Preparation and Characterization of Novel Cationic Copolymer Hydrogels with pH Sensitivity and Thermosensitivity

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ABSTRACT: Cationic hydrogels were synthesized through the copolymerization of *N*-isopropylacrylamide and dimethylaminoethylmethacrylate. *N,N′*-Methylenebisacrylamide was used as a crosslinking agent, and sodium bisulfite/ammonium persulfate was used as an initiator. The equilibrium and dynamic swelling properties were investigated to reveal the pH sensitivity and thermosensitivity of the hydrogels. The conclusion was drawn that the prepared cationic hydrogels demonstrated critical sensitivity at 37°C and pH 7.0–8.0 and that the stronger the acidity was of the buffered solution, the shorter the equilibrium swelling time was of the hydrogels. Drug-release experiments in vitro were carried out at 37°C (close to body temperature), at pH 1.4 (close to the pH of the stomach), and at pH 7.4 (close to the pH of the intestine). The release results indicated that the drug (chloramphenicol) was released more rapidly from the prepared hydrogel in a pH 1.4 buffered solution than in a pH 7.4 one, and this was consistent with the results predicted from the experiments of the swelling kinetics. Moreover, the drug-release process was confirmed by scanning electron micrographs of the hydrogels embedded with chloramphenicol. J Appl Polym Sci 100: 3602–3608, 2006

Key words: hydrogels; copolymerization; crosslinking; FTIR

INTRODUCTION

Hydrogels, defined as crosslinked polymeric networks capable of absorbing a large volume of water while retaining their three-dimensional structures after swelling, have received increasing attention for biomedical applications such as controlled-drug-delivery systems, contact lens materials, catheters, wound dressings, immobilized enzyme reactors, chemical valves, and separation processes.[1,2] Conventional gels show little response to stimuli changes, whereas stimuli-sensitive gels show abrupt volume changes in response to small external stimuli changes in the surrounding conditions, such as the pH,[3–5] temperature,[6–8] ionic strength,[9] specific analyte,[10] and antigen,[11] especially the temperature and pH, because these factors are variables that change in typical physiological, biological, and chemical systems.

Depending on the nature of the side groups along the polymer chains, hydrogels have the ability to respond to environmental changes. In recent years, these stimulus-sensitive hydrogels have become increasingly important carriers for the development of drug-delivery devices. Ionic hydrogels usually contain ionizable pendent groups such as carboxylic acid, sulfonic acid, or quaternized amino units. Because of their ionic nature, these hydrogels have special properties for use in membranes and in pH-sensitive drug-delivery systems. There have been various hydrophilic polymers used as hydrogel constituents. A few examples are poly(methacrylic acid),[12] polyacrylamide,[13] poly(*N*-isopropylacrylamide) (PNIPAAm),[14] poly(2-hydroxyethylmethacrylate),[15] and poly(ethylene oxide).[16]

PNIPAAm gel is a widely studied, temperature-sensitive gel because of its unique properties, displaying a phase transition as the temperature is increased above its phase-transition temperature or lower critical solution temperature (LCST) of 32°C. The tertiary amine groups on *N,N′*-dimethylaminoethylmethacrylate (DMAEMA) are weakly basic and become charged at low pH values, causing the hydrogel to swell. Then, these two monomers can make the hydrogel show pH-sensitive and thermosensitive properties.

In this thesis, one kind of thermosensitive and pH-sensitive hydrogel was prepared through the copolymerization of two monomers: thermosensitive *N*-isopropylacrylamide (NIPAAm) and pH-sensitive, cationic DMAEMA. A serious of experiments were carried out to test such the hydrogel thermosensitivity, pH sensitivity, and swelling kinetic response to environmental stimuli. The conclusion could be

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drawn that this hydrogel had prominent sensitivity at 37°C and pH 7.0–8., and that the stronger the acidity was of the buffered solution, the shorter the equilibrium swelling time was of the hydrogel. The drug-release experiments were carried out under the conditions of 37°C pH 1.4 and pH 7.4, respectively. The release results indicated that chloramphenicol was released more rapidly from the prepared hydrogel in a pH 1.4 buffered solution than in a pH 7.4 one, and this was consistent with the results predicted from the experiments of the swelling kinetics. Moreover, the drug-release process was confirmed with scanning electron microscopy (SEM) micrographs of the hydrogel embedded with chloramphenicol.

