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Temperature-dependent transparency of poly(HPMA-*co*-DMA) hydrogels: effect of synthesis parameters

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Abstract

The acrylic comonomers hydroxypropyl methacrylate (HPMA) and *N*,*N*-dimethylaminoethyl methacrylate (DMA) have been used in several earlier studies to produce pH-responsive hydrogels. However, these same monomers can also be used to prepare hydrogels that are highly responsive to temperature. One manifestation of this temperature sensitivity is a sharp decrease in hydrogel optical transparency that occurs when the temperature exceeds a critical transition value. For example, a hydrogel that exhibits a swelling transition at the physiological pH value of 7.4 has a transition temperature of about 45 °C when the environmental salt concentration is 0.15 M. The value of the transparency transition temperature is shown to depend on hydrogel synthesis parameters such as comonomer mole ratio, crosslinker mole ratio, and even initiator concentration. By reducing the mole ratio of the crosslinker tetraethylene glycol dimethacrylate (TEGDMA), the transition temperature can be lowered by as much as 15 °C. Environmental salt concentration and solvent polarity are also shown to influence the transition temperature.

Keywords: Lower critical solution temperature; Thermo-responsive hydrogel; Hydrophobic interaction

1. Introduction

Polymer hydrogels that exhibit reversible property changes in response to external stimuli such as pH, ionic strength, temperature, or analyte concentration are promising materials for use in chemical sensors [1], controlled drug delivery systems [2–5], ‘intelligent’ windows [6], and numerous other applications [7]. Several research groups have studied hydrogels or polymer solutions that respond both to pH and temperature [8–14]. This dual response can be obtained by synthesizing hydrogels with interpenetrating network structures [8–10], or by combining monomers with different pendant moieties in a random copolymer [11–14]. Here, we investigate pH/temperature-responsive hydrogels which are random copolymers of hydroxypropyl methacrylate (HPMA) and *N*,*N*-dimethylaminoethyl methacrylate (DMA), with tetraethylene glycol dimethacrylate (TEGDMA) as the crosslinker and ammonium peroxydisulfate (APS) as the free radical reaction initiator. The tertiary amine groups on DMA are weakly basic and become charged at low pH values, causing the hydrogel to swell. The transition pH value for DMA homopolymers is approximately nine, but this value can be decreased by copolymerizing DMA with hydrophobic monomers such as HPMA [15]. In particular, crosslinking copolymerization of DMA and HPMA at one of the mole ratios investigated here (30:70) yields a hydrogel with a transition pH near the physiological value of 7.4 [16], which is suitable for biomedical applications such as glucose-dependent insulin release. Hydrogels of a similar type containing either DMA or diethylaminoethyl methacrylate (DEA) have previously been studied in sensors [1,17,18], in insulin pumps [19], and in controlled drug delivery devices [2,4,20–22]. On the microscopic level, Beebe and co-workers have used pH-sensitive hydrogels for autonomous flow control inside microfluidic channels, and Baldi et al. have used glucose-sensitive hydrogels to regulate drug delivery [23,24]. The goal of the current study is to evaluate and enhance the temperature response of pH-sensitive copolymer hydrogels containing DMA, in the belief that this will increase their versatility for device or sensor applications.

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The temperature response of hydrogels containing DMA has been much less studied than the pH or glucose response. Yuk et al. studied the thermal behavior of aqueous solutions containing un-crosslinked random copolymers of DMA and ethyl acrylamide, a hydrophilic monomer [11]. These solutions exhibit a lower critical solution temperature (LCST) attributed to hydrophobic interactions between the pendant ethylamide and/or the pendant (*N*,*N*-dimethylamino)ethyl groups. Presumably the transition temperature reported here for DMA-containing hydrogels is of a similar origin as this LCST. Chen and co-workers presented preliminary results for the temperature-dependent optical transmittance of random copolymer hydrogels containing DMA and butyl methacrylate, a hydrophobic comonomer [12]. These authors mention that the hydrogel properties are highly dependent on the preparation method, but no report was given on the effects of crosslinker mole ratio or initiator concentration. The effect of initiator concentration and other synthesis parameters has been studied for temperature-responsive hydrogels containing *N*-isopropylacrylamide (NIPAM) as the temperature-sensitive moiety [14,25,26]. The linear polymer of NIPAM exhibits an LCST at approximately 32 °C, and the crosslinked hydrogel of NIPAM undergoes a reversible collapse at temperatures above about 33 °C. Synthesis temperature, free radical initiator concentration, and crosslinker mole ratio all affect poly(NIPAM) hydrogel properties, including the hydrogel transition temperature itself in some cases [14,26]. The transition temperature of poly(NIPAM) hydrogels can also be manipulated by incorporating hydrophilic co-monomers such as acrylic acid [27–29].

