Temperature Responsive PEG-based Polyurethanes "*à la carte*"

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Abstract

Temperature responsive polymers able to alter their chemical or physical properties have been extensively investigated. Most of these polymers have an alkane polymer backbone with only carbon-carbon bonds. In this sense, the design of thermoresponsive polymers not only with sharp transition temperatures but also possessing hydrolizable linkages such as esters, carbonates or urethanes in the main backbone are highly desired. Here we show a library of thermoresponsive cationic and anionic polyurethanes synthesized by copolymerization of isophorone diisocyanate with poly(ethylene glycol) and ionic diols. These polyurethanes exhibit lower critical solution temperatures (LCST) that can be easily tuned from 20 °C to 60 °C by altering the polyurethane composition. Our findings show that LCST temperature could be reduced by the using poly(ethylene glycol) of lower molecular weight and/or increasing the hydrophobicity of the employed ionic diol. We also demonstrated that the thermoresponsive behavior could be translated to hydrogels based on those ionic polyurethanes. These "a la carte"

thermoresponsive polyurethanes materials present a great potential in the biomedical field due to the presence of hydrolizable linkages in the main polymer backbone.

Keywords
Polyurethanes, LCST, thermoresponsive, hydrogels

1. Introduction

Polymers are ubiquitous materials in our everyday life and are continuously under development. One of the most interesting classes of polymers are those responding to different stimuli. For instance, functional polymers with thermoresponsive behavior in particular in aqueous media have been considered "intelligent" materials and have been envisioned for a variety of relevant, including drug and gene delivery, tissue engineering, biosensing, and bioseparation or pressure sensitive adhesives [1–5]. The majority of research is based on poly(N-isopropylacrylamide) (PNIPAM), polyvinylcaprolactam and poly(ethyleneglycol) and their copolymers [6–10]. The main reasons for their broad utilization are the simplicity of their synthesis together with the versatility of these polymers to easily tailor their thermal transition temperature close to the body temperature. Nevertheless, in all these cases the main polymer backbone is formed by carbon-carbon linkages which are not susceptible to suffer any hydrolytic degradation in-vivo. As a consequence, there is a great demand for polymers with controllable low critical solution temperature (LCST) that contain hydrolizable linkages in the main backbone. Thus, thermoresponsive polymers composed of hydrolytically degradable units such as polyesters, polyurethanes and polycarbonates have been developed in the last decade. Among them, polyurethanes (PUs) are considered a versatile class of materials. Among different industrial applications in coatings or

foams, they have been implemented in vivo in a variety of devices such as in heart valves, vascular grafts, catheters, prostheses and other blood contacting devices, on account of their versatility and biocompatibility [11–14]. However, there have been very few studies on PUs showing LCST [15–21]. Thus, our group very recently reported the preparation of thermoresponsive PUs by copolymerization of PEG and a second diol 2,2-bis(hydroxymethyl)-propionic acid (bis-MPA) with isophorone diisocyanate [22]. These polyurethanes presented the ability to adjust the LCST modifying the hydrophilic/hydrophobic ratio and to sequester doxorubicin anticancer drug. Nevertheless, we found that only the systems with high PEG content up to 75 mol % were able to promote phase transition near to biologically relevant temperatures.

Hence, in this work we extended the range of polyurethanes showing LCST behavior and having cationic or anionic groups. Polycations are very interesting materials for biomedical applications due to its use as temperature responsive, DNA or protein delivery, and polyanions have been also explored for the encapsulation of amine containing drugs [11,23,24]. In this sense, developing cationic and anionic thermoresponsive polyurethanes with LCST close to body temperature is intriguing. As far as we know, a comprehensive study to understand the factors governing the LCST of ionic polyurethanes has not been presented. We found that depending on the composition of the polymer the LCST temperature could be adjusted. Thus, the effect of the PEG length as well as the nature of the hydrophilicity of the diol in the LCST temperature has been investigated in order to design polyurethanes *in à la carte* fashion. Finally, we showed that this thermoresponsvie behavior could be translated to a three-dimensional structures such as hydrogels showing thermoresponsive behavior.

2. Experimental

2.1. Materials

All materials were purchased from Sigma-Aldrich. PEG diols (PEG_{600}, M_n = 600 Da; PEG_{1000}, M_n = 1000 Da; PEG_{1500}, M_n = 1500 Da) and 2,2-bis(hydroxymethyl)-propionic acid (bis-MPA) were dried by azeotropic distillation in benzene. Isophorone diisocyanate (IPDI) was dried by stirring with CaH_2 in anhydrous dichloromethane (DCM), followed by filtration and solvent removal in vacuo. N-methyldiethanolamine (MDEA) was purified by distillation in vacuo. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was stirred and distilled under reduced pressure over CaH_2 prior to use. Methane sulfonic acid anhydrous (MSA) was used as received. The rest of material were used as received.

2.2. Synthesis of diols with quaternary amines

N-methyldiethanolamine was quaternized with bromoethane and bromohexane, in order to obtain *N*,*N*-bis(2-hydroxyethyl)-*N*-methylbutan-1-aminium bromide (Et-MDEA) and *N*,*N*-bis(2-hydroxyethyl)-*N*-methylhexan-1-aminium bromide (Hex-MDEA) quaternary amine containing diols, respectively.

Et-MDEA was synthesized dissolving MDEA (5 g, 0.042 mol, 1 equiv.) and bromoethane (9.14 g, 0.084 mol, 2 equiv.) in 100 ml of acetonitrile (ACN). The reaction mixture was carried out for 72 h. (Scheme S1 a, Supporting Information). The precipitated product was filtrated, washed with diethyl ether to remove the excess of bromoethane and N-methyldiethanolamine and dried under vacuum. The dried white powder was recrystallized from DMF, and crystals were washed with diethyl ether and dried under vacumm, to give 7.91 g (82 %). The structure was confirmed by ^{1}H NMR

spectroscopy. ^{1}H NMR (300 MHz, DMSO-d6): δ = 5.25 (t, 2H), 3.84 (t, 4H), 3.55 – 3.4 (m, 4H), (m, 2H), 3.08 (s, 3H), 1.26 (t, 3H) (Figure S1, Supporting Information).

Hex-MDEA was prepared in a similar manner mixing MDEA (5 g, 0.042 mol, 1 equiv.) with excess of bromohexane (13.86 g, 0.084 mol, 2 equiv.). The reaction was carried out at 50 ºC during 48 h. (Scheme S1 b, Supporting Information). The product (viscous liquid) was washed several times with diethyl ether, to remove the excess of bromohexane and dried under vacuum, to give 10.32 g (86 %). ^{1}H NMR (300 MHz, DMSO-d6): δ = 5.25 (t, 2H), 3.84 (t, 4H), 3.5 – 3.35 (m, 4H), (m, 2H), 3.1 (s, 3H), 1.7 (m, 2H), 1.3 (m, 6H), 0.89 (t, 3H) (Figure S2, Supporting Information).

