In Situ Thermal Gelation of Water-Soluble Poly(*N*-isopropylacrylamide-*co*-vinylphosphonic acid)

GYOUNG TAE EOM,[1] SEAUNG YOUL OH,[2] TAE GWAN PARK[1]

[1] Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Kusong-dong Yusong-gu, Taejon 305-701, South Korea

[2] College of Pharmacy, Sookmyung Women's University, Chongpa-dong Yongsan-gu, Seoul, 140-742, South Korea

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ABSTRACT: A copolymer based on *N*-isopropylacrylamide (NIPAAm) and vinylphosphonic acid (VP) was synthesized to investigate its thermal gelation behaviors in the presence of calcium ion. The copolymer showed a variety of temperature-sensitive phase transition properties as a function of temperature. In an aqueous solution, it exhibited 3 distinctive phase transitions with gradually increasing the temperature: a transparent solution state, a cloudy and white solution state (sol), and a white and semisolid (gel) state. Particularly, *in situ* reversible sol-gel transition behaviors could be observed over a wide range of temperatures in the presence of varying concentrations of calcium ion. This physical gel that initially was formed homogeneously without changing its dimension in the solution container tended to slowly phase-separate out into a polymer-rich phase and a water-rich phase over the following several days, indicating that a synerisis occurred. The *in situ* thermal gelation behavior was utilized in the controlled release formulation of a model compound. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 70: 1947–1953, 1998

Key words: thermal gelation; poly(*N*-isopropylacrylamide); hydrogel; lower critical solution temperature; drug delivery

INTRODUCTION

During the past decade, intelligent hydrogels that exhibit various physicochemical changes in response to external stimuli have received much attention.[1] Among them, chemically crosslinked temperature-sensitive poly[*N*-isopropylacrylamide (NIPAAm)] has been extensively utilized for the applications of drug delivery, immobilization matrices for enzymes and cells, and bioseparation.[2] Uncrosslinked, water-soluble, and linear poly(NIPAAm) has been used for the conjugation of biomolecules.[3] In general, hydrogels, which are defined as swollen polymer chain networks in an aqueous solution, can be divided, depending on the nature of crosslinking between polymer backbones, into 2 categories: chemical and physical gels.[4] The chemical gels are normally prepared by using a chemical crosslinker, which covalently ties up 2 water-soluble polymer chains together, while the physical gels are formed by noncovalent interactions between the polymer chains. Thus, the former normally exhibits more strong mechanical properties with a relatively better defined structure than the latter. Most synthetic hydrogels have been prepared by chemically crosslinking water-soluble polymer chains. On

Correspondence to: T. G. Park.

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the other hand, natural hydrogels, such as alginates, carrageenan, agar, gellan, pectin, and gelatin, have been formed by noncovalent interactions, such as ionic interaction, hydrophobic interaction, and/or hydrogen bonding.[5] These physical gels can be formed in the presence of counterions or by external environmental variations, such as temperature and pH, which induce the conformational change of the natural polymers. Synthetic physical gels could be similarly made based on water-soluble polymers by molecularly engineering their structures having a self-assembling property. For instances, polypropyleneoxide–polyethyleneoxide–polypropyleneoxide (PPO–PEO–PPO) tri-block copolymers (Pluronic),[6] poly(acrylic acid)-grafted Pluronic,[7] poly(vinyl alcohol),[8] and a modified polyphosphazene[9] showed a variety of gelation behaviors in response to temperature. A delicate balance of hydrophilic/hydrophobicity in the polymer chain, which is believed to be essential for the generation of a temperature-induced self-assembled micelle structure, seems to play a key role in demonstrating an *in situ* thermal gelation.

Uncrosslinked poly(NIPAAm) at high concentration did not demonstrate a reversible and homogeneous self-setting thermal gelation at its lower critical solution temperature (LCST) but showed a phase separation behavior that accompanies with the precipitation of shrunken poly(NIPAAm) gel out of heated solution. Recently, however, it was reported that poly(NIPAAm) containing a small amount of acrylic acid in its backbone demonstrated a thermal gelation behavior at the LCST.[10] This gel formation occurred in the absence of any divalent ions.

