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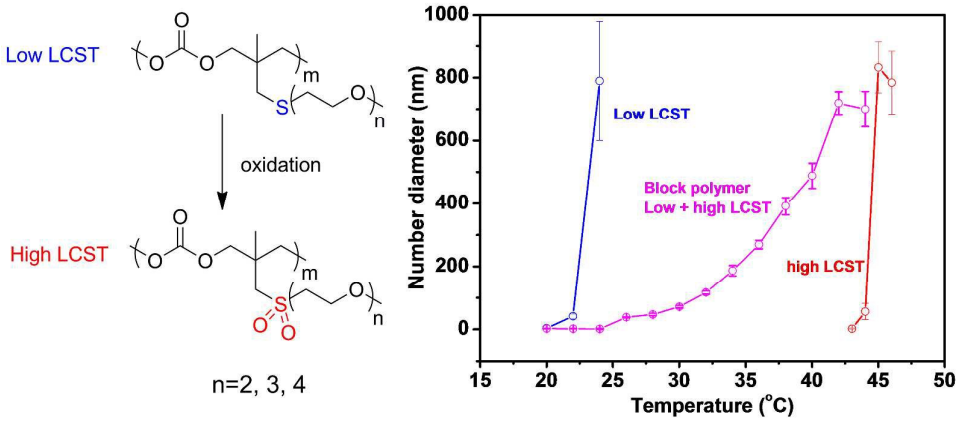
Table of contents graphic



Tunable thermoresponsive aliphatic polycarbonates with oligo ethylene glycol contain thioether and/or sulphone groups.

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ARTICLE

Synthesis and properties of tunable thermoresponsive aliphatic polycarbonate copolymers with oligo ethylene glycol containing thioether and/or sulphone groups

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A series of novel thermoresponsive copolycarbonates were constructed by cyclic trimethylene carbonate (MTC) monomers bearing oligo ethylene glycol (OEG) and with thioether or/and sulphone linkages. They were initiated by 1,4-benzenedimethanol with N-(3,5-trifluoromethyl)phenyl-N'-cyclohexylthiourea (TU)/1,8-diazabicyclo[5,4,0]undec-7-ene) (DBU) as the organic catalyst. The lower critical solution temperature (LCST) properities of the polycarbonates with thioether or sulphone linkage were studied and discussed in regard to molecular weight, salt concentration and polymer concentration. By using monomers of different OEG chain lengths and thioether and/or sulphone linkages, the LCSTs of biodegradable and thermoresponsive polycarbonate copolymers can be easily tuned within a wide temperature window from 0 °C to 46 °C (3 g/L in aqueous solution). In addition, block polycarbonates with two tunable LCSTs were further synthesized and investigated. Results showed that LCST1 was constructed by polycarbonate with hydrophobic thioether linkage, while the LCST2 was achieved by polycarbonate containing hydrophilic sulphone linkage.

Introduction

Polymer materials with LCST attribute are widely applied in the fields of tissue engineering, biosensor, biocatalyst and bioseparation.[1-3] LCST behaviour refers to the dissolution of polymers in aqueous solution below a certain temperature, while dehydration and aggregation occurs above this temperature. Among all the thermoresponsive polymers for biomedical applications, poly(N-isopropylacrylamide)(PNIPAM) is commonly considered as a “golden material” with attractive results.[4] It exhibits a LCST around the body temperature (32 °C) in aqueous solution and shows the merits of little dependence on pH, molecular weight and concentration.[5] In addition, its LCST can be easily modified within a certain temperature range by copolymerization with other monomers or end group decoration.[6-10] However, its cytotoxicity and non-biodegradable property can hinder its bioapplications.[11] In a great demand of polymers with nontoxic, biocompatible and controllable LCST assets, emerging polymers bearing a short OEG side chain can meet this need and show wide in vivo applications. These polymers can be easily achieved by linking OEG to polymerizable monomers followed by further polymerization. The resultant polymers with OEG units are highly biocompatible similar to linear poly ethylene glycol (PEG). Among these polymers, the most common polymer is poly(ethylene glycol methacrylate) (PEGMA) polymerized by ATRP, RAFT, whose LCST properties may compete or even exceed those of PNIPAM.[12, 13] Other thermal responsive polymers such as polyphosphoester,[14] polyamino acid[15-17] and polycaprolactone[18-20] with OEG side group prepared by ring-opening polymerization also exhibit good biocompatibility together with excellent biodegradability.

Recently, synthetic degradable polymers, aliphatic polycarbonates, have been gaining a significant attention in the biomedical field.[21-35] Polycarbonates possess the merits of absence of acidic degradation compounds, which would be detrimental to cells and proteins.[36, 37] In addition, functional cyclic carbonates can be easily achieved by cyclization reactions between dihydroxyl monomers and phosgene or their analogous.[21-23, 38-42] However, highly hydrophobic property, deficient functionality and insufficient biocompatibility may limit their biomedical applications.[21] Therefore, various functional groups have been attempted to be attached to the polycarbonate backbone in order to improve their properties and endow them with multiple functionalities, such as hydrophility, biocompatibility, biodegradability, bioadhesion and stimulative responsive property.[11, 27, 43, 44] Functional group, OEGs, show many merits, such as hydrophilic, biocompatible and thermoresponsive properties. Linking them with polycarbonates by ester and ether groups have made polycarbonates with good biocompatibility and thermoresponsive properties.[11, 24]

Herein, we report the synthesis of a series of thermoresponsive polycarbonates with 2-4 repeating short OEG units attached via a new linkage, thioether or sulphone

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group. The application of it can not only simplify the synthesis of MTC monomers with no need of protection and deprotection process, but also increase the adjustability of the LCST of these polycarbonates. These new thermoresponsive polycarbonates have been prepared by the well-established, metal-free organo-catalyst.[45-47] The LCST of our polycarbonates could be easily adjusted through the control of pendent OEG chain length and thioether/sulphone linkage. In addition, diblock copolymers with two tunable LCSTs were further synthesized. The hydrophobic thioether made LCST of polymer within a low temperature range, while the sulphone linkage could promote the hydrophily of polycarbonate backbone to exhibit a higher LCST.