2. Experimental methods

2.1. Materials

Hydroxypropyl methacrylate (HPMA, Polysciences, Inc.) and *N*,*N*-dimethylaminoethyl methacrylate (DMA, Polysciences, Inc.) were vacuum distilled before copolymerization. Tetraethylene glycol dimethacrylate (TEGDMA, Polysciences, Inc.), ethylene glycol (EG, Aldrich), ammonium peroxydisulfate (APS, Aldrich) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, Aldrich) were used as received.

2.2. Synthesis of hydrogels

Hydrogels of HPMA and DMA were prepared by free radical crosslinking copolymerization in the cavity between two glass plates (100 mm length × 100 mm width × 2 mm thickness) separated by a Teflon spacer (0.4 mm thickness). The polymerization mixture compositions for the pregel solutions are shown in Table 1. The total monomer concentration in the pregel solution (≈99 wt%) was much higher than that used in this laboratory previously (≈30 wt%) in the synthesis of glucose-responsive hydrogels of similar stoichiometry [16]. The pregel monomer system was mixed at room temperature with a magnetic stirrer, and argon gas was bubbled through the mixture to remove oxygen. Freshly prepared free radical reaction initiator APS (4.7 wt% in deionized water) and a small amount of an EG solution containing the reaction activator TEMED were added to the monomer mixture, and the pregel solution was injected into the reaction cavity between the two glass plates. The reactor was surrounded by ambient air at 25 °C during the course of the reaction. After 12–16 h of crosslinking copolymerization, the hydrogel slab was separated from the glass mold and cut into square samples (9 mm length × 9 mm width × 0.4 mm thickness) using a long blade cutter. In order to remove un-crosslinked residues, all hydrogel samples were washed in PBS buffer solution (pH 7.4, ionic strength 0.15 M) for 5 days, with the buffer solution replaced three times per day. After washing, the samples were stored until use at room temperature in a bath solution of deionized water to which 0.15 M of NaCl was added.

2.3. Measurement of transparency transition temperatures and hydrogel swelling ratio

The hydrogel sample was placed in a closed glass vial containing 100 ml of the solution of interest. Initially the vial was cooled to 0 °C, after which the entire vial was placed into a water bath at 60 °C. The heating rate of the sample was approximately 10 °C/min. Periodically the contents of the vial were stirred and the temperature measured, and the onset of opacity in the hydrogel sample was determined visually. For each sample, the transition temperature was measured three times, with reproducibility better than 1 °C. In some cases, a Beckman spectrophotometer (DU Series 65) was used to measure hydrogel absorbance at 400 nm as a function of temperature or salt concentration in order to confirm the visually determined transition temperature.

Swollen hydrogel weights were measured using a blot-and-weigh procedure [16]. At each temperature and salt concentration, swelling measurements were made on three hydrogel samples of identical composition, and the measured sample swelling weights were normalized by the corresponding values at 50 °C and 0.5 M NaCl.

3. Results and discussion

The equilibrium swelling ratio as a function of pH and ionic strength has previously been measured for our ‘base case’ hydrogel system which contains HPMA, DMA, and TEGDMA at the mole ratios 70:30:2, respectively [1,16]. For a hydrogel of the same nominal composition (synthesis conditions TH-II in Table 1), Fig. 1 shows that the optical transparency varies considerably with temperature at fixed

