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Multi-responsive γ -Methylene- γ -Butyrolactone / N-Vinyl Caprolactam Copolymers Involving pH-depend Reversible Lactonization

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Abstract

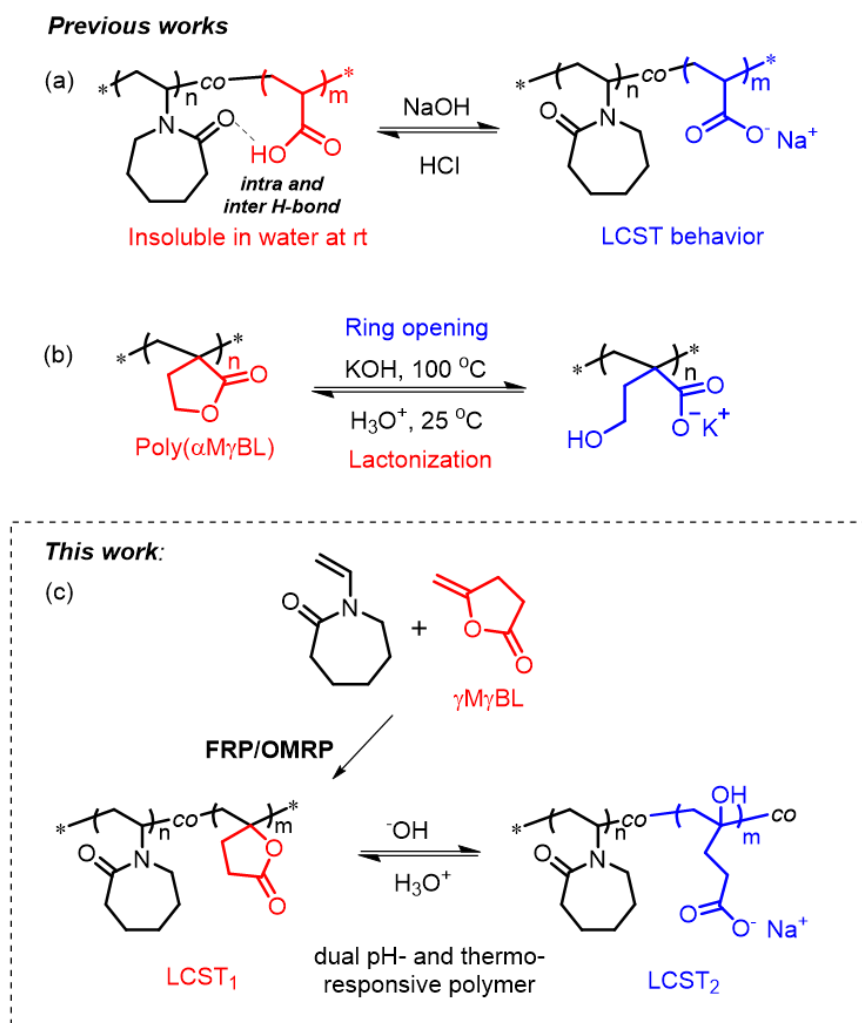
Poly(*N*-vinyl caprolactam) is one of the most popular thermo-responsive polymers used in the biomedical field. For application purposes, its transition temperature can be adjusted by copolymerizing *N*-vinyl caprolactam with comonomers of various hydrophilicity. Insertion of carboxylic acid containing vinyl monomers in PMVCL also imparts some pH-responsiveness to the latter although, at low pH, H-bonding between the protonated carboxylic groups and the amide functions of MVCL induces aggregation phenomena. The present work describes a unique multi-responsive PMVCL system based on γ -methylene- γ -butyrolactone as comonomer involving a peculiar pH-depend reversible lactonization reaction. A series of narrowly distributed statistical MVCL/ γ M γ BL-based copolymers with different molar masses and compositions were prepared via organometallic-mediated radical polymerization. Hydrolysis of the pendant lactones led to the corresponding hydroxy acid-containing copolymers allowing to tune the cloud point temperature of the polymer solution over a wide range of temperature. In contrast to the previously reported acid-bearing PMVCL derivatives, acidification of the hydroxy carboxylate containing MVCL-based copolymers induced the lactonization reaction leading to the regeneration of the starting MVCL/ γ M γ BL-based copolymers, which ultimately prevents aggregation via hydrogen bonding. The synthesis and responsiveness of MVCL/ γ M γ BL-based block copolymers were also considered and discussed. Overall, this unusual multi-responsive methylene lactone-based system constitutes a promising platform for tuning the solution properties of PMVCL, and possibly of other smart polymer sequences, opening new applicative prospects.

Introduction

Polymer foams are versatile materials that allow combining the advantageous properties Driven by the need of controlled drug delivery systems, innovative biosensors and smart actuation devices, there has been an intensive research for the development of stimuli-responsive polymers which can undergo abrupt physiochemical changes in response to slight changes in their external environmental variables such as temperature,¹ pH,² electric³ or magnetic field,⁴ light,⁵ and ionic strength.⁶ Thermo-responsive polymers characterized by a lower critical solution temperature (LCST) are certainly among the most studied stimuli responsive systems.^{7–11} In particular, those characterized by a LCST close to the physiological temperature,^{12–21} such as poly(*N*-vinylcaprolactam) (PMVCL),^{16–19} have attracted a lot of attention especially for biomedical applications. PMVCL is a very popular thermosensitive sequence which exhibits a Type I phase change and a classical Flory-Huggins thermo-responsive phase diagram with a continuous phase transition from about 30 °C to 50 °C, depending on molar mass and concentration.^{16,22,23} Compared to another common LCST polymer, namely poly(*N*-isopropylacrylamide) (PMPAAM), the nitrogen atoms of PMVCL are advantageously connected to carbons of the polymer backbone, prohibiting the release of toxic primary amines upon hydrolysis.^{16,19,24,25} Another major difference with PMPAAM is the absence of H-bond donating group in the molecular structure of PMVCL that is not able to form intra- or interchain hydrogen bonds which limits the hysteresis phenomenon.²³ Given its biocompatibility and coil to globule transition temperature close to the body temperature, PMVCL has been largely considered for biomedical applications,^{26–30} including drug delivery systems and smart surfaces in the forms of micelles,³¹ micro^{27,29,32}/nano gel^{33,34} or multilayer hydrogels.^{35,36}

A classical approach to tune the LCST of PMVCL-based polymers but also to achieve multi-responsive behaviors consists in the incorporation of comonomers in the backbone.^{29,37–39} For example, copolymerizing MVCL with ionizable monomers imparted dual pH- and thermo-sensitivity to the final copolymers.^{40–46} Typical ionizable comonomers which have been associated to MVCL in order to fabricate dual responsive microgels of interest for drug delivery applications are acrylic acid (AA),^{42,46} methacrylic acid (MAA)^{27,43,44} and itaconic acid (IA).^{27,45} At physiological pH, the ionized carboxylate groups predominated, increasing the hydrophilicity of the copolymer as well as its LCST. At pH values lower than pKa, however, carboxylic acid functions were mainly in their protonated state and served as strong hydrogen bond donors for the lactam groups of MVCL units leading to the insolubility of copolymers in water at room temperature (Scheme 1a).⁴⁷ This characteristic impedes the design of a PMVCL-based coil to globule transition system in which LCST can be simply tuned by pH adjustment.

Recently, some methylene heterocyclic compounds have received great interest as monomers notably for the preparation of functional polymers, including carboxylic acid-containing ones. For example, γ -methylene- γ -butyrolactone (γ M γ BL) was copolymerized in a controlled manner with vinyl acetate followed by hydrolysis of both the acetate and lactone functions leading to acid-functionalized poly(vinyl alcohol) with improved water solubility at neutral pH.⁴⁸ As another illustration, saponification of poly(α -methylene- γ -butyrolactone) (poly(α M γ BL)) generated one hydroxyl group and one carboxylate function per repeating unit.⁴⁹ This highly hydrophilic polymer sequence was also used to manufacture superabsorbent materials.^{49–51} Interestingly, relactonization reaction was observed upon acidification of the hydroxy carboxylate-functional polymer at 25°C (Scheme 1b).⁴⁹



Scheme 1. Illustration of the pH-responsive acrylic acid/ γ M γ BL copolymers with intra/inter H-bond formation (a), the reversible lactonization of poly(α M γ BL) (b) and the targeted dual pH- and thermoresponsive γ M γ BL/ γ M γ BL copolymers.

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The present work reports an innovative multi-responsive *NVCL*-based system involving a methylene- γ -butyrolactone compound as comonomer and emphasizing a peculiar pH-dependent ring opening-closure of the pendant lactones (Scheme 1c). Compared to the (meth)acrylic acid-containing *PMVCL* copolymers, aggregation via intra/intermolecular H-bonding should be prevented for such *MBL/NVCL* copolymers thanks to the lactonization reaction in acidic medium. For this purpose, γ -methylene- γ -butyrolactone (γ *M* γ *BL*) was selected as comonomer of *NVCL* since both compounds consists in less activated monomers (LAMs) and should thus exhibit close reactivity favorable to their copolymerization. A series of narrowly distributed *P(NVCL-co- γ M γ BL)* copolymers with different molar masses and compositions were prepared via organometallic-mediated radical polymerization (OMRP), an efficient reversible deactivation radical polymerization (RDRP) method suitable for LAMs^{52–56} including *NCVL*.^{11,57,58} The solution behavior of these copolymers upon pH- and temperature changes was then investigated emphasizing their specific stimuli responsiveness and the key role of the reversible lactonization reaction. The incorporation of γ *M* γ *BL* within *NVCL*-based block copolymers and its effect on the copolymer self-assembly in water was also examined.

