PONTIFICIA UNIVERSIDAD CATÓLICA DEL PERÚ

Escuela de Posgrado

Síntesis y caracterización de nuevos hidrogeles sensibles al pH y la temperatura basados en N-isopropilacrilamida y 2-oxazolinas

Trabajo de investigación para obtener el grado académico de Magíster en Ingeniería y Ciencia de los Materiales que presenta:

Estuardo Job Campos Dávila

Asesor:

Dr. Juan Carlos Rueda Sanchez

Lima, 2021

Designed Monomers and Polymers, 2014
Vol. 17, No. 3, 208–216, http://dx.doi.org/10.1080/15685551.2013.840471

Synthesis and characterization of new pH- and thermo-responsive hydrogels based on N-isopropylacrylamide and 2-oxazolines

Juan Carlos Rueda[a], Estuardo Campos[a], Hartmut Komber[b], Stefan Zschoche[b], Liane Häussler[b] and Brigitte Voit[b]*

[a]*Laboratorio de Polímeros, Sección Física, DGI, Pontificia Universidad Católica del Perú (PUCP), Lima, Box 1761, Perú;* [b]*Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, Dresden 01069, Germany*

(*Received 26 April 2013; accepted 19 June 2013*)

New pH- and thermo-responsive hydrogels (HG) were synthesized by free radical polymerization of N-isopropylacrylamide and a macromonomer, which was a hydrolyzed random copolymer of 2-carboxyethyl- and 2-methyl-2-oxazoline, using a bisacrylamide as crosslinker. The polymerization was carried out in a mixture of water and ethanol at room temperature and was initiated by ammonium peroxodisulfate. The HG showed conformational transitions with variation of temperature and/or pH-value and as a function of hydrogel composition. This property was shown macroscopically as hydrogel contraction or expansion. The HG structures were characterized by high-resolution magic angle spinning (HRMAS) NMR spectroscopy. The thermal properties, in particular the lower critical solution temperatures, were determined by temperature-dependent HRMAS NMR measurements and differential scanning calorimetry. The pH responsibility was determined by swelling experiments in water at different pH values.

Keywords: hydrogels; stimuli-sensitive polymers; networks; graft copolymers; macromonomers; swelling

1. Introduction

In recent years, one of the most intensively investigated fields in polymer chemistry is the synthesis of responsive macromolecules with potential application in the field of biomaterials.[1–4] Polymers suitable for this purpose are, for example, homopolymers, copolymers, and hydrogels (HG) of N-isopropylacrylamide (NiPAAm). These polymers exhibit, in aqueous solution, a conformational transition in a narrow temperature range at which they drastically change their physical properties from hydrophilic to hydrophobic (or vice versa) leading to precipitation (or dissolution) in the aqueous medium.[4–6] This phenomenon is known as lower critical solution temperature (LCST). In the case of poly(NiPAAm), this temperature is around 32 °C, which is close to the human body temperature, and so there is much interest in the use of polymers based on NiPAAm for the preparation of thermo-sensitive biomaterials. The transition temperature of poly(NiPAAm) can be varied toward higher or lower than 32 °C through copolymerization of NiPAAm with hydrophilic or hydrophobic comonomers, respectively, or also by the addition of surfactants substances or inorganic salts.[5,6]

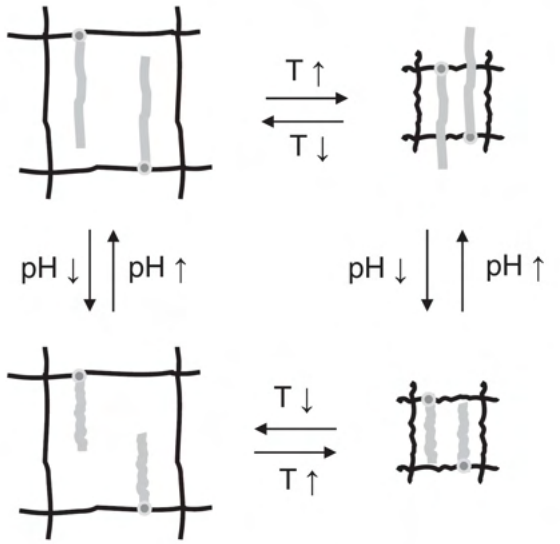
Hydrophilic comonomers of interest are e.g. 2-oxazo lines. 2-Oxazolines are heterocyclic substances whose polymerization has received intensive research till today. [7–27] The polyoxazolines can be prepared through a ring-opening cationic polymerization by initiators, such as Bronsted or Lewis acids, methyl tosylate and triflate, as well as alkyl halides, among others.

Under appropriate reaction conditions (high purity of monomers and solvents, and elimination of nucleophilic substances, such as amines, alcohols, and traces of moisture), the polymerization of 2-oxazolines proceeds in a 'living' fashion without termination or chain transfer reactions, and thus the molecular weight and its distribution, as well as the functionality of these polymers, can be predetermined from the start of polymerization. Likewise, various polymer architectures can be developed, such as block and graft copolymers, star polymers, etc.[10,11]

Some polyoxazolines, as polymethyloxazoline, have hydrophilic character while others, as for example polynonyloxazoline, have hydrophobic character. In recent years, our research group has developed, based on NiPAAm and 2-oxazolines, different polymer structures soluble in water and sensitive to temperature and pH, [20–23] which formed stable and reversible micelles, and the formation of double-responsive nanogels by irradiation with electrons was demonstrated.

HGs provide a unique multi-functionality in the field of micro-electromechanical microfluidic systems.[28,29] Such bi-sensitive HGs are the basic components for

*Corresponding author. Email: voit@ipfdd.de



Scheme 1. Bi-sensitive hydrogel with thermo-sensitive main chains and pH-sensitive side chains.

microfluidic systems as microvalves, micropumps, and hydrodynamic transistors.[29]

In this paper, we present novel HGs (Scheme 1) based on NiPAAm and a 2-oxazoline macromonomer (MM), functionalized with carboxylic acids, with the property of being simultaneously sensitive to temperature and pH.

