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Synthesis and Characterization of a Drug-Delivery System Based on Melamine-Modified Poly(vinyl acetate-*co*-maleic anhydride) Hydrogel

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ABSTRACT: Three-dimensional polymeric networks, which quickly swell by imbibing a large amount of water or deswell in response to changes in their external environment, are called *hydrogels*. These types of polymeric materials are good potential candidates for drug-delivery systems. In this study, we first synthesized poly(vinyl acetate-*co*-maleic anhydride) by free-radical copolymerization. Then, they were modified with different molar ratios of melamine to prepare hydrogels that could be used in drug-delivery systems. The hydrogels were characterized by Fourier transform infrared spectroscopy, ^{1}H-NMR, differential scanning calorimetry, and scanning electron microscopy. In the second step, Ceftazidime antibiotic was loaded on selected hydrogels. The *in vitro* drug release was investigated and compared in three different media (HCl solution at pH = 3 and buffer solutions at pH 6.1and pH 8). © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40389.

KEYWORDS: copolymers; drug-delivery systems; functionalization of polymers

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INTRODUCTION

A hydrogel is a network of polymer chains that are hydrophilic. Sometimes, they are found as a colloidal gel in which water is the dispersion media. A pH-sensitive hydrogel is a network of polymers bearing functional groups in equilibrium with an aqueous solution and a set of mechanical forces. For a pH-sensitive gel, inhomogeneous swelling is affected by the pH and salinity of the external solution. Hydrogels also possess a degree of flexibility similar to that of natural tissue because of their significant water contents.[1] Hydrogels are gaining tremendous importance in a wide variety of applications in medical, pharmaceutical, and related fields, for example, in wound dressings with chitosan-based antibacterial hydrogels,[2] reloadable soft contact lenses based on the molecular imprinting technique,[3] artificial organs, and drug-delivery systems.[4] Among these applications, hydrogel-based drug-delivery systems have become a major subject of research interest.[5–13] It has been demonstrated that hydrogels are excellent candidates for the encapsulation of drugs and bioactive macromolecules, such as peptides and proteins.[14] Hydrogels have been developed as stimuli-responsive materials, which can undergo abrupt volume changes in response to small changes in their environmental parameters, including the temperature, pH, and ionic strength. These unique characteristics of hydrogels are of great interest in drug delivery, cell encapsulation, and tissue engineering.[15–18] Stimuli-responsive polymers play an important role in the development of novel smart hydrogels.[19]

Hydrogels are also of special interest in controlled release applications because of their soft tissue biocompatibility; this leads drugs to disperse in the matrix, and a high degree of control is achieved through the selection of the physical and chemical properties of the polymer network.[20] A variety of synthetic or natural polymeric hydrogels has been used as controlled release systems for drug delivery.[21]

Maleic anhydride (MA) copolymers are very suitable materials for modification and use as hydrogels. These copolymers are usually hydrolyzed in aqueous solutions when two carboxyl groups are formed on the MA unit; this results in a behavior characteristic of dibasic polyacids.[22] MA copolymers are simply crosslinked by different crosslinking agents and have a wide variety of applications, which have been reported previously.[23–25]

Ceftazidime is a third-generation cephalosporin antibiotic. Like other third-generation cephalosporins, it has broad spectrum activity against Gram-positive and Gram-negative bacteria. This antibiotic is usually reserved for the treatment of infections caused by *Pseudomonas aeruginosa;* it is also used in the empirical therapy of febrile neutropenia in combination with other antibiotics. In addition to the syn configuration of the imino side chain, compared to other third-generation cephalosporins,

the more complex moiety (containing two methyl groups and a carboxylic acid group) confers extra stability to the β-lactamase enzymes produced by any Gram-negative bacteria. The extra stability toward β-lactamase increases the activity of Ceftazidime against otherwise resistant Gram-negative organisms, including *P. aeruginosa*. Also, the charged pyridinium moiety increases its water solubility.[26]

The aim of this study was to investigate the ability of a modified poly(vinyl acetate-*co*-maleic anhydride) (PVAMA) hydrogel in a drug-delivery system. The aforementioned copolymer was synthesized and modified with different molar ratios of melamine. One of the modified copolymers was chosen and loaded with Ceftazidime drug to study the *in vitro* drug release. This study was implemented in three different media (HCl solution at pH = 3 and buffer solutions at pH 6.1 and pH 8).

