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ORIGINAL PAPER

# A new photocrosslinkable hydrogel based on a derivative of polyaspartic acid for the controlled release of ketoprofen

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**Abstract** A novel photocrosslinkable and pH-sensitive hydrogel used for drug delivery was developed based on polyaspartic acid. Polysuccinimide (PSI) was modified by hydrazine and acryloyl chloride. The unreacted imide rings of PSI were hydrolyzed. Hydrogels were formed by photocrosslinking without any crosslinker or photoinitiator. Products were characterized by FT-IR and solid-state $^{13}$CNMR analysis. The swelling behaviors of hydrogel in various pH values were studied. Ketoprofen (KP) was chosen as a model drug. Two drug loading methods were compared. The release kinetics of KP was evaluated in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at 37 °C. The results showed that drug-loaded hydrogels were resistant to SGF, and hence they could be useful for oral drug delivery. There would be a wide range of applications for controlled drug delivering system.

**Keywords** Polyaspartic acid · Hydrogel · Photocrosslinking · Drug release · Ketoprofen

## Introduction

Hydrogels have been known as excellent candidates for many biomedical and pharmaceutical applications due to their tunable chemical and three-dimensional physical structure, high water content, good mechanical properties, and biocompatibility. As such, hydrogels have been widely used for drug-delivery carriers [1], tissue engineering templates [2], and biosensors [3]. They can be prepared from

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water-soluble polymers which could be crosslinked either by chemical reaction with crosslinker or irradiation using UV- and $\gamma$-rays [4–6].

It was evident that residues of chemical crosslinker can be toxic for humans and $\gamma$-rays have a higher energy and give rise to the breaking of chemical bonds in a non-selective and are difficult to check way. Photocrosslinking reaction was a technique widely used to synthesize polymers and hydrogels because of its distinct advantages, including a rapid cure rate, low curing temperature in-line production, low energy consumption, and easy process control [7, 8].

Polyaspartic acid (PASP) is a water-soluble synthetic polymer with a protein-like structure, obtained by hydrolyzed reaction of the polysuccinimide (PSI) [9]. Recent studies demonstrated PASP had excellent toxicological and pharmacological properties, i.e., lack of toxicity, antigenicity, and immunogenicity. PASP and its hydrogel were drawing more attention in biomedical fields [10, 11]. It could be chemically crosslinked easily. While during preliminary experiments, PASP did not show any chemical alteration when exposed to the action of UV rays.

In order to improve the photoreaction activity of PASP, Giovanna et al. [12–14] tried to introduce C=C double bonds into PASP. They synthesized a photocrosslinkable PASP derivative by esterification between methacrylic anhydride and hydroxyl group of PSI grafted by ethanol amine. In previous researches, excess ethanol amine relative to imide groups was added. Therefore, the prepared hydrogel had relatively low swelling ratio and poor pH-sensitivity.

In the present article, a novel photocrosslinkable PASP derivate was prepared, containing both C=C double bonds and carboxyl groups. The new macromer was indicated as PASP-Hy-AC. Without any crosslinker or photoinitiators, PASP-Hy-AC was irradiated by UV to get hydrogel. In addition, the potential use of the hydrogel as a novel drug-delivery system was investigated by using Ketoprofen [KP: (*RS*)-2-(3-benzoylphenyl) propionic acid] as a model drug, which is a potent nonsteroidal anti-inflammatory drug [15]. The gastric effects of perorally administered KP may be alleviated, to some extent, by inhibiting its release in the gastric region.

## Experimental

### Materials

L-Aspartic acid was obtained from Changmao Biochemical Engineering Co. Ltd (Zhejiang province, China). PSI with high molecular weight was prepared in our laboratory [16]. KP was purchased from Hubei Wuxue Xunda Pharmaceutical Co., Ltd (Hubei province, China). 85% Hydrazine hydrate, *N*,*N*-dimethylformamide (DMF), 85% phosphoric acid, AC, triethylamine (TEA), and acetone were analytical grade and used without further purification.