EXPERIMENTAL

Materials

DMAEMA was purchased from Aldrich Sigma. The NIPAAm monomer was synthesized according to a method already reported[17] and recrystallized in hexane and toluene (volume ratio = 1 : 1). 2,2′-Azoisobutyronitrile was recrystallized from methanol. All other materials were of reagent-grade purity and were used without further purification.

Preparation of poly(*N*,*N*′-dimethylaminoethylmethacrylate/*N*-isopropylacrylamide) [P(DMAEMA/NIPAAm)]

DMAEMA and NIPAAm were dissolved in water with moderate stirring before N_2 was bubbled to remove dissolved oxygen, and the polymerization of the gels was carried out in glass tubes at room temperature (20°C) for 2 h, with ammonium persulfate (APS) and sodium bisulfite (SBS) as a redox initiator (0.38 mol % based on DMAEMA and NIPAAm) and N,N′-methylenebisacrylamide (BIS) as a crosslinking agent (1.94 mol % based on DMAEMA and NIPAAm). After the polymerization, the synthesized hydrogels were cut into circular disks (10 mm in diameter and 1 mm thick). Then, the gel disk samples were immersed in distilled water at room temperature for 2 days, and the water was refreshed every 12 h to leach out unreacted chemical residue during that period. Swollen gel discs were initially dried under a mild air stream at room temperature for 2 days and then transferred into a vacuum oven for complete drying.

Characterization of the P(DMAEMA/NIPAAm) hydrogel

The synthesized polymers were analyzed with a Bio-Rad model FTS3000 spectrophotometer. The IR samples were prepared as disk-shaped films (10 mm in diameter). The complexes in the form of ground power and predried KBr (IR-grade) were heated to a temperature of 40°C before the two were ground together under an IR lamp to avoid condensation of atmospheric moisture. The mixtures, containing 1–2 wt % of the complexes in KBr, were pressed under a high pressure to form transparent disks, which were loaded onto a sample holder for the IR spectroscopy measurements.

Measurement of the swelling ratio of the P(DMAEMA/NIPAAm) hydrogel

The water contents of the cationic gel discs were determined gravimetrically as a function of the pH value at 37°C and as a function of the temperature at pH 7.4. Different buffer species were used with aqueous solutions of HCl, NaCl, $KHC_8H_4O_4$, NaOH, and Na_3BO_3.

Before the measurements, the dried gels were immersed in an excess amount of deionized water at different temperatures until swelling equilibrium was attained. The weight of the wet sample (W_w) was determined after the surface water was removed via blotting with filter paper. The dry weight (W_d) was determined after the gel was dried in a vacuum oven for 2 days. The water content (wc), based on W_w and W_d, was then calculated:

$$\text{wc}\ (\%) = (W_w - W_d)/W_w \times 100\%$$

Dynamic and equilibrium swelling experiments

The dynamic absorption water measurements of the samples were determined with the gravimetric method. The dried gels were immersed in excess buffer solution at different pH values and different temperatures. The wc (%) was obtained by the weighing of the initial and swollen samples at various time intervals. The amount of water absorbed (M_t) was reported as a function of time, and the equilibrium sorption at an infinitely long time was designated M_∞. R is the radius of the column hydrogel sample. The following equation was used to calculate the diffusion coefficient (D) for $M_t/M_\infty \leqq 0.8$:

$$F = M_t/M_\infty = 4(Dt/\pi R^2)^{1/2} - \pi(Dt/\pi R^2) - (\pi/3)(Dt/\pi R^2)^{3/2} + \ldots$$

Drug-loading and -release experiments

Preparation of the drug-loaded disks

Gel discs with chloramphenicol were prepared under the same conditions described in the previous section. Dried drug-loaded gel discs were incubated in 20-mL buffer solutions of pHs 1.4 and 7.4 at 37°C. At fixed time intervals, the buffer solutions were replaced. To test the effect of temperature, dried hydrogel disks were immersed and equilibrated in a buffer solution,