Experimental section

Materials

Cobalt(II) acetylacetonate ($\text{Co}(\text{acac})_2$) (97%, Acros), 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) (98%, Aldrich), butylamine (99%, Fluorochem) and 2,2'-azobis(4-methoxy-2,4-dimethyl valeronitrile) (V-70, $t_{1/2} = 10$ h at 30 °C) (>98%, Wako) were used as received. *N*-vinyl caprolactam (98%, Aldrich) was purified by flash basic alumina column with *n*-hexane at 40 °C, and then further purified by recrystallization in *n*-hexane at 6 °C. 2,2'-azobisisobutyronitrile (AIBN, $t_{1/2} = 10$ h at 65 °C) (98%, Aldrich) was recrystallized in methanol at 6 °C. Sodium hydroxide (NaOH, $\geq 97\%$, Acros), silica gel for column chromatography (60 Å, ROCC S.A.), tetrahydrofuran (THF, $\geq 99.9\%$, VWR), methanol (MeOH, $\geq 99.8\%$, VWR), *n*-hexane (>99%, VWR), diethyl ether (Et_2O , 100%, VWR) and ethyl acetate ($\geq 99.9\%$, VWR) were used as received. *N,N*-dimethylformamide (DMF, >99%, VWR) was dried over molecular sieves prior to use. Dichloromethane (CH_2Cl_2) was degassed and dried over 4 Å molecular sieves. 1,4-dioxane (99.8%, Aldrich) was purified by distillation under reduced pressure. VAc (>99%, Aldrich) was dried under calcium hydride, purified by distillation under reduced pressure, and degassed by freeze-drying cycle under vacuum. The alkyl-cobalt(III) adduct initiator ($\text{PVAc}_{<4}\text{-Co}(\text{acac})_2$), $[\text{Co}(\text{acac})_2$ -

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(CH(OAc)-CH₂)₄R₀], R₀ being the primary radical generated by 2,2'-azo-bis(4-methoxy-2,4-dimethyl valeronitrile) (V-70, Wako), was prepared as described previously⁵⁹ and stored as a CH₂Cl₂ solution at -20 °C under argon. γ -methylene- γ -butyrolactone (γ M γ BL) was prepared as described previously and stored under argon.⁴⁸ Dialyses were carried out with a Spectra/Por dialysis membrane (pretreated RC tubing 1 kDa).

Characterization

The size exclusion chromatography (SEC) analyses were carried out in DMF containing 0.025 M LiBr at 55 °C with a Waters chromatograph equipped with three columns [PSSGRAM 1000 Å ($\times 2$), 30 Å], a dual λ absorbance detector (Waters 2487), and a refractive index detector (Waters 2414). The system was operated at a flow rate of 1 mL/min, and polystyrene calibration was used. The absolute molar masses of the PVCL, P(MVCL-co- γ M γ BL) and P(MVCL)-*b*-P(MVCL-co- γ M γ BL) block copolymer were determined by SEC equipped with a multiangle laser light scattering (MALLs) detector in DMF/LiBr (0.025 M). The Wyatt MALLs detector (120 mW solid-state laser, $k = 1/4$ 658 nm, DawnHeleos S/N342-H) measures the excess Rayleigh ratio R_h (related to the scattered intensity) at different angles for each slice of the chromatogram. The specific refractive index increment (dn/dc) of polymer was measured by using a Wyatt Optilab refractive index detector ($k = 1/4$ 658 nm). Data were processed with the Astra V software (Wyatt Technology). ¹H nuclear magnetic resonance (¹H NMR) (400 MHz) and power gate proton-decoupled ¹³C NMR (¹³C{¹H} NMR) (101 MHz) spectra were recorded at 298 K with a Bruker AVANCE III HD spectrometer ($B_0 = 9.04$ T) (400 MHz) and treated with MestreNova software. Fourier transform infrared (FT-IR) spectroscopy spectra were recorded on a Thermo Fisher Scientific Nicolet IS5 equipped with an ATR ID5 module using a diamond crystal (650–4000 cm⁻¹). Dynamic light scattering (DLS) and zeta potential analyses were performed on a Delsa NanoC instrument. The transmittance of the aqueous solutions of polymers in MiliQ-water (5 mg/mL) was recorded on a Jasco V630 UV-vis spectrophotometer at 260 nm. Aqueous solution of polymers were placed in a thermostated quartz cuvette. The heating/cooling rate was 2 °C/min between 10 °C and 80 °C. The cloud point temperature (T_{CP}) was defined at the half drop transmittance from the initial transmittance. pH measurements were performed via pH test paper.

Conventional radical copolymerization of MVCL and γ M γ BL. AIBN (0.079 g, 0.48 mmol), MVCL (2.00 g, 14.37 mmol) and γ M γ BL (0.16 g, 1.60 mmol) were placed in a 25 mL Schlenk tube with 1.94 mL of anhydrous 1,4-dioxane. After degassing by bubbling argon for 20 min, the polymerization medium ($f^{\circ}_{\gamma M \gamma BL} = 0.1$) was heated at 65 °C. After 19 h, reaction mixture was very viscous and was diluted with THF. The monomer conversion and the molecular parameters (M_n , \bar{D}) of the polymer were then determined by ¹H NMR in CDCl₃ and SEC in DMF containing 0.025 M LiBr, respectively. The final copolymer was

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purified via repeated precipitations (three times) in hexane and dried under vacuum overnight at 60 °C before characterization by ¹H NMR and ¹³C NMR in CDCl₃. SEC-MALLS measurements were carried out in DMF/LiBr (0.025 M) in order to determine the absolute molar mass of P(NVCL-co- γ M γ BL) (f_{γ M γ BL} = 0.1) ($dn/dc=0.1769$ mL/g).

A similar experiment was carried out with a different initial comonomer feed ratio, namely f_{γ M γ BL} = 0.2, keeping constant all other parameters (65 °C, [comonomers]₀/[AIBN]₀ = 100/3).

The copolymer composition was calculated based on the relative intensity of ¹H NMR signals corresponding to each comonomer using the following equation, where signals **c** and **e** correspond to 2H in α -position of the nitrogen atom and 2H in α -position of the carbonyl group of NVCL units, respectively, whereas signals **g** and **h** correspond together to 4H of the lactone of the MBL units.

$$F_{\gamma$$
M γ BL} = \frac{\frac{\int e, h, g - \int c}{4}}{\frac{\int e, h, g - \int c}{4} + \frac{\int c}{2}}

Organometallic-mediated radical copolymerization of NVCL and γ M γ BL. NVCL (3.60 g, 25.8 mmol) and γ M γ BL (0.28 g, 2.88 mmol) were placed in a 25 mL Schlenk tube, degassed by three freeze-pump-thaw cycles followed by bubbling argon for 10 min at 40 °C. A solution of alkyl-cobalt(III) initiator (PVAc_{<4}-Co(acac)₂) in CH₂Cl₂ (1.82 mL of a 0.063 M stock solution, 0.12 mmol) was placed in a second Schlenk tube followed by the evaporation of CH₂Cl₂ under reduced pressure at room temperature. The NVCL/ γ M γ BL mixture was then transferred via cannula into the Schlenk tube containing the alkyl cobalt initiator followed by polymerization at 40 °C ([NVCL]₀/[γ M γ BL]₀/[PVAc_{<4}-Co(acac)₂]₀=225/25/1, f_{γ M γ BL} = 0.1). During polymerization, samples were regularly taken and added with TEMPO in order to quench the polymerization. The monomer conversion was determined by ¹H NMR in CDCl₃ (1 mg of TEMPO was added per mL of CDCl₃). The molecular parameters (M_n , D) of the polymer were determined by SEC in DMF/LiBr (0.025 M) (samples were dissolved in DMF containing 10 mg of TEMPO/mL). Polystyrene was used as calibration. After 2.5 h, the polymerization mixture was quenched by the addition of 2 mL of TEMPO in THF (0.10 g/mL). The final copolymer was purified by precipitation in hexane three times followed by drying at 60 °C overnight before characterization via ¹H NMR and ¹³C NMR in CDCl₃.

Similar experiments were carried out with different initial comonomer feed ratios, namely f_{γ M γ BL} = 0.2, f_{γ M γ BL} = 0.3, keeping constant all other parameters (65 °C, bulk,

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[comonomers]₀/[PVAc_{<4}-Co(acac)₂]₀ = 250/1). The monomer/initiator ratios were also varied [comonomers]₀/[PVAc_{<4}-Co(acac)₂]₀ = 450/50/1 and 675/75/1, keeping constant all other parameters (65 °C, bulk, $f_{M_7BL} = 0.1$).

Organometallic-mediated radical polymerization of NVCL. NVCL (1.00 g, 7.18 mmol) was placed in a 25 mL Schlenk tube under argon followed by bubbling argon for 10 min at 40 °C. This Schlenk tube was then placed in an ice bath to cool the temperature followed by addition of a solution of alkyl-cobalt(III) initiator (PVAc_{<4}-Co(acac)₂) in CH₂Cl₂ (0.45 mL of a 0.063 M stock solution, 0.029 mmol) and evaporation of CH₂Cl₂ under reduced pressure at room temperature. Subsequently, the reaction mixture was carried out at 40 °C. After 6 h, the polymerization mixture was quenched by the addition of 2 mL of TEMPO in THF (0.10 g/mL) and samples were withdrawn for determining the conversion by ¹H NMR in CDCl₃ and the molecular parameters (M_n , \bar{D}) by SEC in DMF/LiBr (0.025 M). The final copolymer was purified by precipitation in hexane three times followed by drying at 60 °C overnight before characterization via ¹H NMR and ¹³C NMR in CDCl₃.

Synthesis of PMVCL-*b*-(NVCL-co- γ M₇BL) block copolymer by OMRP. NVCL (2.50 g, 18.0 mmol) was placed in a 25 mL Schlenk tube under argon followed by bubbling argon for 10 min at 40 °C. This Schlenk tube was then placed in an ice bath to cool the temperature. A solution of alkyl-cobalt(III) initiator (PVAc_{<4}-Co(acac)₂) in CH₂Cl₂ (0.58 mL of a 0.063 M stock solution, 0.036 mmol) was added followed by the evaporation of CH₂Cl₂ under reduced pressure at room temperature. The reaction mixture was carried out at 40 °C. After 1 h, a sample was picked out of the tube and added with traces of TEMPO (~10 mg/mL) for determining the monomer conversion by ¹H NMR in CDCl₃ and the molecular parameters of the PMVCL precursor by SEC MALLs in DMF/LiBr (0.025 M). Then, γ M₇BL (0.35 g, 3.59 mmol) degassed by three freeze-pump-thaw cycles was injected to the reaction mixture under argon and the polymerization medium was heated at 40 °C. After 6 h, the polymerization mixture was quenched by the addition of 1 mL of TEMPO in THF (0.10 g/mL) and samples were withdrawn for determining the conversion by ¹H NMR in CDCl₃ and the molecular parameters (M_n , \bar{D}) by SEC MALLs in DMF/LiBr (0.025 M) ($dn/dc=0.0938$ mL/g). The final copolymer was purified by precipitation in hexane three times followed by drying at 60 °C overnight before characterization via ¹H NMR and ¹³C NMR in CDCl₃.