Table 1
Feed compositions for hydrogels synthesized in this study

Hydrogel code	Feed composition								
	HPMA		DMA		TEGDMA		APS (mg)	TEMED (mg)	EG (mg)
	g	mole ratio	g	mole ratio	g	mole ratio			
TH-I	1.46	85	0.28	15	0.075	2	0.81	0.0039	7
TH-II	1.15	70	0.54	30	0.075	2	0.81	0.0039	7
TH-III	0.82	50	0.89	50	0.075	2	0.81	0.0039	7
TH-IV	1.15	70	0.54	30	0.019	0.5	0.81	0.0039	7
TH-V	1.15	70	0.54	30	0.037	1	0.81	0.0039	7
TH-VI	1.15	70	0.54	30	0.149	4	0.81	0.0039	7
TH-VII	1.15	70	0.54	30	0.075	2	0.405	0.0039	7
TH-VIII	1.15	70	0.54	30	0.075	2	1.62	0.0039	7
TH-IX	1.15	70	0.54	30	0.075	2	2.43	0.0039	7

pH (pH ≈ 5.6) and ionic strength (0.5 M NaCl). The hydrogel sample, which is transparent at lower temperature, suddenly obtains a milky white color near 25 °C.

Fig. 2 shows the temperature dependence of the equilibrium swelling weight of the same hydrogel at the same fixed pH value (pH ≈ 5.6) and ionic strength (0.5 M NaCl). At this pH value, the amine groups of the DMA comonomer should be fully charged, yet the hydrogel gradually contracts at temperatures above 10 °C. We attribute the contraction of the hydrogel to hydrophobic attractions between polymer chain pendant moieties.

Hydrophobic attractions are a consequence of the presence of water near hydrophobic moieties in aqueous solutions, and the temperature dependence of hydrogen bonding [30,31]. Many water-soluble polymers such as poly(ethylene glycol) (PEG) and random copolymers of DMA and ethyl acrylamide exhibit LCSTs that are attributed to hydrophobic interactions [11,30].

In Fig. 2, the relative weight or volume change is small in comparison to well-studied thermo-responsive hydrogels such as poly(NIPAM) [25,26,28,29]. Furthermore, the gradual variation in swelling weight with temperature makes it difficult to identify a precise value for the hydrogel volume transition temperature. The temperature at which the visual optical transparency changes is much easier to identify. For this reason, and because we are interested in developing visual sensors based on these hydrogels, we focus on hydrogel transparency transition temperatures rather than volume transition temperatures in the remainder of this work. For polymer solutions, there is a well-established connection between turbidity onset and the LCST [11]. However, polymer hydrogels often contain frozen-in microheterogeneity [32], and hydrogel optical transparency is a complex function of the size and number of phase-separated regions [25]. Hence in addition to its dependence on the thermodynamic interactions between the polymer and the solvent, the hydrogel transparency transition temperature may also depend on synthesis parameters such as the free radical initiator concentration [25].

Fig. 3 shows hydrogel absorbance as a function of external salt concentration at fixed temperature, as measured with the Beckman spectrophotometer at 400 nm. By visual appearance the same hydrogel is transparent at low salt concentration, and suddenly changes to a milky white color similar to that shown in Fig. 1 at 0.3 M NaCl.

Consistent with the visual observations, the hydrogel absorbance shown in Fig. 3 increases with salt concentration under the same conditions. The measured absorbance value is 0.25 (arbitrary units) when the external salt concentration is 0.3 M. Hence we use this absorbance value as an empirically determined absorbance criterion for the visual change in transparency.

The influence of comonomer mole ratio and environmental salt concentration on the transparency transition

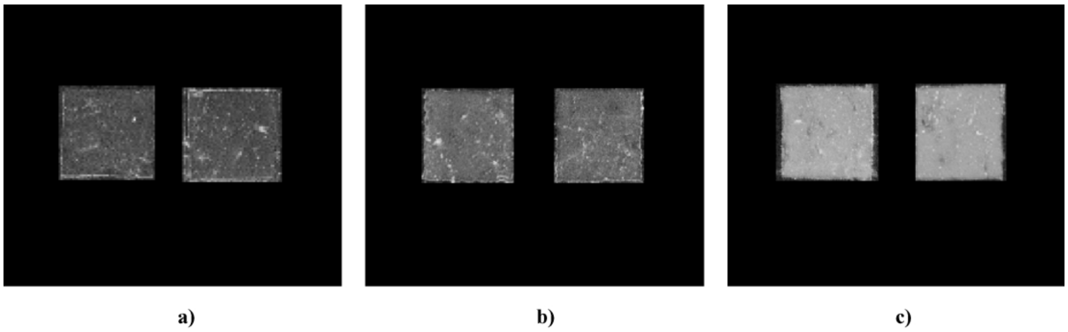
a) b) c)

Fig. 1. Optical photographs of hydrogel containing HPMA/DMA/TEGDMA at mole ratio 70:30:2 at the temperatures: (a) 12 °C, (b) 25 °C, and (c) 35 °C. Environmental salt concentration and pH were fixed at 0.5 M NaCl and 5.6, respectively.

