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Synthesis and Characterization of Novel pH-Responsive Poly(2-hydroxylethyl methacrylate-*co*-*N*-allylsuccinamic acid) Hydrogels for Drug Delivery

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ABSTRACT: In this study, *N*-allylsuccinamic acid (NASA) was synthesized in a single step with a yield of 85%. Carboxylic acid containing NASA was characterized through Fourier transform infrared (FTIR) radiation and ^{1}H-NMR and ^{13}C-NMR analysis, and then it was used for synthesis of poly(2-hydroxylethyl methacrylate-*co*-*N*-allylsuccinamic acid) [p(HEMA-*co*-NASA)] hydrogels. The structure of the obtained pH-responsive p(HEMA-*co*-NASA) hydrogels were characterized with FTIR spectroscopy and scanning electron microscopy analysis, and their swelling characterization was carried out under different drug-release conditions. In the application step of the study, the hydrogels were used for the *in vitro* release of vitamin B12 and Rhodamine 6G, which were selected as model drugs. We determined that the hydrogels used as a drug-delivery matrix could release the drug they had absorbed under different release conditions (phosphate-buffered saline, 0.9% NaCl, and pH 1.2) at high rates for time periods of up to 24 h. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39660.

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INTRODUCTION

Hydrogels are three-dimensional crosslinked polymeric networked structures.[1,2] They can absorb water up to hundreds of times or even thousands of times their mass in aqueous media thanks to functional groups (—OH, —COOH, —NH_2, —$CONH_2$, and —SO_3H) within them.[3,4] In recent times, researchers have concentrated on designing hydrogel systems that are responsive (by swelling or deswelling) to environmental effects such as electric fields, light, pH, temperature, solvent, and the ionic strength of the medium.[5–8] The hydrogels' ability to swell in aqueous media, responsiveness to environmental effects, and biocompatible and biodegradable nature increase their usefulness in the areas of drug-delivery systems, antimicrobial materials, sensors, pharmacology, matrix materials for tissue engineering, catalyst support materials, agriculture, separation, immobilization, and environmental technologies.[9–14]

2-Hydroxyethyl methacrylate (HEMA) hydrogels are biocompatible hydrogels.[12,15] The tunable mechanical features, chemical stability, and especially high biocompatibility of hydrogels containing HEMA have made HEMA a favorite of researchers.[12,15–19] HEMA is generally prepared in the form of copolymer hydrogels with ionic or more hydrophilic monomers. In recent times, researchers at facilities have studied the use of HEMA produced in the form of copolymer hydrogels for many areas, especially in drug-delivery systems and biomedical devices.[12,16,17] Succinamic acid derivatives contain carboxyl groups in their structure. These carboxyl groups can be easily bonded to drugs and the peptide molecules of host materials for biological applications. In addition, succinamic acid derivatives can be used as inhibition agents for yeasts.[20–22]

In this study, an *N*-allylsuccinamic acid (NASA) monomer containing a hydrophilic functional group (—COOH) was synthesized with a yield of 85%, and then it was characterized. The NASA monomer was used in a hydrogel synthesis for the first time. Copolymer hydrogels were prepared with this new type of ionic monomer and with biocompatible HEMA at different molar ratios. Fourier transform infrared (FTIR) radiation, scanning electron microscopy (SEM), and swelling characterization of the resulting new type of poly(2-hydroxylethyl methacrylate-*co*-*N*-allylsuccinamic acid) [p(HEMA-*co*-NASA)] hydrogels were carried out, and their diffusion features were examined in different solutions [distilled water, NaCl, phosphate-buffered saline (PBS), and urea] and conditions. The hydrogels were maintained in a sodium hydroxide solution to be converted into sodium salt to allow the design of p(HEMA-*co*-NASA)

hydrogels as more efficient drug-delivery systems {because the poly(2-hydroxylethyl methacrylate-*co*-*N*-allylsuccinamic acid) hydrogels treated with NaOH [p(HEMA-*co*-NASA–Na)] used during the study cracked during drying when they were initially synthesized as sodium salts, they were converted into sodium salt afterward}. It was determined that the hydrogels could swell at a ratio of 4753 ± 307 wt % as a result of this conversion. Vitamin B12 and Rhodamine 6G (R6G) were used as model drugs in the drug-release step of the study. p(HEMA-*co*-NASA–Na) (1:1) hydrogels, which absorbed the drug to maximum capacity, were used as the drug-delivery matrices for three different release conditions (PBS, 0.9% NaCl, and pH 1.2).

EXPERIMENTAL

Materials

Succinic anhydride (99%), allylamine (99%), and tetrahydrofuran (THF; 99.9%) used in synthesis of NASA were supplied by Fluka and Sigma-Aldrich. 2-Hydroxylethyl methacrylate (HEMA; 97%) used as the monomer was supplied by Aldrich Co. *N,N′*-Methylenebisacrylamide (MBA; 99%) used as cross-linker for the hydrogel synthesis, ammonium persulfate (APS; 98%) used as the initiator, and *N,N,N′,N′*-tetramethylethylenediamine (TEMED; 99%) used as the accelerator were supplied by Sigma-Aldrich and Acros Organics and were used without distillation. Urea and NaCl used for swelling characterization were supplied by Sigma-Aldrich. Vitamin B12 [α-(5,6-dimethylbenzimidazolyl) cyanocobamide; 98%] and R6G (95%), which were used as model drugs, and PBS, which was used as a buffer solution, in the drug-release studies were supplied by Sigma-Aldrich.

All of the solutions and calibration standards (monomer and drug) used in the study were prepared with distilled water. A Consort C864 multi-pH meter was used for the pH measurements.

An ultraviolet–visible (UV–vis) spectrometer (Agilent 8453) was used to determine the released drug amount for the model drugs chosen as a function of time. Calibration graphics were produced at a wavelength of 361 nm for vitamin B12 and at 527 nm for R6G.[23,24] All absorption and release experiments were repeated three times before they were included in the graphics.

Synthesis of NASA

A solution of allylamine (3.43 g; 60 mmol) in dry THF (10 mL) was added dropwise to a stirred solution of succinic anhydride (5.00 g; 50 mmol) in dry THF (150 mL) at 0°C. The mixture was stirred for 12 h at ambient temperature. After the reaction was completed, the solvent was removed under reduced pressure. The obtained solid was recrystallized in acetone to afford NASA as a white solid with an 85% (6.68 g) yield.

M_p: 94–95°C. (Mp assigns Melting of point) FTIR (attenuated total reflectance, cm^{-1}): 3400–2400, 3297, 1688, 1638, 1537. ^{1}H-NMR (300 MHz, hexadeuterated dimethyl sulfoxide): 2.34 (t, $J = 6.9$ Hz, 2H, CH_2CONH), 2.42 (t, $J = 6.9$ Hz, 2H, CH_2COOH), 3.67 (t, $J = 5.4$ Hz, 2H, CONHCH_2), 5.17–4.99 (dq, 2H, CH═CH_2), 5.69–5.83 (m, 1H, CH═CH_2), 8.02 (t, $J = 5.4$ Hz, 1H, NH), 12.06 (br s, 1H, OH). ^{13}C-NMR (100 MHz, hexadeuterated dimethyl sulfoxide): 29.53 (CH_2CONH), 30.42 (CH_2COOH), 41.31 (CONHCH_2), 115.29 (CH═CH_2), 135.85 (CH═CH_2), 171.52 (CONH), 174.34 (COOH).