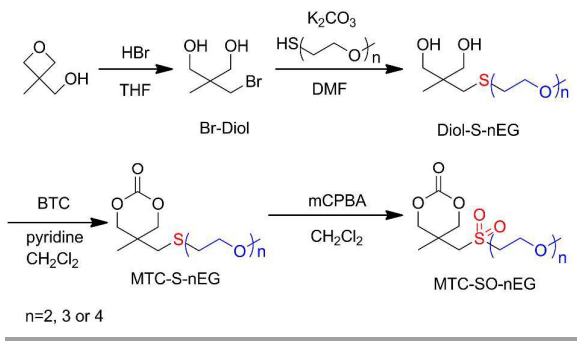
Experiment part

Materials

Mercaptodiethyeneglycolmonomethyl ether (MDiEG), mercaptotriethyeneglycolmonomethyl ether (MTriEG), 1-mercapto-tetraethyleneglycol monomethyl ether (MTeEG) and the thiourea catalyst (TU) were synthesized according to previous methods.[47, 48] 3-Hydroxymethyl-3-methyloxetane (98%, Adams), hydrobromicacid (HBr, 48% in water, Adams) potassium carbonate (K_2CO_3, 99%, Adams), triphosgene (99%, Adams), 3-chloroperoxybenzoic acid (mCPBA, 85%, Adams), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 98%, Adams) and 1,4-benzenedimethanol (99%, TCI) were used as received. Dichloromethane (DCM), N,N-dimethylformamide (DMF) and pyridine were refluxed with calcium hydride and distilled. All other solvents and regents were obtained from Sinoreagent and used as received.

Synthesis

Br-Diol. Br-Diol was synthesized as reported previously.[34] Briefly, 3-hydroxymethyl-3-methyloxetane (6.35 g, 62.3 mmol) was dissolved in THF and cooled in ice temperature, HBr (20.9 ml) was dropped into it slowly. The reaction was allowed to warm to room temperature and stirred for 4 hours. The clear solution was then added with 100 ml deionized water followed by extraction with Et_2O (3*90 ml). Finally, the organic phase was dried over Na_2SO_4 and evaporated to afford the desired product (10.3 g, yield 90%).



Scheme 1. Synthetic routes for MTC-S-nEG and MTC-SO-nEG.

Diol-S-2EG. MDiEG (3.88 g, 28.5 mmol), Br-Diol (3.49 g, 19.0 mmol) and K_2CO_3 (3.93 g, 28.5 mmol) were codissolved in 80 ml DMF. The solution was heated to 55 ^{o}C for 6 hours in the dark. Afterward, the white precipitation was removed by filtration and the crude solution was concentrated followed by adding water (50 ml) and NaCl to saturation. The solution was then extracted with DCM (3*50 ml). The combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuum. The residue was finally purified by column chromatography with hexane/ethyl acetate (1:4) to give the material Diol-S-2EG as pale yellow oil (3.77 g, yield 75%) (Figure S1).

Diol-S-3EG. The procedure was the same as Diol-S-2EG. MTriEG (6.69 g, 37.2 mmol), Br-Diol (4.54 g, 24.8 mmol) and K_2CO_3 (6.16 g, 37.2 mmol) were used. The crude product was then purified by column chromatography with hexane/ethyl acetate (1:8) to give the final material Diol-S-3EG as pale yellow oil (4.77 g, yield 68%) (Figure S2).

Diol-S-4EG. The procedure was the same as Diol-S-2EG. MTeEG (6.60 g, 29.5 mmol), Br-Diol (3.60 g, 19.7 mmol) and K_2CO_3 (6.11 g, 44.3 mmol) were used. The crude product was then purified by column chromatography with ethyl acetate to give the final material Diol-S-4EG as pale yellow oil (3.11 g, yield 48%) (Figure S3).

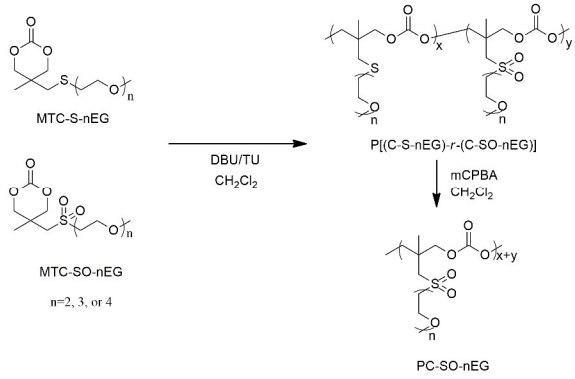
MTC-S-2EG. MTC-S-2EG was synthesized according to the previous report.[42] In brief, triphosgene (2.35 g, 7.92 mmol) in DCM (25 ml) was added dropwise to a mixture of Diol-S-2EG (3.77 g, 15.8 mmol) and pyridine (7.73 ml, 95.0 mmol) in 40 ml anhydrous DCM at -84 ^{o}C over a period of 1 hour, and it was allowed to warm to room temperature. The mixture was then quenched with saturated aqueous NH_4Cl and washed with 1M aqueous HCl (3*70 ml), saturated aqueous $NaHCO_3$ (3*70 mL) and brine (2*70 ml). The organic phase was dried over Na_2SO_4 and evaporated to yield crude product which was purified by column chromatography with hexane/ethyl acetate (1.5:1) to get the desired material MTC-S-2EG as pale yellow oil (3.07 g, yield 89%) (Figure S4).

MTC-S-3EG. The same procedure was used as MTC-S-2EG. Triphosgene (2.51 g, 8.45 mmol), Diol-S-3EG (4.767 g, 16.9 mmol) and pyridine (8.25 ml, 101.2 mmol) were used. The crude product was then purified by column chromatography with hexane/ethyl acetate (1:2) to give the final material MTC-S-3EG as pale yellow oil (3.75 g, yield 72%) (Figure S5).

MTC-S-4EG. The same procedure was used as MTC-S-2EG. Triphosgene (1.42 g, 4.77 mmol), Diol-S-4EG (3.11 g 9.54 mmol) and pyridine (4.66 ml, 57.2 mmol) were used. The crude product was then purified by column chromatography with hexane/ethyl acetate (1:4) to give the final material MTC-S-4EG as pale yellow oil (1.65 g, yield 49%) (Figure S6).