EXPERIMENTAL

Equipment

IR spectra were measured with a Fourier transform infrared (FTIR) spectrophotometer (Nexus-670, Thermo Nicolet). ^{1}H-NMR spectra were recorded on a Bruker Spectrospin Advance ^{1}H-NMR 400 (MHz) spectroscope (Germany). We performed the differential scanning calorimetry (DSC) analysis of the prepared samples with a LENSES STAPT-1000 calorimeter (Germany) by scanning up to 400°C at a heating rate of 10°C/min. The morphology of the particles was studied via scanning electron microscopy (SEM; XL30 Philips Co., The Netherlands), and an ultraviolet–visible (UV–vis) spectrophotometer (T80, PG Instruments, Ltd., United Kingdom) was used to study the drug release.

Materials and Methods

MA (>99%), benzoyl peroxide (BPO; 75%), *n*-hexane (98%), and tetrahydrofuran (THF; >99%) were purchased from Merck and were used without further purification. Vinyl acetate (VA; >99%) was purchased from Fluka and distilled under reduced pressure before use. Triethylamine (99/5%) was purchased from Aldrich, and no further purification was performed. Melamine crystal and Ceftazidime antibiotic were kindly donated by Urmia Petrochemical Complex and Dana Tabriz Pharmaceutical Co., respectively, and they were also used without further purification.

Preparation of PVAMA

Into a 100-mL two-necked, round-bottomed flask equipped with a magnetic stirring bar, dried THF (60 mL), VA (9.2 mL, 0.1 mol), and MA (9.8 g, 0.1 mol) were added, and the contents of the flask were degassed with a nitrogen bubbling system for 20 min. Then, BPO (0.064 g, 0.2 mmol) was added to the flask. The contents of the flask were refluxed under an inert atmosphere for 24 h. After that, the reaction mixture was cooled down to room temperature, and the product was precipitated in *n*-hexane. The obtained sediment was washed several times with *n*-hexane. For further purification, the product was dissolved in THF and precipitated in *n*-hexane once more. Then, it was filtered and dried *in vacuo*. The yield of the product was 94%.

Modification of PVAMA with Melamine

Into a 100-mL, round-bottomed flask equipped with magnetic stirring bar, THF/H_2O (1:1, 60 mL) and PVAMA (2.1 g, 11.4 mmol) were added. The contents of the flask were stirred at room temperature for 15 min. Then, melamine (1.435 g, 11.4 mmol) and triethylamine (1.15 g, 11.4 mmol) were added to the flask. The reaction mixture was stirred at room temperature for 24 h. After 24 h, 50% of the solvent was evaporated with a rotary. This led to complete precipitation of the final product. The precipitate was filtered and washed with water; then, it was dried *in vacuo*. For further purification, the hydrogel was dialyzed for 24 h against distilled water, which was renewed every 6 h, and then, it was filtered and dried *in vacuo*. The yield of the product was 70%. The same procedure was repeated for different molar ratios of melamine (PVAMA/melamine ratios of 1:0.5, 1:1.5, and 1:2).

Table I. Cloud Points of the Prepared Hydrogels

PVAMA/melamine molar ratio	1:0.5	1:1	1:1.5	1:2
Cloud point	7.53	7.63	7.69	7.73

Determination of the Cloud Point Values of the Prepared Hydrogels

The *cloud point* is the pH at which the hydrogel increases to change from a solution form into a nonsoluble gel form. To determine the cloud point of the prepared hydrogels, we added the hydrogel (0.1 g) to distilled water (10 mL), and the pH was adjusted to 10. The mixture was stirred until the hydrogel dissolved completely. Then, the solution was titrated with dilute HCl. The pH at which the solution turned turbid showed the cloud point of the hydrogel. The obtained cloud points are gathered in Table I.