### Instruments

The weight average molecular weight (Mw) of PSI was estimated by a correlation method with Mw of corresponding sodium polyaspartate prepared by hydrolysis of

PSI with NaOH. It was measured in a buffer containing 0.02 M $H_3PO_4$ using HPLC (10-ATVP, SHIMADZU, Japan) by a GF-510 HQ column (7.5 × 300 mm, SHOWA DENKO K.K., Japan)and a detector at UV 206 nm, the molecular weight marker was dextran which was a gift from Uppsala University in Sweden. The C, H, and N contents were detected by elemental analyzer (Vario EL III, ELEMENTAR Analysensysteme GmbH, Germany). Solid-state $^{13}$CNMR spectra were obtained with a NMR instrument (AV-300, Bruker, Germany) and operated at 75 MHz, equipped with a 4-mm triple tuned and MAS probe at room temperature [17]. FT-IR spectra were recorded by a Fourier Transform Infrared Spectrum analyzer (250FTIR, Thermo Nicolet Corporation, USA). UV irradiation was performed by using a UV curing system equipped with a mercury lamp of 1KW at high pressure with an emission at 365 nm (1KW-8.3A, Beijing Lighting Research Institute, China). Freeze-drying took place in a freeze-dryer (Alpha 1-2/LD-2,Martin Christ, Germany). The ionic strength was represented by conductivity measured by a conductometer (DDS-307, Shanghai Precision Scientific Instrument Co., Ltd, China). The spectrophotometric determination of KP was done on a spectrophotometer (Unico2000, Unico (Shanghai) Instruments Co., Ltd., China) at 260 nm [18].

## PSI-Hy synthesis

1.56 mL of 80% hydrazine hydrate (0.0257 mol) in 10 mL DMF was added dropwise to a continuously stirred solution of PSI (5 g, 0.0515 mol PSI repeating unit) in 100 mL DMF. Mw of PSI, determined by GPC, was 86.5 kDa (Mw/Mn = 1.83) [4]. The mixture was maintained at 40 °C for 4 h. During the reaction, a precipitate was formed. This solid material was filtered and washed several times with acetone, then dried under vacuum at 40 °C. 4.93 ± 0.20 g PSI-Hy was obtained and characterized by FT-IR analysis.

## PASP-Hy-AC synthesis

PASP-Hy-AC was obtained by modification of PSI-Hy with AC. 4 g PSI-Hy was dissolved in 80 mL DMF with suitable amount of TEA. And then, a solution of 4 mL AC in 5 mL DMF was added dropwise under stirring at 0 °C. The mixture was kept at 40 °C for 24 h, then precipitated with acetone. The obtained suspension was filtered and washed several times with acetone and dried under vacuum at 40 °C. The dried solid was dispersed in 40 mL distilled water, a suitable amount of 15% NaOH solution was added under stirring at 40 °C until pH was 8. Then the mixture was purified by exhaustive dialysis using ultrafiltration device with molecular weight cut-off of 10,000. The dialyzed solution was freeze-dried; 3.90 ± 0.12 g purified PASP-Hy-AC was obtained. The product was characterized by FT-IR and solid-state $^{13}$CNMR analysis. The degree of derivatization (DD) of AC was evaluated by solid-state $^{13}$CNMR analysis and calculated according to Eq. 1 [19]:

$$\mathrm{DD} = \left(\frac{\text{alkene groups}}{\text{polymer repeating unite}}\right) \times 100\% \quad (1)$$

## Preparation of PASP-Hy-AC hydrogel

2 g PASP-Hy-AC was dissolved in 20 mL double-distilled water. The solution was stirred at 25 °C overnight for homogeneous mixing, poured into a glass Petri dish, dried to a sample of about 2 mm in thickness at 40 °C, and then exposed to long-wavelength UV (intensity: 20 mW/cm$^2$) for 1 h. The UV source was located 10 cm far from the sample. The obtained product was washed with double-distilled water thoroughly to remove unreacted macromers and other impurities. And then the hydrogel was obtained by freeze drying. Thus, 1.64 ± 0.07 g PASP-Hy-AC hydrogel was obtained. The purified hydrogel was characterized by FT-IR and solid-state $^{13}$CNMR analysis.

