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Radiation preparation of drug carriers for dexamethasone and tegafur based on poly(n-isopropylacrylamide) hydrogels

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Abstract: Poly(N-isopropylacrylamide) (PNIPAM) based hydrogels with the lower critical solution temperature (LCST) near the human body-temperature have been obtained from 10% solutions of N-isopropylacrylamide (NIPA) and N,N'-dimethyl acrylamide(DMA) mixture of 90:10 and 85:15 (w/w) by radiation copolymerization and crosslinking using a gamma Co-60 source at a dose of 20 kGy. Water swelling behaviour of the resulting hydrogels was much dependent on the initial ratio of NIPA and DMA. The hydrogels of 85:15 NIPA/DMA was chosen for further investigation for the use as drug cariers.Two kinds of drug carriers were prepared by immerging the hydrogels in solutions containing dexamethasone and tegafur. Then the drug incorporation efficiencies and *in-vitro* release behaviors of the ingredient were analysed. Loading capacities of the hydrogels were about 48.6 and 95.7 mg per g of dried gel for dexamethasone and tegafur, respectively. The results also revealed that the presence of ions in simulated body fluid and solution temperature much affected to the release behaviors of hydrogels for both dexamethasone and tegafur. Release rates of the ingredients were quite fast for both drug models. These drug-loaded hydrogels were biocompatibility without skin irritation suggesting that they may be used as controlled release drug carriers.

Keywords: *poly (N-isopropylacrylamide); hydrogel; dexamethasone; tegafur; loading capacity; drug release*

I. INTRODUCTION

Hydrogel is a network of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining their structure. In practice, high swelling rate is an important property of hydrogels. In this case, a three dimensional network is formed by chemical or physical crosslinking between the polymer chainsunder the hydrogen boding, van der Waals interactions, or physical entanglement. Hydrogels have been intensively studied and applied for development of smart drug carriers, where the hydrogels can protect the drug from hostile environments. They can also be applied as carriers for controlled release drugs by changing the gel structure in response to environmental stimuli, and intended to deliver the drugs with predetermined rates during the certain periods of time. This characteristic has been used to overcome the drawbacks of conventional drugs [1-4]. The hydrogels containing such "sensor" properties can undergo reversible volume phase transitions or gel-sol phase transition upon only minute changes in the environmental condition. Therefore, the stimuli-sensitive hydrogels are ideal candidates for developing drug delivery systems [5]. And temperature-responsive hydrogels are probably the most popular class that was studied and applied as the drug

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carriers. Among that, poly (N-isopropylacrylamide) (PNIPAAm) is the most typical thermo-sensitive hydrogels with lower critical solution temperature (LCST) of about 32°C. Below this temperature, the hydrogel is hydrated and becomes hydrophilic, whereas it would shrink to become a collapsed, dehydrated and hydrophobic form when temperature was above the LCST. This is due to the breakdown of hydrophilic/hydrophobic balance in the network structure [6]. Fortunately, the LCST can be changed by adjusting the ratio of the hydrophilic and hydrophobic segment of the polymer. The most popular way is to synthesize copolymers of hydrophobic (PNIPAAm) and hydrophilic (DMA) monomers with LCST closed to the human body temperature [7-9].

Several techniques have been reported on the synthesis of the hydrogels. The crosslinking network can be formed during radical polymerization or copolymerization of monomers, which act as crosslinking agents. While the chemical polymerization usually requires the presence of additives, which may contaminate the products. Those initiators and catalysts do not require in case of radiation-induced polymerization. During irradiation, radicals produce on the polymer chains by the homolytic scission, such as C-H bonds, even C-C backbones. On the other hand, radiolysis of water molecules generates hydroxyl radicals which can attack polymer chains also resulting in the formation of macro-radicals and then covalent bonds within crosslinked structure [10].