Determination of the Degree of Swelling (DS) Values of the Prepared Hydrogels

To determine the DS values of the hydrogels, the dried copolymer (1 g) was added to relatively acidic water (pH 6, 10 mL). After specific times, the sample was filtered and reweighed. This procedure was continued until a fixed weight was obtained. The DS of each hydrogel was calculated according to the following equation:

$$DS = (M_s - M_0)/M_0$$

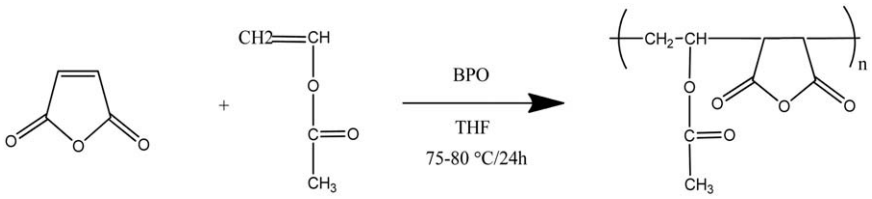
where M_0 and M_s are the weights of the copolymer before and after swelling. Table II shows the DS of the PVAMA/melamine copolymer at a 1:1 ratio.

Preparation of the Drug-Containing Copolymers

The drug-delivery studies have been performed only on the PVAMA/melamine copolymer with a 1:1 ratio. To prepare the drug-containing copolymer, first the copolymer (0.2 g) was dissolved in distilled water through a gentle step-by-step increase in the pH to make an alkaline media (20 mL). The final pH of the copolymer solution was adjusted to 8, which was near the

Table II. DS Values for the Prepared Hydrogel with a 1:1 Molar Ratio of the Two Monomers in the Copolymerization Feed

Time (min)	15	30	45	60	120	180
DS	17	19	22	23	25	26



Scheme 1. Preparation of PVAMA.

cloud point of the hydrogel. In the next step, a Ceftazidime drug solution in water (saturated) was prepared and added to the polymer solution. The final mixture was stirred for 24 h at room temperature. After 24 h, the pH was reduced to 3–3.5, and a white precipitate was obtained. The precipitate was filtered, then washed with distilled water, and finally dried *in vacuo*.

Drug-Release Studies

To investigate the *in vitro* drug release, buffer solutions were used as releasing environments. The release of Ceftazidime was determined with a UV spectrophotometer at a maximum wavelength of 256 nm as a function of time. The procedure was as follows. The drug-containing copolymer (20 mg) was placed in a dialysis tube in a 100-mL container containing 50 mL of carbonate-buffered solutions at pH values of 3, 6.1, and 8 at 25°C. Sampling was started after 30 min and continued at specified time intervals until we obtained a constant absorbance. Because the sampling led to changes in the concentration of the drug in the main container, the sample was returned to the container after we acquired the amount of absorbance.

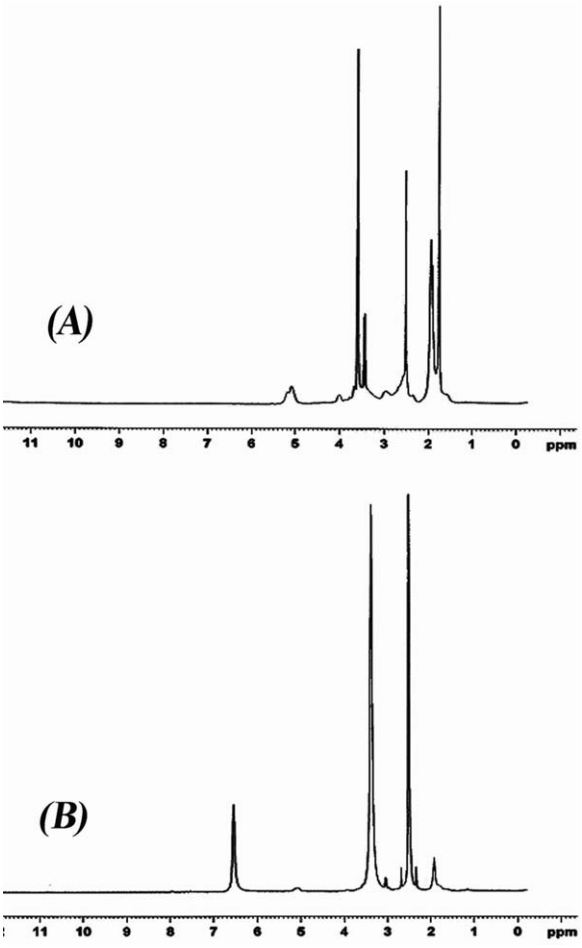


Figure 1. ^{1}H-NMR spectra of (A) PMAVA and (B) modified PVAMA in hexadeuterated dimethyl sulfoxide.

RESULTS AND DISCUSSION

The preparation of PVAMA was performed by the radical copolymerization of VA with MA in the presence of BPO, as it is shown in Scheme 1.