2. Experimental part

2.1. Materials

Chloromethylstyrene (CMS) (Aldrich, mixture of isomers 70 mol% *meta* and 30 mol% *para*) was bi-distilled before use. 2-Methoxycarbonylethyl-2-oxazoline (MEtOxa) was synthesized by a method described in the literature [22,30] and was distilled over calcium hydride before use. N-isopropyl acrylamide (Aldrich) was recrystallized twice in ethanol. Benzonitrile and 2-methyl-2-oxazoline (MeOxa) (Aldrich) were distilled twice over calcium hydride before use. All other substances and solvents were of high purity and were used as received.

2.2. Synthesis of MM

The MM was synthesized via statistical ring-opening cationic copolymerization of MeOxa and MEtOxa initiated by CMS in the presence of sodium iodide. The polymerization was carried out in acetonitrile at 80 °C, similar to the method described in the literature.[18] About 2.51 g (29.5 mmol) MeOxa, 4.63 g (29.5 mmol) MEtOxa, and 1.0 g (6.67 mmol) NaI were dissolved under nitrogen in 15 mL of acetonitrile and to this mixture 0.50 g (3.27 mmol) CMS was added. The reaction mixture was heated for 7 h, and after this time the polymerization was terminated with a solution of KOH in methanol. The obtained MM was purified by standard methods (yield: 98%). The MM was a statistical copolymer of MEtOxa and MeOxa with a polymerization degree of 21 ($M_n = 2400 \pm 150$) and a content of 52.5 mol% of MEtOxa and 47.5 mol% of MeOxa (ratio MEtOxa/MeOxa = 10/11) as determined by ^{1}H NMR spectroscopy.

^{1}H NMR (CD_3OD): δ 2.0–2.25 (CH_3), 2.60 (CH_2COOCH_3), 2.6–2.8 ($COCH_2$), 3.4–3.8 (NCH_2CH_2), 3.65 ($COOCH_3$), 4.68 (Ar–CH_2), 5.25 and 5.82 (CH_2=), 6.75 (=CH–), 7.1–7.5 ppm (H_{ar}).

2.3. Hydrolysis of MM

A mixture of 1.5 g MM, 90 mL 0.1 N aqueous NaOH, and 30 mL methanol was stirred at 55 °C for 7 h. The hydrolyzed macromonomer (HMM) was purified by dialysis using Zellu Trans membrane (Carl Roth GmbH, MWCO: 1000) in deionized water (exchange water three times) at room temperature for three days and then freeze-dried.

^{1}H NMR (CD_3OD): δ 2.0–2.2 (CH_3), 2.47 (CH_2COOH), 2.66 ($COCH_2$), 3.4–3.8 (NCH_2CH_2), 3.67 (CH_2OH), 4.66 (Ar–CH_2), 5.25 and 5.79 (CH_2=), 6.74 (=CH–), 7.1–7.5 ppm (H_{ar}).

^{13}C NMR (CD_3OD): δ 21.3 (CH_3), 30.4 ($COCH_2$), 33.4 (CH_2COOH), 44–48 (NCH_2CH_2), 52.3–54.0 (Ar–CH_2), 61.0–61.5 (CH_2OH), 114–115.5 (CH_2=), 125–131 (both CH_{ar}), 137–140 (both C_{ar}), 137.8 (–CH=), 173.8 ($COCH_3$), 175.7 ($COCH_2$), 180.6 ppm (COOH).

2.4. Synthesis of HG

The typical procedure is as follows: under a dried nitrogen atmosphere, a mixture of 0.27 g macromonomer HMM, 0.50 g NiPAAm, 0.019 g N,N′-methylene bisacrylamide (BIS), 0.015 g N,N,N′,N′-tetramethylethylene diamine (TEMED), and 0.015 g ammonium peroxodisulfate in 3 mL of solvent (water/ethanol 1/1 v/v) was stirred at room temperature until gelation occurred (normally in 10 min or less). The hydrogel was maintained in the reaction medium for 10 h more at room temperature. After polymerization, the hydrogel was purified in a Soxhlet system, successively using ethanol and water as extraction solvents. Finally, the gel was dried at 40 °C under vacuum until weight becomes constant. The experimental details and results are summarized in Table 1.

^{1}H NMR (D_2O): δ 1–2.5 (CH and CH_2 of the backbone), 1.15 ($CH(CH_3)_2$), 2.07 ($COCH_3$), 2.43 (CH_2COOH), 2.59 ($COCH_2$), 3.2–4.0 (NCH_2CH_2), 3.91 (CH), 4.58 (Ar–CH_2), 6.8–8.1 ppm (H_{ar}, residual NH).

Table 1. Composition and characteristics of HG from HMM and NiPAAm.

HG	HMM[a] [mol × 10^5]	NiPAAm[a] [mol × 10^5]	BIS[a] [mol × 10^5]	NiPAAm/HMM Theor.	NiPAAm/HMM Exp.[b]	Gel content[c] [wt.%]	DS[d] [mL/g]
HG1	11.2	442	12.3	39	70	50	75
HG2	8.5	442	12.3	52	100	83	60
HG3	5.6	442	12.3	79	140	89	42
HG4	4.0	442	12.3	111	200	79	21
HG5	2.5	442	12.3	176	310	85	18
HG6	11.2	442	7.1	39	85	87	163
HG7	8.5	442	7.1	52	100	76	130
HG8	5.6	442	7.1	79	150	75	69

[a]Molar feed of HMM, NiPAAm, and BIS in reaction system.
[b]Molar ratio NiPAAm/HMM as calculated from ^{1}H HRMAS NMR signal integrals. Estimated relative error: ±10%.
[c]Obtained insoluble products after extraction in wt.% based on the weight of all starting monomers.
[d]Degree of swelling: DS = $(W_2 - W_1)/\rho W_2$, where W_1 and W_2 are the weights of dried and swollen gel in water, respectively, and ρ is the density of water measured at 25 °C and pH = 5.6.

