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Preparation of Alginate/Poly(*N*-isopropylacrylamide) Hydrogels Using Gamma-ray Irradiation Grafting

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Abstract: To graft *N*-isopropylacrylamide (NIPAAm) onto alginate, varying dosages of γ-rays were irradiated onto alginate films in deionized water and methanol media, which are non-solvents of alginate. We investigated the hydrogels graft ratio, mechanical strength, swelling kinetics and ratio, and behavior with respect to drug release. The graft yield of NIPAAm increased upon increasing the irradiation dose. The use of the aqueous solution increased the graft yield relative to that obtained in methanol. The mechanical strength of the grafted hydrogels increased after grafting with NIPAAm. In a study of the swelling kinetics, we found that all hydrogels reached an equilibrium swollen state within 3 h. The equilibrium swelling ratio of the hydrogels decreased upon increasing the irradiation dose. The swelling ratio of the hydrogels decreased dramatically between 30 and 35 °C because phase separation of NIPAAm occurred at 32 °C. The swelling process, with respect to the temperature change, was repeatable. An NIPAAm-grafted alginate containing a drug sustained its release rate until 3 h after an initial high drug release caused by a burst effect.

Keywords: alginate, gamma-ray irradiation, graft, hydrogel, *N*-isopropylacrylamide.

Introduction

Hydrogels are hydrophilic three-dimensional polymer networks capable of absorbing a large volume of water or other biological fluid within themselves. Stimuli-sensitive hydrogels have the capability to change their swelling behavior, permeability, or mechanical strength in response to external stimuli, such as small changes in pH, ionic strength, temperature and electromagnetic radiation.[1-5] Because of these useful properties, hydrogels have numerous applications and particularly used in the medical and pharmaceutical fields.[1-2,6]

In this study, alginate and *N*-isopropylacrylamide (NIPAAm) were used to prepare temperature and pH sensitive hydrogel. Alginate derived from marine algae is a linear copolymer of 1,4-linked β-D-mannuronate (M) and α-guluronate (G) residues.[7] Alginate has several unique properties and has been much used in medical applications such as wound dressing, scaffolds and drug delivery matrices.[8-9] In addition, NIPAAm gel exhibits phase separation properties in aqueous solution when temperature increases above a certain level. The phase separation temperature is referred to as the lower critical solution temperature (LCST). NIPAAm chains in water hydrated to form an expanded structure when the temperature is kept below the LCST (about 32 °C), with the NIPAAm chains forming a more compact structure by dehydrating when heated above the LCST.[10-15]

To rapidly respond to the stimuli such as pH and temperature, our previous studies designed and compared the comb-type grafted hydrogel with semi-interpenetrating polymer network (semi-IPN) hydrogels.[1,4-5] Results for the response rate of the hydrogel revealed that the comb-type hydrogels kinetically improved their response time in comparison with semi-IPN. To prepare the comb-type grafted hydrogel, the carboxylic groups of alginate main chains were reacted with amino-groups of NIPAAm, which were synthesized using a chain transfer agent.[1] However, it was hard to synthesis the NIPAAm incorporating amino-group at its chain end.

In this study, therefore, gamma-ray irradiation was applied to prepare the stimuli sensitive hydrogel because NIPAAm monomers could be easily polymerized on the alginate main chain. The various irradiation does were applied on the surface of the alginate film in two different solvents such as deionized water and methanol. The properties of hydrogels were evaluated in terms of graft yield, mechanical strength, swelling ratio at various temperatures and behavior of drug release.

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Experimental

Materials. The NIPAAm (Aldrich Chemicals Co., Milwaukee, WI, USA) used was purified by recrystallization from n-hexane/toluene (Duksan Pure Chemicals, Seoul, Korea). The sodium alginate (mannuronate/gluronate (M/G) ratio of the alginate = 1.56) was purchased from Aldrich Chemicals Co. The molecular weight distribution of the alginate was determined using gel permeation chromatography (GPC, Waters Model 510 HPLC pump, Milford, MA, USA) in water using the Millennium software program. The number-average (M_n) and weight-average (M_w) molecular weights were 339,000 and 1,073,000, respectively. The calcium chloride, methanol and ethyl ether (Duksan Pure Chemical) were used as purchased without any further purification. Indomethacin (IMC) was purchased from Sigma Chemicals (St. Louis, MO, USA). The water used in the experiments was purified using a Milli-Q Plus system (Waters, Millipore, MA, USA).

