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SHORT COMMUNICATION

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# A novel sol–gel strategy to prepare temperature-sensitive hydrogel for encapsulation of protein

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**Abstract** A novel sol–gel strategy was proposed to prepare temperature-sensitive hydrogel including the copolymerization of *N*-isopropylacrylamide and 3-methacryloxypropyl-trimethoxy silane and then the hydrolysis and condensation of the linear polymers through the sol–gel process under extra-mild conditions. Bovine serum albumin, as a model protein, was loaded into the hydrogel matrix to investigate the encapsulation and release properties. Experimental results indicated that the preparation conditions were valuable for the loading and release of biomacromolecules.

**Keywords** Hydrogel · Phase separation · Stimuli-sensitive polymers · Sol–gel

## Introduction

Hydrogels are three-dimensional hydrophilic polymer networks that absorb water, but do not dissolve in water. Due to their high hydrophilicity, biocompatibility and similarity to natural tissues, hydrogels have shown many potential applications in the past decade. Stimuli-sensitive hydrogels, which exhibit volume or phase transition in response to slight environmental changes, such as temperature, pH, ionic strength, light, electric and magnetic fields, etc., are called intelligent materials [1–4]. Their fascinating properties allow us to develop a variety of stimuli-sensitive hydrogels for uses in drug delivery [5], gene carriers [6], immobilization of enzyme [7], etc.

Chemically cross-linked poly(*N*-isopropylacrylamide) (PNIPA) is a typical temperature-sensitive hydrogel, which has a transition temperature or lower critical solution temperature (LCST) around 33 °C, below which PNIPA hydrogels swell and retain abundant water within their networks, while gels shrink and become opaque by elevating the surrounding temperature above LCST [8–10]. Because of the protective environment for bioactive molecules and living cells in the polymer networks, adjustable permeability and versatile forms, temperature-sensitive hydrogels have been widely investigated as matrices for many drugs, especially the bioactive macromolecules, such as enzymes, proteins, nucleic acids, etc.

Despite the variety of available methods for protein immobilization in hydrogels, there are relatively few techniques of loading proteins in hydrogels acceptable for drug delivery [11]. The first method involves mixing the drug with monomers/prepolymers and crosslinkers before the formation of hydrogels [12]. Generally, hydrogels are synthesized by polymerizations and crosslinking of vinyl monomers initiated by radicals or polymerization, high-energy irradiation (gamma ray and electron beam) or by addition and condensation reactions of complementary groups (OH, COOH, $NH_2$ and CHO, etc.). All of the above techniques suffer from the possibility of the side reactions that may denature the bioactive reagents as well as the inability to diffuse out of the matrices. An alternative approach is to allow a prepared gel to reswell in a drug-containing solution. Nevertheless, the low drug loading levels (less than 0.1 wt%) resulted by the exclusion of large biomolecules from the gel networks is a serious limitation [13, 14].

Such unfavorable effects can be partly avoided with the use of physically crosslinked gels that are crosslinked by physical interaction and no crosslinking agents are used during the preparation of hydrogels [15, 16]. However, the physical hydrogels are restricted by the weak mechanical strength, lack of long-term stability and strong dependence on the sol polymer precursors. On the other hand, a chemical sol–gel route starting from an alkoxysilane precursor has been developed for the encapsulation of biomolecules and living cells [17–20]. The disadvantages of this chemical sol–gel method include un-mild condition (low or high pH) during the hydrolysis and condensation, and the toxic alcohol as a byproduct from the hydrolysis and condensation reaction in the sol–gel process.

In this communication, we report a novel two-step strategy to prepare thermal-sensitive PNIPA hydrogel combining the copolymerization of vinyl monomers and the sol–gel technique. The model protein, bovine serum albumin (BSA), can be efficiently loaded into the gel matrix and completely released out at the temperature lower than LCST.

## Experimental

### Materials

*N*-isopropylacrylamide (NIPA, Aldrich Chemical Co., Inc., USA) was purified by recrystallization from benzene/*n*-hexane mixed solvent. 3-Methacryloxypropyltrimethoxysilane (MPTMS, Chemical Plant of Wuhan University, Wuhan, P. R. China) was distilled in vacuum. Dibenzoyl peroxide (BPO), 1,4-dioxane, tetrahydrofuran (THF) and diethyl ether were analytical grade and used as received from Shanghai Chemical Co. (China).

