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Carbohydrate Polymers 72 (2008) 479–489
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Synthesis and properties of novel hydrogels from oxidized konjac glucomannan crosslinked gelatin for in vitro drug delivery

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Received 17 April 2007; received in revised form 14 September 2007; accepted 20 September 2007
Available online 29 September 2007

Abstract

This paper describes the use of oxidized konjac glucomannan (DAK) as a macromolecular cross-linker for the preparation of gelatin-based pharmaceutical hydrogels, which crosslinked and gelled in minutes. FTIR, XRD, SEM, swelling and mechanical properties experiments were performed to confirm the effect of DAK and evaluate the relationship of the structure and morphology of the hydrogels. The obtained results indicated that DAK promoted the formation of gelatin network. More interestingly, gelatin hydrogels treated by DAK slowed down prominently the release of the model drug ketoprofen, and the release rate could be tailored by the DAK/GL ratio and pH value of buffer solutions. These results suggest that this process offers an entirely new window of material preparation for controlled release of drugs when compared with traditional preparation of gelatin-based hydrogels crosslinked with small molecules.

Keywords: Gelatin; Polysaccharide; Oxidation; Hydrogels; Drug delivery

1. Introduction

Hydrogels consisted of three-dimensional hydrophilic polymer network, when placed in excess water, they are able to swell and retain large volumes of water without dissolution. Due to their unique properties, a wide range of pharmaceutical and biomedical applications has been described, such as contact lenses, wound dressings, artificial organs, and delivery carriers for the bioactive reagents (Bezemer et al., 2000; Figuly et al., 1997). Many synthetic and naturally derived materials have been reported to form well-characterized hydrogels. Since natural polymers possess better biocompatibility, biodegradability, non-toxicity and easily modified ability than various synthetic materials, more and more researches have focused on natural polymer-based hydrogels (Choi et al., 1999; Fujioka, Maeda, Hojo, & Sano, 1998) using polysaccharides, cellulose derivatives and proteins as drug carrier.

Among natural polymers (preferred for their low toxicity and biocompatibility), gelatin is an attractive candidate as the starting material for preparing hydrogels since it has unique gelling properties. Moreover, due to the large number of functional side groups, gelatin readily undergoes chemical cross-linking, which is very important for its use as a biomaterial. These advantages made gelatin-based controlled release systems possess diverse applications in fields ranging from tissue engineering Okino, Nakayama, Tanaka, and Matsuda (2002) to drug delivery and gene therapy (Koob & Hernandez, 2003; Young, Wong, Tabata, & Mikos, 2005). However, the main limitation of gelatin for the preparation of sustained release hydrogel systems arises from its rapid dissolution in aqueous environments leading to fast drug release profiles at body temperature. In order to overcome this problem, chemical cross-linking procedures which form scarcely or non-soluble products and slow down the release of the loaded drug have been considered. Up to now, glutaraldehyde and formaldehyde

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0144-8617/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2007.09.023

were the most common cross-linking agents used in various gelatin-based biomaterial formulations. Nevertheless, they contain chemical species which are reported to be cytotoxic (Muzzarelli, 1977).

Therefore, it is important to develop a potentially less toxic cross-linking agent for the gelatin-based biomaterial in the interest of human safety.

In this respect, thermal hardening treatments (Esposito, Cortesi, & Nastruzzi, 1996) or 'natural' cross-linking agents have been proposed for the production of gelatin devices to achieve long-term release of the drug. Several polysaccharides (Balakrishnan & Jayakrishnan, 2005; Maia, Ferreira, Carvalho, Ramos, & Gil, 2005; Nishi & Jayakrishnan, 2004) such as dextran, gum arabic and chondroitin sulfate were partially oxidized for medical applications.

