Notes

Preparation of Ideal PEG Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate

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Introduction

Poly(ethylene glycol) (PEG) (also known as poly(ethylene oxide) (PEO) depending on the synthetic conditions) is a cheap, neutral, water-soluble, biocompatible, FDA-approved polymer and thus is probably the most widely applied synthetic polymer in biotechnology and medicine.[1] For instance, PEG is an excellent shielding agent for in vivo delivery of various bioactive compounds. Indeed, PEG allows a good solubility in physiological media and prevents the adsorption of plasma proteins, which can trigger immune response.[2,3] Thus, PEG was extensively used in delivery of either low molecular weight drugs, active peptides, proteins, or genetic material.[4−7]

Different synthetic strategies exist for constructing PEG-based delivery vehicles. A first approach relies on the direct covalent conjugation of an active substance with PEG (a synthetic route commonly known as PEGylation). The covalent linkage between both can be either stable (permanent PEGylation) or labile (prodrug strategy). Another approach relies on the physical entrapment of active substances in the protected internal regions (core, internal layer, hollow) of artificial nanocarriers (e.g., nanospheres, micelles, vesicles or polyplexes). For example, nanocarriers obtained via the aqueous self-assembly of triblock copolymers PEO-*b*-poly(propylene oxide)-*b*-PEO[8] (known commercially as Pluronic) or diblock copolymers PEO-*b*-polypeptide[6,9] have been extensively applied in life science. These classic PEO-based building blocks are typically prepared by anionic ring-opening polymerization. However in the past few years, controlled radical polymerization (CRP) techniques such as atom transfer radical polymerization (ATRP),[10] nitroxide-mediated polymerization (NMP),[10] and reversible addition−fragmentation transfer polymerization (RAFT)[10] have been more and more considered as a straightforward alternative for preparing well-defined building blocks for life science.[11−19] In particular, ATRP was proven to be a very versatile pathway for preparing PEG-based amphiphiles.[13,20−24] However, the possibilities of "PEGylation" are still very limited in ATRP. Two synthetic routes have been used in ATRP for incorporating PEG in macromolecular constructions (Scheme 1). The first one is a macroinitiator approach, in which an ω-hydroxy-PEG segment prepared by anionic polymerization is transformed into an ATRP initiator (Scheme 1a). The latter can be obtained by coupling the hydroxy terminal functionality of PEG with either 2-bromo-2-methylpropionyl bromide[23,25,26] or 2-bromoisobutyric acid.[20,22] Such an approach is not straightforward since it requires to work at the interface between anionic polymerization and CRP.[26] Indeed, commercial α-methoxy-ω-hydroxy-PEG polymers can be used for such synthesis,[20,22,23] but in this case the molecular structure of the PEG segment is only limited to commercially available compounds (i.e., a limited range of molecular weight and α-functionalities can be bought). Another major disadvantage of such approach is the formation of a hydrolyzable ester linkage between the PEG segment and the ATRP initiator (thus, between the PEG segment and the successive block grown by ATRP), which is in most cases problematic for applications in aqueous media.

The second approach directly polymerizes of a radically polymerizable PEG macromonomer such as oligo(ethylene glycol) methacrylate (OEGMA). The pioneering works of Haddleton and co-workers and Armes and co-workers elegantly illustrate that such strategy is a very convenient alternative for incorporating PEG in macromolecular architectures built by CRP.[17,27,28] However, the PEG blocks formed via such a macromonomer approach are macromolecular brushes and therefore possess all the intrinsic disadvantages of this class of macromolecules.[29] First, it is often quite difficult to control precisely the molecular structure of macromolecular brushes (chain length, molecular weight distribution) using CRP techniques.[29] Moreover, short macromolecular brushes often exhibit a compact globular (or starlike) shape in solution, which also hinders further chemical reactions. For instance, in ATRP, it seems difficult to involve the ω-halogen chain-end of macromolecular brushes in transformation reactions (chain-end functionalization or reinitiation).