Preparation of Alginate Film. Alginate was dissolved in doubly deionized water with concentration of 5 wt%. The solution was poured into a petri-dish and dried at 60 °C vacuum oven. To cross-link the alginate film, the sample was immersed in 1 wt% aqueous $CaCl_2$ solution for one hr at room temperature and washed with deionized water three times to remove excess calcium chloride. The cross-linked film was dried at 60 °C.

Grafting of NIPAAm on the alginate film using ^{60}Co γ-ray irraidation. A cross-linked alginate film (size = 1 × 1 × 0.02 cm^3) was put in deionized water and methanol, respectively, containing 5 wt% NIPAAm monomer under N_2 atmosphere. The mixtures were mutual-irradiated by ^{60}Co γ-ray (ACEL type C-1882, Korea Atomic Energy Institute, Daejeon, Korea, irradiation dose: 5-50 kGy, dose rate: 2.15 × 10^5 rad/hr) at room temperature. After the graft reaction had completed, the grafted film was washed thoroughly with deionized water three times. The reactant film was followed by a Soxhlet extraction apparatus in methanol to extract any residuals such as unreacted monomer or homopolymer and then dried at 60 °C in a vacuum oven. Table I showed the designation of each sample prepared in various conditions such as irradiation dose, NIPAAm concentration and nitrogen content, which was measured using electron spectroscopy chemical analysis (ESCA, Thermo VG Scientific Multilab ESCA 3000) to evaluate the PNIPAAm content on the surface of the hydrogel.

The graft ratio, based on the weight change, was calculated using the following equation:

$$\text{Graft ratio (\%)} = \frac{W_g - W_i}{W_i} \times 100 \quad (1)$$

where W_i is the weight of alginate before grafting and W_g, the total weight after grafting NIPAAm onto the alginate main chain.

Mechanical Property and Swelling Behaviors. Mechanical properties of prepared alginate film were determined using a universal testing machine (UTM, INSTRON Model 4465, Canton, MA, USA) according to the American Standard Testing Methods (ASTM) D638. The tensile strength of the water-swollen state was measured five times at a constant deformation rate of 10 mm/min. The standard deviation from the mean was within ± 5%.

A swelling experiment was performed on the alginate-g-NIPAAm hydrogels to observe the behavior as functions of temperature in an aqueous solution. The swelling ratio was calculated using the following formula:

$$\text{Swelling ratio} = \frac{W_s - W_d}{W_d} \quad (2)$$

where W_s is the weight of hydrogel in the swollen state at

Table I. Designation of NIPAAm-grafted Alginate Hydrogels Using Various Irradiation Doses

Sample code[a]	NIPAAm concentration (wt%)	Irradiation dose (kGy)	N (%)[b]	Sample code[c]	NIPAAm concentration (wt%)	Irradiation dose (kGy)
H0-05	0	0	-	M0-00	0	0
H0-05	0	5	-	M0-10	0	5
H0-10	0	10	-	M0-10	0	10
H0-20	0	20	-	M0-20	0	20
H5-05	5	5	4.05 ± 0.52	M5-05	5	5
H5-10	5	10	4.12 ± 0.45	M5-10	5	10
H5-20	5	20	5.81 ± 0.71	M5-20	5	20
H5-30	5	30	8.12 ± 0.25	M5-30	5	30
H5-50	5	50	9.45 ± 0.36	M5-50	5	50

[a]Solvent: water, [b]Nitrogen atom content on the surface of hydrogels was measured using ESCA (Thermo VG Scientific Multilab ESCA 3000). [c]Solvent: methanol

a particular temperature and W_d, the dry weight of the hydrogel. To measure the swelling ratio, pre-weighed dry samples were immersed in deionized water (pH = 5.4). After wiping off the excessive water on the surface of samples, the weight of the swollen samples was measured in the temperature ranging from 20 to 50 °C. The swelling ratio was monitored until there was no further change of weight in the solution.