Base hydrolysis of P(NVCL-co- γ M₇BL). P(NVCL-co- γ M₇BL) (0.26 g, 9500g/mol, $F_{M_7BL}=0.12$) was dissolved in MeOH (2.6 mL). NaOH (0.26 g, 6.5 mmol) in deionized water (2.6 mL) was added to the reaction mixture followed by stirring at room temperature for 48 h. The solution was then dialyzed (membrane 1kDa) against distilled water for 48 h followed by lyophilization. The copolymer, recovered as a white powder, was characterized by FT-IR spectroscopy, ¹H NMR and ¹³C NMR in CDCl₃.

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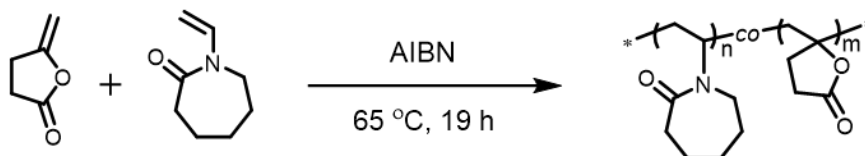
Similar treatments were applied to P(MVCL-co- γ M γ BL) (13000 g/mol, F_{γ M γ BL=0.07), P(MVCL-co- γ M γ BL) (11500g/mol, F_{γ M γ BL=0.10) and PMVCL-*b*-P(MVCL-co- γ M γ BL) (31500 g/mol, F_{γ M γ BL=0.10).

Results and discussion

Conventional radical copolymerization of γ M γ BL and MVCL.

The γ M γ BL/MVCL copolymerization was first performed by conventional radical polymerization in anhydrous 1,4-dioxane at 65 °C using AIBN as initiator (Table 1). Two molar fractions of γ M γ BL (f°_{γ M γ BL = 0.1 and 0.2) were considered in order to produce P(MVCL-co- γ M γ BL) with different compositions. High conversion of both monomers was achieved after 19 h of reaction (> 90 %) leading to copolymers with molar masses around 20000 g/mol according to SEC measurements using a PS calibration or a multi angle light scattering detector (SEC-MALLS) (M_n SEC cal PS = 20000 g/mol, M_n SEC MALLS = 18000 g/mol, entry 1 Table 1).

Table 1. Conventional radical polymerization of MVCL and γ M γ BL.



Entry	f°_{MVCL}	$f^{\circ}_{\gamma M \gamma BL}$	Conv. (%) ^a		M_n SEC (g/mol) ^b	\mathcal{D} ^b	F_{MVCL} ^c	$F_{\gamma M \gamma BL}$ ^c
			MVCL	$\gamma M \gamma BL$				
1	0.9	0.1	99	91	20000	2.0	0.95	0.05
2	0.8	0.2	99	93	19000	2.2	0.92	0.08

Conditions: 65 °C, in anhydrous 1,4-dioxane, 19 h, [comonomers]₀/[AIBN]₀=400/12. ^a Determined by ¹H NMR in CDCl₃. ^b Determined by SEC in DMF/LiBr using PS calibration. ^c Determined by ¹H NMR in CDCl₃ after purification.

The composition of the copolymers was determined via ¹H NMR by comparison of the intensity of signal **c** at 3.24 ppm corresponding to the -CH₂-NC=O- of MVCL and signals between 2.2 and 2.8 ppm containing peaks **h** and **g** of γ M γ BL units and peak **e** of MVCL (Figure 1, see equation in experimental section). The content of γ M γ BL in the copolymer increased from 5 mol% to 8 mol% when the molar fraction of γ M γ BL in the feed increased

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from 0.1 to 0.2. The successful incorporation of γ M γ BL in the copolymer was confirmed by FT-IR analysis showing a peak at 1770 cm⁻¹ assigned to C=O of the pendant lactone groups (Figure S1).

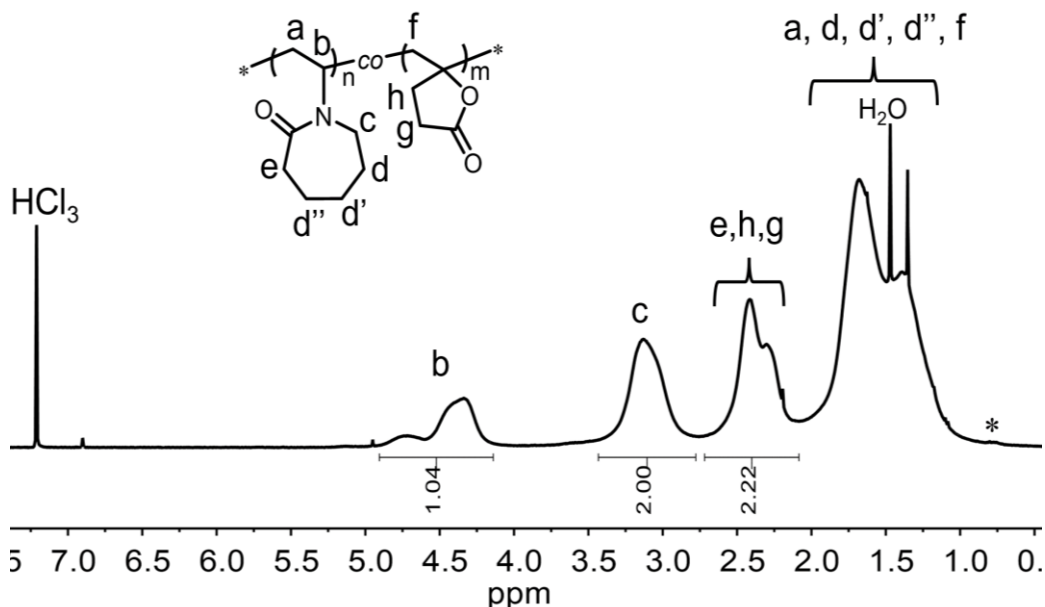
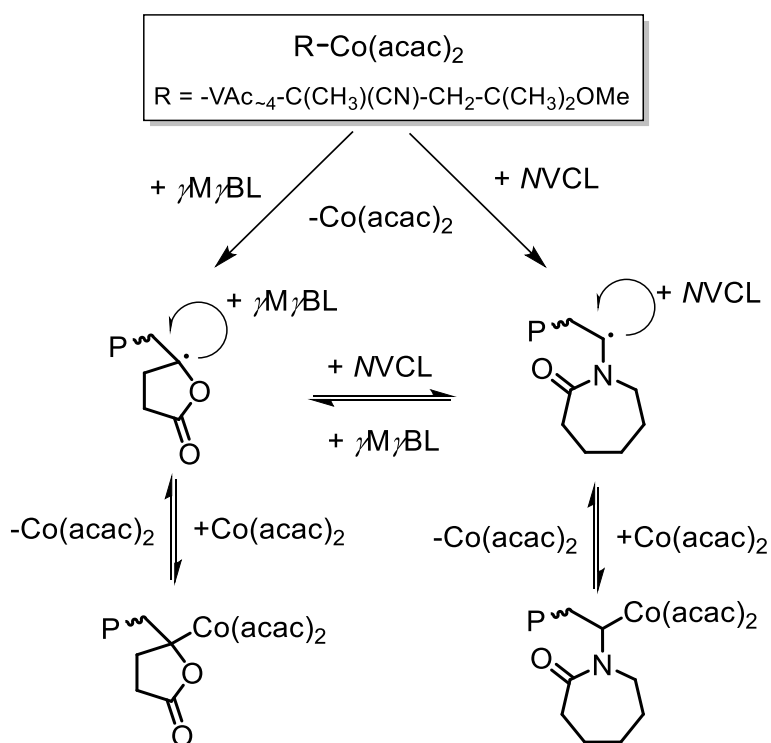


Figure 1. ¹H NMR of P(NVCL-co- γ M γ BL) ($M_n = 20000$ g/mol, $F_{\gamma\text{M}\gamma\text{BL}} = 0.05$) prepared by conventional radical polymerization (Table 1, entry 1). * designates the signal of the methyl groups of the AIBN initiating fragment.

Reversible deactivation radical copolymerization of γ M γ BL and NVCL.

The synthesis of P(NVCL-co- γ M γ BL) statistical copolymers with predictable molar mass, low dispersity and precise composition was then investigated by reversible deactivation radical copolymerization of NVCL and γ M γ BL. For this purpose, the organometallic mediated radical polymerization (OMRP) was selected as RDRP method due to its ability to mediate the radical polymerization of several non-conjugated and less activated monomers (LAMs)^{52,60,61} but also their copolymerization such as VAc/ γ M γ BL,⁴⁸ NVCL/VAc^{57,58} and NVCL/ *N*-vinylamides⁵⁷ copolymerizations. In practice, the OMRP of NVCL and γ M γ BL was conducted in bulk at 40 °C using a previously reported alkyl cobalt(III) species consisting in oligomers of poly(vinyl acetate) end-functionalized by a bis-acetylacetonate cobalt complex (PVAc-₄-Co(acac)₂)⁵⁹ as initiator and controlling agent (Scheme 2 and Table 2).



Scheme 2. Organometallic mediated radical copolymerization of MVCL and $\gamma\text{M}_7\text{BL}$.