In the present study, by using an anionic copolymer of NIPAAm with vinylphosphonic acid (VP), *in situ* thermal gelation was attempted with the aid of counter-ion, calcium. Synthetic polymers containing pendant phopsphonic acid groups have received special attention in the field of dental and orthopedic biomaterials because of their calcium binding affinity.[11] Figure 1 shows a molecular structure of poly(*N*-isopropylacrylamide-*co*-vinylphosphonic acid [poly(NIPAAm-*co*-VP)]. The main objective is to prepare thermally reversible nonshrinking gels useful for drug delivery and *in situ* biocatalyst immobilization. Pendant phosphonic acid groups along the poly(NIPAAm) backbone chain are expected to intermolecularly chelate calcium ions, providing ionic interactions for weak network formation, while more hydrophobic

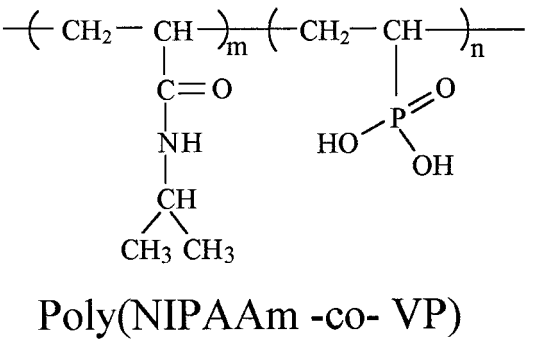


Figure 1 Molecular structure of poly(NIPAAm-*co*-VP).

NIPAAm units in the polymer chain dehydrate, interact, and self-assemble via hydrophobic interaction at their sol-gel transition temperature. The temperature-dependent phase transition behaviors between transparent solution state and white solution state, and the following sol-gel transition are characterized as a function of calcium concentration for poly(NIPAAm-*co*-VP) containing 95/5 molar feed ratio of NIPAAm and VP. Release profile of methylene blue, as a model drug, from *in situ* thermally gelled poly(NIPAAm-*co*-VP) membrane was characterized.

MATERIALS AND METHODS

Materials

N-isopropylacrylamide obtained from Eastman Kodak (Rochester, NY, U.S.A.) was recrystallized from hexane. Vinylphosphonic acid (90% technical grade) purchased from Hoechst Celanese (Charlotte, NC, U.S.A.) was used without further purification. Azobisisobutyronitrile (AIBN) from Aldrich (U.S.A.) was recrystallized from methanol. All other chemicals and solvents were analytical grade.

Methods

Polymer Synthesis

Poly(NIPAAm-*co*-VP) having 95/5 molar feed ratios of NIPAAm and VP was synthesized. Total 10 g of NIPAAm and VP with a molar ratio of 95/5 was dissolved in a mixture of toluene–tetrahydrofuran (180/30 mL), and 50 mg of AIBN was added as an initiator. Under a nitrogen atmosphere, polymerization was carried out at 60°C for 6 h. After evaporating the solvent under reduced pressure,

the copolymer was dissolved in methanol and slowly precipitated in an excess amount of petroleum ether. The collected polymer was dried under vacuum.

Molecular Weight Determination

Molecular weight of the copolymer was determined by using dynamic light scattering with a Malvern Instrument S4700. The copolymer was dissolved in deionized water in varying concentrations ranging from 1.25 mg/mL to 10 mg/mL, and scattering angles were changed from 40 to 140°. The molecular weight was then calculated from the constructed Zimm plot.

Temperature-Dependent Transmittance Change

The copolymer was dissolved with the concentration of 10 mg/mL in 0.1*M* acetate buffer solutions for pH 2–5, and in 0.1*M* tris buffer solutions for pH 6–10. The samples were incubated at different temperatures at least for 30 min to reach an equilibrium. The transmittance at 600 nm was determined at each temperature by using Beckman DU-600 ultraviolet–visible (UV–Vis) spectrophotometer.

Titration Curve of Poly(NIPAAm-co-VP)

1 g of the copolymer was dissolved in 50 mL of deionized water, and the solution was titrated by slowly adding the small volume of 0.5*N* NaOH solution. The change in pH value was continuously monitored. The titration was performed at room temperature.

