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# A pH/Thermo-responsive Injectable Hydrogel System Based on Poly(*N*-acryloylglycine) as a Drug Carrier

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# A B S T R A C T

novel physically cross-linked, injectable poly(N-acryloylglycine) (PNAG) hydrogel was synthesized based on glycine. The molecular structures of the corresponding monomer and polymer have been confirmed by FTIR and <sup>1</sup>H NMR measurements. The sol-gel transition behaviours of PNAG in its aqueous solution were evaluated using test tube-inversion method, as a function of PNAG concentration, pH and ionic strength. PNAG showed a remarkable injectability originating from the H-bonding interaction among amide and carboxyl groups in PNAG chains and water molecules. The swelling behaviours of hydrogel PNAG were systematically investigated at different temperatures and PNAG concentrations. It was found that the PNAG hydrogel demonstrates distinct temperature responsive nature. Using caffeine as a model drug, in vitro drug release behaviour showed that the release rate of caffeine from PNAG hydrogel apparently dropped as PNAG concentration of the system was increased, while the temperature and pH decreased. The amount of caffeine released from PNAG hydrogel, within 120 min, was 69% at room temperature, whereas 88% caffeine was diffused into the medium at 37°C. In addition, PNAG has shown a certain degree of degradability at pH 2.2 of phosphate buffered saline. In this respect, PNAG hydrogel seems to become a promising injectable material in the biomedical field due to its injectability and degradability potentials.

# Key Words:

injectable hydrogel; drug release; poly(*N*-acryloylglycine); physical cross-linked; pH/thermo-responsive.

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# INTRODUCTION

Injectable hydrogels are polymerwater systems which are capable to transform from liquid state to a disordered solid state by formation of a network between the molecules and particles [1]. Generally speaking, the injectable hydrogels usually divide into physical and chemical types. As for the chemical hydrogels constructed by chemical bonds, the cross-linking agents are needed, and a rigorous removal of trace residues is required in some biomedical/pharmaceutical applications [2]. The physical hydrogels formed by intermolecular associations are weak, reversibly produced by Van der Waals forces, electrostatic attraction or H-bonding. Namely, they do not require introduction of an external, chemical cross-linking agent, which usually leads to rapid degradation of the entire hydrogel.

The injectable hydrogels exhibit a series of property changes in

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response to the external conditions including temperature, pH [3,4], light, magnetic field and ionic strength [5-9]. Therefore, the injectable hydrogels have been widely studied in the biomedical/ pharmaceutical applications such as controlled drug release, tissue engineering, regenerative medicine, etc.. Among these systems, pH and thermoresponsive hydrogels are most extensively investigated in the biomedical field because the two important factors can be easily controlled and are applicable both in vitro and in vivo conditions. Compared with conventional drug delivery, the introduction of some injectable materials improves the efficiency of delivery, precise dose control, reduced toxicity and patient compliance. Recently, the injectable stimulisensitive polymer systems were intensively exploited as candidate materials in drug release systems [10-14]. For example, the injectable copolyesters based on lactic acid and castor oil were synthesized by melt condensation and applied in the controlled release of tamsulosin hydrochloride [15]. Also, the injectable drug-delivery systems based on supramolecular hydrogels formed by poly(ethylene oxide)s and  $\alpha$ -cyclodextrin were investigated by Li et al. [16].

In addition, amino acid moieties have been widely introduced into polymer chains or incorporated into the expected degradable polymer materials. The harmless and biodegradable amino acid moieties might be produced during the degradation of the amino acid moiety-mediated polymer materials. For example, in order to prepare a thermo-sensitive copolymer with good biodegradability, glycine ethyl ester was used to modify PNIPAm and polyphosphazene [17]. The introduction of glycine esters to poly(organophosphazenes) chains has been performed to obtain a biodegradable and harmless product as a polymeric drug carrier [18].

To the best of our knowledge, no study has been reported on the injectability of PNAG. Our experimental results, however, have shown an injectability of PNAG under certain conditions. In this study, a new injectable and glycine based pH-sensitive poly(*N*-acryloylglycine) hydrogel was synthesized. PNAG was synthesized by free-radical polymerization. The gelling time was measured by test tube-inversion method in different PNAG concentrations, pH and at various temperatures. The determining factors influencing swelling characteristics of the PNAG hydrogel were investigated in relation to temperature and PNAG concentration. Caffeine as a model drug was entrapped inside the hydrogels and its in vitro release performance from the hydrogels was studied in detail. Moreover, the degradation behaviour was also investigated. The above stated test results confirmed that PNAG was a suitable drug carrier.

