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Haiyang Tian

<https://orcid.org/0000-0001-6603-4949>

Shanqi Qin

1491062726@qq.com

Sichuan University of Science and Engineering <https://orcid.org/0009-0005-6047-4755>

Rui Tan

Yi Liu

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Preparation and Performance Study of a pH/Temperature Dual-Responsive P(NIPAAm-co- β -CEA)/Sodium Alginate Hydrogel with Fully Interpenetrating Double Networks

Haiyang Tian^{a*}, Shanqi Qin^a, Rui Tan^b, Yi Liu^{a*}

^aCollege of Chemical Engineering, Sichuan University of Science & Engineering, Zigong 643000, PR China

^bPangang Group Vanadium Titanium Resources Co., Ltd., Panzhihua 617000, PR China

**E-mail: hytian0102@163.com(Haiyang Tian)*

**E-mail: yiliu@suse.edu.cn(Yi Liu)*

Abstract: The hydrogel in this paper is mainly composed of temperature sensitive material N-isopropyl acrylamide (NIPAAm), pH sensitive material β -carboxyethyl acrylates (β -CEA), sodium alginate (SA), cross-linker N,N'-methylenebisacrylamide (MBA), calcium carbonate (CaCO_3), initiator potassium persulfate (KPS), and co-initiator tetramethyl ethylenediamine (TEMED), and a series of fully interpenetrating double-network hydrogels are prepared. The synthesis conditions of the dual-network hydrogel were optimized through single-factor experiments, and the optimal synthesis conditions were determined by comparing the swelling degree. The hydrogels synthesized under the optimal conditions were evaluated for temperature responsiveness, pH sensitivity, re-swelling, repeated swelling and deswelling, and drug release performance. The results showed that in the temperature response test, at pH=7.4, the swelling degree decreases as the temperature rises, demonstrating that the hydrogel has good thermosensitivity. In the pH sensitivity test, under the condition of T=25°C, the swelling degree increased with the rise in pH, indicating good sensitivity to pH. During the repeated process of deswelling and

swelling, the hydrogel exhibited certain reusability performance at different pH levels and temperatures. Using doxorubicin (DOX) as the model drug, the drug loading and release behaviors of the optimal dual-network hydrogel were evaluated. It was confirmed that the dual-network hydrogel has excellent drug loading performance, and the drug release behavior also exhibits good temperature/pH dual-response performance under different temperatures and pH conditions. This indicates that the hydrogel has good temperature/pH response performance and can achieve intelligent control of the drug release behavior. It has a promising application prospect in targeted and triggered drug delivery systems.

Key words: Hydrogel; Swelling property; Calcium ion; pH-responsive; Temperature-responsive

1. Introduction

In the context of the 21st century, and with the integration of multiple disciplines such as materials science, biology, and medicine, the field of intelligent medical materials has witnessed rapid development.[1] Among them, drug-controlled-release systems based on natural biological materials and intelligent polymers are receiving significant attention from both the academic and industrial communities due to their advantages such as safety, intelligence, and multi-functionality. [2]

Hydrogels as a type of polymer material with a three-dimensional network structure, [3, 4] can maintain their network structure through hydrogen bonds and chemical covalent bonds, and can also retain a large amount of water.[5, 6] This unique structure endows the hydrogel with excellent hydrophilic properties: it exhibits a glassy state at room temperature and undergoes swelling but does not dissolve when exposed to water, maintaining its structural integrity throughout. Particularly noteworthy is the intelligent responsive hydrogel, which can sensitively sense subtle changes in the external environment (such as pH value, temperature, etc.) and make corresponding adjustments. This characteristic makes it an ideal drug-controlled-release carrier.[7]

With the continuous development of hydrogels, single-responsive hydrogels can no longer meet the increasingly complex clinical needs.[8] The development of smart hydrogels with good biocompatibility and the ability to respond simultaneously to multiple external stimuli has become a key direction in the field of drug sustained-release carriers.[9, 10] Therefore, the design of hydrogel drug carriers is rapidly evolving toward multi-responsiveness, precise regulation, and high intelligence. Research on multi-sensitive composite hydrogels holds significant scientific importance and application value. Song[11] developed an injectable pH/temperature dual-responsive nanoparticle-hydrogel composites. In this study, a dual-responsive delivery system loaded with doxorubicin (DOX) was constructed via in situ copolymerization using carboxymethyl chitosan and Pluronic F127 as substrates. The research results showed that in a weakly acidic environment, the cumulative drug release rate of the composite material reached 82.7% within 48 hours, significantly higher than the 34.2% observed under neutral physiological conditions. In terms of temperature responsiveness, when the temperature was increased from 25 °C to 37 °C, the material underwent a sol–gel phase transition, with the gelation time shortening to approximately 90 seconds. In vivo antitumor experiments demonstrated that in the treatment group receiving the DOX-loaded dual-responsive hydrogel, the tumor volume inhibition rate was significantly higher than that in the free DOX administration group, while no obvious damage was observed in the major organs of the mice, indicating that the material possesses good tumor targeting and biosafety. This study fully demonstrates the great potential of pH/temperature dual-responsive hydrogels in the field of tumor therapy and provides an important reference for the research approach of this study.

In this study, a full-interpenetrating double-network hydrogel was synthesized using SA as the raw material, NIPAAm as the thermosensitive monomer, β -CEA as the pH-sensitive monomer, MBA and CaCO_3 as cross-linkers, KPS as the initiator, and TEMED as the co-initiator. NIPAAm was selected as

the thermosensitive monomer due to its favorable physicochemical properties and biological characteristics, as it possesses both hydrophobic isopropyl groups and hydrophilic amide groups,[12] and the hydrophilic/hydrophobic balance between them endows the hydrogel with thermosensitivity.[13] β -CEA was used as the pH-sensitive monomer because the carboxyl groups ($-\text{COOH}$) in its molecular structure can undergo protonation or deprotonation depending on the environmental pH, thereby altering the charge state and swelling behavior of the polymer network and giving the hydrogel pH responsiveness. By adjusting the ratio of the two monomers, the network structure and crosslinking density of the hydrogel can be further modulated, thus optimizing its mechanical properties and elasticity. In this study, a novel dual-sensitive P(NIPAAm-co- β -CEA)/SA hydrogel with both temperature and pH sensitivity was synthesized via the full-interpenetrating polymer network method. The advantages of the full-interpenetrating double-network hydrogel include tunable properties, enhanced mechanical strength and toughness, the ability to incorporate new responsive components, and the capacity to exhibit dual or multiple responsiveness.[14, 15]

2 Experimental

2.1 Materials

N-isopropylacrylamide (NIPAAm, 98%, Shanghai Macklin Biochemical Technology Co., Ltd.), β -(acryloyloxy) propionic acid (β -CEA, 99%, Shanghai Macklin Biochemical Technology Co., Ltd.), sodium alginate (SA, 90%, Shanghai Macklin Biochemical Technology Co., Ltd.), N,N'-methylenebisacrylamide (MBA, Shanghai Macklin Biochemical Technology Co., Ltd.), potassium persulfate (KPS, Chengdu Kelong Chemical Co., Ltd.), N,N,N',N'-tetramethylethylenediamine (TEMED, 99%, Chengdu Kelong Chemical Co., Ltd.), D-gluconolactone (GDL, Shanghai Macklin Biochemical Technology Co., Ltd.), calcium carbonate (CaCO_3 , Chengdu Kelong Chemical Co., Ltd.),

deionized water (prepared in the laboratory), Phosphate buffer solution (PBS prepared with 0.2 mol/L Na_2HPO_4 , pH adjusted to 1.2–7.4 using 0.2 mol/L NaOH and 0.2 mol/L HCl).

2.2 Preparation of Double-Network P(NIPAAm-co- β -CEA)/SA Hydrogel

Different amounts of thermosensitive monomer and pH-sensitive monomer were placed in a beaker. A certain amount of deionized water was added and stirred, then a certain mass of the crosslinker N,N'-methylenebisacrylamide was added. Subsequently, a predetermined quantity of sodium alginate is introduced into the solution and allowed to dissolve completely. A certain mass of the initiator potassium persulfate was weighed and added, and the reaction was continued with constant stirring until completely dissolved. Then, calcium carbonate at a certain percentage relative to the mass of sodium alginate, 0.375% D-gluconolactone, and 40 μL of the co-initiator N,N,N',N'-tetramethylethylenediamine were added and rapidly stirred. The mixture was then poured into pre-prepared disposable test tubes, sealed, and placed in a refrigerator at approximately 10 °C for 24 hours to allow the reaction to proceed. After the reaction, the resulting gel was removed and cut into circular thin pieces. To remove unreacted monomers, the gel pieces were immersed in deionized water for purification for one day, with the deionized water replaced every 4 hours. Finally, the purified gel pieces were dried in a vacuum drying oven at 55 °C until a constant weight was achieved, yielding the dry full-interpenetrating double-network hydrogel. In this study, the single-factor optimization method was employed, wherein one variable was controlled at a time to identify the optimal synthesis conditions.

2.3 Characterization

2.3.1 Fourier Transform infrared spectroscopy (FTIR)

The fully dried hydrogel was ground into powder, and the copolymerized hydrogel was characterized using a PerkinElmer Fourier Transform infrared spectroscopy with a scan number of 32, a

scan range of 4000 cm^{-1} to 400 cm^{-1} , and a resolution of 4 cm^{-1} .

2.3.2 Scanning electron microscope (SEM)

The surface morphology and cross-section of the freeze-dried hydrogel were observed using a field emission scanning electron microscope (VEGA 3 SBU; TESCAN). The hydrogel was swollen to equilibrium in deionized water at 25 °C under pH conditions of 7.4, 4.0, and 1.2, respectively, and then freeze-dried using a freeze dryer. The freeze-dried gel was quenched by liquid nitrogen at low temperature and then gold-coated on its surface, and SEM images were obtained under an accelerating voltage of 15 kV.

2.3.3 Thermogravimetric Analysis (TGA)

Weigh 5-10 mg of the dried hydrogel product and place it in an Al_2O_3 crucible. Use the STA 409PC (from NETZSCH, Germany) thermogravimetric analyzer to measure the thermal stability of the hydrogel sample. Test conditions: In a nitrogen atmosphere, at a heating rate of 10 °C/min, from 30 °C to 600 °C.