MTC-SO-2EG. MTC-S-2EG (1.2 g, 4.5 mmol) was dissolved in DCM (20 ml) and cooled with an ice bath. Subsequently, mCPBA (1.938 g, 11.3 mmol) in DCM (15 ml) was added dropwise and the reaction mixture was stirred overnight to room temperature. Part precipitate of 3-chlorobenzoic acid was filtrated off and the filtrate was evaporated to afford a crude material which was further purified by column chromatography with gradient elution from hexane/ethyl acetate/acetic acid(2:1:0.03) to hexane/ethyl acetate (1:3) to give the final material as pale yellow oil (1.10 g, yield 83%) (Figure S7, Figure S10).

MTC-SO-3EG. A procedure the same as that used above for MTC-SO-2EG was used. MTC-S-3EG (2.80 g, 9.2 mmol) and mCPBA (3.86



Scheme 2. Synthetic routes for polymers.

g, 22.4 mmol) were used. The crude product was purified by column chromatography with gradient elution from hexane/ethyl acetate/acetic acid (2:1:0.03) to ethyl acetate to give the final material as white solid (2.30 g, yield 74%) (Figure S8, Figure S11).

MTC-SO-4EG. A procedure the same as that used above for MTC-SO-2EG was used. MTC-S-4EG (0.97 g, 2.7 mmol) and mCPBA (1.67 g, 9.5 mmol) were used. The crude product was purified by column chromatography with gradient elution from hexane/ethyl acetate/acetic acid (2:1:0.03) to ethyl acetate/acetone (6:1) to give the final material as white solid (0.81 g, yield 78%) (Figure S9, Figure S12).

PC-S-nEG. The polymerization of MTC-S-nEGs were carried out in DCM at room temperature for desired time by using 1,4-benzenedimethanol as an initiator. For the typical example on synthesis of PC-S-2EG, in a glovebox, DBU (6.9 mg, 0.045 mmol) in DCM (0.1 ml) was quickly added to a solution of MTC-S-2EG (600 mg, 2.3 mmol), 1,4-benzenedimethanol (4.5 mg, 0.033 mmol) and TU (25.2 mg, 0.068 mmol) in DCM. After polymerization for 3 h at room temperature, the reaction was quenched by benzoic acid. Finally, the reaction solution was precipitated in a mixture of Et_2O/hexane twice (10:1) to get PC-S-2EG (390 mg) (Figure S13, Figure S14, Figure S15).

PC-SO-nEG. There are two methods for the synthesis of PC-SO-nEGs.

Method (I): The polymerization of MTC-SO-nEGs were carried out in DCM at room temperature for desired time by using 1,4-benzenedimethanol as an initiator. For the typical example on synthesis of PC-SO-2EG, in a glovebox, to a solution of MTC-SO-2EG (400 mg, 1.35 mmol), 1,4-benzenedimethanol (3.6 mg, 0.026 mmol) and TU (15.0 mg, 0.041 mmol) in DCM (0.6 ml), DBU (4.1 mg, 0.027 mmol) in DCM was added. After 2 h polymerization at room temperature, the reaction was quenched by benzoic acid, followed by precipitated in Et_2O twice to get PC-SO-2EG (360 mg).

Method (II): PC-SO-nEG can also be obtained by oxidating PC-S-nEG with mCPBA in DCM at room temperature. Typical example for synthesis of PC-SO-2EG was showed. To a solution of PC-S-2EG (340 mg) in DCM (8 ml) at 0 ^{o}C, mCPBA (668 mg, 3.9 mmol) in 12 ml DCM was added dropwise. The reaction was allowed to room temperature overnight. Then the mixture was concentrated and filtrated to remove 3-chlorobenzoic acid and the filtrate was poured into Et_2O to get desired polymer PC-SO-2EG (330 mg) (Figure S16, Figure S17, Figure S18).

(PC-S-nEG)-*b*-(PC-SO-nEG). The polymerization of (PC-S-nEG)-*b*-(PC-SO-nEG) block copolymer was carried out in DCM at room temperature for desired time by using PC-S-nEG as an initiator. For the typical example on synthesis of (PC-S-4EG)-*b*-(PC-SO-4EG), in a glovebox, to a solution of PC-S-4EG (100 mg) in DCM, MTC-SO-4EG (300 mg, 0.85 mmol), TU (9.6 mg, 0.026 mmol), DBU (2.6 mg, 0.017 mmol) in DCM was added quickly. After polymerization for 2 h at room temperature, the reaction was quenched by benzoic acid, followed by precipitated in Et_2O twice to get the desired product (310 mg).

Cell viability

The biocompatibility of these materials was determined by MTT assay. L929 cells were seeded with DMEM (containing 10% fetal bovine serum) in 96-well plates at a density of 10000 per well and incubated at 37 ^{o}C with 5% CO_2. After 24 h, the culture medium was replaced by fresh medium contain PC-SO-4EG at different concentrations and incubated for 48 h. Afterward, 0.2 ml DMEM containing MTT (0.5 mg/ml) were used to replace the previous medium and incubated for another 4 h. Finally, the medium was completely removed, followed by adding DMSO (0.2 ml) into each well. After gentle shaking for 3 min, absorption of each well was measured with a microplate reader at a wavelength of 490 nm. The relative cell viability was determined by comparing the absorbance of the well containing materials with that of the blank control.

Measurement

^{1}H and ^{13}C NMR spectra were recorded with a BrukerAvance III 400 MHz NMR spectrometer operating at 400 and 100 MHz, respectively. Gel permeation chromatography (GPC) measurements were performed on Tosoh HLC-8320 GPC instrument by using DMF with LiBr (1 g/L) as the eluent at 45 °C relative to methyl methacrylate as standard. The turbidity measurements of materials were recorded with UV/Vis turbidity measurements by using Perkin Elmer Lambda 35 UV/Vis spectrometer equipped with a temperature regulating equipment. All solutions with polymer in water were filtered with 0.45 μm filter before measurements, the transmittance of solutions at 500 nm were recorded with heat at a rate of 1 ^{o}C/min. The sizes of nanoparticles in aqueous solution were measured by Malvern Zetasizer Nano S90, and the solutions (3 mg/ml) were also filtered with 0.45 μm filter before use.

