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PVA-기반 하이드로젤 망상구조 합성: Cefftreiaxon 항생물질의 방출특성 연구

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Synthesis of PVA-based Hydrogels Network: Characterization and Study of Cefftreiaxon Antibiotic Slow Release

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Abstract: Poly(vinyl alcohol) (PVA) is a biocompatible, highly hydrophilic and nontoxic polymer which has great potential for modification and can be use as beneficial hydrogels. In the present work, PVA was firstly modified by bromoacetyl bromide to produce bromoacetylated PVA. Then various diamines such as ethylene diamine, propylene diamine and hexamethlene diamine were used for crosslinking of bromoacetylated PVA to synthesize three-dimensional hydrogels. The swelling behavior of prepared hydrogels was investigated in which ethylene diamine-crosslinked PVA by ethylene diamine showed a fast initial swelling followed by a mild increase before attaining equilibrium. The loading of cefftreiaxon antibiotic on the hydrogels was carried out and *in vitro* drug release of drug loaded hydrogels was investigated. The results show that the drug release rate was enhanced by an increase in pH. The PVA hydrogels were characterized by Fourier transform infrared spectroscopy, thermogravimetric analysis, and scanning electron microscopy.

Keywords: drug delivery systems, hydrogel, poly(vinyl alcohol), cefftreiaxon.

Introduction

Hydrogels are three-dimensional polymeric networks, which quickly swell by imbibing a large amount of water or de-swell in response to changes in their external environment. Degradable hydrogels specially are favorable for a number of applications such as drug delivery system and tissue engineering scaffolds.[1] Stimuli-responsive materials have also been developed by hydrogels, which can undergo sudden volume changes in response to small changes in their environmental parameters, including the temperature, pH, and ionic strength. These unique features of hydrogels are of great interest in drug delivery, cell encapsulation, and tissue engineering.[2-5] Due to lack of control, drug release from conventional drug formulations in response to physiological requirements have resulted in the development of controlled drug delivery systems.[6-8] A variety of synthetic and naturally derived materials have been reported to form well-characterized hydrogels useful as controlled release systems for drug delivery.[9,10]

Poly(vinyl alcohol) (PVA), is biocompatible, water-soluble, highly hydrophilic and nontoxic polymer with excellent film-forming property, and therefore very suitable for modification and hydrogels.[11-13] PVA films have high mechanical strength, low fouling potential, long-term temperature and pH stability.[14,15] These characteristics of PVA have led to their use in bioseparation, biotechnology, biomedical and pharmaceutical industry.[16-18] It can be easily prepared with excellent chemical resistance.[19] The resistance against organic solvents and aqueous solubility make it adaptable for many applications.[20] In order for PVA to be useful for a wide variety of application, it must be crosslinked, specifically in the areas of medicine and pharmaceutical sciences. In addition, various derivatives of

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PVA hydrogels have been prepared, and a large number of medical applications have been reported by several authors. Peppas *et al*.[21,22] conducted some of the earliest work in considering PVA hydrogels as biomaterials. Morimoto *et al*.[23] examined the controlled release of several drugs from PVA hydrogel carriers for rectal administration. Swamy *et al*.[24] synthesized hydroxypropyl methyl cellulose and PVA blend microspheres by a water-in-oil emulsion method, and ciprofloxacin hydrochloride was loaded into interpenetrating polymer network microspheres crosslinked with glutaraldehyde.

Ceftriaxone is an antibiotic, which is useful for the treatment of a number of bacterial infections. It is a third-generation cephalosporin.[25] Like other third-generation cephalosporins, it has broad-spectrum activity against Gram-positive bacteria and expanded Gram-negative coverage in comparison to second-generation agents. In most cases, it is considered to be equivalent to cefotaxime regarding safety and efficacy. It is on the world health organization's list of essential medicines, a list of the most significant medication needed in a basic health system. Immunologic ceftriaxone side effects have included life-threatening and fatal cases of immune hemolytic anemia, with symptoms of pallor, tachycardia, hypotension, dyspnea, and severe back pain. Most of these patients had preexisting hematologic or immunodeficiency disorders, and Crohn's disease.[26,27] Moreover the stability of ceftriaxone in different pH has already been examined.[28] So that injection of the antibiotic should be under the supervision of qualified personnel. The use of this medication with controlled release characteristics can overcome this problem. However, it is necessary to investigate the biocompatibility of the improved PVA hydrogels.