#### **EXPERIMENTAL**

#### Materials

Glycine (Gly) was purchased from Kermel Chemical Reagent Factory (Tianjin, China). Potassium persulphate (KPS) and sodium bisulphite (SBS) were supplied by Beijing Chemical Factory and Taixing Reagent Factory (Tianjin, China), respectively. Acryloyl chloride was prepared by the reaction between benzyl chloride and acrylic as described in a published report [19]. The phosphate buffered saline (PBS) with different pH values was made according to the standard method (ionic strength: 0.1 mol/kg). The model drug, caffeine, was extracted from tea leaves, and recrystallized twice with acetone and petroleum ether. All other chemicals were analytical grades and used without any further purification and treatment.

### **Instruments and Measurements**

<sup>1</sup>H NMR Spectrum was recorded on a Bruker Avance 400 (Bruker, Switzerland) with D<sub>2</sub>O as a solvent and tetramethylsilane (TMS) as an internal reference. Fourier transform infrared (FTIR) spectra of NAG and PNAG were measured with a Vector 22 FTIR spectrophotometer (Bruker, Switzerland) in KBr pellet in the range of 500-4000 cm<sup>-1</sup>. Gel permeation chromatography (Viscotek270, America) was used to determine the molecular weight of PNAG. The determined samples were dissolved in freshly filtered sodium nitrate solution (0.1 M). NaNO<sub>3</sub> solution was eluted at a rate of 1.0 mL/min and the external column temperature was kept at 35°C. The molecular weights of PNAG were calculated from polyethylene oxide standard with narrow molecular weight distribution. The amount of released caffeine was



Scheme I. Preparation of poly(N-acryloylglycine).

measured by monitoring the optical transmittance at 272 nm using a Unico UV-2000 spectrophotometer.

#### Synthesis of N-Acryloylglycine

N-Acryloylglycine (NAG) was prepared by a modified method according to the procedure described by Bentolila et al. [20]. In a flask, glycine (10.00 g, 133.3 mmol) was dissolved in NaOH solution (6 M, 50 mL). The mixture was soaked in an ice bath for 20 min. Then, acryloyl chloride (11.00 mL, 136.0 mmol) dissolved in tetrahydrofuran (25 mL) was added dropwise to the flask with vigorous stirring over 2 h. Then, the mixture was allowed stirring unceasingly at the same temperature for 1 h. Finally, the solution was acidified with 4 M HCl to pH 2, saturated with NaCl, and extracted with ethyl acetate. The organic layer was dried with anhydrous sodium sulphate overnight, filtered, concentrated under reduced pressure. An amount of 11.70 g crude product (68%) was recrystallized with ethyl acetate: diethyl ether (1:1); mp 132-132.5°C; IR (cm<sup>-1</sup>, in KBr): 3323.7 ( $v_{N-H}$ ), 1724.2 ( $v_{O-C=O}$ ), 1656.4 (ν<sub>N-C=O</sub>), 1612.4 (ν<sub>C=C</sub>), 1551.46 (γ<sub>N-H</sub>).

#### Synthesis of PNAG Hydrogel

Specified quantities of NAG and  $H_2O$  were added into a test tube with a magnetic stirring rod. KPS was added into the above solution after NAG was dissolved. Then, sodium bisulphite was adhered onto the test tube wall. The test tube was pretreated with standard cycles of evacuation with nitrogen gas to remove oxygen. Then, sodium bisulphite was washed into the mixture in the tube with agitation. A gel-like mixture was obtained after the test tube was placed at room temperature for 2 h. Then, the mixture was turned into solution when it was warmed to 50°C. The hot solution was added dropwise into the cold acetone with vigorous stirring to precipitate crude PNAG. The pure PNAG was obtained after several dissolutions and precipitations [21].

A specified quantity of PNAG and de-ionized water were added into a test tube, and were heated to 50°C with stirring to make PNAG dissolved. The PNAG hydrogel was obtained after the solution was refrigerated. Similarly, the drug-loaded PNAG hydrogel for release study was also prepared by introduction of caffeine in advance according to the procedures reported previously [22]. Caffeine was fixed at specific proportion with respect to the weight of PNAG. The synthetic scheme for PNAG is illustrated in Scheme I.