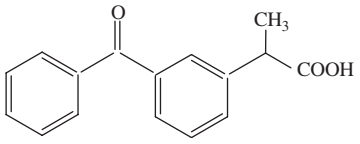
In this paper, we propose developing oxidized konjac glucomannan as a macromolecular crosslink agent for gelatin to prepare a composite hydrogel drug carrier. KGM is a high-molecular weight water-soluble non-ionic natural polysaccharide. Due to its good biocompatibility, biodegradability as well as excellent gel-forming properties, KGM shows promise in controlled release systems (Chen, Liu, & Zhuo, 2005; Du et al., 2004; Liu, Hu, & Zhuo, 2004). Chemically it is a linear random copolymer of β-(1,4), linked D-mannose and D-glucose in a molar ratio of 1.6:1 (Kato & Matsuda, 1969)with about 1 in 19 U being acetylated (Maekaji, 1978). Being a β-(1,4) linked polysaccharide, KGM can be oxidized by reacting with sodium periodate. Simultaneously, the carbon–carbon bonds of the cisdiol group in the KGM molecular chain are cleaved and generate reactive aldehyde functions, which can develop chemical cross-linking action with gelatin via imino bonds or Schiff's linkage between free amino groups and the aldehyde groups of oxidized polysaccharide. So combining properties of gelatin and KGM-derived materials would generate a hydrogel that obtains advantages from both KGM- and gelatin-based biomaterials.

The aims of this work were (a) the production and characterization of cross-linked gelatin hydrogels by using oxidized KGM and (b) the evaluation of the effect of oxidized KGM cross-linking on the physicochemical and release characteristics of the gelatin matrix.

2. Experimental

2.1. Materials

Konjac glucomannan (KGM) was purchased from Huaxianzi Konjac Corp. (ShiYan, Hubei, China). The content of glucomannan is above 95%. The weight-average molecular weight (M_w) was 9.80×10^5 (according to the manufacturer's specification). Gelatin in powder form was purchased from Shanghai Chemical Reagent Co., (Shanghai, China) with the number-average molecular weight (M_n) of about 8.7×10^4. The isoelectric point is 4.0–5.0. Ketoprofen was a biochemical reagent (used as model drug) and commercially obtained from Sinopharm Chemical Reagent Co., Ltd., China. Scheme 1 shows the chemical structure of ketoprofen. Dialysis tubing (molecular weight cut-off, 10,000–8000) was commercially available, and other reagents were all of analytical grade.



Scheme 1. Chemical structure of ketoprofen.

2.2. Preparation of oxidized konjac glucomannan

Konjac glucomannan was oxidized using sodium periodate. Into 300 mL of a 2% (w/v) aqueous dispersion of KGM (0.037 mol) was introduced 1.58 g (0.0074 mol) of sodium periodate for obtaining 20% theoretical oxidation degree of polysaccharide, and the mixture was stirred vigorously at 30 °C in the dark for 12 h. Ethylene glycol (5 mL) was then added to the reaction mixture to reduce any unreacted periodate and stirred for another 2 h. The reaction mixture was dialyzed against distilled water with several changes of water over 3 days until the dialysate was free from iodate (checked with silver nitrate). Reaction product (DAK) dispersions were centrifuged for 10 min at 3000 rpm, and then the DAK was recovered from the supernatant by precipitation with acetone three times. The dried samples were stored in vacuum desiccators over P_2O_5 for further use. The extent of oxidation was determined according to Bouhadir, Hausman, and Mooney, (1999). The final yield of the product was also calculated. The number-average molecular weight (M_n) of DAK was determined by size exclusion chromatography combined with static laser light scattering (SEC-LLS) (Dawn DSP, Wyatt Technology Corporation).

2.3. Synthesis of hydrogels and drug loading

Dialdehyde KGM (DAK) was dissolved to a final concentration of 9.0% (w/v). Desired quantities of gelatin (GL) were dissolved in hot distilled water to obtain a transparent solution of the same concentration. Afterwards, the two aqueous solutions were mixed by stirring vigorously. The weight ratios of DAK/GL in the three different hydrogel formations were 2/1, 1/1, and 1/2, respectively. Some physical properties of the composite hydrogels are given

Table 1
Compositions and characters of the DKG-GL hydrogels

Samples	DAK (g)	Gelatin (g)	DAK/GL (%)	ρ (dry state) (g/cm^3)
1DKG	1	0.5	200	1.457
2DKG	1	1	100	1.418
3DKG	1	2	50	1.414

in Table 1. The hydrogels formed within about 3 min and were aged in a refrigerator at 4 °C for 24 h. The above three different ratio hydrogel samples were coded as 1DKG, 2DKG and 3DKG. The gels were dried under ambient temperature to constant weight and stored for further use. The model drug ketoprofen was first dissolved in the 9.0% (w/v) gelatin aqueous solution; the hydrogels loaded with ketoprofen were prepared by the mixture of above gelatin solution with 9.0% (w/v) DAK aqueous solution, followed by drying at ambient temperature. The amount of drug loaded in the dry gels is 2% (w/w).