Figure 1(a) shows the ^{1}H-NMR spectrum of the PVAMA. The peak at 1.75–1.92 ppm was related to the protons of CH_2 in the polymer chain. Also, the peak at 2.50 ppm was attributed to the protons of the methyl group connected to the carbonyl. The peaks at 3.50–4.00 and 5.08–5.17 ppm were related to the protons of the two CH groups of the anhydride ring and the proton of the CH group bonded to the oxygen, respectively.

The ^{1}H-NMR spectrum of the melamine-grafted PVAMA [Figure 1(b)] showed a peak around 1.91 ppm; this was attributed to the protons of the repeated methylene group. The peak related to the protons of the methyl group bonded to the

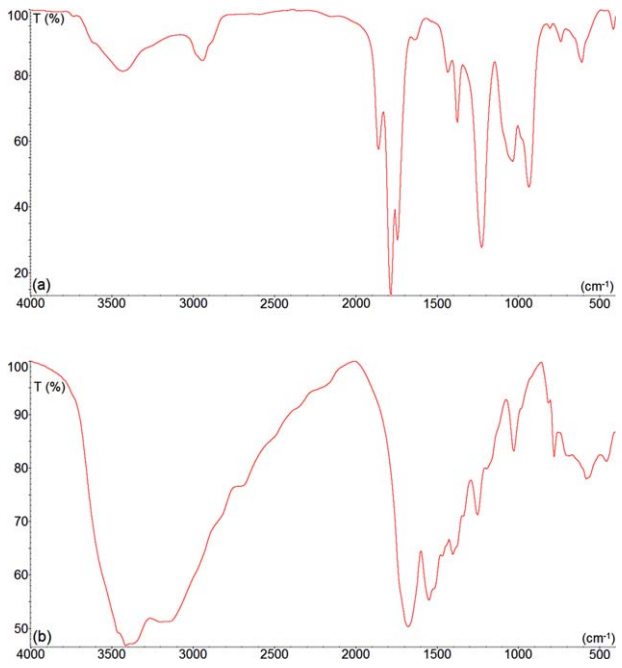


Figure 2. FTIR spectra of (a) PVAMA and (b) modified PVAMA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

carbonyl was apparent at 2.50 ppm; this overlapped with that of the dimethyl sulfoxide protons. The peak at 3.36 ppm was related to the protons of the two CH groups bonded to the carbonyls of the amidic groups and the carbonyls of the acidic groups. The peak at 5.06 ppm was attributed to the protons of the CH groups connected to the oxygen. The protons of the NH_2 groups of grafted melamine showed a peak around 6.53 ppm (Scheme 2).

In the FTIR spectrum of PVAMA [Figure 2(a)], the peak around 2930 cm^{-1} showed the stretching vibrations of the aliphatic C—H groups. Also, the carbonyls of the anhydride had sharp absorption bonds at 1785 and 1860 cm^{-1}. There was another sharp peak at 1225 cm^{-1}, which was attributed to the C—O stretching vibrations. Other absorption bonds between 609 and 738 cm^{-1} were related to C—H out-of-plane bending vibrations.

The FTIR spectrum of the 1:1 copolymer is shown in Figure 2(b). As shown in this figure, there was a wide absorption peak at 2500–3500 cm^{-1}, which was related to the stretching vibrations of the acidic hydroxyl groups and which overlapped with the NH stretching vibrations. Also, the signal at 1677 cm^{-1} was attributed to the amidic carbonyl groups. The peaks at 1467 and 1251 cm^{-1} showed the C—H bending and C—O stretching vibrations, respectively. The N—C vibrations showed a peak at 1195 cm^{-1}.

The thermal analysis behavior of the samples was evaluated by DSC analysis. The data obtained from the thermal analysis of PVAMA showed the melting point to be at 220°C. The

