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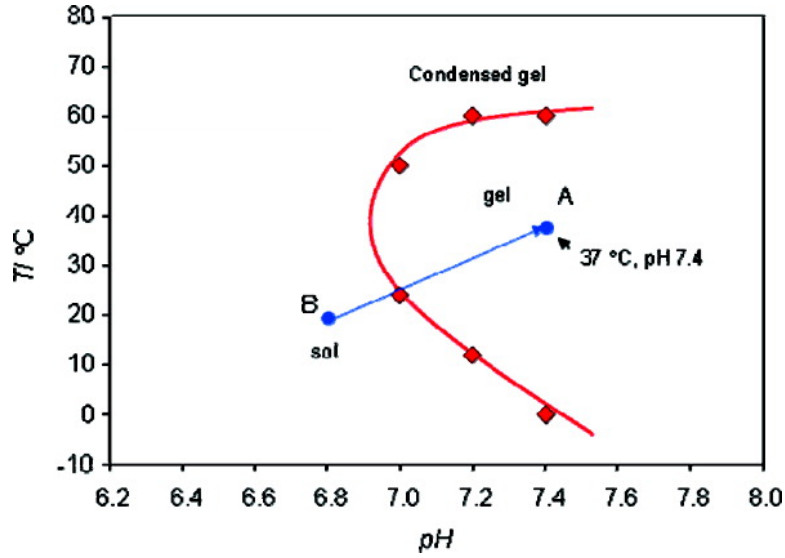

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Injectable Poly(amidoamine)-poly(ethylene glycol)-poly(amidoamine) Triblock Copolymer Hydrogel with Dual Sensitivities: pH and Temperature

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A novel triblock copolymer for use in an injectable pH- and temperature-sensitive hydrogel is synthesized by conjugating poly(amidoamine) (PAA) to poly(ethylene glycol): poly(amidoamine)-poly(ethylene glycol)-poly(amidoamine) (PAA-PEG-PAA). The polymer was characterized with ^{1}H NMR and gel permeation chromatography in the diluents $CDCl_3$ and $CHCl_3$, respectively. The PAA block acts as a pH- and temperature-sensitive block. The PAA-PEG-PAA copolymer in aqueous solution (12.5 wt %) underwent a sol−gel transition as a function of pH and temperature. After injection into a rat, the copolymer solution (12.5 wt %) was immediately changed to a gel.

In situ forming polymeric hydrogels have attracted considerable interest in recent decades because of their potential biomedical and pharmaceutical applications.[1−6] Thermosensitive hydrogels such as poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-*co*-glycolic acid)[7,8] and polycaprolactone-poly(ethylene glycol)-polycaprolactone[9] are typical examples of in situ forming polymeric hydrogels. These copolymers exhibit a reversible sol−gel−sol transition in aqueous media with increasing temperature. The gelation mechanism of these hydrogels has been investigated intensively: these amphiphilic block copolymers assemble first into micelles and bridged-micelles at low temperatures, and then the ordered packing of bridged micelles is triggered at a higher temperature and a macroscopic gel forms.[7−10] A thermosensitive polymer solution (sol) is injected into the body using a syringe and it is converted to a gel as a result of an increase in temperature caused by body temperature. The increase in temperature during injection might cause the gelation inside the needle, thus, blocking the needle. Therefore, their practical use is inconvenient.[9,11] In addition, their neutrality limits their usefulness in the delivery of ionic proteins/peptides.

To overcome these problems, pH- and temperature-sensitive pentablock copolymer hydrogels such as sulfamethazine-poly(lactide-*co*-caprolactone)-poly(ethylene glycol)-poly(lactide-*co*-caprolactone)-sulfamethazine (OSM-PCLA-PEG-PCLA-OSM)[11,12] and poly(β-amino ester)-poly(caprolactone)-poly(ethylene glycol)-poly(caprolactone)-poly(β-amino ester) (PAE-PCL-PEG-PCL-PAE)[13] have been investigated. These hydrogels can be injected into the body without gelling or blocking the needle during injection at room temperature. The association of bridged micelles has been proposed as the gelation mechanism of these pentablock copolymers in solution. In addition, the gel windows of these two hydrogels are appropriate to physiological conditions (37 °C, pH 7.4), and are thus favorable to practical applications. The OSM-based hydrogel is an anionic hydrogel. After injecting the polymer solution (15 wt %, pH 8.0) into the body, the solution is converted to a gel due to the increase in temperature to 37 °C and the decrease in pH to 7.4. In contrast to the OSM-based hydrogel, the PAE-based hydrogel is a cationic hydrogel. A polymer solution (30 wt %, pH 7.0) was injected into the body at room temperature and was found to convert to a gel due to the increases in pH and temperature to 7.4 and 37 °C, respectively.

