



Ultrasonic assisted microwave synthesis of poly (Chitosan-co-gelatin)/polyvinyl pyrrolidone IPN hydrogel

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ABSTRACT

The hydrogels based on gelatin cross-linked with chitosan (CS) and polyvinyl pyrrolidone (PVP) were synthesized using microwave and ultrasonic coupling technique in this study. This interpenetrating polymer network (IPN) hydrogels were cross-linked by glutaraldehyde and 1,2-Epoxy-4-vinylcyclohexane. The presence of function groups in the structure of hydrogel films were confirmed using Fourier transform infrared spectroscopy (FT-IR), thermal stability was measured by DSC, and the swelling behaviors were measured gravimetrically in distilled water at the temperature of 27 °C. At last, the mechanical properties were tested. The results showed that the hydrogel prepared with microwave and ultrasonic exhibited the highest tensile strength (86.68 MPa), comparing with the hydrogel prepared with traditional method and only microwave reactive field. The FT-IR and XRD results showed that the chemical reactions occurred between the $-NH_2$ of chitosan and the $-COOH$ of gelatin, and the introduction of ultrasound can improve the reaction rate. The hydrogel film gained in microwave and ultrasonic coupling field has the best combination properties. Therefore, the new microwave-ultrasonic coupling technique is the potential technology to prepare the new hydrogel due to less synthesis time.

1. Introduction

Hydrogels are three-dimensional cross linked network structures that are able to swell in water or other fluid without dissolving. Because of its high moisture content, excellent flexibility and outstanding viscoelasticity, the hydrogel has been widely used in drug controlled release, artificial organs, material separation, tissue engineering and so on. Polyacrylic acid derivatives, polyacrylamide, polyvinyl alcohol and other hydrophilic polymers with some long train structures are used to produce hydrogels that are the raw materials from petroleum based chemicals which are wastage materials. Therefore, the natural polymers are available in large quantities in nature and these biopolymers are also biodegradable and non-toxic, so many attempts have been made to develop new biopolymer based hydrogels, such as gelose, giantarum, protein [1–3]. The structural versatility available in synthetic hydrogels has given them distinctive properties, which in turn have enhanced their practical utility. Therefore, a combination of natural and synthetic polymers can modify the properties of hydrogel.

The polymer for preparing the hydrogel must meet the following conditions, one is the characteristic network structure, such as the distinct network of hydroxyethyl methacrylate (HEMA), poly(N-isopropyl-acrylamide) and its derivative, poly (N-vinyl-pyrrolidone) (PVP)

and so on [4–7], and the other is plenteous hydrophilic groups in its main or side chain. Therefore, the hydrogel materials based on the peptide, protein and other natural biopolymers attend much attention [8,9], such as chitin and collagen. Chitin, a poly- β -1,4-N-acetylglucosamine, is one of most abundant biopolymers in nature, and it is the main component of the exoskeleton of arthropods and a structural polysaccharide in fungal cell walls, such as insects, arachnids and crustaceans [10]. And chitosan (β -1,4)-2-amino-2-deoxy-D-glucose) is the deacetylated product of chitin. Most of the naturally occurring polysaccharides, e.g. cellulose, dextran, pectin, alginic acid, agar, agarose and carragenans, are neutral or acidic in nature, while chitin and chitosan are examples of highly basic polysaccharides. As the only natural cationoid polysaccharides, chitin and chitosan has been widely used as a biocompatible material, such as artificial skin [11], scaffold materials [12], coating materials [13], drug delivery [14], wound dressing [15], and so on.

As an important component of the extracellular matrix, collagen guarantees the spatial structure and biological properties of the extracellular. Its special three-dimensional helical skeletal structure, and the hydrophilic and hydrophobic groups of the amino acid residue ensures the collagen can be used the raw material to prepare hydrogel as drug carrier, cell culture and body repair. Gelatin is a biodegradable

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polymer, which can be obtained by thermal denaturation or physical and chemical degradation of collagen, so it exhibits excellent biocompatibility, plasticity and adhesiveness, good film and particle forming material. Its most frequent used in the biomedical field include hard and soft capsules, microspheres, sealants for vascular prostheses, wound dressing and adsorbent pad for surgical use, as well as three dimensional tissue regeneration [16]. However, sometimes the applications of pure gelatin hydrogel films are limited because they exhibit relatively low mechanical properties. Nowadays, some methods have been developed to improve the strength of hydrogel, such as increasing the crosslinking density, adding fibrous materials, copolymerization with the monomer of better mechanical performance. And the mentioned solutions would damage the intrinsic properties of hydrogel, such as hydrophilicity, transparency, penetration and so on. Formation of interpenetrating polymer network (IPN) is a way for the blending two polymers, which can be possible to improve the mechanical properties of the compound materials [17,18]. It is called a full-IPN when the both polymers are cross linked, and the structure is called semi-IPN, if only one is cross linked in the presence of another cross linked without any chemical bonds between the two polymers.

