in our earlier communication[20] so in the present study we focus instead on the behavior of the MPC−DPA diblock copolymers. These copolymers can be molecularly dissolved in dilute acidic solution since the DPA block is protonated and hence hydrophilic under these conditions. On adjusting the copolymer solution to around pH 6−7, the DPA becomes deprotonated and hence hydrophobic, leading to the formation of micelles with dehydrated DPA cores and MPC coronas. ^{1}H NMR spectroscopy studies of an MPC_{30}−DPA_{60} diblock copolymer (see Figure 8) confirmed that the protonated DPA residues are fully solvated at pH 2.3 (see signal "c"). At pH 7.1 the DPA residues are only partially protonated and hence signal "c" is attenuated, while at pH 10.8 the fully deprotonated DPA block is now hydrophobic and signal "c" disappears completely. For a fixed Dp of 30 for the MPC block, the intensity-average diameter of the micelles formed at around pH 7.1−7.3 depends on the DPA block length and increases from 27 nm (Dp = 30) to 44 nm (Dp = 60) to 55 nm (Dp = 100). In each case the micelles obtained between pH 7 and pH 8 were near-monodisperse, with polydispersities ranging from 0.08 to 0.10. Similar results were obtained for the MPC_{30}−DEA_n diblocks, where $n = 30$, 60, or 100. These observations are in good agreement with several other reports that the length of the core-forming block dictates the size and aggregation number of the micelles.[24]

Pyrene fluorescence is a very sensitive technique for detecting the formation of block copolymer micelles. Pyrene is highly hydrophobic and has very low solubility in water so it migrates preferentially into the hydrophobic micelle cores. Thus, a red shift is observed in the pyrene fluorescence spectra, and there are also changes in relative peak intensities in the vibrational fine structure.[25,26] In the present work the protocol based on the analysis of pyrene emission spectra reported by Gast and co-workers is employed.[24] Figure 9 shows the variation in the intensity ratio (I_1/I_3) vs solution pH for three MPC-based diblock copolymers. As the solution