To gain insight into this copolymerization, both the monomer/alkyl cobalt molar ratio and the feed composition were changed. First, the comonomers/R-Co(acac)₂ molar ratio was varied from 250 to 750 while keeping an initial MVCL/ $\gamma\text{M}_7\text{BL}$ feed composition of 0.9/0.1 (Table 2, entries 1-3). The comonomer conversion and the molecular parameters of the copolymers were determined throughout the polymerization by ¹H NMR and SEC, respectively. The structure and composition of the copolymers were determined by ¹H NMR and confirmed by COSY and HSQC analyses (Figure S2). Similar polymerization kinetics were observed for these experiments and total conversion exceeded 60 % after few hours in all cases (Figure 2b). As confirmed by the regular shift of the SEC chromatograms towards lower elution volume (Figure S3), the *M_n* of the P(MVCL-co- $\gamma\text{M}_7\text{BL}$) copolymer increased linearly with the conversion (Figure 2a) confirming the controlled character of the copolymerization. The dispersity of the copolymers remained rather low along the polymerization (*D* ~ 1.1-1.35) and only slightly increased above 50% conversion when higher molar mass was targeted probably due to the occurrence of some irreversible termination reactions (Table 2 entry 3). As expected, for similar monomer conversion, the molar mass of the copolymer increased with the comonomers/R-Co(acac)₂ molar ratio (e.g. 13000 g/mol and 33000 g/mol for [comonomers]₀/[R-Co(acac)₂]₀ of 250 and 750, respectively, compare entries 1 and 3 in Table 2). Copolymers

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with similar composition were formed ($F_{\gamma M \gamma BL} = 0.05 \sim 0.07$) in these experiments involving the same comonomer feed composition ($f^{\circ}_{\gamma M \gamma BL} = 0.1$).

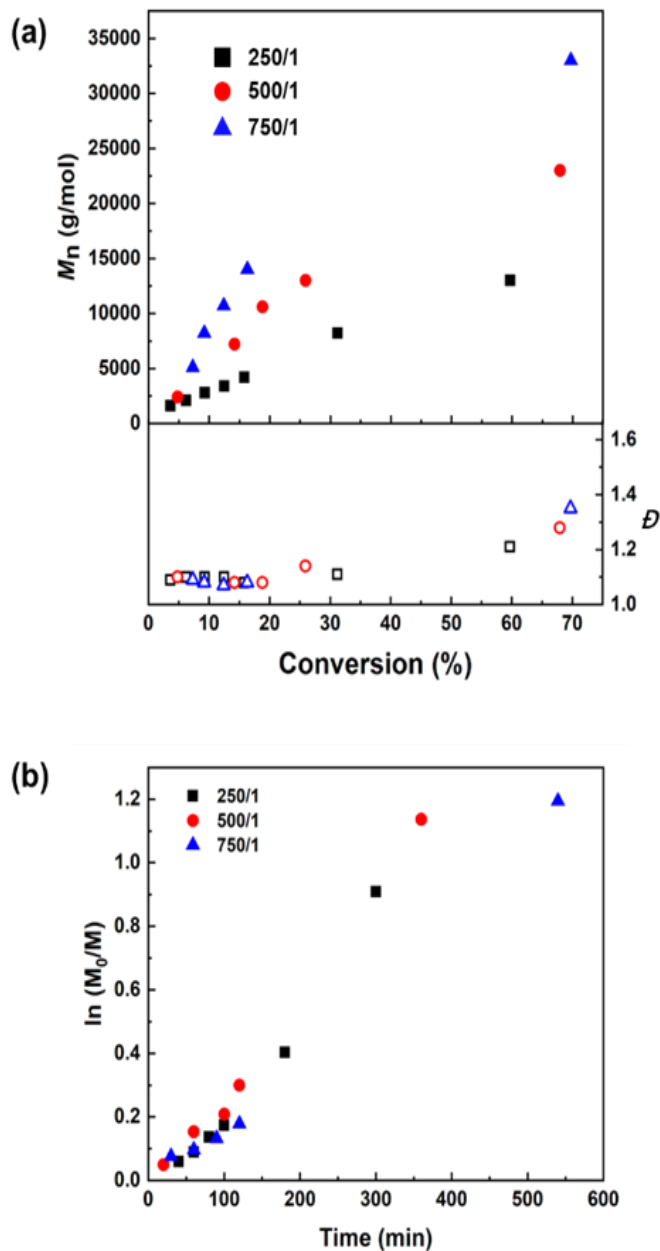


Figure 2. (a) Dependence of M_n (full symbols) and \bar{D} (hollow symbols) on the total comonomers conversion and (b) time dependence of $\ln(M_0/M)$ for the OMRP of MVCL and $\gamma M \gamma BL$ with different $[\text{monomers}]_0/[\text{R-Co}(\text{acac})_2]_0$ ratio: \blacksquare $[\text{monomers}]_0/[\text{R-Co}(\text{acac})_2]_0=250/1$, \bullet $[\text{monomers}]_0/[\text{R-Co}(\text{acac})_2]_0=500/1$, \blacktriangle $[\text{monomers}]_0/[\text{R-Co}(\text{acac})_2]_0=750/1$. ($f^{\circ}_{\gamma M \gamma BL} = 0.1$, Table 2 entry 1-3)

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Table 2. Organometallic mediated radical copolymerization of *NVCL* and $\gamma M\gamma BL$.

Entry	[<i>NVCL</i>] ₀ /[$\gamma M\gamma BL$] ₀ / [R-Co(acac) ₂] ₀	$f^{\circ}_{\gamma M\gamma BL}$	Time (min)	Conv. (%) ^a		Conv. total (%)	M_n^{SEC} (g/mol) ^b	\mathcal{D}^b	$F_{\gamma M\gamma BL}^c$
				<i>NVCL</i>	$\gamma M\gamma BL$				
1	225/25/1	0.1	20	4	0	4	1600	1.09	
			40	6	4	6	2100	1.10	
			60	9	5	9	2800	1.10	
			80	13	11	13	3400	1.10	
			100	16	14	16	4200	1.08	
			180	33	35	33	8200	1.11	
			300	61	48	60	13000	1.21	
2	450/50/1	0.1	20	4	12	5	2400	1.10	
			60	14	16	14	7200	1.08	
			100	19	17	19	10600	1.08	
			120	26	25	26	13000	1.14	
			360	70	49	68	23000	1.28	
3	675/75/1	0.1	30	7	10	7	5100	1.09	
			60	9	11	9	8200	1.08	
			90	12	16	12	10700	1.07	
			120	16	19	16	14000	1.08	
			540	74	31	70	33000	1.35	
4	200/50/1	0.2	30	7	1	6	2000	1.11	
			60	12	3	10	3000	1.09	
			90	17	4	14	4100	1.09	
			128	25	10	22	5500	1.08	
			300	63	46	60	11500	1.18	
5	175/75/1	0.3	30	2	1	2	2000	1.07	
			66	7	3	6	3200	1.10	
			98	14	4	11	4100	1.10	
			120	18	4	14	4900	1.10	
			300	55	25	46	9500	1.15	
6	250/1	0	300	60	/	60	28000	1.18	0

Conditions: 40 °C, bulk. ^a Determined by ¹H NMR in CDCl₃. ^b Determined by SEC in DMF/LiBr using PS calibration. ^c Determined by ¹H NMR in CDCl₃ after purification.

Next, the $\gamma M\gamma BL$ /*NVCL* feed ratio was changed ($f^{\circ}_{\gamma M\gamma BL}$ between 0.1 ~ 0.3) while keeping a [comonomers]₀/[R-Co(acac)₂]₀ ratio equal to 250 (Table 2, entries 1,4-5). The copolymerization slowed down when the $f^{\circ}_{\gamma M\gamma BL}$ was increased, i.e. the global comonomer conversion reached 46 % after 5 hours when $f^{\circ}_{\gamma M\gamma BL}$ was increased to 0.3 (entry 5 in Table 2, Figure S4b). Nevertheless, all these copolymerizations took place in a controlled manner (Figures S4a and S5). Ultimately, P(*NVCL-co- $\gamma M\gamma BL$*) copolymers of low dispersity (1.15~1.21) and molar mass between 9500 g/mol and 13000 g/mol were collected. The copolymer composition was successfully tuned by adjusting the $\gamma M\gamma BL$

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content in the copolymerization feed, namely $F_{\gamma\text{M}\gamma\text{BL}}$ increased from 0.07 to 0.12 as $f^{\circ}_{\gamma\text{M}\gamma\text{BL}}$ increased from 0.1 to 0.3.

Hydrolysis of the $\gamma\text{M}\gamma\text{BL}/\text{NVCL}$ statistical copolymers.

The copolymerization of NVCL with hydrophobic or hydrophilic monomers is a widespread approach to modulate its LCST and meet the requirements of specific applications.¹⁸ In this respect, P(NVCL-co- $\gamma\text{M}\gamma\text{BL}$) is of particular interest since the ring strain of butyrolactone and the electrophilicity of its carboxyl group enable a wide variety of post-polymerization modifications that are likely to change the hydrophilicity of the copolymer, and so its thermo-responsive behavior. In particular, we considered the hydrolysis of the lactones of P(NVCL-co- $\gamma\text{M}\gamma\text{BL}$) in order to generate hydroxy acid functions along the PVNCL backbone (Figure 3a). For this purpose, the P(NVCL-co- $\gamma\text{M}\gamma\text{BL}$) was treated with NaOH in a MeOH/H₂O (1/1:v/v) mixture for 48h at room temperature in order to hydrolyze the pendant lactone and produce pH responsive copolymers containing hydroxy carboxylate functions, namely P(NVCL-co-HPEA). The successful saponification of the butyrolactone was notably confirmed by FT-IR (Figure 3b) as assessed by the disappearance of the characteristic signal of the ester functions (C=O ester) at 1769 cm⁻¹ and the appearance of a signal at 1568 cm⁻¹ corresponding to carboxylate groups. The formation of P(NVCL-co-HPEA) was further confirmed by ¹H NMR, COSY and HSQC analyses (Figure S6).