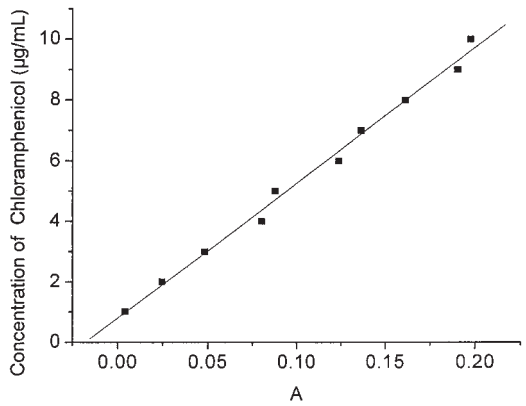


Figure 1 Standard curve of chloramphenicol.

dried at room temperature, place in the drier, and then weighed. The water contents were calculated.

Standard curve of chloramphenicol

Chloramphenicol was weighted accurately and dissolved in 20% ethanol to make a 500-mL solution. Distilled water was added to part of that solution. After the solution was cooled, the absorbance (A) of a series of standard solutions with different concentrations (C) at 293.00 nm was determined with a WFZ-26A ultraviolet spectrophotometer (Tianjin, China). The standard curve of chloramphenicol was based on the graph of C and A (Fig. 1), and then an equation of the standard curve was obtained: C (μg/mL) $= 44.65396A + 0.79213$ ($R = 0.99651$).

Drug release of the hydrogels

The drug-loaded disks were placed in 20-mL buffer solutions (pHs 1.4 and 7.4).The studies were carried out at 37°C. Part of the buffer solution was taken at every set interval; the released concentration was analyzed at 293.00 nm with a UV spectrophotometer. Then, the weight of the released drug was calculated with the equation of the standard curve.

For SEM (X-650, Hitachi, Japan) observations, chloramphenicol-loaded polymer disks were immersed in buffer solutions (pHs 1.4 and 7.4) for 8 h at 37°C. At every set interval, samples were taken out and then gold-coated with a sputter coater for SEM observations.

RESULTS AND DISCUSSION

Synthesis and characterization of the polymers

All the polymers were prepared by copolymerization between NIPAAm and DMAEMA in the presence of *N,N′*-methylenebisacrylamide as a crosslinker and SBS–APS as an initiator.

Fourier transform infrared spectroscopy was used to confirm the structure of the PNIPAAm and P(DMAEMA/NIPAAm) hydrogels. Figure 2 shows their IR spectra. Vinyl group peaks appeared at 995–905 cm^{-1} in the spectra of NIPAAm. After polymerization and crosslinking, there was no obvious peak appearing around 995–905 cm^{-1} in the spectra of crosslinked PNIPAAm. These data suggest that NIPAAm monomers were successfully polymerized and crosslinked. The characteristic peaks of DMAEMA appeared at 1728 cm^{-1}. This suggests that most groups of DMAEMA reacted with NIPAAM.

Determination of the swelling ratio of P(DMAEMA/NIPAAm)

The equilibrium degree of swelling for each hydrogel was expressed as the ratio of the wet weight to the dry weight. However, it is difficult to fully explain the swelling behavior of these species. The complexity arises from the fact that the swelling behavior involves several parameters, including the degree of crosslinking and the ionic character of the gel. A high degree of crosslinking reduces swelling. In contrast, a high ion concentration in the gel increases swelling because of increased water flow into the gel by osmosis. Furthermore, the repulsion of charges within the gel causes it to expand more, and this increases swelling. The swellability of the gels also depends on the properties of the swelling medium, such as the pH, ionic strength, and charge of the cation present in the medium.

Effect of pH

The crosslinked polymers were allowed to swell in buffer solutions with pHs 1–11 at 37°C and ionic strength (I) = 0.1M. From Figure 3, we can see that the PNIPAAm hydrogel had no pH sensitivity, but the P(DMAEMA/NIPAAm) hydrogel had higher degrees

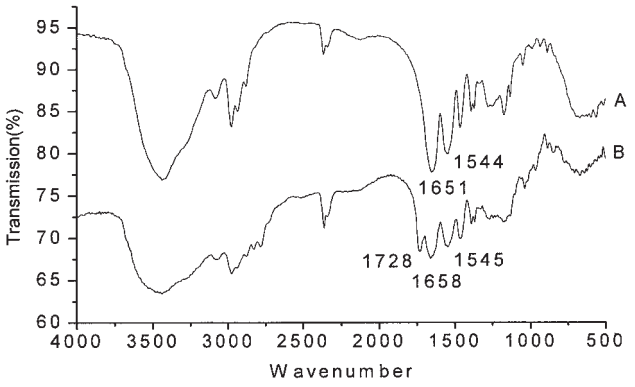


Figure 2 IR spectra of (A) the PNIPAAm hydrogel and (B) P(DMAEMA/NIPAAm) hydrogel.