2.4. The characterization of hydrogels

2.4.1. FT-IR analysis

To confirm the formation of the cross-linked structure and also to find the chemical stability of the drug in hydrogels, Fourier transforms infrared (FTIR) spectra data of gelatin, DAK, composite gels, ketoprofen and ketoprofen-loaded gels were recorded with spectrometer (Nicolet MX-1E, USA) using a KBr method.

2.4.2. X-ray diffraction studies

Wide-angle X-ray diffraction patterns for various samples were analyzed using a Shimadzu XRD-6000 (Japan) diffractometer equipped with a CuKα target at 40 kv and 30 mA with a scan rate of 4°/min. The diffraction angle ranged from $2\theta = 3°$ to $2\theta = 40°$.

2.4.3. Morphology observations

The surface morphology and internal structure of DKG were observed by a scanning electron microscope (SEM S-450, Hitachi, Japan).

The samples were freeze-dried at −55 °C to maintain the porous structure without any collapse.

The average distance of pores and wall thicknesses (i.e., the average distance between neighboring pores) were measured by geometrical observation on screen.

2.5. Formation of 2-D gel films and mechanical properties studies

Gelatin and DAK were dissolved in distilled water to obtain transparent solutions of 3% (w/v) concentration. Appropriate proportions of these solutions were mixed by stirring vigorously to obtain various blend ratios (the weight ratio of DAK/GL is 2/1, 1/1, and 1/2, respectively). The homogeneous mixtures of DAK and gelatin were degassed and spread over a Teflon plate of 10 cm × 10 cm and water was allowed to evaporate at room temperature for 3 days. Then DKG composite gel membranes' tensile strength and elongation at break were determined by using a versatile electron tensile tester (CMT-6503, Shenzhen SANS Test Machine Co. Ltd., China) with a tensile rate of 3 mm/min. The film strips were 70 mm in length, 10 mm in width, and the initial grip separation was set at 40 mm. Prior to tensile testing, film specimens were conditioned for 3 days in an environmental chamber at 25 °C and 50% RH. Three parallel measurements were performed, and the mean was obtained for the films.

2.6. Studies of equilibrium swelling

The known weight DKG dry gels were immersed in pH 4.0, pH 9.0 buffer solutions, respectively, and kept at 25 °C for 48 h until equilibrium of swelling had been reached. The swollen gels were taken out and immediately weighed with microbalance after the excess of water lying on the surfaces was absorbed with a filter paper. The equilibrium swelling ratio (SR) was calculated using the following equation:

$$\mathrm{SR} = (W_s - W_d)/W_d \times 100\% \quad (1)$$

where W_s and W_d are the weights of the gels at the equilibrium swelling state and at the dry state, respectively. Experiments were repeated in triplicate for each gel specimen and mean value was obtained.

2.7. Measures of water retention by the hydrogels

As a part of the characterization study, the water retaining capacity of hydrogels was investigated as a function of time of exposure in 30% relative humidity at 25 °C.

Pieces of swollen hydrogels of approximately 15 cm^3 of volume were put on Petri plates. The highly swollen hydrogels were weighted and the decreases in their weights measured as a function of time by gravimetry. The values of water retention (WR) were obtained by the following equation:

$$\mathrm{WR}\ (\%) = (M_s - M_t)/M_s \times 100 \quad (2)$$

where M_s is the initial weight of the hydrogel in water and M_t is its weight after loss of water at each time.