Therefore, the present study was designed to synthesize three new hydrogels by crosslinking of bromoacetylated poly(vinyl alcohol) (BAPVA). In the current study, the aim of this work is to synthesize novel hydrogel networks based on PVA and to examine their ability in Ceftriaxone slow release systems. For this propose the PVA was bromoacetylated and then crosslinked by three types of aliphatic diamines, ethylenediamine (EDA), propylendiamine (PDA) and hexamethylenediamine (HMDA). The main difference between the present project and the past similar works lies in non-solvent method to modify PVA prior to crosslinking stage. This matter (the seperation of solvent from the product) in turn will be economically beneficial. In addition, the ceftriaxone antibiotic was loaded on the hydrogels to investigate *in vitro* drug release and water sorption behaviors at different pH[28] in different media (buffer solutions at pH 3, 7.4 and 8.5).

Experimental

Materials. PVA (M_w=72000, degree of hydrolysis > 98%), *N*-methyl-2-pyrrolidone (NMP), sodium hydrogen carbonate, sodium hydroxide and hydrochloric acid (37%) were obtained from merk (Germany). Bromoacetyl bromide (≥ 98%), triethylamine (99/5%), ethylenediamine (≥ 99), 1,3-diaminopropan (≥ 99) hexamethylenediamine (98%) ethyl acetate and tetrahydrofourane (THF) (≥ 99) were purchased from Sigma-Aldrich and were used without further purifications. Ceftriaxone was kindly donated by Dena Tabriz Pharmaceutical Co.

Measurements. IR spectra were measured with a Fourier transform infrared spectrophotometer (FTIR) (Perkin Elmer, Spectrum two, USA). The thermogravimetric analysis (TGA) of the prepared samples was performed using the (TGA; L81-I LENSES Co., Germany) by scanning from room temperature up to 400 ºC with the heating rate of 10 ºC/min. Moreover, the morphology of samples was observed from scanning electron microscopy (SEM) images which were obtained using a (FESEM; TSCAN Co., Czech Republic) scanning electron microscope. The UV-Vis spectrophotometer (JENWAY 6305, UK) was used to study the drug release.

Preparations of BAPVA. The PVA (0.2 g, 0.0027 mmol) and triethylamine (0.5 g, 4.9 mmol) were added to a 50 mL of Masonry mortar. Then bromoacetyl bromide (1 g, 4.95 mmol) was added to the above mixture dropwise along with grinding at the room temperature for 2 h. Finally, the mixture was poured into 100 mL of THF, and then filtered and washed several times with THF followed by drying in a vacuum oven at 40 ºC.

Synthesis of the Hydrogel Networks. The crosslinking of BAPVA with EDA (c-PVA/EDA) was prepared in a 50 mL, one-necked, round-bottomed flask equipped with a reflux condenser and a magnetic stirrer bar. For this purpose, a mixture of the BAPVA (0.15 g, 0.002 mmol), triethylamine (0.2 g, 1.9 mmol), EDA (1 g, 1.6 mmol) and NMP (10 mL, 103 mmol) were added and stirred at room temperature for 15 h. Subsequently, the mixture was heated up to 95 ºC under constant stirring until the formation of a homogeneous mixture. The homogeneous mixture was refluxed at 95 ºC for 8 h, then cooled down to room temperature. The resultant mixture was precipitated in excess ethyl acetate and washed several times with ethyl acetate. The precipitate was dried in vacuum for 24 h (yield=82%).

For preparation of crosslinked PVA with PDA (c-PVA/PDA) and HMDA (c-PVA/HMDA) the same procedure was repeated

(yield of c-PVA/PDA=79% and yield of c-PVA/HMDA=74%).

Degree of Swelling (DS) Determination for the Prepared Hydrogels. To determine the DS values of the hydrogels, 20 mg of each dry hydrogel was immersed in various pH environments (pH 3, 7.4, and 8.5) at room temperature. After specific times, the samples were filtered and reweighed. This procedure was continued until a fixed weight was obtained. The DS of each hydrogel was calculated according to the following eq. (1)[29,30]

$$DS = \frac{W_2 - W_1}{W_1} \quad (1)$$

where W_1 and W_2 are the weights of the hydrogel before and after swelling. Table 1 shows the DS of the PVA hydrogels.

