Facile Synthesis of Well-Defined, Biocompatible Phosphorylcholine-Based Methacrylate Copolymers via Atom Transfer Radical Polymerization at 20 °C

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It is well-known that phosphorylcholine-based polymers can be used to produce surfaces which are remarkably resistant to protein adsorption and cell adhesion.[1,2] On the basis of this approach, many biomedical applications have been developed, including high performance surgical stents and extended-wear contact lenses.[3] However, there have been no reports of the direct, controlled polymerization of phosphorylcholine-based monomers such as 2-methacryloyloxyethyl phosphorylcholine [MPC]. One problem is that such monomers are usually 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. Recently, Matyjaszewski and others have developed a new form of pseudo-living free radical chemistry known as atom transfer radical polymerization (ATRP).[4−7] This chemistry has gained a deserved reputation for excellent tolerance of both monomer functionality and protic sources.[6,7]

We recently reported that ATRP is particularly effective for hydrophilic monomers in aqueous media under mild conditions.[8] In view of these results, and given that MPC is insoluble in many organic solvents, we investigated the feasibility of polymerizing MPC via ATRP in aqueous media (see Figure 1). An unexpected problem was encountered in our preliminary experiments, since the MPC monomer (donated by Biocompatibles, UK) polymerized spontaneously in aqueous solution at 20 °C.[9]

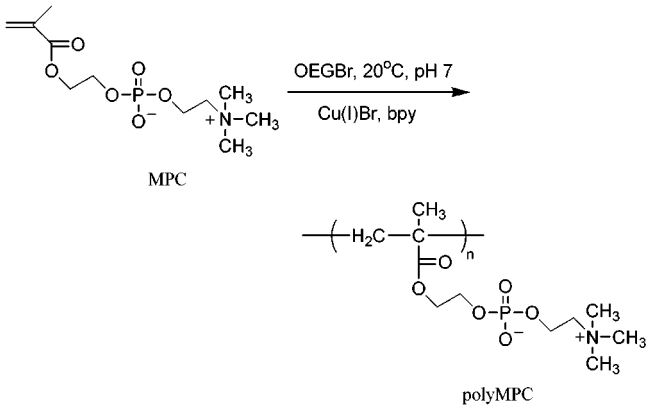

Figure 1. Reaction scheme for the atom transfer radical polymerization of MPC in aqueous media at 20 °C.

In initial syntheses this problem was exacerbated because we had elected to add the ATRP initiator last to an aqueous (and already polymerizing!) solution of MPC. GPC analysis of the resulting polymer indicated a bimodal molecular weight distribution, with the higher mass peak corresponding to the uncontrolled spontaneous polymerization of MPC. Fortunately, later experiments confirmed that adding MPC last as a solid to an aqueous solution containing the ATRP initiator and catalyst produced a very fast rate of ATRP, with over 90% conversion being achieved within 5 min at 20 °C. Since the rate for the autopolymerization of MPC is much slower (conversions of 50% required 5 h for a 17 wt % solution of MPC at 20 °C; see Figure 1 in Supporting Information), essentially only controlled ATRP occurs, and well-defined (co)polymers with unimodal distributions are readily obtained.

A typical protocol for the controlled polymerization of MPC by aqueous ATRP is as follows. A water-soluble ATRP initiator (OEGBr, 413 mg, 0.67 mmol, 1 equiv) was synthesized as reported previously[10] and dissolved in doubly distilled, deionized water (10 mL). After purging with nitrogen for 30 min, Cu(I)Br catalyst (96 mg, 0.67 mmol, 1 equiv) and bpy ligand (208 mg, 1.30 mmol, 2 equiv) were added to the stirred solution under nitrogen. MPC (2.0 g, 6.7 mmol, 10 equiv) was then added as a solid to the reaction mixture under nitrogen. The reaction mixture immediately became dark green and progressively more viscous. Exotherms of 2−4 °C were typically observed, indicating that polymerization was occurring. After the exotherm had abated, the resulting MPC homopolymer was precipitated into THF, then redissolved in water, and passed through a silica column to remove residual ATRP catalyst.

Molecular weight distributions were assessed using GPC (1.0 M NaCl solution with 50 μM Trizma buffer, Superdex 200 column, PEO standards, RI detector). The kinetics of polymerization were monitored by ^{1}H NMR spectroscopy 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. GPC analysis indicated narrow, unimodal molecular weight distributions, with polydispersities (M_w/M_n) of around 1.18 to 1.45 (see Table 1). The initiator was used as an "end-group" to determine the degrees of polymerization of the homopolymers by ^{1}H NMR spectroscopy. In these calculations it was assumed that the initiator efficiency was 100%, chain transfer was negligible, and that every polymer chain contained an oligo(ethylene glycol) fragment. The latter assumption was confirmed by the following experiment. An aqueous solution of MPC homopolymer (degree of polym-

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Table 1. Synthesis Conditions, Molecular Weight Data, and Conversions for the Homopolymerization of MPC via ATRP at 20 °C in Either Water (entries 1−6) or Methanol (entries 7, 8)

MPC wt %	initiator mM	target Dp	expt Dp[a]	expt M_n[b]	M_w/M_n[b]	conv. (%)
17	0	c	c	c	c	53[c]
17	67	10	11	4,710	1.18	>96
17	40	17	18	6,900	1.28	>96
40	222	10	10	4,230	1.23	>96
40	111	20	20	7,550	1.39	>96
40	73	30	29	10,720	1.45	>96
17	67	10	11	3,800	1.12	>96
40	73	30	29	8,640	1.41	>96