PNIPAAm can be synthesized from NIPA by radiation polymerization in both solid state and solution [10]. In aqueous solution, PNIPAAm can easily absorb water to become hydrogels. And the properties of obtained PNIPAM hydrogels can be modified by adjusting monomer concentrations and irradiation conditions [11]. Copolymerization of NIPA and other monomers results in the copolymers with more versatile properties, such as faster responsibility when heating and thermo-sensitive hydrogels with additional stimuli. In our previous studies, some PNIPAAm based hydrogels have been prepared in aqueous solution by gamma irradiation [8,12].

To be a glucocorticosteroid with anti-inflammatory and immuno-suppressant effects, dexamethasone (DEX) has been recently applied to treat some cancer, but its conventional prolonged administration usually causes the undesired side effects [13].

Tegafur is a chemotherapeutic fluorouracil prodrug used in the treatment of cancers that can transfer to 5-fluorouracil during metabolism. The major side effects of tegafur are similar to fluorouracil and include myelo-suppression, central neurotoxicity and gastrointestinal toxicity, which is the dose-limiting side effect of tegafur. Therefore, the use of these prodrugs should be controlled in order to reduce their side effects.

In this study, poly(PNIPAAm-co-DMA) copolymerhydrogelswere obtained from the NIPA/DMA solutions by gamma irradiation at 20 kGy as indicated in our previous studies [7]. The swelling degrees of the resulting hydrogelswere investigatedwith NIPA/DMA ratios in distilled water at room temperature. Their loading capacities and release behaviors ofdexamethasone and tegafuras were determined in distilled water and simulated body fluid using UV-Vis spectroscopy.The influence of the solution temperature on the release rate of the products was also investigated with time at 25, 37 and 40ºC under constant shaking rate. *In vitro* skin irritation test for these drug hydrogels were determined with OECD (TG 404) at National Institute of Drug Quality Control.

II. EXPERIMENTS

A. Materials

N-isoprpylacrylamide 98% (NIPA), N,N-dimethylacrylamide (DMA) monomers were purchased from Wako Pure Chemical Inc., Japan. Nitrogen gas at industrial-grade purity was obtained from DucGiang Chemical company, Vietnam. Other chemicals for preparation of artificial body fluid were bought from Merck, Germany. Dexamethasone sodium phosphate and tegafur, chemotherapy prodrugs used in anti-cancer treatments were obtained from the national institute of drug quality control. All chemicals were used as received.

B. Sample preparation and irradiation

The aquoeus solutions of 10% NIPA/DMA monomer mixture with predetermined weight ratios of 90:10 and 85:15 were divided into clean glass ampoules of 5 mL, degassed by bubbling with nitrogen gas for 10 min, sealed off for sampling. Each sample was irradiated at 20 kGy under gamma ray at Hanoi Irradiation Center as described in our previous studies [8]. Absorbed dose was measured with ECB dosimeters attached to the samples. After irradiation, the obtained P(NIPAAm-co-DMA) hydrogels were collected and cut into 1 cm length. The hydrogel disks were immerged in excess distilled water at 60°C for a week to remove non-crosslinked polymers/monomers, dried at room temperature overnight, then under vacuum.

C. Measurements

Swelling degree of hydrogel

Dried hydrogel disks were immersed in distilled water in glass beaker, which were put in water bath at room temperature (25 ± 0.2°C), for 72 h to reach the equilibrium of swelling. After that, the swollen hydrogel was taken, blotted out the excess water using paper tissue, and then weighed directly. Water swelling ratios of hydrogels were calculated as follow:

$$Water\ swelling\ ratio\ (g/g) = \frac{m_s - m_0}{m_0} \times 100$$

where m_0 and m_s are the weights of initial and swollen hydrogel. In this experiment, each value was calculated as an average of three separate measurements.