Moreover, these pentablock copolymers consist of pH-sensitive moieties that exhibit an ionic nature and can thus form ionic linkages with counter-ionic drugs or proteins. In a study of the PAE-based hydrogel,[13] a negatively charged protein (insulin) was encapsulated with positively charged PAE through ionic interactions. The 14-day slow release of insulin in vitro was attributed to the degradation of PAE. However, these pentablock copolymers contain complicated structures, which enable pH-sensitive moieties, such as OSM and PAE, to be introduced into the thermogelling triblock copolymers.

In this study, we designed a novel triblock copolymer for use in an injectable pH- and temperature-sensitive hydrogel. This triblock copolymer has the same gelation characteristics as a function of pH and temperature as PAE-based pentablock copolymers, but it also has a similar cationic function.[13] In addition, the viscosity increases more than 5 orders of magnitude after injection of a polymer solution to the body, leading to formation of a stronger gel than other pH and temperature pentablock hydrogels.[12,13] To design this simple structure, poly(amidoamine) (PAA) was conjugated to poly(ethylene glycol) to create a triblock copolymer: poly(amidoamine)-poly(ethylene glycol)-poly(amidoamine) (PAA-PEG-PAA). PAA synthesized by carrying out the Michael-addition polymerization of diacrylamide and multifunctional amines is known to be biodegradable,[14,15] pH-sensitive,[16,17] and also to exhibit less cytotoxicity[15] than other synthetic copolymers.

Scheme 1 shows the chemical structure of the triblock PAA-PEG-PAA. Its molecular structure and molecular weight were characterized with ^{1}H NMR and gel permeation chromatography in the diluents $CDCl_3$ and $CHCl_3$, respectively (see the Supporting Information). These data are listed in Table 1.

The sol−gel transition was recorded using the test inverting method.[13] Each polymer solution was considered to be a gel if there was no flow observed within 1 min after inverting the vial. The sol-to-gel transition of this copolymer in an aqueous

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Scheme 1. PAA-PEG-PAA Triblock Copolymer Structure

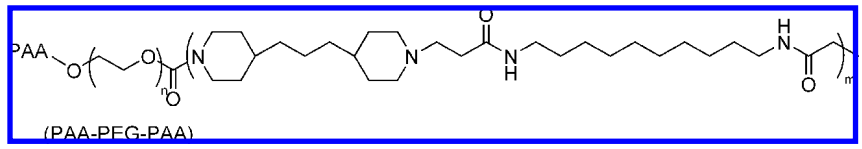


Table 1. Characteristics of the Triblock Copolymer PAA-PEG-PAA

sample	PEG[a]	M_n[b]	M_w/M_n[b]
1580−4600−1580	4600	7760	1.43

[a] The number-average molecular weight (M_n) of PEG was provided by Aldrich. [b] The M_n of the triblock copolymer and its polydispersity index were measured with GPC.

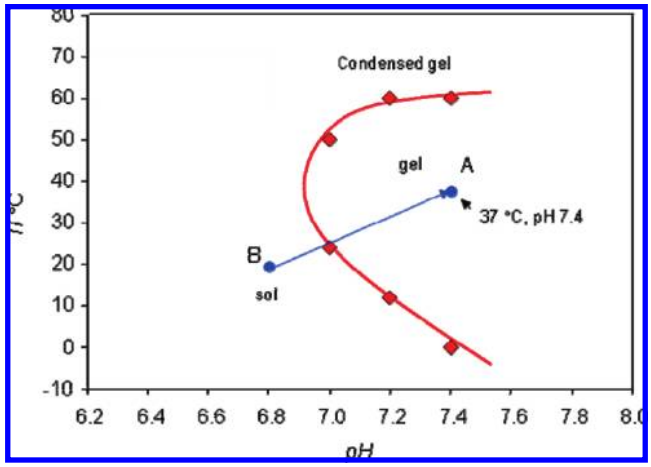


Figure 1. pH and temperature phase diagram of the 12.5 wt % PAA-PEG-PAA triblock copolymer in an aqueous medium.

medium (12.5 wt %) is illustrated as a function of pH and temperature in Figure 1. This phase diagram is the same as the phase diagram of the PAE-PCL-PEG-PCL-PAE pentablock copolymer. Two kinds of block copolymers, including a temperature-sensitive block (PCL) and a pH-sensitive block (PAE), have previously been combined to obtain bifunctionality in a pentablock copolymer.[13] However, in the PAA-PEG-PAA triblock copolymer, PAA has dual functionality in that it is sensitive to both pH and temperature: PAA is converted from a hydrophilic state to a hydrophobic state when the pH or temperature increase, as discussed in more detail below.