## pH sensitivity of the PASP-Hy-AC hydrogel

The equilibrium swelling ratio of the hydrogel was conducted at 25 °C by a tea-bag method [4]. The hydrogel was immersed in various pH solutions (pH 2.0, 3.6, 5.0, 7.0, and 7.4). After 24 h, the equilibrium swelling ratio of hydrogel was measured. All of the buffer solutions were adjusted to same ionic strengths by adding sodium chloride to eliminate the influence of ionic strengths.

## Drug loading procedure

Method 1: Drug loading by soaking after crosslinking. Dried hydrogels were immersed into a concentrated solution of KP in ethanol and left soaking at room temperature under stirring for 24 h. Then, the solvent was removed by filtration and the sample was rapidly washed with ethanol in order to remove KP adsorbed on the surface and dried by freeze drying.

Method 2: Drug loading during crosslinking. At first, the stability of KP to UV light was tested by exposure of drug powder to irradiation for 1 h. The UV and IR spectra of KP had no obvious difference during irradiation (data not shown). Suitable amount KP was added to solution of PASP-Hy-AC (10% w/v). The process of photocrosslinking was the same as the method described above. After UV induced polymerization, the drug-loaded hydrogels were collected, rapidly washed with deionized water and ethanol, respectively, and then freeze-dried to a constant.

## Determination of drug amount entrapped in PASP-Hy-AC hydrogel

50 mg drug-loaded hydrogel was immersed in 50 mL ethanol at 25 °C under continuous stirring. After 5 h, the hydrogel was filtrated and the amount of released KP was determined by UV analysis. The hydrogel was added into ethanol (50 mL) again and the extraction was repeated until no further detectable KP was found in the fluid.

Drug release profiles from hydrogel

Drug release was studied in SGF and SIF. Each drug-loaded hydrogel (400 mg) was introduced into 1000 mL medium solution maintained at 37 °C and stirred at 150 rpm in a thermostatized glass reactor. Aliquots (5 mL) were withdrawn at appropriate time intervals to measure absorbance at 260 nm, then returned into the glass reactor. The release ratio was calculated according to Eq. 2.

$$\text{Release ratio (wt\% )} = \frac{C_i V_0}{M_0} \tag{2}$$

where, $C_i$ is the KP concentration of released media at the $i$th sample time, $V_0$ is the initial volume of solution, $M_0$ is the amount of loaded drug.

## Results and discussions

Synthetic route of PASP-Hy-AC was shown in Fig. 1.

As seen in Table 1, the C content in PSI-Hy decreased compared to PSI, whereas N and H content increased. It was because that a hydrazine molecule was added to polymer chains. Then PSI-Hy reacted with AC and 15% NaOH to product PASP-Hy-AC subsequently. Owing to alkaline hydrolysis, the unreacted imide rings of PSI-Hy were hydrolyzed to form carboxyl groups. During this step, a number of