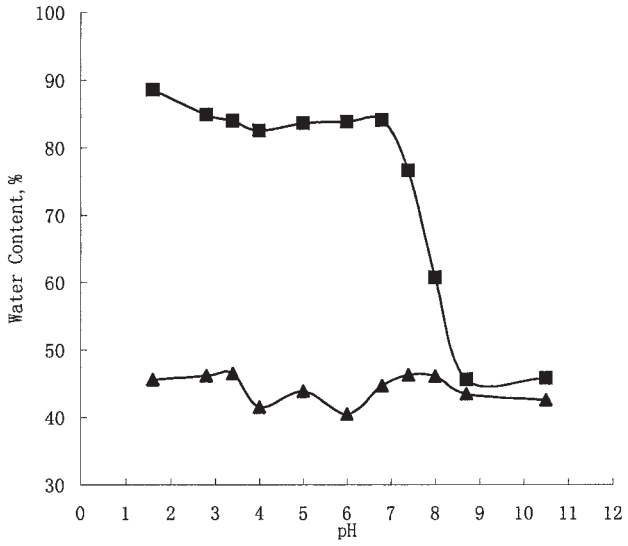


Figure 3 Swelling behavior of the hydrogels at 37°C as a function of the pH: (▲):PNIPAAm hydrogel and (■) P(DMAEMA/NIPAAm) hydrogel.

of swelling at an acidic pH than at a basic pH. The range of the hydrogel's pH sensitivity was 7–8.

Figure 3 shows the pH dependence on the gel equilibrium SR for our synthesized poly(DMAEMA/NIPAAm) gels at 37°C. The DMAEMA groups hydrolyzed into carboxylic acid groups, which promoted an increase in the SR (see Fig. 3). The anionic polymeric networks containing carboxylic acid groups were imidized as the pH of the external swelling medium increased; meanwhile, the hydrophilicity was increased and enhanced the SR. From these results, we could determine the sensitivities of the gels to pH and discovered that the SR of the gels had a negative correlation to the ratio of NIPAAm to DMAEMA under a higher pH condition.

This phenomenon can be explained as follows, In the lower pH region, there are ionized amino groups and strong charge repulsion in P(DMAEMA/NIPAAm). Under that condition, the P(DMAEMA/NIPAAm) hydrogel may have a more extended form by the protonated amino groups and the positive charge repulsion among them. In the higher pH environment, it is difficult for the P(DMAEMA/NIPAAm) hydrogel to produce enough ionized amino groups, so the hydrogel takes a contracted form. This can be seen clearly in Figure 4.

Effect of the temperature

The effect of the temperature on the SR of the P(DMAEMA/NIPAAm) copolymer gels in a buffer solution (pH 7.4) is shown in Figure 5. The results show that the polymeric networks exhibited a deswelling behavior at higher temperatures. This was because the

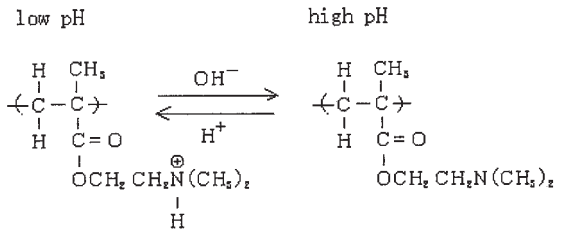


Figure 4 pH-dependent ionization of P(DMAEMA/NIPAAm).

amino group of NIPAAm in the polymer formed an intermolecular hydrogen bond with surrounding water at low temperatures, which turned into an intramolecular hydrogen bond over its gel-transition temperature (LCST = 32°C). This phenomenon caused the gel's hydration capability to decrease. At the same time, when NIPAAm was copolymerized with DMAEMA, the hydrophilic groups of the gel increased. These result made the state of the water molecule in the gel change from free water to bound water and made the SR of the gel increase over its gel-transition temperature (32°C) and reach a new LCST of about 37°C.