Results and Discussion

Synthesis of MTC-S-nEG and MTC-SO-nEG

Esterification and click reaction have been widely used to attach functional groups to carbonate.[25, 33, 39, 49, 50] However, more efficient and easier methods are still in great demand. In this work, thioether group was chosen as a linkage to connect MTC and OEG (Scheme 1) for the following reasons: 1) the products can be easily obtained by highly efficient thio-bromo reaction with rather high yield under a mild condition; 2) thioether shows reactive oxygen species (ROS) responsive

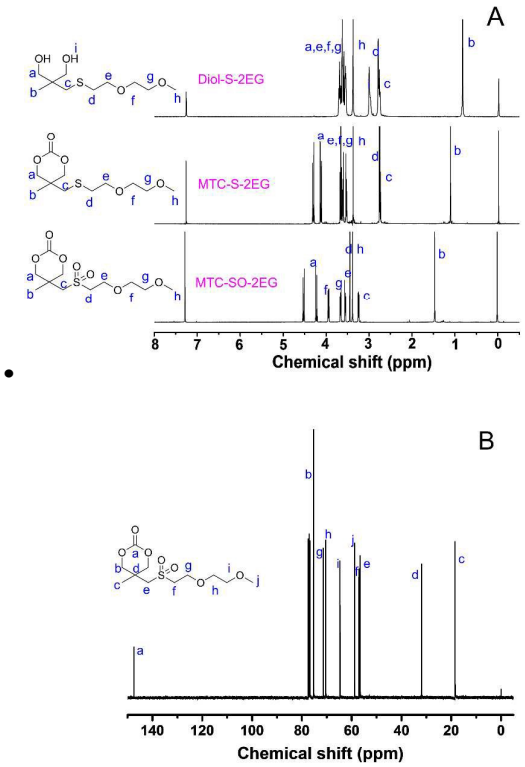


Figure 1. (A)^{1}H NMR spectra of the monomers containing thioether or sulphone groups in CDCl3. (B)^{13}C NMR spectra of MTC-SO-2EG in $CDCl_3$.

behavior, combined with thermal responsive OEGs, which can make the resultant polycarbonates as potential multi-responsive carriers; 3) thioether can be further converted to alkylated tertiary sulfonium system, which may have potential applications in the field of biomedical materials; 4) thioether can be oxidized to hydrophilic sulphone group and copolymerizing monomers with thioether or sulphone group easily realize the adjustment of LCSTs.

As shown in Scheme 1, in the presence of K_2CO_3 as a base, the thioetherification between Br-Diol and nEG with ratio of 1:1.5 gave the key intermediate, Diol-S-nEG. The structures of Diol-S-nEG, verified by ^{1}H NMR (Figure 1A, Figure S1, Figure S2, Figure S3), showed the expected resonance signals of thioether group (peaks of d and c). Then, triphosgene was used to mediate the intra-molecular cyclization of Diol-S-nEG. The successful synthesis of MTC-S-nEGs (Figure 1A, Figure S4, Figure S5, Figure S6) was confirmed by the appearance of new peaks at 4.15 ppm and 4.30 ppm which belonged to the methylene protons of MTC. With 3-chloroperoxybenzoic acid (mCPBA) as an oxidant, MTC-SO-nEGs were synthesized at room temperature in a few hours with no ring opening of MTCs. In ^{1}H NMR (Figure 1A, Figure S7, Figure S8, Figure S9), the downfield shift of the protons around the sulphone group witnessed the successful synthesis of the final monomers. ^{13}C

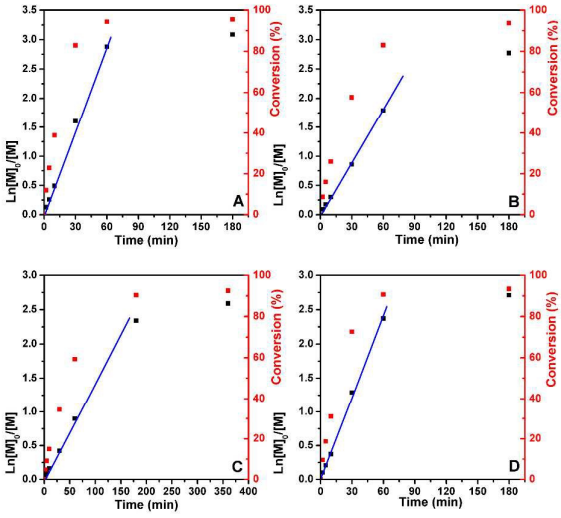


Figure 2. Polymerization kinetics and conversion plots for (A) MTC-S-2EG, (B) MTC-S-3EG, (C) MTC-S-4EG, (D) MTC-SO-3EG.

NMR spectra of MTC-SO-nEG (Figure 1B, Figure S10, Figure S11, Figure S12) also proved their structures.

Synthesis of polycarbonates

Polycarbonates were prepared by using 1,4-benzenedimethanol as an initiator and TU/DBU catalyzed ring opening polymerization (Scheme 2). The polymerization kinetics of MTC monomers were firstly studied by ^{1}H NMR spectra. The feed ratio of monomers to initiator and monomers to DBU/TU were 60/1 and 60/1.2/1.8 respectively, while the monomer concentrations were set at 2M. With the elapse of time, the peak intensity of monomers decreased with the enlargement of that of the peak belonged to the backbone of polycarbonates. By calculating the ratio of peak area of monomers (4.30 ppm and 4.42 ppm for MTC-S-nEG, 4.51 ppm and 4.23 ppm for MTC-SO-nEG) to that of polycarbonates (4.08 ppm for PC-S-nEG, 4.25 ppm for PC-SO-nEG), we can get the first-order kinetic plots below a conversion of 95% (Figure 2). In addition, the final products showed a polymer dispersion index (PDI) around 1.3 (Table 1). These results indicated that the polymerization of MTC-S-nEG or/and MTC-SO-nEG was governed by well controlled condition by DBU/TU. However, when the conversion was above 95%, the polymerization deviated from linearity, which may be due to the transesterification of polymers or other side reactions. What's more interesting, with the increase of repeating unites of EO from 2 to 4, the rate of polymerization obviously decreased, possibly because of a higher steric hindrance. In addition, PC-SO-3EG showed a more rapid conversion compared with PC-S-3EG, which might be due to the fact that sulphone group made cyclic carbonates of PC-SO-3EG with higher H-bond basicity and binding constants of ester with the thiourea.