2.3. Synthesis of diol with a pendant methacrylic group (bis-MPA-methracrylate)

Bis-MPA-methacrylate was synthesized in three steps (Scheme S2, Supporting Information). In the first step, a mixture of bis-MPA (10.0 g, 1 equiv.), 2,2-dimethoxypropane (13,8 mL, 1.35 equiv.), and PTSA (0.7 g 5 %wt.) was stirred in 50 mL of acetone overnight at room temperature. To the reaction mixture 1mL of a solution 1:1 NH_4OH 30%:EtOH was added. The reaction mixture was evaporated to dryness and the residue was redissolved in 150 mL of DCM and rinsed three times with distilled water (20 mL), dried with Na_2SO_4. The solution was filtered and dried under vacuum. A white powdery residue was recovered 12 g (93 %). The ^{1}H NMR spectrum was compared against reported literature and found to be identical.

The second step consisted on the esterification of protected diol. To a solution of protected acid (3 g, 1.0 equiv.) in 150 mL of anhydrous DCM, 2-hydroxyethylmethacrylate (2.24 g, 0.9 equiv.) was added and the reaction mixture was kept 15 minutes in an ice bath. DIC (2.39 g, 1.1 equiv.) and DMAP (0.21 g, 0.1 equiv.) were then added and stirred for 48 h at room temperature, letting the ice melt slowly.

After the reaction completion, the reaction mixture was filtered, to eliminate the urea salts precipitated during the reaction. The product containing filtrate was diluted with extra 250 mL of DCM and rinsed 3 times with 100 mL of water. The organic phase was dried over Na_2SO_4, and concentrated. The concentrated product was loaded onto a silica gel column and purified by flash column chromatography using a mixture of hexanes/EtOAc (7:3 v/v), Rf=0.69, to give 6.65 g (84 %) as yellowish liquid. ^{1}H NMR (400 MHz, DMSO-d6) δ 6.04 (s, 1H), 5.71 (s, 1H), 4.34 (s, 4H), 4.03 (d, J = 11.6 Hz, 2H), 3.61 (d, J = 11.6 Hz, 2H), 1.88 (d, J = 1.3 Hz, 3H), 1.36 (s, 3H), 1.24 (s, 3H), 1.07 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 174.34 , 167.13 , 136.40 , 126.83 , 98.15 , 65.73 , 62.89 , 42.07 , 25.85 , 22.89 , 18.85 .

The third and last step is the deprotection of the acetonide moiety. 1.9 g of the protected diol methacrylate were added to 400 mL of MeOH, together with 9.5 g of DOWEX® 50W-X8, and let stirred overnight at room temperature. Filtration of the suspension and drying under vacuum of the solvent lead the diol methacrylate as pure compound in quantitative yield (~100%). Rf=0.05 hexanes/EtOAc (7:3 v/v). ^{1}H NMR (400 MHz, DMSO-d6) δ 6.05 (s, 1H), 5.70 (s, 1H), 4.66 (t, J = 5.5 Hz, 2H), 4.39 – 4.15 (m, 4H), 3.74 – 3.40 (m, 4H), 1.89 (s, 3H), 1.04 (s, 3H) (Figure S3, Supporting Information). ^{13}C NMR (101 MHz, DMSO-d6) δ 174.53 , 166.40 , 135.69 , 126.00 , 63.74 , 62.27 , 61.52 , 50.22 , 17.92 , 16.76.

2.4. Synthesis of thermoresponsive PUs

All PUs were synthesized according to Scheme 1 using IPDI as diisocyanate, 50 mol % of PEG diol as soft segment and 50 mol % of functional monomer. The synthesis of thermally responsible PUs was performed in a glove box. Ten PUs were synthesized by copolymerization of PEG of different molecular weight (PEG_{600}, PEG_{1000} and PEG_{1500}),

with other diols such as bis-MPA, MDEA, Et-MDEA and Hex-MDEA (Table 1).

In a typical polymerization, diols (1 equiv., 2.60 mmol) and the diisocyanate (1.00 equiv., 2.60 mmol) were dissolved in anhydrous solvent (1 M). MSA catalyst (5 mol %) was employed in the experiments with anionic diol (bis-MPA), while DBU catalyst (5 mol %) was used in the polymerizations with cationic diols (MDEA, Et-MDEA and Hex-MDEA). The polymerizations were carried out at 30 °C until full conversion and monitored by FTIR and ^{1}H NMR. The resulting polymers were purified by precipitation in diethyl ether and dried under vacuum.

2.5. Free-radical UV crosslinking with pendant methacrylic groups

In a nitrogen-purged glovebox, a 20-mL vial was charged with PEG_{1000} diol (1.261 g, 1.261 mmol, 0.485 equiv.), IPDI (0.58 g, 2.60 mmol, 1 equiv.), bis-MPA (0.169 g, 1.261 mmol, 0.485 equiv.), bis-MPA-methacrylate (0.0192 g, 0.078 mmol, 0.03 equiv.) and 5.3 ml dichloromethane (1 M). MSA (0.012 g, 0.13 mmol, 0.05 equiv.) was added to the stirred solution. The reaction was carried out until full conversion monitored by ^{1}H NMR. The resulting polymers were purified by precipitation in diethyl ether and dried under vacuum. Afterwards, 0.3 g of polyurethane and 0.15 mg of 2 hydroxy-2 methylpropiophenone photoinitiators were dissolved in 3 ml of DCM and casted onto a silicon mold. The mold was then placed in a UV chamber and photopolymerized using a Dymax UV-5 conveyor belt system with an intensity of 800 mW/cm^2 operating at a wavelength of 365 nm. The tape speed was set at 0.9 m/min, giving a sample exposure time under the UV lamp of 7 seconds, and 3 repetitive cycles were applied to ensure complete photopolymerization.

2.6. Characterization

^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded at room temperature on a Bruker Avance 300 spectrometer operating at 300 MHz. Samples were dissolved in deuterated dimethyl sulfoxide (DMSO-d6).

Fourier transform infrared spectroscopy (FTIR) was used to confirm the polymerization process, using a Nicolet Magna 560 spectrometer at a resolution of 2 cm^{-1}, and a total of 64 interferograms were signal-averaged. Samples were prepared by solution-casting the reaction mixture onto the KBr plate.

The molecular weight distribution and averages of PUs were determined by Size exclusion chromatography (SEC). PUs with tertiary and quaternary amines in the composition were characterized using a Agilent Technologies PL-GPC 50 Integrated GPC system, with a Shodex KD-806M column. DMF with a 10mM concentration of LiBr at 50 °C was used as the solvent and toluene as a marker. On the other hand, the molecular weight of PUs with carboxylic acid groups were determined in tetrahydrofuran (THF) at 30 °C using a Waters chromatograph equipped with four 5 mm Waters columns (300 mm × 7.7 mm) connected in series with increasing pore size (100, 1000, 10^5, 10^6 Å). Polystyrene of different molecular weights, ranging from 2100 g mol^{-1} to 1920000 g mol^{-1}, were used for the calibration of the SEC.