Dynamic and equilibrium swelling experiments

To investigate the influence of external conditions on the dynamic and equilibrium behavior of anionic networks, thin disks of P(DMAEMA/NIPAAm) were tested in various buffer solutions. Figure 6 presents the dynamic water uptake of glassy copolymers in an aqueous buffer, at a constant ionic strength of 0.1*M* at different temperatures, as a function of the pH of the external swelling medium. When the temperature in-

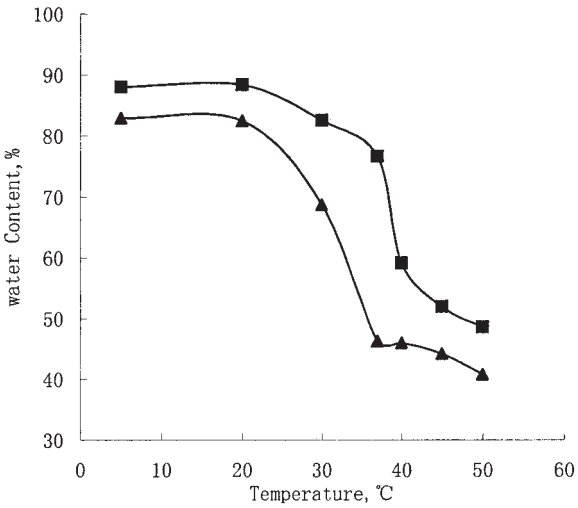


Figure 5 Swelling behavior of the hydrogels at pH 7.4 as a function of the temperature: (▲) PNIPAAm hydrogel and (■) P(DMAEMA/NIPAAm) hydrogel.

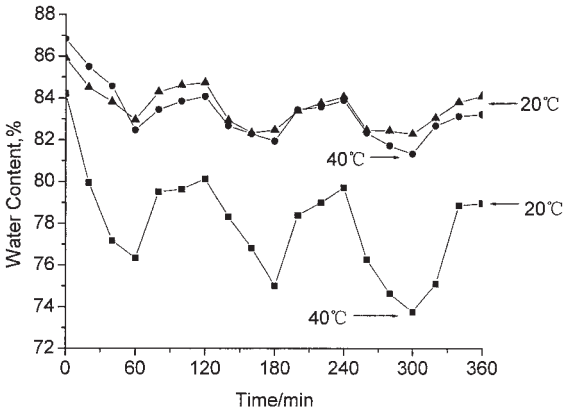


Figure 6 Swelling behavior of the P(DMAEMA/NIPAAm) hydrogel as a function of time: (■) pH 4.0, (●) pH 5.6, and (▲) pH 6.8.

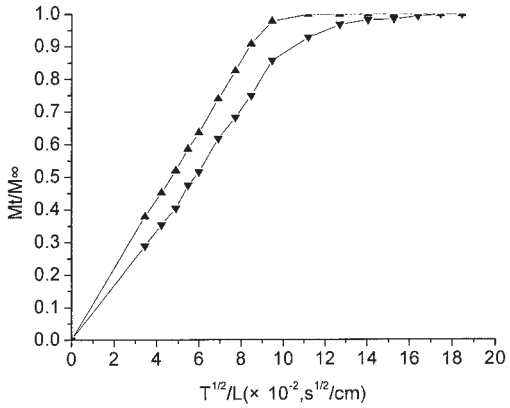


Figure 8 Dynamic swelling behavior of the P(DMAEMA/NIPAAm) hydrogel in environments of different pHs: (▲) pH 4.0 and (▼) pH 6.8.

creased from 20 to 40°C, the water content of the hydrogel also increased (and vice versa). This phenomenon shows that P(DMAEMA/NIPAAm) hydrogels have good reversibility. On the other hand, the water content of the hydrogel increased when the pH of the medium decreased. This may have been caused by the shrunken structure network of P(DMAEMA/NIPAAm) in the lower pH environment and the extended structure in the higher pH. In the beginning, some amino groups of the hydrogel were hydrolyzed, and this enhanced the swelling ratio. When the gels were transferred to a lower pH, there were electrostatic repulsive forces among the groups, and this promoted a decreased swelling ratio.