Table I. Synthesis Compositions of the p(HEMA-*co*-NASA) Hydrogels

HEMA (mL)	NASA (g)	Monomer feed ratio (mol)	Solvent volume (mL)	TEMED (μL)	Yield (%)
2.5	3.14	1:1	3.6	50	79
2.5	1.35	7:3	1.8	50	84
2.5	0.78	8:2	1.2	50	86
2.5	0.35	9:1	0.8	50	90
2.5	—	1:0	0.5	50	93

APS = 1% mol, MBA = 0.5% mol (with respect to total monomer amount).

Synthesis of the p(HEMA-*co*-NASA) Hydrogels

The p(HEMA-*co*-NASA) hydrogels were synthesized as a series with 2.5 mL of HEMA (HEMA was directly used without water addition during the polymerization process) with the redox polymerization technique, as illustrated in Table I. HEMA and NASA, in the amounts shown in Table I, were crosslinked with MBA at ratios ranging between 0.5 and 1.5%. A volume of 50 μL of TEMED was added to the solution containing HEMA, NASA, and MBA after was stirred until it became homogeneous. The solution was stirred again until it was homogeneous, and then the initiator dissolved in 0.5 mL of distilled water (APS, 1 mol % with respect to the monomer) was added to it. Then, pipettes were filled with the solution with a diameter of 5 mm with the help of an injector. They were maintained for 6 h to complete polymerization. Then, the hydrogels extracted from the pipettes were cut to a diameter of 5 mm and rinsed in distilled water for 24 h. The p(HEMA-*co*-NASA) hydrogels were dried in an oven at 40°C to be stored after the rinsing process. Furthermore, the hydrogels were maintained in a 1*M* NaOH solution for 24 h to convert the —COOH groups within the p(HEMA-*co*-NASA) hydrogels into sodium salt, and they were rinsed with distilled water until they reached neutral pH. Then, they were dried and stored to be used in swelling characterization and drug-release experiments.

Instrumental Characterization

The ^{1}H-NMR and ^{13}C-NMR spectra used in the characterization of NASA were recorded by a Bruker-300 device, whereas the FTIR spectra used in the characterization of NASA and the hydrogels were recorded with a PerkinElmer Spectrum 100 instrument and an attenuated total reflectance unit (4000–650 cm^{-1}). The hydrogels were coated with a palladium–gold alloy before SEM analysis (QUANTA 400F) for the characterization of surface morphology.

Swelling/Deswelling Kinetics of the Hydrogels

Studies of the water absorption kinetics of the p(HEMA-*co*-NASA) and p(HEMA-*co*-NASA–Na) hydrogels produced as a series were carried out in distilled water. Furthermore, swelling characterization was carried out in NaCl (0.9%), urea (0.05*M*), and PBS solutions, which were used in biomedical applications.

In addition, the maximum equilibrium swelling (S) characterization of the hydrogels was carried out at different pH values (pH 2–12). The solutions with different pH values required for this process were prepared by the addition of 0.1M NaOH and 0.1M HCl in drops into distilled water.

For studies on swelling kinetics of the hydrogels as a function of time, approximately 100 mg of hydrogel was placed in distilled water, and the increase in mass was measured at specified times. For pH swelling characterization studies and the determination of S (%), approximately 100 mg of hydrogel was placed into the solution (NaCl, urea, and PBS), and the increase in their mass after 24 h was measured. All swelling characterizations of the hydrogels were carried out at room temperature.

Deswelling characterization of the hydrogels was carried out as follows. Approximately 100 mg of hydrogel was maintained in distilled water for 24 h to ensure that it retained water at its maximum capacity. Water absorbed by the hydrogel was calculated in percentage by mass [eq. (1)], and it was maintained in an oven at 37°C. The mass of the hydrogel was measured at specified times, and the deswelling amount was calculated with eq. (1):[25]

$$S\% = [(M_S - M_D)/M_D] \times 100 \quad (1)$$

where M_S is the weight of the swollen hydrogel and M_D is the weight of the dried hydrogel.

Drug-Loading and Drug-Release Experiments

The hydrogels produced with HEMA were used in the controlled release of vitamin B12 and R6G as model drugs to investigate their biomedical application. To load the drugs into the hydrogels, we maintained 250 mg of hydrogel in 100 mg/L (100 mL) of a model drug solution for 24 h. The drug amount absorbed by the hydrogel at the end of this time period was determined with a UV–vis spectrophotometer.

After the hydrogels had absorbed the drugs, the release studies were carried out in three different media (PBS, 0.9% NaCl, and pH 1.2) at 37°C. The hydrogel (250 mg), which had absorbed the drug to maximum capacity, was transferred into the medium (PBS, 0.9% NaCl, and pH 1.2, 50 mL) and stirred at 250 rpm for the release studies. During the studies on drug delivery, the samples collected from the release media were measured with a UV–vis spectrophotometer and drug concentrations were calculated.

The maximum amount of absorption/release (q_e) was calculated with eq. (2):[26]

$$q_e = (C_I - C_e)V/M \quad (2)$$

where C_I and C_e are the initial and equilibrium concentrations (of vitamin B12 and R6G; mg/L), respectively; V is the volume of vitamin B12 and R6G solutions; and W is the weight of the p(HEMA-*co*-NASA) (1:1) hydrogels.

RESULTS AND DISCUSSION

Synthesis and Spectral Characterization

As shown in Figure 1(a), NASA was synthesized in THF media in a single step with a yield of 85% and characterized by FTIR spectroscopy and ^{1}H-NMR and ^{13}C-NMR analysis. According to the FTIR spectrum of NASA given in Figure 1(b), the characteristic O—H and N—H stretching bands for the compound were observed at 3400–2400 and 3297 cm^{-1}, whereas the C=O stretching frequencies of the compound were observed at 1688 and 1639 cm^{-1} for carboxylic acid and amide groups, respectively. The characteristic N—H bending band for amide compounds was observed at 1538 cm^{-1}.

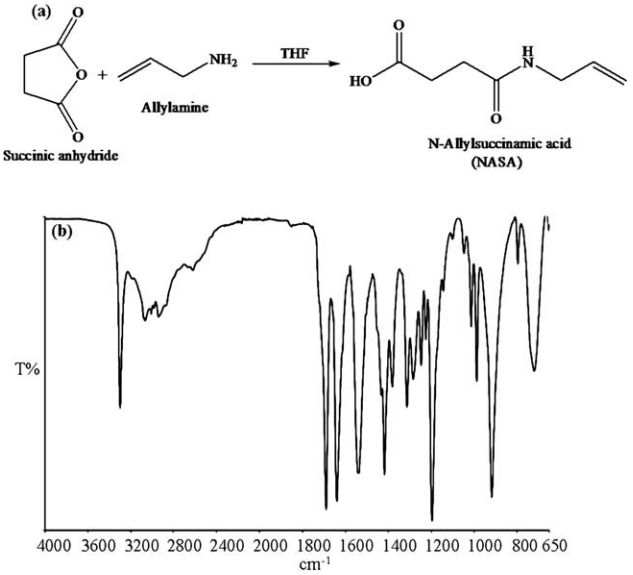

Figure 1. (a) Synthesis of the NASA monomer and (b) FTIR spectrum of NASA.

