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ORIGINAL ARTICLE

Thermoresponsive and Biodegradable Amphiphilic Block Copolymers with Pendant Functional Groups

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Abstract

BACKGROUND: To develop the biodegradability and thermoresponsive hydrogel, in this work we designed a pendant-functionalized, thermoresponsive, amphiphilic block copolymer.

METHODS: Methoxy poly(ethylene glycol) (MPEG)-*b*-[poly(ε-caprolactone)-*ran*-poly(ε-caprolactone-3-one)-*ran*-poly-lactic acid] (MCL) and (MPEG-*b*-[PCL-*ran*-POD-*ran*-PLA]) [MCL-(CO)] block copolymers were prepared by ring-opening polymerization of ε-caprolactone, OD and lactide monomers. The subsequent derivatization of MCL-(CO) provided MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-COOH)-*ran*-PLA] [MCL-(COOH)] with COOH pendant groups and MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-NH_2)-*ran*-PLA] [MCL-(NH_2)] with NH_2 pendant groups.

RESULTS: The measured segment ratios of MCL-(CO), MCL-(COOH), and MCL-(NH_2) agreed well with the target ratios. The abundances of the COOH and NH_2 groups in the MCL-(COOH) and MCL-(NH_2) copolymers were determined by ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy, and agreed well with the target abundances. MCL-(CO), MCL-(COOH), and MCL-(NH_2) formed homogeneous, white, opaque emulsions at room temperature. Rheological analysis of the block copolymer suspensions indicated a solution-to-hydrogel phase transition as a function of temperature. The solution-to-hydrogel phase transitions and the biodegradation of MCL-(CO), MCL-(COOH), and MCL-(NH_2) were affected by varying the type (ketone, COOH, or NH_2) and abundance of the pendant groups.

CONCLUSION: MCL-(CO), MCL-(COOH), and MCL-(NH_2) with ketone, COOH, and NH_2 pendant groups showed solution-to-hydrogel phase transitions and biodegradation behaviors that depended on both the type and number of pendant groups.

Keywords Thermoresponsive · Amphiphilic block copolymers · Pendant group · Solution-to-hydrogel phase transitions · Biodegradation

1 Introduction

Recently, several thermoresponsive amphiphilic block copolymers exhibiting solution-to-hydrogel phase transitions, as a function of temperature, have been reported [1, 2]. If amphiphilic block copolymer solutions (as thermoresponsive hydrogel) can be prepared at room temperature, biologics, such as drugs and cells, can easily be mixed into the copolymer [3]. Therefore, various amphiphilic block copolymers have been employed as candidates for use as thermoresponsive polymers [4–10]. The thermal

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response is thought to occur via the formation of individual micelles, which then coaggregate and form hydrophobic domains; this causes the solution-to-hydrogel phase transition [3].

Recently, several groups, including ours, have reported thermoresponsive amphiphilic block copolymers consisting of poly(ethylene glycol) (PEG) and biodegradable polyesters—such as poly(ε-caprolactone) (PCL), polylactic acid (PLA), and polyglycolic acid (PGA) [or their copolymers, such as poly(lactic-*co*-glycolic acid)] [11–13]. Recently, we prepared amphiphilic block copolymers with functional groups at the chain-end and pendant positions via ring-opening copolymerization (ROP) of a 3-benzyloxymethyl-6-methyl-1,4-dioxane-2,5-dione, 2-chloride-ε-caprolactone, or 4-benzyloxy-ε-caprolactone, monomer with ε-caprolactone (CL), glycolide (GA), or L-lactide (LA); we then measured the thermal response as a function of temperature [14–19]. The pendant functional groups on the amphiphilic block copolymers altered the formation of coaggregated hydrophobic domains via intra- and intermolecular hydrophobic aggregation.

In this study, to develop a novel, pendant-functionalized, thermoresponsive, amphiphilic block copolymer, we prepared the 2-oxepane-1,5-dione (OD) monomer (Fig. 1) [20]. CL, OD, and LA monomers were co-polymerized with methoxy poly(ethylene glycol) (MPEG) in various ratios to prepare MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-one)-*ran*-PLA] [MCL-(CO)] block copolymers. MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-COOH)-*ran*-PLA] [MCL-(COOH)] and MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-NH_2)-*ran*-PLA] [MCL-(NH_2)] were prepared by the subsequent derivatization of MCL-(CO). We measured the thermal responses of MCL-(CO), MCL-(COOH), and MCL-(NH_2) with ketone, COOH, and NH_2 pendant groups. Finally, we examined the biodegradability of MCL-(CO), MCL-(COOH), and MCL-(NH_2). Thus, we investigated the solution-to-hydrogel phase transitions and biodegradabilities of MCL-(CO), MCL-(COOH), and