2.5. Swelling properties

The swelling equilibrium in water of the obtained hydrogel was determined as follows: 0.10 g of dried hydrogel was immersed in 50 mL of deionized water at room temperature for 24 h. The swollen hydrogel was weighed after gently removing excess water with filter paper. The swelling degree was calculated from equation $(W_2 - W_1)/\rho W_2$, where W_1 and W_2 are the weights of dried and swollen gel, respectively, and ρ is the density of water which is near 1 g mL^{-1}.

2.6. Measurements of geometrical dimension of hydrogel in function of pH

The height of a piece or fragment of hydrogel was measured at different pH values at 25 °C. Thus, the expansion or contraction of hydrogel at different pH values was determined approximately in comparison to the hydrogel height at pH = 5.6.

2.7. Analytical measurements

The NMR measurements were carried out on a Bruker Avance III 500 NMR spectrometer operating at 500.13 MHz for ^{1}H and 125.75 MHz for ^{13}C. The spectra were acquired at a temperature of 30 °C and referenced to the solvent peak (CD_3OD: $\delta(^1H)$ = 3.31 ppm, $\delta(^{13}C)$ = 49.0 ppm). A 4 mm ^{1}H/^{13}C/^{2}H high-resolution magic angle spinning (HRMAS) gradient probe was used for measurements on swollen HGs. Here, the sample (ca. 3–4 mg) was packed into a 4 mm HRMAS rotor (90 µL sample volume) and D_2O was added to the hydrogel directly inside the rotor. In all experiments, samples were spun with 4.5 kHz. The spectra were referenced on the signal of the CH_3 group of the NiPAAm comonomer ($\delta(^1H)$ = 1.15 ppm).

The temperature in the probe was controlled by the Bruker variable temperature accessory BVT-3000. Temperature-dependent measurements on a HRMAS rotor filled with 80% 1,2-ethanediol in DMSO-d_6 were carried out to determine the temperature in the rotor from the chemical shift difference of the OH and CH_2 protons' signals according to Ref. [31].

The differential scanning calorimetry (DSC) measurements were performed on a DSC 204 F1 Phoenix of Netzsch in the temperature range of −20–80 °C at a scan rate of ± 2 K min^{-1} in screwed steel pans to prevent the overlapping process of vaporization of water. The transition temperatures T_{tr} were determined using the peak maxima of the 2nd heating run.

3. Results and discussion

3.1. Synthesis of MM

The MM was synthesized by ring-opening cationic polymerization of an equimolar mixture of MeOxa and MEtOxa initiated by CMS in acetonitrile in the presence of sodium iodide (Scheme 2).

As already discussed in a previous paper for a similar polymerization system, in this case, it is postulated that the real initiator species is iodomethylstyrene which is formed *in situ* as a product of reaction between CMS and sodium iodide.[18] The iodomethylstyrene initiates the polymerization of MEtOxa and MeOxa, and because the reactivity of these monomers is similar [21–23] finally a statistical copolymer of these monomers is formed which contains a vinyl group at the one chain end (starting group) and a OH group at the other chain end, because the polymerization has been terminated by KOH in methanol.[32] The structure of MM was confirmed by ^{1}H NMR spectroscopy.

The ester groups of macromonomer MM were hydrolyzed under mild conditions with aqueous sodium hydroxide solution (0.1 N NaOH, 55 °C, 7 h) to avoid the hydrolysis of the amide groups (Scheme 2).

Complete hydrolysis was verified by NMR spectroscopy. Thus, the disappearance of the methylester signal at 3.65 ppm in the ^{1}H NMR spectrum (Figure 1(a)) and at 52.2 ppm in the ^{13}C NMR spectrum [22] confirms successful hydrolysis. The copolymer composition was determined from signal integrals of H_f and H_g (acid form, 2-carboxyethyl-2-oxazoline (CEtOxa)) and H_e (MeOxa) giving a content of approximately 47.5 and 52.5 mol%, respectively, for both monomers, fitting well to the equimolar ratio used for polymerization. Relating these integrals to the integrals of the styryl-type starting group resulted in a degree of polymerization of $n \sim 21$ ($M_n = 2400 \pm 150$ g/mol) which is in good agreement with the theoretical value ($n = 18$). This result evidences the living copolymerization of both monomers. The relatively low reaction temperature (80 °C), a relatively short reaction time, and the dilute reaction mixture prevent the premature polymerization of the vinyl groups.[18]

After hydrolysis, the carboxylated groups of the MM (HMM) were neutralized to pH of 5.6 which corresponds to a carboxylate/carboxylic acid ratio of about 1 (degree of neutralization 50%).[23]

3.2. *Synthesis of HG*

The HGs were obtained by free radical copolymerization of MM HMM, NiPAAm, and BIS keeping the molar content of the HMM between 0.5 and 3 mol% and BIS below 3 mol% (Table 1). The polymerization was carried out at room temperature in an equal per volume mixture of water and ethanol and was initiated by a redox initiator system based on ammonium peroxodisulfate in the presence of the catalysator TEMED (Scheme 3). Different HGs were synthesized with variations in the molar ratio of NiPAAm/HMM. The gelation was always relatively rapid and produced transparent HGs in 10 min or less, but the HGs were maintained in the reaction medium for additional 10 h. The reaction product was extracted to remove all soluble fractions. Thus, usually 75–89 wt.% of insoluble hydrogel was obtained with