Drug loading and control release test

The resulting P(NIPAAm-co-DMA) hydrogels were loaded with dexamethasone or tegafur by solution loading method [14].In which, a certain amount of dried hydrogel (W_g) was equilibrated in an aqueous solution containing 5000 ppm of the drug at room temperature [15]. After 72 h incubation, the drug-loaded hydrogel (carrier) was taken out, washed with cold water. The optical absorbance of the solution with remaining drug was determined by a Shimadzu UV-2450 spectrophotometer. The drug concentration was extrapolated using the calibration curve corresponding to each drug. The loading drug content and loading capacity were determined by difference of the initial or administered dose (W_{Di}) and the remaining (W_{Da}) drug or residual dose in the drug solution (%) as following equations:

$$Loading\ drug\ content\ (mg/g\ dried\ hydrogel) = \frac{W_{Di} - W_{Da}}{W_g} \quad (2)$$

$$Loading\ capacity\ (\%) = \frac{W_{Di} - W_{Da}}{W_{Di}} \times 100 \quad (3)$$

About 50 mg drug carriers were transferred in a glass beaker containing 200 mL of distilled water or simulated body fluid for the *in vitro* release behavior studies. All beakers were put in shaker bath that keep at a certain

temperature. After predetermined time periods, aliquots of 3 mL were withdrawn and their optical absorbance values were measured by UV spectrometer at 241.5 and 271 nm for dexamethasone and tegafur, respectively. The drug release was reported as percentage release as follow:

$$\%\ drug\ release\ at\ t\ time = \frac{D_{Rt}}{D_{total}} \times 100 \quad (4)$$

where D_{Rt} and D_{total} are the weight of released drug at time t and total drug loaded in the drug carries.

In vitro skin irritation test

Biocompatibility of the drug carriers were analyzed by the *in vitro* skin irritation test, followed OECD standard test guideline TG 404 of the National Institute of drug quality control. For this, the drug loaded hydrogels is applied in a single dose to a small area of skin (~ 6 cm^2) of an experimental animal (rat), untreated skin area of the test animal serve as the control.

kGy was chosen for radiation preparation of the hydrogels. But the initial ratio of monomers also affected to their morphology as observed in the Figure (1c & d). This is due to the mobility of network increased with the increasing of hydrophilic constituent of DMA domain in the hydrogels [15].

B. Swelling behavior

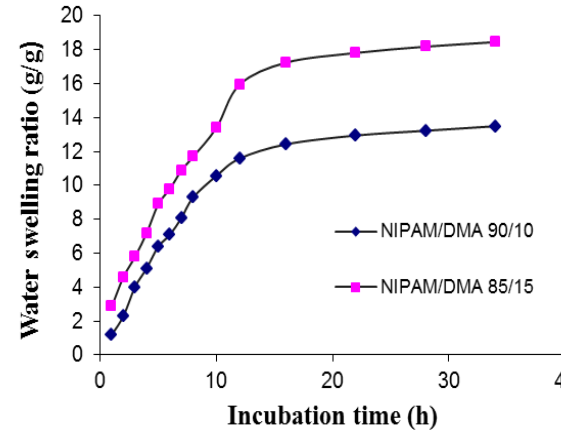

Fig. 2. Swelling behavior of P(NIPAAm-co-DMA) hydrogels

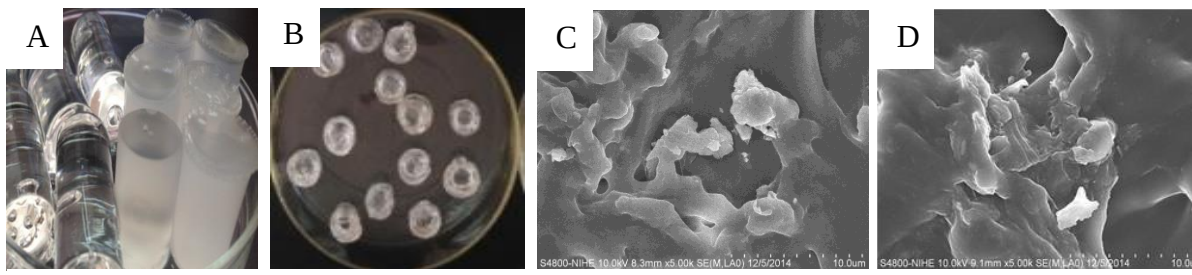

Fig. 1. Resulting hydrogels from the irradiated solutions at swollen (A); dried state (B); and SEM images of hydrogels from 90:10 (C) and 85:15 NIPA/DMA by weight ratio (D)