The radiation synthesis is a kind of new technology which was high effective and eco-friendly. And because of its high temperatures for attack the solution with relatively short times and thus creating reactions faster than conventional thermal conditions, microwave irradiation has been used for preparation of the hydrogel in several researches [19,20]. Such as, ultrasound assisted has been investigated for the initiation of polymerization reactions to prepare polymer hydrogel since it can be used for both dispersion of monomer droplets and for generation of free radicals [21,22]. The use of ultrasonic irradiations during the hydrogel synthesis not only can control the molecular weights, but also can improve the swelling ratio [23]. Li et al. found ultrasonic wave can loosen the agglutination of collagen in the water, and increase the yields when extract the bovine tendon collagen with enzyme [24]. Wang et al. introduce the ultrasound wave into the fabrication of lignin-g-poly (acrylamide-co-N-Isopropyl acrylamide) hybrid hydrogel, and the hydrogel has excellent sorbent for dye removal from wastewater [25]. Liao et al. prepared the gelatin hydrolysis by collagenase, the results found that the reaction rate constant and enzyme inactivation constant were increased by 27.5% and 27.8% with ultrasonic pretreatment [26]. Therefore, the polymerization by solo microwave irradiation and ultrasound wave method can improve the efficiency.

In this present study, the P(CS-co-gelatin)/PVP interpenetrating polymer network hydrogels were synthesized via free radical polymerization by ultrasonic assisted microwave irradiation method, the hydrogels were separately prepared by using only microwave irradiation and conventional thermal conditions. The hydrogels compositions were characterized by Fourier transform infrared spectroscopy (FT-IR). Thermal stability of the IPN hydrogel was determined by DSC, and the mechanical properties and swelling kinetics of the IPN hydrogel samples were studied by tensile testing and gravimetric measurement method.

2. Experimental

2.1. Materials

Gelatin, chitosan (CS, deacetylation degree > 95%), ammonium persulfate (APS, 99%), polyvinylpyrrolidone (PVP, 99.5%), glutaraldehyde (GA, 25%), 1,2-Epoxy-4-vinylcyclohexane (UVR6105, 98%) and acetic acid (HAc, 99%).

2.2. Preparation of hydrogel films

Chitosan was dissolved in 20 mL 1% acetic acid solution, 1.0 g gelatin was added into the solution to make the ratio of gelatin and

chitosan is 2:1. And then about 0.05 g 1,2-Epoxy-4-vinylcyclohexane (UVR6105) was dropped into the three-necked bottle. 1.0 g PVP powder was dissolved in 10 mL distilled water with glutaraldehyde as the crosslinking agent. And the resulting solution was reacted at three kinds of conditions. One is the conventional thermal condition with water bath taking APS as the initiator, and the synthesized hydrogel named with CTH. The second method is the microwave radiation, and power of microwave is 500 W, the gained sample was named with MRH. The third reaction condition is the coupling reaction field with microwave and ultrasonic wave (XH-300UL Microwave-Ultrasonic-UV synthesis, Beijing Xianghu Technology Limited Co., China), and the power of microwave and ultrasonic wave was 300 W and 500 W, respectively, the sample was named with UMH. The mixture solution was processed at 60 °C, and the reaction time is 120 min, 30 min and 15 min for these three methods. And then the obtained thick dispersion was poured onto a petri dish. The solvent was allowed to evaporate at room temperature for 2 days and the resulting films were further dried in vacuum at 50 °C for 8 h.

2.3. Characterization

2.3.1. FT-IR analysis

The FTIR spectra were used to analyze compositions of the hydrogel samples that gave information about functional groups presented in hydrogel structure. The PVP/gelatin blend hydrogel films samples were characterized by Fourier transform infrared (FT-IR) spectroscopy using Nicolet 6700FT-IR instrument (Thermo Equipment Co., USA) in the wave number range 400–4000 cm^{-1} with resolution 4 cm^{-1} at room temperature. The FTIR spectrum was taken in a transmittance mode.