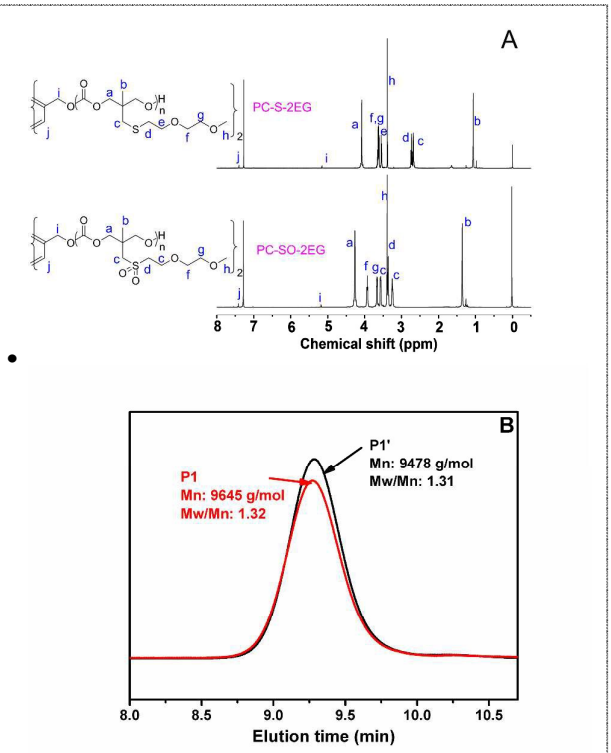


Figure 3. (A)^{1}H NMR spectra of polycarbonates containing thioether or sulphone group, PC-S-2EG and PC-SO-2EG in $CDCl_3$ respectively. (B) GPC traces of PC-S-2EG (P1') and PC-SO-2EG (P1).

For PC-SO-nEG, method (II) can also prepare the target polymers. After oxidation of PC, most of the peaks moved to lower chemical shift (Figure 3A, Figure S16-S18). The protons in thioether group at 2.68 ppm and 2.73 ppm completely disappeared and shifted to 3.25 ppm and 3.32 ppm, which indicated the complete conversion of thioether to sulphone groups. Additionally, in the GPC traces (Figure 3B), the peaks of P1 slightly moved to less elution time compared to its unoxidated polymer P1' and the two polymers showed the same molecular weight distributions, which might imply that no degradation or transesterification happened under the oxidation of mCPBA. All these results confirmed the successful achievement of products by oxidation without occurrence of side reactions.

In ^{1}H NMR spectra, by integrating the signal intensity of the methyl protons located on the polycarbonate backbone (4.08 ppm for PC-S-nEG, 4.25 ppm for PC-SO-nEG) against that of methylene protons situated on initiator (5.16 ppm), the average number molecular weight (Mn) of polycarbonates can be calculated, which were higher than the values got from GPC (Table 1). It was possibly a consequence of the unusual solution behavior of macromolecular brushes.[51]

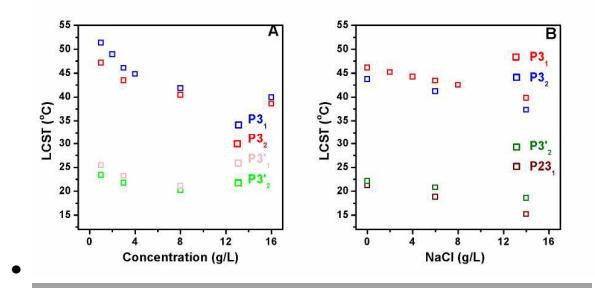


Figure 4. (A) Plots of LCST as a function of concentration of $P3_1$, $P3_2$, $P3'_1$, $P3'_2$ in aqueous solution. (B) Plots of LCST as a function of NaCl concentration of $P3_1$, $P3_2$, $P3'_2$ and $P23_1$ (3 g/L in aqueous solution).

Effects of both molecular weight and polymer concentrations on LCST

In the following, the LSCT behaviors of polymer with thioether or sulphone linkage were compared. Generally speaking, the copolymers based on the same structure should have similar thermoresponsive properties, PC-S-4EG and PC-SO-4EG were selected as an example. The dehydration process of 4EG side chains was firstly observed by ^{1}H NMR technique (Figure S21). Around the LSCT, the chemical shift of 4EG in PC-SO-4EG obviously moved to a higher field, which revealed the destruction of hydrogen bond between H_2O and 4EG chain upon heating. Meanwhile, the dehydration process was also presented in the dynamic light scattering (DLS) results (Figure S22). Below the LCST, the polymers showed the diameter below 5 nm, which was expected for hydrodynamic radius of a single copolymer chain. With raising the temperature above the LCST, the diameter of particles quickly increased to around 1 μm with a PDI=1, which indicated that the heat induced large aggregations with wide distribution and they were further proved by TEM results (Figure S23). Further heating slightly decreased the size of aggregates, which was possibly due to the further dehydration of OEG chains. These polycarbonates showed a slight hysteresis in a heating/cooling cycle because the OEG chains could only be the H-bond accepters in solution during the hydration process.[12]

Molecular structure can greatly affect the LCST properties of polycarbonates in solution. In Figure 4A, $P3'_1$and $P3'_2$ showed a relatively lower LCST. The difference of LCST for $P3'_1$ was about 5.2 ^{o}C from 1 g/L to 8 g/L, and the decrease of the LCST difference was observed for $P3'_2$ with a higher molecular weight. Meanwhile, with a sulphone linkage, the LCST of $P3_1$ decreased from 51.4 ^{o}C to 41.7 ^{o}C with the increase of concentration from 1 g/L to 8 g/L. The decrease magnitude of LCST was as large as 10 degree. For higher molecular weight polymer $P3_2$, the LCST decreased from 47.0 ^{o}C to 40.3 ^{o}C with the increase of concentration from 1 g/L to 8 g/L. Therefore, the sulphone group on one hand increased the LCST of polycarbonate to the body temperature range, on the other hand made the change of LCST easier under different polymer concentrations. It might be because of the fact that the highly hydrophilic sulphone makes OEG harder to dehydrate, which clearly