Drug Loading. A total of 20 mg of hydrogel was allowed to equilibrate at the cefftreiaxon solution (1 g, cefftreiaxon in 2 mL water) for 24 h. After that, the hydrogel was taken out, dried, and the percent of loading was calculated using the following eq. (2):

$$\text{Percent of loading} = \frac{W_2 - W_1}{W_1} \times 100 \quad (2)$$

where W_2 and W_1 are weight of drug loaded and dry hydrogel, respectively.

Drug Release Studies. In order to measure equilibrium drug release in the release medium, the standard calibration curve of the absorbance as a function of the ceftriaxone drug concentration was drawn at 293 nm on the UV spectrophotometer. For the drug release evaluation, drug-loaded hydrogel (16 mg) was placed in a dialysis tube in 50 mL of carbonate-buffered solutions at various pH values (3, 7.4 and 8.5) at 35 °C. At planned time intervals, 3 mL of the release medium was removed with a syringe, and we recorded the absorbance at a wavelength of 293 nm in a UV-visible spectrophotometer. Then the solution was returned back to the medium.

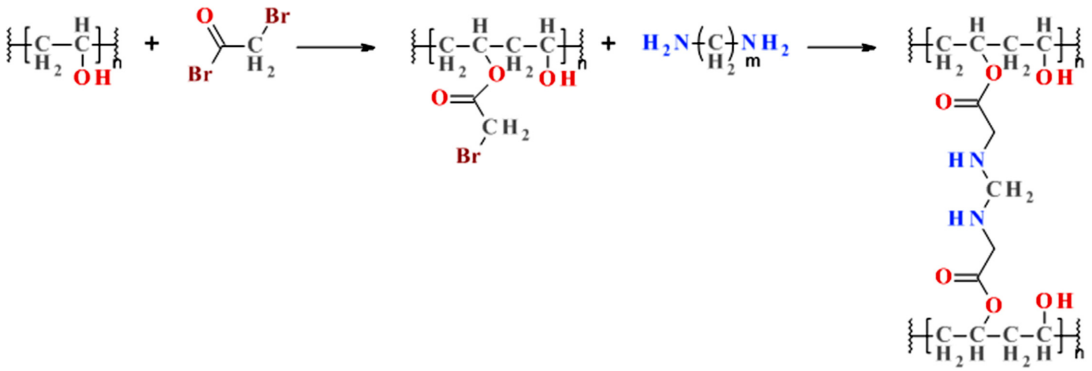
Results and Discussion

Synthesis of the Hydrogel Networks. Three kinds of hydrogels were synthesized according to the synthetic path which is portrayed in Scheme 1. The hydrogels were synthesized via processing in two steps. In the first step, the PVA was modified by bromoacetyl bromide to produce BAPVA. Slow addition of the bromoacetyl bromide to the mixture of PVA and triethylamine was carried out along with grinding at the room temperature for 2 h. The crosslinking was carried out in the second step of the reaction. In this step the reaction was carried out between BAPVA and various diamines in the presence of triethylamine as the base in the NMP as a solvent.

Fourier Transform Infrared Spectroscopy. Figure 1(a)

Table 1. DS Values of the Prepared Hydrogels

Time (min)		15	30	60	120	240	480	1440
DS (c-BAPVA/EDA)	pH 3	3.6	4	5.92	6.46	6.66	7.06	7.66
	pH 7.4	2.68	3.06	4.14	4.14	5.14	5.94	6.94
	pH 8.5	1.8	2.4	3	3.44	3.7	4.2	5.7



Scheme 1. Synthetic path for the preparation of PVA hydrogels (n=2-3,6).