III. RESULTS AND DISCUSSIONS

A. Radiation preparation of the P(NIPAAm-co-DMA) hydrogels

Figure 1 showed an example of P(NIPAAm-co-DMA) hydrogels obtained from the solutions of 90:10 and 85:15 of NIPA/DMA (w/w) by gamma irradiation. As described in our previous study, the radiation dose of 20

Water swelling behaviors of P(NIPAAm-co-DMA) hydrogels were investigated at room temperature. As presented in Figure 2, swelling degrees of all hydrogels increased with incubation time, reach to an equilibrium swelling values of about 13 and 18 g/g for the hydrogels obtained from NIPA/DMA of 90:10 and 85:15, respectively. These swelling ratios are rather high suggested that the hydrogels can

also absorb larger amount of drug or further be modified to become effective controlled release carriers. The swelling degree of hydrogel also increased with DMA, a hydrophilic constituent in the hydrogel as mentioned in our last study [11]. This may be due to a higher water content swollen by hydrophilic interaction of DMA with water.

C. Formation of a drug carrier and drug loading

Table I. Uptake and loading capacity of desamethasone by the hydrogel

Sample	Dried hydrogel mass (g)	Drug uptake calculated from swelling (mg)	Drug uptake measured by UV (mg)	Loading drug content (mg/g dried gel)	Loading capacity(%)
1	0.210±0.002	11.64 ±0.26	10.56±0.25	50.53±0.98	72.56±0.32
2	0.210±0.001	10.74±0.14	9.65±0.16	47.08±0.57	71.89±0.32
3	0.210±0.002	10.97±0.07	9.97 ±0.05	48.14±0.22	72.65±0.23
Average	0.210±0.016	11.12 ±0.16	10.06 ±0.52	48.59±0.59	72.36±0.29

In order to prepare a drug carrier, the hydrogels were incubated in a drug solution. As presented in Table I and Table II, total drug loading in practice are lower than that calculated from the initial and remaining weight of the drug in the solution. Loading capacity were 99.4 and 88.6 % for dexamethasone and tegafur, respectively.

Table II. Uptake and loading capacity of tegafur by the hydrogel

Sample	Dried hydrogel mass (g)	Drug uptake calculated from swelling (mg)	Drug uptake measured by UV (mg)	Loading drug content(mg/g dried gel)	Loading capacity (%)
1	0.210±0.003	23.63 ±0.12	20.86 ±0.13	95.56±0.59	97.45±0.39
2	0.220±0.005	24.07 ±0.12	21.27 ±0.06	94.79 ±0.74	97.43±0.43
3	0.210±0.002	23.77±0.03	21.26±0.07	96.94 ±0.60	98.06±0.42
Average	0.210±0.038	23.82 ±0.09	21.16 ±0.09	95.75 ±0.64	97.60 ±0.41

Maximum amounts of the loaded drugs were 48.6 and 95.7 mg/g dried hydrogels for dexamethasone and tegafur, respectively. In the same conditions, tegafur showed higher loading capacity. The amount of incorporated drug and interaction type of drug and hydrogels much depend on the structures and properties of both drug and hydrogel. These results may be explained by the higher water solubility of tegafur. However, further studies should be carried out to estimate their applicability in practice.