Drug release behaviors. IMC (1-[*p*-chlorobenzoyl]-5-methnowl-2-methylindole-3-acetic acid) was used and loaded as a model drug in the hydrogel. Swelling-loaded technique was used to load the drug into dried hydrogels. An appropriate amount of IMC was saturated with ethyl alcohol and stirred to dissolve at room temperature. Each sample (size = 1 × 1 × 0.02 cm^3) was soaked into aqueous drug solution for two days at 25 °C. The film containing drug solution was blotted with filter paper to eliminate the surface water and dried at room temperature.

Drug-released behaviors were evaluated in deionized water under gentle stirring. The amount of released drug was periodically analyzed by using a UV spectrophotometer (Shimatzu, Model UV-2101PC, Koyto, Japan). The UV absorbance of IMC was measured at λ_{max} = 320 nm. Solutions with known concentrations of IMC in deionized water were used to calibrate and to obtain a quantitative curve equation, which was C = 0.00288 A + (-1.61167 × 10^{-4}), where *A* is absorbance and adequately describes the increment in IMC concentration from 0.32 to 15.23 mg/mL actual dose.

Statistical Analysis. The data were analyzed by ANOVA using SAS (Release 6.12, SAS Institute Inc., Cary, NC, USA) and differences among mean values were processed by Duncan's multiple range tests. Values of $p < 0.05$ were statistically considered.

Results and Discussion

Graft Ratio of the Hydrogels. Irradiation technique was widely used in biomaterials science for surface modification and sterilization to improve their properties.[16-18] The exposure of a polymer to radiation can lead to chain graft without catalyst or additives because absorbed energy into the main chain of polymer initiated a free radical process. In particular, carboxylic acids in alginate generated high yields of CO_2 by exposed γ-ray and produced free radicals.[19] The generated radicals on the surface of alginate film could react with NIPAAm monomers dissolved in water or methanol.

Figure 1 shows the graft ratios of the hydrogels under various media and irradiation doses. The graft ratio of hydrogels in water increased with the radiation dose. At 50 kGy of irradiation dose, NIPAAm monomers were grafted on the alginate with graft ratio of 18.7%. On the other hand, graft ratio in methanol medium was lower than that in deionized water and did not increase with irradiation dose. Table I shows the surface elemental analysis using ESCA, indicating

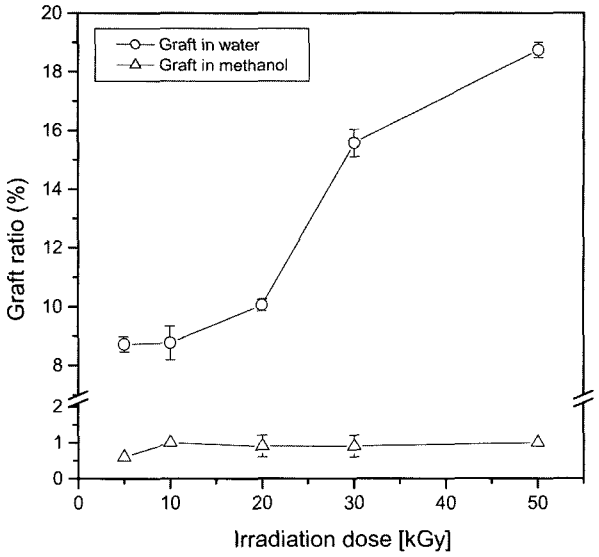

Figure 1. Graft ratios of the hydrogels under various irradiation doses in deionized water and methanol.

that the presence of nitrogen atom on the surface of the hydrogel could confirm the graft of NIPAAm on the alginate. The results for ESCA revealed that the nitrogen content increased with the irradiation dose and water rather than methanol improved the graft yield.

Graft yield is dependent on irradiation conditions, such as radiation dose, dose rate, reaction temperature, solvent, monomer concentration and proton concentration. It has already proved that the grafting percentage increased with irradiation dose.[17,18,20] In addition, the differences of graft ratio in solvents might be closely related with the swelling properties of hydrogels in the respective solvent. The graft yield at the same irradiation dose was improved in the deionized water because the film in deionized water exhibited high surface area due to the higher swelling ratio in comparison with methanol, which was a non-solvent to alginate. In addition, the graft yield in the methanol might be restricted because the radicals formed inside of the alginate film at the high irradiation dose could not encounter with the sufficient amounts of NIPAAm monomer due to the low swelling ratio of the alginate film in the methanol.