#### **Sol-gel Transition Behaviour**

The thermo-sensitive sol-gel phase transition of PNAG was studied using test tube-inversion method described by Kim et al. [23]. In this investigation, the inner diameter of the test tube was 12 mm and the volume was 12 mL. The sol and gel were defined as 'flow liquid sol' and 'non-flow solid gel' within 1 min, respectively. The final gelling time in this study was obtained from the average value of three recorded results.

#### **Swelling Studies of PNAG**

The classical gravimetric method was used to measure the swelling ratios (SR) of the hydrogels. The swelling ratio (SR) of PNAG was determined by immersing the dried hydrogel in phosphate buffer solutions (PBS) with different pH at room temperature and 37°C. The hydrogels were removed from the aqueous solution, weighed after the removal of surface water with a filter paper at a definite time

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interval. The degree of swelling, SR, is expressed as the amount of absorbed water per gram dry polymer during a definite time interval: the SR is calculated using the following equation:

$$SR = (W_s - W_d) / W_d$$

where  $W_d$  and  $W_s$  represent the weights of hydrogels before and after swelling [24].

### In Vitro Drug Release Behaviour

In a flask, the caffeine-loaded PNAG hydrogels (about 0.03 g) were immersed into 40 mL PBS (pH 7.4 or 2.2) simulated to the gastric fluid and neutral intestine. The release system was thermostated in a shaking bath at a designated temperature. At specific intervals, 3 mL sample solution was withdrawn from the release medium. The caffeine released from hydrogel was measured by the absorption at 272 nm using a UV spectrometer. In this study, 3 mL fresh release medium was added into the flask to maintain the unchanged volume. Finally, the amount of the released caffeine from PNAG hydrogel was calculated according to the standard calibration curve.

# Degradation

Separate solutions were made each by an amount of 0.1 g PNAG powder dissolved in 3 mL PBS of pH 2.2 (ionic strength: 0.1 mol/kg). All solutions were placed at the temperature of  $37^{\circ}$ C. The molecular weight of PNAG in different degradation times was determined by GPC method. In GPC measurement, polyethylene oxide was used as standard and HR-3 (NaNO<sub>3</sub>) column was selected.

# **RESULTS AND DISCUSSION**

# Synthesis and Characterization of PNAG Hydrogel

As one of the injectable hydrogels, the physical hydrogels constructed with the intermolecular association do not require introduction of an external cross-linking agent. Usually, the intermolecular association contains some weak interactions such as Van der Waals forces, electrostatic attraction or H-bonding, etc.. Therefore, the phase transition of the



**Figure 1.** Photograph of PNAG/water system at different temperatures (X: 40°C; Y: room temperature).

injectable hydrogel is reversible and the unique degradability is often observed in its application. In this study, a novel physically cross-linked glycinebased, injectable poly(*N*-acryloylglycine) (PNAG) hydrogel was synthesized. The injectability of PNAG actually originates from hydrophilic interaction (H-bonding among amide, carboxyl groups on PNAG chains and water molecules) and hydrophobic interaction (CH, CH<sub>2</sub> and CH<sub>3</sub>). As it is well established the hydrophilic/hydrophobic interaction is affected by temperature changes. PNAG aqueous solution flows freely at higher temperature, but turns into gel at lower temperature without any need for stirring. The PNAG solution in sol phase is a free-flowing injectable liquid. The sol-gel transition



Figure 2. FTIR Spectrum of PNAG.

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Figure 3. <sup>1</sup>H NMR Spectrum of PNAG (in D<sub>2</sub>O).

behaviour of PNAG is affected by many factors, including the temperature, pH and ionic strength in its aqueous solution. Figure 1 shows the photograph of PNAG/water system at different temperatures. At 40°C (X in Figure 1), PNAG in water exhibited a freely-flowing solution, although PNAG/water system turned to be a solid hydrogel phase at room temperature (Y in Figure 1).

FTIR and <sup>1</sup>H NMR measurements were used to characterize the chemical structure of PNAG. The FTIR of PNAG in Figure 2 shows, the peaks at about 3430 cm<sup>-1</sup> and 3650 cm<sup>-1</sup> which are assigned to the N-H and O-H stretching vibrations, while the bending peak related to N-H was observed at 1546 cm<sup>-1</sup>. The peaks at 1732 cm<sup>-1</sup> and 1641 cm<sup>-1</sup> were ascribed to the characteristics stretching vibrations of carbonyls from the carboxyl and amide groups, respectively. The peaks attributed to C-H stretching vibration were observed at 2850 cm<sup>-1</sup> and 3200 cm<sup>-1</sup>.