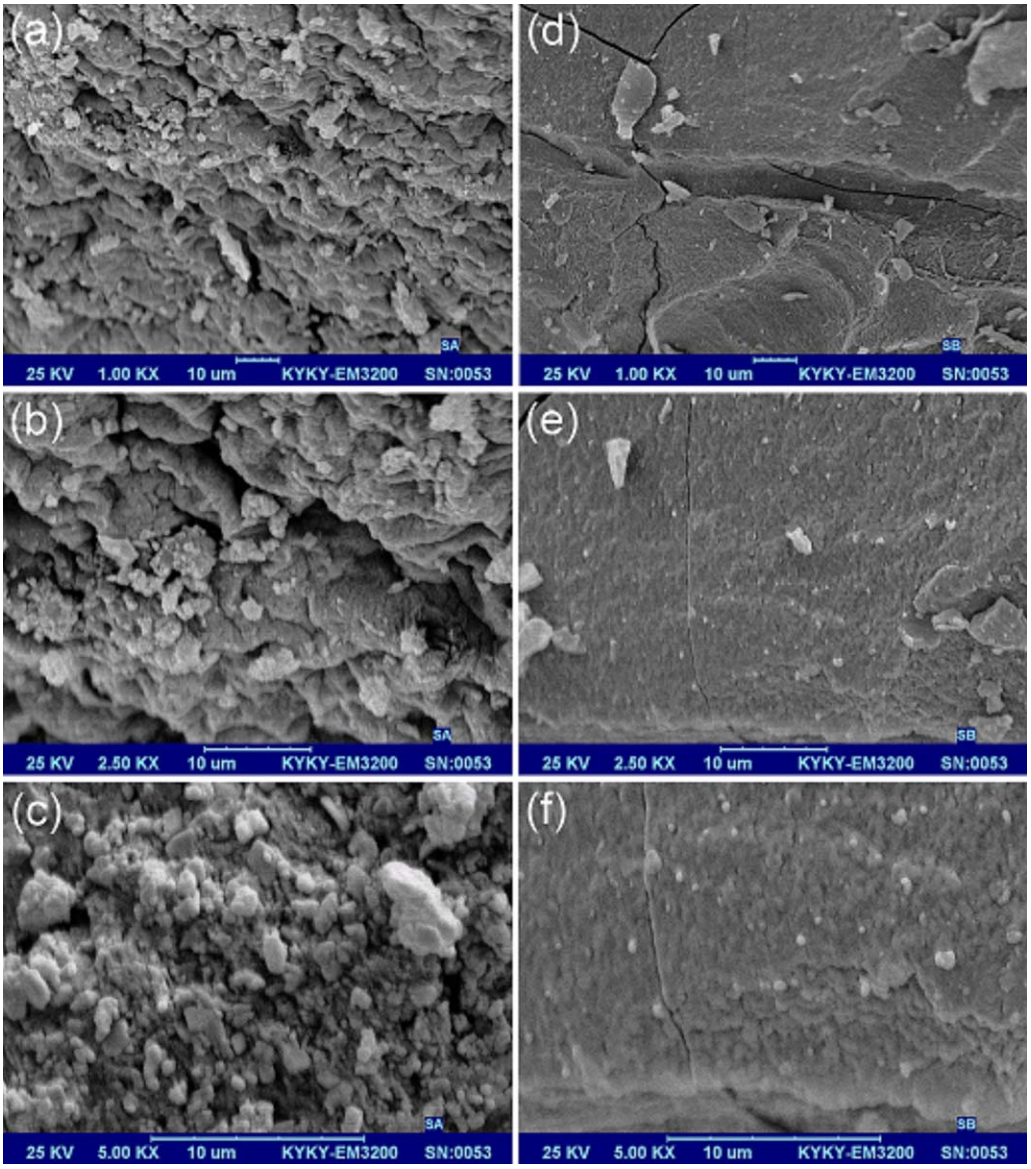


Figure 3. SEM images of the modified hydrogel: (a–c) before drug loading and (d–f) after drug loading. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

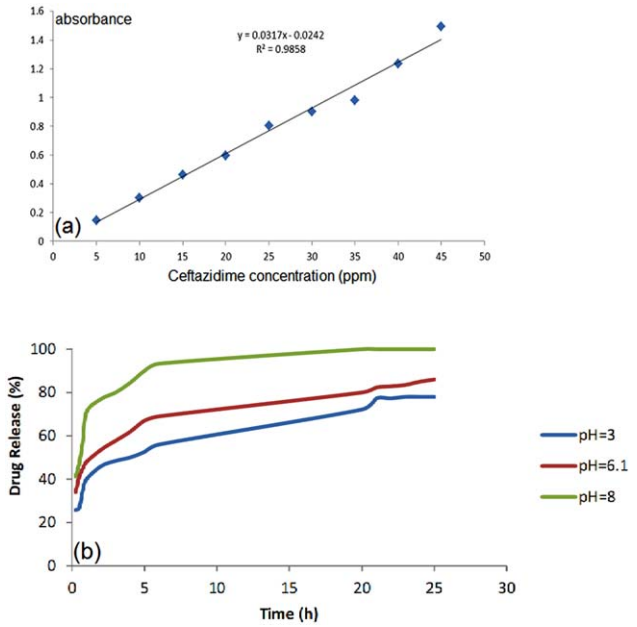


Figure 4. (a) Calibration curve of ceftazidime and (b) drug-release curve. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