The results of the water diffusion coefficient change in the P(DMAEMA/NIPAAm) hydrogel and dynamic swelling behavior at 37°C in media of different pHs are shown in Figures 7 and 8, respectively. From Figure 7, we can see that the environment's pH had a large effect on the coefficient. During the swelling of the P(DMAEMA/NIPAAm) hydrogel in buffer solutions, the water diffusion coefficient changed with the time that the hydrogels were immersed in aqueous solutions. At the beginning of the swelling procedure, the coefficient increased with the swelling time and abruptly changed with the pH of the solutions. The reason may be that under lower pH conditions, it took the macromolecular chains of the P(DMAEMA/NIPAAm) hydrogel less time to redisperse in buffer solutions. This can be confirmed in Figure 8: in pH 4.0, the time for the hydrogel to reach swelling equilibrium was 3.5 h, whereas in pH 6.8, the time was 8.5 h.

Drug-loading and -release experiments

One reason for the current interest in ionic hydrogels for both biological and nonbiological uses is the possibility that the ease of diffusion of small molecules through the gel can be controlled by the pH or ionic strength. The release of chloramphenicol from the crosslinked polymers in different pH buffer solutions

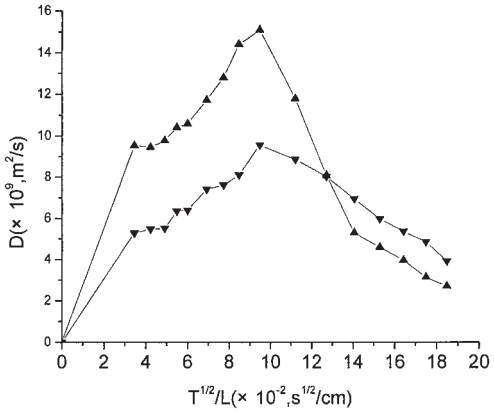


Figure 7 Change in the diffusion coefficient of water (D) in the P(DMAEMA/NIPAAm) hydrogel swelling in media of different pHs: (▲) pH 4.0 and (▼) pH 6.8.

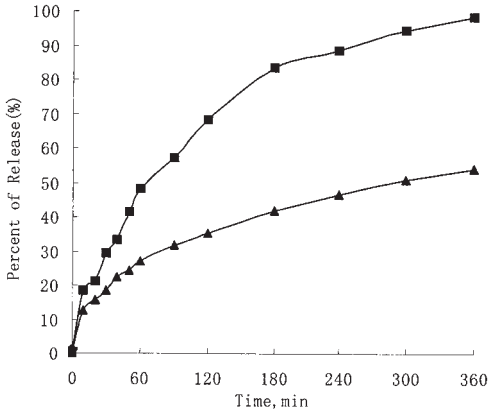
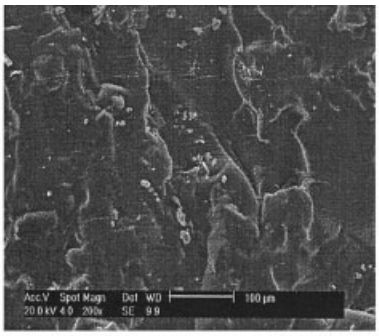
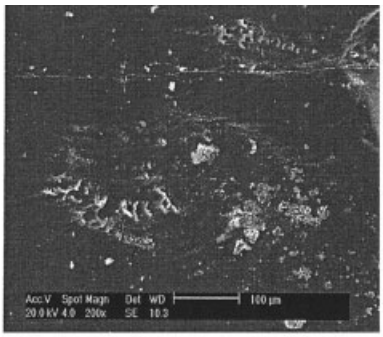


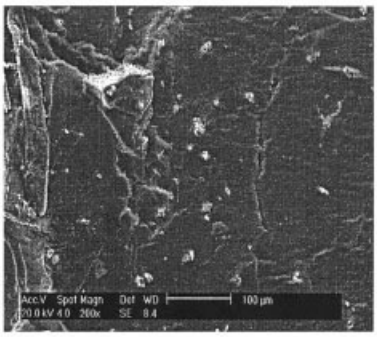
Figure 9 Release of chloramphenicol in the P(DMAEMA/NIPAAm) hydrogel: (■) pH 1.4 and (▲) pH 7.4.