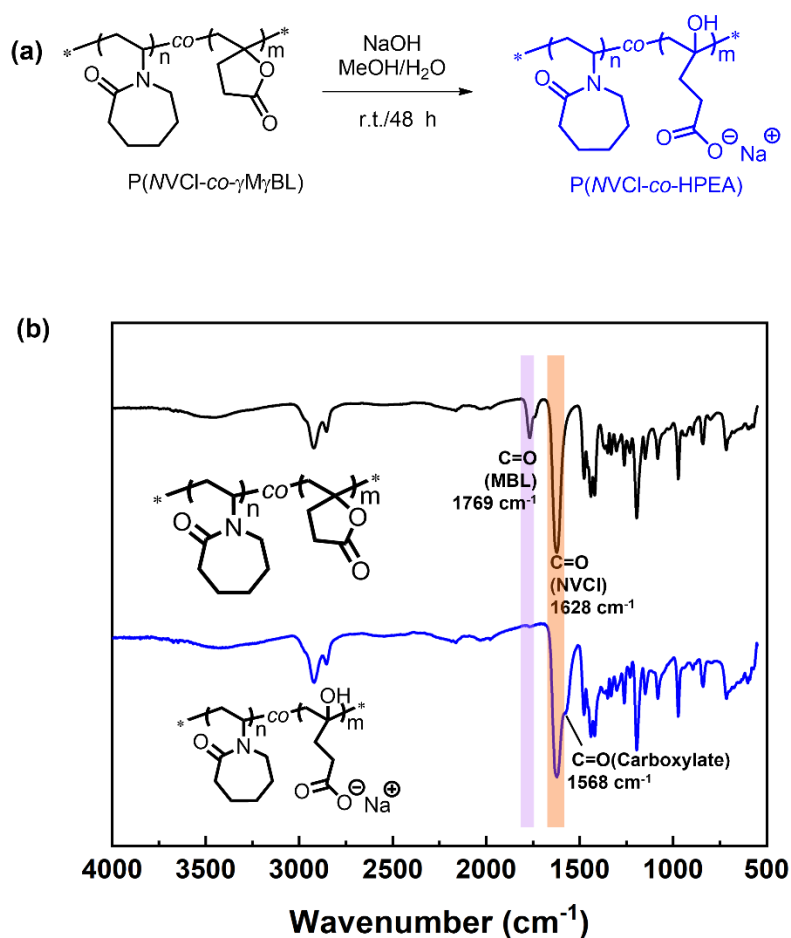


Figure 3. (a) General conditions for the conversion of P(NVCL-co- γ M γ BL) into P(NVCL-co-HPEA) and (b) FT-IR spectra of P(NVCL-co- γ M γ BL) (sample from Table 2, entry 4) before (black) and after hydrolysis (blue) of the lactone functions.

Thermo-responsiveness of the γ M γ BL/NVCL and HPEA/NVCL statistical copolymers.

The effect of the incorporation of γ M γ BL in PNVCL and of the hydrolysis of the pendant lactones on the solution behavior of the copolymers was then studied. The cloud point temperature (T_{CP}) of the copolymers aqueous solutions (5 mg/mL) were measured by turbidimetry. A heating rate of $2 \text{ }^\circ\text{C}/\text{min}$ was used and T_{CP} was defined as the temperature at which half of the transmittance of the solution was lost. First, we examined the thermal response of a series of P(NVCL-co- γ M γ BL)s with a γ M γ BL molar fraction around 0.07 and a molar mass ranging from 13000 to 33000 g/mol (Table 2, entries 1-3). A PNVCL of 10000 g/mol characterized by a T_{CP} of $36.9 \text{ }^\circ\text{C}$ served as reference. According to the

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overlay of the transmittance vs temperature plots, the incorporation of hydrophobic γ M γ BL in the PMVCL decreased the T_{CP} by few degrees to 34.2 °C (Figure 4a). The transition temperature of the P(NVCL-co- γ M γ BL) with similar composition (Figure 4a) also slightly decreased from 34.2 °C to 33.1 °C as the molar mass of the copolymer increased from 13000 to 33000 g/mol in line with the typical Flory–Huggins behavior of PMVCL-based copolymers.¹⁸ In addition, increasing the γ M γ BL content of the copolymer from 7 to 12 mol% while keeping similar molar masses (9500-13000 g/mol, Table 2 entries 1,4,5) decreased the T_{CP} from 34.2°C to 31.1 °C (Figure 4b). In all cases, hysteresis of only few degrees was observed.

According to the previous section, the hydrophobic γ M γ BL units of P(NVCL-co- γ M γ BL)s were converted into more hydrophilic hydroxy carboxylate functions upon treatment with NaOH. The T_{CPS} of the resulting P(NVCL-co-HPEA)s, measured at pH 6 to ensure proper deprotonation of the carboxylic groups, were superior to those of their P(NVCL-co- γ M γ BL)s precursors (Figure 4c). For example, the transition temperature of the P(NVCL-co-HPEA) containing only 7 mol% of hydroxy carboxylate functions was 24.1 °C higher compared its γ M γ BL counterpart (Table 2, entry 1). Moreover, no precipitation of the P(NVCL-co-HPEA) was observed between 10 °C and 80 °C when the HPEA content reached 10 and 12 mol%. Overall, the introduction γ M γ BL within PMVCL and the post-polymerization modification of the lactones constitute a promising platform for tuning the T_{CP} of the copolymer solution over a wide temperature range.

Dynamic light scattering (DLS) analyses also revealed different solution behaviors for P(NVCL-co- γ M γ BL) and P(NVCL-co-HPEA) ($F_{\gamma M \gamma BL} = F_{HPEA} = 0.07$) above their T_{CP} (Figure 5). A transition from free chains to particles was observed when the aqueous solution of the γ M γ BL -containing copolymer exceeded the T_{CP} , namely 34.2 °C. In this case, the diameter of the particles increased from 500 nm to several microns as temperature increased from 35 °C to 55 °C due to dehydration and aggregation of P(NVCL-co- γ M γ BL) that is deprived of any stabilizing group. For P(NVCL-co-HPEA), however, only submicron particles (200-400 nm) were observed above the T_{CP} , namely 61.0 °C. In contrast to P(NVCL-co- γ M γ BL), the carboxylate functions of P(NVCL-co-HPEA) provided electrostatic stabilization to the particles and prevented their further aggregation. It is noteworthy that the particle size first increased up to about 400 nm at 75 °C before decreasing to 220 nm at higher temperature. As an explanation, particles formed at 75 °C were still swollen by water and underwent further dehydration of their core when the temperature was increased leading to the reduction of their size to about 200 nm. For both copolymers, no more objects were detected by DLS after cooling the solutions to room temperature proving the perfect reversibility of the process.

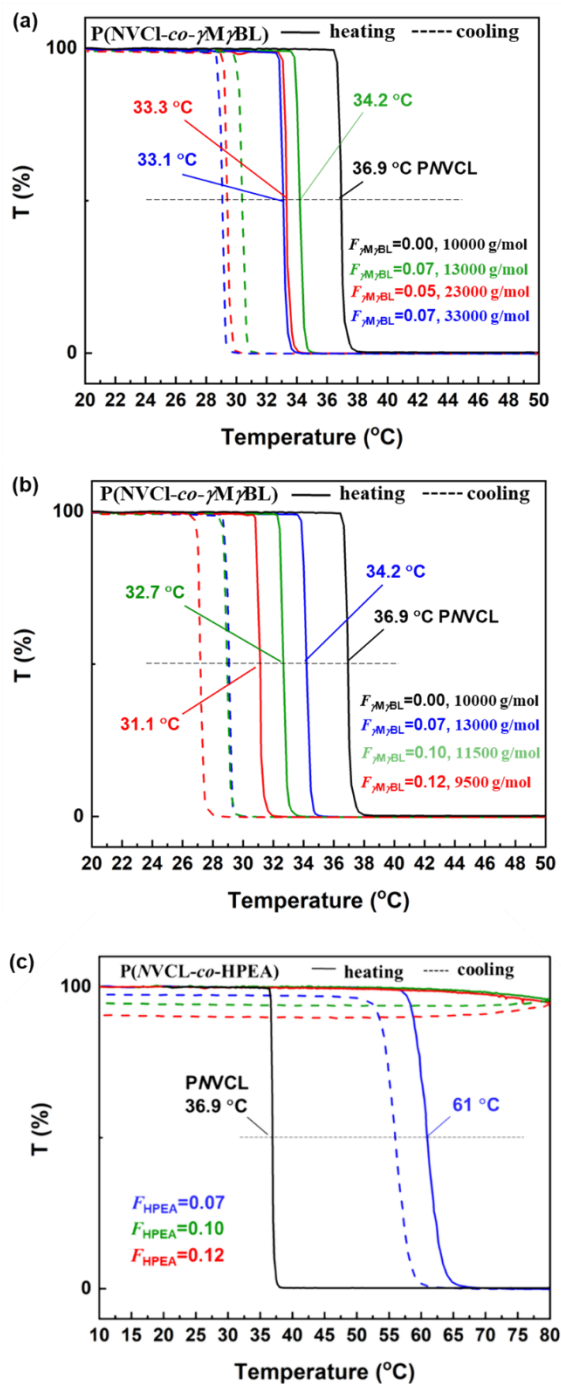


Figure 4. Turbidimetry analysis of aqueous solutions of (a) P(NVCL-co- γ M γ BL)s with different molar masses (Table 2, entries 1-3), (b) P(NVCL-co- γ M γ BL)s with different compositions (Table 2, entries 1, 4, 5), (c) P(NVCL-co-HPEA)s with different compositions ($F_{HPEA} = 0.07 \sim 0.12$) (samples derived from precursors in Table 2, entries 1, 4, 5) at a concentration of 5 mg/mL.