2.3.2. Thermal analysis

The thermal analysis was performed using a German NETZSCH 204DSC under nitrogen purge. The samples (3.0–5.0 mg) were sealed hermetically in the DSC sample pan. Samples were heated from 30 °C to 300 °C at a heating rate of 10 °C min^{-1} using an empty pan as a reference.

2.3.3. Crystallization characteristics

The X-ray diffraction was used to analyze the crystallization characteristics of the hydrogel films using a Bruker D8 advance X-ray diffraction meter. The scan was taken in the 2θ range, 5–60° with a scanning speed and step size of 1°/min and 0.01°, respectively.

2.3.4. Swelling behavior

The gravimetric method was used to measure the equilibrium swelling ratio of the hydrogel samples. The dry gels were immersed in distilled water at 27 °C at predetermined time intervals. Then, the hydrogels were drawn from water and eliminate non-adsorbed water on the samples surface with filter paper. The equilibrium water swelling of hydrogel samples were defined the following equation:

$$SR = \frac{W_s - W_d}{W_d} \times 100\%$$

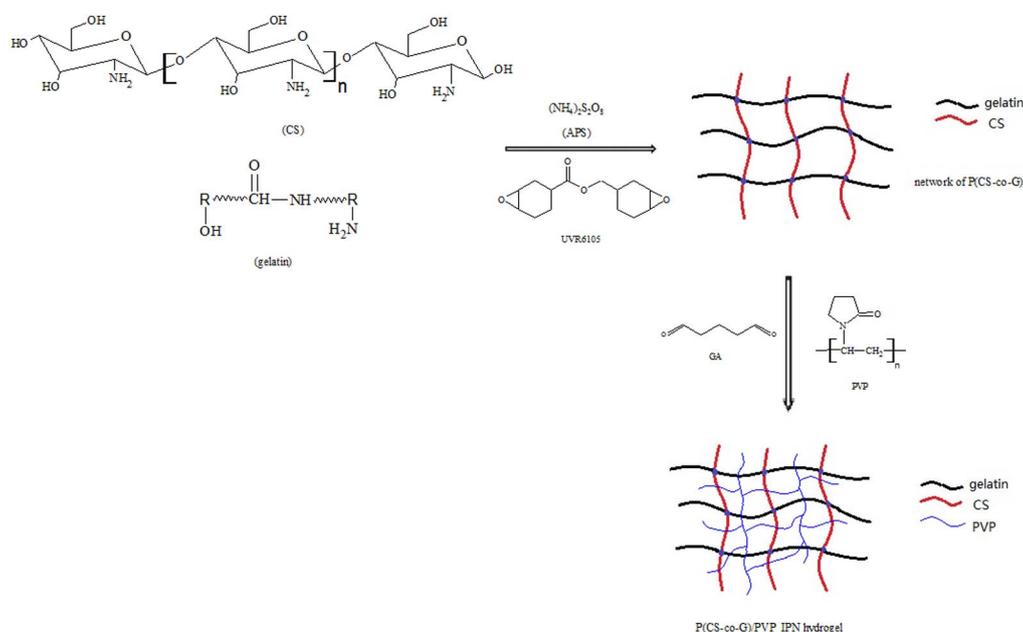
where W_d was the weight of dried gels, and W_s was the weight of hydrogel samples in the swollen equilibrium at different time. The swelling rate of hydrogel samples were obtained according to the equation:

$$v = \frac{W_{t_2} - W_{t_1}}{W_d(t_2 - t_1)}$$

where t_1 and t_2 mean the swelling time, and W_{t_2} and W_{t_1} were the weight of the sample at t_2 and t_1 .

2.3.5. Mechanical characterization

Tensile strength and elongation at break testing were performed to evaluate the mechanical properties of the dry hydrogel films. Stress-strain curves of strip shaped films equilibrated in a constant



Scheme 1. The sketch of structure of the P (CS-co-gelatin)/PVP IPN hydrogel.

temperature dryer for 72 h (relative humidity 75%) were recorded using CMT4204 Tensile Machine (MTS Industrial Systems Co. LTD). The thickness of the specimens was tested primordially using a manual micrometer with a resolution of 0.01 mm. And each specimen of length 4 cm and width 1 cm was gripped between the two jaws of the tensile machine, then the hydrogel films were uniaxially stretched in the vertical direction at a constant speed of 5 mm/min. And finally the values of tensile strength and elongation at break were obtained. Each test was conducted on three samples and the average was calculated.