At low pH values and low temperatures (such as pH 6.8 and 20 °C, i.e., state B) the ionization of PAA meant that the polymer was soluble. At a low pH (pH 6.8), the hydrophobicity of the PAA segments increased with increasing temperature and the number of micelles grew. However, the number of micelles was not sufficient for gel formation, and the polymer solution exhibited a sol state up to 60 °C. At pH 7.0, PAA was partially deionized to form micelles and bridged micelles. However, the PAA blocks still exhibited hydrophilicity at temperatures below 24 °C and, thus, the solution was present in the sol phase. Above 24 °C, the increase in the hydrophobicity of the copolymer segments accelerated the association of the micelles and bridged micelles and a gel was formed.

At pH 7.4, the polymer solution became a gel due to the high degree of deionization of the PAA blocks and the association of the bridged micelles, even at 0 °C. An increase in the hydrophobicity of the PAA blocks at a higher temperature (37 °C, i.e., state A) lead to the tightly ordered packing of bridged micelles and a strong gel was formed. When the temperature was increased above the gel-to-condensed gel transition temperature, a significant amount of water was squeezed out from the block copolymer due to the partial dehydration of the PEG blocks. In addition, the sol−gel transition of the triblock copolymer solutions could be tailored by varying the PEG block

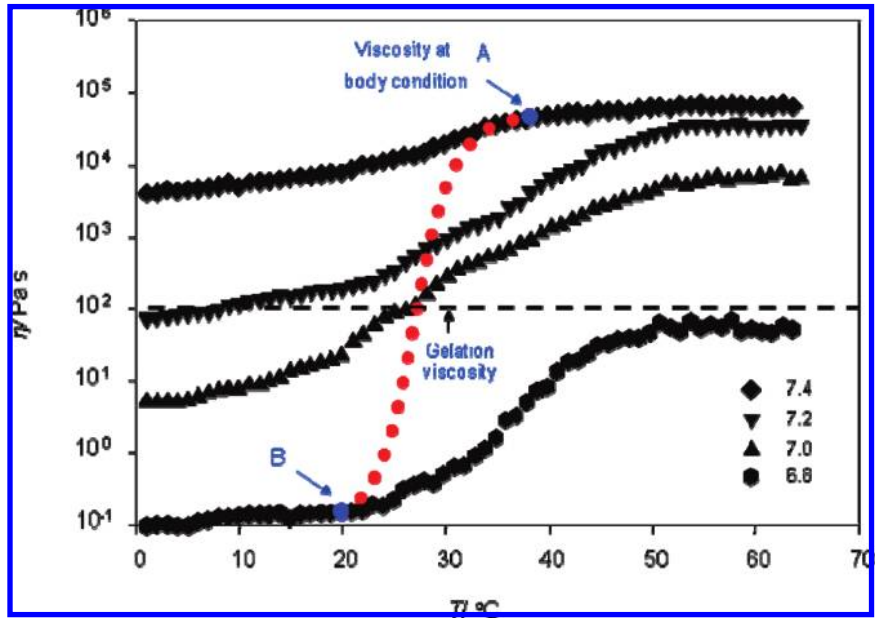


Figure 2. Log viscosity η of the PAA-PEG-PAA triblock copolymer in an aqueous solution (12.5 wt %) as a function of pH and temperature, obtained with dynamic rheological measurements under a controlled stress (0.4 Pa) at a frequency of 1.0 rad s^{-1}. The heating rate was 0.2 °C min^{-1}. The *x* axis is the temperature and the *y* axis is the log scale of the viscosity (η).

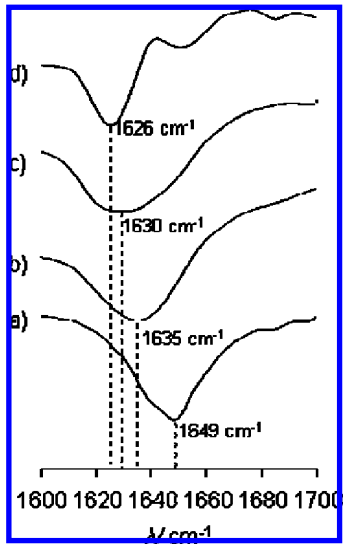


Figure 3. Infrared spectral shifts of the amide carbonyl region of the PAA-PEG-PAA triblock copolymer in $CDCl_3$ and D_2O for various values of the pH: (a) homogeneous solution in $CDCl_3$; (b) in D_2O at pH 7.0; (c) in D_2O at pH 7.2; (d) in D_2O at pH 7.4.