Phase Diagram Construction

The copolymer (10% w/v) dissolved in 0.1*M* tris buffer, pH 7, containing different calcium chloride concentrations, were equilibrated in a temperature-controlled water bath. By raising temperature 1–2°C at every 1 h, the physical appearance was visually observed by a naked eye. For the convenience of visual observation, the following states were classified: transparent fluid, white fluid, white gel, and phase-separated white gel. The formation of homogeneous gel was confirmed by the observation of a whole-volume gelation and no fluid flow when inverting the sample vial for 5 min. The formation of phase-separated gel was confirmed by the visualization of the 2 phases composed of a polymer-rich phase (shrunken and precipitated gel) and a water-rich phase.

Temperature-Dependent Viscosity Change

Poly(NIPAAm-*co*-VP) having 95/5 molar feed ratio of NIPAAm/VP was dissolved in 0.1*M* tris buffer, pH 7, containing 900 m*M* calcium chloride. After adjusting the polymer concentration at 10% (w/v), the solution was charged into the tube of a falling-ball viscometer (Gilmont Instrument Co). The flow time of a glass ball was recorded in duplicate between 2 lines marked on the tube at different temperatures. The solution viscosity was then calculated.

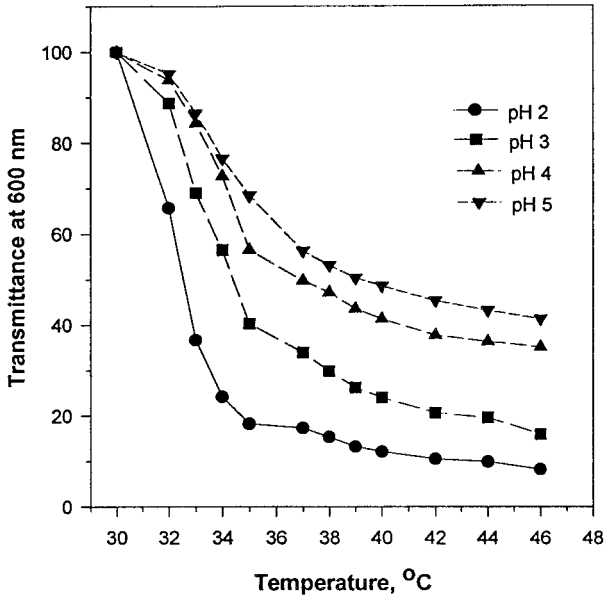
Drug Release Experiment

The copolymer solution with the concentration of 10% (w/v) dissolved in 0.1*M* tris buffer (pH 7) containing 900 m*M* calcium chloride, and 10 m*M* methylene blue was prepared and injected into a disposable dialysis cassette having a 3-mL internal volume within a double-side dialysis membrane (molecular weight cutoff, 10,000) (Pierce, U.S.A.). The dialysis cassettes after equilibrating at 37°C in an oven incubator were placed in 100 mL of the same buffer at 37°C. The sample was withdrawn at predetermined time intervals, and the release amount was analyzed by measuring absorbance at 600 nm.

RESULTS AND DISCUSSION

The molecular weight of poly(NIPAAm-*co*-VP) determined from a dynamic light scattering was 168,000. The value of radius gyration was 24.6 nm. Figure 2 shows the temperature-dependent transmittance change of poly(NIPAAm-*co*-VP) as a function of pH. It can be seen that with increasing the pH, the LCST shifts to higher temperature, and the extent of transmittance change becomes broader. This is a typical trend of poly(NIPAAm)-based copolymers that contain hydrophilic comonomers in the poly(NIPAAm) backbone.[12] Since poly(NIPAAm-*co*-VP) has phosphonic acid groups in its polymer chain, which can be ionized to monobasic and dibasic phosphonic acids with an increase in the pH, the LCST shifts to higher temperature region due to the concomitantly increased hydrophilicity in the polymer backbone. It can be also noted that the degree of the LCST shift greatly depends on the buffer species used. At the low-pH region where acetate buffer was used, more broad transmittance change can be found in a gradually increas-

ing manner; whereas at the high-pH region with tris buffer, their LCSTs are lower, and the transmittance changes are sharper than those of the acetate buffer pH values.[13] This is likely to be related to the association of positively charged tris ions with the negatively ionized phosphonic acid groups. Figure 3 shows the titration curve of