2.3.4 X-ray photoelectron spectroscopy (XPS)

The X-ray photoelectron spectroscopy (XPS) analysis in this study was performed using a Thermo Scientific K-Alpha⁺ spectrometer equipped with a monochromatic Al $K\alpha$ X-ray source. Powder samples (≥ 10 mg) were prepared by dry pressing onto conductive tape. During the measurement, the charge neutralization system was activated. A survey spectrum (pass energy of 150 eV) was conducted for elemental screening, and high-resolution fine spectra (pass energy of 50 eV) of C, O, N, and Ca were collected to analyze their chemical states. All spectra were corrected using the C 1s binding energy, 284.8 eV, as the standard.

2.4 Performance testing

2.4.1 Swelling measurements

The dried hydrogel prepared in the experiment was weighed using an analytical balance, and the recorded data were designated as M_0 . The weighed dried hydrogel was placed into a beaker, and 20 mL of pH 7.4 PBS was measured using a graduated cylinder. The beaker was then placed in a water bath at 25 °C for heating. At intervals of 1 hour, the hydrogel was removed from the beaker, gently wiped dry with filter paper, and precisely weighed using an analytical balance; the recorded data were designated as M_1 . The swelling ratio (SR) was then calculated. The weight measurement was performed three times, and the average value of the three measurements was taken:

$$SR = \frac{(M_1 - M_0)}{M_0} \quad (1)$$

2.4.2 Response performance measurements

For the temperature response test, under the condition of pH 7.4, a certain mass of dried hydrogel was placed in PBS at temperatures of 25 °C, 37 °C, and 42 °C, and then allowed to swell at constant temperature. At intervals of one hour, the hydrogel was taken out, the surface moisture was wiped off with a moistened filter paper, and it was weighed using an analytical balance. The data were recorded to calculate the swelling ratio of the hydrogel. For the pH response test, under the condition of 25 °C, a certain mass of dried hydrogel was placed in PBS with pH values of 1.2, 4.0, and 7.4. The swelling degree was measured in the same manner, and the data were recorded to calculate the swelling ratio of the hydrogel.

2.4.3 Deswelling and Repeated Deswelling measurements

After the hydrogel was fully swollen in a PBS at pH 7.4 and 25 °C, it was then placed into a PBS at pH 7.4 and 42 °C, as well as a PBS at pH 1.2 and 25 °C, to undergo deswelling. The hydrogel was taken

out every hour, weighed, and the data were recorded to calculate the water retention (WR) of the hydrogel:

$$WR = \frac{W_1 - W_0}{W_2 - W_0} \quad (2)$$

W_1 and W_2 are the masses of the hydrogel at swelling time t and at swelling equilibrium, respectively, and W_0 is the initial weight of the dry gel.

Two pieces of hydrogel were first immersed in a PBS at pH 7.4 and 25 °C until swelling equilibrium was reached. They were then separately placed into a PBS at pH 7.4 and 42 °C, and a PBS at pH 1.2 and 25 °C, respectively, for deswelling for one hour. After one hour, both pieces of hydrogel were transferred back to the original PBS (pH 7.4, 25 °C). This cycle was repeated. The swelling ratio was plotted against time to obtain the repeated swelling–deswelling performance of the hydrogel.

2.4.4 Drug releasing studies

Doxorubicin (DOX) was selected as a model drug to evaluate the drug loading capacity and drug release capability of the samples. The drug loading process was as follows: a drug solution with a concentration of 2 mg/mL was prepared using a PBS at pH 7.4. The absorbance of the original drug solution was measured as A_1 . The dried hydrogel was then immersed in the solution until swelling equilibrium was reached. After that, the hydrogel was removed and dried, and the absorbance of the drug solution after loading was measured as A_2 . The drug loading content was calculated based on the difference between the two absorbance values. The drug release process was as follows: the dried drug-loaded hydrogel was placed into PBS at pH 1.2 and pH 7.4, respectively. At intervals of 1 hour, 5 mL of the release medium was taken to measure the absorbance, and an equal volume of fresh blank PBS was added to maintain a constant volume. The concentration of DOX was calculated based on the absorbance measurements. Finally, the cumulative release rate of DOX was calculated according to the corresponding standard curve.

$$LC(\mu\text{g}/\text{mg})=W_e/W_m \quad (3)$$

$$Q_n(\%)=(M_t/M_0)\times 100\% \quad (4)$$

Where M_t and M_0 represent the amount of drug released at time t and the amount of drug loaded in the dry gel, respectively; LC is the drug loading capacity; and $Q_n(\%)$ is the cumulative drug release rate.

2.4.5 Cytotoxicity assay

A 100 μL suspension of 293T cells (derived from Human Embryonic Kidney 293 Cells) in good growth condition was seeded into 96-well plates at a density of 5,000–10,000 cells per well and cultured for 24 h. After cultivation, the prepared double-network hydrogel was immersed in Dulbecco's Modified Eagle Medium (DMEM, containing 10% fetal bovine serum, FBS) for 24 h to obtain the hydrogel extract. Subsequently, 2 mL of the extract was co-cultured with 293T cells for 24 h, while the control group was cultured in complete DMEM. Cytotoxicity was assessed using the CCK-8 assay. After incubation, the absorbance of each group was measured at 450 nm in the dark, and the cell viability was calculated.

3 Results and discussion

3.1 Influence of various factors on the swelling behavior of hydrogels

3.1.1 The influence of β -CEA content on the hydrogels swelling performance

Fig. 1 shows the swelling curves of hydrogels with different β -CEA contents (as a percentage of NIPAAm mol%) of 5% (0.0637 g), 10% (0.1274 g), 15% (0.1911 g), 20% (0.2548 g), and 25% (0.3185 g) in a PBS at pH=7.4 and 25 °C.

Based on 1 g of NIPAAm, the amounts of the other components are shown in Table 1 (mass fraction of SA relative to NIPAAm; mass fraction of MBA relative to the total mass of NIPAAm and β -CEA; mass fraction of KPS relative to the total mass of NIPAAm and β -CEA; mass fraction of Ca^{2+} relative to SA; mass fraction of D-gluconolactone relative to SA).

Table 1 The contents of all components except β -CEA

Fig. 1 Swelling kinetic curves of hydrogels with different β -CEA contents (pH=7.4, T=25°C)

As shown in Fig. 1, all samples exhibited similar swelling kinetic behavior: in the initial swelling stage (first 50 minutes), the swelling rates were similar and mainly controlled by the diffusion rate of water molecules; subsequently, a rapid swelling stage occurred within 200 minutes; finally, equilibrium was approached after approximately 600 minutes.

When the β -CEA content increased from 5% to 20%, the equilibrium swelling degree of the hydrogel showed a continuous upward trend, reaching a maximum value of 16.9 at 20%. This is because the β -CEA molecular chains contain a large number of carboxyl groups (-COOH). In the PBS at pH 7.4, these carboxyl groups ionize and convert into negatively charged carboxylate ions (-COO⁻). On the one hand, -COO⁻ is a strongly hydrophilic group, which enhances the hydrophilicity of the hydrogel; on the other hand, the electrostatic repulsion between the fixed negative charges on the polymer chains further expands the three-dimensional network, allowing it to accommodate more water molecules.[16] Therefore, increasing the β -CEA content within a certain range can effectively improve the water absorption and swelling capacity of the hydrogel. However, when the β -CEA content further increased to 25%, the equilibrium swelling degree decreased to 14.5, which is comparable to that of the sample with low β -CEA content. This is because the excess β -CEA molecules contain a large number of carboxyl groups, which tend to form strong intermolecular hydrogen bonds with each other as well as with amide groups. As a result, the network structure becomes uneven and dense, the effective pore size decreases, and the expansion of polymer chains as well as the entry of water molecules are restricted, thereby inhibiting swelling.[17]

In summary, the introduction of β -CEA can enhance the swelling performance of the hydrogel by increasing hydrophilic groups and electrostatic repulsion. However, its content has an optimal range (20%

in this study). Beyond this range, an excessively high monomer actually hinder the swelling process.

3.1.2 The influence of SA content on the hydrogels swelling performance

Fig. 2 shows the swelling curves of hydrogels with different SA contents at pH 7.4 and T=25 °C.

Table 2 The contents of all components except SA

Table 3 The contents of SA, Ca²⁺ and GDL

Fig. 2 Swelling kinetic curves of hydrogels with different SA contents (pH=7.4, T=25°C)

From the Fig. 2, within the concentration range of 4% to 18%, the swelling degree of the hydrogel rises sharply in the first 200 minutes. And in the range of 4% to 16%, as the amount of SA goes up, the swelling degree keeps increasing, peaking at 18.0 when the SA content hits 16%. This is because SA chains carry plenty of carboxyl groups, which turn into negatively charged carboxylate ions once in the PBS. The increase in SA content introduces more negative charges into the network.[18] On one hand, the strong affinity between these charged groups and water molecules directly enhances the hydrophilicity of the hydrogel; on the other hand, the increased electrostatic repulsion between chains causes the network structure to become more expanded and the pore size to enlarge, thereby accommodating more water and leading to a higher swelling degree.[19] However, when the SA content is further increased to 18%, the equilibrium swelling degree becomes lower than that of the sample with 16% SA. This may be because the excess SA molecular chains, which are rich in carboxyl and hydroxyl groups, readily form a dense hydrogen bond network between the chains, creating a physical barrier.[20] Although hydrophilicity continues to increase, the overly dense network structure limits network expansion, becoming the dominant factor for the decreased swelling. This indicates that a higher SA content does not necessarily lead to a higher swelling degree; instead, it may reduce it. There exists an optimal range, beyond which performance may decline. In this study, the optimal SA content is 16%.

3.1.3 The influence of MBA content on the hydrogels swelling performance

Fig. 3 shows the swelling curve of with different MBA contents at pH 7.4 and 25 °C, and Table 4 lists the amounts of the other components.