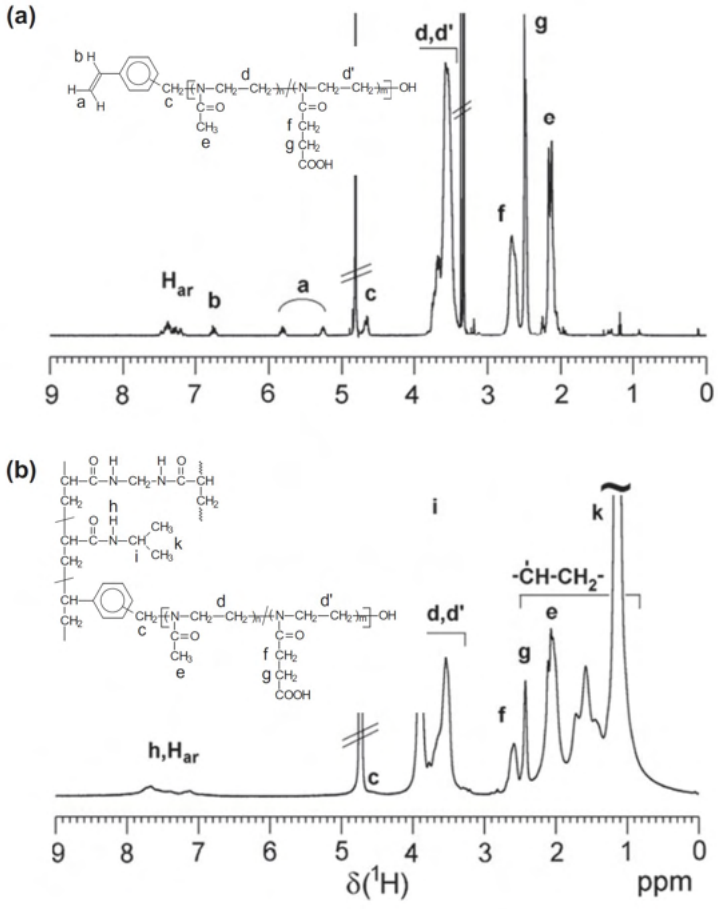
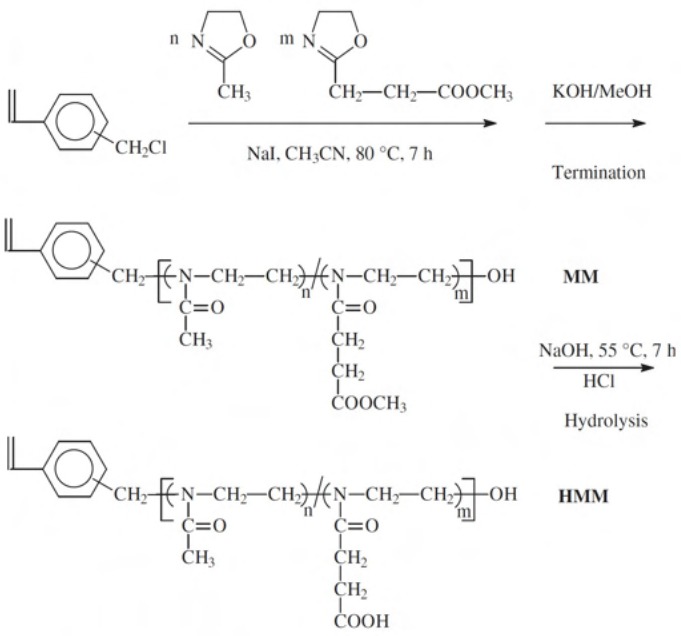
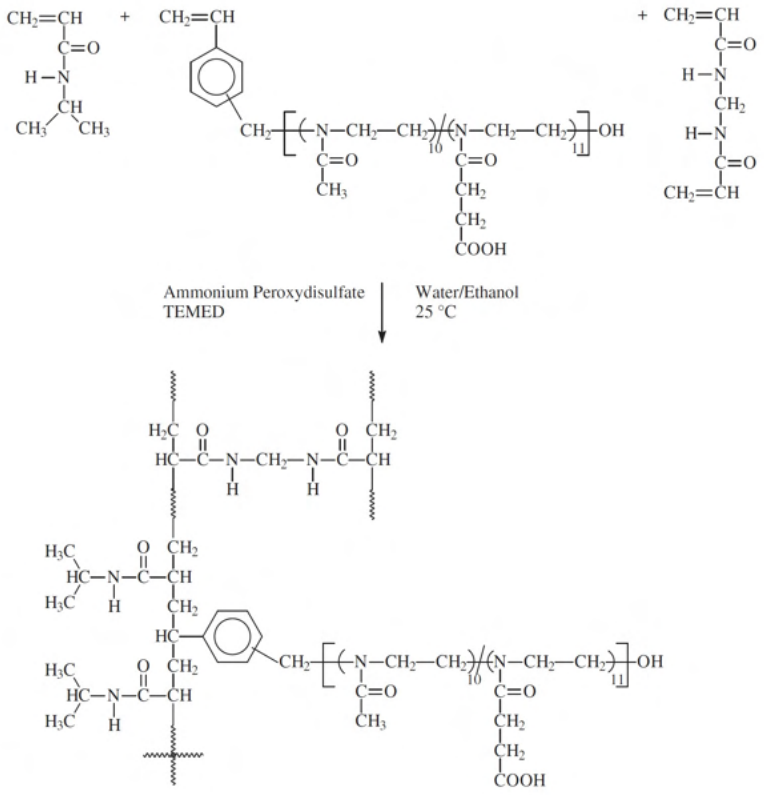


Figure 1. (a) ^{1}H NMR spectrum of the hydrolyzed macromonomer (HMM; solvent: CD_3OD) and (b) ^{1}H HRMAS NMR spectrum of hydrogel 1 (HG1; solvent: D_2O). No signals of the crosslinking units could be observed.



Scheme 2. Synthesis and subsequent hydrolysis of MM.



Scheme 3. Synthesis of hydrogel (HG).

regard to the starting material. Table 1 summarizes the details and the results.