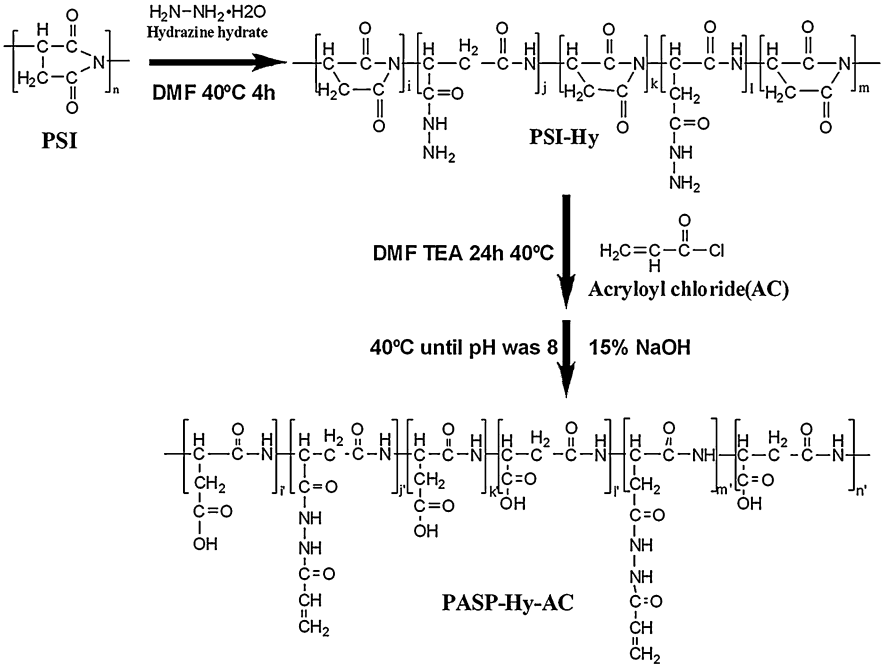


**Fig. 1** Synthetic route of PASP-Hy-AC

**Table 1** Elemental analysis of PSI, PSI-Hy, and PASP-Hy-AC

| | C (%) | N (%) | H (%) |
|---|---|---|---|
| PSI | 47.73 | 13.83 | 3.771 |
| PSI-Hy | 39.01 | 20.73 | 5.170 |
| PASP-Hy-AC | 44.97 | 16.43 | 5.564 |

water molecules and residues of AC were added to polymer chains, which led to the increase of H and C content and the decrease of N content.

Solid-state $^{13}$CNMR spectra of PASP-Hy-AC and its hydrogel were shown in Fig. 2. The C=O carbon appeared at 172.1 ppm, the C$\alpha$ carbon and C$\beta$ carbon at 50.6 and 35.6 ppm, the C=C carbon at 128.5 ppm. Compared Fig. 2a with Fig. 2b, the