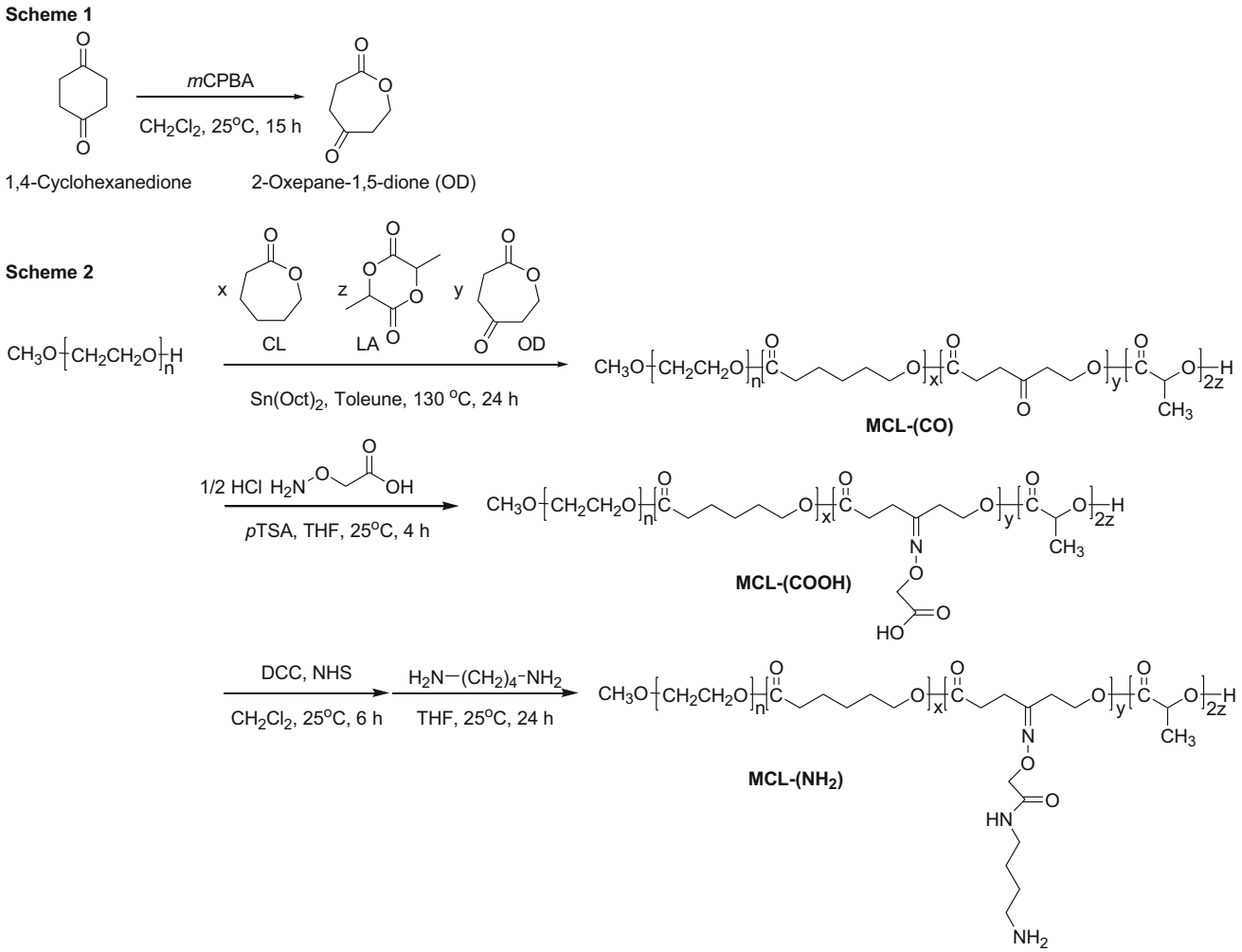

Fig. 1 Synthesis of 2-oxepane-1,5-dione (OD) (Scheme 1) and preparation of MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymers (Scheme 2)

MCL-(NH_2) with different species and numbers of pendant groups.

2 Experimental

2.1 Materials

MPEG (number-average molecular weight, M_n, = 750; Sigma, MO, USA), tin(II) 2-ethylhexanoate ($Sn(Oct)_2$; Sigma, MO, USA), 1,4-cyclohexanedione (Sigma, Mo, USA), *meta*-chloroperoxybenzoic acid (*m*CPBA; Sigma, MO, USA), carboxymethoxylamine hemihydrochloride (TCI, Tokyo, Japan), *p*-toluenesulfonic acid monohydrate (*p*TSA; TCI, Tokyo, Japan), *N*-hydroxysuccinimide (NHS; Sigma, MO, USA), *N*,*N*′-dicyclohexylcarbodiimide (DCC; Sigma, MO, USA), and diaminobutane (Sigma, MO, USA) were used as received. CL (TCI, Tokyo, Japan) was distilled over CaH_2 under reduced pressure prior to use. LA (Boehringer Ingelheim, Germany) was recrystallized twice from ethyl acetate prior to use. Dichloromethane (DCM) was distilled sequentially, under nitrogen, over $CaCl_2$ and CaH_2, before use.

2.2 Characterization

^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were measured using a Mercury Plus 400 spectrometer (Varian); $CDCl_3$ and D_2O were used as solvents and tetramethylsilane as the internal standard. The molecular-weight distributions of each copolymer were measured at 40 °C using a Futecs gel permeation chromatograph (GPC; RI-201H, refractive index detector) and polystyrene gel columns (Shodex K-802, K-803 and K-804). $CHCl_3$ was used as the eluent at a flow rate of 1.0 mL/min. Polystyrene was used as the calibration standard. The hydrodynamic diameters of the particles formed from each copolymer were measured by dynamic light scattering (DLS; ELSZ-1000, Otsuka Electronics, Osaka, Japan) at room temperature.

2.3 Preparation of 2-oxepane-1,5-dione (OD)

Prior to use, all glassware was dried by heating under vacuum and then flushed with dry nitrogen. All reactions were conducted under dry nitrogen. First, 1,4-cyclohexanedione (5.00 g, 43.80 mmol) was dissolved in DCM (200 mL) and stirred for 2 h at 50 °C. Then, *meta*-chloroperoxybenzoic acid (10.60 g, 61.33 mmol) in DCM (60 mL) was added to the solution dropwise and the mixture was stirred at 25 °C. After 15 h, the solution was concentrated by rotary evaporation of the DCM. The reaction mixture was then washed thrice with diethyl ether. The organic phase was extracted and the solvent removed by evaporation, yielding a white power (OD) (2.69 g, 20.99 mmol, 48% yield). ^{1}H-NMR ($CDCl_3$): 4.41 (*t*, 2H, –CH_2O)–), 2.83 (*t*, 2H, –CH_2–COO–), 2.74 (*m*, 4H, –CH_2-CO–). Elemental analysis results: abundances calculated for $C_6H_8O_3$: C: 56.24, H: 6.29; measured: C: 55.95, H: 6.28.