A very interesting new alternative was recently suggested by Ishizone and co-workers, who investigated the anionic polymerization of various methacrylates possessing rather short oligo(ethylene glycol) side chains.[30] In particular, they shown that, although possessing only two ethylene oxide units as side groups, polymers of 2-(2′-methoxyethoxy)ethyl methacrylate (MEO_2MA) were soluble in water at room temperature. Hence, MEO_2MA would be also a particularly tempting monomer for preparing water-soluble segments by ATRP (or more generally by CRP) since this methacrylate is radically polymerizable, commercially available, possesses a chemical structure resembling PEG, and moreover leads to polymers with a linear architecture. Unfortunately, poly(2-(2′-methoxyethoxy)ethyl methacrylate) ($PMEO_2MA$) has a major limitation: it exhibits a lower critical solution temperature (LCST) in water around 26 °C,[30] which indeed completely prohibits in vivo applications. On the other hand, POEGMA macromolecular brushes usually exhibit a much higher LCST in water due to their longer PEG side chains (typically LCST is around 90 °C for POEGMA with side chains of 8/9 ethylene oxide units).[31] Therefore, it is also

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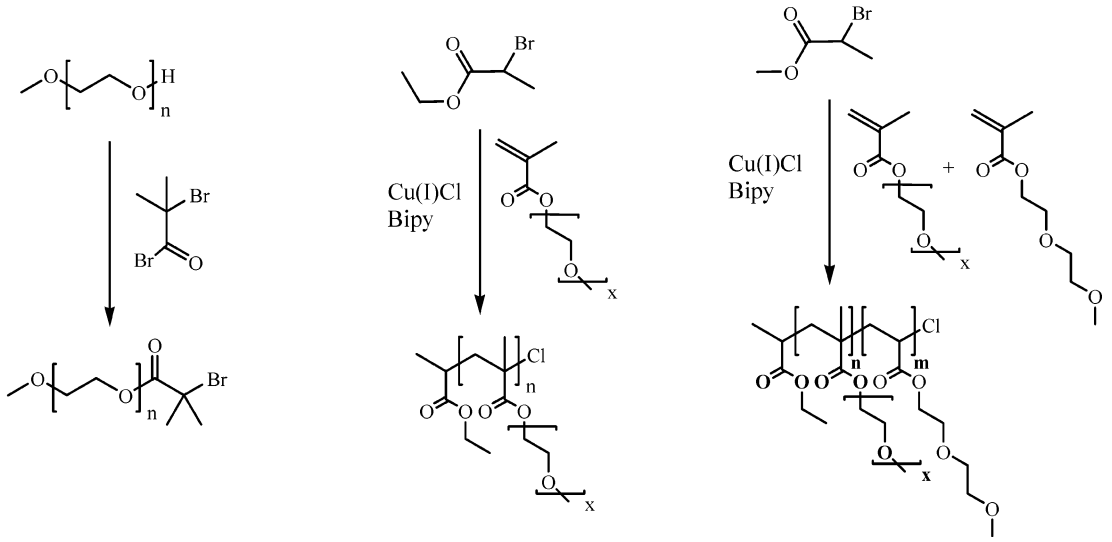
Scheme 1. Various Synthetic Strategies Used in Atom Transfer Radical Polymerization for Incorporating Poly(ethylene glycol)



expectable that copolymers of MEO_2MA and OEGMA possess higher LCST than 26 °C. The latter was recently suggested by Kitano and co-workers.[32] However, up to now, no systematic studies of these copolymers has been reported.

The target of the present note is to illustrate that copolymers of MEO_2MA and OEGMA could be ideal water-soluble blocks for life science applications. Thus, we studied herein the ATRP of a series of P(MEO_2MA-*co*-OEGMA) containing increasing amounts of OEGMA. In particular, the properties in aqueous solution of P(MEO_2MA-*co*-OEGMA) copolymers were investigated.

Experimental Part

Chemicals. 2-(2-Methoxyethoxy)ethyl methacrylate (Aldrich, 95%), poly(ethylene glycol) methyl ether methacrylate (Aldrich, M_n = 475 g mol^{-1}), methyl 2-bromopropionate (MBP) (Aldrich, 98%), and 2,2′ bipyridyl (Bipy) (Fluka, 98%) were used as received. Copper(I) bromide (Aldrich, 98%) and copper(I) chloride (Acros, 95%) were washed with glacial acetic acid in order to remove any soluble oxidized species, filtered, washed with ethanol, and dried.