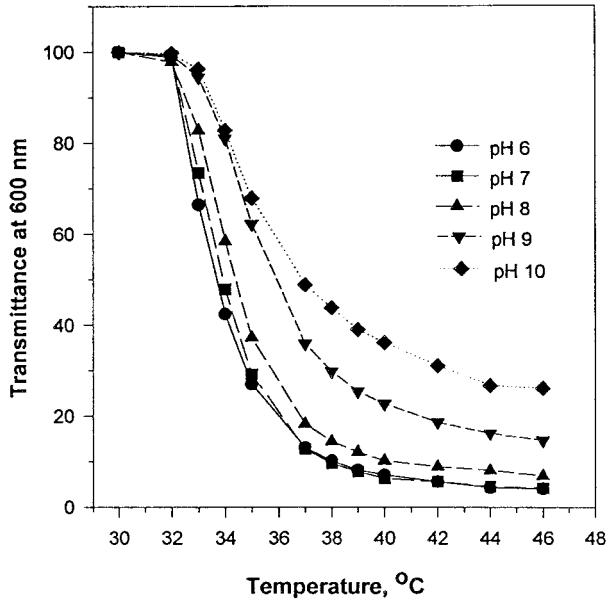


Figure 2 Temperature-dependent transmittance change of the copolymers as a function of pH. An acetate buffer was used for pH 2–5, and a tris buffer was used for pH 6–10.

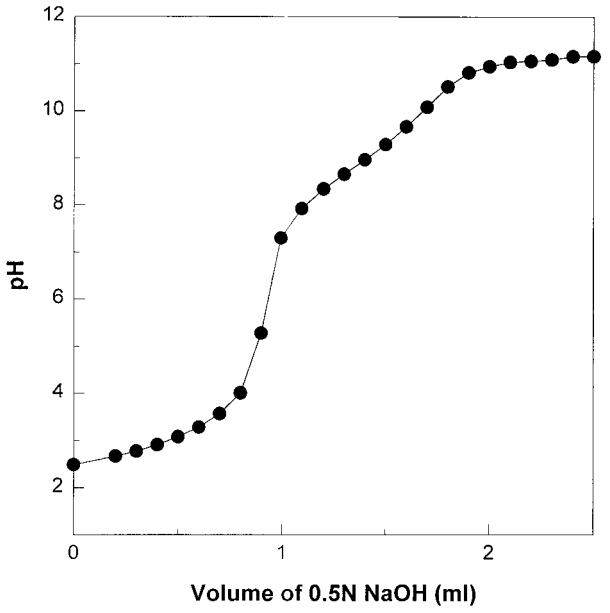


Figure 3 Titration curve of poly(NIPAAm-*co*-VP) at room temperature.

the copolymer, where 2 inflection points can be observed. From the titration curve, it can be estimated that the phosphonic acid groups in the copolymer dissociate to monobasic and dibasic species around the pH values of 3.1 (pK_1) and 9.0 (pK_2), respectively, which substantiate the pH-dependent transmittance change profiles in the vicinity of the 2 pK values in Figure 2. It should be noted that the obtained pK values of the copolymer are only valid at room temperature since an increase in the temperature, the copolymer becomes more hydrophobic with a concomitant loss of water, which affects the pK value to a large extent.[14]

Figure 4 shows the temperature-dependent phase diagram of 10% (w/v) aqueous solution of the copolymer with varying concentrations of calcium chloride at pH 7. It can be observed that there are 3 distinctive states of the copolymer: transparent fluid, white fluid, and white gel, depending on both temperature and calcium concentration. The phase diagram was constructed after incubating the copolymer solution for 1 h at a specific temperature. Each phase state was determined until no further change of fluid states was noticed up to the 1 h period. The physical appearances of the 3 states are demonstrated in Figure 5. Thermally self-setting gel is clearly observed above the sol-gel temperature. The copolymer without calcium ions transformed its fluid state from transparent to white at