The optical transmittance of the polymer aqueous solution of various temperatures was measured using a UV–vis spectrometer (UV-2550 Shimadzu) equipped with a temperature-controllable cell. Polymer solutions (1 mg mL^{-1} unless otherwise mentioned) were prepared in Phosphate Buffer Saline (PBS, 150 mM) and left at 4 °C overnight to ensure complete dissolution and equilibration. The measurements were performed with a wavelength of 600 nm, and the sample solutions were equilibrated for

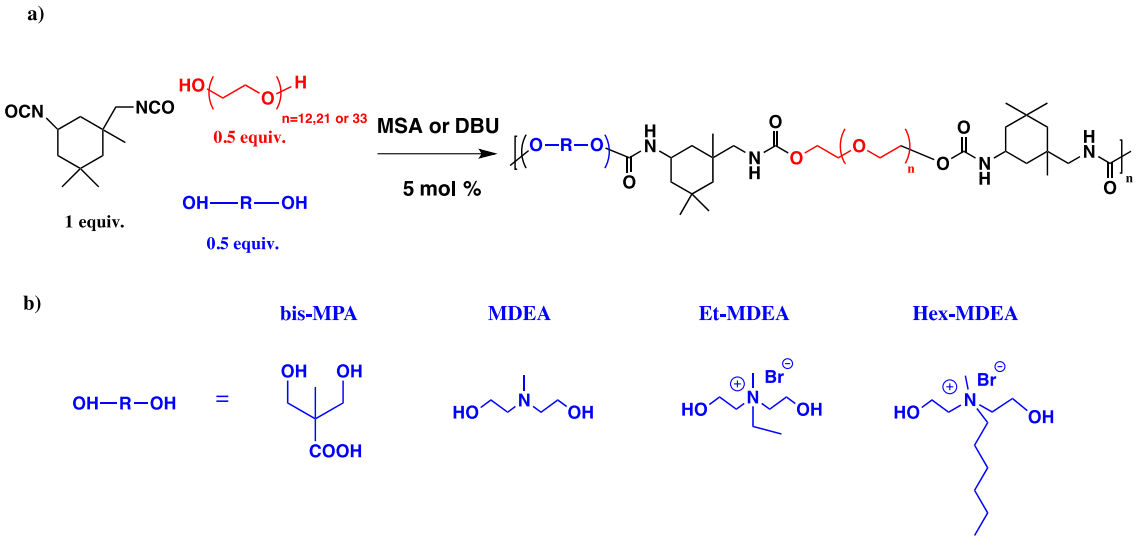
10 min before each measurement. The cloud point temperature (Tcp) was defined as the temperature at which the change in absorbance started decreasing. The measurements of Tcp were carried out in triplicates, the arithmetic means and the standard deviations are reported.

Rheology measurements on the gels were conducted on Anton Paar Physica MCR 101 rheometer using oscillatory tests with parallel plate geometry to plot G' and G" versus frequency.. Angular frequency sweeps from 0.0628 s^{-1} to 314s^{-1} at constant strain amplitude (γ = 1%) were applied at 25 °C and 35 ºC.

3. Results and discussion

3.1. Synthesis of temperature responsive ionic polyurethanes

In the presented work, we explore the preparation of thermally responsible polyurethanes. The polymerization was accomplished by dissolving equimolar amounts of diisocyanate and diols. In order to study the effect of the ionic structure in the LCST, IPDI was polymerized with low molecular weight PEG and with 4 different ionic diols (Scheme 1)



Scheme 1. General organocatalyzed synthesis of PUs from IPDI, PEG and diols with different functionality.

Bis-MPA was chosen as anionic diol and MDEA, Et-MDEA and Hex-MDEA were chosen as cationic diols. In the presence of bis-MPA a strong Brønsted acid such as MSA, which activates the isocyanate group (electrophilic activation), was used as catalyst, while in the case of cationic diols (MDEA, Et-MDEA and Hex-MDEA), a superbase such as DBU was employed to activate the alcohol group (nucleophilic activation). The choice of catalyst was logical based on the mutual compatibility of the functional group and catalyst [22,25]. In addition, three different molecular weight PEG diol (600, 1000 and 1500) were used to study the effect of the molecular weight in the LCST. Table 1 summarized the 9 linear polyurethanes synthesized for this study.

The polymerizations were monitored by FTIR and ^{1}H NMR until full conversion (>98 mol% in all reactions). Figure 1 shows the FTIR spectra of the initial reaction mixture (t = 0) for PU1 where appears the characteristic band of isocyanate stretch (N=C=O) at 2265 cm^{-1}. As the reaction proceeded, there was a decrease in the intensity of the peak at 2265 cm^{-1}, followed by a complete disappearance of the band, while the intensities of absorptions assigned to urethane groups emerged at 1720 cm^{-1} and at 1550 cm^{-1}, confirming successful urethane linkage formation. Similar behavior was observed for the 9 polyurethanes.

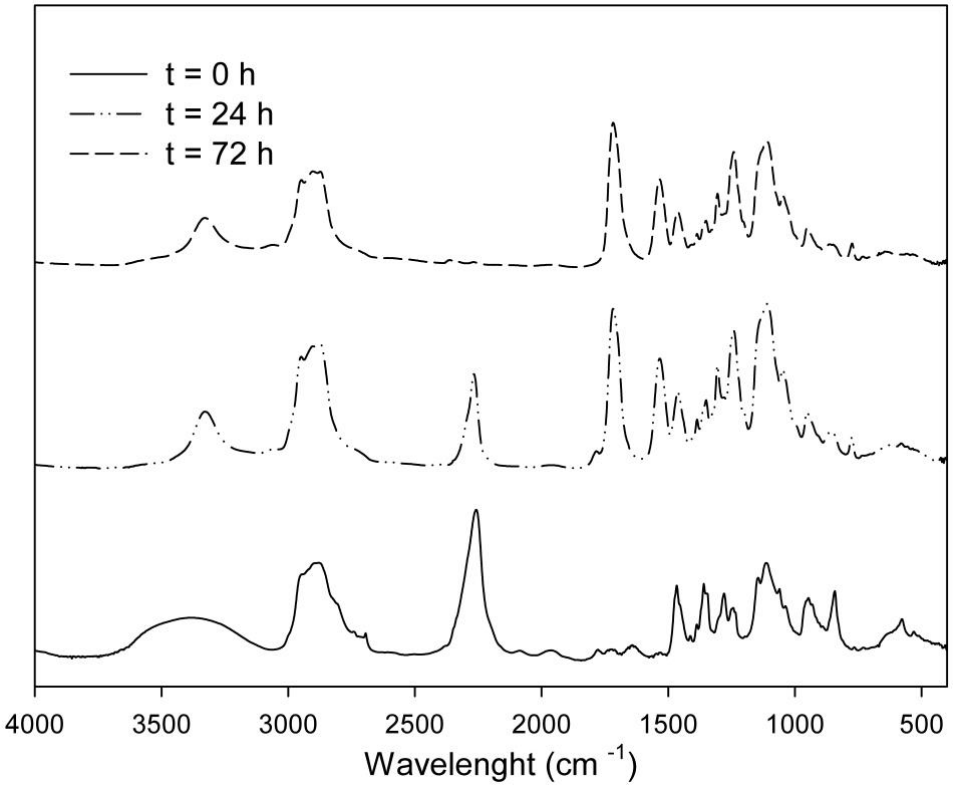


Figure 1. Infrared spectra recorded at different reaction times for **PU-bis-MPA-PEG$_{600}$.**

To further verify the polymerization process, ^{1}H NMR studies were also carried out (Figure S4, Supporting Information). As observed, when the reaction proceeded, characteristic signals (δ = 3.10 ppm) of the methylene protons proximal to the isocyanate disappeared and a new signal due to methylene protons linked to the urethane groups appeared at δ = 4.20 ppm. This demonstrates the complete consumption of the diisocyanate and the polyurethane formation. SEC analysis was also performed to verify the formation of high-molecular weight polymers. The weight average molecular weight (Mw) and polydispersity index (Đ) of PUs are listed in Table 1. All PUs presented high molecular weight between 26,000 and 47,000 g/mol and Đ between 1,5 and 1,8 in good agreement with those reported for isocyanate-alcohol polymerizations catalyzed with MSA and DBU [25,26]. Additionally, for all synthesized PUs, the calculated molar ratios of PEG/ionic diol were close to the original feed ratio (Table 1).