Example of Atom Transfer Radical Copolymerization of MEO_2MA and OEGMA. Copper bromide (13 mg, 0.09 mmol) and 2,2′ bipyridyl (28.3 mg, 0.18 mmol) were added to a Schlenk tube sealed with a septum. The tube was purged with dry argon for a few minutes. Then, a degassed mixture of 2-(2-methoxyethoxy)ethyl methacrylate (1.53 g, 8.1 mmol), poly(ethylene glycol) methyl ether methacrylate (431 mg, 0.9 mmol) and 2.4 mL of ethanol was added through the septum with a degassed syringe. Last, methyl 2-bromopropionate (15.1 mg, 0.09 mmol) was added with a microliter syringe. The mixture was heated at 60 °C in an oil bath for 3 h. After several hours, the experiment was stopped by opening the flask and exposing the catalyst to air. The final mixture was diluted in ethanol and passed through a short silica column (60−200 mesh) in order to remove copper catalyst. Then, the filtered solution was diluted with deionized water and subsequently purified by dialysis in water (Roth, ZelluTrans membrane, molecular weight cutoff: 4000−6000). Last, water was removed by azeotropic distillation with ethanol. The purified polymer appeared as a clear oil (M_n = 24 300 g mol^{-1}; M_w/M_n = 1.57)

Measurements and Analysis. *Size Exclusion Chromatography (SEC).* Molecular weights and molecular weight distributions were determined by SEC performed at 25 °C in tetrahydrofuran (THF) as eluent, using three 5 μ-MZ-SDV columns with pore sizes of 10^3, 10^5, and 10^6 Å (flow rate 1 mL min^{-1}). The detection was performed with a RI (Shodex RI-71) and a UV detector (TSP UV 1000; 260 nm). For calibration, linear polystyrene standards (PSS, Germany) were used.

Cloud Point Measurements. The cloud points of the polymer solutions in water were measured on a Tepper TP1 photometer (Mainz, Germany). Transmittance of polymer solutions in deionized water at 670 nm was monitored as a function of temperature (cell path length: 12 mm; one heating/cooling cycle at rate of 1 °C min^{-1}).

^{1}H NMR. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker DPX-400 operating at 400.1 MHz. Monomer conversions were calculated from ^{1}H NMR spectra by comparing the integrations of the vinyl protons of the remaining monomers (5.56 and 6.12 ppm) to the overall integration of the region 3.90−4.40 ppm where resonate 2 protons of the remaining monomers and 2 protons of the formed polymers. An overall monomer conversion was calculated since both monomers MEO_2MA and OEGMA were assumed to possess comparable reactivities. The molar fraction of OEGMA in the P(MEO_2MA-*co*-OEGMA) copolymers was also calculated from ^{1}H NMR spectra by comparing the overall integration of the methoxy protons of both MEO_2MA and OEGMA (3.35−3.41 ppm) to the overall integration of the region 3.55−3.65 ppm where resonate around 30 protons of OEGMA and 2 protons of MEO_2MA.

Results and Discussion

Table 1 shows the molecular structure and the properties of a series of P(MEO_2MA-*co*-OEGMA) copolymers prepared by atom transfer radical copolymerization of MEO_2MA and OEGMA. In this series, the initial molar fractions of MEO_2MA and OEGMA monomers was variable, but an overall average degree of polymerization of 100 was targeted in all cases.

The copolymers were prepared at 60 °C in ethanol solution in the presence of the homogeneous ATRP catalytic system copper(I) bromide/2,2′-bipyridyl. These synthetic conditions were first selected as a fast screening system since bromide-based catalysts and polar protic solvents are both known to generate very fast ATRP kinetics.[10,28] As expected, in such conditions, homopolymers or copolymers of MEO_2MA and POEGMA could be obtained in high yields in a rather short time. However, the results of Table 1 underline major differences between homopolymers of OEGMA (POEGMA) and P(MEO_2-MA-*co*-OEGMA) copolymers. Indeed, in the case of POEGMA, experimental molecular weights measured by size exclusion chromatography were found to be much lower than theoretical values. The latter is a consequence and a good illustration of the unusual solution behavior of macromolecular brushes.[29]