The ^{1}H-NMR spectrum for NASA is given in Figure 2(a). The —OH and —NH protons resonated to be a 12.06-ppm singlet and an 8.02-ppm triplet for the compound. The olefin protons related to *CH*=CH$_2$ and CH=*CH*$_2$ groups were observed at 5.69–5.83 and 4.99–5.17 ppm as multiples and a doublet in quartets, respectively. The aliphatic protons resonated at 3.67, 2.42, and 2.34 ppm as triplets for CONH*CH*$_2$, *CH*$_2$COOH, and *CH*$_2$CONH groups, respectively. According to the ^{13}C-NMR spectra, seven signals related to the compound were observed at 174.34 (C=O, carboxylic acid), 171.52 (C=O, amide), 135.85 (C=C), 115.29 (C=C), 41.31, 30.41, and 29.53 (aliphatic carbons) ppm [Figure 2(b)].

p(HEMA-*co*-NASA) hydrogels containing —OH functional groups related to HEMA and —COOH groups related to the NASA monomer were synthesized under the conditions given in Table I and as shown in Figure 3(a) with MBA as the crosslinker. Herein, NASA could not be synthesized alone as a homopolymer hydrogel. As shown in Table I, although the p(HEMA-*co*-NASA) (1:0) hydrogel could be synthesized with a yield of 93%, NASA, which was added to the structure, decreased the yield of the hydrogel. However, despite the decrease in yield, the p(HEMA-*co*-NASA) (1:1) hydrogel was synthesized with a yield of 79%. The IR spectra of poly(2-hydroxyethyl methacrylate) [p(HEMA)] and p(HEMA-*co*-NASA) (1:1) are given in Figure 3(b). According to the IR spectra of the p(HEMA) hydrogels, the bands related to —OH and C=O stretching were observed at 3384 and 1708 cm^{-1}, respectively. In the FTIR spectra of p(HEMA-*co*-NASA) (1:1), C=O stretching for the carboxylic acid group of NASA overlapped with the band of C=O

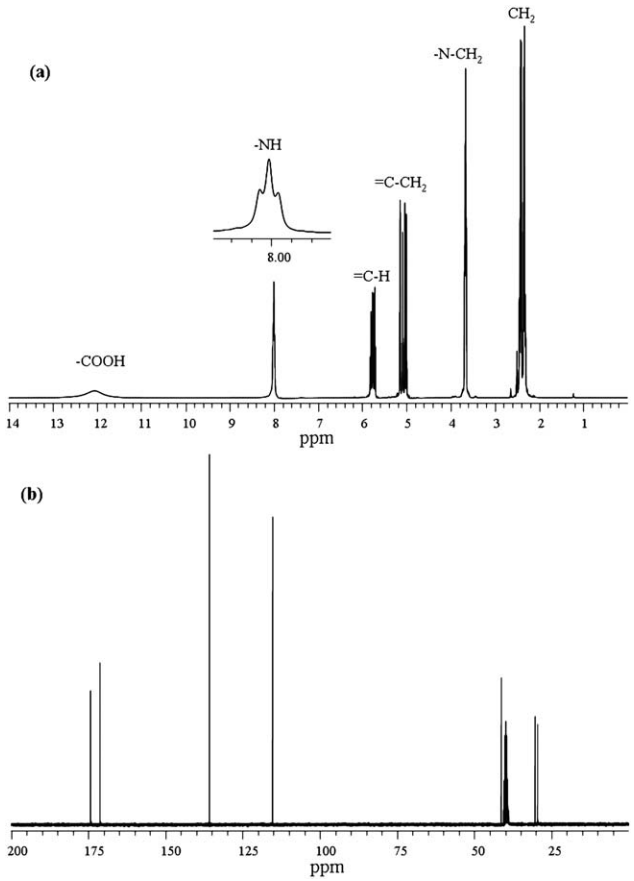

Figure 2. (a)^{1}H-NMR and (b)^{13}C-NMR spectra of the NASA monomer.

stretching of the ester group of HEMA at 1708 cm^{-1}. In the spectrum, the C═O stretching and N—H bending bands of the amide group of NASA were observed at 1651 and 1546 cm^{-1}, respectively. Thus, according to these spectral assessments, the p(HEMA-*co*-NASA) hydrogels were successfully synthesized.

Figure 4(a–e) shows SEM images of the p(HEMA-*co*-NASA) hydrogels, which were synthesized at different molar ratios (1:0, 9:1; 8:2; 7:3, and 1:1) and were not converted into sodium salt. Considering the morphological features of the hydrogels, we observed that their surfaces were heterogeneous. We noted that the porous structure increased with the increasing amount of NASA included in the hydrogel. The water amount that was retained by the hydrogel increased with increasing porous structure of the surface.

Swelling Characterization of the p(HEMA-*co*-NASA) Hydrogels

Hydrogels, called *superabsorbents,* may be used in many areas. The reason for the diversity of their application areas is the fact that they can respond to environmental effects by swelling or deswelling. The medium in which such a response is produced depends on the functional groups within the hydrogel. NASA is a monomer containing —COOH groups. Carboxylic acid groups in hydrogels ionize in alkaline medium (pH > 7) so that they absorb more water. Thus, the hydrogel responds to variations in pH by swelling. Figure 5 shows the swelling characteristics of the p(HEMA-*co*-NASA) hydrogels in media with various pH values. The p(HEMA-*co*-NASA) (1:0) hydrogel used as control in the swelling characterization experiments, depending on the pH of the solution, was not responsive to pH because it consisted of only neutral HEMA monomers. The p(HEMA-*co*-NASA) hydrogels become pH-responsive upon their inclusion of the ionic NASA monomer. According to Figure 5, the p(HEMA-*co*-NASA) (1:0) hydrogel swelled at a ratio of 70 ± 11 mass % within a range of pH of 2–12. On the other hand, the p(HEMA-*co*-NASA) (9:1) hydrogel, which was synthesized by the addition of NASA at a ratio of 10% into the hydrogel, swelled at a ratio of 142 ± 29 mass % at pH 12. When the NASA ratios added to the hydrogel were 20, 30, and 50%, the swelling amounts at pH 12 were 314 ± 49, 441 ± 50, and 839 ± 72 mass %, respectively. As shown in Figure 5, the p(HEMA-*co*-NASA) (1:1) hydrogel swelled at a ratio of 209 ± 44 mass % at pH 6, whereas it swelled fourfold more upon when the alkalinity of the medium was increased. Thus, it may be said that NASA, which ionized in the form of —COONa, was added to the network structure of the hydrogels. The easy polymerization of the HEMA monomer with a high yield is one of its essential features. However, the p(HEMA-*co*-NASA) hydrogels containing an anionic group swelled less than expected because they did not retain water in very high amounts in the form of the hydrogel. Figure 6(a) shows the swelling characteristics of the p(HEMA-*co*-NASA) hydrogels in distilled water and those of p(HEMA-*co*-NASA) hydrogels maintained in the 1*M* NaOH solution. The p(HEMA-*co*-NASA) hydrogels were maintained in NaOH solution for 24 h and rinsed with distilled water until they reached a neutral pH to convert their —COOH groups into —COONa ones. The