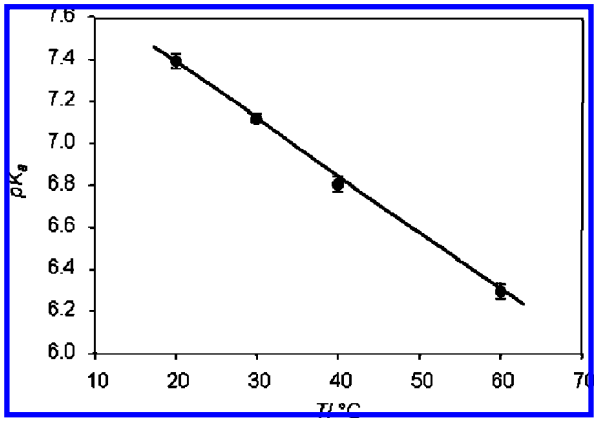
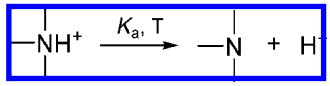


Figure 4. pK_a of the triblock copolymer in an aqueous solution as a function of temperature.

Scheme 2. Release of Protons of the Remaining Protonated Tertiary Amines at Higher Temperatures



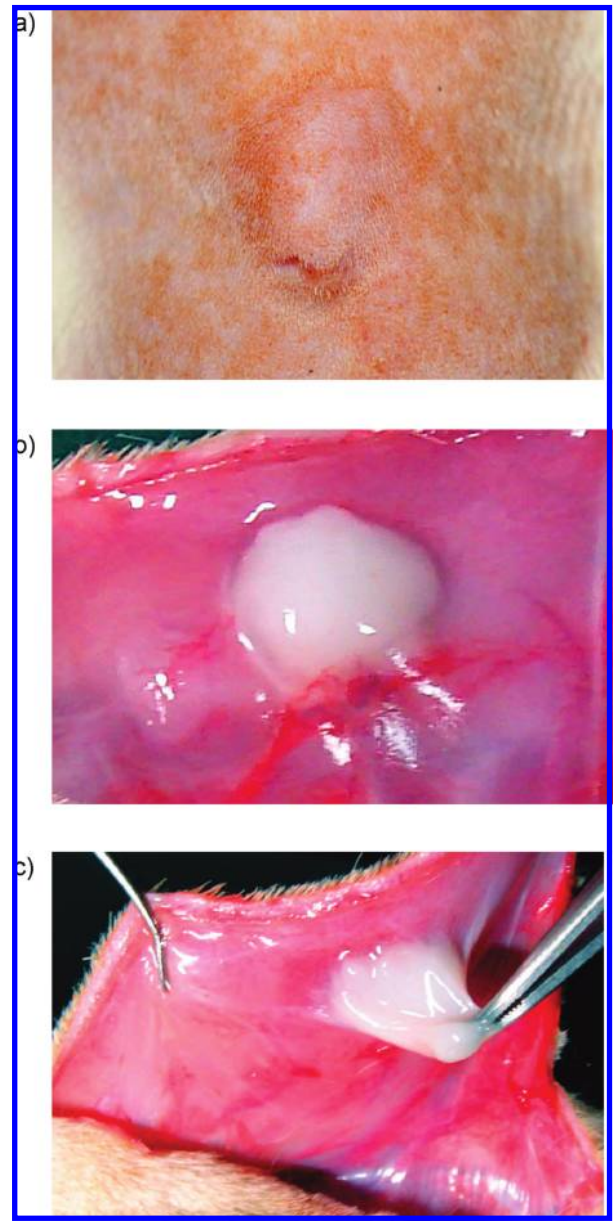


Figure 5. Gel morphology in vivo after a subcutaneous injection of 12.5 wt % copolymer solution at pH 6.8 into an SD rat: (a) after injection of 200 μL polymer solution; (b) the resulting hydrogel was found to be adhered to the SD rat tissue after only 1 min; (c) the stretched hydrogel has bioadhesive properties with respect to SD rat tissue.

length and the PAA molecular weight (see the Supporting Information).