As shown in Figure 3, the peaks at 1.4-1.7 ppm and 3.9-4.1 ppm in <sup>1</sup>H NMR spectrum of PNAG are assigned to methylene protons from  $-CH_2-CH$ - and  $-NH-CH_2-C=O$ , respectively. The sharp peaks at 2.1-2.2 ppm were attributed to methine protons from  $-CH_2-CH$ -. Meanwhile, the ratio of the exact

integrated area of the hydrogen atoms among the three groups gave an indication to the expected structure of PNAG. The peak at 4.6 ppm is assigned to protons from water. It is necessary to state that the peaks due to protons from carboxyl and amide groups did not appear due to the deuteration of  $D_2O$ .

#### **Sol-gel Transition Behaviour**

Sol-gel transition phenomenon itself directly relates to many physical and chemical processes. In fact, the sol-gel phase transition is entirely due to coupling through chemical bonds between the molecules (covalent and ionic bonds) or weak interactions (hydrogen bonding, hydrophobic or hydrophilic interactions, etc.). In this work, the thermo-responsive hydrogel system was linked together by weak physical interactions. Thus the phase transition is reversible. The gelling time of PNAG in different conditions is very important for its application as an injectable material such as in situ drug-loading carrier. Before drug encapsulation and release behaviour studies, the gelling time of PNAG was evaluated by test tube inversion method.

# *Effect of PNAG Concentration and Temperature on Gelling Time*

The effect of PNAG concentration and temperature on gelling time is shown in Table 1. From the data, we can see a higher polymer concentration has resulted in shorter gelling time. When PNAG concentrations were set at 7.83, 14.04, 14.73 and 18.04%, the gelling times at 0°C were in the respective order of 32.5, 6.5, 2.5 and 1.5 min. Notably, the gelling time was shorter as the PNAG concentration was increased. At higher PNAG concentration, a more cross-linking spot was formed by H-bonding interaction among carboxyl, amide from PNAG chains and water molecules. Thus, the rate of transformation from sol to gel was much faster. In addition, at too low PNAG

PC <sub>PNAG</sub> (%w/w)	0°C (min)	9°C (min)	13°C (min)	18°C (min)	25°C (min)
7.8	32.5	90.0	272.0	-	-
14.0	6.5	15.5	44.5	186.0	-
14.7	2.5	4.0	6.0	16.5	115.0
18.0	1.5	2.5	3.5	5.5	20.5

 Table 1. Effects of PNAG concentration and temperature on gelling time.

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	Gelling time							
Concentration . (mol/L)	0°C		13°C		18°C		25°C	
	Nal (min)	NaCl (min)	Nal (min)	NaCl (min)	Nal (min)	NaCl (min)	Nal (min)	NaCl (min)
0.0	10	1.0	1.5	1.5	2.0	2.0	5.5	5.5
0.4	1.5	1.5	2.0	2.5	3.5	4.5	6.5	8.5
0.6	2.0	1.5	2.5	2.0	4.0	4.5	8.5	11.5
0.8	2.5	3.0	4.0	6.5	6.0	17.0	16.5	21.5
1.0	3.5	4.0	8.5	6.0	153.0	15.5	-	17.0

Table 2. Effects of Nal and NaCl concentrations on gelling time.

concentration (7.83%) the gelation did not occur above 18°C even after several hours.

As it is seen from Table 1, the obvious dependency of gelation time on temperature was also found when lower PNAG concentration was fixed. The gelling time of PNAG hydrogel was prolonged with an increase in temperature. In PNAG solution, the hydrophobic and hydrophilic interactions (Hbonding) dominate in intermolecular interactions involved which are influenced by the external temperature [25]. At higher temperature, the hydrophobic interaction is usually more significant than H-bonding, where PNAG/water system exhibits fluidic state. At lower temperature, the H-bonding is strengthened and it is dominant in interactive amides, carboxyls and water molecules and as a result the PNAG/water system is a solidified hydrogel state. Additionally, the molecular motion of PNAG was decreased as the temperature was lowered; thus accelerating the transition of PNAG from "gel" to "sol" phase.

