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Temperature- and pH-responsive behaviour of poly(2-(2-methoxyethoxy)ethyl methacrylate-*co*-*N*,*N*-dimethylaminoethyl methacrylate) hydrogels

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ABSTRACT

Hydrogels with pH/temperature responsiveness and high water uptake have been synthesized by the free radical polymerization of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) with *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA) in a low proportion. The amphiphilic character of the biocompatible MEO_2MA provides thermo-sensitivity at low temperature. On the other hand, DMAEMA units incorporate ionisable amino groups and hydrophobic moieties, leading by themselves to a dual pH and thermo-sensitive system. Therefore, the combination of both monomers yields an interesting system with tuneable pH/temperature responsiveness and swelling capacity, which depends on composition and ionic strength. Thus, the volume transition temperature (VTT) is suppressed at low pH due to the basic character of DMAEMA. However, at basic pH, where amino groups are not charged, lower swelling capacities and narrow thermal volume transitions were obtained. At neutral pH, higher modulation of both the swelling achieved and VTT was observed.

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1. Introduction

Stimuli-responsive polymers are able to experiment large changes in their physical or chemical properties as a function of small changes in the environmental conditions. This is due to secondary interactions that are magnified or reduced with changes in pH, temperature, ionic strength, solvent composition, electric field, or by the presence of specific molecules [1,2]. Negative thermo-sensitivity in water is an interesting phenomenon exhibited by several polymers [2,3]. They undergo a collapse when reaching a critical temperature, called lower critical solution temperature (LCST). The polymers which exhibit this behaviour usually incorporate hydrophilic and hydrophobic moieties in their structure. Thus, the critical balance between the water hydrogen bond bridges and the hydrophobic interactions modulates this LCST. In the case of polymer gels, a considerable volume change takes place at a temperature close the LCST of the linear polymers; it is known as volume transition temperature (VTT).

Undoubtedly, linear and crosslinked poly(*N*-isopropylacrylamide [P(*N*-iPAAm)] is the most widely studied thermo-responsive system, due to the sharpness of their LCST and VTT and because the values of these transitions are close to body temperature, which is very relevant for biomedical applications [3,4]. However, thermo-responsive copolymers that do not contain acrylamides are gaining increasing attention [5]. In particular thermo-responsive systems based on 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) are being highly investigated in recent years [6–11]. Some of these studies indicate that copolymers based on MEO_2MA present thermo-sensitivity similar to P(*N*-iPAAm) but with the advantage of its biocompatibility [12,13].

Another thermo-responsive polymer is the poly(*N*,*N*-dimethylaminoethyl methacrylate) [P(DMAEMA)], which is also a weak polyelectrolyte and therefore, exhibits

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pH sensitivity. The LCST of P(DMAEMA) depends on the molecular weight and pH [14,15]. At basic pH, the amino groups are protonated and therefore, the electrostatic repulsions between the polymer chains impeded the collapse. At neutral pH, the thermal transition appears, being the LCST value of around 78 °C [14,15], rather high for a biomedical application. Furthermore, polymers based on DMAEMA have been matter of interest, because they are cationic polyelectrolyte, which can be used to synthesize gene or drug carriers based on interaction of charges. Thus, their polyelectrolyte complexes have been found to transfect a wide variety of cell types due to the ability of the polymer to condense pDNA, buffer lysosomal acidity, and transiently disrupt lipid bilayer membranes [16–18]. Moselhy et al. [19] have proved the efficiency of P(*N*-iPAAm-*co*-DMAEMA) copolymers in transfection enhancement activity of modified vectors in nasopharyngeal cancer cells in culture. In addition, Kurisawa et al. [20–22] have advanced the functional role of copolymers of *N*-iPAAm, butyl methacrylate (BMA) and DMAEMA for gene transfection applications.

Taking all these facts into account, in this contribution we explore the thermo- and pH-sensitivity of a new kind of copolymeric hydrogels based mainly on MEO_2MA, and a low proportion of DMAEMA. MEO_2MA units will provide biocompatibility and thermo-sensitivity at low temperatures, while ionisable DMAEMA units will provide pH-sensitivity [23] and amino groups, which could interact with drugs, peptides or genes. Therefore, we anticipate that this new system will be interesting for biomedical applications. The chemical structures of the monomers employed for the preparation of the corresponding P(MEO_2MA-*co*-DMAEMA) copolymeric hydrogels are represented in Scheme 1.