shows the FTIR spectrum of the PVA and indicates the presence of OH groups due to the bands that appeared in the region between 3300-3416 cm^{-1}. The band at 2933 cm^{-1} was due to C-H stretching of the aliphatic hydrogen groups. In addition, the band at 1010 cm^{-1} was due to C-O groups of PVA. By comparing the spectrum corresponding to the BAPVA [Figure 1(b)] with PVA, the O-H stretching band at 3416 cm^{-1} was shifted to 3422 cm^{-1} as a result of the partial esterification of the O-H and the decrease in hydrogen bond. The appearance of a sharp peak at 1735 cm^{-1} showed the formation of esteric carbonyl band. Also, the appearance of the band at 793 cm^{-1} corresponds to the carbon–bromine stretching vibrations and this confirms the effectiveness of bromoacetylation. Figure 1(c) shows the spectrum of the c-PVA/EDA hydrogel. In this figure the intensity of the C-H vibration bond around 2940 cm^{-1} was slightly increased, because of the crosslinking reaction and the introduction of more C-H in the crosslinked form. Also, the comparison of the spectra for the BAPVA with crosslinked hydrogel shows that the strength band about 3400 cm^{-1} was decreased. It may be due to increasing hydrogen bonds as the result of higher number of the N-H bonds. We also noted that the band present at 787 cm^{-1} in the spectrum of BAPVA was completely omitted because of the removal of the bromine in the BAPVA structure after crosslinking with diamine. Also the strength/intensity of C-H aliphatic stretching vibration was decreased from crosslinked hydroglel by HMDA to EDA due to the decrease in number of aliphatic hydrogens. At last, these results confirm the successful synthesis of hydrogels network.

Thermogravimetric Analysis (TGA). Thermogravimetric analysis was performed to examine the thermal stability of the PVA, c-PVA/EDA and c-PVA/PDA hydrogels at 10° C/min

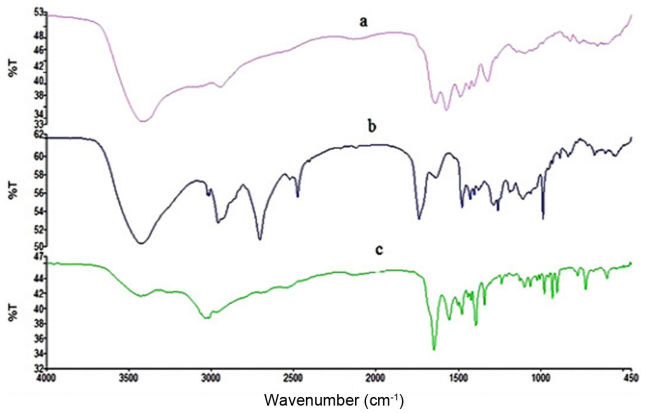


Figure 1. IR spectra of (a) PVA; (b) BAPVA; (c) c-PVA/ EDA.

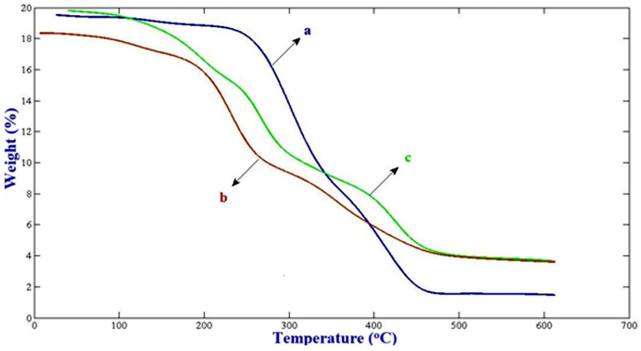


Figure 2. TGA of PVA and hydrogels (a) PVA; (b) c-PVA/EDA; (c) c-PVA/PDA.

under an N_2 flow as shown in Figure 2. These curves distinguish the differences between the PVA, c-PVA/EDA and c-PVA/PDA. The thermogram of PVA shows a decomposition step as a pure material which starts from 240 °C (Figure 1(a)). The thermogram of the c-PVA/EDA shows three decomposition steps. The first step starts from 100 °C related to evaporation of absorbed water from hydrogel backbone. The second step is related to the breaking of ester and amine linkages in the hydrogel. The third step starting from 240 °C is related to the decomposition of PVA backbone. Also the thermogram of c-PVA/PDA shows three decomposition steps similar to c-PVA/EDA. The first step starting from 120 °C is related to evaporation of adsorbed humidity in hydrogel matrix. The second step starting from 180 °C is related to breaking of crosslinking agent. The third step which starts from 250 °C is related to decomposition of PVA main chain. These observations shows the clear difference between synthesized hydrogel with pure PVA.