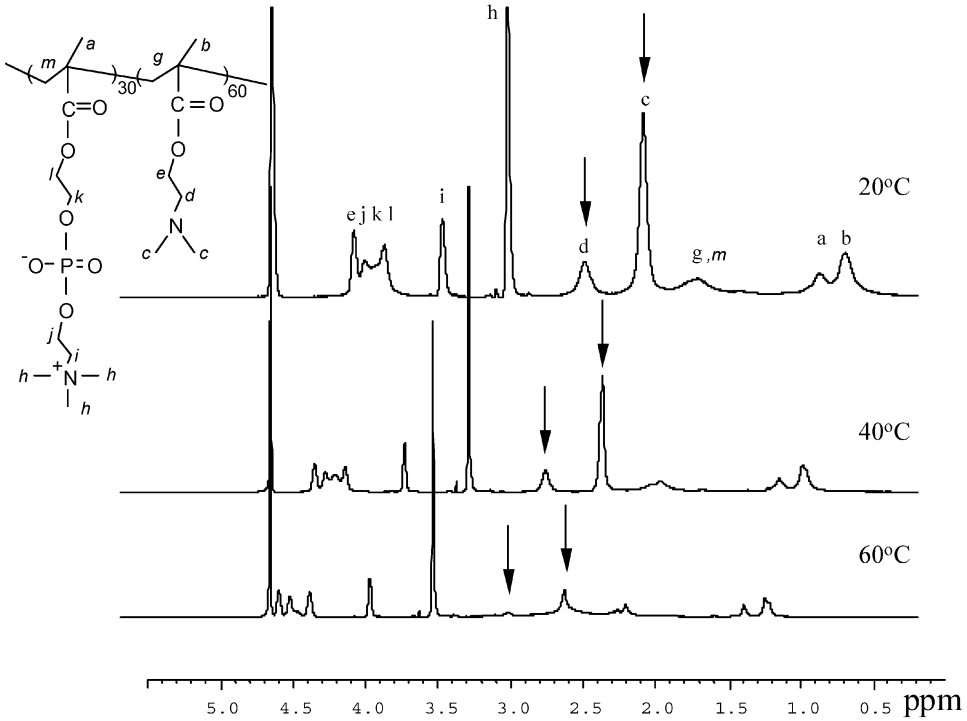

Figure 7. Variable temperature ^{1}H NMR studies of the thermoresponsive MPC_{30}−DMA_{60} diblock copolymer. Note the attenuation of the NMR signals assigned to the DMA residues.