2. Experimental

2.1. Materials

The monomers 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA, Aldrich 95%) and *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA, Aldrich) were purified by passing by neutral alumina column to remove the antioxidant inhibitor. The activator *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, Fluka 99%), the crosslinker tetraethylene glycol dimethacrylate (TEGDMA, Fluka 90%), the initiator ammonium peroxodisulfate (APS, Fluka 98%) and the solvents ethanol (Normapur, analytical reagent), methanol (Fluka 99.8%), 2-propanone (Merck 99.8%) and chloroform (Sds 99.9%) were used as received. Water for all reactions, solutions for swelling experiments and hydrogel purification was Milli-Q from water purification facility (Millipore Milli-U10). Phosphate buffer solutions (PBS) were prepared employing sodium dihydrogen phosphate anhydrous (Fluka ⩾99%), disodium hydrogen phosphate (Panreac ⩾98%), phophoric acid (Panreac 85%) and sodium chloride (Panreac ⩾99.5%) to keep constant and controlled the ionic strength (μ).

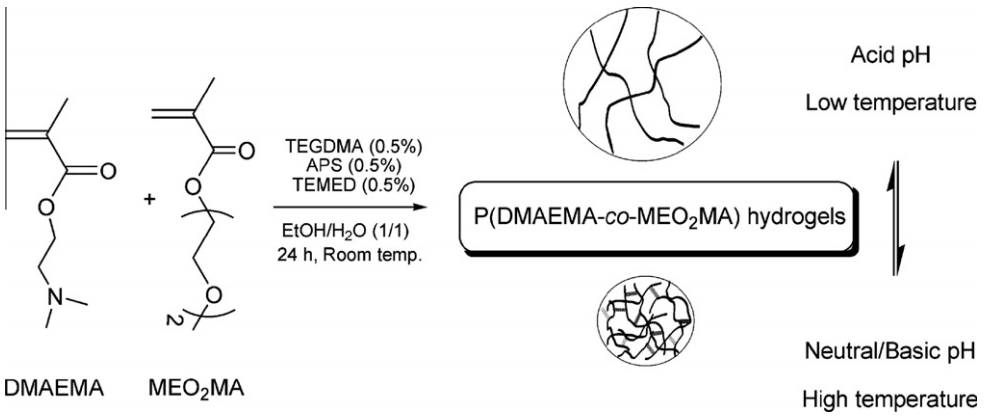
2.2. Syntheses of P(MEO_2MA-*co*-DMAEMA) hydrogels

P(MEO_2MA-*co*-DMAEMA) copolymeric hydrogels were synthesized by free-radical cross-linking random polymerization employing the monomer feed ratios given in Table 1. As it is shown in Scheme 1, polymerizations were carried out using a monomer/solvent ratio of 1 g mL^{-1} being the solvent a water–ethanol (1:1, v/v) mixture. In all cases, crosslinker TEGDMA, activator TEMED and initiator APS were used with an initial weight ratio of 0.5 wt.% with respect to total monomer.

The procedure to obtain disk shaped gels was as follows: the mixture solution was cast on glass plates

Table 1
Comonomeric composition for P(MEO_2MA-*co*-DMAEMA) copolymers determined by elemental analysis.

Feed MEO_2MA composition (mol-%)	Experimental MEO_2MA composition (mol-%)	
	Hydrogels	Uncrosslinked
100	100	100
95	92.9	92.4
90	89.0	88.3
82	82.3	79.5
75	75.7	75.9
62	63.6	65.0
50	50.1	52.2



Scheme 1. Synthetic conditions for the preparation of the P(MEO_2MA-*co*-DMAEMA) hydrogels.

enclosed by a rubber framework-spacer with 1 mm thickness and sealed off with other glass plate in order to avoid air contact during the polymerization (24 h, room temperature). Afterwards, the gel sheets were removed from the glass plate and uniform disks with a diameter of 6 mm were punched out the gel sheet using a stainless steel cork borer. Then, the disks were immersed in freshwater for, at least, 3 days to remove the unreacted chemicals. During this time the water was replaced several times. After that, the hydrogels were dried at room temperature until constant weight. In all cases, the polymerizations showed conversions values between 58% and 70%. These values were not proportional to the monomeric composition and they were calculated gravimetrically.

In order to evaluate the crosslinking effect on the LCST, uncrosslinked P(MEO_2MA-*co*-DMAEMA) copolymers were also synthesized using the procedure and feed monomeric composition previously described for crosslinked copolymers (see Table 1). In these cases, the conversion values of the polymerizations, calculated gravimetrically from the weight of the dried polymers, were between 65% and 80%.

2.3. Polymer characterization

The chemical composition of the copolymers was estimated by organic elemental analysis in a Heraeus CHN-O Rapid Analysis. The values obtained are given in Table 1. In addition, solution ^{1}H NMR spectra of uncrosslinked polymers were recorded in a Varian INOVA 400 MHz spectrometer in $CDCl_3$ at 25 °C using the typical acquisition parameters.