Table 1. Properties of Copolymers of OEGMA and MEO_2MA Prepared by ATRP[a]

	$[OEGMA]_0/[MEO_2\text{-}MA]_0$	conversion[b]	F_{OEGMA}[c] (%)	M_n[d]	$M_{n\ th}$[e]	M_w/M_n[c]	LCST[f] (°C)
1	100:0	0.91	100	10 000	43 200	1.18	90
2	30:70	0.93	33	35 000	25 500	1.22	59
3	20:80	0.91	20	29 700	22 300	1.33	49
4	15:85	0.88	16	19 900	20 300	1.57	44
5	10:90	0.92	10	24 300	19 900	1.57	39
6	08:92	0.90	8	24 800	19 000	1.45	37
7	05:95	0.86	6	18 300	17 400	1.66	32
8	0:100	0.90	0	16 700	16 900	1.72	28

[a] Experimental conditions: 3 h, 60 °C; in ethanol solution (monomer/ethanol = 1:1.25 (v/v)); $([OEGMA]_0 + [MEO2\text{-}MA]_0)/[MBP]_0/[CuBr]_0/[Bipy]_0 = 100/1/1/2$. [b] Overall monomer conversion measured by ^1H NMR. [c] Calculated by ^1H NMR. [d] Measured by SEC in THF. [e] $M_{n,th}$ = conversion $(475[OEGMA]_0 + 188[MEO_2\text{-}MA]_0)/[MBP]_0$. [f] Measured for aqueous solutions with a concentration of 3 mg mL^{-1}.

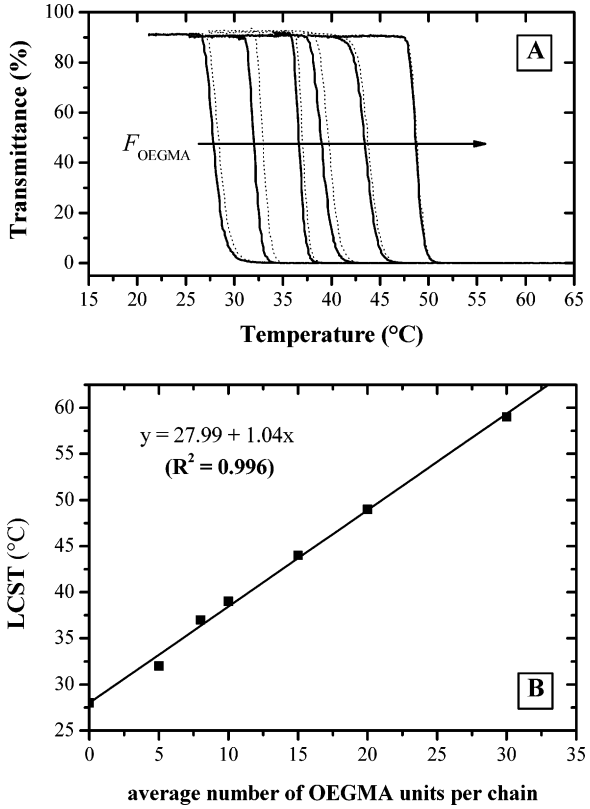


Figure 1. (A) Plots of transmittance as a function of temperature (670 nm, 1 °C mn^{-1}) measured for aqueous solutions (3 mg mL^{-1}) of a series of copolymers of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2-MA) and oligo(ethylene glycol) methacrylate (OEGMA) with an increasing OEGMA content (Table 1). Solid lines: heating cycles; dotted lines: cooling cycles. (B) Plots of the measured lower critical solution temperature (LCST) as a function of the theoretical average number of OEGMA units per chain for P(EO_2MA-*co*-OEGMA) copolymers.