Entry	sample	Molar feed ratio M1':M2':M3':M1:M2:M3:I[a]	Polymerization time [hour]	DP[b] M1':M2':M3':M1:M2:M3:I	Mn[c] [kDa]	Mw/Mn
P1[d]	PC-SO-2EG	60:0:0:0:0:0:1	-	0:0:0:55:0:0:1	9.6	1.32
P1'	PC-S-2EG	60:0:0:0:0:0:1	2	55:0:0:0:0:0:1	9.5	1.31
$P13_1$	P[(C-SO-2EG)-*co*-(C-SO-4EG)] 1	0:0:0:27:0:33:1	2	0:0:0:22:0:29:1	9.4	1.34
$P13_2$	P[(C-SO-2EG)-*co*-(C-SO-4EG)] 2	0:0:0:18:0:42:1	3	0:0:0:18:0:39:1	11.2	1.34
$P13_3$	P[(C-SO-2EG)-*co*-(C-SO-4EG)] 3	0:0:0:6:0:54:1	3	0:0:0:5:0:51:1	12.0	1.33
P2[d]	PC-SO-3EG	0:60:0:0:0:0:1	-	0:0:0:0:51:0:1	10.6	1.29
P2'	PC-S-3EG	0:60:0:0:0:0:1	2	0:51:0:0:0:0:1	10.3	1.29
$P23_1$	P[(C-SO-3EG)-*co*-(C-SO-4EG)] 1	0:0:0:0:48:12:1	2	0:0:0:0:37:9:1	8.7	1.30
$P23_2$	P[(C-SO-3EG)-*co*-(C-SO-4EG)] 2	0:0:0:0:36:24:1	3	0:0:0:0:28:19:1	9.5	1.30
$P23_3$	P[(C-SO-3EG)-*co*-(C-SO-4EG)] 3	0:0:0:0:27:33:1	3	0:0:0:0:20:26:1	9.6	1.30
$P23_4$	P[(C-SO-3EG)-*co*-(C-SO-4EG)] 4	0:0:0:0:15:45:1	3	0:0:0:0:13:31:1	9.1	1.31
$P3_1$[d]	PC-SO-4EG 1	0:0:60:0:0:0:1	-	0:0:0:0:0:46:1	10.0	1.27
$P3'_1$	PC-S-4EG 1	0:0:60:0:0:0:1	3	0:0:46:0:0:0:1	9.7	1.29
$P3_2$[d]	PC-SO-4EG 2	0:0:100:0:0:0:1	-	0:0:0:0:0:79:1	15.7	1.29
$P3'_2$	PC-S-4EG 2	0:0:100:0:0:0:1	3	0:0:79:0:0:0:1	15.2	1.28
$P3'3_1$	P[(C-S-4EG)-*co*-(C-SO-4EG)] 1	0:0:18:0:0:42:1	3	0:0:14:0:0:34:1	9.8	1.28
$P3'3_2$	P[(C-S-4EG)-*co*-(C-SO-4EG)] 2	0:0:42:0:0:18:1	3	0:0:35:0:0:14:1	10.6	1.29

Table 1. Synthesis of polycarbonates by ROP with different ratios of monomers
[a]M1', M2', M3', M1, M2, M3 and I are MTC-S-2EG, MTC-S-3EG, MTC-S-4EG, MTC-SO-2EG, MTC-SO-3EG, MTC-SO-4EG and initiator respectively. [b]DP: degree of polymerization, DP was calculated by ^{1}H NMR spectra. [c]TheMn and Mw/Mn were achieved from GPC. [d]These polymers were obtained from oxidation of their corresponding PC-S-nEGs.

showed in lower concentration and lower molecular weight condition. Fortunately, it could be alleviated by increasing the molecular weight of polymers. Longer polycarbonate chain provided higher hydrophobic environment and offset the hydrophilic charter of sulphone under a dilute condition. Obviously, the increase of polycarbonate molecular weight more or less lowered the LCST of the polymers. For example, with the increase of Mn from 10.0 kDa ($P3_1$) to 15.7 kDa ($P3_2$), the LCST of PC-SO-4EG decreased. This phenomenon was more significant at a low concentration. At 1 g/L, the decreasing amplitude reached 3.7 ^oC! However, with the increase of sample concentration to 8 g/L, it rapidly reduced to 1.4 ^oC. Apparently, at higher concentrations, the intermolecular interactions are more likely to dominate and offset the molecular weight effect. At a certain high concentration, the LCST of two polymers with different molecular weight might possibly be the same.

Effect of NaCl concentrations on LCST

The ionic strength of solution also affected the LCST of polymers in aqueous solution (Figure 4B). Herein, NaCl was used as an additive salt in our systems. With increased concentration of NaCl, the LCST of polycarbonates decreased. This tendency was same as previous report.[52, 53] It was due to the rather high interfacial water structuring normally associated with OEG.[52] Specifically, with NaCl concentration increasing from 0 to 14 g/L, the LCST of $P3_2$ dropped from 43.6 ^oC to 37.3 ^oC and that of $P3'_2$ changed from 22 ^oC to 18.5 ^oC. The variation range of LCST in PC-SO-4EG was larger than that of PC-S-4EG. It might due to the reason that sulphone group showed

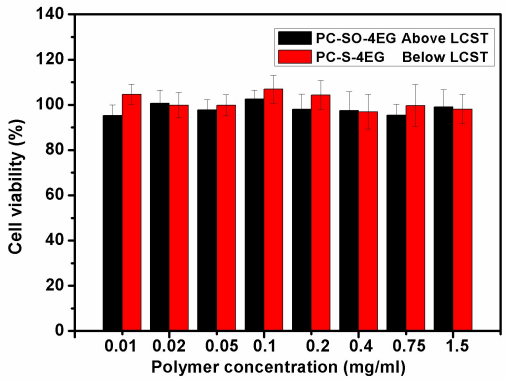


Figure 5. Cell viability studies of thermoresponsive polycarbonates. The LCST of PC-S-4EG and PC-SO-3EG were lower than 37 °C, they were in a state of aggregates. The LCST of PC-SO-4EG was higher than 37 °C.