The characterization of the HG swollen in D_2O by ^1H HRMAS NMR spectroscopy confirms the expected hydrogel structure. Figure 1(b) depicts the spectrum of hydrogel 1 (HG1). Whereas the low content of crosslinker BIS which could not be proved due to its restricted mobility at strained crosslinking points, the interjacent poly(NiPAAm) chains and the polyoxazoline side chains can be well observed. The ratio of NiPAAm and HMM units was calculated from the signal integrals of H_i (NiPAAm) and $H_{d,d'}$ (HMM). Despite the relatively large error caused, for example by line shape and signal overlap, it is obvious that for all HG the content of HMM is significantly lower than expected from the monomer feed ratio (Table 1). This result was expected because the NiPAAm, as low molar mass monomer, polymerizes more rapidly than the macromonomer HMM although the styrenic chain end is probably slightly more reactive than NiPAAm.[20] The styrene group is the starting group and a part of each HMM chain. Therefore, incomplete styrene functionalization cannot be the reason for the low incorporation of HMM into the hydrogel.

Thus, HGs resulted where the molar ratio NiPAAM/HMM varied between 310 and 70 which correspond to the weight contents of HMM in the hydrogel between 6.2 and 22.3 wt.% (Table 2).

Next, absorption tests in water were done with the HG (Table 1). So, it was determined that water absorption increases with the decrease of molar ratio NiPAAm/HMM (cp., e.g., HG1 and HG5), because the hydrophilicity of macromonomer HMM with polar carboxylate and carboxylic groups is higher than that of poly(NiPAAm). Also, for samples with the same molar ratio NiPAAm/HMM (cp., e.g., HG1 and HG6), the water absorption of HG was higher with a decrease of BIS, because in this case the crosslinking density of the hydrogel is lower. In some cases (HG6 and HG7), water absorption of more than 100% was in the range of superabsorbent materials.

The HG showed sensitivity to temperature because of the poly(NiPAAm) segments, and in addition they are also pH-sensitive because of the hydrolyzed polyMEtOxa segments. The conformational transitions associated with these responses were detected by a macroscopic change in the HG geometrical dimensions by volume contraction or expansion. The dimension of change was dependent on the molar ratio NIPAAm/HMM inside the hydrogel.

The thermal conformational transitions of the HG were measured with ^1H HRMAS NMR spectroscopy and DSC (Table 2). The study of phase transition of aqueous polymer solutions and gels and the determination of LCST temperature by NMR spectroscopy is a well-introduced method [33] for poly(NiPAAM)-based polymers containing polyoxazolines.[20,22,23] The ^1H NMR spectra of the HG swollen in D_2O show no restricted mobility neither for the poly(NiPAAm) nor for the polyoxazoline moieties at temperatures $\leqslant 33\,^{\circ}C$ (Figures 1(b) and 2). With increasing temperature, the transition of the coiled structure of the poly(NIPAAm) moiety to a globular-like structure results in a marked decrease in the integrated intensity of all poly(NIPAAm) signals (Figure 2(a), –CHR-CH_2– backbone and H_k), whereas the polyoxazoline side chain signals remain almost unaffected ($H_e - H_g$). This phase transition can be best followed for the methyl groups' signal *k* (Figure 2(b) and (c)). The transition temperature T_{tr} was determined at 50% height of signal *k* compared to the signal height at 26 °C (Figure 2(c), Table 2). Furthermore, the temperature at 10% height was determined (Figure 2(c), Table 2). The temperature difference between T_{tr} and $T_{10\%}$ gives a measure for the steepness of the phase transition.

The transition temperatures T_{tr} show a slight dependency on the HMM content, going up from 33 to

Table 2. Conformational transitions of the HG in dependence of the composition as determined by ^1H HRMAS NMR spectroscopy and DSC.

HG	HMM content [wt.%]	BIS content[a] [mol%]	Degree of swelling[b] [mL g^{-1}]	T_{tr} (NMR)[c] [°C]	$T_{10\%}$ (NMR)[c] [°C]	ΔT (NMR)[c] [K]	T_{tr} (DSC)[d] [°C]
HG1	22.3	2.7	74	36.5	44	7.5	38
HG2	16.2	2.7	60	36	43	7	37
HG3	12.6	2.7	42	35	40	5	37
HG4	9.1	2.7	21	36	41	5	36.5
HG5	6.2	2.7	18	33	36	3	35.5
HG6	19.6	1.6	163	37	45	8	38.5
HG7	16.9	1.6	130	36	46	10	36
HG8	12.5	1.6	69	35	40	5	37

[a]Molar feed of HMM, NiPAAm, and BIS in reaction system.
[b]Degree of swelling: DS = $(W_2 - W_1)/\rho W_2$, where W_1 and W_2 are the weights of dried and swollen gel in water, respectively, and ρ is the specific density of water. Measured at 25 °C and pH = 5.6.
[c]Determined from temperature-dependent ^1H HRMAS NMR measurements (Figure 2(c)): T_{tr} at 50% maximum signal height; $T_{10\%}$ at 10% maximum signal height; $\Delta T = T_{10\%} - T_{tr}$.
[d]DSC: T_{tr} = the peak maximum temperature (2nd heating).