Mechanical Strength of the Hydrogels. Alginate is a typical degradable material under comparatively high dose of irradiation due to the scission of glycoside bonds by irradiation energy.[17] The generated radicals induced the main-chain scissions, resulting in the reduction of its mechanical strength.[21-24]

The degradation was investigated in terms of mechanical strength of grafted hydrogels. Figure 2 shows the mechanical strength of grafted hydrogels swollen in deionized water. Tensile strength was about 2.8 MPa at initial state and improved after irradiation. Thus, the applied irradiation dosage

did not negatively affect on the mechanical strength of grafted hydrogels meaning that the grafted NIPAAm chain might compensate the reduced mechanical strength of alginate main chain. In particular, the mechanical strength of swollen hydrogels was also affected on the swelling ratio of the hydrogel. The mechanical strengths of H5-10 and H5-20 were improved as the increase of irradiation dose due to the increased graft yield of PNIPAAm (see Figure 1) and the decreased equilibrium swelling ratio in comparison with the same without irradiation (see sample H0-00 in Figure 3). The sample H5-30 showed the enhanced mechanical strength in comparison with H5-10 and H5-20 because of higher graft yield and lower swelling ratio.

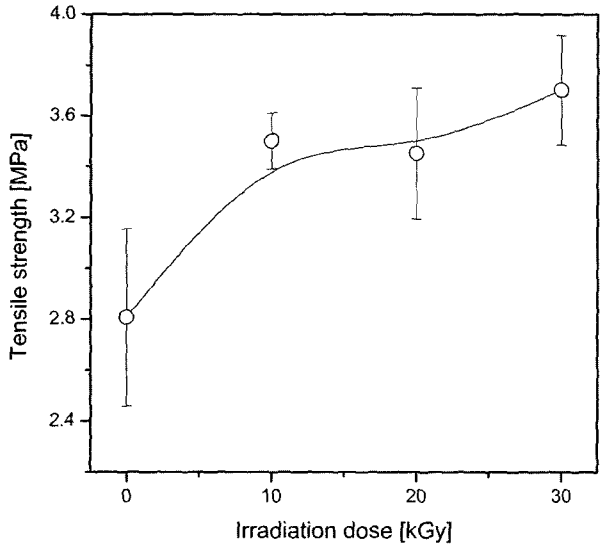

Figure 2. Mechanical strength of the grafted hydrogels swollen in deionized water (ASTM D638, deformation rate of 10 mm/min).

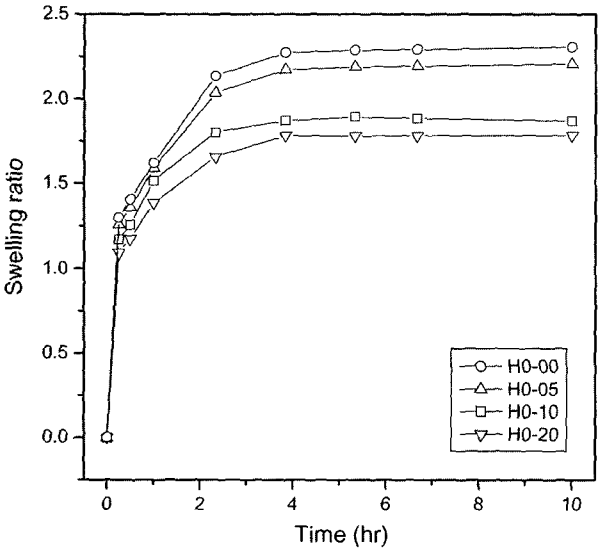

Figure 3. Swelling kinetics of the alginate films which were treated at various irradiation doses in the deionized water (25 °C and pH = 5.4)

Swelling Kinetics and Ratio. Figures 3 and 4 show the swelling kinetics of alginate-g-NIPAAm in water and pH = 5.4. In the swelling kinetics, both original and NIPAAm-grafted hydrogels reached an equilibrium after about 3 hrs.

The equilibrium swelling ratio of the hydrogels without NIPAAm decreased with the increasing irradiation dose (see Figure 3), because the number of carboxylic group also decreased with increasing irradiation dose. The radicals generated during the irradiation transformed the carboxylic groups, which could interact with water, into CO_2 gas.