[a] From ^{1}H NMR (see text for details). [b] From aqueous GPC analysis. [c] Auto-polymerization with no initiator, therefore a theoretical target Dp could not be calculated. The stated conversion was obtained after 5 h. Aqueous GPC column limits were exceeded, rendering analysis meaningless.

erization, Dp = 20) was precipitated into THF, which is a good solvent for the oligo(ethylene glycol)-based initiator. No change in the Dp of the precipitated homopolymer was detected by NMR, which confirmed that all of the initiator groups were covalently attached to the polymer chains, as expected. Only relatively low Dp's were targeted in this study, because of the known exclusion limits of our GPC column.[11]

In all cases the aqueous ATRP of MPC was rapid: high yields (>96%) were obtained within 10 min at 20 °C and 17 wt % MPC concentration. At 40 wt % MPC, conversions of more than 96% were obtained within 3 min. However, polydispersities were a little higher at 1.23−1.45, indicating reduced control under these conditions. The semilogarithmic plot for the homopolymerization of MPC was linear up to around 75% conversion (see Figure 2a in Supporting Information). At higher conversions nonlinear behavior was observed, which indicated that the polymer radical concentration was no longer constant, probably due to some premature chain termination. On the other hand, the molecular weight versus conversion was linear up to 95% (see Figure 2b in Supporting Information).

Although ATRP was sufficiently fast in aqueous media that autopolymerization was negligible, it was nevertheless decided to examine the feasibility of polymerizing MPC in methanol at 20 °C, since MPC does not spontaneously polymerize in this solvent. Well-controlled ATRP occurred much more slowly under these conditions, with only 70% conversion after 4 h.[12] Aqueous GPC analysis indicated a final polydispersity of only 1.12 at 17 wt % MPC. However, aqueous ATRP has the advantage that high yields are achieved more efficiently.

MPC-based diblock copolymers can also be synthesized via ATRP. For example, addition of 2-(diethylamino)ethyl methacrylate [DEA] to an MPC homopolymerization at high conversion in methanol resulted in further chain growth and the formation of an MPC−DEA diblock copolymer. ^{1}H NMR studies indicated that this block copolymer comprised 77 mol % DEA, which is in excellent agreement with the target block composition. This diblock copolymer dissolved molecularly in acidic solution due to protonation of the DEA residues but formed micelles at pH 8 on addition of NaOD, see Figure 2. ^{1}H NMR studies confirmed

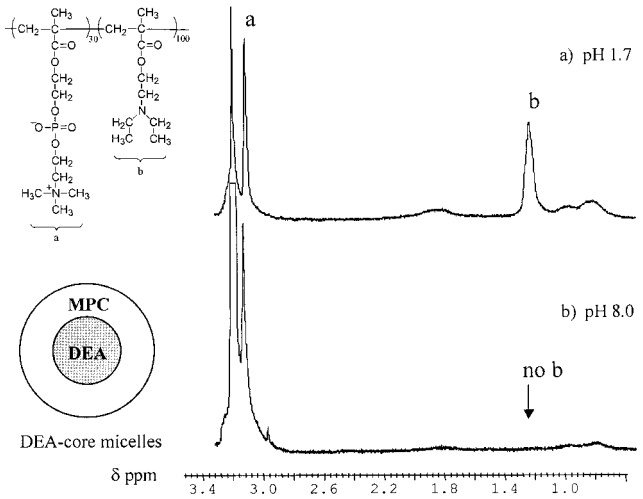

Figure 2. ^{1}H NMR spectra of the MPC−DEA diblock copolymer: (a) at pH 1.7 (DCl/D_2O); (b) pH 8.0 (+ NaOD).

that the deprotonated, hydrophobic DEA residues formed the dehydrated micelle cores (disappearance of signal b at δ 1.2) and the MPC residues formed the solvated coronas, as expected. Dynamic light-scattering studies indicated a narrow, unimodal size distribution, with an intensity-average micelle diameter of 43 nm.

Such micelles are expected to act as "stealthy" nanoparticles, since the MPC block should minimize protein adsorption and hence prevent phagocytosis. To examine this hypothesis, the MPC−DEA diblock copolymer was spin-coated onto a PET substrate from ethanol, and an ELISA fibrinogen assay was conducted (see Supporting Information).[13] The MPC−DEA diblock copolymer coating gave a 76% reduction in fibrinogen binding compared to that with an uncoated PET substrate. Even greater fibrinogen reduction (up to 85%) was obtained using another MPC−DEA diblock copolymer. Thus, planar surfaces coated with MPC-based copolymers prepared via ATRP are rendered highly biocompatible. This indicates that MPC−DEA diblock copolymer micelles are also most likely biocompatible and therefore show considerable promise for drug delivery applications.

In summary, the controlled polymerization of MPC has been demonstrated for the first time. Well-defined copolymers are readily prepared via ATRP at 20 °C in both water and methanol. Aqueous ATRP allows high conversions to be achieved more efficiently, although polydispersities are higher. This advance will allow the synthesis of a wide range of new biocompatible copolymers for biomedical applications.

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Supporting Information Available: Conversion vs time curves for the auto-polymerization of MPC in water and the controlled ATRP of MPC in both water and methanol; conversion vs time data, semilogarithmic plot, and M_n vs conversion plot for the aqueous ATRP of MPC at 20 °C; fibrinogen reduction ELISA assay details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) More recently, we have achieved target degrees of polymerization of up to 200 via methanolic ATRP, with polydispersities of around 1.35. Semilog kinetic plots are more linear for methanolic syntheses (up to 95% conversion in some cases), which is consistent with the lower polydispersities observed under these conditions.

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