2.4 Synthesis of MPEG-*b*-[poly(ε-caprolactone)-*ran*-poly(L-lactide)] (PCL-*ran*-PLA) diblock copolymer (MCL)

A solution of MPEG (1 g, 1.33 mmol) in toluene (65 mL) was azeotropically distilled to remove the water present in the MPEG; around 30 mL of toluene was removed. CL (2.81 g, 24.59 mmol) and LA (0.39 g, 2.71 mmol) were added to the solution. These were followed by $Sn(Oct)_2$ in dry toluene (1.6 mL, 0.1 M), which was added to the solution at 25 °C, under a nitrogen atmosphere. After polymerization for 24 h at 130 °C, the reaction solution was added to a mixture of *n*-hexane and diethyl ether (v/v = 4/1) to precipitate MCL. The precipitated polymer was removed from the solution by filtration and dried under vacuum to yield a colorless solid.

2.5 Synthesis of MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-one)-*ran*-PLA] [MCL-(CO)]

Prior to use, all glassware was dried by heating under vacuum and then flushed with dry nitrogen. A typical polymerization process, used to synthesize MCL-$(CO)_{10}$ with a CL:OD:LA ratio of 80:10:10 and using MPEG as an initiator, was as follows: A mixture of MPEG (1.88 g, 2.50 mmol) and toluene (65 mL) was azeotropically distilled to remove any water; around 30 mL of toluene was removed. CL (4.63 g, 40.56 mmol), OD (0.65 g, 5.07 mmol), and LA (0.73 g, 5.06 mmol) were added to the solution. These were followed by $Sn(Oct)_2$ in dry toluene (3 mL, 0.1 M), which was added at 25 °C under a nitrogen atmosphere. After polymerization for 24 h at 130 °C, the reaction solution was added to a mixture of *n*-hexane and diethyl ether (v/v = 4/1) to precipitate MCL-$(CO)_{10}$. The obtained MCL-$(CO)_{10}$ was dissolved in DCM. The resultant solution was reprecipitated to cold methanol. MCL-$(CO)_3$, MCL-$(CO)_5$, and MCL-$(CO)_{15}$ with molecular weights of 2400 g/mol were prepared according to the same protocol. The ratio of the poly(CL), poly(OD), and poly(LA) segments in the MCL-(CO) copolymers was determined by comparing the intensity of the carbonyl peaks at 173.8 ppm, 198.1 ppm, and 170.2 ppm (poly(CL), poly(OD), and poly(LA), respectively) in the ^{13}C-NMR.

2.6 Synthesis of MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-COOH)-*ran*-PLA] [MCL-(COOH)]

MCL-$(CO)_{10}$ (4 g, 1.27 mmol), carboxymethoxylamine hemihydrochloride (0.03 g, 0.29 mmol) and *p*-toluenesulfonic acid (15 mg, 0.09 mmol) were dissolved in anhydrous THF (40 mL) and stirred under nitrogen for 4 h at 25 °C. To precipitate MCL-$(COOH)_{10}$, the reaction solution was added to a mixture of *n*-hexane and ethyl ether (v/v = 4/1). MCL-$(COOH)_3$, MCL-$(COOH)_5$, and MCL-$(COOH)_{15}$ were synthesized according to the same procedure.

2.7 Synthesis of MPEG-*b*-[PCL-*ran*-poly(ε-caprolactone-3-NH_2)-*ran*-PLA] [MCL-(NH_2)]

MCL-$(COOH)_{10}$ (3 g, 0.90 mmol) was dissolved in anhydrous DCM (25 mL). *N*,*N*′-dicyclohexylcarbodiimide (0.04 g, 0.21 mmol) and *N*-hydroxysuccinimide (0.02 g, 0.21 mmol) were added to the MCL-$(COOH)_{10}$ solution; the mixture was stirred for 6 h at 25 °C and then filtered. Diaminobutane (0.04 g, 0.45 mmol) was added to the solution, which was then stirred for 24 h at 25 °C. The reaction solution was precipitated by adding it to a mixture of *n*-hexane, diethyl ether, and methanol (v/v = 8/1.5/0.5; 1 L). The precipitated mixture was solubilized in DCM and washed three times with distilled water (DW). The organic phase was extracted, dried over anhydrous $MgSO_4$ and the solvent was removed by evaporation to obtain MCL-$(NH_2)_{10}$. MCL-$(NH_2)_3$, MCL-$(NH_2)_5$, and MCL-$(NH_2)_{15}$ were prepared according to the same procedure.

2.8 Determination of sol-to-gel phase-transition via rheological measurement

The viscosities of the MCL, MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymer emulsions (40 mg in 160 μL of DW) were measured using a DV-III Ultra viscometer equipped with a programmable rheometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) and a TC-502P circulating bath with a programmable controller (Brookfield Engineering Laboratories, Middleboro, MA, USA). Using a T-F spindle (Brookfield Engineering Laboratories, Middleboro, MA, USA) rotating at 0.2 rpm, the viscosity of each copolymer emulsion was measured at temperatures from 10 to 50 °C in 1 °C increments.