Table 4 The contents of all components except MBA

Fig. 3 Swelling kinetic curves of hydrogels with different MBA contents (pH=7.4, T=25°C)

As shown in Fig. 3, with the increase in MBA content (from 1% to 8%), the final equilibrium swelling degree of the hydrogels exhibits a significant decreasing trend. Moreover, the time required for the hydrogels to reach swelling equilibrium also shortens as the content increases (the curves for MBA contents above 4% level off after approximately 600 min, while those for 1% and 2% still show an upward trend).

This is because MBA, as a chemical crosslinker, directly affects the crosslinking density of the hydrogel. The higher the MBA content, the greater the degree of crosslinking, the tighter the connections between polymer chains, and the smaller the pore size formed. Therefore, an appropriate crosslinker density facilitates the formation of a uniform network, while either too high or too low a density leads to network inhomogeneity. Excessively high crosslinker density causes local aggregation, whereas too low a density results in defects or even dissolution and dispersion.[21] It is precisely this characteristic that, under high crosslinking conditions, leads to an inhomogeneous and overly dense network, which physically restricts water molecule penetration. Although the hydrogel with 1% MBA exhibits a higher swelling ratio compared to others, its crosslinking density is insufficient, making it prone to breakage upon swelling and resulting in local defects. Therefore, to achieve an optimal balance between crosslinking density and swelling degree, a crosslinker concentration of 2% is preferable, which provides adequate crosslinking density while still retaining a certain level of swelling performance.

3.1.4 The influence of KPS content on the hydrogels swelling performance

Fig. 4 shows the swelling curve of hydrogels made with different KPS contents, and Table 5 lists the amounts of the other components.

Table 5 The contents of all components except KPS

Fig. 4 Swelling kinetic curves of hydrogels with different KPS contents (pH=7.4, T=25°C)

Hydrogels with different KPS contents all exhibit an increase in swelling degree over time. After 600 min, the swelling degree of hydrogels with higher KPS content gradually reaches equilibrium. As shown in Fig. 4, the swelling degree first increases and then decreases with increasing KPS concentration. The reason for this change may be that the initiator concentration has complex, even competing effects on the polymerization process and the formation of the final network structure. At low concentrations, insufficient initiation may lead to an imperfect network; at moderate concentrations, the initiation efficiency and chain growth are good, forming a network structure conducive to water absorption, thereby achieving a higher swelling degree (with 6% being optimal). When the concentration is too high, excessive initiator leads to shorter polymer chains and increased chain termination, resulting in more polymer chains that are mainly short, which severely restricts the entry of water molecules and causes a significant decrease in the swelling degree.[22] Therefore, reasonable control of the initiator content plays a crucial role in preparing hydrogels with specific swelling properties.

3.1.5 The influence of Ca^{2+} content on the hydrogels swelling performance

Fig. 5 shows the swelling curve of the hydrogels with different Ca^{2+} contents, and Table 6 lists the amounts of the other components.

Table 6 The contents of all components except Ca^{2+}

Fig. 5 Swelling kinetic curves of hydrogels with different Ca^{2+} contents (pH=7.4、T=25°C)

As shown in Fig. 5, hydrogels with different Ca^{2+} contents all exhibit an increase in swelling degree

over time, reaching an equilibrium swelling state at 400 minutes. The swelling degree decreases with increasing Ca^{2+} content, with the most pronounced swelling observed at a Ca^{2+} content of 0.1%, reaching a swelling degree of 20.0.

Ca^{2+} acts as a physical crosslinker in the hydrogel, forming ionic bridges with anionic groups on polymers like SA to build up the three-dimensional network.[23] The Ca^{2+} content affects the crosslinking density. Swelling degree and crosslinking density usually show a negative correlation.[24] Although the swelling performance at Ca^{2+} contents of 0.1% and 0.2% is higher than that at 0.3%, these lower concentrations are more likely to result in a loosely crosslinked network or even an incomplete three-dimensional network structure. In such cases, a sparser network allows more water molecules to enter, leading to a higher swelling degree. Therefore, the higher swelling degrees observed at 0.1% and 0.2% Ca^{2+} contents cannot be considered optimal synthesis conditions. In this system, a Ca^{2+} content of 0.3% is the optimal choice. At this concentration, the crosslinked network achieved a good balance between swelling capacity and mechanical properties.

3.2 Characterization results

3.2.1 FTIR analysis

Fig. 6 shows the FTIR spectrum of the double-network hydrogel prepared under the optimal synthesis conditions (1 g NIPAAm, 20 mol% β -CEA, 0.16 g SA, 2% MBA, 6% KPS, 0.3% Ca^{2+}).

Fig. 6 FTIR spectrum of the double-network hydrogel

The peak at 3431.0 cm^{-1} corresponds to the O-H stretching vibration, which originates from the hydroxyl groups (-OH) on the polymer chains, namely the SA component in the hydrogel. This peak is broad and has a high intensity, indicating that there are a large number of hydroxyl groups in the sample.[6] The peak near 2971.3 cm^{-1} corresponds to C-H stretching vibrations.[25] The range of 1510--

1680 cm^{-1} is the characteristic region for amide I and II bands, where the strong peak near 1652.2 cm^{-1} mainly corresponds to the C=O stretching vibration, and the peak near 1545.4 cm^{-1} mainly corresponds to the N-H bending vibration.[26] The appearance of these two peaks corresponds to the -COOH and amide groups present in the sample, confirming the successful copolymerization of NIPAAm and β -CEA. The absorption peaks in the range of 1300–1000 cm^{-1} are mainly attributed to the stretching vibration of the C-O single bonds in the sugar ring of SA. The peak in the low-wavenumber region near 496.4 cm^{-1} is generally associated with skeletal vibrations, lattice vibrations, or vibrations of heavier atoms; its appearance here indicates the interaction between calcium ions and the polymer chains in this hydrogel containing metal-ion crosslinking. This spectrum demonstrates that the hydrogel is composed of amide-group-containing polymers, namely NIPAAm, β -CEA, SA, and calcium ions, confirming the successful synthesis of the double-network hydrogel.

3.2.2 SEM analysis

Fig. 7 shows SEM images of the double-network hydrogel synthesized under the optimal formulation. The images were taken after the hydrogel had reached swelling equilibrium in PBS at pH=7.4, pH=4.0, and pH=1.2 at 25 °C, followed by freeze-drying. From the images, it can be seen that at pH=7.4, the hydrogel exhibits a porous three-dimensional network structure composed of a polymer backbone and pores. These pores are interconnected, forming internal microchannels within the hydrogel. This porous structure is key to absorbing and retaining large amounts of water, and its connectivity is very important for the diffusion of water molecules inside the hydrogel as well as for the release of drugs or other substances. At pH=4.0, no obvious pore size is observed, and the network structure becomes denser. This is because some carboxyl groups are in an un-ionized state, enhancing hydrogen bonding between polymer chains and restricting network expansion. In the strongly acidic environment at pH=1.2,

the hydrogel structure further contracts, the surface becomes rough, and the pores almost disappear, indicating a high degree of carboxyl protonation, weakened electrostatic repulsion, and dominant hydrophobic interactions, leading to severe network collapse.[27] The morphological differences seen at different pH values directly reflect the pH-responsive behavior of the hydrogel.

Fig. 7 SEM images of the double-network hydrogel at pH (a) 7.4, (b) 4.0, and (c) 1.2

3.2.3 TGA analysis

Fig. 8 shows the TGA analysis results of the dried hydrogel, looking at how its thermal stability changes with temperature.

Fig. 8 TGA and DTG curves of the double-network hydrogel

Fig. 8 shows the TGA and DTG curves of the double-network hydrogel. In the low temperature stage (30 °C to 100 °C), the mass drops initially from 100% down to about 90%. This loss comes from evaporation of adsorbed or bound water in the hydrogel, and the percentage of mass loss reflects the amount of bound water present in the sample before testing. The main decomposition stage occurs between 200 °C and 400 °C, where the hydrogel undergoes a severe mass loss, with the curve dropping rapidly from 90% to below 10%. The loss in the 230 °C to 370 °C range is due to thermal decomposition of the polymer chains in the hydrogel. Different polymers have different decomposition temperatures.[19] SA and NIPAAm are the main polymer components of the hydrogel, and their decomposition temperatures are above 200 °C. In the figure, the decomposition peak between 200 °C and 300 °C corresponds to SA, while the peak between 300 °C and 400 °C corresponds to the thermal decomposition of NIPAAm. In the high temperature stage (above 400 °C), the mass loss becomes very slow and the curve levels off. At 600 °C, the remaining mass is about 8%, which may be carbon residue from polymer decomposition or inorganic salt components.[11, 26] This figure, through the changes in decomposition temperature and mass loss percentage, shows that the material has decent thermal stability. Below 200 °C,

the material remains relatively stable in terms of mass.

3.2.3 XPS analysis

The elemental composition and surface chemical state of the double-network hydrogel were characterized by XPS, as shown in Fig. 9.

Fig. 9 XPS analysis of the hydrogel: (a) full spectrum spectrum, (b) C 1s, (c) O 1s, (d) N 1s, (e) Ca 2p

The XPS survey spectrum (Fig. 9a) shows that the hydrogel surface contains four elements: C, O, N, and Ca, confirming the presence of NIPAAm, β -CEA, SA, and Ca^{2+} crosslinked structures. The C 1s spectrum (Fig. 9b) can be fitted as C–C/C–N (284.8 eV), C=O (287.5 eV), C–O–C (286.1 eV), etc., corresponding to the polymer main chain/amide bond, carbonyl group, and C–O–C bond in the alginate sugar ring. The O 1s spectrum (Fig. 9c) shows that 531.4 eV and 531.3 eV belong to C=O and C–O bonds respectively. The N 1s spectrum (Fig. 9d) at 399.5 eV/399.2 eV peaks belong to the amide group (–CONH–), indicating that NIPAAm has successfully polymerized. The Ca 2p spectrum (Fig. 9e) shows the Ca 2p_{3/2} and Ca 2p_{1/2} peaks at 347.5 eV and 350.7 eV, [17, 28, 29] confirming that Ca^{2+} forms ionic crosslinks with the carboxyl groups of SA. These XPS results further verify the chemical composition and crosslinking structure of the hydrogel.

3.3 Performance analysis

3.3.1 The pH response performance

Fig. 10 shows the swelling degree of the hydrogel under the optimal formulation as a function of time at different pH.