3.2. Characterization of temperature responsive ionic polyurethanes

Thermoresponsive polymers exhibit a solution transition at a certain temperature, which causes a sudden change in the solvation state. Polymers, which become insoluble upon heating, have a so-called "Low Critical Solution Temperature" (LCST). Below the LCST, the polymer is solvated in water corresponding to a random coil structure of the polymer chain. However, around LCST, the intermolecular interactions become favored, and the polymer is dehydrated. As a consequence the polymer chains change their conformation to a dense globular structure. For this kind of thermoresponsive polymers, the transitions temperatures are not absolute LCST, because the transitions are concentration dependent [15]. Therefore, these transition temperatures are more appropriately referred to as ''cloud points temperature'' (Tcp), because is the temperature at which occurs the formation of PU aggregates. In this study we analyzed the Tcp temperature in PBS because the osmolarity and ion concentrations of the solutions match those of the human body.

Table 1. Functional diol and PEG employed in the PUs synthesis. Characterization of synthesized PUs.

Entry	Entry	Functional Diols	PEG Molar mass	Funct. diol/ PEG molar ratio[a)]	Mw (kDa)/ Đ[b)]	Tcp (°C)[c)]
1	**PU-bis-MPA-PEG$_{600}$**	bis-MPA	600	50/50	27.9/ 1.6	35.6 ± 0.7
2	**PU-bis-MPA-PEG$_{1000}$**	bis-MPA	1000	50/50	28.6/ 1.7	42.4 ± 0.1
3	**PU-bis-MPA-PEG$_{1500}$**	bis-MPA	1500	49/51	27.6/ 1.6	56.8 ± 0.6
4	**PU-MDEA-PEG$_{1000}$**	MDEA	1000	48/52	26.5/ 1.7	22.6 ± 0.6
5	**PU-MDEA-PEG$_{1500}$**	MDEA	1500	50/50	36.8/ 1.5	37.1 ± 0.6

6	**PU-Et-MDEA-PEG$_{1000}$**	Et-MDEA	1000	44/56	47.1/ 1.5	60.3 ± 0.4
7	**PU-Et-MDEA-PEG$_{1500}$**	Et-MDEA	1500	46/54	71.6/ 1.6	63.1 ± 0.3
8	**PU-Hex-MDEA-PEG$_{1000}$**	Hex-MDEA	1000	44/56	29.8/ 1.8	38.5 ± 0.6
9	**PU-Hex-MDEA-PEG$_{1500}$**	Hex-MDEA	1500	47/53	58.4/ 1.6	41.7 ± 0.2

[a)]determined by ^{1}H NMR; [b)]determined by GPC; [c)]determined from turbidimetry measurements

a) Effect of the chemical nature of the functional diol

The copolymers of an ionic monomer and a non-ionic monomer not only can affect to the thermal transition but also can incorporate functionality for specific applications. As amphiphilic balance has been shown to play a pivotal role in the LCST properties, therefore a series of polymers was synthesized by reacting the 4 different precursor diols using 1500 PEG diol and their Tcp behavior was investigated (Figure 2). As observed the Tcp is increased in the following order: Et-MDEA>bis-MPA>Hex-MDEA>MDEA being the MDEA the lowest (37.1 ºC) and Et-MDEA the highest (63.1 ºC). As expected, MDEA possessed the lowest Tcp in PBS. According to several works the pKa of the analogous poly(N,N-dimethylaminoethylmethacrylate) is located in the range 7–7.5 [27]. Thus, MDEA based polyurethanes are expected to be non-charged in PBS, being more hydrophobic than the other 3 that are charged in PBS (pH =7.4). Comparing Et-MDEA and Hex-MDEA the Tcp of PU containing Et-MDEA is 21.4 ºC higher than that with Hex-MDEA. This substantial increase in the Tcp temperature can be explained taking into account the much higher hydrophobicity of hexyl side chain rather than ethyl side chain. Finally the bis-MPA based diol at 7.4 is fully deprotonated meaning that in PBS the diol is quite hydrophilic. Therefore, in the presence of PEG$_{1500}$ its Tcp is quite high (56.8 ºC).

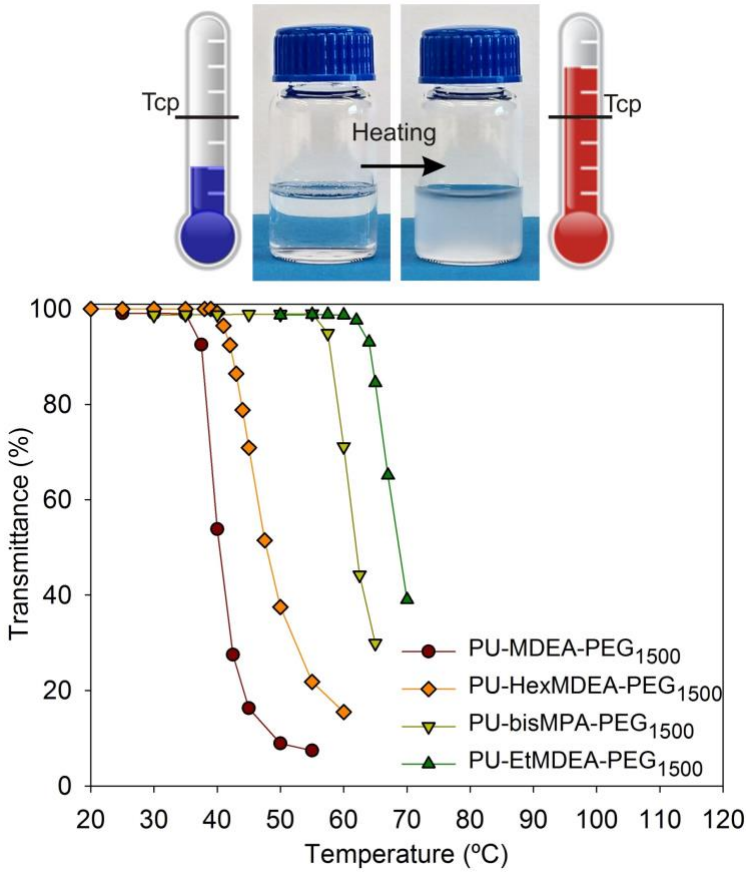


Figure 2. Turbidity curves for PU-MDEA-PEG$_{1500}$, PU-Et-MDEA-PEG$_{1500}$, PU-Hex-MDEA-PEG$_{1500}$, PU-bis-MPA-PEG$_{1500}$ in PBS, with a PU concentration of 1 mg/ml.