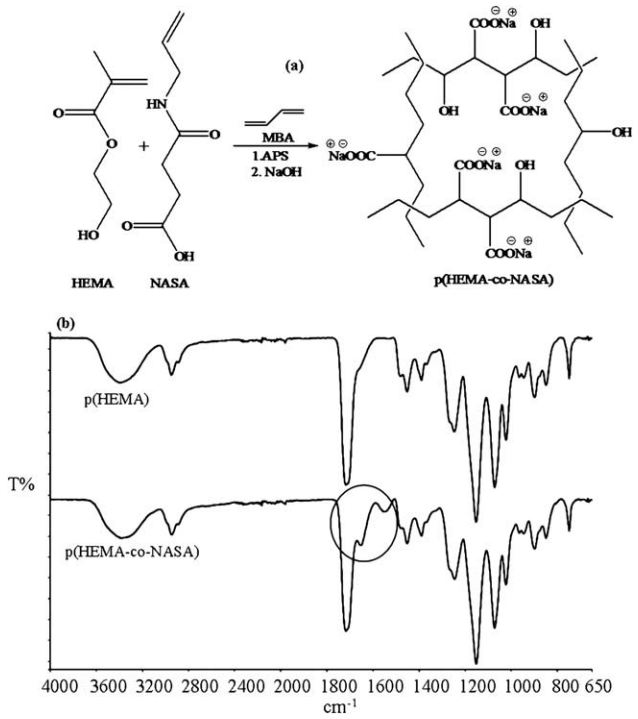

Figure 3. (a) Schematic representation of the synthesis of the p(HEMA-*co*-NASA) hydrogels with different molar ratios and (b) FTIR spectra of the p(HEMA) and p(HEMA-*co*-NASA) (1:1) hydrogels.

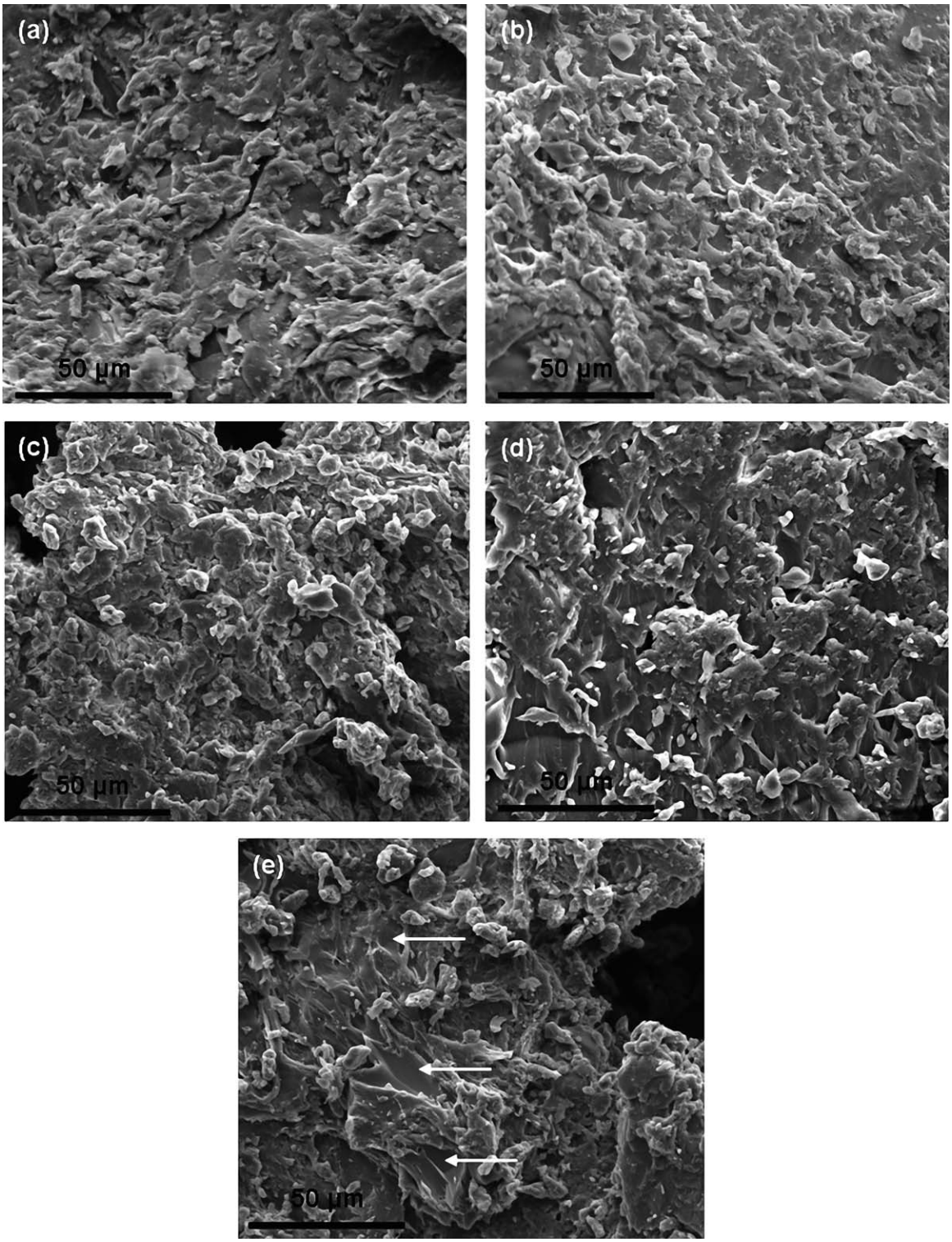

Figure 4. SEM images of the (a) p(HEMA), (b) p(HEMA-*co*-NASA) (9:1), (c) p(HEMA-*co*-NASA) (8:2), (d) p(HEMA-*co*-NASA) (7:3), and (e) p(HEMA-*co*-NASA) (1:1) hydrogels.

p(HEMA-*co*-NASA–Na) hydrogels, containing —COONa groups in the form of sodium salt, retained more drug and water because of their ability to ionize and because of the charges in their structure. According to Figure 6(a), the 1:0, 9:1, 8:2, 7:3, and 1:1 p(HEMA-*co*-NASA) hydrogels with increasing NASA content swelled in distilled water at ratios of 77 ± 9, 93 ± 12, 128 ± 14, 149 ± 18, and 217 ± 22 mass %, respectively, whereas the p(HEMA-*co*-NASA–Na) hydrogels with increasing NASA contents (1:0, 9:1, 8:2, 7:3, and 1:1) swelled in distilled water at ratios of 74 ± 11, 351 ± 9, 1092 ± 93, 1978 ± 166, and