### Synthesis and characterization of linear poly(*N*-isopropylacyamide-*co*-3-methacryloxy propyltrimethoxy silane) P(NIPA-*co*-MPTMS)

An amount of 2 g of NIPA and 0.3 mL of MPTMS were dissolved in 15 mL 1,4-dioxane, and then 50 mg of BPO was added to the mixture. Nitrogen was bubbled for 30 min at room temperature, then the temperature was increased to 80 °C to initiate the polymerization. The reaction was carried out at 80 °C for 4 h. After the temperature decreased to ambient temperature, 1,4-dioxane was evaporated in vacuum. The residue was dissolved in THF and precipitated in a large amount of diethyl ether. The polymer was further purified by dissolving in THF, and reprecipitated in petroleum ether. The molecular weight and molecular weight distribution of the obtained copolymer were determined by gel permeation chromatography (GPC) equipped with Waters 2690D separations module, a Waters 2410 refractive index detector and waters styragel HR1& styragel HR4 guard column using polystyrene standards. DMF was used as eluent at a flow rate of 0.3 mL/min. The weight-average molecular weight was 45,000 and the polydispersity was 3.2. $^1$H-NMR spectra were performed on Mercury VX-300 spectrometer using tetramethylsilane (TMS) as internal reference and chloroform-*d* ($CDCl_3$) as a solvent. $^1$H-NMR ($CDCl_3$,δ, ppm): δ 3.90–4.05 (m, $CH(CH_3)_2 + OCH_2(CH_2)_2$), 3.47 (s, $Si(OCH_3)_3$), 2.1–2.3 (m, $CHCO$), 1.83 (m, $CH_2CH_2CH_2$–), 1.6–1.7 (m, $CH_2CHCO$), 1.0–1.2 (d, $CH(CH_3)_2$, C–$CH_3$). The molar ratio of NIPA to MPTMS in P(NIPA-*co*-MPTMS) is 19:1, which is determined by calculating the integral area of the signals at 3.47 and 1.0–1.2, which represent the –$OCH_3$ in MPTMS and –$CH_3$ in MPTMS and NIPA, respectively.

### Preparation of the hydrogel and measurement of LCST

An amount of 200 mg P(NIPA-*co*-MPTMS) was dissolved in 1.0 mL water. Upon addition of 0.5 mL citric acid/phosphate buffer solution to modulate pH to 6, hydrolysis and condensation of the siloxane give rise to a transparent hydrogel with the diameter of 25 mm and the thickness of 3 mm. The gel formed was allowed to age for 24 h, then immersed in distilled water for 3 days at room temperature with water being changed repeatedly to remove chemical residues.

The swelling ratio (SR) of the hydrogel is defined as SR = Ws/Wd, where Ws is the weight of water in a swollen hydrogel at a certain temperature and Wd is the weight of the hydrogel after vacuum drying. Ws was measured gravimetrically after blotting the excess surface water with moistened filter paper, after incubating the gel in distilled water for at least 24 h.

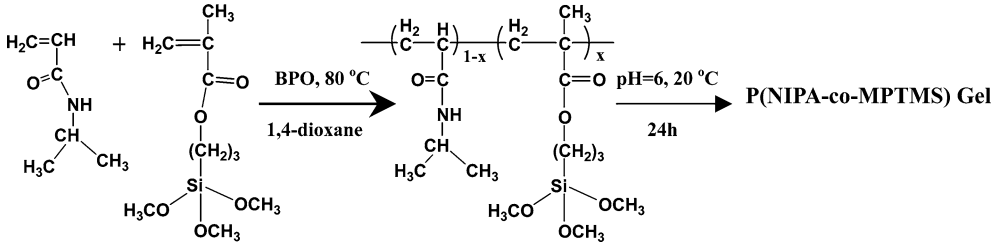
### Protein loading and release

Protein-encapsulated gel was prepared as depicted above except that 50 mg BSA was added to the solution before cross-linking. The drug-loaded gel was washed three times with distilled water. The protein release studies were carried out at 20 °C. The gel entrapping BSA was put into a beaker containing 20 ml water. At fixed time intervals, the concentrations of released BSA were measured and calculated by the absorption at 278 nm using a UV-spectrometer (PERKIN ELMER, Lamda Bio40) and the water was replaced. Drug loading is defined as $A/B$, where $A$ is the weight of BSA loaded in the hydrogel and $B$ is the weight of dried gel.

### Gel electrophoresis

Gel electrophoresis was performed to determine whether the protein encapsulated and released from the hydrogel

**Scheme 1** The formation scheme of P(NIPA-*co*-MPTMS) hydrogel




was modified. The original and released BSA were analyzed by electropheresis on an SDS-PAGE containing 10% (w/v) following an established procedure [21]. Protein was visualized by strain with coomassie brilliant blue. The results demonstrated that no change of BSA was observed during the encapsulation or release process.