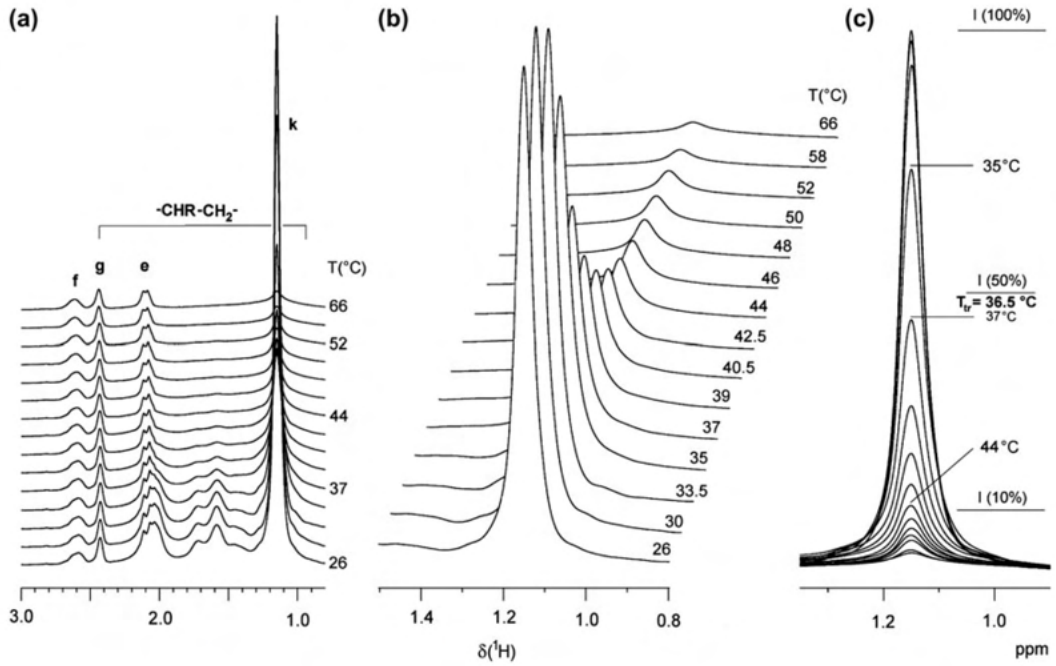


Figure 2. Temperature-dependent ^{1}H HRMAS NMR spectra of hydrogel 1 (HG1) swollen in D_2O. (a) Region showing the different effects on signals of the polyoxazoline side chains (H_e – H_g) and of the main chain containing 95 mol% NiPAAm units (–CHR-CH_2–, H_k). (b) and (c) expanded region of the methyl protons' signal, H_k, with indicated signal heights of 100, 50, and 10%. T_{tr} was determined at 50% signal height. The temperatures given in (b) were applied for all samples. Additionally, measurements were carried out at 28, 32, 55, and 62 °C (not shown).

37 °C. With the increase in the number of hydrophilic side chains (HMM), the transition temperature of PNiPAAm segments in the hydrogel increased, as already discussed in a previous paper for a similar but not crosslinked system.[20] But here, we have a theoretical length of poly(NiPAAm) segments between two crosslinks of 18 (HG1–HG5) and 31 (HG6–HG8) monomer units, respectively. Only less than 30% of the poly (PNiPAAm) segments statistically contain a hydrophilic HMM side chain. We can assume that the temperature of the collapse of main chain segments is caused not only directly by the NiPAAm copolymer chains but also by the concentration of HMM in the closed volume of the HG. The hydrophilic HMM side chains reduce, without covalent connection to the NiPAAm units, the thermal transition of the poly(PNiPAAm) segments since they provide a more polar environment. At constant BIS content, the amount of incorporated HMM in the hydrogel determines strongly the degree of swelling. Thus, the highest degree of swelling (measured at pH 5.6) of 163 was observed for 1.6 mol% BIS and 19.6 wt.% HMM (HG6), whereas the lowest DS with 18 corresponds to HG5 with 2.7 mol% BIS and only 6.2 wt.% HMM.

The broad distribution of segment length in the network and the statistical spatial distribution of HMM segments cause broadening of the transition range (see Figures 2 and 3).

The conformational transitions were also measured by means of DSC (Table 2 and Figure 3). The T_{tr} values were in agreement with the NMR values and all trends observed were the same.

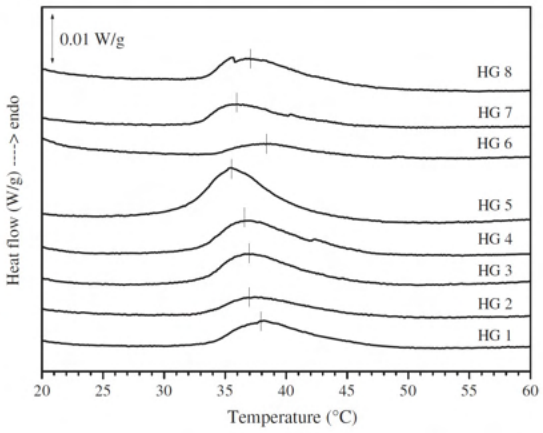


Figure 3. DSC measurements: the LCST behavior of HGs HG1–HG8 in hermetic closed sample pans determined from the 2nd heating run.

Table 3. Swelling characteristics (DS) of HG at variable HMM content.

HG	HMM content [wt.%]	DS 25 °C, pH 5.6 [mL g^{-1}]	DS 60 °C, pH 5.6 [mL g^{-1}]	DS 25 °C, pH 1 [mL g^{-1}]	DS 25 °C, pH 11 [mL g^{-1}]
HG1	22.3	75 (1)	32.5 (0.5)	14 (0.19)	30 (0.4)
HG2	16.3	60 (1)	–	15 (0.25)	30 (0.5)
HG3	12.6	42 (1)	–	13 (0.31)	15 (0.36)
HG4	9.1	21 (1)	4 (0.19)	11 (0.52)	10.5 (0.5)
HG5	6.2	18 (1)	–	10 (0.56)	10 (0.56)

Note: Value in parentheses: ratio $DS_{X\,°C,\,pHY}/DS_{25\,°C,\,pH\,5.6}$, relative DS with reference value of DS at 25 °C and pH 5.6.

All HGs showed a volume contraction when the temperature was increased and this contraction was stronger with a higher ratio NIPAAm/HMM. Two examples were determined at constant pH 5.6 (Table 3). HG1 and HG4 had a contraction of 50 and 81%, respectively, when the temperature was increased from 25 to 60 °C.