3. Results and discussions

3.1. Preparation of P(CS-co-gelatin)/PVP hydrogel film

In the microwave and ultrasound coupling field, the copolymerization of chitosan and gelatin in PVP aqueous solution was prepared via a free radical polymerization. The possible reaction of P(CS-co-gelatin)/PVP IPN hydrogel has been illustrated in Scheme 1 [25]. In this work, the initiator of APS was used to initiate the free radical polymerization reaction for the traditional method, GA and UVR6105 are cross linker. In the first step, the P(CS-co-gelatin) network can be obtained from copolymerization of gelatin and chitosan in solution by using UVR6105 as a crosslinker. The ring-opening reaction of epoxy compounds can be catalyzed by APS to form hydroxyl radical in the conformation of UVR6105, and then the high active can link with the $-\text{NH}_2$ of CS and $-\text{COOH}$ of gelatin. Then, the P(CS-co-gelatin) polymer networks were crosslinked with PVP using glutaraldehyde (GA) as a crosslinker. GA can react with the $-\text{OH}$, $-\text{NH}_2$ and the $-\text{CO}-\text{NH}-$ of the CS and gelatin [27]. Meanwhile, taking GA as cross linker, the intact network between the CS and PVP also can be obtained. And the cross linking reactions not only will occur between the aldehyde and primary amine, but also between the aldehyde and hydroxyl [28,29].

3.2. FT-IR analysis

FT-IR measurement is an important method to study the intermolecular specific interaction in the polymer blends. It is believed that the integrated triple helix conformation of collagen is directly related with some feature amide bands in IR spectra, especially their typical characteristic bands of amide A, B, I and II. Generally, the amide A and B bands are mainly associated with the stretching vibrations of N–H

groups at the wave number of $3400\text{--}3300\text{ cm}^{-1}$ and $1650\text{--}1550\text{ cm}^{-1}$ [30]. The literature has reported that the amide I band of collagen is centered at 1690 cm^{-1} , which predominantly is originated from the proteinic C=O stretching vibrations coupled to N–H bending vibration. And the amide II bands located at around 1250 cm^{-1} are attributed to the amide N–H bending vibrations and C–N stretching vibrations [31]. As the hydrolysis product of the collagen, gelatin remains the characteristic structure of the triple helix and proper band of the collagen [30], as shown in Fig. 1a. Meanwhile, the main characteristic absorption bands of chitosan at 1655, 1585 and 1300 cm^{-1} can be assigned to the amide I, amide II and $-\text{CH}_2$ bending vibration, respectively [32]. Accordingly, for the PVP, the strong bands at 1634 cm^{-1} and 1698 cm^{-1} were assigned to the stretching vibration of C=O group, and the apparent absorption peaks at 1273 cm^{-1} and 1150 cm^{-1} are attributed to the stretching vibration of C–N.

The chemical structures of synthesized hydrogels studied by using FT-IR spectroscopy are shown in Fig. 1b. The spectra of all samples present the same characteristic peaks. The lately literatures have confirmed that the specific intermolecular interactions between PVP and collagen is the hydrogen bonds [33]. Moreover, there are no any new bands can be detected in the spectra of the PVP-collagen blends. As shown in Fig. 1b, the strong absorption band of the hydrogel films located at $1690\text{--}1630\text{ cm}^{-1}$ is attributed to the C=O group stretching vibration. At the same time, the peaks at $1200\text{--}1500\text{ cm}^{-1}$ were closely linked with the triple helical structure of collagen. And when the A_{1240}/A_{1470} was 1.0, the triple helical structure is unbroken [34]. Further, the absorption peaks at around 2360 cm^{-1} in the spectra of the three hydrogel samples are attributed to the characteristic band of the collagen, which indicated that the triple helix backbone conformation of gelatin does not change obviously [30]. Compared with the spectrum of solo gelatin, the absorption band at around 1650 cm^{-1} is more obvious for the hydrogel samples, which means the amide group is more in the structure of the synthesized hydrogel. Therefore, the results indicated there are chemical reactions occurred between the $-\text{NH}_2$ of chitosan and the $-\text{COOH}$ of gelatin. Nevertheless, the amide I and II bands are slightly shifted to lower wave numbers with respect to the pure gelatin, which may be interpreted as the formation of the intermolecular covalent bond between the P(CS-co-gelatin) and PVP. Meanwhile, the peak intensity at 1420 cm^{-1} , 1520 cm^{-1} and 1650 cm^{-1} are stronger for UMH sample. This phenomenon can be explained that the ultrasound can relax the fiber structure of gelatin leading to the sufficient