2.9 *In vitro* degradation test

Aqueous solutions, with concentrations of 1 wt%, of each copolymer were prepared at 80 °C. These were incubated at 37 °C, with shaking at 100 rpm, for 1, 2 and 4 weeks. After the specified incubation time, the solutions were freeze-dried. Immediately after lyophilization, the molecular weights were measured by GPC, and compared to those of the unincubated copolymers.

3 Results and discussion

3.1 Preparation of 2-oxepane-1,5-dione (OD)

The synthesis of OD was performed according to Fig. 1 (Scheme 1). The ketone, 1,4-cyclohexanedione, was oxidized to its ester (Baeyer–Villiger oxidation) by *m*CPBA. The yield was 48% and the structure was confirmed by ^{1}H-NMR and elemental analysis (see Scheme 2).

3.2 Preparation of MCL, MCL-(CO), MCL-(COOH) and MCL-(NH_2) copolymers

We have previously reported the preparation of MCL copolymers and studied the effect of the CL:LA ratio on their thermal response [11]. MCL copolymers with a CL:LA ratio of less than 90:10 were almost water-soluble; they showed no phase transition at body temperature (ca. 37 °C), but did show a phase transition to a reduced-viscosity phase above 40 °C. Thus, in this study, we synthesized MCL copolymers with a fixed ratio of [CL + OD]:LA of 90:10, while varying the CL:OD ratio; we also synthesized polyester segments of the same molecular weight (2400 g/mol).

MCL-(CO) was synthesized by ROP with various ratios of CL:OD (Fig. 1, Scheme 2). MCL with a CL:LA ratio of 90:10 was prepared as a control sample. The properties of the MCL and MCL-(CO) copolymers with different CL:OD ratios are summarized in Table 1.

The colorless MCL-(CO) copolymers were obtained with yields of over 90%. All the MCL-(CO) copolymers exhibited a monomodal GPC curve. ^{1}H- and ^{13}C-NMR analyses of the MCL-(CO) copolymers showed the characteristic peaks of MPEG, poly(CL), poly(OD), and poly(LA) (Figs. 2, 3). ^{13}C-NMR signals corresponding to the carbonyl groups of poly(CL), poly(OD), and poly(LA) were observed at 173.8 ppm, 198.1 ppm, and 170.2 ppm, respectively. The CL:OD ratios calculated from the proton- and carbon-integration agreed well with the targeted values.

The molecular weights, CL:OD ratios, and [CL + OD]/LA:MPEG ratios of the samples are summarized in Table 1. The MCL-(CO) copolymers exhibited the targeted molecular weights, around 2400 g/mol, and feed ratios of [CL + OD]:LA, as determined from the ^{1}H- and ^{13}C-NMR spectra. This indicates that the ROP of CL, OD, and LA

Table 1 Properties of MCL, MCL-(CO), MCL-(COOH) and MCL-(NH_2) copolymers

Polymer	[CL/OD]/[LA] molar ratio[a] (%)	$M_{n,\ calcd}$ MPEG-polyester	$M^{a,b}_{n,\ NMR}$ MPEG-polyester	Yield (%)	M_w/M_n^c	Hydrodynamic diameter (nm)[d]
MCL	[91/0] [9]	750–2400	750–2390	92	1.31	–
MCL-$(CO)_3$		750–2400	750–2470	91	1.53	255
MCL-$(COOH)_3$	[91/2] [7]	750–2540	750–2540	92	1.55	324
MCL-$(NH_2)_3$		750–2610	750–2620	80	1.59	184
MCL-$(CO)_5$		750–2400	750–2460	92	1.59	287
MCL-$(COOH)_5$	[89/4] [6]	750–2600	750–2590	93	1.62	266
MCL-$(NH_2)_5$		750–2730	750–2730	86	1.63	180
MCL-$(CO)_{10}$		750–2400	750–2390	93	1.52	332
MCL-$(COOH)_{10}$	[84/8] [8]	750–2560	750–2570	89	1.53	215
MCL-$(NH_2)_{10}$		750–2740	750–2730	82	1.55	177
MCL-$(CO)_{15}$		750–2400	750–2410	90	1.62	484
MCL-$(COOH)_{15}$	[83/13] [4]	750–2620	750–2630	91	1.64	196
MCL-$(NH_2)_{15}$		750–2850	750–2860	88	1.66	2066

[a]Determined from ^{13}C-NMR spectra

[b]Determined from ^{1}H-NMR spectra

[c]Measured by means of gel-permeation chromatography (based on standard polystyrene)

[d]Measured by DLS

yielded the targeted MCL-(CO) copolymers, with ketone group abundance controllable between 3 and 15 mol%.

The MCL-(CO) ketone group was reacted with carboxymethoxylamine to give MCL-(COOH)—MCL with COOH pendant groups. Finally, the MCL-(COOH) copolymer was activated with DCC and NHS and reacted with diaminobutane to give MCL-(NH_2) copolymers. The MCL-(COOH) and MCL-(NH_2) copolymers exhibited ^{1}H NMR peaks characteristic of COOH and NH_2 (Fig. 2). The abundances of the COOH and NH_2 in the MCL-(COOH) and MCL-(NH_2) copolymers were determined from the ^{1}H NMR peaks, and agreed well with the targeted abundances. This indicates that the MCL-(COOH) and MCL-(NH_2) diblock copolymers contained the targeted abundance of COOH and NH_2 pendant-groups, between 3 and 15 mol% depending on the sample. Collectively, this indicates that the COOH and NH_2 pendant groups were added to the MCL segment with controllable abundances.