b) Effect of PEG molecular Weight

It is known that the key parameter that determines the thermoresponsive behavior of PEG-based PUs is the balance between hydrophilic and hydrophobic moieties in the PU molecular structure [15,16,22,28–30]. First we investigated the Tcp of entries 1,2 and 3 (PU-bis-MPA$_{600}$, PU-bis-MPA$_{1000}$ and PU-bis-MPA$_{1500}$) prepared using bis-MPA diol. As observed in Figure 3a, the Tcp temperature is dependent on the molecular weight of PEG units. As we increased the molar mass of the PEG end-capped diol from 600 to 1500, the Tcp temperature increased from 35.6 to 56.8 ºC. Similar behavior was also observed by Lutz et al.[8,9] for poly(ethylene glycol) methyl ether methacrylates, where they found that when increasing the molar mass of PEG acrylates the Tcp temperature increases in a linear manner as a function of the methylene units. They found that below

cloud point the ether oxygens of PEG form stabilizing H-bonds with water**,** able to solubilize the polymer at room temperature, because the balance between favorable polymer-water interactions and unfavorable hydrophobic interactions is sufficient to allow solubilization. Meanwhile above the Tcp, polymer–polymer interactions are thermodynamically favored over polymer-water interactions and the polymer precipitates. They found that this Tcp increases as they increased the number of repeating units in the oligoethlyene glycol monomeric unit. Similar effect was observed for the other 3 monomers MDEA (Figure 3b), Et-MDEA (Figure 3c) and Hex-MDEA (Figure 3d). Hence, the Tcp temperature of PU-MDEA-PEG_{1000} (22.6 ºC), PU-Hex-MDEA-PEG_{1000} (38.5 ºC) and PU-Et-MDEA-PEG_{1000} (60.3 ºC) prepared using PEG of 1000 could be raised to PU-MDEA-PEG_{1500} (37.1 ºC), PU-Hex-MDEA-PEG_{1500} (41.7 ºC) and PU-Et-MDEA-PEG_{1500} (63.1 ºC), respectively.

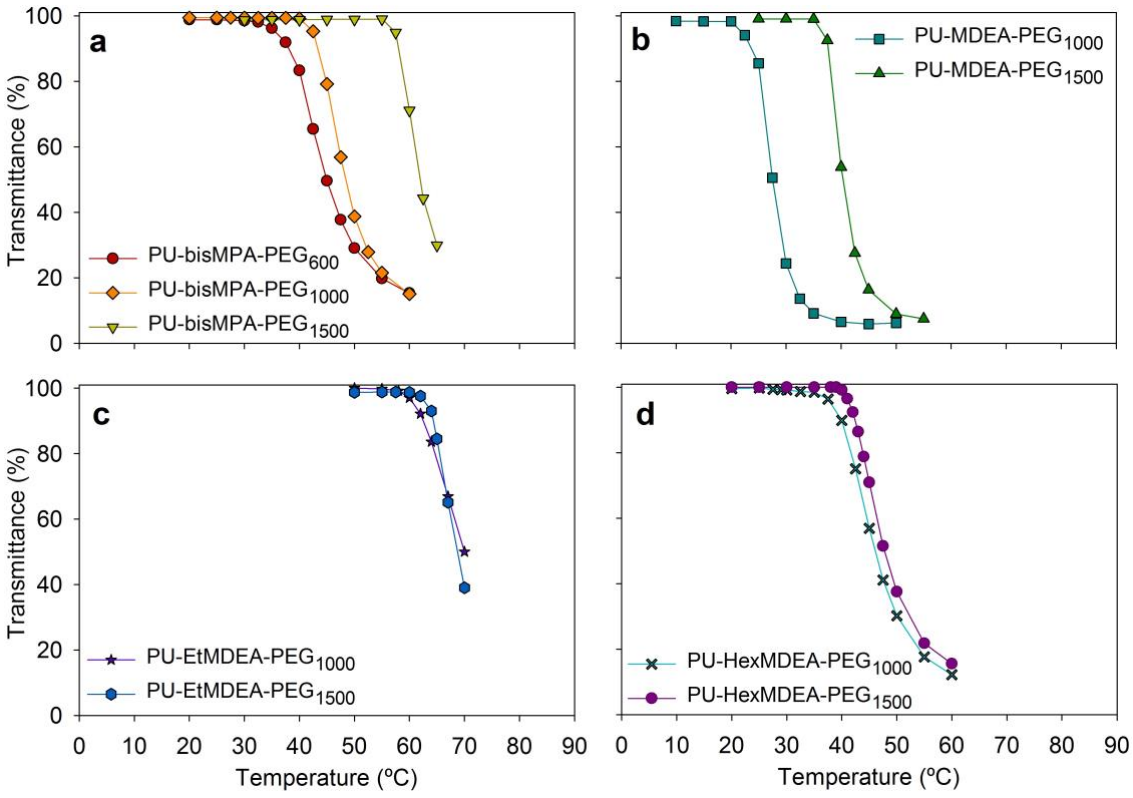


Figure 3. a) turbidity curves for polyurethanes prepared using bis-MPA diol with different PEG-s, b) turbidity curves for polyurethanes prepared using MDEA diol with

different PEG-s c) turbidity curves for polyurethanes prepared using Et-MDEA diol and d) turbidity curves for polyurethanes prepared using Hex-MDEA diol with different PEG-s. All the PU were dissolved at 1 mg/ml concentration in PBS.

c) Effect of PU concentration and heating-cooling cycles

The effect of PU concentration on Tcp, for PU2 (PEG_{1000}-bis-MPA-IPDI) in PBS solution is presented in Figure 4. Changes in the transmittance of aqueous mixtures were determined by UV-vis spectroscopy. As expected, increasing de PU2 concentration the Tcp was considerably reduced with a faster change in the sample transmittance. This result is in good agreement with other thermoresponsive systems and can be explained due to the higher ability of polymers to form aggregates at higher polymer concentrations.

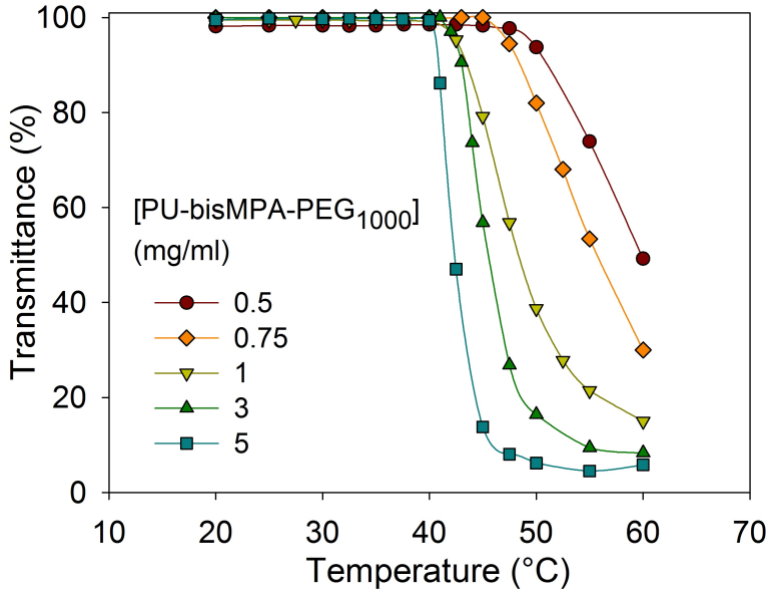


Figure 4. Turbidity curves for different concentrations of PU-bis-MPA-PEG_{1000} in PBS.