# Effect of the Additive Salt on Gelling Time of PNAG

NaCl of 'salting-out' electrolyte and NaI as 'salting-in' electrolyte would both affect the sol-gel transition behaviours [26]. These effects are attributed to competing interactions among each salt, water and polymer chains. Salting-out and salting-in effects on gelling time may be explained by the impact effects of salts on the structured water around the polymer [27]. In order to investigate the effect of inorganic salts on the gelling time of PNAG, two series of NaCl and NaI (0.4-1.0 mol/L) were added into PNAG aqueous solution, and their gelling time was measured. Table 2 presents the influence of NaCl and NaI on sol-gel transition of PNAG/water system as PNAG concentration was controlled at 9.4%. In Table 2, the gelling time was prolonged by the addition of NaI, for example, when NaI concentration was fixed at 0.0, 0.4, 0.8 and 1.0% at 18°C, the gelling time of PNAG was in the respective order of 2.0, 3.5, 4.0, 6.0 and 153.0 min. For NaCl, the addition of NaCl showed a different effect as evident in this table. At low concentration, NaCl made the gelling time to be longer but it turned shorter when the concentration was much above 0.8 mol/L.

# Effect of Molecular Weight on the Gelling Time of PNAG

Table 3 presents the effect of molecular weight of PNAG on the gelling time. As the molecular weight of PNAG was increased, the gelling time was decreased. For instance, the gelling time of PNAG at  $18.0^{\circ}$ C reached 186.0, 2.0 and 2.0 min, when the molecular weights were 1.42, 1.94 and  $2.32 \times 10^5$ . Obviously, the gelling time of PNAG has shown a remarkable dependency on its molecular weight. At the same PNAG concentration and temperature, the effective cross-linking density in PNAG networks was clearly increased with increase in PNAG molecular weight, leading to more rapid transition of sol to gel.

# Swelling Studies of PNAG Hydrogel

Swelling behaviour is an important criterion in drug delivery system. Figure 4 shows the swelling behaviour of PNAG hydrogel at different PNAG concentrations (10.67% and 19.55%) and temperatures. As it is evident in Figure 4 the swelling

₩ <sub>w</sub> (×10 <sup>5</sup> )	0°C (min)	13°C (min)	18°C (min)	25°C (min)
1.42	6.5	44.5	186.0	-
2.32	1.0	1.5	2.0	8.0 5.5

Table 3. Effect of molecular weight on gelling time (PNAG concentration: 9.40 w/w%).

ratio increases with PNAG concentration from 10.67% to 19.55%. In addition, the hydrogels give indication of temperature rise from 14°C to 37°C. The hydrogel exhibits high swelling ratio at higher temperature. This behaviour may be attributed to the following fact: PNAG chain contains a hydrophilic



**Figure 4.** The swelling behaviour of PNAG at: (a) pH 2.2, temperature: 14°C and (b) pH 2.2, PNAG: 19.55%.

group (-NHCO) and the hydrophilic groups in the polymer structure form stronger intermolecular Hbond with water molecules at low temperature. When the temperature is increased, the intermolecular H-bonds are weakened, and even they are disappeared. At the same time, water molecules penetrate into the gel, leading to higher swelling ratio.

#### In Vitro Drug Release Behaviour of PNAG

Drug release from polymeric hydrogel is closely related to many factors such as swelling behaviour of hydrogel, drug affinity to polymer chains, drug solubility in water, etc. [28]. In this study, caffeine as a model drug was trapped during the preparation of PNAG hydrogel.

Figure 5 depicts the in vitro release profile of caffeine from PNAG hydrogel at different temperatures. Compared to 14°C, the accelerated caffeine release was found at 37°C. On one hand, the caffeine release was affected by H-bonding interactions between the drug molecule and polymeric chains, as the intermolecular H-bonding interactions of -N= and -C=O groups of caffeine develop with the carboxyl and amide groups of PNAG chains. As it is well known, the H-bonding interactions are weakened or disappeared when the temperature is increased. Compared to temperature of 14°C, the weaker intermolecular H-bonding interaction at 37°C consequently accelerates the release of caffeine. As a result, the release rate of caffeine at 37°C is faster than that at 14°C in pH 7.4 PBS. On the other hand, the release behaviours of caffeine entrapped inside PNAG were also related to the swelling of hydrogel. At higher temperature, the cross-linking locations were reduced to some extent by H-bonding in PNAG hydrogel. The higher swelling of PNAG inevitably produces much more voids and channels for caffeine to be released. In this

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**Figure 5.** The effect of temperature on caffeine release behaviour. Release conditions: pH 7.4, PNAG 19.55% and caffeine 0.12%.

case, the resistance was greatly decreased during caffeine release. Therefore, more caffeine diffused out of the PNAG hydrogel at 37°C at equal time intervals.