The HG also showed, at room temperature, a volume contraction or expansion with a change of pH (Table 3). The volume change contraction was determined in relation to the hydrogel volume at pH 5.6. For example, when the pH value was decreased from 5.6 to 1.0, the HGs suffer a volume contraction of 81, 75, 69, 47, and 44% for HGs: HG1, HG2, HG3, HG4, and HG5, respectively. The contraction was considerably lower for higher ratios NiPAAm/HMM in the hydrogel. The contraction of hydrogel at lower pH is possibly due to the hydrogen bond formation between the amide and acid groups [34–37] inside the hydrogel and the lower solubility of the HMM chain in its acid form. The change in contraction is in accordance with the change in ratio of NiPAAm/HMM. The hydrogel volume expansion from pH 1.0 to pH 5.6 is probably due to the mutual repulsion of increasing number of carboxylate groups.

Interestingly, at relatively high pH values, as for example at pH 11–12, the HG again showed a contraction. When the pH changed from 5.6 to 11.0, the contractions for HG1, HG2, HG3, HG4, and HG5 were 60, 50, 65, 50, and 45%, respectively. This phenomenon can possibly have a relation to the total high ionic charge inside the hydrogel at relatively high pH. Remarkably, the difference in the DS between pH 1 and pH 11 strongly decreases with lower content of HMM.

4. Conclusions

New HGs with sensitivity to temperature and pH have been synthesized by radical copolymerization of NiPAAm and polyoxazoline MM based on 2-methyl- and CEtOxa in the presence of a crosslinker (bisacrylamide). The MM synthesis proceeded as 'living' polymerization of the 2-oxazolines at relative low reaction temperature. Hydrolysis of methylester groups in the originally used MEtOxa comonomer under mild reaction conditions allowed the synthesis of an MM containing about 21 repeating units with a styryl end group and about 50 mol% statistically distributed carboxylic acid groups in CEtOxa along the chain. By this, we have produced a network of crosslinked temperature-sensitive poly(NiPAAm) main chains and pH-sensitive side chains of poly(MeOxa + CEtOxa) inside the network. The absolute swelling at pH 5.6 is strongly dependent on the HMM content at the same ratio of NiPAAm/BIS.

HRMAS NMR spectroscopy and differential scanning calorimetry (DSC) allowed the determination of thermal transition of the poly(NiPAAm) segments in the inner part of the HG. The temperature of conformational transition of hydrogel increased slightly with the increase in the relative content of hydrophilic MM, but the determined values ranging from 33 to 37 °C stayed in the interesting physiological regime of around 36 °C.

However, the volume change at the transition temperature was significantly increased at lower MM content and higher NiPAAm content. Contrarily, the volume change by changing the pH from 5.6 to 1 increased at higher MM content and lower NiPAAm content. The observed contraction of the hydrogel at lower pH (at room temperature) is caused by hydrogen bonds between the amide and acid groups inside the hydrogel.

Thus, bi-sensitive HG could be realized using the design principle of a thermo-sensitive network with variable sensitive side chains based on polyoxazolines synthesized in a quasi 'living' manner. These new pH- and thermo-sensitive HG are interesting for use as actuator and sensor gels in smart chemo-mechanical valves, pumps, or chemical transistors.

Acknowledgment

The authors wish to thank the Academic Department of Research (DGI) of Pontifical Catholic University of Peru for the financial support for this research. The assistance of Dr M Edelmann in the DSC studies and A Korwitz in NMR measurements is gratefully acknowledged.

References

[1] Weber C, Hoogenboom R, Schubert U. Temperature responsive bio-compatible polymers based on poly(ethylene oxide) and poly(2-oxazoline)s. Prog. Polym. Sci. 2012;37:686–714.
[2] Cohen M, Huck W, Genzer J, Mueller M, Ober C, Stamm M, Winnik F. Emerging applications of stimuli-responsive polymer materials. Nat. Mater. 2010;9:101–113.

[3] Langer R, Tirrell DA. Designing materials for biology and medicine. Nature. 2004;428:487–492.

[4] Okano T, editor. Biorelated polymers and gels. San Diego (CA): Academic Press; 1998.

[5] Schild HG. Poly(*N*-isopropylacrylamide): experiment, theory and application. Prog. Polym. Sci. 1992;17:163–249.

[6] Dimitrov I, Trzebicka B, Müller A, Dworak A, Tsvetanov C. Thermosensitive water-soluble copolymers with doubly responsive reversibly interacting entities. Prog. Polym. Sci. 2007;32:1275–1343.

[7] Bloksma M, Weber C, Perevyazko I, Kuse A, Hoogenboom R, Schubert U. Poly(2-cyclopropyl-2-oxazoline): from rate acceleration by cyclopropyl to thermoresponsive properties. Macromolecules. 2011;44:4057–4064.

[8] Park JS, Kataoka K. Comprehensive and accurate control of thermosensitivity of poly(2-alkyl-2-oxazoline)s via well-defined gradient or random copolymerization. Macromolecules. 2007;40:3599–3609.

[9] Huber S, Jordan R. Modulation of the lower critical solution temperature of 2-Alkyl-2-oxazoline copolymers. Colloid Polym. Sci. 2008;286:395–402.

[10] Aoi K, Okada M. Polymeritation of oxazolines. Prog. Polym. Sci. 1996;21:151–208.

[11] Velichkova RS, Christova DC. Amphiphilic polymers from macromonomers and telechelics. Prog. Polym. Sci. 1995;20:819–887.

[12] Saegusa T, Kobayashi S. Cyclic imino ethers – polymeri zation chemistry and polymer characteristics. Makromol. Chem. Macromol. Symp. 1986;1:23–37.

[13] Dworak A. The role of cationic and covalent active centers in the polymerization of 2-methyl-2-oxazoline initiated with benzyl bromide. Macromol. Chem. Phys. 1998;199:1843–1849.