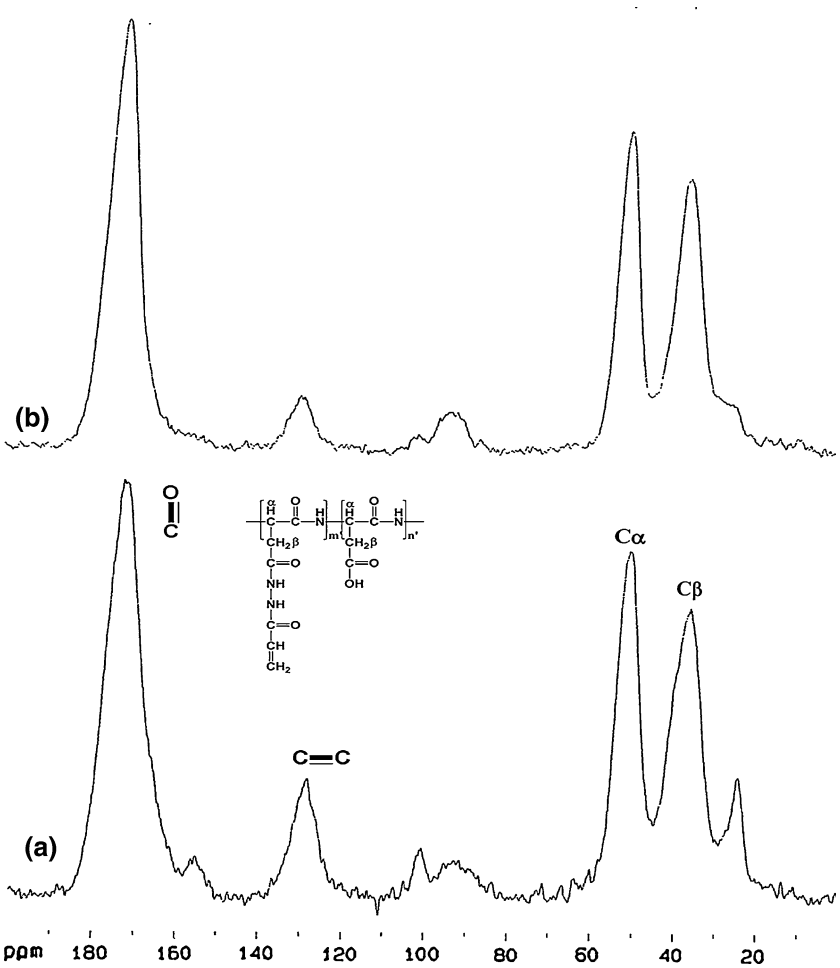


**Fig. 2** Solid-state $^{13}$CNMR spectra of **a** PASP-Hy-AC and **b** the hydrogel at room temperature

peak of C=C of PASP-Hy-AC hydrogel decreased relative to that of PASP-Hy-AC. This could identify the consumption of C=C bonds during crosslinking process. DD was calculated from Fig. 2a by comparing the integral of the peak related to C=C at 128.2 ppm awardable to AC with the integral related to Cα carbon at 50.8 ppm awardable to –*CH*–$CH_2$–CO–NH– [17, 20]. DD was 18.5 ± 2.1 mol% of linked AC for PASP-Hy-AC.

Figure 3 showed the FT-IR spectra of (a) PSI-Hy, (b) PASP-Hy-AC, and (c) PASP-Hy-AC hydrogel. Compared with spectrum of (a), spectrum of (b) showed that the absorption peak of imide rings (1716 $cm^{-1}$) became much smaller, while new peaks standing for amide (1654 $cm^{-1}$, 1523 $cm^{-1}$) were more obvious due to hydrolysis of the imide rings. Furthermore, spectra of (b) and (c) showed the typical double bond absorption bands at 985 $cm^{-1}$. This confirmed the successful incorporation of C=C into the polymer framework. The peak of C=C became weak in the spectrum of (c), and this could be used to identify the consumption of C=C bonds during photocrosslinking process.

In Fig. 4, the increasing pH led to gel swelling. It's well known that the pH of the solution where an ionic polymer is swollen affects the extent of swelling. At low pH, protons effectively suppressed the ionization of carboxylic acid groups of the hydrogel, and consequently, flexibility of the chain was rather low. As pH increased, carboxylic acid groups were ionized, the polymer chains extended more as the ionic groups repel each other. This result was observed in the higher swelling ratios seen as the pH increased from 2.0 to 7.4.