Dried disks were left to swell in PBS at different experimental condition during 24 h. Then, samples were taken out, wiped superficially with blotting paper and weighed. The equilibrium swelling (Q_∞) was calculated in grams of water per grams of dry gel using the following expression:

$$Q_\infty = (m_\infty - m_0)/m_0 = W_\infty/m_0 \quad (1)$$

where m_∞ is the weight of the swollen gel at equilibrium, m_0 is the weight of the dry polymer gel, and W_∞ is the weight of the solvent uptake at equilibrium. VTT or LCST were defined as the inflection point in the Q_∞, *versus* temperature plot.

When increasing the temperature above the volume transition temperature of the copolymer hydrogels and at the LCST of the linear copolymers, a decrease of the transparency occurs as a consequence of the polymer collapse, so that determining the change in the optical transmittance at 500 nm of the system in buffer solutions as a function of temperature, the VTT and LCST can be estimated. The analysis was made using a Cary 3 BIO-Varian UV–visible spectrophotometer. Temperature was raised from 15 to 20 °C above the transition temperature at a rate of 1 °C min^{-1}. The VTT or LCST were defined as the temperature at the inflection point in the absorbance versus temperature curve. For the LCST determination of uncrosslinked polymers, a buffer solution at pH 7 with a polymer concentration of 5 mg mL^{-1} was prepared. In addition, the phase transition behaviour of copolymer hydrogels was also determined from the temperature dependence of the gravimetric swelling ratio from 5 to 65 °C in PBS at pH 7.

3. Results and discussion

The first part of our investigation was conducted to evaluate the composition of each sample. In this sense, microanalysis and NMR were employed. From elemental analysis and taking into account that only DMAEMA incorporates nitrogen in its structure, the monomeric composition values, shown in Table 1, were determined. It is observed that the experimental compositions are quite similar to those employed in the feed. This experimental result together with the fact that conversion values between 58% and 70% were achieved seems to indicate that random monomeric distributions are obtained and therefore, the hydrogels present compositional homogeneity. It is also important to mention, at this point, that only copolymers up to 50 mol-% content of DMAEMA have been investigated in this contribution because higher contents are expected to show too high LCST or VTT values. On the other hand, ^{1}H NMR of the soluble polymers indicated the correct structure formation. Thus, as an example, Fig. 1 shows the spectrum of the linear P(MEO_2MA-*co*-DMAEMA) with 25 mol-% of DMAEMA including the signal assignment.

3.1. Effect of composition, temperature and pH on the swelling behaviour of P(MEO_2MA-co-DMAEMA) hydrogels

Fig. 2 shows the equilibrium swelling for the whole range of compositions investigated under acid and neutral pH at selected temperatures. This figure mainly illustrates the effect of the composition on the swelling degree. As it can be seen, the hydrogels with higher DMAEMA content (50–30 mol-%) exhibit noticeable swelling ratios, in particular at low pH, where the amino groups of DMAEMA are ionized and the contribution of the osmotic pressure is

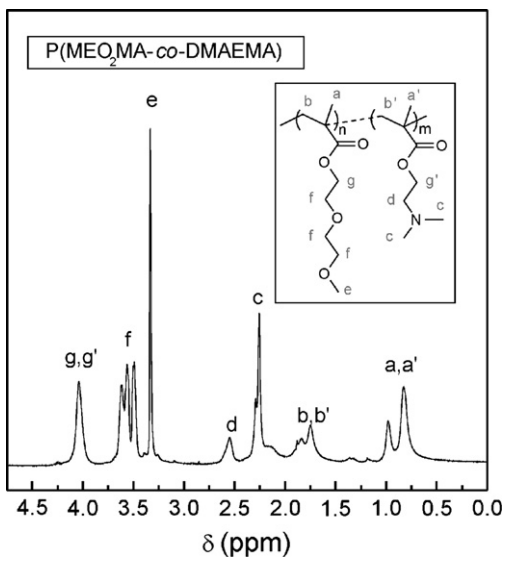


Fig. 1. ^{1}H NMR spectrum in $CDCl_3$ at 25 °C of a linear P(MEO_2MA-*co*-DMAEMA) with feed composition of 25 mol-% of MEO_2MA.