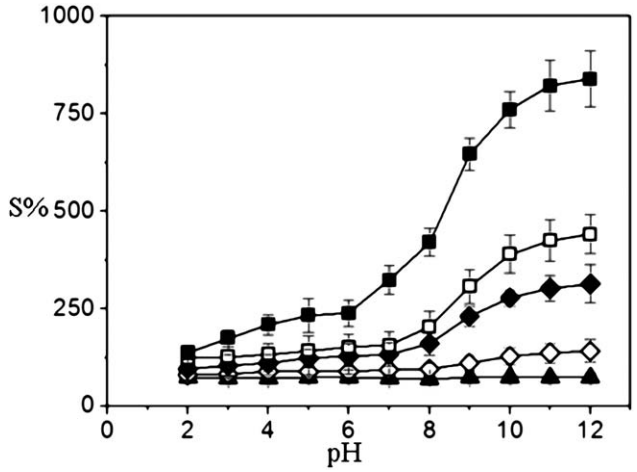

Figure 5. pH dependence of the *S* values of the p(HEMA-*co*-NASA) hydrogels: (■) p(HEMA-*co*-NASA) (1:1), (□) p(HEMA-*co*-NASA) (7:3), (◆) p(HEMA-*co*-NASA) (8:2), (◆) p(HEMA-*co*-NASA) (9:1), and (▲) p(HEMA-*co*-NASA) (1:0). The pH was adjusted with 0.1*M* HCl and 0.1*M* NaOH.

4753 ± 307 mass %, respectively. The variation in the crosslinker ratio changed the mechanical features of the hydrogel, and furthermore, it had a strong effect on its water-retention capacity. As shown in Figure 6(b), the p(HEMA-*co*-NASA–Na) (1:1) hydrogels were produced with four different MBA ratios (0.5, 0.75, 1, and 1.5%). Accordingly, the p(HEMA-*co*-NASA–Na) (1:1) hydrogels, which were synthesized in the form of sodium salt with 0.5 mol % (according to total monomer amount) of crosslinker, swelled in distilled water at a ratio of 4753 ± 307 mass %. However, an increasing MBA ratio resulted in more frequent crosslinking of the polymer chains. Such intensive crosslinking caused a reduction in the swelling ability of the hydrogel and made it more fragile. The reduced swelling amounts according to the MBA percentage ratio (0.5, 0.75, 1, and 1.5%) used in synthesis of the hydrogel were 4753 ± 307, 2764 ± 222, 2150 ± 164, and 1014 ± 121%, respectively. Herein, when the crosslinker ratio exceeded 0.75%, the p(HEMA-*co*-NASA–Na) (1:1) hydrogels deformed rapidly and were spontaneously broken down during the drying process. However, the hydrogels synthesized with MBA at a ratio of 0.5 mol % were

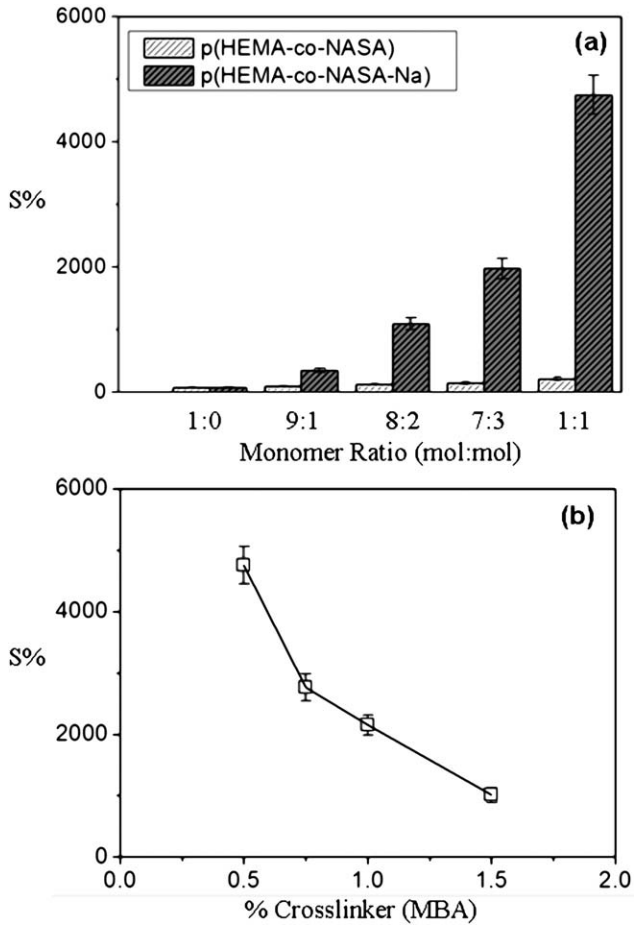

Figure 6. (a) Equilibrium swelling ratios of the p(HEMA-*co*-NASA) and p(HEMA-*co*-NASA–Na) hydrogels in distilled water and (b) effect of the amount of MBA on the equilibrium swelling ratio in distilled water [p(HEMA-*co*-NASA–Na) (1:1), APS = 1%].

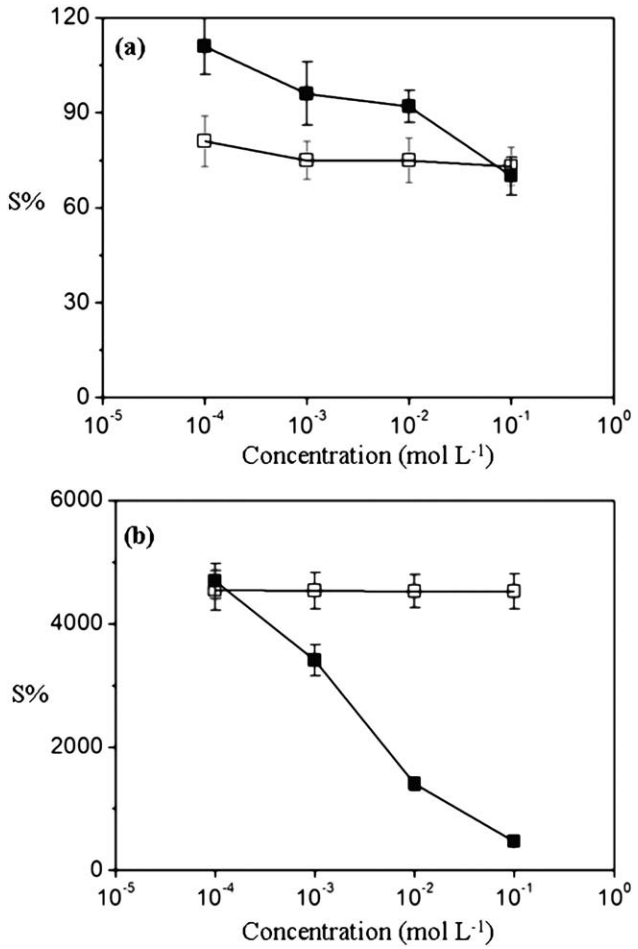

Figure 7. Equilibrium swelling ratios of the hydrogels (0.5% crosslinker) immersed in solutions of NaCl and urea at 37°C: (a) p(HEMA-*co*-NASA) (1:1) [(■) p(HEMA-*co*-NASA)/NaCl and (□) p(HEMA-*co*-NASA)/urea] and (b) p(HEMA-*co*-NASA–Na) (1:1) [(■) p(HEMA-*co*-NASA–Na)/NaCl and (□) p(HEMA-*co*-NASA–Na)/urea)].