D. In vitro drug release

The drug release from the hydrogel was measured with incubation time, and Figure 3 showed the cumulative release of drug as functions of time at 25, 37 and 40°C. It was observed that the release rates were quite fast for both drug models, and most drugs released

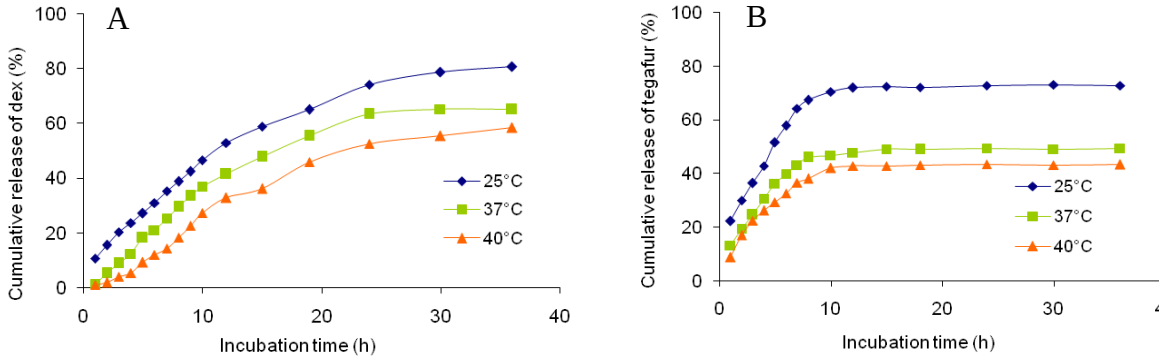

Fig. 3. Cumulative release of dexamethasone (A) and tegafur (B) from P(NIAAm-co-DMA) hydrogels in simulated body fluid at various temperatures

from the hydrogels after first 24 h. After that, the drug seemed not release any more for tegafur or released at very slow rate for dexamethasone. At the same temperature, dexamethasone gradually released at first 12 h, slower released after 24 h, and then equilibrated after 36 h, whereas tegafur quickly release during first 12 h, then reach to equilibrium state. The release of drug through hydrogels may cause by diffusion of the drug trapped on hydrogels, morphological, structural and characteristic changes in hydrogel, degradation of polymer etc. However, it is very difficult to predict the drug release, because it was governed by some parameters such as nature of drug, degradability of polymer hydrogels, polymer-drug interaction.

Figure 3 also revealed that the content of released drugs reduced with increasing of the solution temperature. This is due to the hydrogels shrinking when heated through their LCST, when the drug diffused from inside to surface, but do not released from the hydrogels. As the result, the drug release reduced by heating. There is a large amount of drug still remained in the hydrogels after 36 h immersed in the simulated human body fluid. This may due to a fact that both drug models are more or less hydrophilic, and the hydrophilic interaction prevent the drug release from the hydrogels.

3.5. *Biocompatibility*

In vitro skin irritation test showed no any skin irritation can be observed in the tested animals during investigation period, suggesting that the dexamethasone and tegafur loaded P(NIPAAm-co-DMA) hydrogels can be applied as the drug carriers for further experiments.

IV. CONCLUSION

Two kinds of P(NIAPAm-co-DMA) hydrogels were prepared by simultaneously gamma radiation copolymerization and crosslinking. The resulting hydrogels were thermo-sensitive with swelling behavior that are suitable to use as drug carriers and their equilibrium swelling degree increased with the increase of the hydrophilic segments of DMA in the initial solution.

The drug models were easily incorporated into the hydrogels followed solution loading method by the same loading process. The drug-loaded hydrogels can be applied as drug carriers and their loading capacities were 48.6 and 94.7 mg/g (w/w) dried hydrogel for dexamethasone and tegafur, respectively.

In vitro release test showed that drug release from the carriers of dried P(NIPA-co-DMA) hydrogels were quite fast, and the

release of tegafur was faster than that of dexamethasone in distilled water as well as simulated human body fluid. The results also revealed that the drug release rate was dependent on temperature. There are no any skin irritation caused by hydrogels observed for the product, meant that the hydrogels can be applied as subcutaneous or hypodermic drug carriers.

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