## Results and discussion

The formation schedule of hydrogel was illustrated in Scheme 1. Firstly, linear P(NIPA-*co*-MPTMS) was synthesized by radical copolymerization of NIPA and MPTMS, which was initiated by BPO at 80 °C. Secondly, the linear polymer was employed as the sol precursor for gel preparation under extra-mild conditions (pH = 6), under which the solution gradually became viscous and a transparent hydrogel formed via the hydrolysis of the Si–OMe to generate Si–OH and the condensation of Si–OH to produce Si–O–Si crosslinking points.

The swelling behavior as a function of temperature is an important parameter to evaluate the hydrogels, which can suggest the thermal-sensitivity of the novel hydrogel. As shown in Fig. 1, the gel exhibits higher water absorption and becomes swollen at lower temperature. Upon increasing the temperature, hydrogel loses water and shrinks in volume. The SR decreases dramatically when the temperature increases beyond 29 °C, which is regarded as the LCST of the hydrogel. It is well investigated that the specific balance of hydrophilic and hydrophobic groups existing in the PNIPA network is the main mechanism to explain the LCST behavior. At the temperature lower than the LCST, H-bonds interactions between water and the side chains behave cooperatively to form a stable shell around the hydrophobic groups and allow the hydrogel to be well swollen. When the temperature is increased above LCST, the delicate balance will be disrupted and the interactions among the hydrophobic groups begin to play a dominant role, as a result of which, the polymer chains aggregate together [22, 23]. Macroscopically, the hydrogel will squeeze entrapped water out and collapse. For conventional homo-PNIPA hydrogel, the LCST is considered as 32–34 °C. However,

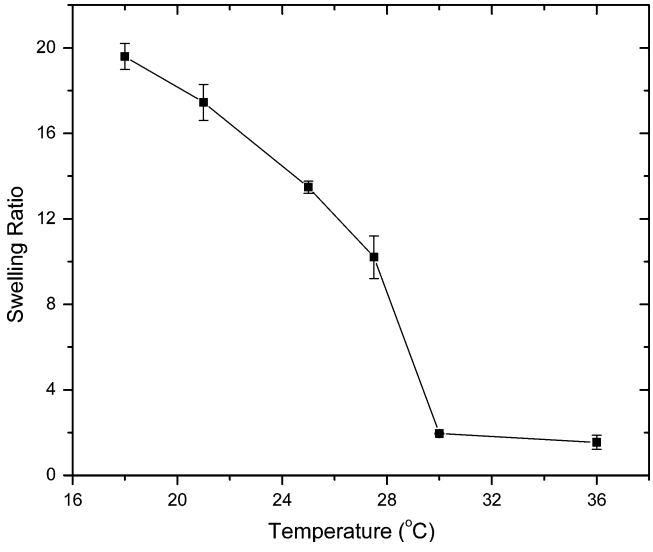



**Fig. 1** Equilibrium swelling ratio of the novel hydrogel as a function of temperature in the range from 18 °C to 36 °C

the LCST of the novel hydrogel thus synthesized was found as 29 °C, which is lower than that of conventional PNIPA hydrogel. It is reported that LCST of PNIPA hydrogel could be modulated by controlling the copolymer composition. Copolymerization of NIPA with hydrophobic monomers such as butyl acrylate and methyl methacrylate decreases the LCST of the formed hydrogel, while hydrophilic comonomers, such as acrylamide or acryl acid are proven to shift LCST to a higher point [24]. In this work, MPTMS is more hydrophobic than NIPA; therefore, incorporation of hydrophobic MPTMS into hydrogel network probably reduces the hydrophilicity of hydrogel, which leads to a decreased LCST.

Just as mentioned above, when hydrogels are utilized as drug delivery systems, drug loading and release are two important and difficult aspects to control. The recently developed sol–gel technology is simple, versatile and widely used in encapsulating biomolecules [17, 18]. However, the current sol–gel method using alkoxysilane as the sol precursors still has some serious limitations. Firstly, the low solubility and reactivity of the sol precursors (e.g., $Si(OCH_3)_4$, $Si(OCH_2CH_3)_4$) generally necessitate non-aqueous solvents and catalysts (such as