Fig. 10 The swelling kinetics curves of the dual-network hydrogel at different pH values (T=25°C;

pH=1.2, 4.0 and 7.4)

Under all tested pH conditions, the swelling degree increases with time and eventually levels off to

reach equilibrium swelling. In strongly acidic pH 1.2 and weakly acidic pH 4.0, the equilibrium swelling degrees are both very low, though the value at pH 4.0 is slightly higher than that at pH 1.2. At pH 7.4, the equilibrium swelling degree rises sharply to a maximum of over 21. The swelling degree shows a clear trend of increasing as the pH goes up. This responsiveness mainly comes from the carboxyl groups ($-\text{COOH}$) present in β -CEA and SA, both of which are components of the hydrogel. The reason for this behavior is that under strongly and weakly acidic conditions, most of the carboxyl groups remain protonated, leaving very few negative charges on the polymer chains. In contrast, at pH 7.4, most of the carboxyl groups become deprotonated and turn into negatively charged carboxylate ions ($-\text{COO}^-$).^[30] As a result, the number of negative charges in the network greatly increases. This shows that the pH responsiveness of the hydrogel is primarily governed by the degree of ionization of the carboxyl groups, which in turn strongly depends on the environmental pH. This significant pH sensitivity enables targeted drug delivery in different pH environments along the gastrointestinal tract.

3.3.2 The temperature response performance

Fig. 11 shows that at temperatures of 25 °C, 29 °C, 33 °C, 37 °C, and 42 °C, the swelling degree of the hydrogel increases with time and levels off after 600 to 800 minutes, reaching an equilibrium swelling state.

Fig. 11 The swelling kinetics curves of the dual-network hydrogel at different temperature (pH=7.4; T=25 °C, 29 °C, 33 °C, 37 °C, and 42 °C)

The swelling rate is relatively fast in the initial stage. It can be seen from the figure that as the temperature rises, the final equilibrium swelling degree of the hydrogel drops significantly. At 25 °C and 29 °C, the swelling degree reaches its highest value, over 21. At 42 °C, the equilibrium swelling degree falls to its lowest, around 17.5, and at 33 °C and 37 °C, the swelling degree also decreases with increasing temperature. This behavior occurs because the PNIPAAm component has a lower critical solution

temperature (LCST) characteristic.[31] Below the LCST, the amide groups and isopropyl side chains on the PNIPAAm segments can form many hydrogen bonds with water molecules. The polymer chains become highly hydrophilic, remain extended, and the network absorbs a large amount of water, leading to swelling. Above the LCST, as the temperature rises, the hydrogen bonding between water molecules and the polymer chains weakens.[32] At the same time, hydrophobic interactions among the isopropyl groups and other hydrophobic moieties on the NIPAAm chains become stronger. When the temperature exceeds the LCST, hydrophobic interactions dominate, the polymer chains undergo a conformational transition, squeezing water molecules out of the network. The chains collapse, the network shrinks, and the swelling degree decreases.[33] The hydrogel, which is composed of NIPAAm, β -CEA, and SA, is a composite system. Its temperature sensitivity comes mainly from NIPAAm, while SA and β -CEA contribute basic hydrophilicity and possibly charges. These factors affect the swelling degree at different temperatures and may also shift the effective LCST of PNIPAAm.[34] The temperature range shown in Fig. 11 (25 °C to 42 °C) spans both the LCST of pure PNIPAAm (around 32 °C) and physiological temperature (37 °C).

3.3.3 The deswelling properties

Fig. 12 shows the deswelling curves of the hydrogel under different temperatures and pH conditions. It can be seen from the figure that the hydrogel is pH/temperature dual-responsive, and its swelling and deswelling behaviors can be adjusted by changing the pH and temperature.

Fig.12 The swelling-deswelling kinetics curve of the dual-network hydrogel

In Fig. 12(a), after the hydrogel had been fully swollen at 25 °C and pH 7.4, it was then placed under different conditions to undergo deswelling. At 25 °C and pH = 1.2, the hydrogel rapidly undergoes intense re-swelling, and its water retention performance is extremely poor. This is because under low pH, the

polyanionic components become protonated, losing their main driving force for water uptake and maintaining the swollen state. Even though 25 °C favors the hydrophilicity of NIPAAm, the hydrogel still deswells quickly and retains very little water. In Fig. 12(b), at 42 °C and pH 7.4, the elevated temperature causes the NIPAAm component to become hydrophobic and contract, while the near-neutral pH leads to extensive ionization of the polyanionic components. The competition between these two opposing effects reaches a balance, resulting in partial deswelling of the hydrogel,[35] yet it still maintains a certain performance (retaining more than 80% of its swelling degree after 400 min). This indicates that the temperature response of the hydrogel is weaker than its pH response.

3.3.4 The repeated swelling and deswelling properties

Fig. 13 shows the swelling kinetic curves of the double-network hydrogel under different temperatures and pH conditions.

Fig. 13 The repeated swelling-deswelling kinetics curves of the dual-network hydrogel under different conditions (a) T=25°C, pH=7.4/1.2; (b) pH=7.4, T=25°C/42°C

After the hydrogel had been fully swollen at 25 °C and pH 7.4, it was then placed separately into two PBS: one at 25 °C and pH 1.2, and the other at 42 °C and pH 7.4. Once swelling equilibrium was reached (every 48 hours), both samples were transferred back into the 25 °C, pH 7.4 buffer, and this cycle was repeated. As can be seen from Fig. 13(a), when the gel was placed in the 25 °C, pH 1.2 buffer, it underwent a drastic deswelling, dropping to a swelling degree of around 1. Then, over 48 hours, it slowly re-swelled to about 19.1. After repeated deswelling and swelling cycles, the hydrogel was still able to recover to roughly 19.0. In Fig. 13(b), when the gel was placed in the 42 °C, pH 7.4 buffer, its swelling degree decreased from 19.7 to 16.4 over 48 hours, a difference of 3.3. After repeated cycling, it consistently returned to a swelling degree of about 19.4. These results demonstrate that within the range

of 25 °C to 42 °C and pH 1.2 to 7.4, the hydrogel exhibits decent reversible deswelling-swelling performance, can be used multiple times, and shows reproducible swelling/deswelling cycles.

3.3.5 Drug release studies

Fig. 14 Standard curves of DOX at different pH values: (a) pH=4.0, (b) pH=7.4

The maximum UV absorption wavelength of doxorubicin hydrochloride (DOX) is 480 nm,[36] At this wavelength, standard curves for DOX at different pH values were constructed with mass concentration as the horizontal axis and absorbance as the vertical axis. Linear fitting of the data points gave the standard equations, as shown in Fig. 14.

Fig. 15 Cumulative drug release rate curves of the double-network hydrogel over time at different temperatures and pH values. (a) pH=7.4, T=25°C/37°C/42°C; (b) T=25°C, pH=7.4/4.0/1.2

DOX was used as a model drug to evaluate the drug loading and release behavior of the double-network hydrogel prepared with the optimal formulation. The drug loading measurement showed that the hydrogel achieved a loading capacity (LC) of 149.99 µg/mg for DOX, indicating good drug loading capability.

As shown in Fig. 15(a), at a fixed pH of 7.4, the cumulative drug release curves at 25 °C, 37 °C, and 42 °C were compared. The drug release exhibited a clear temperature dependence: the cumulative release rate was lowest at 25 °C (about 34.3%), increased at 37 °C (about 38.1%), and reached the highest at 42 °C (about 43.2%). This is due to the thermosensitive nature of the PNIPAAm component. Below the LCST, the polymer chains are hydrophilic and extended, the network is loose, and drug diffusion is slow. Above the LCST, the chains become hydrophobic and collapse, the network shrinks, and an “expulsion” effect,[37] accelerates drug release. As shown in Fig. 15(b), at a constant temperature of 25 °C, the release behavior displayed a clear pH responsiveness. In acidic environments at pH 1.2 and

4.0, the release was faster and more complete (about 103.6% and 62.7%, respectively), while under neutral conditions at pH 7.4, the release was slower. This originates from the ionization behavior of the carboxyl groups in β -CEA and SA at different pH values. Under acidic conditions, the carboxyl groups are protonated,[38] which weakens the electrostatic interaction between the carboxyl groups and the positively charged DOX, thus promoting rapid drug release.[39] Under neutral conditions, the carboxyl groups are ionized, electrostatic repulsion increases, the network is highly swollen, and strong electrostatic adsorption occurs between the positively charged DOX and the negatively charged network backbone, thereby slowing down the release.[40] In summary, this hydrogel exhibits good temperature/pH dual responsiveness, enabling intelligent regulation of drug release behavior, and shows promising application prospects in targeted and triggered drug delivery systems. Moreover, the release reaches equilibrium at around 50 hours, demonstrating good sustained release characteristics that are favorable for long-term drug delivery.

3.4 Cell toxicity test

The cytotoxicity of the hydrogel extract was evaluated using 293T cells.[41] The hydrogel extract was diluted to different concentrations (155 mg/mL, 77.5 mg/mL, and 62 mg/mL, corresponding to dilution factors of 1 $\times$, 0.5 $\times$, and 0.4 $\times$, respectively) and then co-cultured with the cells. Cell viability was determined using the CCK-8 assay.[33] The results showed that at the diluted concentrations of 62 mg/mL and 77.5 mg/mL, cell viability remained above 95%, with no significant difference from the control group ($p > 0.05$). Even at the concentration of 155 mg/mL, cell viability was still higher than 90%. These findings indicate that the hydrogel extract has no obvious toxicity toward 293T cells within the tested concentration range and exhibits good biocompatibility.