Figure 5 presents the turbidity curves during the heating-cooling cycle for different type of PUs. All the samples showed thermal reversibility when they were heated and subsequently cooled and no significant hysteresis was presented. These data indicate that the temperature-induced phase transition is stable and PUs does not undergo significant degradation or changes in their solubility during the experimental time.

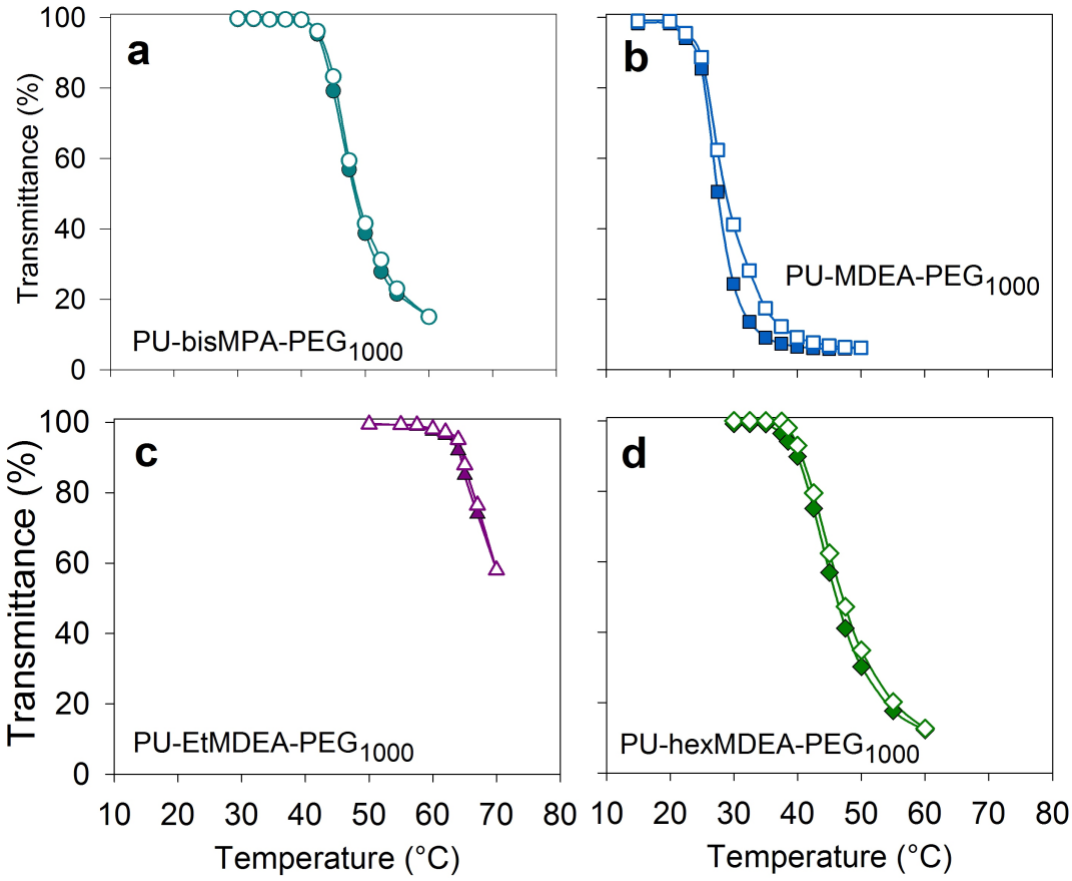


Figure 5. Turbidity curves during the heating-cooling cycle for a) PU-bis-MPA-PEG$_{1000}$ b) PU-MDEA-PEG$_{1000}$ c) PU-Et-MDEA-PEG$_{1000}$ d) PU-Hex-MDEA-PEG$_{1000}$. All the PU were dissolved at 1 mg/ml concentration in PBS.

All this aside, these results suggests that using a judicious choice in the structure of the functional diol and PEG molecular weight thermoresponsive polyurethanes can be prepared *à la carte.* Not only the Tcp temperature could be adjusted but also different cationic and anionic functional groups could be incorporated. All these systems are reversible and they do not present any significant hysteresis. A summary of the different ionic PUs and its Tcp are represented in Figure 6.

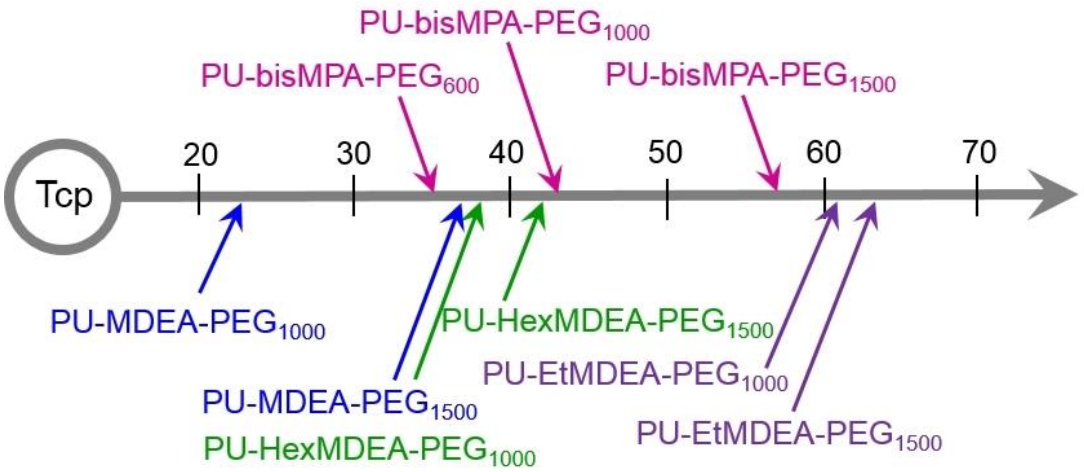
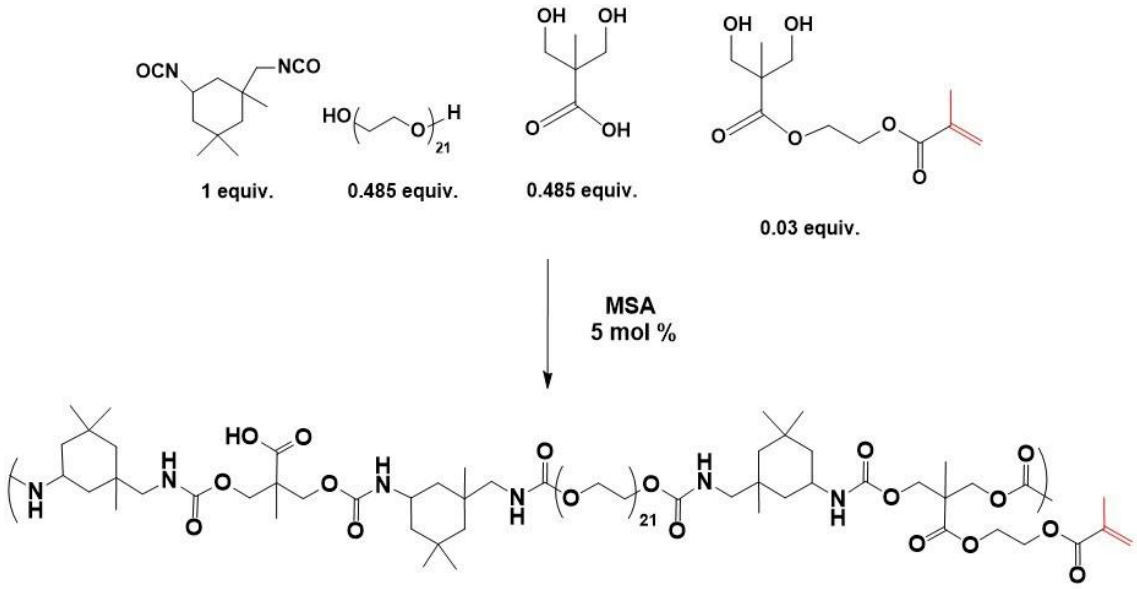