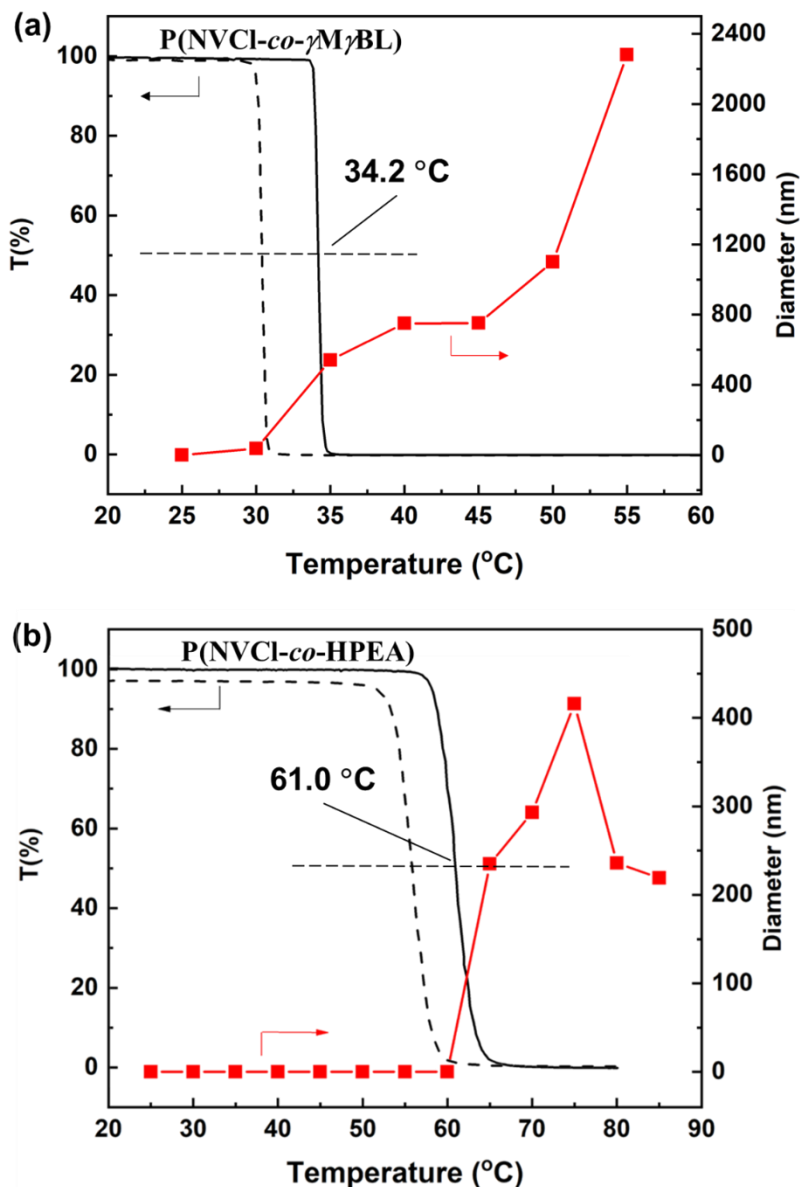


Figure 5. DLS measurements of object diameter (red line) and turbidimetry (black lines) in water at 5 mg/mL as a function of temperature for (a) P(NVCL-co- γ M γ BL) ($M_n = 13000$ g/mol, $F_{\gamma M \gamma BL} = 0.07$) and (b) the corresponding hydrolyzed P(NVCL-co-HPEA) ($F_{HPEA} = 0.07$) at pH 6.

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pH-responsiveness of HPEA/NVCL statistical copolymers

The pH-responsive behavior of carboxylic acid-containing P(NVCL-co-HPEA) was also explored. As mentioned above and illustrated in Figure 6a, at 25 °C and pH 6, the P(NVCL-co-HPEA) containing 12 mol% of HPEA units was mainly in its deprotonated state and completely soluble in water. After acidification with HCl to pH 2, the solution became instantaneously turbid. This phenomenon was reported for other acid-functionalized PNVL such as P(NVCL-co-AA),⁴⁷ P(NVCL-co-MAA)^{43,62} and P(NVCL-co-IA)⁴⁵ and rationalized by the formation of intra and intermolecular H-bonding between the protonated carboxylic acid of HPEA and the amide functions of the NVCL units. In sharp contrast to the previously reported acid-functionalized PNVL, however, the P(NVCL-co-HPEA) completely dissolved in water after 12 h without extra stimuli. This specific behavior of P(NVCL-co-HPEA) compared to other acid-containing PNVL derivatives was attributed to the re-lactonization reaction and the regeneration of the pristine P(NVCL-co- γ M γ BL) copolymer. This assumption was confirmed by FT-IR analyses. As shown in Figure 6b, right after acidification of P(NVCL-co-HPEA), the signal at 1568 cm⁻¹ corresponding the carboxylate moieties was replaced by a peak characteristic of the protonated carboxylic group at 1720 cm⁻¹. After 12h at room temperature and pH 2, this COOH signal spontaneously disappeared and the lactone signal reappeared at 1769 cm⁻¹ confirming the regeneration of the γ M γ BL units. A very similar behavior was observed for the copolymer containing a lower HPEA content, namely 7 mol%.

Finally, the thermo-responsiveness of the reconstructed P(NVCL-co- γ M γ BL) at pH 2 was investigated and compared to the P(NVCL-co-HPEA) before acidification (pH 6) and to the initial P(NVCL-co- γ M γ BL). After acid treatment of P(NVCL-co-HPEA) containing 7 mol% of HPEA and 12 h, the T_{CP} of the copolymer markedly decreased from 61.0 °C to 38.6 °C, so close to the T_{CP} of the starting P(NVCL-co- γ M γ BL) (Figure 7a). For the P(NVCL-co-HPEA) with higher content of HPEA (12 mol%), the acidification essentially restored the LCST behavior of the starting P(NVCL-co- γ M γ BL) copolymer which further supports the relactonization reaction in acidic medium (Figure 7b). Note however that the transition temperatures of the initial and the reconstructed P(NVCL-co- γ M γ BL)s differ by few degrees. The slight discrepancy might be due to residual traces of alcohol and acid functions in the final copolymer rendering the latter slightly more hydrophilic than the starting P(NVCL-co- γ M γ BL)s. It is also worth remembering that salts generated during the acid/base treatments might also affect the LCST behavior of the copolymer. In the end, the P(NVCL-co- γ M γ BL) copolymer exhibits a peculiar dual pH- and thermo-responsive behavior involving a unique reversible delactonization reaction.

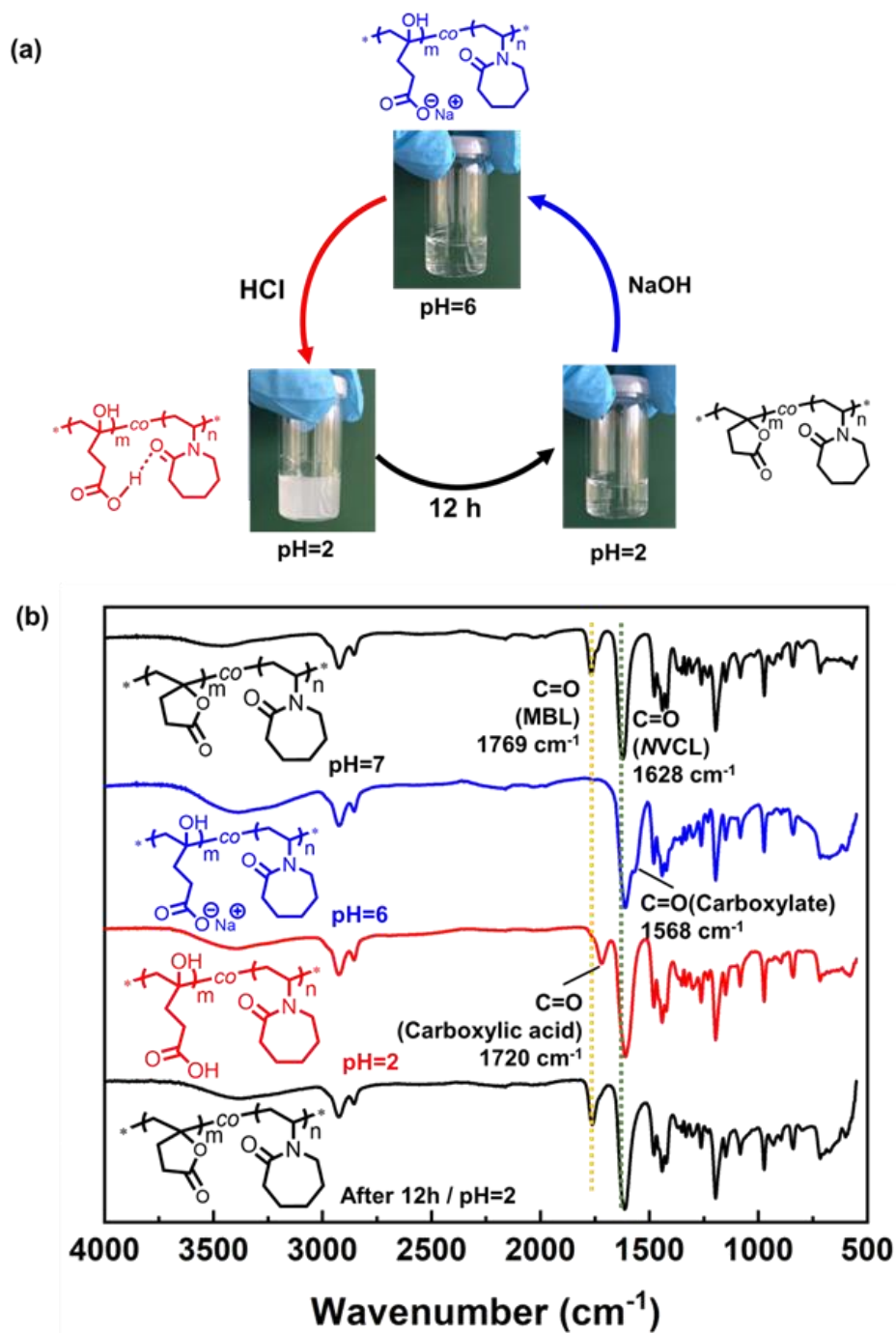


Figure 6. (a) Illustration of the solution behavior of P(MVCL-co-HPEA) ($F_{M,BL} = 0.12$, Table 2 entry 5) at different pH. (b) FT-IR spectra of the initial P(MVCL-co- γ M β BL) copolymer and P(MVCL-co-HPEA) after different acid-base treatments.