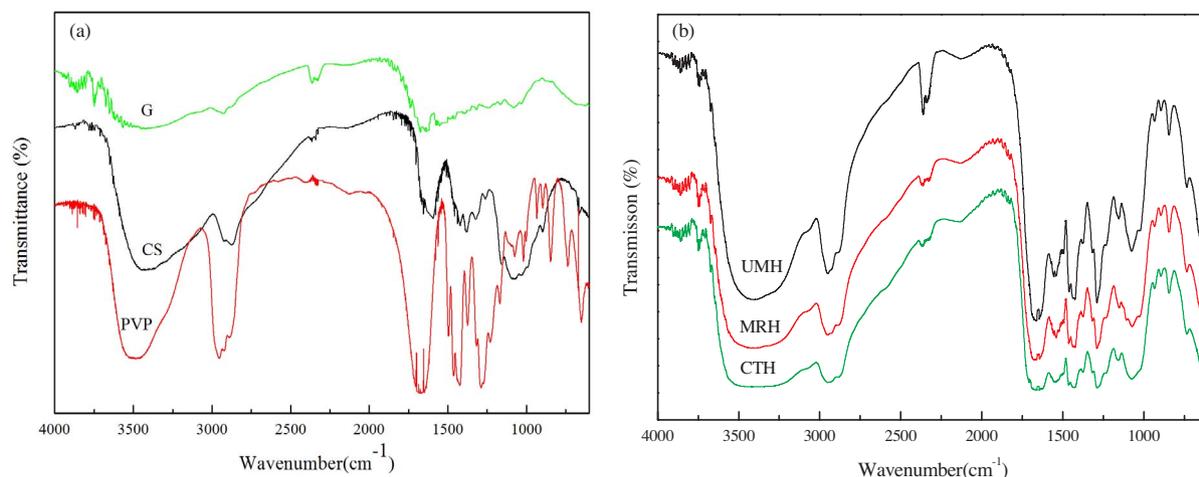


Fig. 1. FT-IR spectroscopy of CS, gelatin, PVP and hydrogel films. The samples were characterized by Fourier transform infrared (FT-IR) spectroscopy using Nicolet 6700 FT-IR instrument (Thermo Equipment Co., USA) in the wave number range 400–4000 cm^{-1} with resolution 4 cm^{-1} at room temperature.

cross linking between the CS and gelatin. In a word, the blending of the chitosan, gelatin and PVP does not destroy the natural structure of gelatin, which is the fundamental for maintaining its specific biological activity.

3.3. Thermal analysis

There are two methods to prepare the multicomponent polymer blends. One is to blend the components by melt blending, the other is to blend through solution casting method by using a cosolvent. However, one of the biggest disadvantages of biomaterials is their thermal stability. The biodegradable polymer may undergo decomposition more or less during melt blending process. So, the P(CS-co-gelatin)/PVP hydrogel films were prepared by using solution casting method in this work, and Fig. 2 shows the DSC traces of solo components and the three hydrogel films.

In case of gelatin, the glass transition temperature (T_g) reported has been explained by the block copolymer model for amino acid content of gelatin [35]. From Fig. 2, the T_g of gelatin occurs at 70 °C and is associated with the glass transition of α amino acid blocks in the peptide chain. Besides, the gelatin sample exhibited a T_m (melting transition temperature, endothermic peak) at about 183.5 °C, which can be attributed to the crystalline domains, the gelatin film helix-coil transition

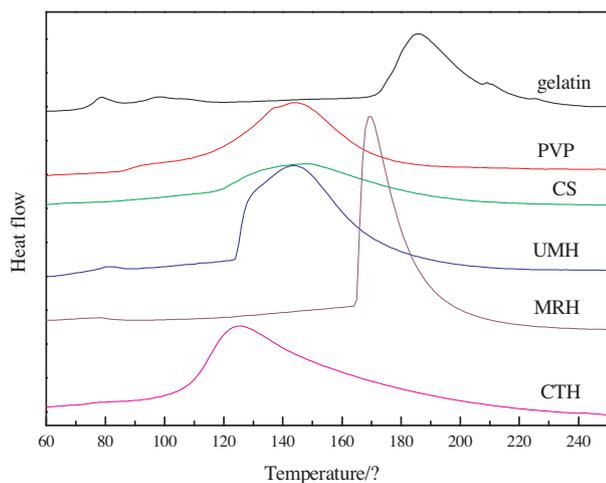


Fig. 2. DSC trace of samples. The thermal analysis was performed using a German NETZSCH 204DSC under nitrogen purge. The samples (3.0–5.0 mg) were heated from 30 °C to 300 °C at a heating rate of 10 °C min^{-1} using an empty pan as a reference.