Fig. 16 293T cell viability

4 Conclusion

In this study, a temperature/pH dual-sensitive double-network hydrogel was prepared by incorporating N-isopropylacrylamide (NIPAAm) and β -CEA as copolymer monomers with sodium alginate (SA) via a full-interpenetrating network technique. The structure, swelling performance, pH sensitivity, temperature sensitivity, repeated deswelling-swelling behavior, and drug release properties of the hydrogel were investigated. Through optimization of the experimental conditions, it was found that the optimal synthesis conditions for the double-network hydrogel are as follows: β -CEA content of 20 mol%, SA content of 16 wt%, MBA content of 2 wt%, KPS content of 6 wt%, and Ca^{2+} content of 0.3 wt%. Under gradually increasing temperatures, the swelling degree of the synthesized double-network hydrogel decreased accordingly, indicating that this double-network hydrogel exhibits obvious thermosensitivity. At a constant temperature of 25 °C, the swelling degrees of the hydrogel at pH 1.2 and 4.0 were relatively low (0.68 and 2.73, respectively), whereas at pH 7.4 the swelling degree increased significantly to 21.8, demonstrating clear pH sensitivity. Drug release tests using DOX as a model drug confirmed that this full-interpenetrating double-network hydrogel has excellent drug loading and release performance, with a maximum drug loading capacity of 149.99 $\mu\text{g}/\text{mg}$. The release profiles exhibited good sustained release characteristics, which are favorable for long-term drug delivery. Moreover, the drug release showed excellent temperature/pH dual sensitivity: at pH 7.4, the cumulative drug release rate increased from 34.3% to 43.2% as the temperature rose from 25 °C to 42 °C; at 25 °C, the cumulative release rate increased from 34.3% to 103.6% as the pH decreased from 7.4 to 1.2. This enables the hydrogel to meet different administration environments and targeted release requirements, achieving intelligent regulation of drug release behavior. Cytotoxicity test results showed that the hydrogel extract had no obvious toxicity toward 293T cells within the tested concentration range, with cell viability above

90% in all cases, indicating good biocompatibility. In summary, this double-network hydrogel holds promising application prospects in the field of smart drug delivery materials.

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Declarations

Competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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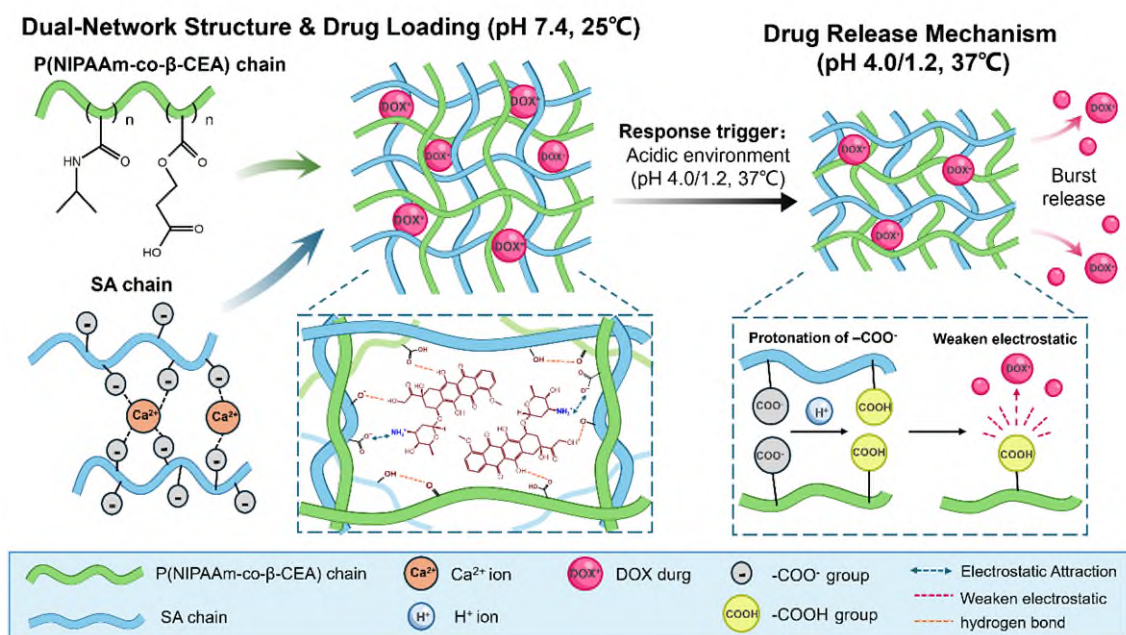
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Graphical Abstract

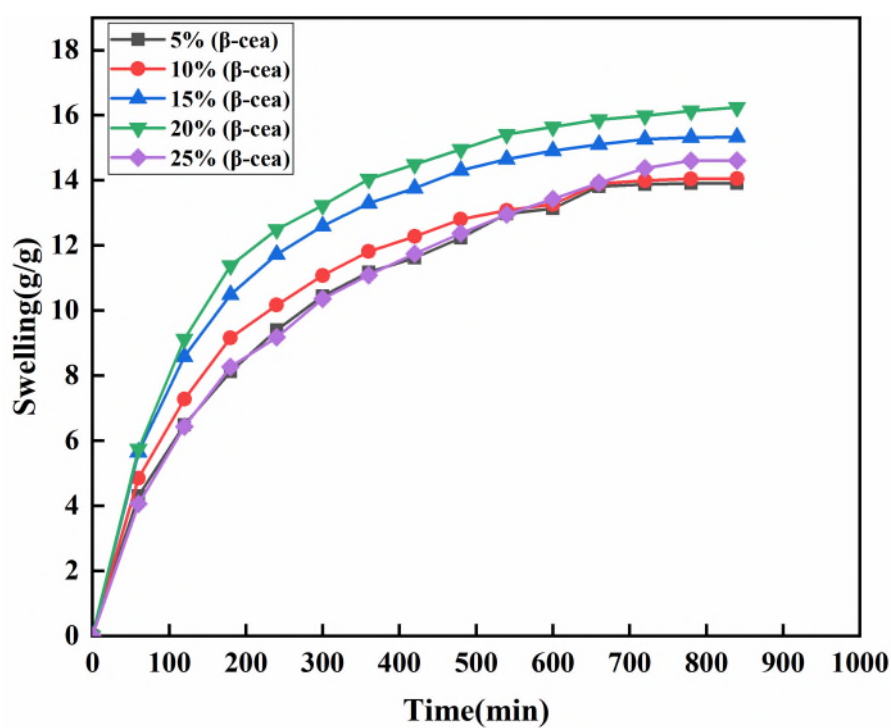


Fig. 1 Swelling kinetic curves of hydrogels with different β-CEA contents (pH=7.4, T=25°C)

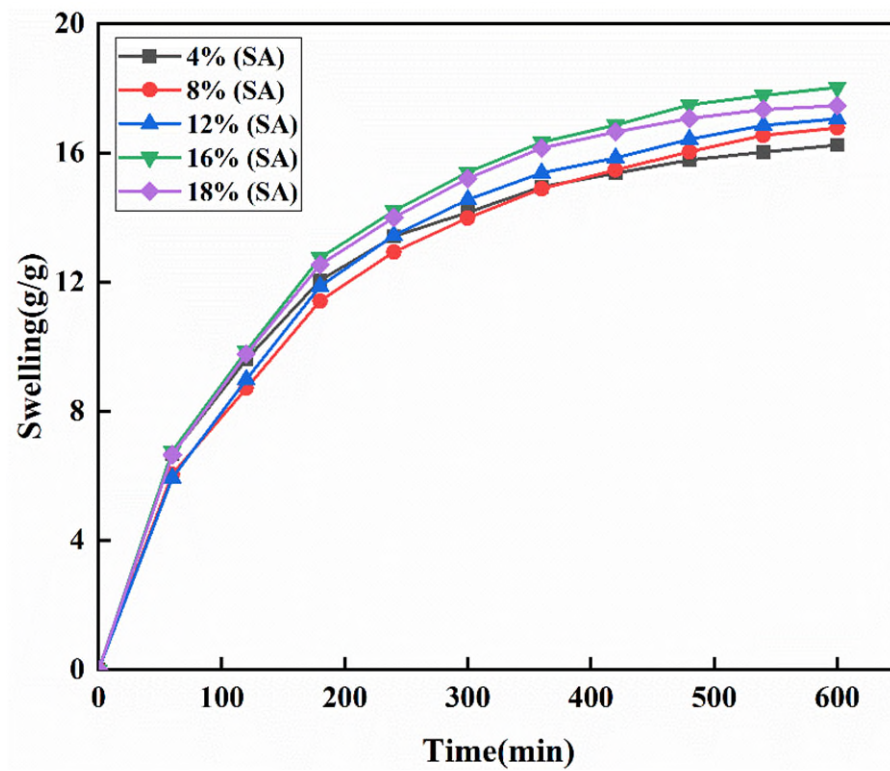


Fig. 2 Swelling kinetic curves of hydrogels with different SA contents (pH=7.4, T=25°C)

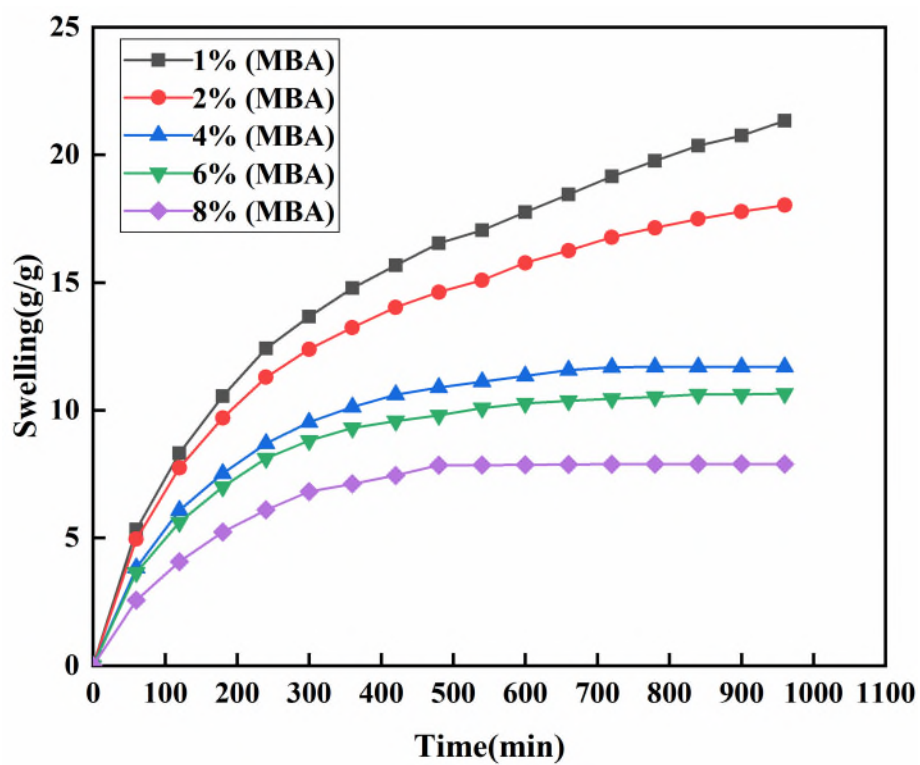


Fig. 3 Swelling kinetic curves of hydrogels with different MBA contents (pH=7.4, T=25°C)