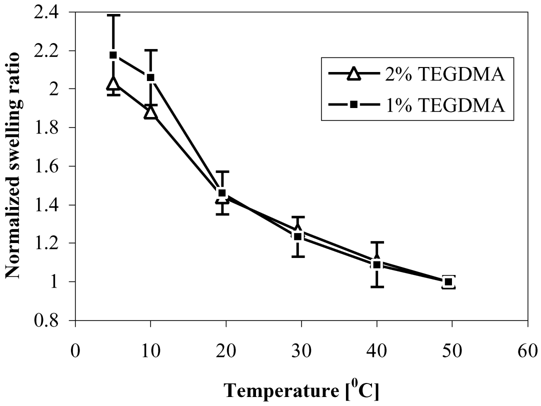

Fig. 2. Equilibrium weight as function of temperature, divided by the weight for the same sample at 50 °C, for hydrogels containing HPMA/DMA at the mole ratio 70:30 and crosslinker TEGDMA at the mol% given in the legend. Environmental salt concentration and pH were fixed at 0.5 M NaCl and 5.6, respectively.

temperature is shown in Fig. 4 at a fixed environmental pH value of 5.6. Also shown are transition temperatures measured via the spectrophotometer using the empirical criterion just discussed; these are in good agreement with the visual measurements. An increase in salt concentration sharply decreases the transition temperature, which is not surprising since salt has a similar effect on the LCST of water-soluble polymers like PEG [30]. Variation in the comonomer mole ratio affects the transition temperature by altering the hydrophobic/hydrophilic balance [27]. Both HPMA and DMA are relatively hydrophobic compared to most hydrogel monomers at high pH, with HPMA/DMA hydrogels only containing about 50 wt% of water at equilibrium [16]. By contrast, poly(NIPAM) hydrogels contain over 90% water at 20 °C [25]. However, DMA becomes a charged hydrophilic monomer at pH values below the transition pH value. The transition pH value for DMA is approximately nine in hydrogels containing 100 mol% DMA, and approximately 7.4 for hydrogels containing 30 mol% DMA and 70 mol% HPMA [16]. We estimate that the transition pH value is approximately 6.5 for hydrogels containing 15 mol% DMA and 85 mol% HPMA, based on results for similar hydrogels [15]. Therefore, the DMA comonomers were probably charged and hydrophilic for both hydrogel compositions under all the conditions shown in Fig. 4. An increase in DMA content is seen to increase the transparency transition temperature (at fixed salt concentration). This is consistent with the trends reported by Feil et al. [28,29], who observed that incorporation of ionizable acrylic acid monomers into poly(NIPAM) hydrogels at 10 mol% increased the volume transition temperature from 33 to 50 °C.

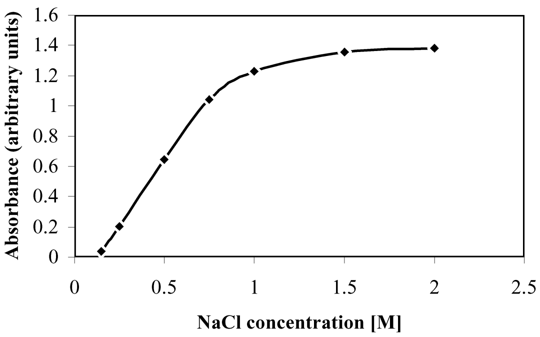

Fig. 3. Absorbance at 400 nm vs. external salt concentration at 23 °C, for a hydrogel containing HPMA/DMA/TEGDMA at the mole ratio 85:15:2. The value of the salt concentration giving a visual change in transparency is 0.3 M.