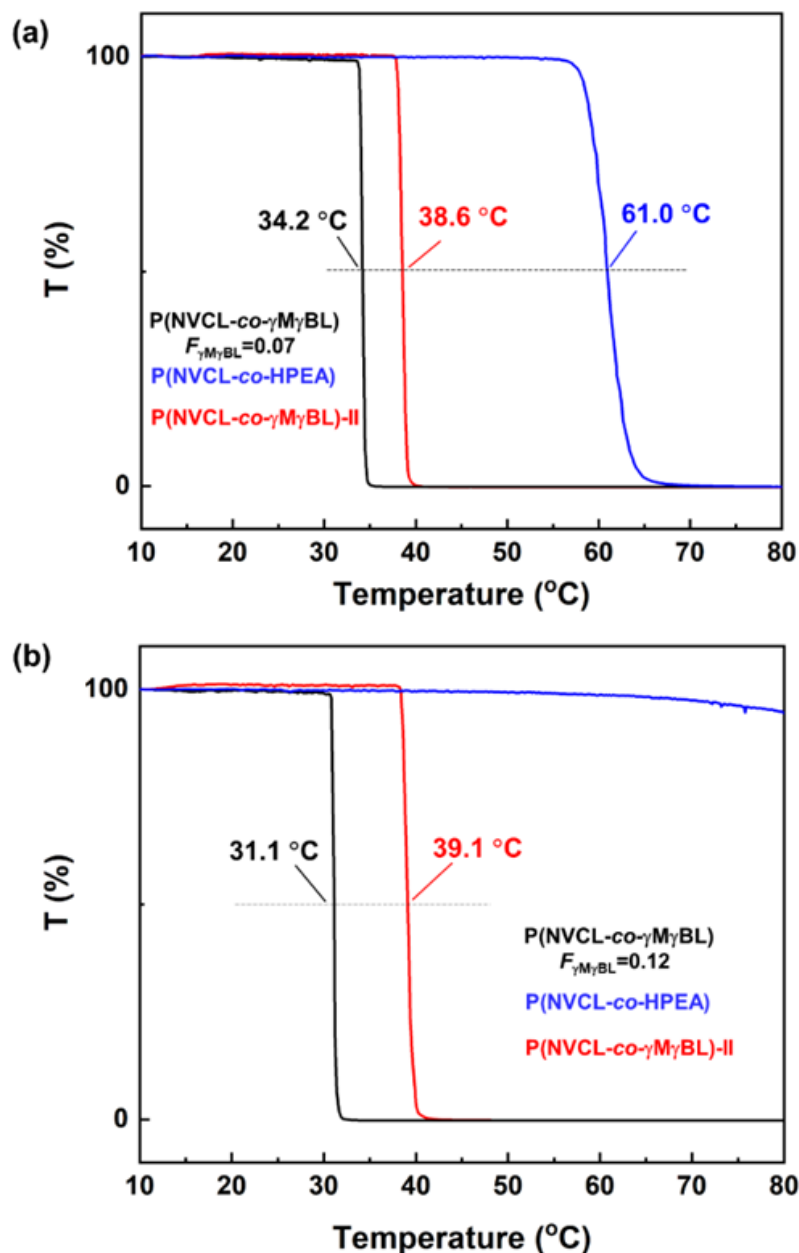


Figure 7. Turbidimetry analysis of aqueous solutions (5mg/mL) of the P(NVCL-co- $\gamma M \gamma BL$) precursors (a) $F_{\gamma M \gamma BL} = 0.07$ and (b) $F_{\gamma M \gamma BL} = 0.12$ (at pH 7, and their corresponding P(NVCL-co-HPEA)s (pH 6) and P(NVCL-co- $\gamma M \gamma BL$)-II copolymers resulting from the relectonization reaction (pH 2).

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Synthesis and stimuli-responsiveness of γ M γ BL- and HPEA-based block copolymers.

Stimuli-responsive polymers often deliver their full potential when incorporated in more complex macromolecular architectures leading to specific solution behaviors such as multistep assembly.^{11,57} In this perspective, we considered the synthesis of a *P*(NVCL-*b*-*P*(NVCL-*co*- γ M γ BL)) block copolymer and its *P*(NVCL-*b*-*P*(NVCL-*co*-HPEA)) derivative before studying their thermal response in water. In theory, the incorporation of more hydrophobic γ M γ BL or more hydrophilic HPEA units in one of the sequence of the copolymer could induce distinct transition temperatures for each block and possibly a peculiar assembly of the copolymer in solution.

Taking advantage of the controlled character of the OMRP of NVCL (Table 2, entry 6) and of its copolymerization with γ M γ BL, we synthesized well-defined *P*(NVCL-*b*-*P*(NVCL-*co*- γ M γ BL)) by sequential organometallic-mediated radical polymerization of NVCL and copolymerization of NVCL/ γ M γ BL in one-pot (Figure 8a). First, the OMRP of NVCL was performed in bulk at 40 °C using a [NVCL]/[R-Co(acac)₂] molar ratio of 500. After 1h, the NVCL conversion reached 13% leading to a *P*(NVCL-Co(acac)₂) precursor with M_n MALLs of 12600 g/mol and a dispersity of 1.04. Without prior purification, an γ M γ BL was added to this medium in order to reach a [NVCL]/[γ M γ BL] feed ratio equal to 82/18 and the copolymerization was conducted at 40 °C for 6h. As assessed by the clear shift of the SEC chromatograms towards lower elution time (Figure 8b), the chain extension polymerization was successful with an almost complete consumption of the *P*(NVCL) precursor. As a result, a well-defined *P*(NVCL-*b*-*P*(NVCL-*co*- γ M γ BL)) block copolymer (M_n MALLs = 31500 g/mol, $D=$ 1.19) was synthesized. The composition of the block copolymer was determined based on the M_n MALLs of the *P*(NVCL) precursor as well as the M_n MALLs and ¹H NMR of the final copolymer (Figure S7), namely *P*(NVCL)₉₁-*b*-*P*(NVCL)₁₁₉-*co*- γ M γ BL₂₃). Following the hydrolysis procedure described in the previous section, the γ M γ BL-containing block copolymer was successfully converted into *P*(NVCL)₉₁-*b*-*P*(NVCL)₁₁₉-*co*-HPEA₂₃).

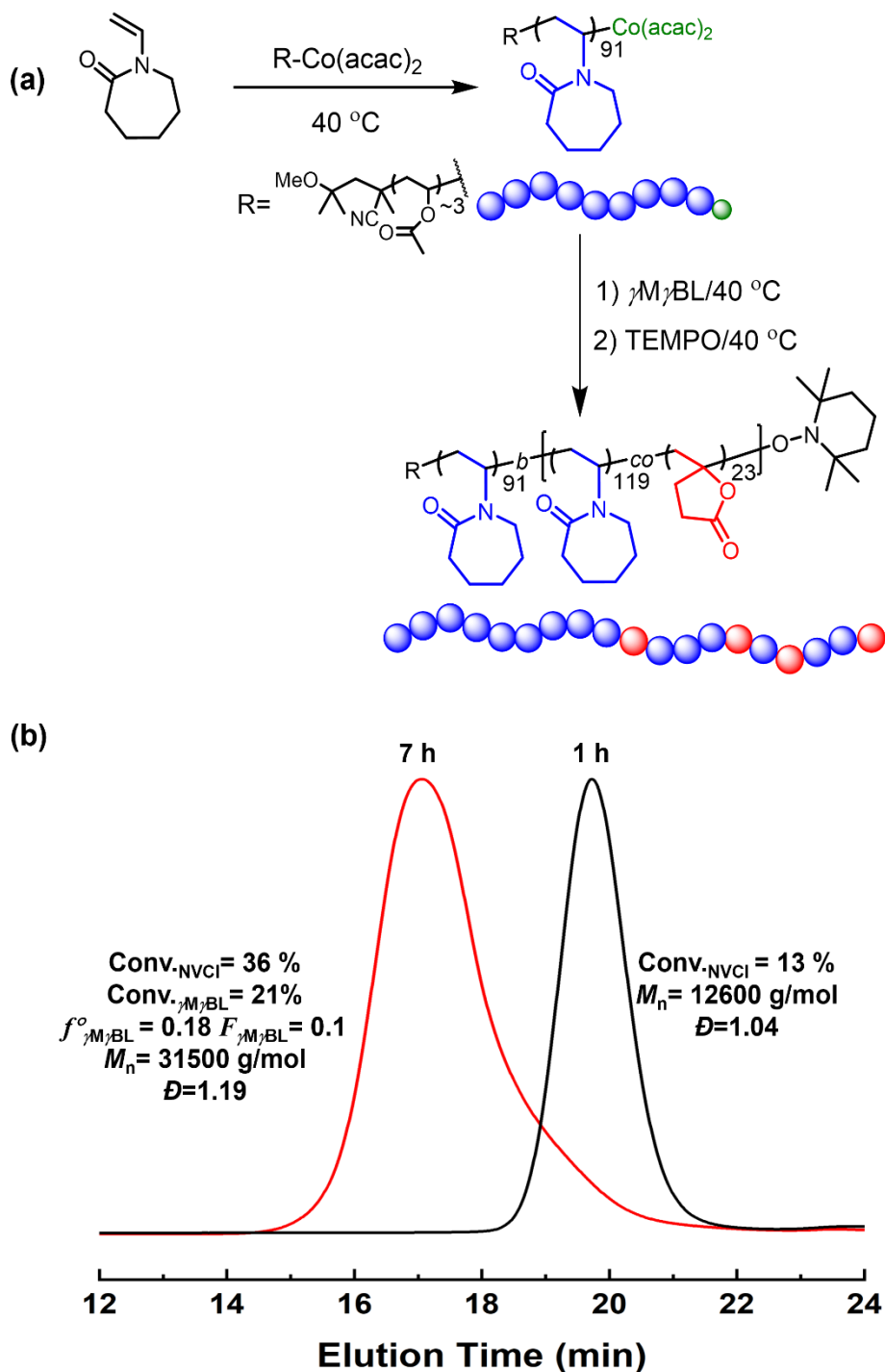


Figure 8. General strategy (a) and overlay of SEC traces (b) for the synthesis of the PMVCL-*b*-P(NVCL-*co*- γ M₂BL) block copolymer by OMRP. First step: [NVCL]₀/[R-Co(acac)₂]₀=500/1, second step: [NVCL]₀/[γ M₂BL]₀/[PMVCL-Co(acac)₂]₀=410/90/1).