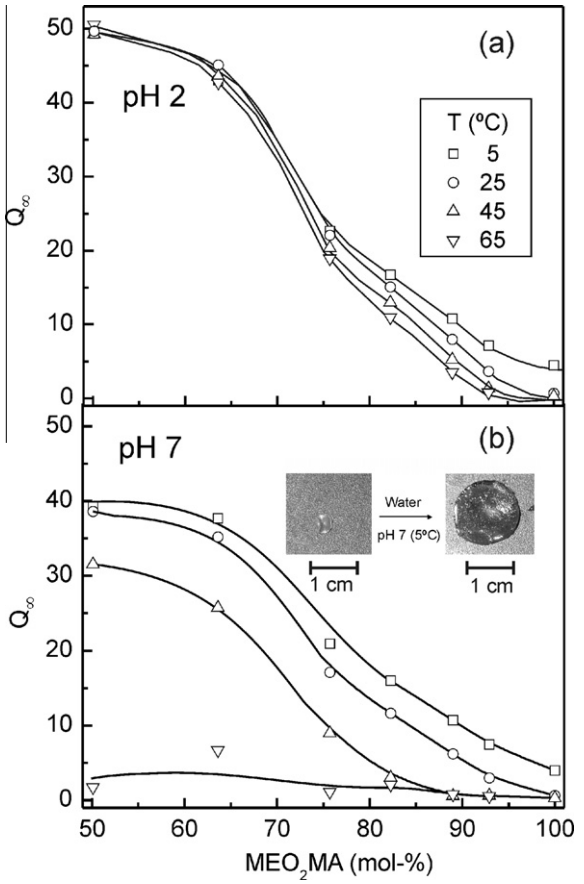


Fig. 2. Equilibrium swelling for P(MEO$_2$MA-*co*-DMAEMA) hydrogels as a function of MEO$_2$MA composition and temperature at (a) pH 2 and (b) pH 7.

considerable. Thus, hydrogels are able to increase 40 times their weight, which is a huge absorbent capacity.

Another interesting fact observed in this figure is that, for pH 2 and high DMAEMA compositions, Q_∞ is independent on temperature. Nevertheless, at pH close to the pK_a of the amino groups, strong temperature dependence is accounted for. In contrast to P(MEO$_2$MA), copolymers with DMAEMA exhibit a dramatic change in the equilibrium swelling degree between the collapse and the expanded state. In Fig. 3, equilibrium swelling as function of both composition and temperature are shown as three-dimensional plots for pH 2, 4, 7 and 9 (Fig. 3a–d, respectively). At a first sight, it can be observed that these surfaces, obtained from the adjustments of the experimental points (solid circles), are very different. At low pH (Fig. 3a and b), amino groups are positively charged, so that the hydrogels behave as polyelecrolytes absorbing large amounts of water. Therefore, it was detected that Q_∞ strongly increases with increasing the DMAEMA content in the hydrogel. Moreover, the electrostatic repulsions between the DMAEMA units are in opposite to the hydrophobic interactions so that the thermo-induced collapse is suppressed. Thus, at these conditions, the collapse is only observed for the P(MEO$_2$MA) hydrogel. At pH 9 (Fig. 3d), above the pK_a, the amino groups are uncharged resulting in an increase of the hydrophobic interactions for the whole range of compositions. That causes lower values of Q_∞ than those obtained at acid pH and thermal volume transitions occurring in a narrow temperature range (from 25 to 45 °C, approximately). At this pH, there is also a dependence of Q_∞ with composition. Although DMAEMA is unionized, it still establishes better interactions with water than MEO$_2$MA, resulting in higher both Q_∞ and VTT with increasing DMAEMA content. This result is in agreement with the hypothesis suggested by Feil et al. [24], who indicated that the phase separation temperature is determined by the overall hydrophilicity of the polymer, and thus related to the Q_∞ value.

The analysis of the composition and temperature dependence behaviour at pH 7 is shown in Fig. 3c. At this neutral pH medium, close to the pK_a of DMAEMA, a nice modulation of the VTT as a function of composition is observed. With increasing DMAEMA content in the copolymer, the collapsing temperature moves around 40 °C, from 25 °C to 65 °C, approximately. At the same time, hydrogels in the swollen state present high Q_∞ values, only slightly bellow to those obtained at low pH (see Fig. 3a and b). Therefore, these compounds could be interesting for biomedical applications because the VTT occurs with a considerable change in the hydrogel volume at physiological pH and, on the other hand, this temperature can be modulated by changing copolymer composition.