and the disruption of molecular ordered structure [36]. Meanwhile, the T_m of synthesized hydrogel sample is lower than that of gelatin, due to the formation of interaction between CS, PVP and gelatin under stirring conditions using ultrasound vibration or mechanical agitation, which can damage the triple helix structure of gelatin in a way. During the three samples, MRH sample was gained only using microwave irradiation, and its T_m is the most close the T_m of gelatin, which also meant stirring can loose the collagen bundles.

3.4. XRD analysis

The crystal structure of CS, PVP, gelatin and hydrogel film were determined by XRD, as shown in Fig. 3.

It is reported that the typical X-ray diffractogram of collagen consists of two different diffraction peaks: a relatively sharp peak yielded around 7.6°, indicating the intermolecular lateral packing distance among the three peptide chains of collagen molecules, a broad peak centered at around 20°, assigning to the intensity of the reconstructed triple helix structure of collagen [37]. The regular structure of chitosan ensures its strong crystallinity and compactness because of the hydrogen bond. As shown in Fig. 3, there are two obvious diffraction peaks at 10.5° and 20.1° for CS sample, and according to the results that there are Form I (at 10°) and Form II (at 20.6°) crystalline forms for

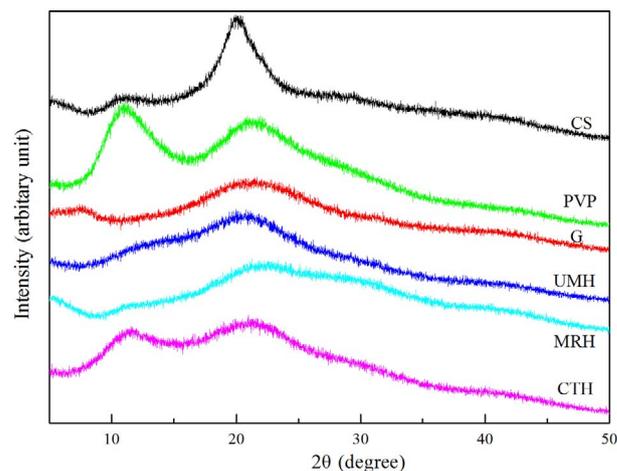


Fig. 3. X-ray diffraction patterns of CS, gelatin, PVP and hydrogel films. The X-ray diffraction was used to analyze the crystallization characteristic of the hydrogel films using a Bruker D8 advance X-ray diffraction meter. The scan was taken in the 2θ range, 5–60° with a scanning speed and step size of 1°/mm and 0.01°, respectively.

Table 1
Tensile strength and elongation of the synthesized hydrogel films.

Sample	Tensile strength/MPa	Elongation at break/%
UMH	86.68	8.47
MRH	26.97	33.24
CTH	15.52	10.92