Table II. Diffusion Parameters of the p(HEMA-*co*-NASA–Na) Hydrogels in Distilled Water

Hydrogel	n	k	R^2
p(HEMA-*co*-NASA) (1:0)	0.4755	0.0252	0.9982
p(HEMA-*co*-NASA) (9:1)	0.4998	0.0249	0.9982
p(HEMA-*co*-NASA) (8:2)	0.6424	0.0143	0.9987
p(HEMA-*co*-NASA) (7:3)	0.6710	0.0129	0.9945
p(HEMA-*co*-NASA) (1:1)	0.7899	0.0089	0.9993

mechanically strong enough. Furthermore, the hydrogels synthesized with MBA at a ratio of 0.5 mol % were chosen for further steps (swelling characterization and drug-release experiments) of the study because they also had a maximum water-retention capacity.

Hydrogels are expected to swell in different media (PBS, NaCl, and urea) for the diversification of biomedical usage areas. Figure 7 shows the swelling characteristics of the p(HEMA-*co*-NASA) (1:1) and p(HEMA-*co*-NASA–Na) (1:1) hydrogels in NaCl and urea solutions prepared at different concentrations (10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} *M*). According to Figure 7(a), the maximum swelling amount of the p(HEMA-*co*-NASA) (1:1) hydrogel in urea was 77 ± 8%. The hydrogels' maximum swelling amount did not change, despite variation in the urea concentration (10^{-4} to 10^{-1} *M*). Considering the results in the NaCl solution, which was another swelling medium, for the p(HEMA-*co*-NASA) hydrogels, the swelling ratio of the hydrogels decreased from 111 ± 9 to 70 ± 6% with the increase in the NaCl concentration. Figure 7(b) shows the swelling characterization of the p(HEMA-*co*-NASA–Na) (1:1) hydrogels, which were converted to the form of sodium salt in urea and NaCl solutions. According to this, the p(HEMA-*co*-NASA–Na) hydrogels' swelling ratios did not change, although the urea concentration changed, and they swelled at a ratio of approximately 4530 ± 310 mass %. The swelling ratios of the p(HEMA-*co*-NASA–Na) hydrogels in the NaCl solutions in different concentrations changed inversely with NaCl concentration. The reduced swelling amounts of the p(HEMA-*co*-NASA–Na) (1:1) hydrogels, depending on the changing NaCl concentrations (10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} *M*), were 4695 ± 288, 3409 ± 256, 1402 ± 111, and 472 ± 67 mass %, respectively.

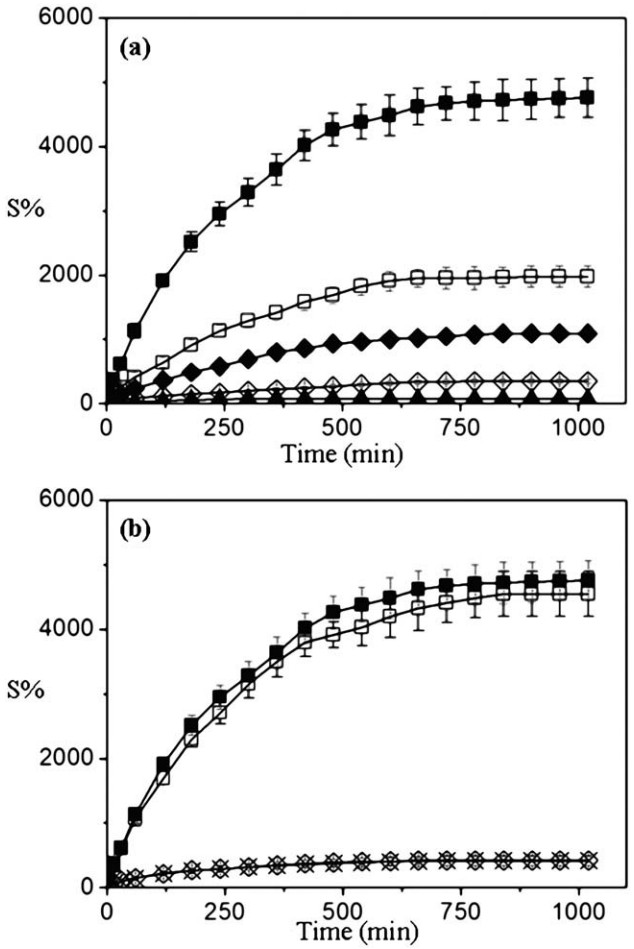

Figure 8. (a) Swelling isotherms of the hydrogels with time in distilled water [(■) p(HEMA-*co*-NASA–Na) (1:1), (□) p(HEMA-*co*-NASA–Na) (7:3), (◆) p(HEMA-*co*-NASA–Na) (8:2), (◆) p(HEMA-*co*-NASA–Na) (9:1), and (▲) p(HEMA-*co*-NASA–Na) (1:0)] and (b) swelling isotherms of the p(HEMA-*co*-NASA–Na) (1:1) hydrogel with time in (■) distilled water, (□) a urea solution (0.05*M*), (◆) PBS, and (×) an NaCl solution (0.9%).

The time period in which a hydrogel achieved *S* is very important in the absorption and release processes. According to Figure 8(a), the p(HEMA-*co*-NASA–Na) hydrogels' swelling ratios increased in direct proportion to the increase in the amount of NASA monomer that was included in the structure of the hydrogels. The hydrogels became anionic with the effect of the hydrophilic —COONa groups within the NASA monomer; these groups are converted into sodium salt. The superabsorbent p(HEMA-*co*-NASA–Na) hydrogels' swelling amounts by mass according to the molar ratios of 1:0; 9:1; 8:2; 7:3, and 1:1 were 74 ± 11, 351 ± 31, 1092 ± 93, 1978 ± 166, and 4753 ± 307% (in distilled water), respectively, because of their anionic characteristics. As shown in Figure 8(a), the p(HEMA-*co*-NASA–Na) (1:0) hydrogel achieved *S* in 240 min, whereas the p(HEMA-*co*-NASA–Na) (1:1) hydrogel achieved *S* in 840 min. Figure 8(b) shows the swelling isotherm curves for the p(HEMA-*co*-NASA–Na) (1:1) hydrogel in 0.9% NaCl (isotonic serum medium), 0.05*M* urea (the urea concentration in blood), and PBS (a drug-release solution). According to this, the p(HEMA-*co*-NASA–Na) (1:1) hydrogels achieved equilibrium in 650 min in PBS and NaCl solutions and swelled at a ratio of 419 ± 72 and 418 ± 76 mass %, respectively. On the other hand, the p(HEMA-*co*-NASA–Na) (1:1) hydrogels swelled in the 0.05*M*