3.3 Thermoresponsiveness of the MCL, MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymer suspensions

Aqueous solutions of MCL-(CO), MCL-(COOH), and MCL-(NH_2), with functional group concentrations between 3 and 15 mol%, were prepared as homogeneous emulsions, which were white and opaque. The control MCL suspension also formed an opaque emulsion. To examine the phase transition behavior of the block copolymer suspensions, rheological analysis was performed between 10 and 50 °C (Fig. 4A).

Below 39 °C, MCL formed a homogeneous solution with a viscosity of 1 cP below; this increased to a maximum of 4.7×10^3 cP at 42 °C. The MCL-(CO) copolymer suspensions also exhibited a viscosity of almost 1 cP at low temperatures, indicating a homogeneous suspension due to complete dissolution of the copolymers. With increasing temperature, the viscosity of the MCL-(CO) copolymer suspensions increased until they displayed gel-like behavior. The viscosities and phase-change-onset temperatures of each MCL-(CO) copolymer suspension depended on the functional group content of the sample. The onset temperatures of the MCL-(CO) copolymer suspensions were 33 and 38 °C at 3 and 5 mol% ketone groups, respectively. However, the phase change did not occur with more than 10 mol% ketone groups. The MCL-(CO) copolymer with the highest viscosity was that with 3 mol% ketone groups, at 5.4×10^3 cP, which decreased to 2.9×10^3 cP at 5 mol%, 950 cP at 10 mol%, and 260 cP at 15 mol%; this indicates that viscosity is inversely correlated with the abundance of the ketone group.

The onset temperatures of the MCL-(COOH) copolymers were 19 and 26 °C for the 3 and 5 mol% COOH pendant groups, respectively (Fig. 4B). The onset temperatures of the MCL-(COOH) copolymers were lower than those of the MCL-(CO) copolymers, but the maximum viscosities were higher. The maximum viscosities of MCL-(COOH) were 1.3×10^4 cP and 9.6×10^3 cP at 3 and

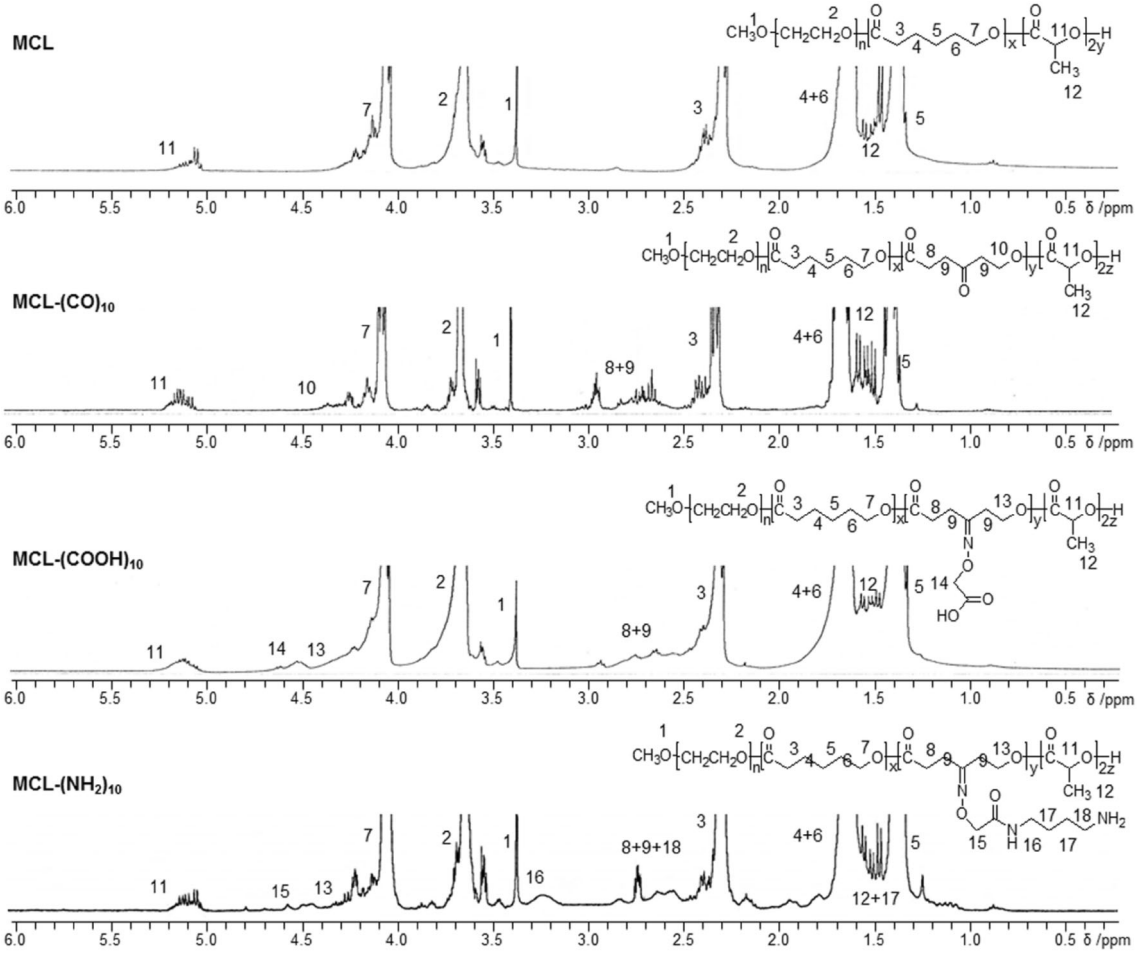

Fig. 2 ^{1}H-NMR spectra of **A** MCL, **B** MCL-$(CO)_{10}$, **C** MCL-$(COOH)_{10}$ and **D** MCL-$(NH_2)_{10}$ copolymers in $CDCl_3$

5 mol%, respectively. At ketone and COOH pendant group abundances of 3 and 5 mol%, MCL-(COOH) exhibited higher maximum viscosities than MCL-(CO); this is attributed to the intra- and intermolecular hydrogen bonding interactions between the COOH groups.