As it was expected, the swelling properties of these hydrogels depend on temperature but moreover, we have seen that the thermo-responsiveness also depends on the pH. In order to show the pH sensitivity of the hydrogels, in the three-dimensional plots shown in Fig. 4, the swelling pH-dependence is analyzed for the whole range of composition at various temperatures. It can be seen that Q_∞ increases at low pH and at higher DMAEMA content. At 5 and 25 °C, temperatures bellow the LCST of the copolymers, the behaviour is quite similar. Only, it seems that collapse slightly moves to low pH. This fact becomes more evident with increasing temperature. In fact, at 45 °C, the pH-induced collapse decreases for copolymers with higher MEO$_2$MA content and, at much higher temperature (65 °C), this effect is already relevant for the whole range of compositions. A plausible explanation for this behaviour was given by Siegel and Firestone [25] who found for hydrogels of DMAEMA copolymerized with hydrophobic comonomers, a systematic reduction in pH at which the swelling transition occurs as the hydrophobic comonomer increased. They suggested that the transition will occur when a particular balance of osmotic and hydrophobic forces is achieved. As network hydrophobicity increases, a greater degree of ionization is required to enable the transition. This happened with increasing temperature or with increasing MEO$_2$MA content. Other authors have reported a dependence of the pK_a of DMAEMA on temperature.[14,24] Thus, Feil et al. [24] reported a decrease of the pK_a of P(N-iPAAm-*co*-BMA-*co*-DMAEMA) terpolymers with increasing temperature, that was attributed to a decrease of the basicity. On the other hand, Plamper et al. [14] found a decrease of the pH with increasing temperature in buffer-free solutions of P(DMAEMA) and also a change in the slope of the pH–temperature curve at the LCST. This result

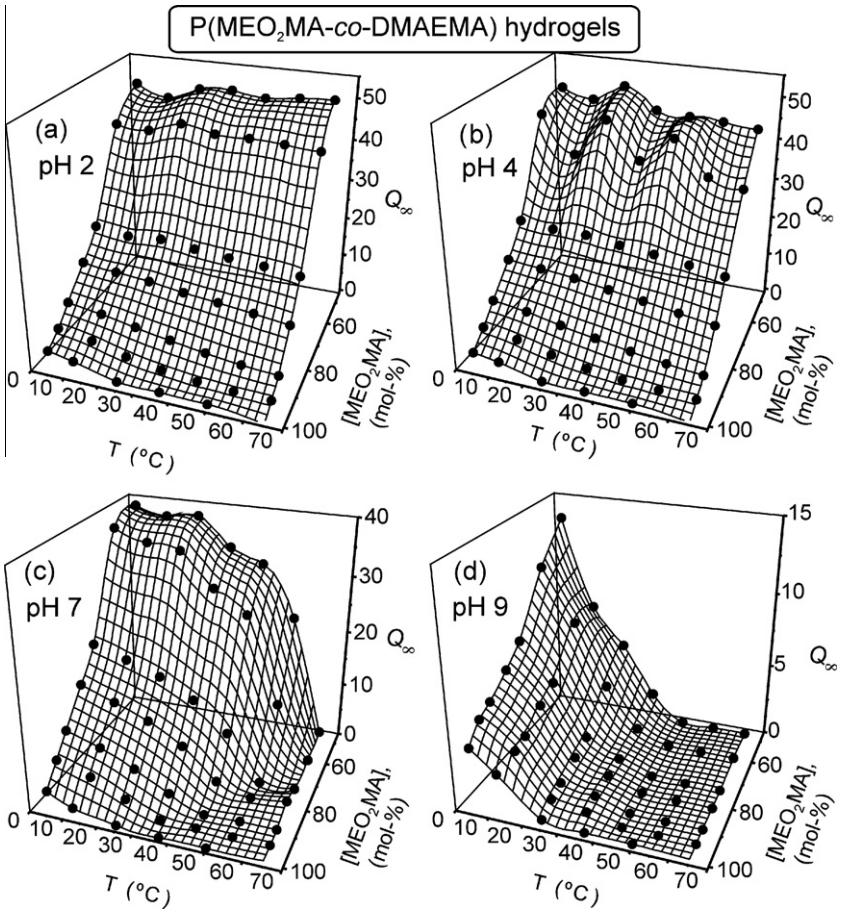


Fig. 3. Three-dimensional plots presenting the equilibrium swelling as a function of MEO_2MA composition and temperature of the P(MEO_2MA-*co*-DMAEMA) hydrogels at (a) pH 2, (b) pH 4, (c) pH 7 and (d) pH 9.

was attributed to a hindering of the amino groups ionization due to an increase in the local density (intra- and intermolecular aggregation).

3.2. VTT and LCST of P(MEO_2MA-co-DMAEMA) copolymers

The VTT of hydrogels and LCST of linear copolymers can be estimated by measuring the change in light absorbance trough them as a function of temperature in a swollen state or in solution, respectively. In Fig. 5, the VTT values determined in this way are shown as function of hydrogel composition at pH 7, 8 and 9. As it was observed in the swelling experiments, VTT increases as the DMAEMA content increases in the copolymer, according with the higher hydrophilicity of this comonomer. Moreover a strong dependence on pH is demonstrated. For the whole range of copolymeric compositions, an increase of the VTT is observed when the pH decreases, indicating the role of the charge of the amino groups on the VTT values. It is remarkable the good agreement between the VTT values estimated by this technique and those observed gravimetrically in Fig. 4.