Figure 6. Represents the Tcp temperature of the prepared different cationic, anionic and non-charged PEG based polyurethanes.

3.3. Thermoresponsive gel based on thermoresponsive polyurethanes

In order to expand the scope of these thermoresponsive polyurethanes we intended to show that this chemistry can be translated to three dimensional systems such as thermally responsible hydrogels. Environmentally responsive hydrogels have found numerous applications especially in the biomedical field. In our case, we prepared PUs that could be cross-linked by simply incorporating a diol that contain a methacrylic group in the initial synthetic formulation (Scheme 2). The incorporation of methacrylic units in the polymer backbone was confirmed by ^{1}H NMR observing the characteristic signals of methacrylic protons at δ = 6.05 ppm and δ = 5.7 ppm (Figure S13, Supporting Information).



Scheme 2. MSA catalyzed synthesis of PU10 from IPDI, PEG_{1000}, bis-MPA and bis-MPA-methacrylate.

A cross-linked PU film was easily prepared by curing under a high intensity UV-lamp this methacrylic PU in the presence of a photoinitiator. In a second stage, this film could be swollen with water or PBS forming a hydrogel. We found that at 20 ºC below the Tcp, the cross-linked film was swollen in PBS. As expected, below the Tcp of the PU the polymer is hydrophilic absorbing a large amount of water. But, increasing the temperature above the Tcp (35 ºC), the polymer chains collapse and water molecules are expelled. This is clearly seen in the pictures of the hydrogel and the collapsed film in Figure 7. These results indicates the potential of these thermoresponsive cross-linkable PUs to produce hydrogels where the LCST temperature could be easily adjusted with a proper selection of the PU composition. To further confirm the formation of 3-D structures, the rheological behaviors of the gels at room temperature and at high temperatures were characterized using oscillatory tests (SI section). In both cases the elastic modulus (G') was greater than the viscous (G") modulus for all the hydrogels

confirming the formation of a crosslinked hydrogel which modulus increases slightly in the collapsed state at 35 ºC (Figure S14).

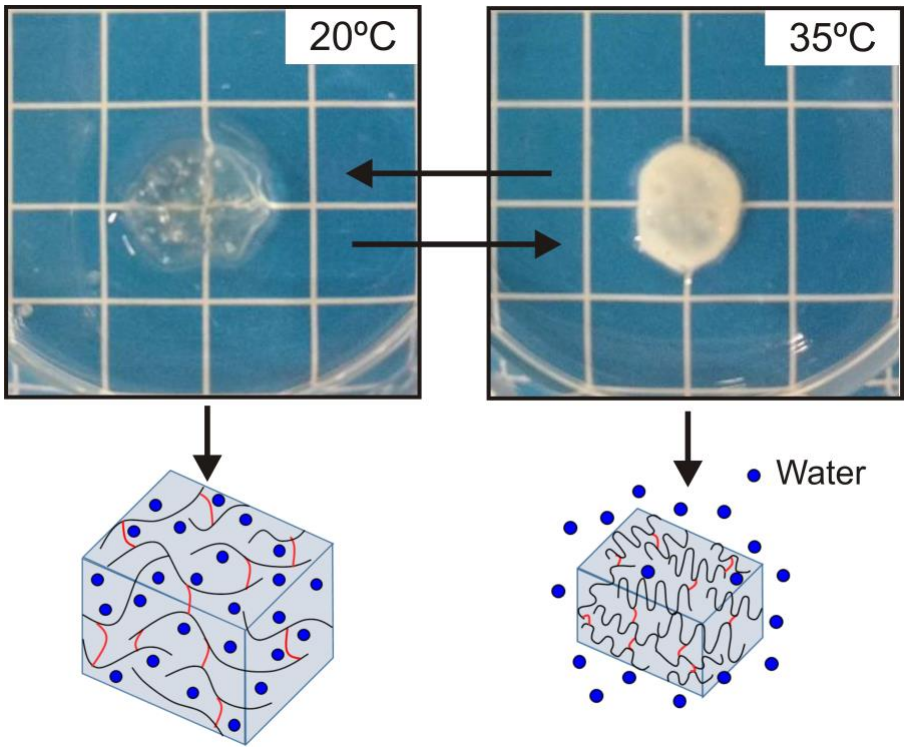


Figure 7. Schematic representation of PU hydrogel behavior below and above the Tcp.

4. Conclusions

New functionalized thermoresponsive PUs were synthesized by copolymerization of PEG of different molecular weights, with diols that provide carboxylic acid groups, tertiary and quaternary amines, and IPDI, using organocatalysis. Successful polymerization was confirmed by FTIR, ^{1}H NMR, and SEC. All PUs synthesized with functional diols exhibited a reversible thermoresponsive behavior in PBS. In addition, the Tcp values of different functionalized PUs were tuned modifying the PEG molecular weight, and the hydrophobicity of functional diols. In this form, PUs with

Tcp close to the body temperature could be obtained. Finally using the versatility of polyurethanes, we added a little amount of a diol with pendant methacrilyc group. Thus, we were able to produce thermally responsible PU hydrogels. It is envisioned that this facile synthetic approach to prepare temperature-responsive materials will provide a versatile and convenient platform for a variety of applications.

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6. References

[1] S. Mura, J. Nicolas, P. Couvreur, Stimuli-responsive nanocarriers for drug delivery, Nat. Mater. 12 (2013) 991–1003.

[2] L. Klouda, Thermoresponsive hydrogels in biomedical applications: A seven-year update, Eur. J. Pharm. Biopharm. 97 (2015) 338–349.