Table III. Diffusion Parameters of the p(HEMA-*co*-NASA–Na) (1:1) Hydrogels in Various Swelling Media

Swelling medium	n	k	R^2
Distilled water	0.7899	0.0089	0.9993
Urea (0.05*M*)	0.7621	0.0094	0.9984
PBS	0.5643	0.0283	0.9997
NaCl (0.9%)	0.5162	0.0358	0.9992

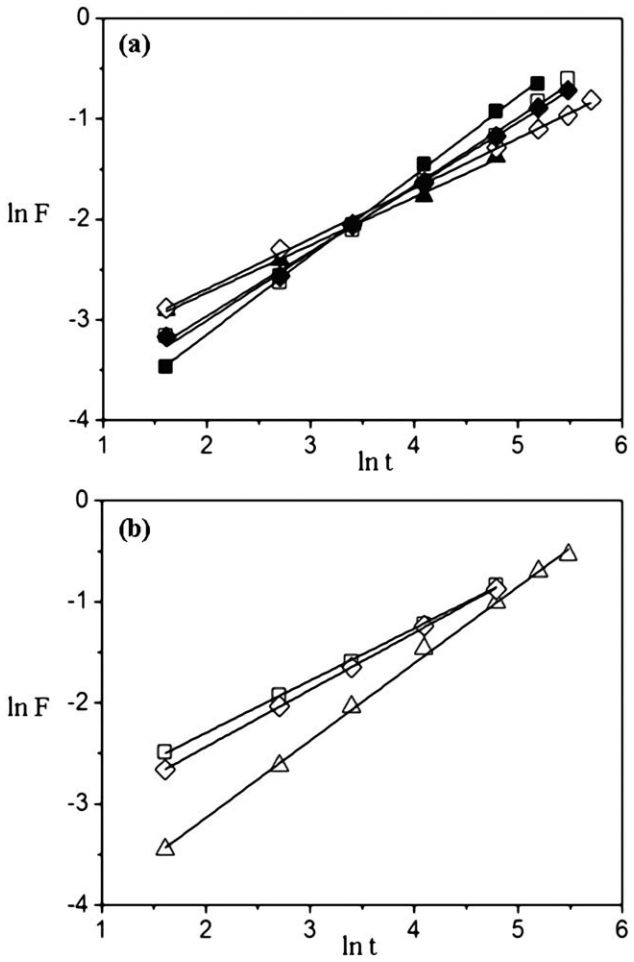

Figure 9. Plots of ln F versus ln t for (a) p(HEMA-*co*-NASA–Na) hydrogels in distilled water [(■) p(HEMA-*co*-NASA–Na) (1:1), (□) p(HEMA-*co*-NASA–Na) (7:3), (◆) p(HEMA-*co*-NASA–Na) (8:2), (◇) p(HEMA-*co*-NASA–Na) (9:1), and (▲) p(HEMA-*co*-NASA–Na) (1:0)] and (b) the p(HEMA-*co*-NASA–Na) (1:1) hydrogel in urea and NaCl solutions [(□) NaCl (0.9%), (◆) PBS, and (△) urea (0.05*M*)].

urea solution at a ratio of 4543 ± 351% and achieved equilibrium in 780 min.

With the obtained swelling values in Figure 8, we observed that the p(HEMA-*co*-NASA–Na) (1:1) hydrogel swelled approximately 65 times more than the p(HEMA-*co*-NASA) (1:0) hydrogel. It may be said that the hydrogels were synthesized successfully, even if only the swelling values are considered.

To determine the nature of the diffusion of the solutions (distilled water, PBS, NaCl, and urea) into the p(HEMA-*co*-NASA–Na) hydrogels, the following equation was used:[27–29]

$$F = W_t / W_\infty = kt^n \quad (3)$$

where F is the fractional uptake at time t; W_t and W_∞ represent the amounts of medium absorbed (NaCl, PBS, and urea) by the p(HEMA-*co*-NASA–Na) hydrogels at time t and at equilibrium, respectively; k is a diffusion constant; and n is a diffusion exponent that takes into account the mode of water transport [eq. (3) is available for the first 60% of the fractional uptake].

The swelling characteristics of the p(HEMA-*co*-NASA–Na) hydrogels were studied by analysis of the diffusion rate versus time (Figure 8). The n values of the p(HEMA-*co*-NASA–Na) hydrogels were estimated from linear curves (Figure 9). The n values depended on the geometric shape of the hydrogels (cylinder-shaped hydrogels were used in this study), which affected the diffusion mechanism. The n values given in Tables II and III represent the absorption profiles (in distilled water, PBS, NaCl, and urea) with the variation in the amount of NASA monomer in the hydrogel. These profiles were compared with the p(HEMA-*co*-NASA–Na) (1:0) hydrogel. The n values ranged from 0.4755–0.7899, and this supported the non-Fickian diffusion model, with a tendency toward macromolecular structure relaxation. In addition to the obtained k values, the coefficients of determination (R^2's) are summarized in Tables II and III. A great number of p(HEMA)-based copolymeric hydrogels have been synthesized, as discussed in the literature, and these hydrogels have been characterized in terms of their swelling properties.[30–35] The S values of the synthesized hydrogels as copolymeric with different monomers are given in Table IV.

The deswelling time of the hydrogels is as significant as their water-retention capacity. The deswelling time for the hydrogels at 37°C to be used as a controlled drug-delivery system was expected to be longer. Figure 10 shows deswelling times of the p(HEMA-*co*-NASA–Na) hydrogels, which were planned for use as drug-delivery matrices. According to this, the deswelling time for p(HEMA) was 150 min. The deswelling time got longer when the hydrogel's NASA content was increased (10, 20, 30, and 50 mol %). Thus, the deswelling times, depending on the increased NASA content, were 270, 300, 480, and 540 min, respectively.