In order to study the crosslinking effect on the thermal transition of P(MEO_2MA-*co*-DMAEMA) copolymers, the cloud points of the uncrosslinked samples were also estimated by measuring the absorbance at 500 nm in a buffer solution at pH 7. The results are included in Fig. 6, in which a similar behaviour to that observed for the crosslinked polymers can be seen. That is a rapid increase of the LCST up to 10 mol-% of DMAEMA, followed by a slow increase of the cloud point temperature with a further increase of DMAEMA comonomer. The LCST values of linear polymers lie below VTT corresponding to hydrogels. This is an expected result due to the crosslinking and it has been observed in other thermo-sensitive polymers, such as P(N-iPAAm) [1].

3.3. Effect of the ionic strength on the swelling behaviour and VTT of P(MEO_2MA-co-DMAEMA) hydrogels

It is well known that the salt concentration exerts a great influence on the swelling ratio of ionisable hydrogels. Thus, Fig. 7a shows that the swelling ratio of P(MEO_2MA-*co*-DMAEMA) hydrogels in PBS at pH 7 decreases with increasing NaCl concentration. The effect of the ionic strength on the swelling of these ionized hydrogels is similar to that observed elsewhere for macroscopic hydrogels [26] and microgels [27], and it is explained by using

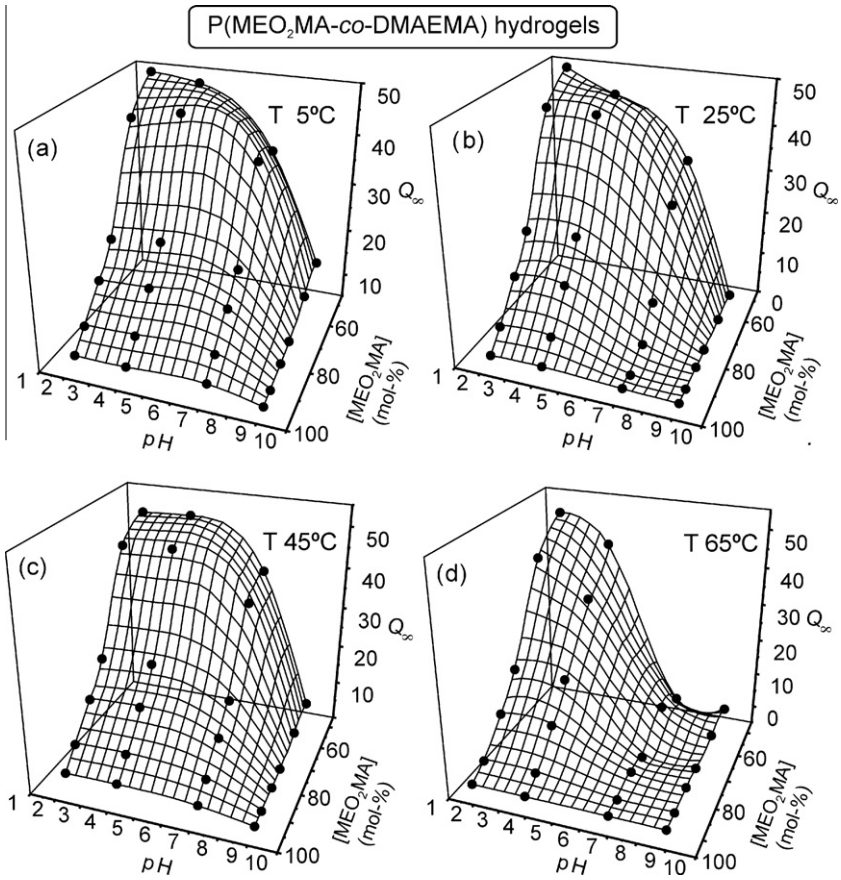


Fig. 4. Three-dimensional plots presenting the equilibrium swelling as a function of MEO_2MA composition and pH of the P(MEO_2MA-*co*-DMAEMA) hydrogels at (a) 5 °C, (b) 25 °C, (c) 45 °C and (d) 65 °C.