[3] A. Gandhi, A. Paul, S.O. Sen, K.K. Sen, Studies on thermoresponsive polymers: Phase behaviour, drug delivery and biomedical applications, Asian J. Pharm. Sci. 10 (2015) 99–107.

[4] K. Nagase, T. Okano, Thermoresponsive-polymer-based materials for temperature-modulated bioanalysis and bioseparations, J. Mater. Chem. B. 4 (2016) 6381–6397.

[5] F.H. Meng, Z.Y. Zhong, J. Feijen, Stimuli-Responsive Polymersomes for Programmed Drug Delivery, Biomacromolecules. 10 (2009) 197–209.

[6] D. Roy, W.L. Brooks, B.S. Sumerlin, New directions in thermoresponsive

polymers, Chem Soc Rev. 42 (2013) 7214–7243.

[7] S. Hocine, M.-H. Li, Thermoresponsive self-assembled polymer colloids in water, Soft Matter. 9 (2013) 5839-5861.

[8] J.F. Lutz, Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials, J. Polym. Sci. Part A Polym. Chem. 46 (2008) 3459–3470.

[9] J.F. Lutz, Ö. Akdemir, A. Hoth, Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: Is the age of poly(NIPAM) over?, J. Am. Chem. Soc. 128 (2006) 13046–13047.

[10] G. Vancoillie, D. Frank, R. Hoogenboom, Thermoresponsive poly(oligo ethylene glycol acrylates), Prog. Polym. Sci. 39 (2014) 1074–1095.

[11] M. Ding, J. Li, H. Tan, Q. Fu, Self-assembly of biodegradable polyurethanes for controlled delivery applications, Soft Matter. 8 (2012) 5414–5428.

[12] W. He, X. Zheng, Q. Zhao, L. Duan, Q. Lv, G.H. Gao, S. Yu, pH-Triggered Charge-Reversal Polyurethane Micelles for Controlled Release of Doxorubicin, Macromol. Biosci. (2016) 925–935.

[13] M. Ding, N. Song, X. He, J. Li, L. Zhou, H. Tan, Q. Fu, Q. Gu, Toward the next-generation nanomedicines: Design of multifunctional multiblock polyurethanes for effective cancer treatment, ACS Nano. 7 (2013) 1918–1928.

[14] M. Ding, X. Zeng, X. He, J. Li, H. Tan, Q. Fu, Cell internalizable and intracellularly degradable cationic polyurethane micelles as a potential platform for efficient imaging and drug delivery, Biomacromolecules. 15 (2014) 2896–2906.

[15] X. Sun, H. Gao, G. Wu, Y. Wang, Y. Fan, J. Ma, Biodegradable and temperature-responsive polyurethanes for adriamycin delivery, Int. J. Pharm. 412

(2011) 52–58.

[16] H. Fu, H. Gao, G. Wu, Y. Wang, Y. Fan, J. Ma, Preparation and tunable temperature sensitivity of biodegradable polyurethane nanoassemblies from diisocyanate and poly(ethylene glycol), Soft Matter. 7 (2011) 3546–3552.

[17] C.T. Huynh, M.K. Nguyen, D.P. Huynh, S.W. Kim, D.S. Lee, PH/temperature-sensitive 4-arm poly(ethylene glycol)-poly(amino urethane) copolymer hydrogels, Polymer (Guildf). 51 (2010) 3843–3850.

[18] C.T. Huynh, Q.V. Nguyen, S.W. Kang, D.S. Lee, Synthesis and characterization of poly(amino urea urethane)-based block copolymer and its potential application as injectable pH/temperature-sensitive hydrogel for protein carrier, Polym. (United Kingdom). 53 (2012) 4069–4075.

[19] Z. Li, Z. Zhang, K.L. Liu, X. Ni, J. Li, Biodegradable hyperbranched amphiphilic polyurethane multiblock copolymers consisting of poly(propylene glycol), poly(ethylene glycol), and polycaprolactone as in situ thermogels, Biomacromolecules. 13 (2012) 3977–3989.

[20] A. Wang, H. Gao, Y. Sun, Y.L. Sun, Y.W. Yang, G. Wu, Y. Wang, Y. Fan, J. Ma, Temperature- and pH-responsive nanoparticles of biocompatible polyurethanes for doxorubicin delivery, Int. J. Pharm. 441 (2013) 30–39.

[21] Q. Song, H. Chen, S. Zhou, K. Zhao, B. Wang, P. Hu, Thermo- and pH-sensitive shape memory polyurethane containing carboxyl groups, Polym. Chem. 7 (2016) 1739–1746.

[22] H. Sardon, J.P.K. Tan, J.M.W. Chan, D. Mantione, D. Mecerreyes, J.L. Hedrick, Y.Y. Yang, Thermoresponsive random poly(ether urethanes) with tailorable LCSTs for anticancer drug delivery, Macromol. Rapid Commun. 36 (2015) 1761–1767.

[23] K. Kim, W.C.W. Chen, Y. Heo, Y. Wang, Polycations and their biomedical applications, Prog. Polym. Sci. 60 (2016) 18–50.

[24] E. Haladjova, N. Toncheva-Moncheva, M.D. Apostolova, B. Trzebicka, A. Dworak, P. Petrov, I. Dimitrov, S. Rangelov, C.B. Tsvetanov, Polymeric nanoparticle engineering: From temperature-responsive polymer mesoglobules to gene delivery systems, Biomacromolecules. 15 (2014) 4377–4395.

[25] Q. Xu, H. Sardon, J.M.W. Chan, J.L. Hedrick, Y.Y. Yang, Polyurethane-coated silica particles with broad-spectrum antibacterial properties, Polym. Chem. 6 (2015) 2011–2022.

[26] H. Sardon, A.C. Engler, J.M.W. Chan, J.M. García, D.J. Coady, A. Pascual, D. Mecerreyes, G.O. Jones, J.E. Rice, H.W. Horn, J.L. Hedrick, Organic acid-catalyzed polyurethane formation via a dual-activated mechanism: Unexpected preference of n-activation over o-activation of isocyanates, J. Am. Chem. Soc. 135 (2013) 16235–16241.

[27] G. Aguirre, J. Ramos, J. Forcada, Advanced design of t and pH dual-responsive PDEAEMA-PVCL core-shell nanogels for siRNA delivery, J. Polym. Sci. Part A Polym. Chem. 54 (2016) 3203–3217.

[28] J.F. Lutz, A. Hoth, Preparation of ideal PEG analogues with a tunable thermosensitivity by controlled radical copolymerization of 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate, Macromolecules. 39 (2006) 893–896.

[29] A. Dong, Y. Zhai, L.I. Xiao, H. Qi, Q.I. Tian, L. Deng, R. Guo, Thermosensitive Behavior of Poly (ethylene glycol)/ Poly (2- (N , N - dimethylamino) ethyl methacrylate) Double Hydrophilic Block Copolymers, 48 (2010) 503–508.

[30] J.P. Swanson, M.R. Martinez, M.A. Cruz, S.G. Mankoci, P.J. Costanzo, A. Joy,

N. Yan, Y. Kou, Z.-C. Li, A coacervate-forming biodegradable polyester with elevated LCST based on bis-(2-methoxyethyl)amine, Polym. Chem. 7 (2016) 4693–4702.