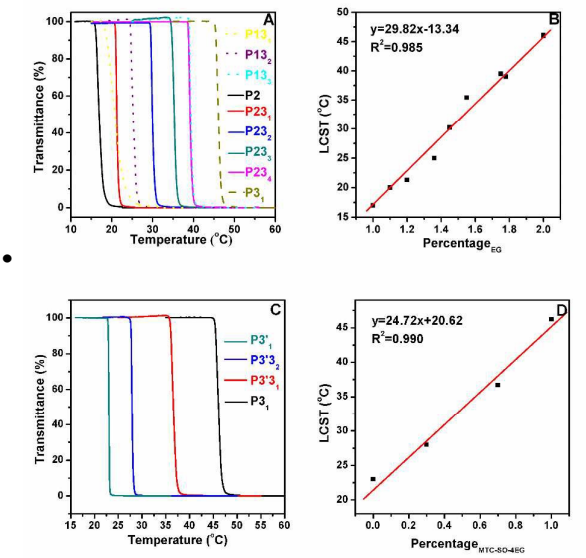


Figure 6. (A) Turbidity measurement for aqueous solution of polycarbonates with different ratios of MTC-SO-nEGs (3 g/L in aqueous solution). (B) Plot of LCST as a function of the percentage of EG unit in copolymer. (C) Turbidity measurement for aqueous solution of polycarbonates with different ratios of MTC-SO-4EG to MTC-S-4EG (3 g/L in aqueous solution). (D) Plot of LCST as a function of the percentage of MTC-SO-4EG in copolymer.

lower hydration at a higher salt concentration, which accelerated the process of polymer chain dehydration around the LCST of polymers. In addition, the molecular weight had little effect on the change of temperature range at different salt concentrations.

Cell Viability Assays

Cell viability of PC-S-nEG/PC-SO-nEG was studied using L929 cells with a MTT assay, the results were shown in figure 5. All polymers showed no obvious cytotoxicity to L929 cells at all test concentrations up to 0.5 mg/ml, which was the same as the OEG based polycarbonates reported by Hedrick.[11] Therefore, the thioether and sulphone linkages had little influence on the biocompatibility of these materials.

Modulate the polycarbonate with the desired LCST

The detailed LCST of these polymers listed in Table 1 were analyzed and the concentration was set at 3 g/L. Due to the high hydrophobicity of polycarbonate, PC-SO-2EG could not dissolve in water even at 0 °C, and the LSCT of PC-SO-2EG was about -15 °C in water according to Equation 2. PC-SO-3EG showed a LCST around 15 °C, below that of analogue linked by ether,[24] which indicated that sulphone showed less hydrophilicity than ether group. However, both of them could obviously increase the hydrophilicity of thermoresponsive polycarbonate compared with ester linkage.[11] Interestingly, the homopolymer of PC-SO-4EG exhibited a LSCT of about 45 °C in aqueous solution. Predictably, by copolymerizing these monomers with different ethylene oxide units, the LCST of the target polycarbonate could be adjusted easily below the LCST of PC-SO-4EG (Figure 6A). The observed LCST behaved a liner mathematical correlation with the percentage of 4EG at the same concentration (Figure S19, Figure S20) and the same molecular weight condition. For 4EG and 3EG copolymers, their LCSTs could be predicted from Equation 1. Equally, Equation 2 described the change of LCST by modulating the ratio between 4EG and 2EG. Further on, connecting the two equations (Figure 6B), the resulting Equation 3 described the LCST of the copolymer of MTC-SO-2EG, MTC-SO-3EG and MTC-SO-4EGwith different ratios. In general, for copolymer with an average of 100 monomer units, one unit of EG contributed 0.298 °C increase of LCST in the resultant copolymer. For homopolymer PC-SO-3EG, the $DP_{Percentage\ of\ EG}$ was 100%, in a similar way, the $DP_{Percentage\ of\ EG}$ of PC-SO-4EG was 200%. According to the equation 3, the polycarbonate with LCST at 37 °C (body temperature) or a little higher temperature could be easily achieved by copolymerization. Therefore, these polycarbonates show great potential in constructing thermoresponsive and biodegradable drug delivery carriers.

$LCST=30.25Percentage_{4EG}+16.7$ (1)

$LCST=59.24Percentage_{4EG}-13.7$ (2)

$LCST=29.82DP_{Percentage\ of\ EG}-13.3$ (3)

In addition, copolymerization of MTC-S-nEG with different OEG lengths could also result in polycarbonates with modulating LCST under 24 °C.

Besides, the LCST of the polycarbonates could also be adjusted by copolymerizing MTC with thioether or sulphone groups. For example, with different ratios of monomers, the LSCT of