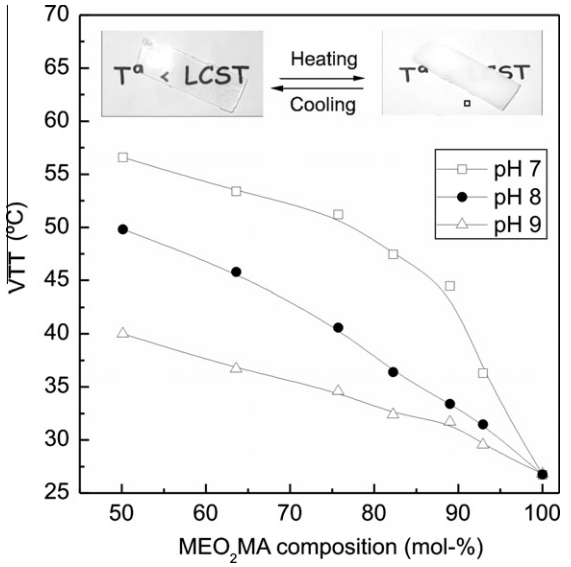


Fig. 5. VTT values obtained by absorbance at 500 nm of swollen P(MEO_2MA-*co*-DMAEMA) hydrogels as a function of composition in PBS at pH 7, 8 and 9. Photographs showing the change of transparency of a hydrogel slice at temperature below (left) and above (right) the VTT have been included.

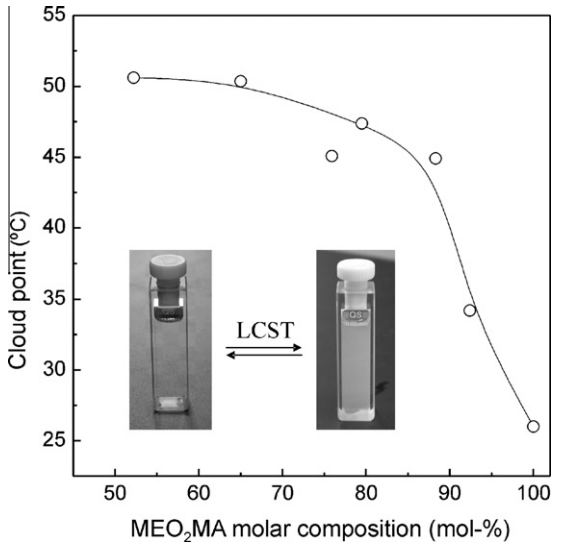


Fig. 6. LCST values obtained by absorbance at 500 nm of solutions of P(MEO_2MA-*co*-DMAEMA) linear copolymers as a function of composition in PBS at pH 7.

Donnan equilibrium arguments. As ionic strength rises, the difference in concentration of mobile ions between the gel and solution is reduced, thereby decreasing the osmotic swelling pressure of these mobile ions inside the gel.

On the other hand, the salt concentration should have an effect on the VTT of thermo-sensitive polymers with ionisable comonomers, as it has been reported for *N*-iPAAm copolymers [27,28], where NaCl concentrations higher than 0.1 M decrease the VTT values. In Fig. 7b the experimental VTT values obtained from absorbance measurements in PBS at pH 7 and with NaCl concentration of 0.035, 0.5 and 1 mol L^{-1} are represented. As it was expected, VTT decreases with increasing the NaCl concentration. In addition, a surprising experimental result was observed, the sharpness of the transition depends on the monomeric composition, since it was broader as higher was the DMAEMA content. In fact, for hydrogels with compositions higher than ~20 mol-% the VTT disappears or cannot be determined in the temperature range under study (5–70 °C). For this reason the VTT values for these compositions are missing in the figure. We hypothesize that this absence of VTT could be a consequence of a stabilization of the charged amino groups of DMAEMA at this pH, induced by the higher ion concentration.