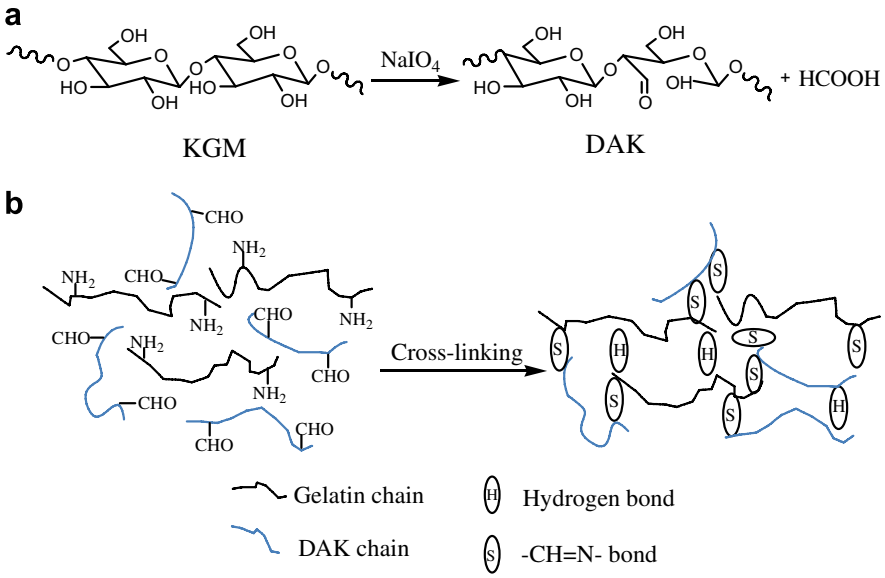
2.8. Release studies

The drug releases from the hydrogels were carried out in a shaker incubator at a shaking speed of 50 rpm at 37 °C. The desired quantity of drug-loaded hydrogels (0.2 g) was immersed in 100 mL of release medium. pH 9.0 and pH 4.0 buffer solutions were used as the dissolution media. At scheduled time intervals, 5 mL solution was withdrawn and equal volume of the same dissolution medium was added back to maintain a constant volume. The amount of ketoprofen released from the hydrogels was determined by UV–Visible spectrophotometer measurements at 259 nm (Shimadzu UV-1 60A, Japan) and calculated from a previously calibrated standard curve. The results are means of two determinations.

3. Results and discussion

3.1. Synthesis and characterization of oxidized KGM

KGM is a linear random copolymer of β-(1,4) linked D-mannose and D-glucose, the vicinal hydroxyl groups of



Scheme 2. (a) Periodate oxidation of konjac glucomannan (KGM). (b) Formation of DAK-GL composite hydrogels via the Schiff-base reaction and hydrogen bonds.

KGM can be cleaved by periodate oxidation to form dialdehyde derivatives (shown in Scheme 2a). At the same time, non-specific oxidative cleavage of glycosidic bonds in the KGM backbone leads to significant decrease in molecular weight (Domb, Linden, Polacheck, & Benita, 1996).

In our research, we found that DAK with the higher oxidation degree shows a faster reaction rate with gelatin than DAK with a lower degree of oxidation. However, lower DAK with a lower degree of oxidation can preserve most of the other properties of the pure KGM and maintain a relatively higher molecular weight. Taking these factors into consideration, in this paper, KGM was oxidized to obtain a theoretical degree of oxidation assuming each α-glycol group consumes one molecule of periodate. The determination of the actual aldehyde content of DAK revealed an extent of oxidation of $17.2 \pm 0.21\%$, the yield of the DAK is $71.87 \pm 2.40\%$ and the number-average molecular weight M_n is 1.06×10^5.

Clearly, the low yield of DAK is due to small chain fragments generated by non-specific oxidation which pass through the dialysis membrane. The oxidization reaction of KGM by sodium periodate can be monitored with FT-IR. Fig. 1 shows the FTIR spectra of pure KGM and DAK. In the spectrum for pure KGM (see curve a), It can be seen that the absorption band of carbonyl of acetyl groups was at $1734\ cm^{-1}$ and the intense peak at $1645\ cm^{-1}$ is attributed to the in-plane deformation of the water molecule (Zhang et al., 2001). However, the oxidation leads to the appearance of two characteristic bands of DAK (see curve b) around 1730 and $880\ cm^{-1}$ regions. The former is also ascribed to the aldehyde symmetric vibrational band (carbonyl), which changed from a small shoulder in pure KGM to a distinct peak in DAK. The latter can be assigned to the hemiacetal structure between the aldehyde groups and neighboring hydroxyl groups (Kim, Kuga, Wada, Okano, & Kondo, 2000).