The release behaviours of caffeine in different PBS pH were performed in this study. As shown in Figure 6, the cumulative release quantities of caffeine in pH 7.4 PBS is found to be 92% while that of pH 2.2 PBS was 67% in 140 min. In acidic release medium, the carboxyl groups from PNAG chains exist in the form of -COOH. The stronger H-bonding among PNAG chains dominates as intermolecular interactions, resulting in higher cross-linking density in PNAG network. Thus, compared with the neutral medium (pH 7.4 PBS), a slower release of caffeine behaviour was observed in this study. At the same time, caffeine molecules were ionized in acidic condition due to caffeine's weak organic base property. The strong ionic interaction prevents caffeine from diffusing into the external medium.

In addition, PNAG hydrogels showed an initial 30% burst release within the first 20 min. The initial burst release of drugs from other drug carriers system is also reported [29]. The possible explanation is that one part of caffeine was adsorbed on the surface of the hydrogel during the gelation process, and it diffused rapidly when the hydrogel came into contact with the release medium. The drug concentration gradient was another driving force for drug diffusion in this investigation. The higher caffeine concentration



**Figure 6.** The effect of pH on caffeine release behaviour. Release conditions: temperature: 14°C, PNAG 10.67% and caffeine 0.22%.

gradient between PNAG hydrogel surface and the release medium at the early stage led to higher initial burst and fast release rate.

The effect of PNAG concentration on caffeine release was conducted in PBS of pH 7.4 at room temperature. As shown in Figure 7, both the rate and the cumulative amount of caffeine release are inversely proportional to PNAG concentration. After 1 h, more than 71% of caffeine is released from hydrogel having 10.6% PNAG, whereas only 46% is released from 19.55% PNAG containing hydrogel.



**Figure 7.** The effect of PNAG concentration on caffeine release behaviour. Release conditions: pH 7.4, temperature:  $14^{\circ}$ C, and caffeine 0.22%.



Figure 8. Degradation of PNAG in pH 2.2 buffer solution.

When PNAG concentration was higher, the Hbonding interactions were stronger at the same temperature, leading to an increase in cross-linking density in PNAG network. Thus, the narrowing channel for the drug molecules diffusion into the surroundings resulted in slower release of caffeine. As it is well known, the degradability also plays a critical role in determining the overall release profiles.

# Degradation

The degradation behaviour is very important in many drug carriers in practical applications. In order to investigate the degradability of PNAG in in vitro, the hydrogels were incubated in pH 2.2 buffer solution at 37°C simulated as gastric fluid. In vitro degradation behaviour of PNAG was evaluated by tracking the molecular weight change. As it is observed from Figure 8, by prolonging the degradation time, the molecular weight of PNAG is decreased step by step. After 17, 30 and 45 days, the molecular weight of PNAG decreased to 8.6, 4.5 and  $3.2 \times 10^5$  from its initial molecular weight of 9.5×10<sup>5</sup>. This experimental result indicated the biodegradability potential of PNAG.

# CONCLUSION

In this study, a new injectable PNAG hydrogel was successfully prepared by free-radical polymerization with glycine. The gelling time for PNAG was apparently affected by many factors including the temperature, pH and ionic strength in its aqueous

solution. The gelling time of PNAG rapidly decreased as PNAG concentration was increased, and when the temperature decreased. The swelling measurements of PNAG hydrogel clearly demonstrated the temperature-responsive nature of the materials. The in vitro release profiles of caffeine from PNAG hydrogel were evaluated as a function of pH, PNAG concentration and temperature. Compared with the acidic release medium (pH 2.2), a remarkable increase in caffeine release was observed in the PBS basic pH 7.4. Additionally, the release rate was faster at 37°C than that at 14°C due to the occurrence of PNAG hydrogel phase transition. These preliminary investigations have shown that PNAG hydrogels, as it was expected, are promising injectable materials in the biomedical field.

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