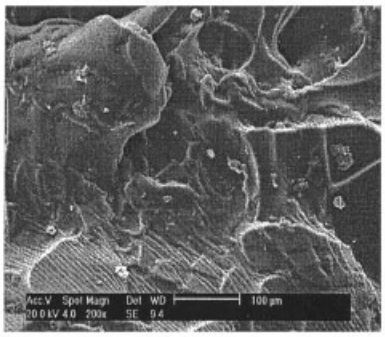
pH=1.4
(hydrogel loading with chloramphenicol)



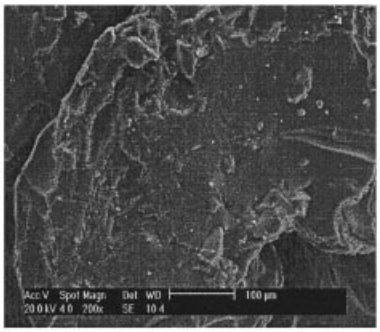
pH=7.4
(hydrogel loading with chloramphenicol)



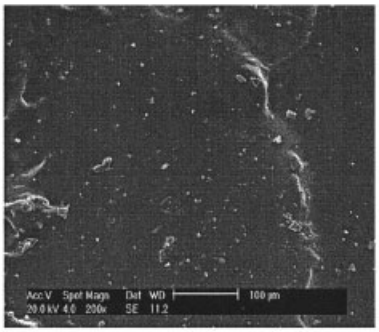
pH=1. 4
(releasing time 0.5h,
releasing ratio 29.32%)



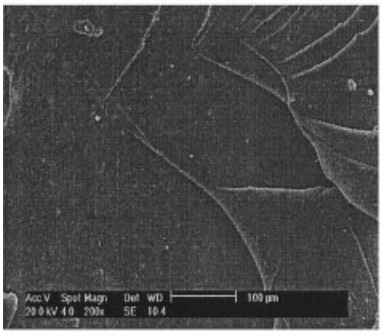
pH=7. 4
(releasing time 0.5h,
releasing ratio 18.59%)



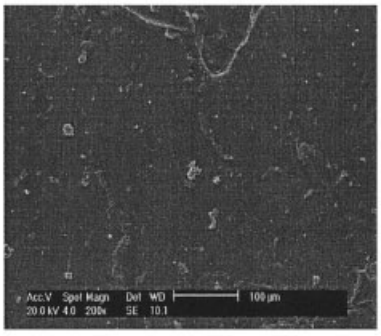
pH=1. 4
(releasing time 1.0h,
releasing ratio 48.35%)



pH=7. 4
(releasing time 1.0h,
releasing ratio 27.01%)



pH=1. 4
(releasing time 6.0h,
releasing ratio 98.41%)



pH=7. 4
(releasing time 6.0h,
releasing ratio 54.28%)

Figure 10 SEM images of P(DMAEMA/NIPAAm) hydrogels loaded with chloramphenicol.

was studied at 37°C as a model for imitating drug-release conditions in the body. The results shown in Figure 9 show that in a pH 7.4 buffer solution, only a small fraction of the drug was released from the hydrogel even after 6 h. The rate of drug release from the hydrogel was significantly higher under a lower pH condition than under a higher condition. This was attributed to the larger number of amino groups present in P(DMAEMA/NIPAAm) (mostly protonated) and the higher swellability of the gels in a pH 1.4 buffer. The linear release profile of chloramphenicol in different buffers was confirmed by SEM of the drug release from the gels during this period, as shown in Figure 10. In Figure 10, we can clearly see the procedure of the P(DMAEMA/NIPAAm) hydrogel containing chloramphenicol releasing the drug in different periods. As time went on, the number and diameter of the chloramphenicol particles decreased. These release profiles are comparable to those of drugs released from natural and synthetic hydrogels and can easily be modified by changes in the pH conditions.

CONCLUSIONS

The synthesis of crosslinkable P(DMAEMA/NIPAAm) that bears amino-functional groups allows for the preparation of a new class of pH-sensitive hydrogels. These hydrogels provide advantages over systems that have been studied in the past in the sense that the hydrogels have pH sensitivity with a sensitivity range of pH 7–8 and a temperature sensitivity with LCST at 37°C. These properties provide the hydrogels with great potential for use in controlled-drug-release systems.

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