Scanning Electron Microscopy (SEM). In general, the scanning electron microscopy (SEM) shows microstructure morphologies of hydrogels. The SEM of the synthesized PVA hydrogel before and after drug loading is shown in Figure 3(a)-(d). These images verify that the synthesized PVA-based hydrogel has porous and fiber structure. Also it is clearly observable that the drug loaded hydrogel has a smooth surface and indeed after the drug was loaded, their pores were filled with the loaded drug.

Swelling Behavior. The swelling behavior of the hydrogels in water was investigated and the results were shown in Figure 4. The prepared hydrogels, c-PVA/EDA, c-PVA/PDA, and c-PVA/HMDA were tested for swelling behavior and the swelling ratio was calculated according to eq. (1). The PVA/PDA, and the c-PVA/HMDA were dissolved in the buffer solutions

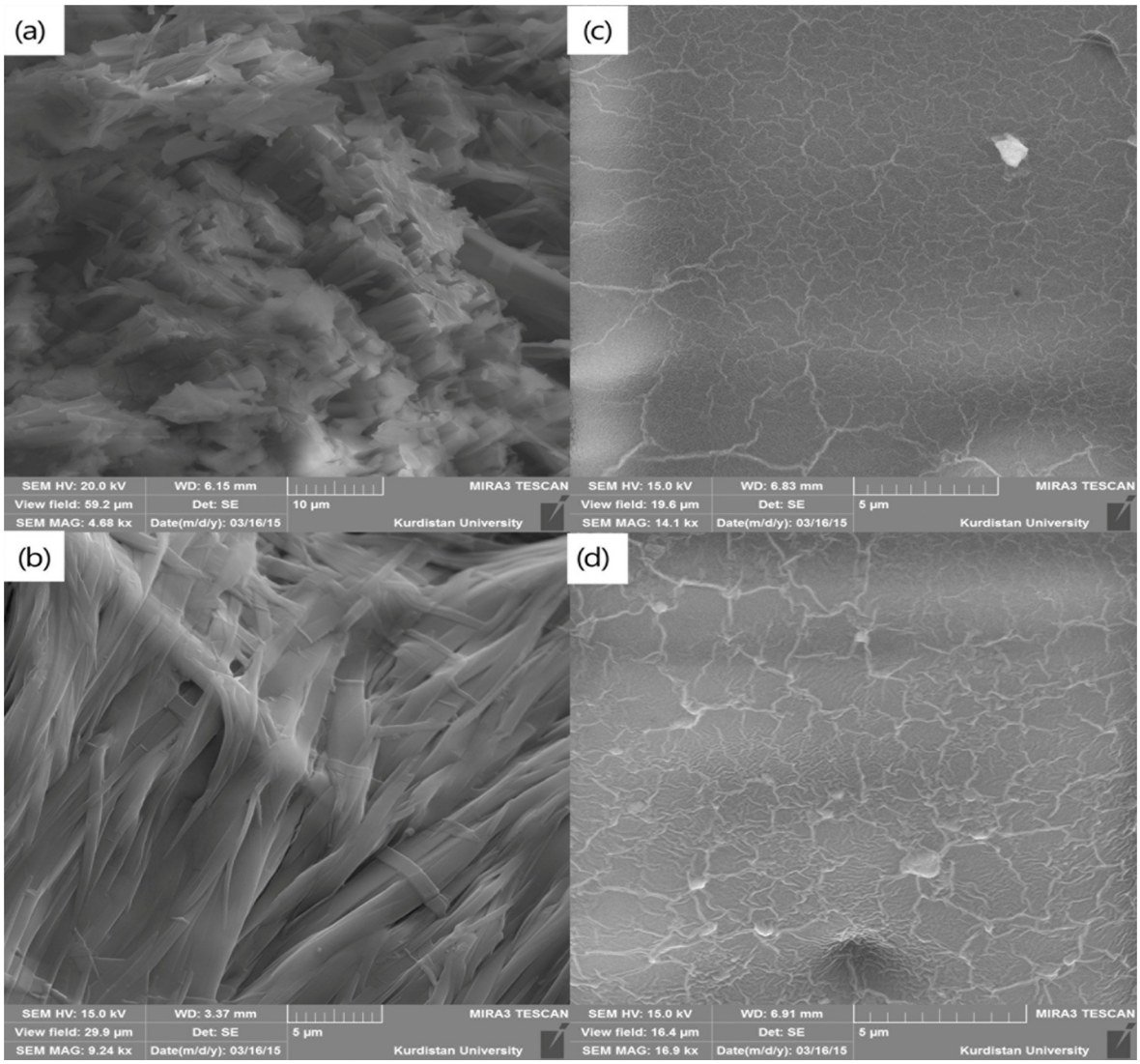


Figure 3. SEM images of the modified hydrogels: (a-b) before drug loading; (c-d) after drug loading.

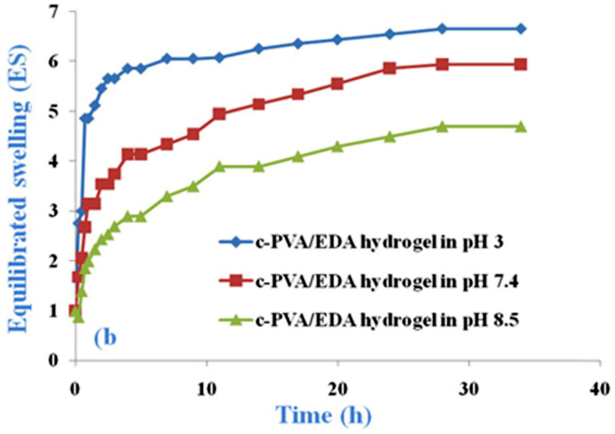


Figure 4. Swelling behavior of the c-PVA/EDA hydrogels in pH 3, 7.4, and 8.5.

after 1 h so their swelling ratio couldn't be measured. Only the c-PVA/EDA hydrogel was not dissolved and its swelling behavior was examined in buffer solutions at pH 3, 7.4 and 8.5 (Figure 4). In the c-PVA/EDA hydrogel with increase of pH, a decrease in the swelling ratio was observed. Also, the hydrogel network displayed a fast initial swelling followed by a mild increase until attaining equilibrium. The fast initial swelling was due to the hydrophilic nature of the hydrogel network.