Table IV. Maximum Equilibrium Swelling Ratios of the HEMA-Based Hydrogels in Different Media and at Different pHs

Hydrogel	Swelling ratio (g/g)	Reference
Poly(*N*-isopropylacrylamide-*co*-*N*-acryloxysuccinimide-*co*-2-hydroxyethyl methacrylate)	2.45	30
Poly(2-gydroxyethyl methacrylate-*co*-acrylic acid-*co*-sodium acrylate)	6	31
Poly[2-hydroxyethyl methacrylate-*co*-2-(vinyl-1-pyridiniumpropane sulfonate)]	12	32
Poly(*N*-isopropylacrylamide-*co*-2-hydroxyethyl methacrylate)/cellulose	10.6	33
Poly(*N*-isopropylacrylamide-*co*-*N*-hydroxymethylacrylamide-*co*-2-hydroxyethyl methacrylate)	18	34
Poly(*N*-isopropylacrylamide-*co*-acrylamide-*co*-2-hydroxyethyl methacrylate)	37	35
p(HEMA-*co*-NASA-Na)	46.5	This study

Effect of the Release Medium on the Controlled Drug Release

The p(HEMA-*co*-NASA–Na) (1:1) hydrogel was chosen for *in vitro* drug-release studies because it had the maximum swelling capacity among the hydrogels synthesized in this study. The maximum absorption amounts of p(HEMA-*co*-NASA–Na) (1:1) for vitamin B12 and R6G drugs, which were chosen as model drugs, were 11.3 and 8.6 mg/g, respectively [the same absorption amounts for the p(HEMA-*co*-NASA) (1:1) hydrogel were 7.1 and 4.9 mg/g, respectively).

In vitro release studies for the vitamin B12 and R6G drugs were carried out in three different media. These media were PBS, 0.9% NaCl (isotonic serum), and pH 1.2 (pH in stomach).[6] According to the release graph for vitamin B12 in Figure 11(a), the fastest percentage cumulative release occurred at pH 1.2 with a ratio of 96.8 ± 2.6%. Furthermore, the study of the release of vitamin B12 in the PBS medium showed that it was complete in approximately 14 h with a ratio of 95.8 ± 3.5%. We observed that efficiency in release was lower in serum medium compared to the other media (93.7 ± 4.4%), and the release was complete in approximately 15 h. Figure 11(b) shows the cumulative release amounts as a function of the time and the times for R6G. According to this, the *in vitro* release ratios for PBS, 0.9% NaCl, and pH 1.2 were 97.8 ± 1.7, 94.6 ± 3.1, and 98.9 ± 0.8%, respectively, while the release times were 22, 30, and 12 h.

CONCLUSIONS

In this study, NASA, which was planned for use as a monomer for hydrogel synthesis, was synthesized in a single step with a yield of 85%. NASA, whose characterization was carried out through FTIR spectroscopy and ^{1}H and ^{13}C-NMR spectroscopy, was used in synthesis of p(HEMA-*co*-NASA) hydrogels with redox polymerization for the first time reported in the literature.

FTIR spectroscopy, SEM, and swelling characterization of p(HEMA-*co*-NASA) hydrogels containing carboxylic acid groups were carried out. The pH-responsive p(HEMA-*co*-NASA) hydrogels had a higher (water and drug) absorption capacity when they were treated with alkalis. The p(HEMA-*co*-NASA) (1:1) hydrogels swelled in distilled water at a ratio of 217 ± 22 mass %, whereas the p(HEMA-*co*-NASA–Na) (1:1) hydrogels swelled in distilled water at a ratio of 4753 ± 307 mass %. Furthermore, the usefulness of the p(HEMA-*co*-NASA–Na) hydrogels, which could also swell in PBS, isotonic serum, and urea solutions, as controlled drug-release systems was studied. The p(HEMA-*co*-NASA–Na) hydrogels, which were produced from HEMA selected as a comonomer because of its biocompatible nature, were used for *in vitro* release studies of vitamin B12 and R6G, which were chosen as model drugs. According to the release studies carried out in three different media (PBS, 0.9% NaCl, and pH 1.2), the obtained drug-release ratio was minimum at 93.7 ± 4.4%.

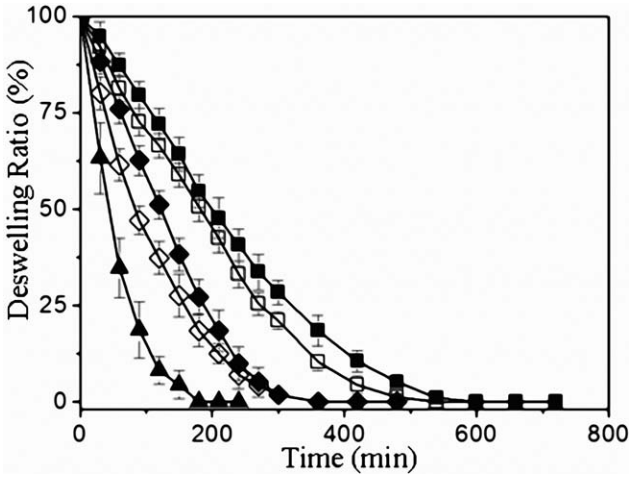

Figure 10. Deswelling kinetics of the p(HEMA-*co*-NASA–Na) hydrogels at 37°C: (■) p(HEMA-*co*-NASA–Na) (1:1), (□) p(HEMA-*co*-NASA–Na) (7:3), (◆) p(HEMA-*co*-NASA–Na) (8:2), (◇) p(HEMA-*co*-NASA–Na) (9:1), and (▲) p(HEMA-*co*-NASA–Na) (1:0).

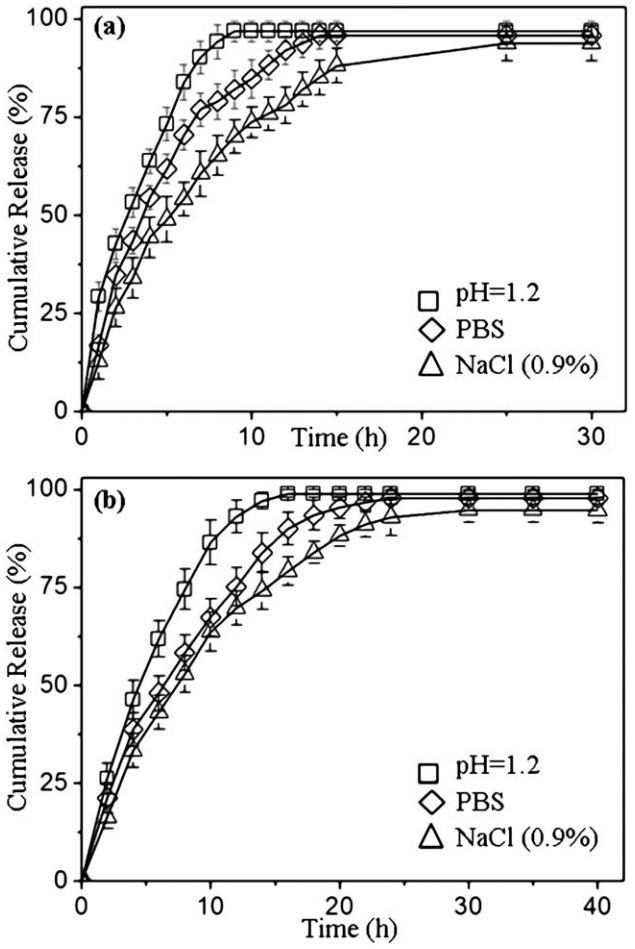

Figure 11. *In vitro* pH-responsive drug-release behavior of (a) vitamin B12 and (b) R6G with the p(HEMA-*co*-NASA–Na) (1:1) hydrogels at 37°C.

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