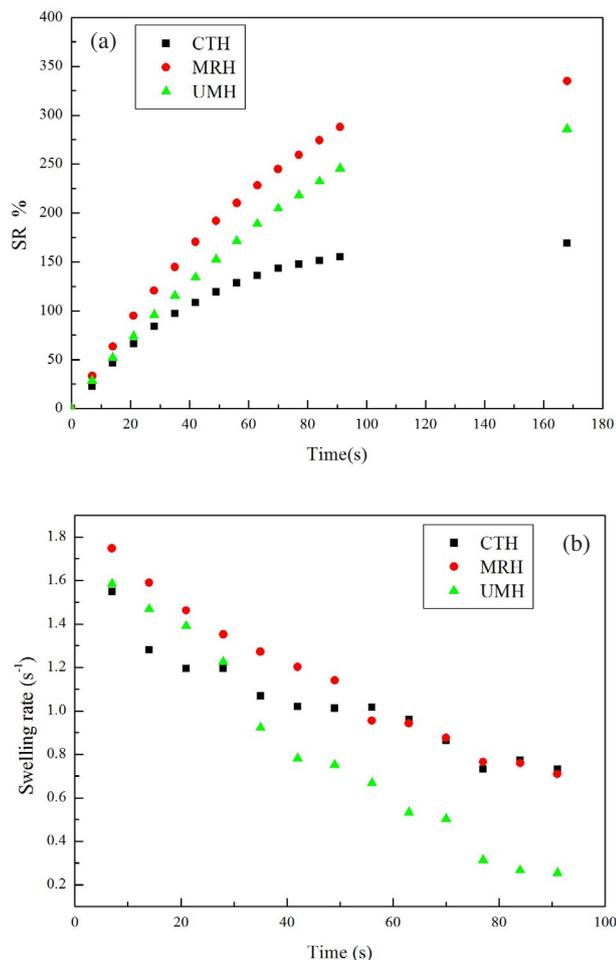


Fig. 4. The swelling ratio and swelling rate of the hydrogel vs time. The gravimetric method was used to measure the equilibrium swelling ratio of the hydrogel samples.

chitosan [38]. Combined with the intensity of every diffraction peak of CS in Fig. 3, it can be found the form II crystallinity is mainly exist [39]. Comparing the diffraction patterns of the three hydrogel samples, it can be found that the spectra of CTH is similar with PVP, while the UMH and MRH keep the helical structure of gelatin. Therefore, the mechanical properties of sample synthesized with microwave should be better than that of sample gained with conventional thermal method, which also can be confirmed by the data in Table 1.

3.5. Swelling behavior

Generally, the chemical structure and aggregate structure can both affect the water absorbability of polymer. Fig. 4 shows the swelling ratio and swelling rate of the synthesized hydrogels with different methods obtained from gravimetric method.

From Fig. 4a, it can be seen that the percentage swelling of the samples were higher than 150%. And the UMH sample synthesized with ultrasound and microwave showed the highest mass swelling (> 300%). The percentage mass swelling of the CTH sample gained

with traditional stirred was significantly lower than that of MRH samples synthesized with microwave irradiation. The different in percentage mass swelling of hydrogel could be due to the different in crosslink density. By comparing the curves in Fig. 4a, it can be found that the percentage mass swelling of sample 3 was remarkable lower than UMH sample and MRH sample, which indicated the crosslink density is better for the hydrogel sample obtained with irradiation. According to Fig. 4b, the swelling rates of all hydrogel samples were decreased and then gradually became into an equilibrium value, which means the swelling process will be poised with a short time for the gelatin based hydrogel. Therefore, it can be used as a quick absorbent.

3.6. Mechanical properties

Because polymer materials such as films may be subjected to various kinds of stress during its used, so suitable mechanical properties play a crucial role in the biomedical application. Table 1 listed the tensile strength and elongation at break of the synthesized gelatin based hydrogel films.

Comparing the tensile strength of the three samples, it can be found that the tensile strength of the sample synthesized with microwave irradiation is bigger than traditional stirring. And this phenomenon indicated that the crosslinking density and degree of reaction between the components is better for microwave irradiation, which supported the results of IR spectra. According to the data in Table 1, the introduction of ultrasound can enhance the tensile strength of the hydrogel film obviously. However, DSC show the triple-helix structure of the gelatin had been partly destroyed for UMH sample, and the special triple-helix of collagen ensure its excellent mechanical properties. Therefore, it can be presumed that the tensile strength of the hydrogel is mainly related to the crosslinking density, and the ultrasound can relax the collagen fibers leading to the sufficient cross link. At the same time, because of the rupture of the peptide chain, the elongation at break has been decreased.

4. Conclusions

Interpenetrating polymer networks (IPN) hydrogel films were successfully synthesized via free radical polymerization using glutaraldehyde (GA) and 1,2-Epoxy-4-vinylcyclohexane (UVR6105) as crosslinkers with microwave and ultrasound assisted. FT-IR spectra confirmed the compositions of hydrogels in the IPN structure. And the swelling capacity and mechanical properties of synthesized hydrogel film with microwave and ultrasound are better than that of traditional thermal gained sample. Due to the microwave heating process has high temperatures for attack the solution with relatively short times and without the initiator, it's more green and potential than conventional thermal conditions. Taken as a whole, these results may pave a way for developing a novel preparation method of hydrogel based gelatin for rapid water absorbent.

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