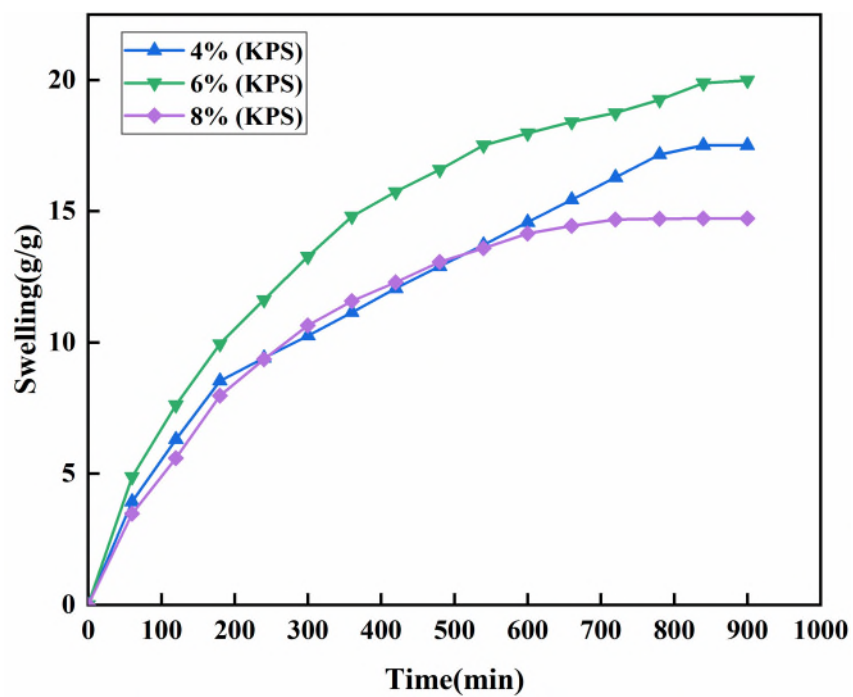


Fig. 4 Swelling kinetic curves of hydrogels with different KPS contents (pH=7.4, T=25°C)

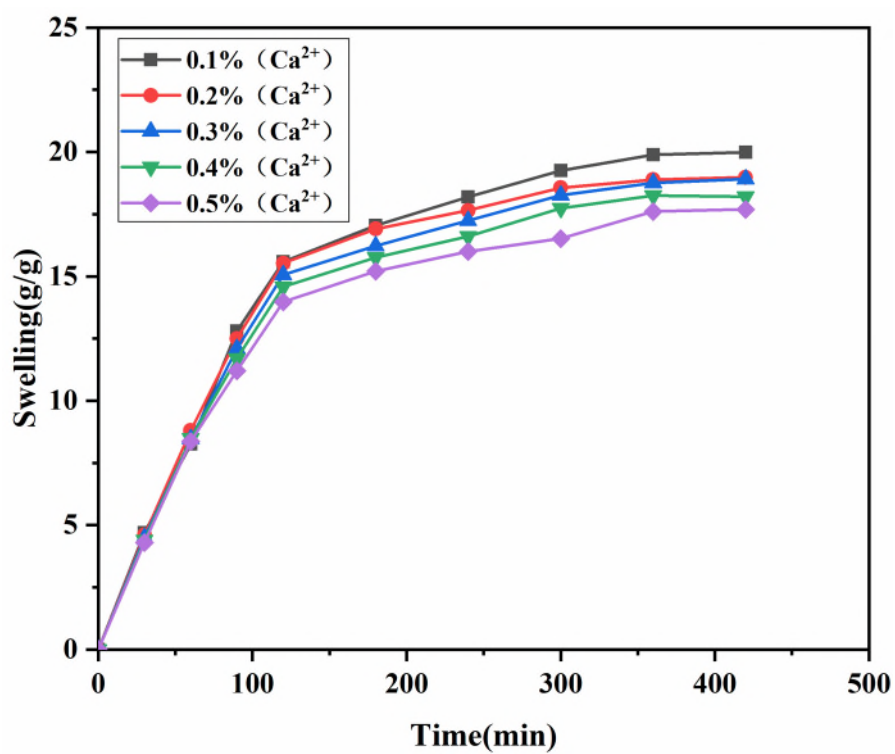


Fig. 5 Swelling kinetic curves of hydrogels with different Ca^{2+} contents (pH=7.4, T=25°C)

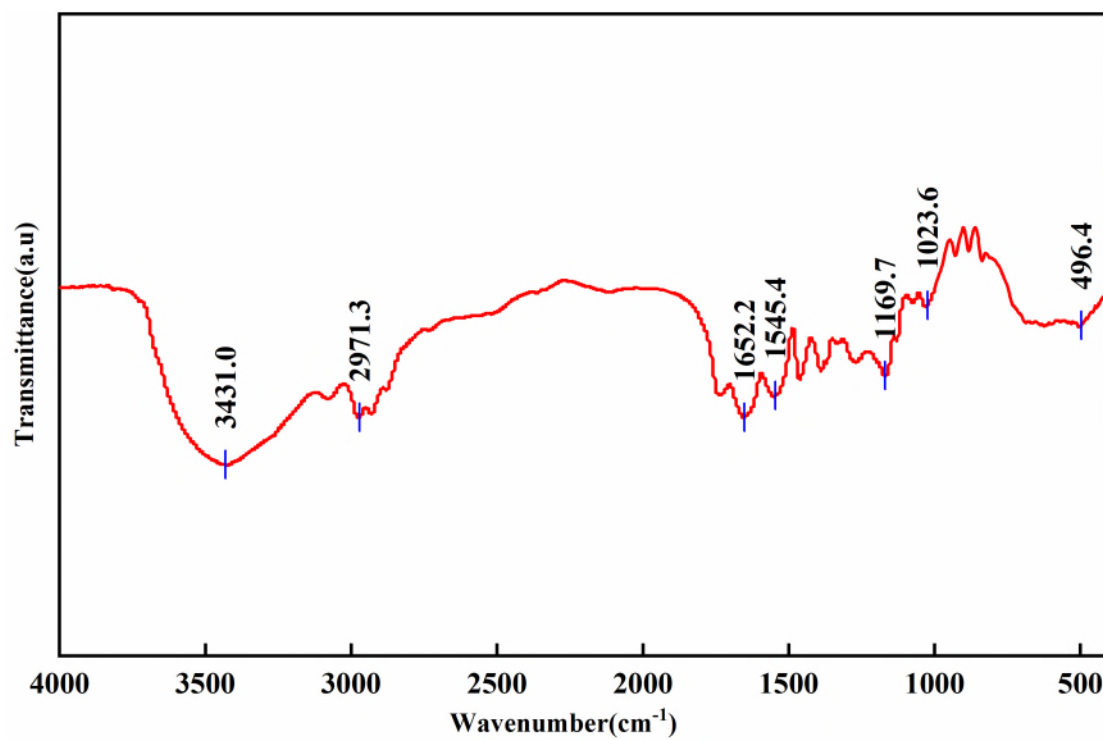


Fig. 6 FTIR spectrum of the double-network hydrogel

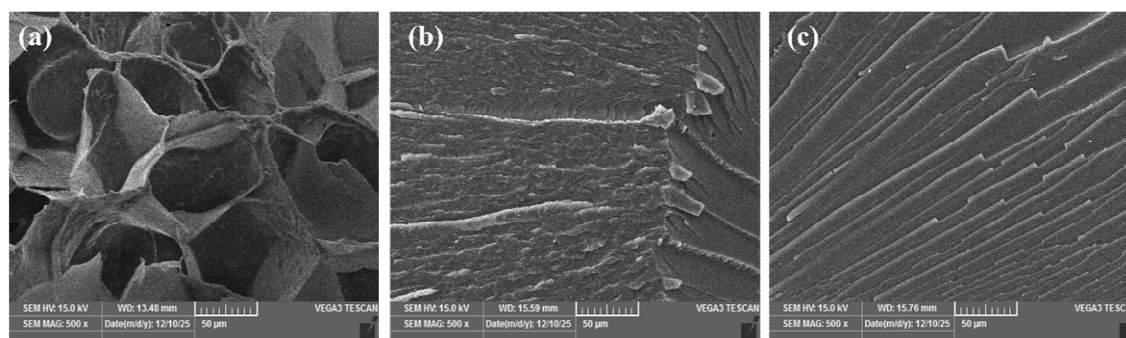


Fig. 7 SEM images of the double-network hydrogel at pH (a) 7.4, (b) 4.0, and (c) 1.2