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Next, the thermal response of $P(WCL_{91}-b-P(WCL_{119}-co-\gamma M\gamma BL_{23}))$ and the corresponding $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ was investigated by turbidimetry and DLS (Figure 9). Figure 9a shows the evolution of the transmittance upon heating the aqueous solution of the $P(WCL_{91}-b-P(WCL_{119}-co-\gamma M\gamma BL_{23}))$ copolymer. In this case, the LCSTs of the $P(WCL_{91}-b-P(WCL_{119}-co-\gamma M\gamma BL_{23}))$ and the $P(WCL_{119}-co-\gamma M\gamma BL_{23})$ segments were too close to be distinguished. Only one transition temperature appeared around 34 °C and DLS emphasized a transition from free chains to particles at this temperature (Figure 9a). Similarly to its statistical counterparts, the size of the $P(WCL_{91}-b-P(WCL_{119}-co-\gamma M\gamma BL_{23}))$ particles increased beyond the micron when the solution is further heated inducing sedimentation of the copolymer.

The thermal response of $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ was then studied in water at pH 6 (Figure 9b). According to the previous section, outside of a block copolymer type structure, $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ should exhibit a phase transition around 37 °C whereas the $P(WCL_{119}-co-HPEA_{23})$ in its ionized state should remain soluble at least until 80 °C. In the light of these considerations, when increasing the solution temperature above the critical transition temperature of the $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ block, one might expect the self-assembly of the copolymer from free chains to micelles composed of a dehydrated $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ core stabilized by a charged $P(WCL_{119}-co-HPEA_{23})$ shell. In practice, no transition appeared around 37-40 °C but a progressive decrease of transmittance was observed at 70.5 °C. This transition corresponded to the formation of particles with a diameter ranging from 440 nm at 75 °C to 520 nm at 85 °C. In this case, the T_{CP} of the $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ block might be shifted to higher temperature (from 34 °C to 70 °C) due to its combination with a much hydrophilic $P(WCL_{119}-co-HPEA_{23})$ block. Nevertheless, a classical self-assembly of the block copolymer into micelles by selective dehydration of the $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ block at 70.5 °C is quite unlikely considering the rather large size of the objects. Instead, the $P(WCL_{91}-b-P(WCL_{119}-co-HPEA_{23}))$ most probably follows a complex particle formation pathway upon heating with a preferential location of the $P(WCL_{119}-co-HPEA_{23})$ segment at the outer region of these nanoobjects. In this respect, zeta potential analyses confirmed the presence of negative charges at the surface of the particles (from -40 to -55 mv, Table S1) which contribute to their stabilization preventing further aggregation. Again, DLS confirmed the complete dissolution of the objects upon cooling and so the reversibility of the transition phenomena.

Overall, this block copolymerization proves that $P(WCL-co-\gamma M\gamma BL)$ and $P(WCL-co-HPEA)$ segments can be incorporated in more complex polymer architectures. In the future, the latter could be associated to other hydrophilic, hydrophobic, thermoresponsive or pH-sensitive sequences paving the way to unique stimuli-responsive materials.

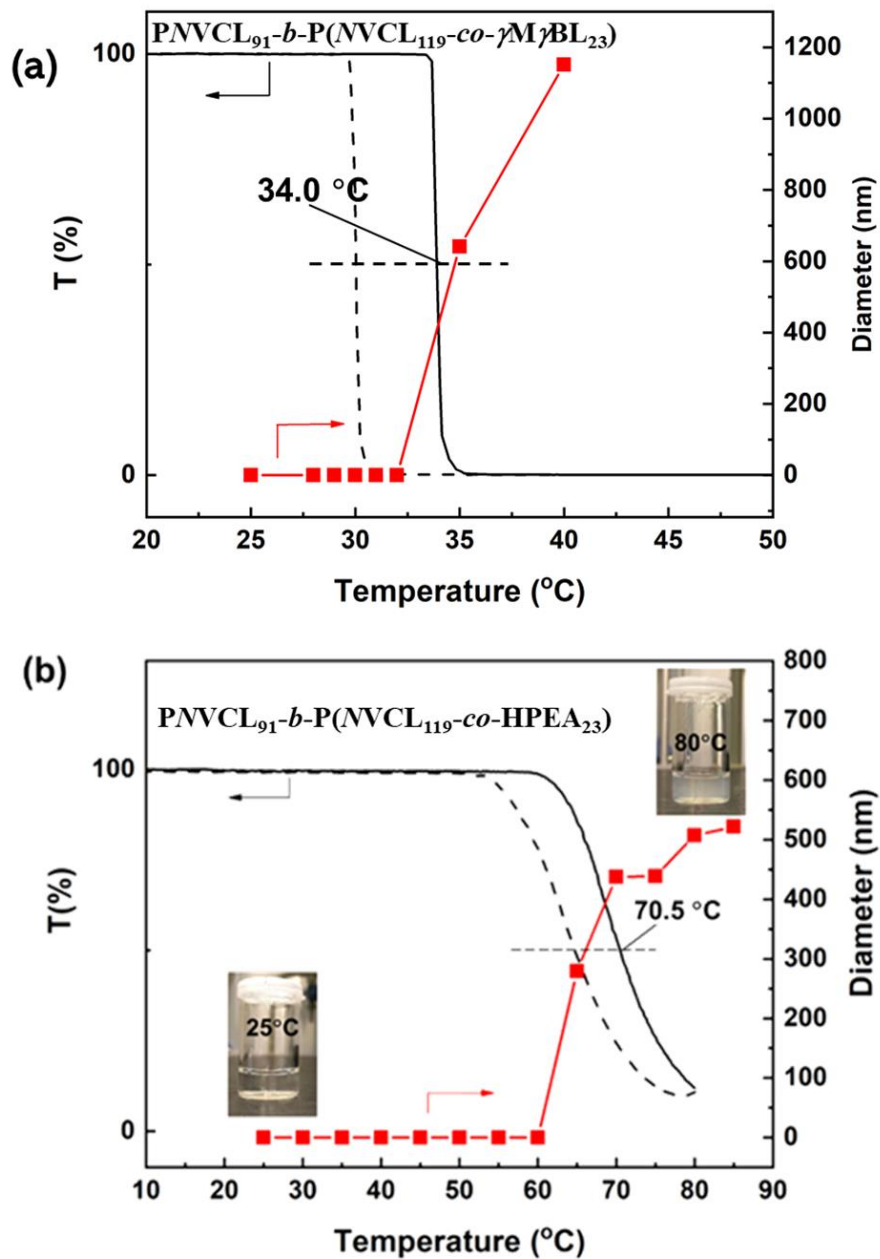


Figure 9. Turbidimetry (black lines) and object diameter measured by DLS (red lines) in water at 5 mg/mL as a function of temperature for (a) $\text{PNVCL}_{91}\text{-}b\text{-P}(\text{NVCL}_{119}\text{-}co\text{-}\gamma\text{M}\gamma\text{BL}_{23})$ and (b) the corresponding hydrolyzed $\text{PNVCL}_{91}\text{-}b\text{-P}(\text{NVCL}_{119}\text{-}co\text{-HPEA}_{23})$ at pH 6. The dotted line correspond to the cooling curve.

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Conclusions

In this work, we reported the successful statistical radical copolymerization of *N*VCL with a methylene lactone monomer, namely γ M γ BL, which constitutes a unique and promising platform for the design of multi-responsive *N*VCL-based materials. A series of low dispersity *N*VCL/ γ M γ BL-based copolymers with different molar masses and compositions were produced via organometallic-mediated radical polymerization, a reversible deactivation radical polymerization known for its efficiency to mediate the radical polymerization of less activated monomers (LAMs), a category comprising the comonomers under investigation here. The synthesized *N*VCL/ γ M γ BL-based copolymers displayed a T_{CP} in water which decreases when increasing the copolymer molar mass and \square M \square BL content. As post-polymerization modification, we described the successful hydrolysis of the pendant lactones leading to the corresponding ring opened hydroxy acid PN*N*VCL-based copolymers. This chemical transformation allowed to tune the cloud point temperature of the copolymer solution over a wide range of temperatures (from 27 °C to >80 °C). The pH responsiveness of the hydroxy acid functional *N*VCL-based copolymer was examined in more details. At pH 6, so in its deprotonated state, the copolymer composed of 7 mol% of hydroxy carboxylate monomer units showed a T_{CP} of 61 °C and no transition was observed below 80 °C when this content was increased to 10 mol%. Upon reacidification at pH 2, the hydroxy acid-functional PN*N*VCL solution first became opaque due to the protonation of the carboxylate groups and the formation of acid/amide H-bonds but, in sharp contrast with the previously reported acid-functional PN*N*VCL copolymers, this solution cleared up spontaneously over time at room temperature. This phenomenon was demonstrated to be due to the relactonization reaction and the regeneration of the initial *N*VCL/ γ M γ BL-based copolymers exhibiting the original LCST behavior. Finally, the stimuli responsiveness of a well-defined PN*N*VCL-*b*-P(*N*VCL-*co*- γ M γ BL) block copolymer and the corresponding PN*N*VCL-*b*-P(*N*VCL-*co*-HPEA) was investigated by turbidimetry, DLS and zeta potential analyses. Overall, this unusual multi-responsive lactone-functionalized PN*N*VCL system involving a pH dependent and reversible delactonization reaction constitutes a promising platform for tuning the solution properties of PN*N*VCL, and possibly of other smart polymer sequences, opening up new prospects and innovation possibilities.

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