[14] Hoogenboom R, Fitjen MWM, Schubert US. Parallel kinetics investigation of 2-oxazoline polymerizations with different initiators as basis for designed copolymer synthesis. J. Polym. Sci., Part A: Polym. Chem. 2004;42: 1830–1840.

[15] Nuyken O, Rueda-Sánchez J, Voit B. Synthesis of amphiphilic graft copolymers by ring-opening polymerization of 2-methyl-2-oxazoline initiated by poly [isobutene-co-(p,m-chloromethylstyrene)]macroinitiators. Macromol. Rapid Commun. 1997;18:125–131.

[16] Christova D, Velichkova R, Loos W, Goethals EJ, Du Prez F. New thermo-responsive polymer materials based on poly(2-ethyl-2-oxazoline) segments. Polymer. 2003;44: 2255–2261.

[17] Rueda J, Suica R, Komber H, Voit V. Synthesis of new polymethyloxazoline hydrogels by the 'macroinitiator' method. Macromol. Chem. Phys. 2003;204:954–960.

[18] Rueda J, Komber H, Cedrón JC, Voit B, Shevtsova G. Synthesis of new hydrogels by copolymerization of poly(2-methyl-2-oxazoline) bis(macromonomers) and N-vinylpyrrolidone. Macromol. Chem. Phys. 2003;204: 947–953.

[19] Hoogenboom R, Thijs H, Jochems M, Van Lankvelt B, Fitjen M, Schubert U. Tuning the LCST of poly(2-oxazo line)s by varying composition and molecular weight: alternatives to poly(N-isopropylacrylamide)? Chem. Commun. 2008;44:5758–5760.

[20] Rueda J, Zschoche S, Komber H, Schmaljohann D, Voit B. Synthesis and characterization of thermoresponsive graft copolymers of NIPAAm and 2-alkyl-2-oxazolines by the 'grafting from' method. Macromolecules. 2005;38: 7330–7336.

[21] Zschoche S, Rueda J, Boyko V, Arndt KF, Krahl F, Voit B. Thermo-responsive nanogels based on poly[NIPAAm-graft-(2-alkyl-2-oxazoline)]s crosslinked in the micellar state. Macromol. Chem. Phys. 2010;211:1035–1042.

[22] Rueda J, Zschoche S, Komber H, Krahl F, Arndt KF, Voit B. New thermo-sensitive graft copolymers based on a poly(N-isopropylacrylamide) backbone and functional polyoxazoline grafts with random and diblock structure. Macromol. Chem. Phys. 2010;211:706–711.

[23] Zschoche S, Rueda JC, Binner M, Komber H, Janke A, Arndt KF, Voit B. Reversibly switchable pH- and thermoresponsive core–shell nanogels based on poly (NiPAAm)-*graft*-poly(2-carboxyethyl-2-oxazoline)s. Macromol. Chem. Phys. 2012;213:215–226.

[24] Hoogenboom R. Poly(2-oxazoline)s: a polymer class with numerous potential applications. Angew. Chem. Int. Ed. 2009;48:7978–7994.

[25] Luxenhofer R, Han Y, Schulz A, Tong J, He Z, Kabanov AV, Jordan R. Poly(2-oxazoline)s as polymer therapeutics. Macromol. Rapid Commun. 2012;33:1613–1631.

[26] Kelly AM, Wiesbrock F. Strategies for the synthesis of poly(2-oxazoline)-based hydrogels. Macromol. Rapid Commun. 2012;33:1632–1647.

[27] Dargaville TR, Forster R, Farrugia BL, Kempe K, Voorhaar L, Schubert US, Hoogenboom R. Poly(2-oxazoline) hydrogel monoliths via thiol-ene coupling. Macromol. Rapid Commun. 2012;33:1695–1700.

[28] Kuckling D, Richter A, Arndt KF. Temperature and pH-dependent swelling behavior of poly(N-isopropylacrylamide) copolymer hydrogels and their use in flow control. Macromol. Mater. Eng. 2003;288:144–151.

[29] Richter A. Hydrogels for actuators. In: Gerlach G, Arndt KF, editors. Hydrogel sensors and actuators. Berlin: Springer; 2009. p. 221–248.

[30] Zarka T, Nuyken O, Weberskirch R. Amphiphilic polymer supports for the asymmetric hydrogenation of amino acid precursors in water. Chem. Eur. J. 2003;9: 3228–3234.

[31] Berger S, Braun S, editors. 200 and more NMR experiments, a practical course. Weinheim: Wiley-VCH; 2004. p. 146.

[32] Nuyken O, Maier G, Gross A, Fischer H. Systematic investigations on the reactivity of oxazolinium salts. Macromol. Chem. Phys. 1996;197:83–95.

[33] Spěváček J. NMR investigations of phase transition in aqueous polymer solutions and gels. Curr. Opin. Colloid Interface Sci. 2009;14:184–191.

[34] Chourdakis M, Bokias G, Staikos G. Poly(*N*-isopropylacrylamide) grafted to a strongly charged backbone: thermoresponsive behavior in aqueous solution. J. Appl. Polym. Sci. 2004;92:3466–3470.

[35] Garay MT, Llamas MC, Iglesias E. Study of polymer-polymer complexes and blends of poly (N-isopropylacrylamide) with poly(carboxylic acid): 1. Poly(acrylic acid) and poly(methacrylic acid). Polymer. 1997;38:5081–5096.

[36] Garay MT, Alava C, Rodriguez M. Study of polymer–polymer complexes and blends of poly(*N*-isopropylacrylamide) with poly(carboxylic acid). 2. Poly(acrylic acid) and poly(methacrylic acid) partially neutralized. Polymer. 2000;41:5799–5807.

[37] Weber C, Becer CR, Guenther W, Hoogenboom R, Schu bert US. Dual responsive methacrylic acid and oligo (2-ethyl-2-oxazoline) containing graft copolymers. Macromolecules. 2010;43:160–167.