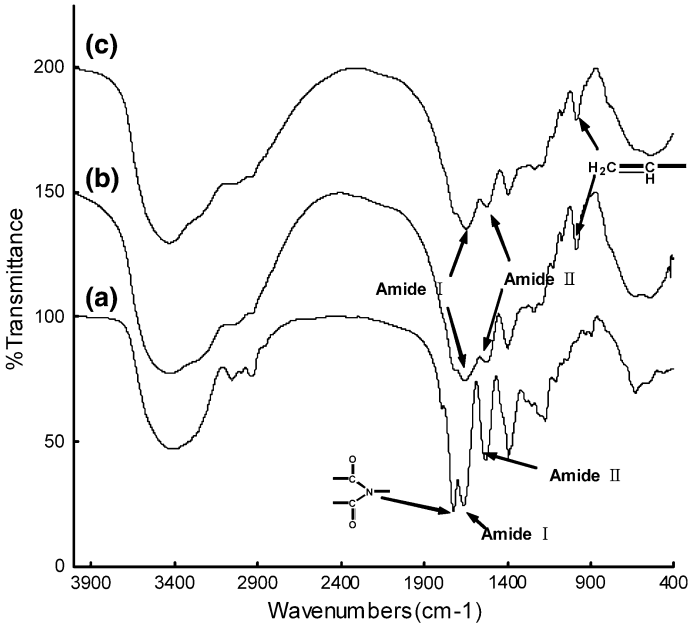


**Fig. 3** FT-IR spectra of (*a*) PSI-Hy, (*b*) PASP-Hy-AC, (*c*) PASP-Hy-AC hydrogel

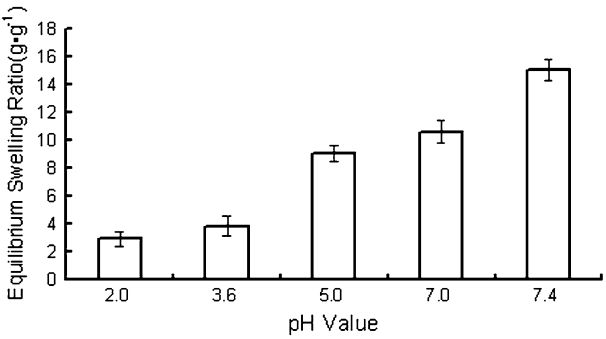


**Fig. 4** The equilibrium swelling ratio of the PASP-Hy-AC hydrogel at various pH values (solution conductivity was adjusted to approximate 750 us/cm)

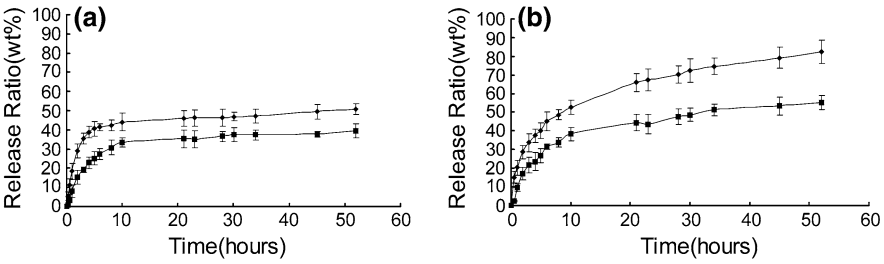


**Fig. 5** Release profiles of KP from drug-loaded hydrodel (pH 1.2 (*filled square*), pH 6.8 (*filled diamond*), 37 °C). **a** KP loaded during irradiation, the drug-loaded amount 9.34% (w/w), **b** KP loaded by soaking, the drug-loaded amount 8.52% (w/w)

A preliminary study was undertaken to ascertain the ability of the prepared networks to physically entrap drug molecules, and release them in simulated gastrointestinal fluid. The amount of KP incorporated during the irradiation process was 9.34% (w/w) for hydrogel. The soaking method had been carried out in such a way as to produce amount of drug entrapped corresponding to 8.52% (w/w) for hydrogel. To test the ability of hydrogel to delivery the entrapped drug, release experiments were performed in SGF (pH 1.2) and SIF (pH 6.8).

Figure 5 depicted the release of KP (as percent of total entrapped dose) as a function of the release time. As seen in Fig. 5a, the release rate of KP loaded during irradiation was 15% in SGF at 2 h. After 50 h, the release rate was 39% in SGF and 51% in SIF. The release rate depended on pH conditions. The drug release in SIF was faster as compared with that in SGF independent of drug-loaded method. The same way was observed in Fig. 5b, the release rate of KP loaded by soaking was 17% in SGF at 2 h. After 50 h, the release rate was 55% in SGF and 83% in SIF. There were two reasons for the restriction of KP release at acidic solution. PASP-Hy-AC hydrogel was shrunk to form a compact structure at acidic solution, and amines of PASP-Hy-AC hydrogel were protonated and therefore could interact with oppositely charged drug ions at acidic pH. Besides, experimental data evidenced a more rapid release of drug from samples prepared by soaking procedure than that from samples

in which KP was loaded during the photocrosslinking, probably because in the former case the drug molecules were not tightly entrapped in the network.

## Conclusions

A derivative of PASP (PASP-Hy-AC) containing acrylate moieties was synthesized. The value of derivative degree was 18.5 ± 2.1 mol%. PASP-Hy-AC hydrogel was obtained by UV irradiation without toxic crosslinker and photoinitiator. Due to carboxylic acid groups in the matrix, the hydrogel had predominant pH-sensitive swelling property. The release rate of KP from the hydrogel depended on pH conditions and had shown to be one of the sustained KP release in SIF for 50 h. The study will be useful in designing and developing novel controlled delivery systems.

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