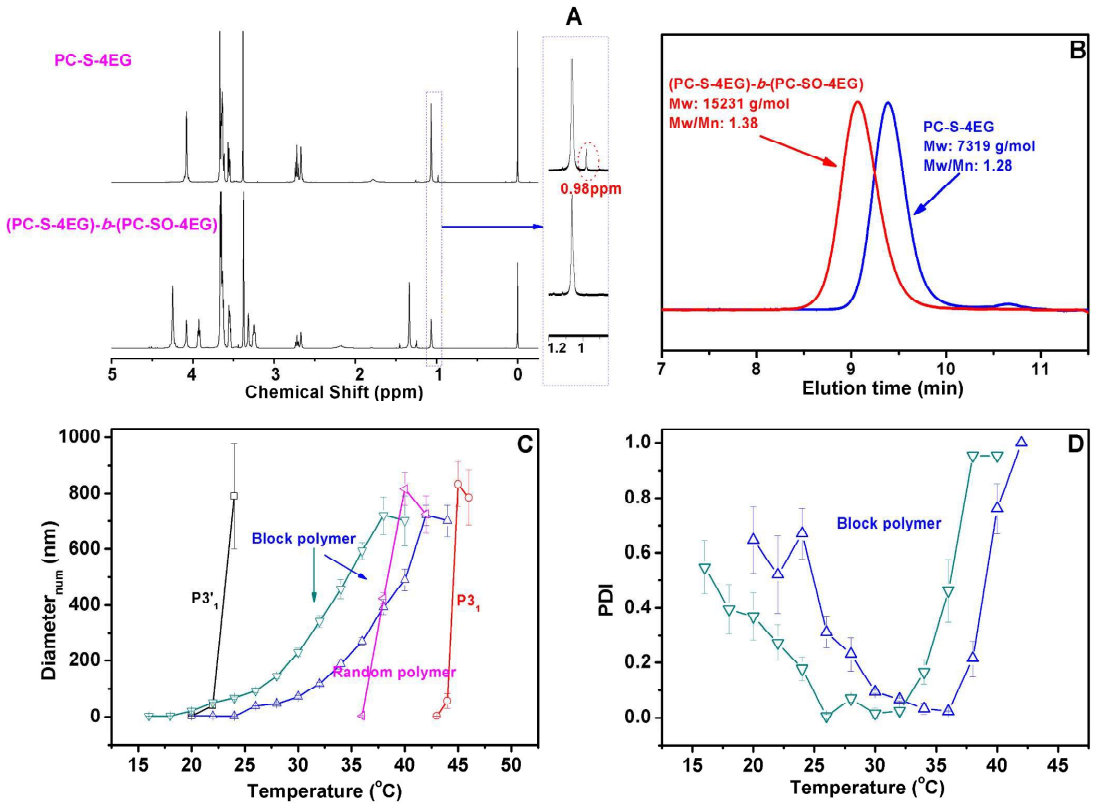


Figure 7. (A) ^{1}H NMR spectra of polycarbonates, PC-S-4EG and (PC-S-4EG)-*b*-(PC-SO-4EG), in $CDCl_3$ respectively. (B) GPC traces of PC-S-4EG and (PC-S-4EG)-*b*-(PC-SO-4EG). (C) Temperature dependence of the diameter change for aqueous solutions of PC (3g/L) in heat (PC-S-3/4EG)-*b*-(PC-SO-3/4EG): olive green, (PC-S-4EG)-*b*-(PC-SO-4EG): blue, P[(C-S-4EG)-*r*-(C-SO-4EG)]: pink). (D) Temperature dependence of the PDI for aqueous solutions of PC (3g/L) in heat (PC-S-3/4EG)-*b*-(PC-SO-3/4EG): olive green, (PC-S-4EG)-*b*-(PC-SO-4EG): blue).

copolymers containing MTC-S-4EG and MTC-SO-4EG could be modulated within the range from 23 °C to 45 °C (Figure 6C). With the increased amount of MTC-SO-4EG, the LCST of copolymers also increased linearly (Figure 6D). In a short summary, the introduction of functional groups to the monomers might have provided another way to modulate the LCST of polymers.

Block polycarbonate with two tunable LCSTs

In this section, the block polycarbonates with two tunable LCST were studied. For one thing, the low LCST can facilitate micelle formation and drug encapsulation in hydrophobic core, which could be varied from 0 °C to 24 °C by copolymerization of monomers with hydrophobic thioether linkage. For another thing, the high LCST helps enhance the aggregation of micelle at the target with hyperthermia and increase the passive targeting capability, which could be achieved by copolymerizing monomers with hydrophilic sulphone linkage. The block polymers were thus achieved by polymerizing MTC-SO-nEGs with PC-S-nEGs as a macroinitiator. In order to diminish the transesterification effect as much as possible, the conversion of MTC-SO-nEGs was kept under 70%. From ^{1}H NMR spectrum, the peak at 0.98 ppm belonging to the end group of MTC-S-nEGs backbone disappeared, which indicated that PC-SO-nEGs had been initiated from PC-S-nEGs (Figure 7A). The GPC results also confirmed this conclusion (Figure 7B). Further, the LCST behaviors of desired polycarbonates were studied by DLS results. Compared to P[(C-S-nEG)-*r*-(C-SO-nEG)]s with one LCST, (PC-S-nEG)-*b*-(PC-SO-nEG)s showed two tunable LCSTs by modulating the MTC-S-nEGs/MTC-SO-nEGs with different OEG lengths (Figure 7C). However, between the two LCSTs, the gradual increase of the particle sizes was observed under continuous heating. These phenomena might be due to the presence of poor structures of block polycarbonates during the polymerization of PC-SO-nEG. In kinetic results, the polymerization of PC-S-nEGs and PC-SO-nEGs showed a good control with DBU/TU. However, the transesterification might still happen during the polymerization even at a low conversion, which might cause detrimental damage to the structure of block copolymer. Therefore, the hydrophobic PC-S-nEG fragment might insert into the PC-SO-nEG block and lowered the LCST of partly hydrophilic PC-SO-4EG. Although the particle sizes gradually increased under heating, the PDI kept around 0.1, indicating that the particles were rather stable during this process, and the polycarbonates might still showed the character of two LCST. The transesterification effect is hard to be eliminated, the optimization research on the structure of block polycarbonate by

polymer–polymer conjugation via click chemistry is still on-going in our lab and will be presented in our future work.

Conclusions

A series of MTC monomers bearing OEGs with thioether or/and sulphone linkages were synthesized firstly through a sequence of highly-efficient thio-bromo reaction, cyclization and oxidation. The polymerization kinetics of the monomers and GPC results of the products have indicated a good control of polymerization by using DBU/TU as the catalyst. Then, the correlations between the LSCT and molecular weight of polymer, concentration of polymer as well as ionic strength were studied in detail. Results showed that oxidation of thioether to sulphone increased the LSCT of polycarbonate within body temperature range and made it slightly sensitive to the change of solution condition. Cytotoxicity assays indicated that these polycarbonates have little toxicity. By copolymerizing MTCs with different length of OEGs and linkages (thioether or sulphone), the LCST of polycarbonates can be accurately tuned on demand by changing the initial compositions of monomers, and showed a linear function of the monomer compositions. In addition, the block polycarbonates with two tunable LCSTs were constructed. LCST1 and LCST2 were achieved by polymerization of monomers with hydrophobic thioether and hydrophilic sulphone linkages, respectively. With these unique properties, our novel biodegradable and thermoresponsive polycarbonates are promising candidates for applications in biomedical field.

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