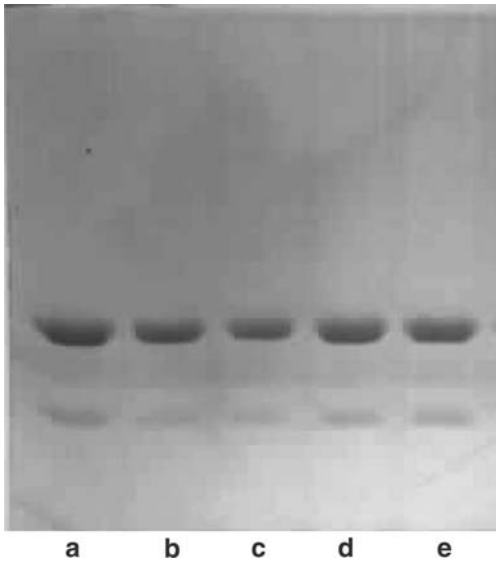



**Fig. 2** Gel electrophoresis of BSA. **a** Control BSA. **b** Released BSA (2 h). **c** Rreleased BSA (5.5 h). **d** Released BSA (11 h). **e** Released BSA (25.5 h)

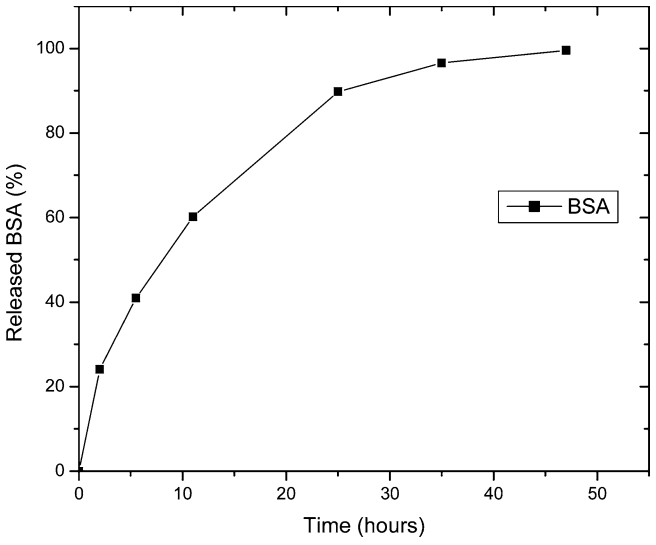



**Fig. 3** Cumulative release of BSA from the novel hydrogel as a function of time at 20 °C. Data points indicated the mean values of three experiments

strong acids and strong bases), both of which may be harmful to the bioactive reagents. Secondly, large amounts of alcohol generated as byproduct of the hydrolysis and condensations reaction of the alkoxyde precursors has detrimental effects on the structure and activities of biomolecules. Finally, it is difficult for the proteins loaded in hydrogels to diffuse out.

Compared with the traditional alkoxysilane sol–gel methods, the novel sol–gel technique we described here has several notable features. Firstly, the mole ratio of MPTMS/NIPA in P(NIPA-*co*-MPTMS) is low and adjustable, we have synthesized the other copolymers with the molar ratio of NIPA to MPTMS as 34:1 and 58:1 by modulating the ratio of monomers. P(NIPA-*co*-MPTMS) is well soluble in both water and organic solvents at room temperature, which means that organic solvents are unnecessary in gelation process and the amounts of the alcohol generated is much less than that of the gels prepared from alkoxysilanes. Secondly, citric acid/phosphate buffer solution (pH 6.0) was used for the sol–gel process, which is extra-mild for encapsulation of bioactive molecules. As a model protein, 50 mg of BSA was added to the reaction mixture before the addition of citric acid/phosphate buffer. The gelation reaction was carried out under the same conditions to obtain the protein-loaded hydrogel. The gel electrophoresis of the original BSA and released BSA are shown in Fig. 2. From the figure, we found that the migration of released BSA is the same as that of the control BSA, which confirmed that the molecular weight of BSA was not changed and it was not chemically modified during the encapsulation or release process.

At last, the release ability and release rate of BSA from the hydrogel is a noteworthy problem. Because BSA was added into the reaction mixture before gelation, it was loaded within the matrix after the formation of hydrogel. In this study, the amount of encapsulated BSA is 38.74 mg, and the drug loading is 24.2%. BSA was released from the hydrogel in distilled water at 20 °C. The results in Fig. 3 show that 40% of encapsulated BSA was released within 5 h with a "burst" way, and almost all of the BSA loaded in the hydrogel matrix can be released out within 48 h. The amount of the unloaded BSA and the released BSA is almost the same as the amount of the protein added, which indicates that the BSA can be effectively loaded inside the hydrogel and completely released from the hydrogel.

## Conclusions

In conclusion, a novel two-step sol–gel strategy was adopted to prepare temperature-sensitive hydrogel for the efficient loading and release of BSA under mild conditions. No change of the BSA was observed in the process of loading and release. We believe that this novel sol–gel technique will show potential in encapsulation of bioactive molecules and living cells.

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