MCL-(NH_2) copolymer suspensions showed an onset temperature of 27 °C and a maximum viscosity of 1.7×10^5 cP at only 3 mol% (Fig. 4C). The suspensions of the MCL-(NH_2) copolymers with 5 mol% of pendant group exhibited a viscosity of almost 1 cP at low temperatures, indicating a homogeneous suspended phase due to complete dissolution.

The 3 mol% pendant group samples showed increasing viscosity in the order MCL-$(CO)_3 \ll$ MCL-$(COOH)_3 \ll$ MCL-$(NH_2)_3$. Collectively, the sol-to-gel phase transition of the MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymers depended on the type and concentration of the pendant groups. Thus, it is conjectured that the pendant groups on the MCL copolymers affect the stability of the hydrophobic aggregates of MCL copolymers.

3.4 Solution properties of the MCL, MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymer suspensions

As the MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymers formed homogeneous suspensions, 1 mg/mL copolymer solutions were prepared to compare the solution properties of the different pendant-group-functionalized MCL copolymers.

Figure 5 shows the room-temperature ^{1}H NMR spectra for the MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymers in D_2O. In D_2O, the MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymers exhibited broad peaks, assignable to the polyester blocks, but, in $CDCl_3$, the same copolymers showed narrow resonance peaks (Fig. 2). This is due to limitations on the molecular motion of the polyester blocks when dissolved in D_2O.

The mean hydrodynamic diameters of the MCL-(CO) copolymer solutions increased as the pendant group abundance increased (Table 1). However, the MCL-

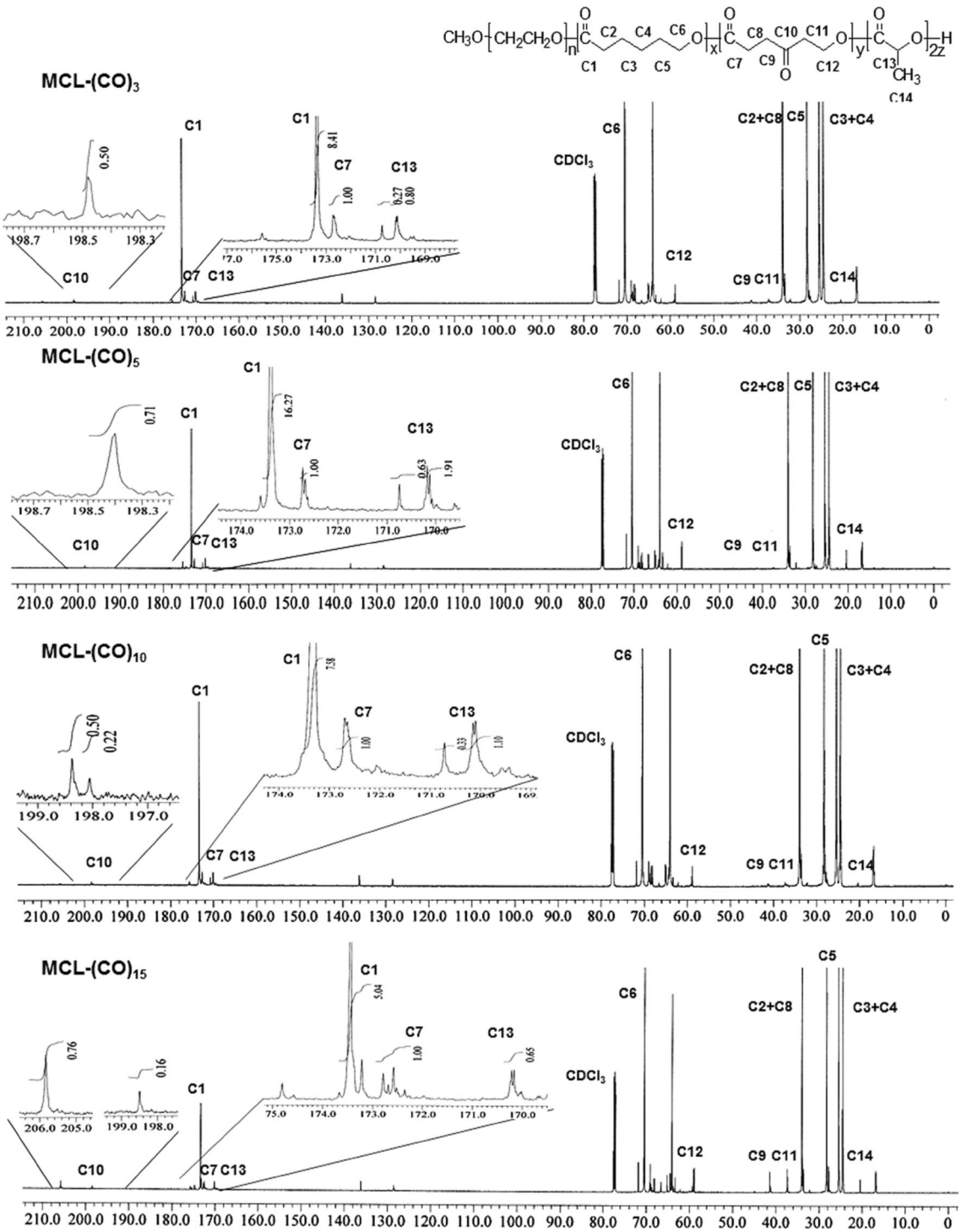

Fig. 3 ^{13}C-NMR spectra of MCL-(CO), with different amounts of OD, in $CDCl_3$

(COOH) and MCL-(NH_2) copolymer solutions showed a decrease in mean hydrodynamic diameters as the pendant group abundance increased, except in the case of MCL-$(NH_2)_{15}$.