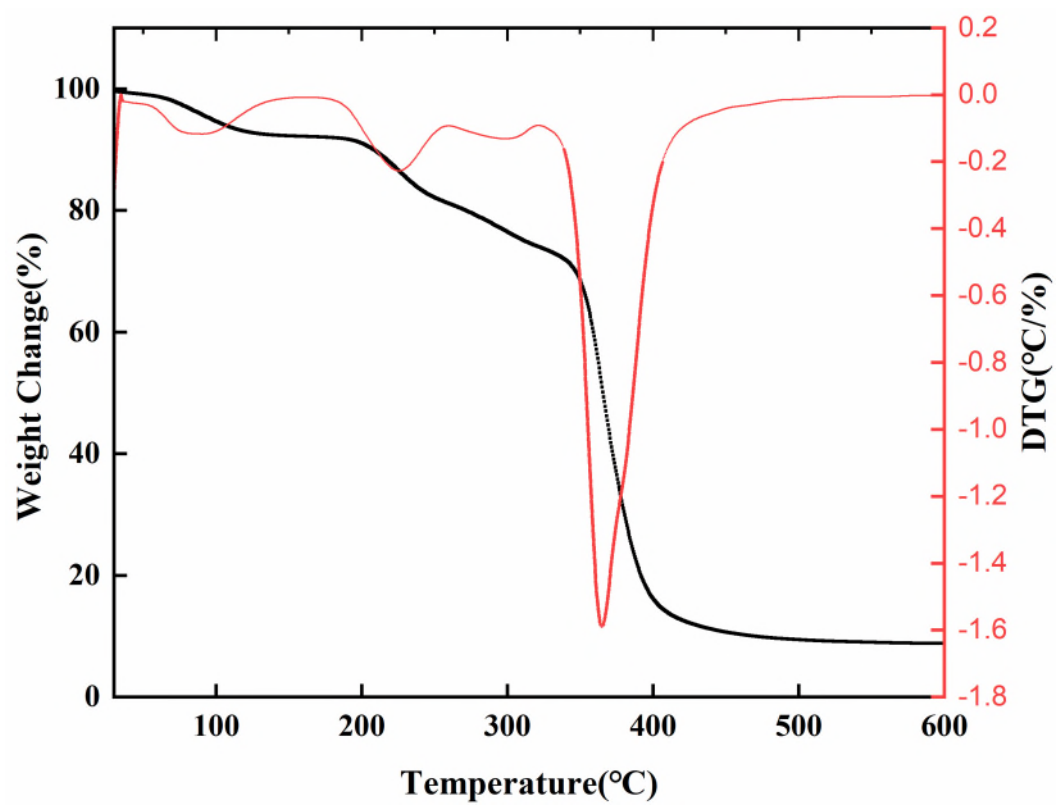


Fig. 8 TGA and DTG curves of the double-network hydrogel

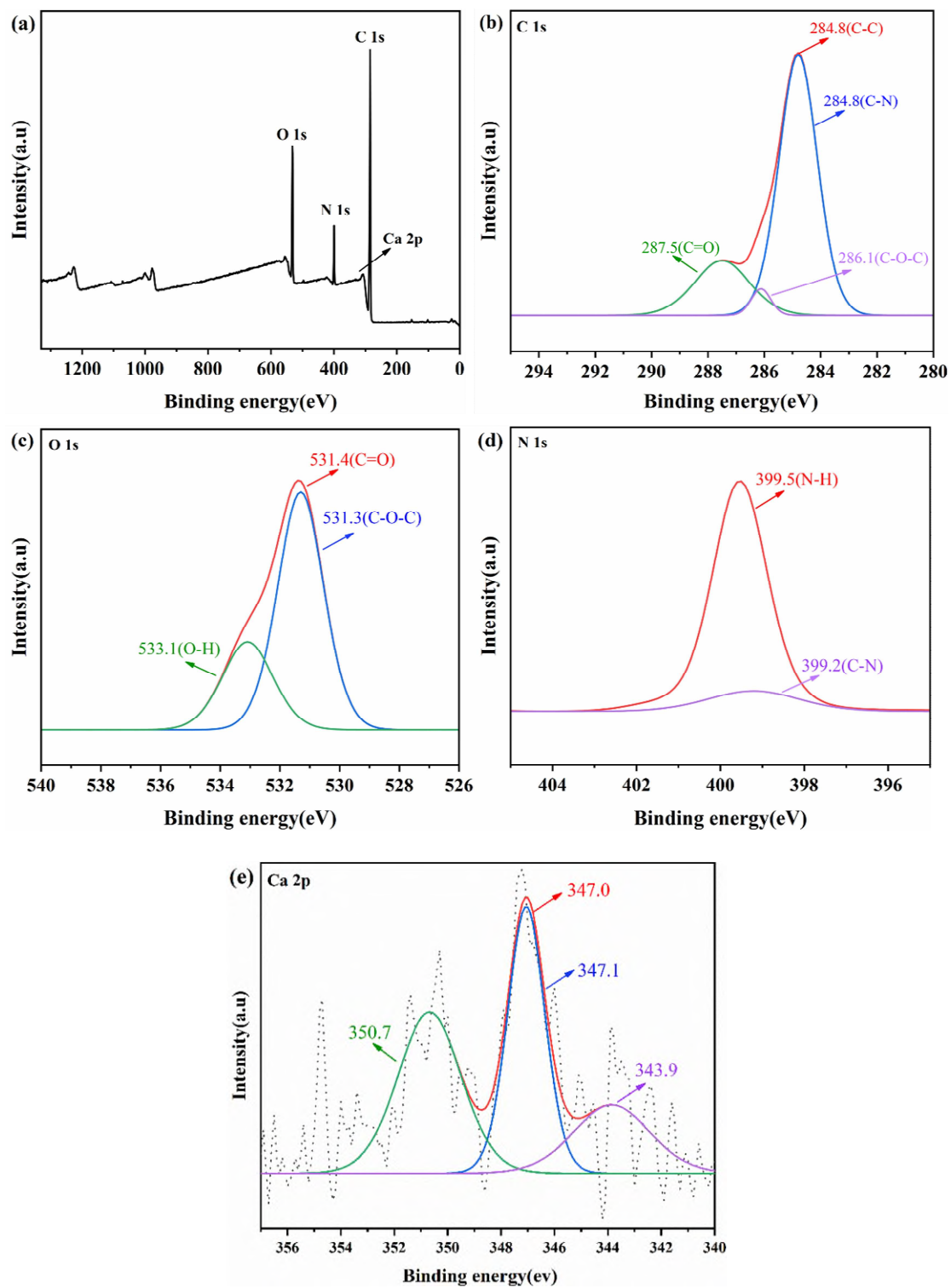


Fig. 9 XPS analysis of the hydrogel: (a) full spectrum spectrum, (b) C 1s, (c) O 1s, (d) N 1s, (e) Ca 2p

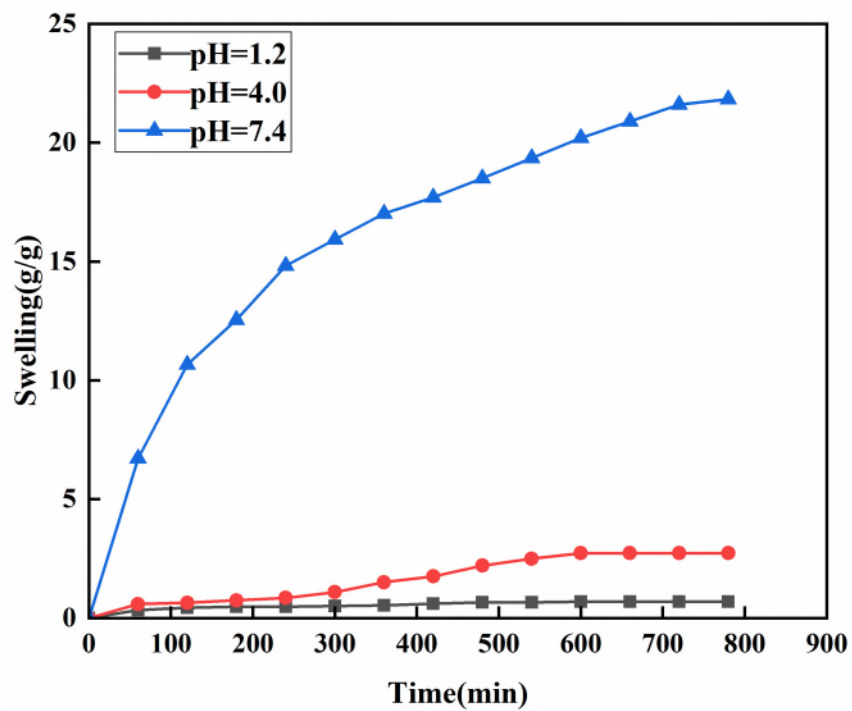


Fig. 10 The swelling kinetics curves of the dual-network hydrogel at different pH values ($T=25^{\circ}\text{C}$;
pH=1.2, 4.0 and 7.4)

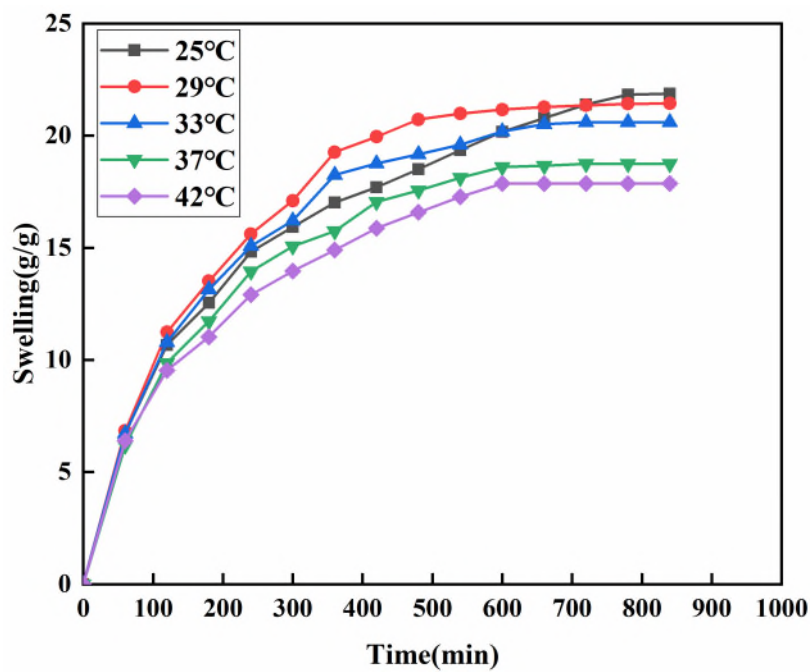


Fig. 11 The swelling kinetics curves of the dual-network hydrogel at different temperature (pH=7.4;
 $T=25^{\circ}\text{C}$, 29°C , 33°C , 37°C , and 42°C)