Figure 4(a) shows the swelling behaviors of PNIPAAm-

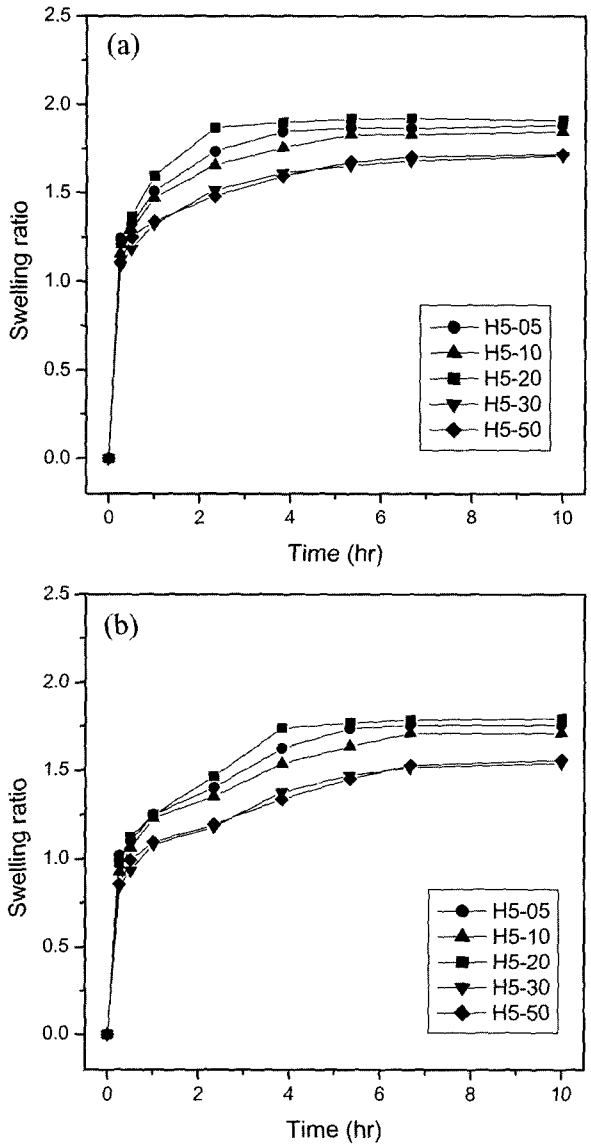

Figure 4. Swelling kinetics of alginate-g-NIPAAm hydrogels in deionized water (pH = 5.4). (a) 25 °C and (b) 37 °C.

grafted hydrogels at 25 °C. The swelling ratio of the H5-20 was the highest among the grafted hydrogel due to the high graft yield of PNIPAAm and relatively proper irradiation dose. If only graft yield was considered on the swelling ratio, the swelling of H5-30 and H5-50 should be higher than that of H5-20. However, despite of high graft ratio as shown in Figure 1, the swelling ratios of H5-30 and H5-50 were lower than other hydrogels because the very high irradiation dose might induce not only the graft reaction with NIPAAm but also the excess carboxylic group deformation, which decreased the swelling ratio of the hydrogel.[19] In addition, an increase of swelling ratio caused by free volume of graft chain was not expected because NIPAAm chains were mainly grafted on the surface of hydrogels. Figure 4(b) shows the swelling behaviors at 37 °C. The equilibrium swelling ratio at 37 °C decreased and swelling rate of the hydrogel was slower than those of 25 °C due to the phase-separated PNIPAAm on the surface of hydrogel.

Temperature-dependant Swelling Behavior. Figure 5 shows the temperature-dependant swelling behavior of the hydrogels, when the temperature of the aqueous media increases from 20 to 45 °C. The swelling ratio of hydrogels decreased between 30 and 35 °C due to the phase separation of NIPAAm at 32 °C. Temperature sensitivity of NIPAAm was due to the dissociation of ordered water molecules surrounding hydrophobic N-isopropyl groups in NIPAAm. As a result, the grafted hydrogels composed of alginate and NIPAAm underwent a volume phase transition in water at around the LCST of PNIPAAm, because PNIPAAm chains hydrate to form expanded structures in water when the solution temperature is below its LCST but becomes compact structure by dehydration when heated up above the LCST.