Experimental molecular weights measured for P(MEO_2MA-*co*-OEGMA) copolymers were much closer to theoretical values, which indicates that such copolymers most likely adopt a random coil conformation in THF solution. The latter confirms that P(MEO_2MA-*co*-OEGMA) might be much more practical platforms for macromolecular engineering that POEGMA. Nevertheless, P(MEO_2MA-*co*-OEGMA) copolymers were found to exhibit extremely interesting solution properties in water. Table 1 and Figure 1 report the values of LCST measured for aqueous solutions of either the homopolymer of MEO_2MA ($PMEO_2MA$), P(MEO_2MA-*co*-OEGMA), or POEGMA (studied reference concentration was 3 mg mL^{-1}).

For homopolymers $PMEO_2MA$ and POEGMA, cloud points of respectively 28 and 90 °C were measured, which are in good agreement with previously reported values.[30−32] For P(MEO_2-MA-*co*-OEGMA) samples, measured LCST values were in between aforementioned limits (i.e., comprised in the range 28−90 °C). As expected, LCST values increase with the molar fraction of OEGMA F_{OEGMA} in the copolymer (and also with f_{OEGMA}, the fraction of OEGMA in the initial comonomer feed, since F_{OEGMA} and f_{OEGMA} were found to be nearly equal in all cases). Moreover, the mathematical correlation between the observed LCST and the theoretical average number of OEGMA units per chain DP_{OEGMA} was found to be extremely simple and useful (Figure 1B). Indeed, in the studied range of composition ($0 < f_{OEGMA}$, $F_{OEGMA} < 0.3$; $0 < DP_{OEGMA} < 30$), the LCST of P(MEO_2MA-*co*-OEGMA) is a linear function of DP_{OEGMA} with a slope of nearly unity. Therefore, the LCST of P(MEO_2-MA-*co*-OEGMA) copolymers can be precisely predicted from DP_{OEGMA} (eq 1).

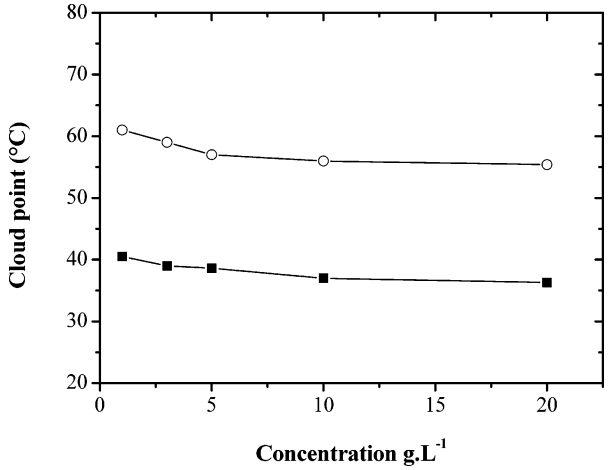


Figure 2. Evolution of the cloud point as a function of concentration for aqueous solutions of copolymers of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate (OEGMA) possessing either 10% (■) or 30% (○) of OEGMA per chain (Table 1, entries 2 and 5).

$$LCST = 28 + 1.04DP_{OEGMA} \quad (1)$$

Thus, in P(MEO_2MA-*co*-OEGMA) copolymers possessing on average 100 monomer units, each unit of OEGMA in the chain leads approximately to an increase of LCST of 1 °C (this law is indeed only valid in the studied range of compositions). For example, a well-defined copolymer P(MEO_2MA-*co*-OEGMA) possessing a LCST of 37 °C (body temperature) could be synthesized from an initial monomer feed possessing 8% of OEGMA (Table 1, entry 6). Similarly, LSCT could be tuned up to temperatures corresponding to high fevers (39−40 °C) by simply using a slightly higher f_{OEGMA} (Table 1, entry 5). These results clearly illustrate that P(MEO_2MA-*co*-OEGMA) copolymers possess a very high potential for biomedical applications. Moreover, the LCST of P(MEO_2MA-*co*-OEGMA) copolymers was not found to very sensitive to their concentration in water, which is a very important issue for applications. Figure 2 shows the influence of concentration on the cloud points measured for two different samples. In the studied range of

Table 2. Influence of the Catalyst System on the Molecular Structure of P(MEO_2MA-*co*-OEGMA) Prepared by ATRP[a]