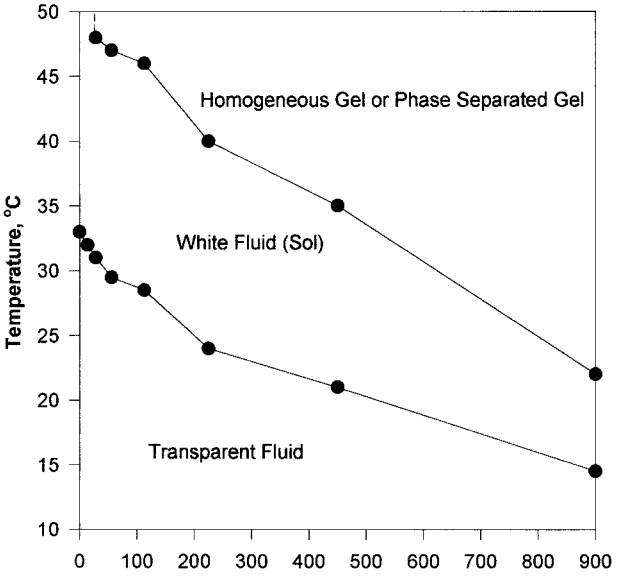


Figure 4 Temperature-dependent phase diagram of 10% (w/v) copolymer solution in the presence of varying calcium chloride concentrations.

its LCST but never exhibited homogeneous or phase-separated (shrunken) gel formation with increasing the temperature. Here, the term "homogeneous" means a macroscopic definition as judged from the visual appearance, not a microscopic one on the basis of molecular level. With the addition of calcium, however, the gel formation occurred. This indicates that the calcium ions can bridge 2 monobasic phosphonic acid groups together and intermolecularly crosslink the 2 polymer chains. Additionally, they might interact intramolecularly to form a more compact copolymer structure. The addition of divalent calcium ions could increase the effective molecular weight of the copolymer, which induced a greater tendency of the gel formation. For the formation of physical gels, multiple junction sites in the polymer chain are necessary for noncovalent interactions.[15] Therefore, the formation of gel in the presence of calcium can be explained by an extended chain length of the copolymer. The phase transition between transparent and white fluid states, and the sol-gel transition between fluid and semisolid gel states occurred in 2 different temperature regimes. The former can be understood as a conventional phase transition behavior, and its temperature can be regarded as the LCST due to its coincidence with the transmittance change in Figure 2. The latter can be thought as a true thermal sol-gel transition observed in several natural polysaccharides.[4] Both of the thermal transition temperatures decrease with increasing the calcium concentration, suggesting that calcium ions might affect the hydrophobic interaction between the polymer chains. The calcium ions possibly exerted a salting-out effect on the copolymer, lowering the LCST. It has been reported that the addition of inorganic species lowered the LCST of poly(NIPAAm).[16] When the copolymer became the gel state above the sol-gel transition temperature, the