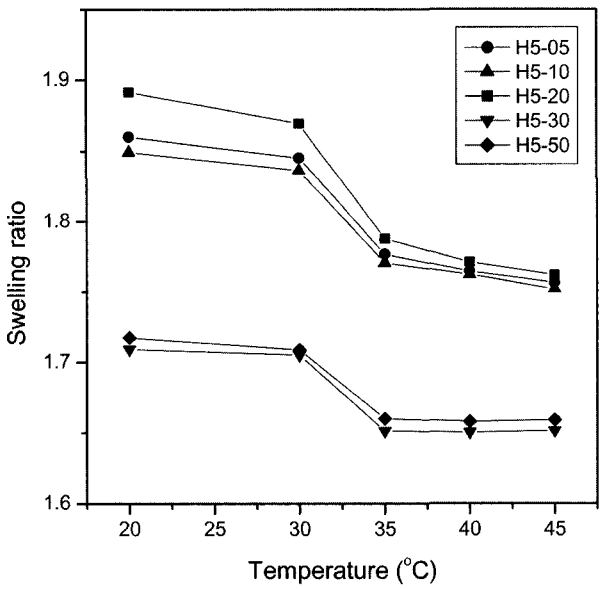

Figure 5. Swelling ratios of alginate-g-NIPAAm hydrogels in deionized water (pH = 5.4) as a function of temperature.

Pulsatile Stimuli-response Swelling Behavior. A step-wise swelling behavior was observed in temperatures alternating between 25 and 40 °C, as shown in Figure 6. The pulsatile swelling periods were 4 hrs because the swelling ratio of hydrogels reached an equilibrium swollen state within 3 hrs. The swelling process proved to be repeatable, in accordance with the temperature changes. The NIPAAm grafted hydrogels responded to temperature change, whereas the swelling ratio of the alginate alone was not changed during the swelling and deswelling processes due to the absence of NIPAAm. However, the swelling ratio of grafted hydrogels was slightly changed with the pulsatile temperature changes due to the low graft yield of NIPAAm onto the alginate.

IMC Release from the Prepared Hydrogels. IMC release was performed in deionized water at 25 °C by UV-spectro-

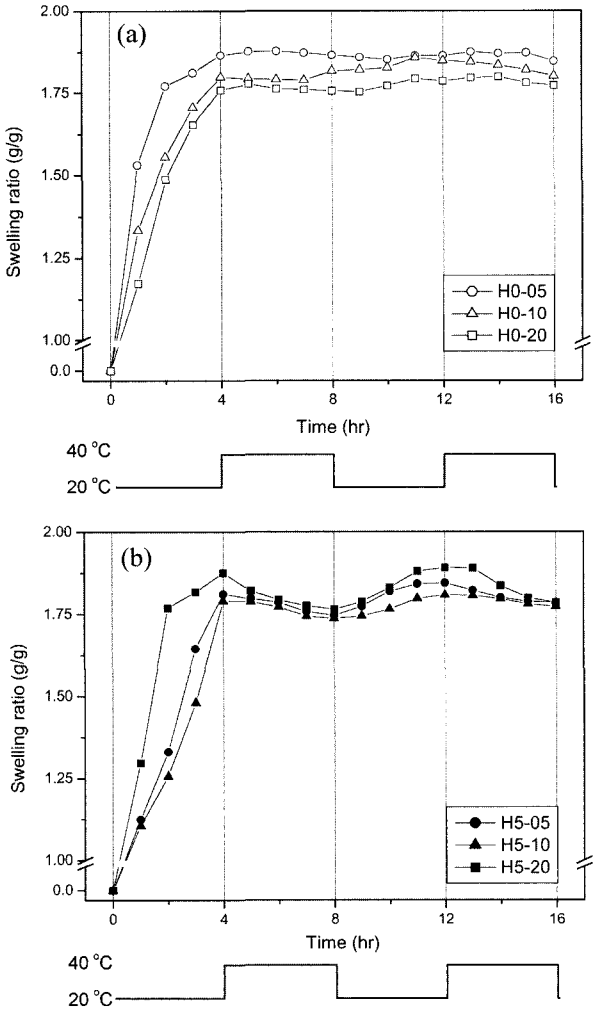

Figure 6. Pulsatile swelling ratio of alginate-g-NIPAAm hydrogels in deionized water (pH = 5.4) in response to the temperature changes between 25 and 40 °C: (a) not grafted hydrogels and (b) NIPAAm-grafted hydrogels.