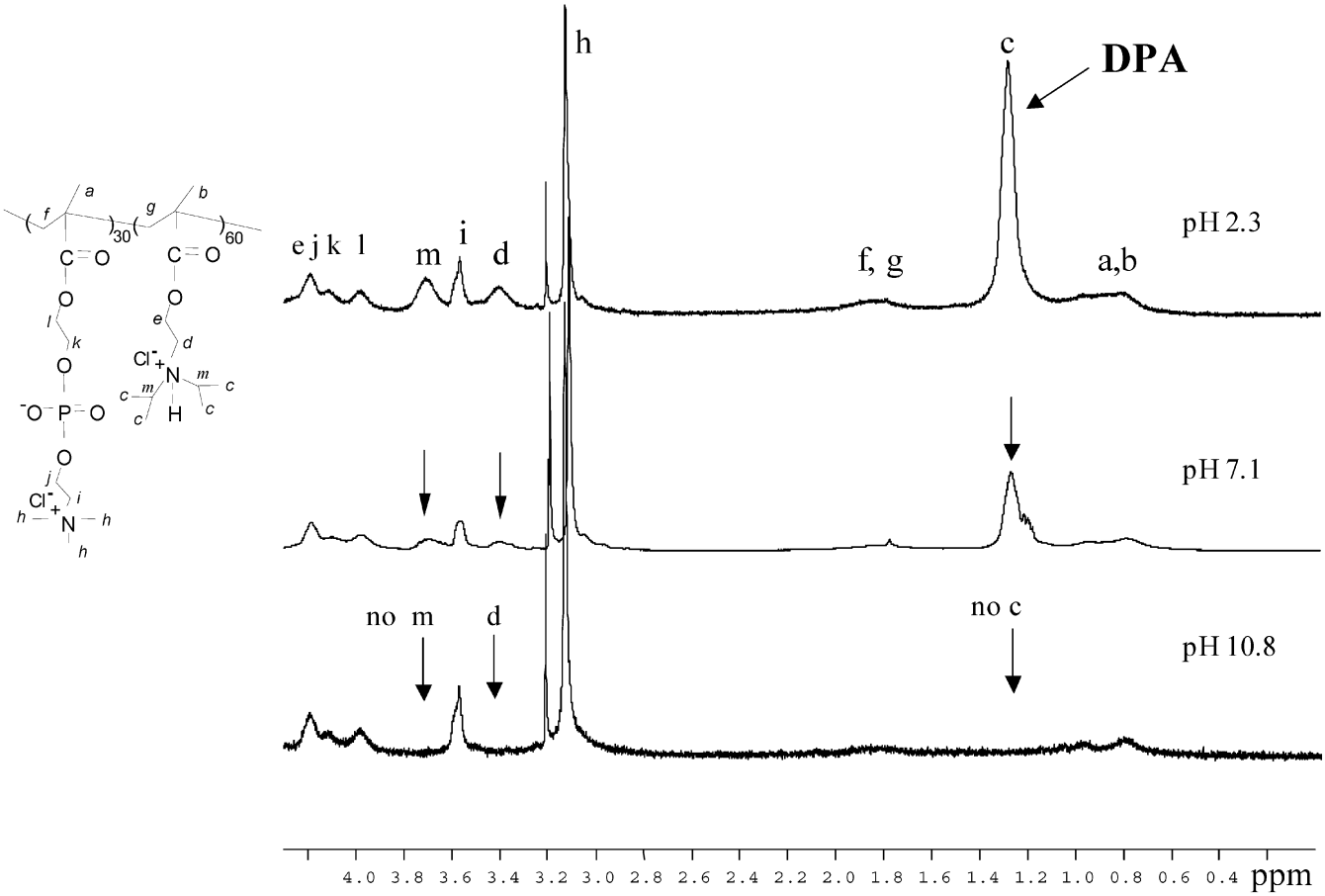

Figure 8. ^{1}H NMR study of the pH-induced micellization behavior of the MPC_{30}−DPA_{60} diblock copolymer. Note the disappearance of the NMR signals assigned to the DPA residues.

pH is increased, the tertiary amine methacrylate residues become progressively deprotonated. The critical pH for micellization was estimated from the reduced I_1/I_3 ratio, which indicates a more hydrophobic (micellar) environment for the pyrene probe. (In the case of the MPC_{30}−DMA_{60} diblock copolymer, there was very little reduction in the I_1/I_3 ratio; hence, either no micelles are formed under these conditions or the micelles are not

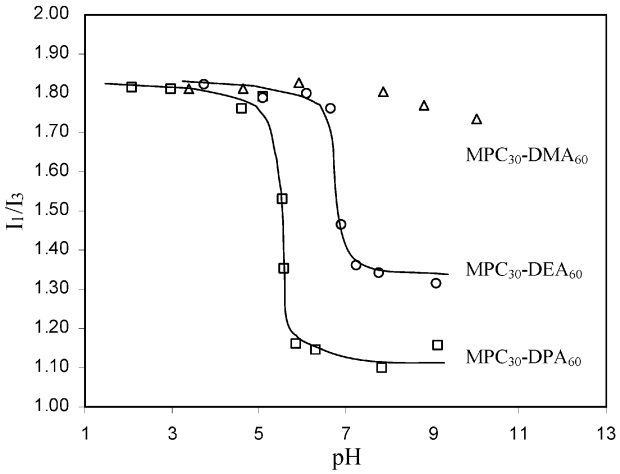

Figure 9. Determination of the critical pH for micellization of the MPC_{30}−DMA_{60}, MPC_{30}−DEA_{60}, and MPC_{30}−DPA_{60} diblock copolymers at 25 °C from pyrene emission spectra (excitation wavelength λ_{ex} = 333 nm; the diblock copolymer concentration was 0.50 g L^{-1} in each case).

hydrophobic enough to ensure efficient pyrene uptake.) The critical micellization pH values estimated for the MPC_{30}−DPA_{60} and MPC_{30}−DEA_{60} diblock copolymers using this method are pH 5.6 and pH 6.9, respectively. These values correlate quite well with the known pK_a's of 6.0 and 7.3 for DPA and DEA homopolymer reported by Bütün et al.[27] It is also noteworthy that the plateau value for the I_1/I_3 ratio observed at high pH is indicative of the relative hydrophobicity of the micelle cores. The plateau value of approximately 1.15 obtained for the MPC_{30}−DPA_{60} diblock copolymer micelles is comparable to that observed by Wilhelm et al. for polystyrene-core micelles and suggests that highly hydrophobic micelle cores are formed.[25] In contrast, the micelles formed by the MPC_{30}−DEA_{60} diblock copolymer clearly have significantly less hydrophobic character. This hypothesis was supported by further fluorescence studies in order to determine the degree of pyrene partitioning within the micelles. Here the protocol reported by Eisenberg and co-workers[28] was adopted. Assuming that the DEA and DPA micelle core densities are around 1.0 g cm^{-3}, the pyrene partition coefficients for the MPC_{30}−DEA_{60} and MPC_{30}−DPA_{60} diblock copolymer micelles were calculated to be 3.2×10^4 and 1.1×10^5, respectively. Thus, the pyrene partition coefficient for the DPA-core micelles is close to the value of $(1.9-2.4) \times 10^5$ reported for highly hydrophobic polystyrene micelle cores.[28] On the basis of our fluorescence studies we anticipate that, compared to the MPC−DEA diblock copolymers, the MPC−DPA diblocks should form more stable micelles with higher loading capacities for the encapsulation of various "actives" such as hydrophobic drugs. We are now examining this prediction in our laboratories.[29]