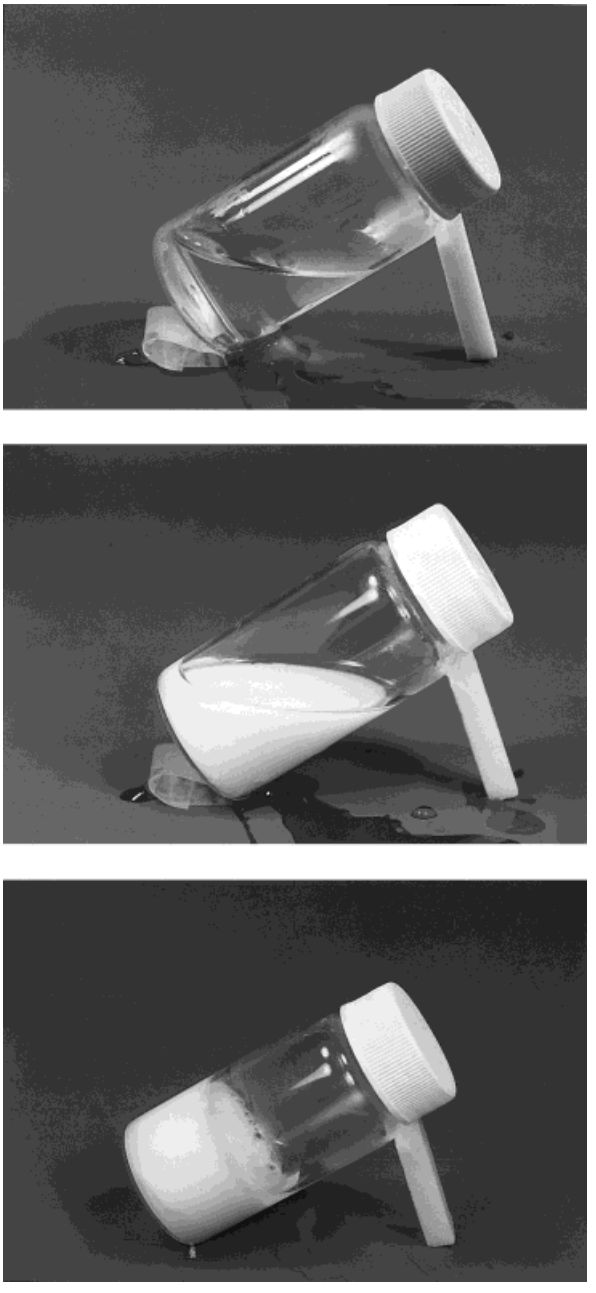


Figure 5 Photographic demonstration of transparent fluid (top), white fluid (sol; middle), and white gel (bottom).

formation of a semisolid gel occurred instantaneously and homogeneously throughout a whole fluid volume in the vial, and there was no fluid flow when the sample vial was inverted. However, the homogeneous gel state gradually turned into a phase-separated gel state over the following several days of incubation, resulting in the formation of a polymer-rich shrunken gel phase and a water-rich phase. This phase-separation behavior can be understood as a syneresis phenomenon, which means that the separation of water from the polymer network is due to the polymer relaxation.[17] The syneresis occurs in most physical thermoreversible gels that maintain their crosslinking between the polymer chains by weak noncovalent interactions. The polymer relaxation time is very important in determining the syneresis rate. Thus, the copolymer gel in this study is likely to have a relatively short relaxation time compared to other naturally occurring thermogels, such as agarose that has a very long relaxation time. The gel formation region in the phase diagram, strictly speaking, is under a nonequilibrium state, in which viscoelastic properties of the copolymer gel continuously change over time. The copolymer started to exhibit a thermoreversible gel behavior above its concentration of 5% (w/v). More compact, rigid, longer relaxed thermogels can be generated when increasing the copolymer concentration.

The viscosity of 10% (w/v) copolymer solution in the presence of 900 m*M* calcium chloride was measured as a function of temperature. Figure 6 shows the viscosity change at different temperatures. The viscosity continuously decreases up to the sol-gel transition temperature and increases infinitely beyond that temperature. It can be also noted that the viscosity starts to decrease sharply near the transparent-white fluid transition temperature (LCST), suggesting that the copolymer underwent a conformational phase transition from a random coil to a globular structure at that temperature, as suggested by previous studies. The reduced hydrodynamic size of individual molecule with a concomitant self-aggregation at the LCST was likely to lower the effective copolymer concentration in the solution and might result in a more decreased viscosity. The temperatures at which the gel formation and the gel melting take place are different, meaning that a hysteresis exists. This could be attributed to the time-dependent change of viscoelastic properties of the gel, which was affected by the polymer relaxation.[5]

The gel formation at the sol-gel transition temperature which occurs well above the LCST, indicates that more energy is needed to hydrophobically self-associate the polymer chains. The development of intermolecular multiple junction zone required for the physical crosslinks is induced by hydrophobic interaction that is known as an entropy-driven process. The homopoly(NIPAAm) solution, when heated, became a phase-separated gel at the LCST, and never generated a homogeneous and whole-volume gel. In the case of poly(NIPAAm-*co*-VP), an individual copolymer molecule having phosphonic acid groups in the backbone can collapse and shrink intramolecularly by hydrophobic interaction of NIPAAm units, while hydrophilic and ionizable phosphonic acid residues might be forcefully oriented out towards the aqueous solution, like a single molecular micelle structure. These micellar molecules might be ionically bridged by calcium ions, and subsequently aggregated to some extent, but still had a fluidity. Upon further heating beyond the sol-gel transition temperature, the aggregated micellar structures are postulated to self-assemble hydrophobically, resulting in a semisolid gel formation. In other words, repeating NIPAAm segments in the polymer chain could form multiple junction zones by hydrophobic interaction, while weakly interconnected phosphonic acid residues via calcium ions might have water-contacting capacities and maintain a network structure throughout a whole volume.