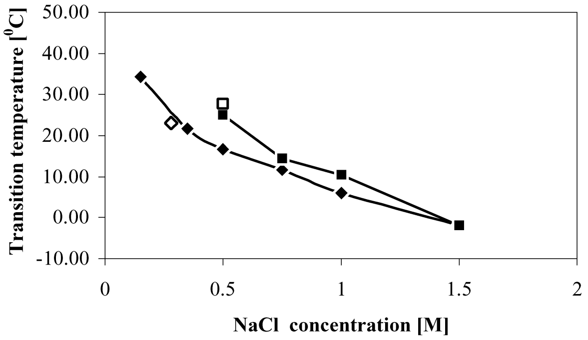

Fig. 4. Transparency transition temperature vs. external salt concentration at pH 5.6, with diamonds corresponding to hydrogels containing HPMA/DMA/TEGDMA at the mole ratio 85:15:2, squares corresponding to hydrogels containing HPMA/DMA/TEGDMA at the mole ratio 70:30:2, and unfilled symbols corresponding to spectrophotometric measurements. Errors bars are smaller than the symbol size ($n = 3$).

Much more surprising are experimental trends shown in Figs. 5 and 6. In Fig. 5, the transparency transition temperature increases by more than 15 °C with increasing nominal mole ratio of crosslinker TEGDMA (at fixed ratio of HPMA to DMA). Relatively high levels of crosslinker are of interest in our laboratory, because we physically entrap glucose-responsive enzymes in highly crosslinked pH-responsive hydrogels in order to fabricate glucose sensors [16]. The crosslinker used, TEGDMA, contains PEG-like units and is hydrophilic. Thus one possible explanation for

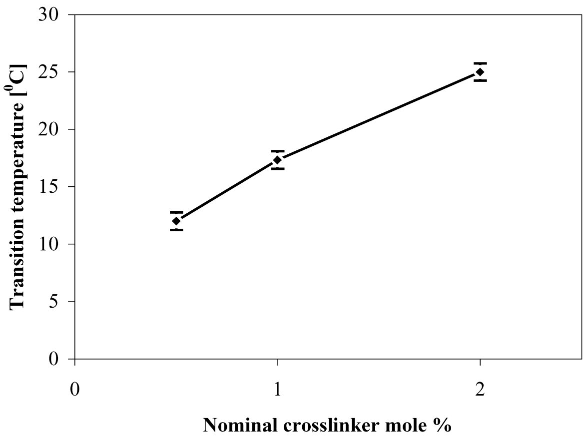

Fig. 5. Transparency transition temperature vs. nominal crosslinker mol% for copolymer hydrogels containing HPMA and DMA in the mole ratio 70:30. Environmental salt concentration and pH were fixed at 0.5 M NaCl and 5.6, respectively.

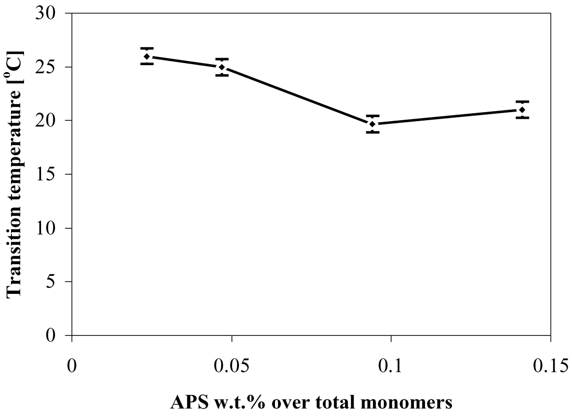

Fig. 6. Transparency transition temperature of hydrogels containing HPMA/DMA/TEGDMA in the mole ratio 70:30:2 vs. wt% of free radical initiator APS used during synthesis with fixed amount of reaction activator TEMED ($n = 3$). Transition temperature was measured at fixed environmental salt concentration (0.5 M) and fixed pH (5.6).

the effect of TEGDMA shown in Fig. 5 is that incorporation of large amounts of crosslinker into the network alters the hydrophilic/hydrophobic balance, thereby increasing the hydrogel transition temperature. Alternatively, the more highly crosslinked hydrogels are stiffer, and this stiffness might prevent hydrophobic interactions between some of the pendant hydrophobic moieties. Somewhat similar behavior has recently been reported for the volume phase transition temperature of poly(NIPAM) hydrogels by Kim et al. [14]. Kim et al. report that the hydrogel volume collapse temperature depends on the chemical identity of the crosslinker (poly(ethylene glycol) diacrylate vs. *N*,*N*′-methylenebisacrylamide (MBAAm)), and that the volume transition is broader for hydrogels containing larger nominal amounts of crosslinker (MBAAm).