In collaboration with Stolnik and co-workers, we have recently shown that PEO−DMA diblock copolymers are very effective synthetic vectors for the efficient condensation of calf thymus DNA.[30] Here the cationic DMA residues interact electrostatically with the anionic phosphate residues on the DNA backbone, leading to efficient condensation with the PEO chains, providing an effective steric barrier to further particle aggregation. The result is colloidally stable DNA-loaded micelles with dimensions of less than 100 nm. Clearly, it will be of interest to see whether the MPC−DMA diblock copolymers can also act as synthetic vectors for DNA condensation. Preliminary control experiments are encouraging: MPC homopolymer does not form interpolyelectrolyte complexes with synthetic anionic polyelectrolytes so the MPC component of the diblock copolymer should not interfere with the electrostatic complexation required to achieve DNA condensation. One potentially significant advantage of the MPC block over the PEO block is that the ATRP chemistry used to prepare the former should allow the use of functional ATRP initiators for the polymerization of MPC. This should allow the preparation of biocompatible micelles with appropriate functional groups (e.g., primary amines) decorating the outside of the micelles, which is a prerequisite for the exploitation of cell-targeting strategies.[31]

Finally, it is noteworthy that simply treating aqueous solutions of these MPC-based block copolymers with silica enables the residual ATRP catalyst to be reduced to just a few ppm in most cases (see final columns in Tables 1−3). Although even lower catalyst levels are probably desirable for biomedical applications, we believe that these new biocompatible diblock copolymers show great promise for a range of biomedical applications.[32]

Conclusions

In summary, we have synthesized a wide range of novel, well-defined, biocompatible MPC-based diblock copolymers using ATRP in protic media. Macroinitiators were employed to prepare PEO−MPC, PPO−MPC, and PDMS−MPC diblocks, and the sequential monomer addition route was exploited for the block copolymerization of MPC with 12 different methacrylic comonomers. Generally high conversions were achieved under mild conditions, and polydispersities were relatively low (M_w/M_n = 1.1−1.3). The PPO−MPC diblocks exhibited thermoresponsive behavior in dilute aqueous solution: molecular dissolution was achieved at low temperature, with large, hydrated PPO-core micelles being formed at elevated temperature. The MPC−DMA diblock copolymer also proved to be thermoresponsive as judged by ^{1}H NMR spectroscopy and dynamic light scattering studies. The MPC−DEA and MPC−DPA diblocks were pH-responsive: molecular dissolution was achieved at low pH due to protonation of the tertiary amine groups, and micellization occurred between pH 7 and 9, as the DEA or DPA blocks became deprotonated and hence hydrophobic. In the case of the MPC−DPA diblock, the pyrene partition constant for the DPA-core micelles was within a factor of 3 of that reported previously for polystyrene-core micelles. This augurs well for the efficient loading of MPC−DPA micelles with hydrophobic drugs. In all cases micellization of these biocompatible stimuli-responsive diblock copolymers was fully reversible: on reverting to the original solution temperature or pH the micelles dissociate rapidly to give individual copolymer chains. Analyses for residual ATRP catalyst using ICP−AES indicated typical Cu levels of 1−2 ppm in the final purified diblock copolymers (see Tables 1−3).

Selected MPC-based diblock copolymers are now being evaluated in various biomedical applications in our laboratory. These include novel synthetic vectors for gene therapy, stimuli-responsive biocompatible micelles for hydrophobic drugs, and precursors for the preparation of new biocompatible shell cross-linked micelles.

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