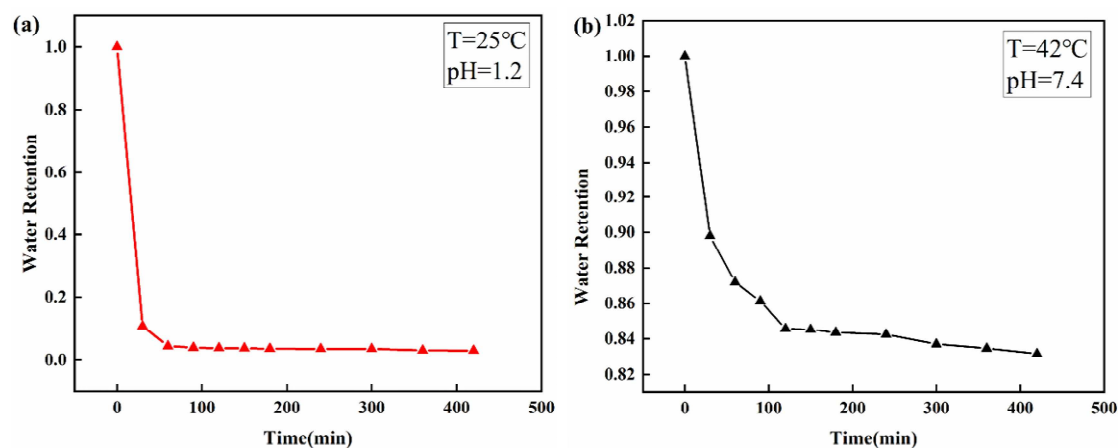


Fig.12 The swelling-deswelling kinetics curve of the dual-network hydrogel

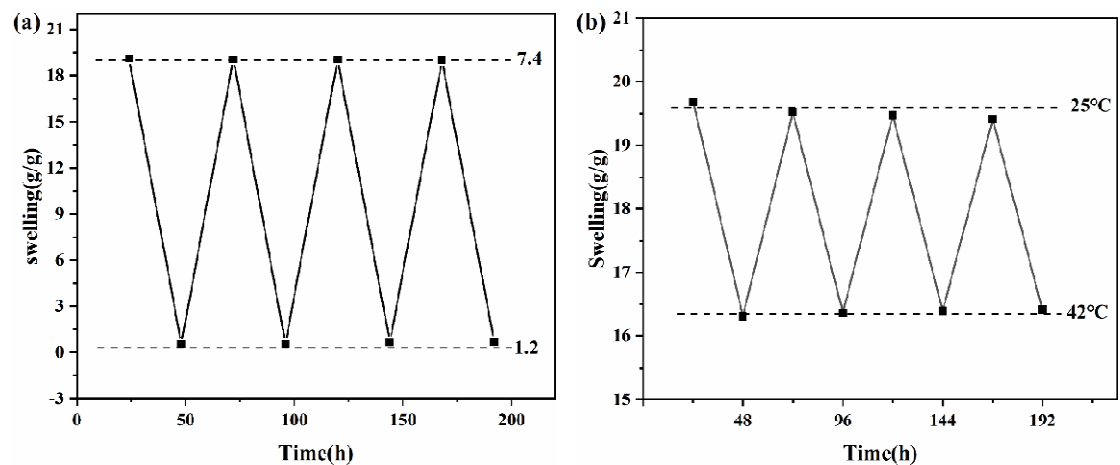


Fig. 13 The repeated swelling-deswelling kinetics curves of the dual-network hydrogel under different conditions (a) $T=25^{\circ}\text{C}$, $\text{pH}=7.4/1.2$; (b) $\text{pH}=7.4$, $T=25^{\circ}\text{C}/42^{\circ}\text{C}$

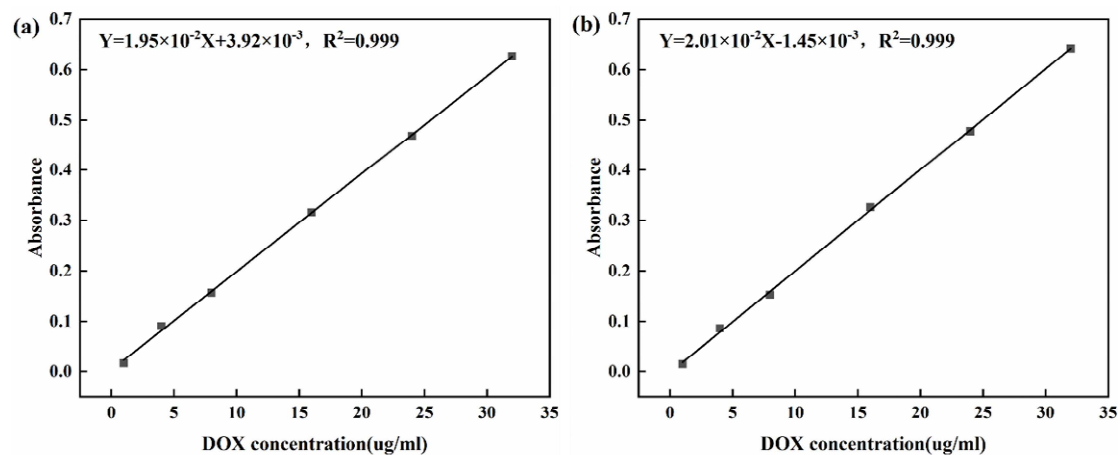


Fig. 14 Standard curves of DOX at different pH values: (a) $\text{pH}=4.0$, (b) $\text{pH}=7.4$

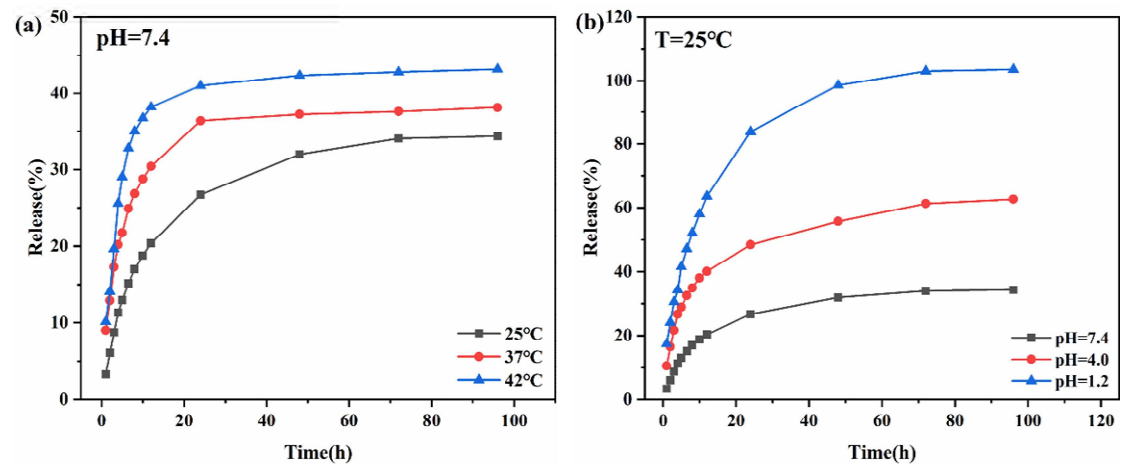


Fig. 15 Cumulative drug release rate curves of the double-network hydrogel over time at different

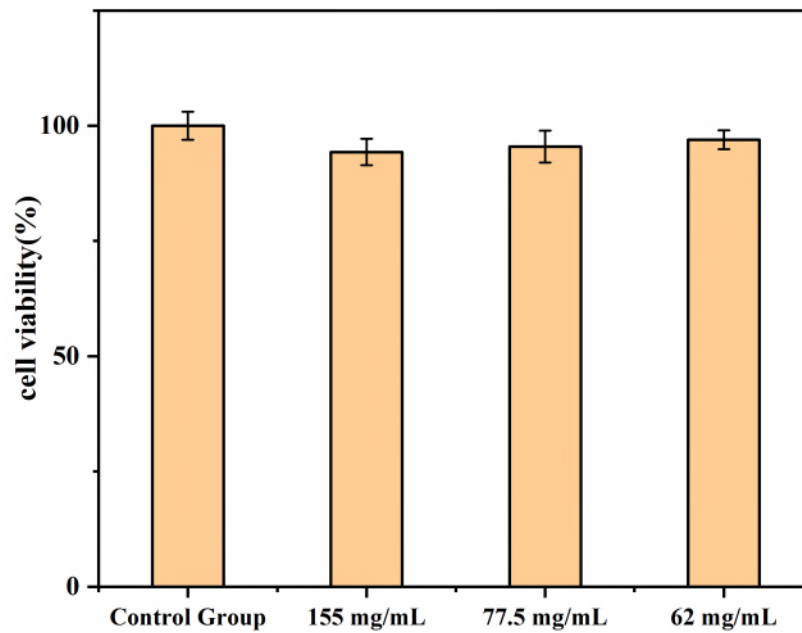


Fig. 16 293T cell viability

Table 1 The contents of all components except β -CEA

Sample	SA	MBA	KPS	Ca ²⁺	GDL
Content	4 %	2 %	2 %	0.1 %	0.375 %
Amount	0.04 g	0.0212 g	0.0212 g	0.04 mg	0.15 mg

Table 2 The contents of all components except SA

Sample	β -CEA	MBA	KPS
Content	20%	2%	2%
Amount	0.2548 g	0.0212 g	0.0212 g

Table 3 The contents of SA, Ca²⁺ and GDL

The content of SA	The amount of SA	The amount of Ca ²⁺	The amount of GDL
4%	0.04 g	0.04 mg	0.15 mg
8%	0.08 g	0.08 mg	0.3 mg
12%	0.12 g	0.12 mg	0.45 mg
16%	0.16 g	0.16 mg	0.6 mg
18%	0.18 g	0.18 mg	0.675 mg

Table 4 The contents of all components except MBA

Sample	β -CEA	SA	KPS	Ca ²⁺	GDL
Content	20%	16%	2%	0.1%	0.375%

Amount	0.2548 g	0.16 g	0.0212 g	0.04 mg	0.6 mg
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Table 5 The contents of all components except KPS

Sample	β -CEA	SA	MBA	Ca^{2+}	GDL
Content	20%	16%	2%	0.1%	0.375%
Amount	0.2548g	0.16g	0.212g	0.04mg	0.6mg

Table 6 The contents of all components except Ca^{2+}

Sample	β -CEA	SA	MBA	KPS	GDL
Content	20%	16%	2%	6%	0.375%
Amount	0.2548g	0.16g	0.025g	0.0752g	0.6mg