	$[OEGMA]_0/[MEO_2\text{-}MA]_0$	catalyst	time (h)	conversion[b]	M_n[c]	$M_{n\,th}$[d]	M_w/M_n[c]
1	100:0	CuBr/Bipy	3	0.91	10 000	43 200	1.18
2	100:0	CuCl/Bipy	10	0.83	15 900	39 400	1.07
3	30:70	CuBr/Bipy	3	0.93	35 000	25 500	1.22
4	30:70	CuCl/Bipy	10	0.90	22 200	24 700	1.23
5	10:90	CuBr/Bipy	3	0.92	24 300	19 900	1.57
6	10:90	CuCl/Bipy	9	0.95	18 500	20 600	1.27
7	0:100	CuBr/Bipy	3	0.90	16 700	16 900	1.72
8	0:100	CuCl/Bipy	10	0.97	21 200	18 200	1.27

[a] Experimental conditions: 60 °C; in ethanol solution (monomer/ethanol = 1:1.25 (v/v)); $([OEGMA]_0 + [MEO2\text{-}MA]_0)/[MBP]_0/[CuX]_0/[Bipy] = 100/1/1/2$. [b] Overall monomer conversion measured by ^1H NMR. [c] Measured by SEC in THF. $M_{n,th}$ = conversion $(475[OEGMA]_0 + 188[MEO2\text{-}MA]_0)/[MBP]_0$.

concentration (1−20 mg mL^{-1}), the LCST was found to only increase of a few degrees with high dilution.

ATRP in ethanol was found to be a very straightforward method for preparing P(MEO_2MA-*co*-OEGMA) copolymers. However, in the presence of the ATRP catalyst CuBr/Bipy broad molecular weight distributions were obtained for the copolymers. For POEGMA homopolymers, very narrow molecular weight distributions were obtained with CuBr/Bipy (these low polydispersities are maybe just apparent due to the globular shape of the macromolecular brushes or can be due to the fact that termination reactions are usually disfavored with macromonomers). On the other hand, for P(MEO_2MA-*co*-OEGMA), it appears from Table 1 that the polydispersity index increases with the molar fraction of MEO_2MA in the copolymer. Chloride-based ATRP catalysts usually lead to a much better control of the radical polymerization of methacrylates than bromide-based catalysts.[10] Thus, the catalytic system CuCl/Bipy was tested for preparing POEGMA, P(MEO_2MA-*co*-OEGMA), and PMEO_2MA samples (Table 2). As expected, this catalyst allowed a much better control of the molecular structure of the polymers. Experimental molecular weights of P(MEO_2MA-*co*-OEGMA) and PMEO_2MA were found to be close to theoretical values in all cases (for POEGMA, molecular weights measured by GPC were always found to be underestimated, independently of the catalyst structure). Nevertheless, a narrow molecular weight distribution was obtained for all samples ($M_w/M_n < 1.3$). For copolymers of comparable composition, molecular weight distribution was found to have no significant effect on the LCST. The cloud points measured for the polymers prepared using CuCl/Bipy were identical to those reported in Table 1 for their analogues synthesized with CuBr/Bipy.

Conclusion

A new class of water-soluble copolymers based on 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate P(MEO_2MA-*co*-OEGMA) was investigated. ATRP at 60 °C in ethanol was found to be a very straightforward method for preparing these copolymers in high yields. In the presence of the ATRP catalytic system CuCl/bipy, well-defined P(MEO_2MA-*co*-OEGMA) samples with a controlled chain length and a narrow molecular weight distribution could be prepared. Moreover, P(MEO_2MA-*co*-OEGMA) copolymers were found to exhibit extremely interesting solution properties in water. Indeed, the LCST of these copolymers could be accurately tuned by adjusting the fraction of OEGMA units in the copolymer chains. Thus, LCST can be predicted on demand by adjusting the initial composition of the comonomer feed. For example, LCST of 37 or 39 °C could be precisely obtained by using either 8 or 10% of OEGMA in the initial comonomer mixtures. Moreover, the LCST of P(MEO_2MA-*co*-OEGMA) samples was not found to be very dependent on polymer concentration. Therefore, these new copolymers can be considered as ideal segments for constructing amphiphiles by CRP and moreover as very high potential candidates for application in biotechnology.

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