Collectively, the mean hydrodynamic diameters of the MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymers depended on the types and concentrations of the pendant groups. This is attributed to the effects that the pendant groups may have on the formation of MCL micelles.

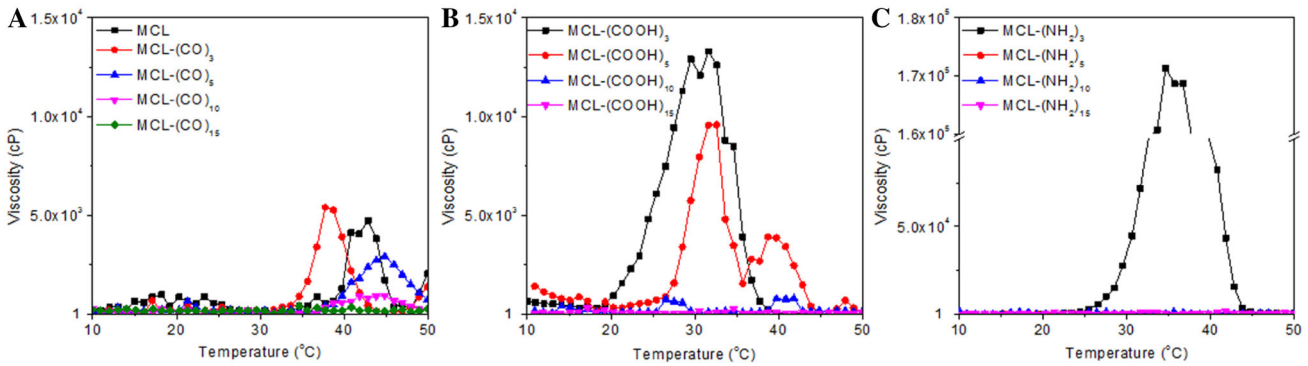

Fig. 4 Viscosity *vs* temperature curves of **A** MCL-(CO), **B** MCL-(COOH), and **C** MCL-(NH_2) copolymers with 3–15 mol% of pendant groups

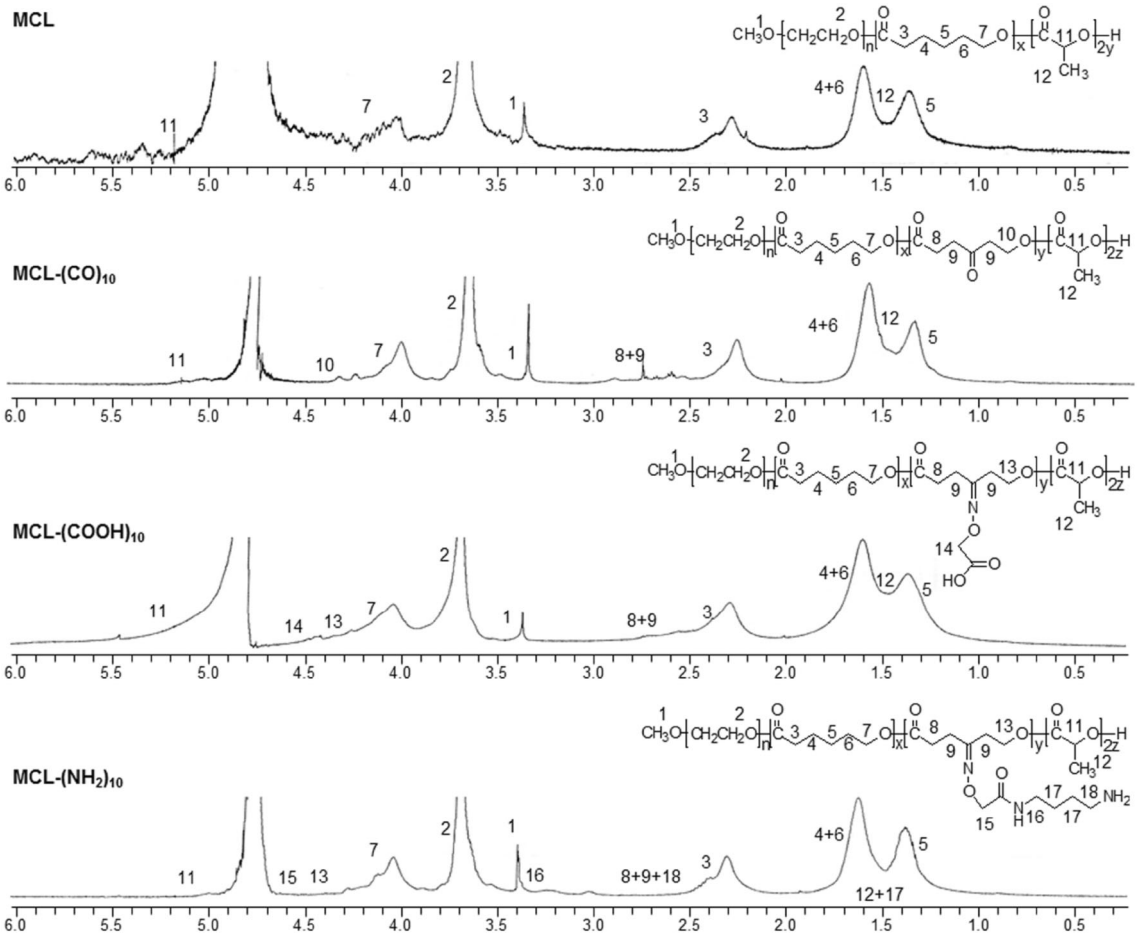

Fig. 5 ^{1}H-NMR spectra of **A** MCL, **B** MCL-(CO), **C** MCL-(COOH), and **D** MCL-(NH_2) copolymers in D_2O