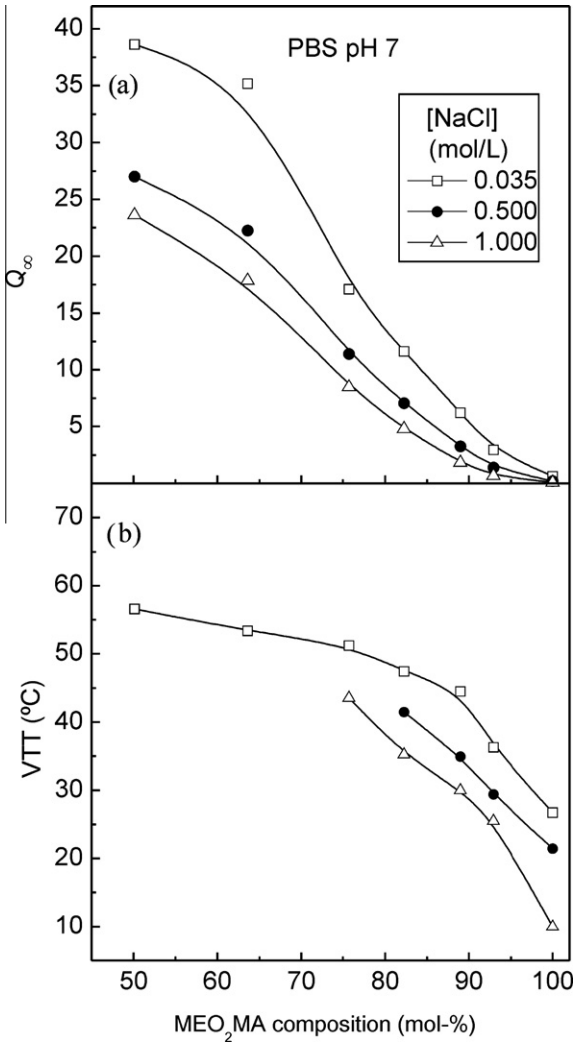


Fig. 7. (a) Equilibrium swelling at 25 °C and (b) VTT values obtained by absorbance at 500 nm of swollen P(MEO$_2$MA-*co*-DMAEMA) hydrogels as a function of MEO$_2$MA composition in PBS at pH 7 for different NaCl concentrations.

3.4. Effect of the solvent on the swelling behaviour of P(MEO$_2$MA-co-DMAEMA) hydrogels

Stimuli responsive hydrogels have been used in biomedical applications. In particular the controlled release of drugs is one of the most studied subjects [29]. Until this point, the swelling of these copolymers has been investigated in aqueous solution. However, for these applications the load of the drug in the hydrogel is the first step, and it is frequently limited by the low water solubility of the drug. The imbibition of the gel into a solution of the drug is one of the most employed methods to load the drug into the polymer matrix. This procedure often requires an organic solvent or water–solvent mixture to facilitate drug solubility and enhance polymer swelling. For this reason, the equilibrium swelling as function of MEO$_2$MA composition was studied for three different easily evaporated solvents, such as chloroform, methanol and 2-propanone. Fig. 8 shows Q_∞ values obtained for these solvents and those determined for the hydrogels in PBS at pH 7 and pH 9, included for comparison. The swelling achieved is inversely proportional to the solvent polarity. Thus, as higher is the polarity the lower is the equilibrium swelling. This result indicates that chloroform could be a good solvent for drug loading, taking also into consideration that drugs are often hydrophobic. Other noticeable result extracted from Fig. 8 is that there is not significant difference between the results obtained with methanol and 2-propanone. Both solvents have the same polarity and therefore, the protic character of the methanol does not seem to have

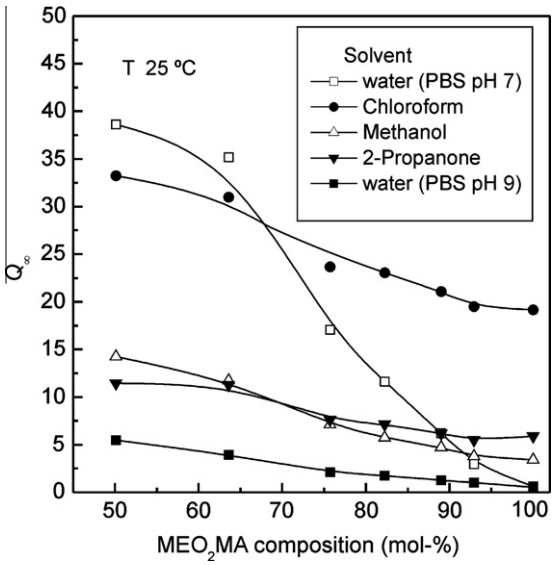


Fig. 8. Equilibrium swelling as a function of the MEO$_2$MA composition of the P(MEO$_2$MA-*co*-DMAEMA) hydrogels using different solvents.

a relevant influence on the swelling capacity. Finally, it should be noted that, in all cases, an increase of the achieved swelling is observed when the DMAEMA content increases. This result could be attributed either to a slight difference in the crosslinking density of the hydrogels or, as we believe, to more favorable interactions between the DMAEMA units with any of the solvent under study.

4. Conclusion

Thermo- and pH-responsive hydrogels with high swelling capacity can be easily synthesized by the free radical polymerization of MEO_2MA and DMAEMA as comonomer. The additional contribution of the osmotic pressure with increasing amount of DMAEMA causes a higher equilibrium swelling for the P(MEO_2MA-*co*-DMAEMA) copolymers than in neutral P(MEO_2MA) hydrogels. The VTT of the copolymers can be modulated by changing composition, pH and ionic strength. The pH responsiveness of the copolymers depends also on the hydrophobic balance, thus a shift to lower collapsing pH has been detected with increasing temperature and with increasing MEO_2MA content. This dual responsiveness together with the large extent of the transition from the collapsed hydrophobic state to the hydrophilic state, makes this system promising for biomedical applications.

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