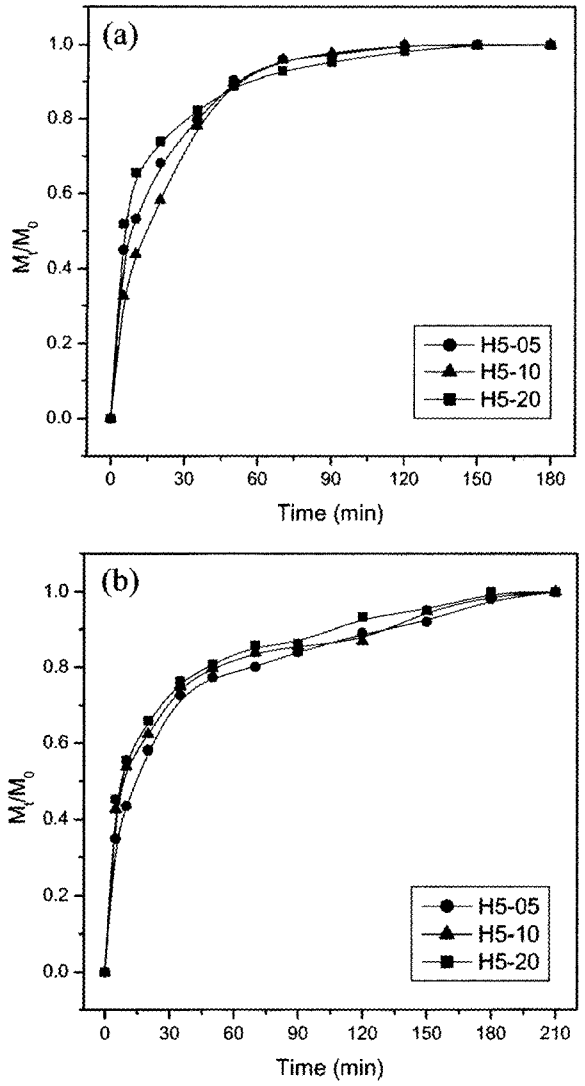

Figure 7. Drug release behaviors of alginate-g-NIPAAm hydrogels containing IMC in deionized water (pH = 5.4) at (a) 25 °C and (b) 37 °C. M_t: release amount of each time and M_o: release amount of drug at equilibrium state.

photometer. Figure 7(a) showed the profiles of IMC released from NIPAAm-grafted hydrogels in deionized water at 25 °C. It took about 3 hrs to reach at 80% of equilibrium release amount. An initial burst effect was shown in all samples. The H5-20 hydrogel showed the fast release of drug until 15 min and sustained release rate until 3 hrs, whereas drug-release behaviors of H5-05 and H5-10 exhibited relatively low amount during the initial burst period but fast release rate. Figure 7(b) showed the release profiles of IMC at 37 °C, which is above the LCST. All samples at 37 °C relatively sustained release rate rather than at 25 °C because the grafted PNIPAAm formed the barrier on the surface of the hydrogel due to the phase-separation of PNIPAAm above the LCST. The phase-separated PNIPAAm retarded the release of IMC from the hydrogel.

Conclusions

The hydrogels composed of NIPAAm and alginate were prepared using γ-ray radiation graft technique. The graft ratio increased with irradiation dose and improved in deionized water in comparison with methanol. The applied irradiation dosage did not negatively affect on the mechanical strength of grafted hydrogels which in turn increased with grafting NIPAAm. All hydrogels reached an equilibrium swollen state within three hours. The equilibrium swelling ratio of hydrogels decreased with the increasing irradiation dose because of the reduction in the number of carboxylic groups. The swelling ratio of hydrogels decreased between 30 and 35 °C due to the phase separation of NIPAAm at 32 °C. The swelling process proved to be repeatable, in accordance with the temperature changes. In the drug release, it took about 3 hrs to reach at 80% of equilibrium release amount at 25 °C, whereas the hydrogel at 37 °C relatively sustained release rate due to the phase-separation of PNIPAAm above the LCST.

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