Dynamic rheological analysis was used to show the effects of varying the pH and temperature on the viscosity of the triblock copolymer in aqueous media (Figure 2). At pH 7.0 and low temperatures, the viscosity increased very slowly with increases in the temperature, but increased rapidly above 20 °C. This trend might be due to the strong interactions between the PAA segments, which are caused by increases in the hydrophobicity of the PAA blocks with increases in temperature. At pH 7.4, the strong hydrophobic interactions of the PAA segments meant that the viscosity was high even at 0 °C. When the temperature was increased from 0 to 37 °C, the viscosity increased from 4.2 to 43.6 kPa s. At the gel-to-condensed gel temperature and higher temperatures (Figure 1), the viscosity did not change (Figure 2) due to the strong hydrogen bonding between polymer chains at high temperatures.[16,17] The sol-to-gel temperatures in the vials (see Figure 1) were 24 (at pH 7.0), 8 (at pH 7.2), and 0 °C (at pH 7.4), indicating that the gelation of the copolymer solution did not depend on an abrupt increase in the viscosity but on a critical value of the viscosity (the gelation viscosity; see the square-dotted line in Figure 2, $\eta \sim$ 0.1 kPa s). At pH 6.8, an appreciable increase in the viscosity was observed in the range 25−50 °C. However, this increase was not sufficient for the formation of a gel.

The line of red-closed circles in Figure 2 provided an estimate of the change in viscosity after injection of the solution into the body. The low viscosity copolymer solution (pH 6.8, 20 °C, state B in Figure 1) was converted to a gel (pH 7.4, 37 °C, state A) after injection. The viscosity of the polymer solution increased rapidly by more than five orders of magnitude ($\eta \sim$ from 0.16−43630 Pa s), leading to formation of a strong gel.

Fourier transform infrared spectroscopy (FTIR)[18−21] of the PAA-PEG-PAA triblock copolymer hydrogels (12.5 wt %) was

carried out in D_2O at various pH values and the results are presented in Figure 3. The peaks present at 1635, 1630, and 1626 cm^{-1} at pH 7.0, 7.2, and 7.4, respectively, were assigned to the amide carbonyl stretch. The peaks of the hydrogels at these pH values were shifted to lower energies with respect to the conventional carbonyl stretching centered at 1649 cm^{-1},[22] corresponding to the formation of well-developed hydrogen bonding β-sheet networks (1620−1640 cm^{-1}),[22,23] which suggests that the hydrogen-bonding networks between the polymer chains are well-ordered at these pH values.

Potentiometric titration was carried out to further elucidate the variation of the properties of solutions of the triblock copolymer in aqueous media with increasing temperature, and the results are shown in Figure 4. Note that the pK_a of the PAA-PEG-PAA triblock copolymer decreased linearly with increases in temperature, from 7.4 at 20 °C to 6.8 at 40 °C and 6.3 at 60 °C.[24,25] K_a is the ionization constant of a cationic acid (Scheme 2). This result indicates that it is energetically preferable for the PAA segments in aqueous media to release protons at higher temperatures.[24] Therefore, PAA becomes more hydrophobic at higher temperatures.

To investigate the injectability and in vivo spontaneous gel formation of the triblock copolymer solution, a 200 μL triblock copolymer solution (12.5 wt %) at pH 6.8 was subcutaneously injected into a SD rat with a syringe needle. The block copolymer solution was found to form an irregularly shaped skin protrusion (Figure 5a). One minute after injection, the SD rat was sacrificed to determine the gel morphology. As shown in Figure 5b, a white gel was found; this gel formed rapidly at the injection site due to the increase in the pH and temperature. This result suggests that the triblock copolymer solution can easily be injected into the body and forms an in situ gel within a short time. It was difficult to remove the gel from the SD rat because the gel had adhered to its tissues, but it was carefully removed with scissors and tweezers (Figure 5c).

In summary, this paper reports a novel, injectable triblock copolymer hydrogel based on poly(amidoamine) synthesized via Michael-addition polymerization. This triblock copolymer forms a pH- and temperature-sensitive hydrogel in aqueous media, which is similar to the PAE-based pentablock copolymer hydrogel. This gelation in aqueous media occurs as a result of self-assembly, the stabilization provided by interactions such as β-sheet-like hydrogen bonding networks,[22,23] and the hydrophobic interactions in the gelation pH and temperature range (7.0, 7.2, 7.4). This triblock copolymer solution is stable for more than two weeks (see the Supporting Information). In addition, the adhesion of the hydrogel to rat tissue, degradability,[14,15] and its excellent cytotoxicity (see the Supporting Information) suggest that it has bioadhesive applications.

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Supporting Information Available. Text giving detailed experimental procedures and supplementary figures showing ^1H NMR, sol−gel transition, gel stability, and schemes showing the structures and synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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