decomposition of the copolymer chains occurred from 280 to 400°C. For the melamine-grafted PVAMA, the DSC data showed the melting point to be at 180°C; melting was followed by the release of the acetate groups. The degradation of the sample started at 245°C and continued in two different steps. In 340°C, the melamine groups started to degrade, and total degradation of the sample started from 355°C. The thermal stability of the grafted copolymer was altered in regard to that of the unmodified one.

Figure 3(a–c) shows the SEM images of the hydrogel before drug loading in three different resolutions. The images after drug loading are shown in Figure 3(d–f). It was clearly observable that the unloaded hydrogel had a porous surface compared with the loaded hydrogel. After the drug was loaded, it have been the pores that were filled with the drug, and its surface was smoothed compared to that of the unloaded hydrogel.

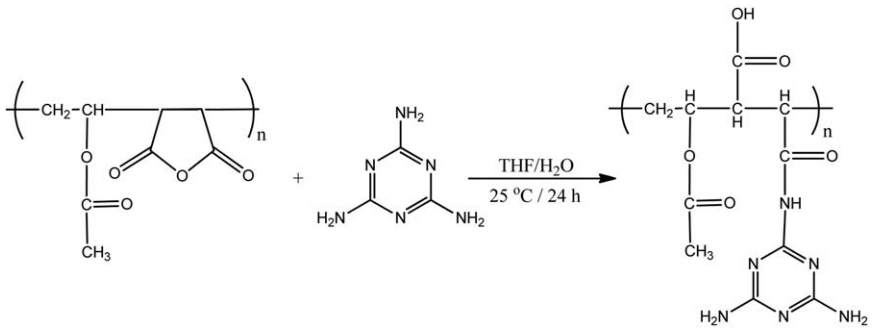
In many cases, the Lambert–Beer law is followed during UV–vis absorption by polyatomic substances. The most straightforward way to use the Lambert–Beer law for quantitative analysis is to measure the absorbance of the sample solution at a wavelength in which the species in solution is known to absorb radiation. The calibration curve of the Ceftazidime drug as a function of the concentration is shown in Figure 4(a). It was apparent that the slope of the plot should have been the product of the molar absorptivity and the cell path length. Because the working curve was linear and went through the origin, it could be used to determine concentrations from the measured absorption values.[27]

The loading of the drug on the hydrogel was done by a physical absorption method; indeed, the interaction of the hydrogel functional groups with drug functional groups caused a better dispersion of drug in the hydrogel matrix. The release profile of Ceftazidime antibiotic from the loaded hydrogel was studied in three different media. The drug-release curves, which show the drug-release percentages at different times, are shown in Figure 4(b).

It was clearly observable that the drug-release rate was enhanced when the pH was increased. This was a confirmation seal for the pH sensitivity of the hydrogel. At pH 3, the absorbance started from 0.06 and showed upward growth for next 20 h. After that, the drug release was almost finished, and the absorbance showed an approximately fixed value. As the figure shows, the drug release at pH 6.1 showed a higher rate compared with the previous one, but the gradient of the release was almost equal. At pH 8, the diagram showed that almost 90% of the drug was released during the first 10 h. Also, the release rate was specifically increased. This theorem determined that the drug-release rate from such hydrogels could be controlled by the pH, depending on the target cell demand.

CONCLUSIONS

PVAMA copolymer was successfully synthesized and modified with melamine to the obtained pH-sensitive hydrogels, which could be used in drug-delivery systems. The investigation of the drug-release ability of the modified hydrogel was implemented with Ceftazidime antibiotic. The drug-release experiments were performed and compared in three different media. The results show that the drug-release rate was enhanced by an increase in the pH, a consequence that provides us with a suitable way to control the release rate in various treatments.



Scheme 2. Modification reaction of PVAMA with melamine.

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