Drug Release Study. 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 resulted in better dispersion of drug in the hydrogel matrix. The release profile of Cefftiaxone antibiotic from the loaded hydrogel was studied in three different media.

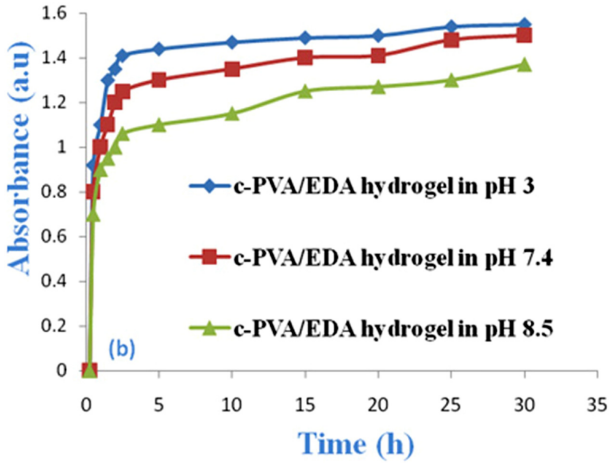


Figure 5. Percentage of cumulative release c-PVA/EDA at different pH.

The drug release curves, which show the drug release percentages at different times, are shown in Figure 5.

It was clearly observable that the drug release rate was enhanced when the pH was decreased. 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 the next 5 h. After that, the drug release was almost finished, and the absorbance showed an approximately fixed value. As shown in Figure 5, the drug release at pH 7.4 showed upward growths for next 25 h, then, the absorbance showed an approximately fixed value. The drug release at pH 8.5 showed over growth the first 15 h and 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

In the present work, we report the development of novel PVA hydrogels suitable for drug delivery applications. The modified PVA was synthesized by bromoacetylation of the PVA. Then PVA hydrogel networks were successfully synthesized from the modified PVA and different derivatives of diamines as crosslinking agent. The investigation of the drug release ability of the modified hydrogel was implemented with cefftriaxone 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 a suitable way to control the release rate in various treatments.

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