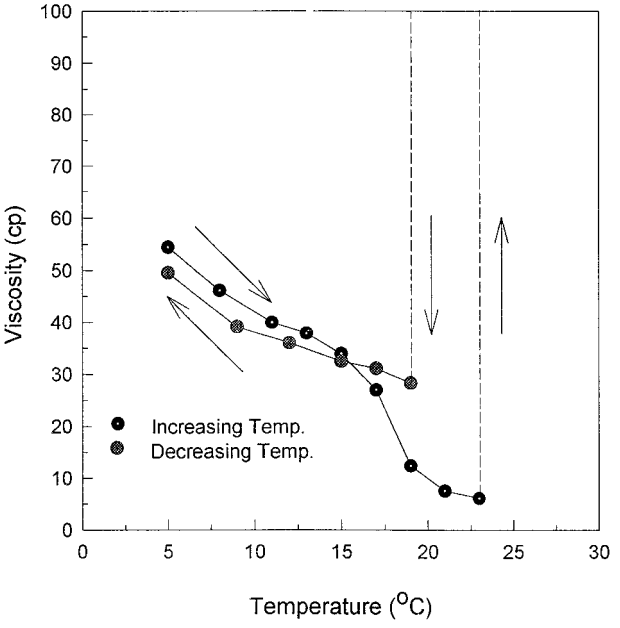


Figure 6 Viscosity change of 10% (w/v) copolymer solution as a function of temperature.

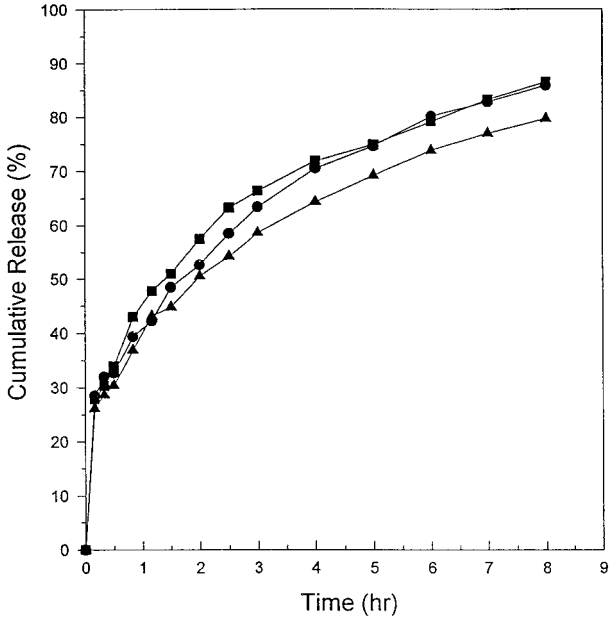


Figure 7 Methylene blue release profile, in triplicate, from the *in situ* formed gel at 37°C.

The *in situ* sol-gel transition behavior was applied for the controlled release of methylene blue used as a model drug. The copolymer solution was mixed with methylene blue below the LCST, and this solution was incubated in the buffer solution containing no calcium ions above the gelation temperature (37°C). The release profile from the *in situ* formed gel is shown in Figure 7. During this release period, the gel maintained a homogeneous state without demonstrating the phase separation, even in the absence of calcium ion in the buffer medium. It can be seen that the release of methylene blue sustains for 8 h with an initial burst, following a typical diffusion-controlled release. This kind of *in situ* gelation polymer system can be potentially utilized for ophthalmic bioadhesive drug delivery systems where prolonged retention and sustained release of a drug at the site of application is beneficial to increased bioavailability.[18]

In summary, it has been demonstrated that the aqueous solution of poly(NIPAAm-*co*-VP) in the presence of calcium ions exhibited the *in situ* sol-gel transition behaviors. For the thermoresponsive gel formation, the presence of phosphonic acid groups in the poly(NIPAAm) chain and the polymer concentration were important. The addition of calcium ions could adjust the gelation temperature over a wide range of temperatures.

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