3.5 Degradation of the MCL-(CO), MCL-(COOH), and MCL-(NH_2) copolymers

The copolymer suspensions were stirred to examine, *in vitro*, their degradation behavior in phosphate-buffered saline at 37 °C for 4 weeks. At the end of the process, their molecular weights were measured by GPC. Figure 6A shows GPC traces, recorded at several points throughout the degradation process, for MCL-(CO), MCL-(COOH), and MCL-(NH_2). The remaining molecular weights of MCL-(CO), MCL-(COOH), and MCL-(NH_2) versus implantation time were plotted and are shown in Fig. 6B. The GPC traces of the degraded samples show several peaks at higher retention times; the intensity of these peaks increases as a function of time, indicating increasing degradation. Over the degradation process, there is a

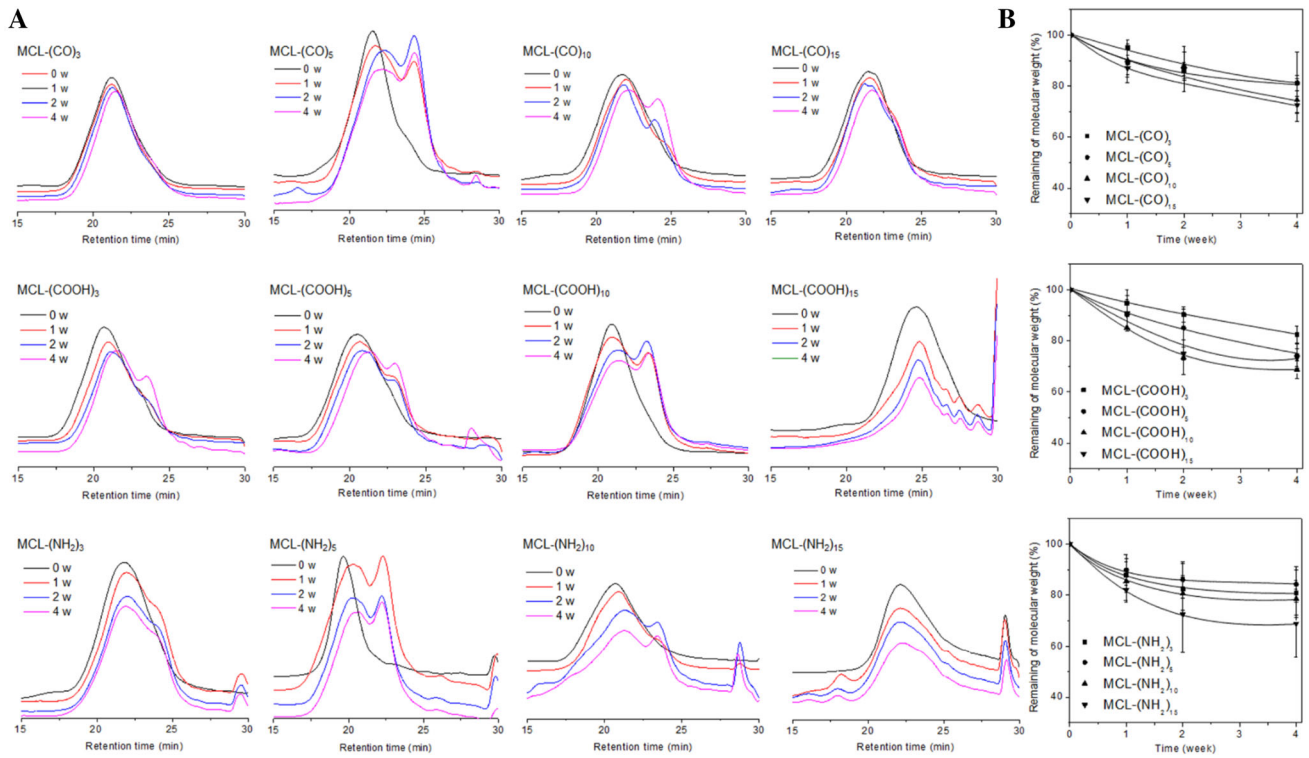

Fig. 6 **A** Gel permeation chromatograms (GPC) charts and **B** the remaining molecular weight versus time for MCL, MCL-(CO), MCL-(COOH) and MCL-(NH_2) copolymers after 1–4 weeks degradation testing

gradual shift to low-molecular-weight peaks with higher retention times; these are assignable to the degraded species, as the degradation process results in decreases in molecular weight. In this work, the findings indicated that degradation was changed by both the type and number of pendant groups.

4 Conclusions

In this study, we successfully synthesized the thermoresponsive, amphiphilic block copolymers MCL-(CO), MCL-(COOH), and MCL-(NH_2), which have ketone, COOH, and NH_2 pendant groups, respectively. The solution-to-hydrogel phase transition and biodegradation behavior were affected by both the type and number of pendant groups. Although we found that MCL-(CO), MCL-(COOH), and MCL-(NH_2) can be utilized as potential thermoresponsive materials, further studies will be needed to provide a rationale as drug and as cell delivery carriers in animal model. Additionally, we believe that investigation for biocompatibility of the copolymers will be needed as future work.

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Compliance with ethical standards

Conflict of interests The authors declare no competing financial interests.

Ethical statement There are no animal experiments carried out for this article.

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