Even when the hydrogels are prepared with fixed mole ratios of all comonomers, including crosslinker, the hydrogel transparency varies with the concentration of free radical initiator APS during synthesis. Use of higher initiator concentration gives a greater concentration of induced reaction centers and a faster reaction rate [32]. According to the results shown in Fig. 6, this leads to hydrogels with lower transparency transition temperatures over most of the concentration range of APS studied. In earlier studies, free radical initiator concentration has been found to affect the microheterogeneity of polyacrylamide hydrogels [32], and the transparency of poly(NIPAM) hydrogels [25]. In the latter study, Gehrke and co-workers found that the temperature of the pregel solution increases significantly during the course of the reaction, with a larger temperature rise associated with higher initiator concentration. If the increase in temperature during reaction exceeds the LCST of the pregel solution, the resulting hydrogel may contain microscopic phase-separated regions that scatter light and reduce transparency. We speculate that the mechanism identified by Gehrke et al. is also responsible for the decrease in transparency transition temperature with increase in initiator concentration in our hydrogels, which were synthesized under nearly bulk polymerization conditions in ambient air. For unknown reasons, at the highest APS concentration shown in Fig. 6, the transition temperature appears to reach a plateau or possibly begins to increase with further increase in initiator concentration.

Lastly, we consider the effect of the dielectric constant of the surrounding solvent on the transparency of the pH/temperature responsive hydrogels (Fig. 7). If the solvent dielectric constant is reduced due to the addition of either EG or ethanol (dielectric constant 24.3, Ref. [33]), the transparency transition temperature increases by an amount that depends on the mole fraction of additive. This trend may be attributed to an increase in electrostatic repulsion between charged DMA groups due to the reduction in solvent dielectric constant.

4. Conclusions

Random copolymer hydrogels containing acrylic comonomers HPMA and DMA, which have previously been shown to swell at low pH values [16], are shown here to shrink with increasing temperature. As these hydrogels shrink they become opaque. The change in transparency can be observed visually and is confirmed by spectrophotometric measurements at 400 nm. The hydrogel transparency transition temperature has a strong dependence on copolymer composition and preparation method. Surprisingly, the transparency transition temperature decreases with decreasing mole ratio of crosslinker or with increasing concentration of free radical initiator during synthesis. As shown previously, pH-sensitive hydrogels of similar copolymer composition containing immobilized enzyme glucose oxidase can be used to detect glucose, because glucose oxidase converts glucose to gluconic acid, thereby lowering the pH value within the hydrogel [2,16,18,20–22]. In our earlier study, the presence of glucose was detected from the increase in hydrogel swelling volume that occurred without change in transparency [16]. However, we envision using

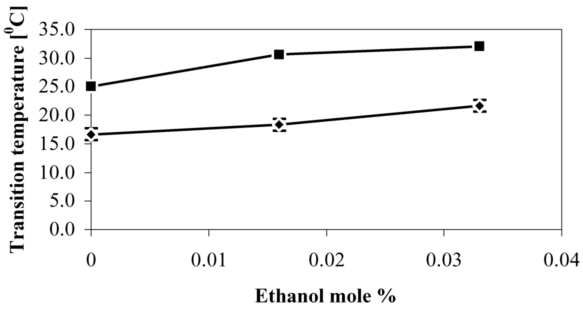

Fig. 7. Transparency transition temperature vs. ethanol mol% in mixed solvent of water and ethanol at pH 5.6 and salt concentration 0.5 M NaCl. Diamonds correspond to measurements on hydrogels with HPMA/DMA/-TEGDMA mole ratio 85:15:2, and squares correspond to measurements on hydrogels with HPMA/DMA/TEGDMA mole ratio 70:30:2 (error bars shown).

the results of the current study to adjust the hydrogel transition temperature so that it is slightly below the use temperature of a glucose sensor. In this case, lowering of the pH value by glucose will shift the hydrogel transition above the use temperature by increasing the mole ratio of charged DMA groups (Fig. 4). Hence the presence of glucose will be quickly detectable from a visual change in the hydrogel from opaque to transparent.

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