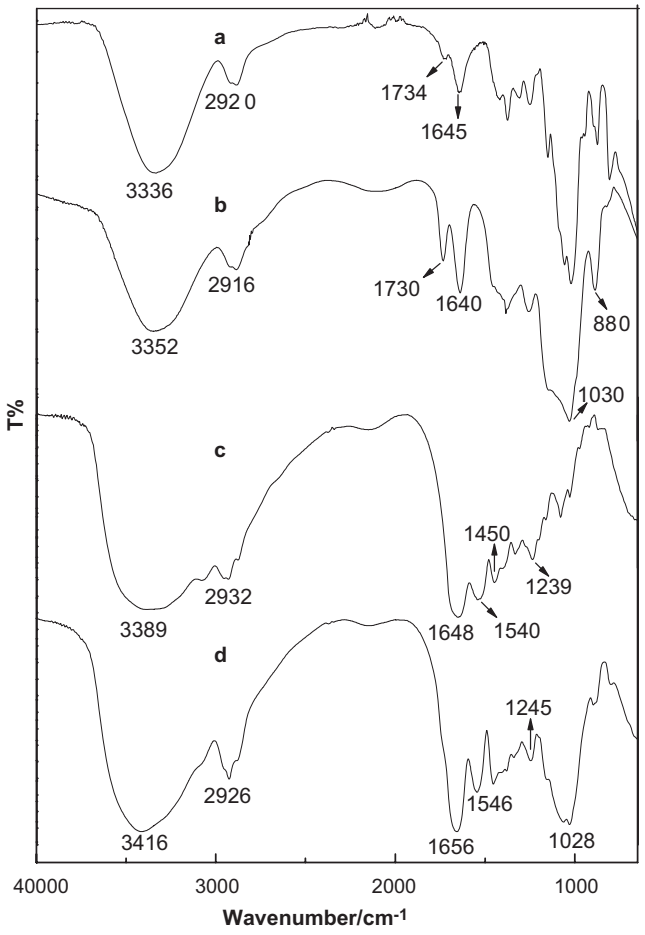


Fig. 1. FTIR spectra of native KGM (a), DAK (b), gelatin (c) and blankcomposite gel DKG (d).

3.2. Characterization of hydrogels

The DAK/GL composite hydrogels were prepared by Schiff's base cross-linking reaction between the amine group of gelatin and aldehyde of DAK (Scheme 2b). The hydrogen bonds also contribute to gels' cross-linking structure, but the Schiff base formation is predominant, what prevents dissolution and excessive swelling of the polymer matrix in the presence of water.

3.2.1. FT-IR analysis

In order to confirm the cross-linking of gelatin chains by DAK and possibility of structural change of drug-loaded composite gel, FTIR spectra of the gelatin, DAK, and blank composite gel DKG are compared in Fig. 1; whereas Fig. 2 shows the FT-IR spectra of blank composite gel DKG, ketoprofen and drug-loaded composite gel DKG.

In the case of native gelatin (Fig. 1c), the wide absorption band around 3389 cm^{-1} was due to the stretching vibration of O—H bonded to N—H. The band appearing at 1648 cm^{-1} indicates amide I band (C—O) was attributable to both a random coil and α-helix conformation of gelatin reported by Prystupa and Donald (1996) and Muyonga, Cole, and Duodu (2004).

The N—H bending vibration is indicated by a band observed at 1540 cm^{-1} (amide II), while bands at 1239 cm^{-1} indicate the C—N bond stretching vibrations. From the FT-IR spectra of composite gel DKG in Fig. 1d, we can see that the characteristic absorption bands at 1648 and 1540 cm^{-1} of gelatin shifted to higher wave number at 1656 and 1546 cm^{-1}. The absorption band around 3389 cm^{-1} also shifted to a higher wave number at 3416 cm^{-1}. At the same time, the characteristic peaks of DAK at 1730 and 880 cm^{-1}disappeared in Fig. 1d. All these suggested that a strong interaction existed between gelatin and DAK. In addition, due to overlapping with the peak of amide I band of gelatin, there was little difficulty in finding the new bond at about 1656 cm^{-1} which is due to the C=N stretching vibration of the imine group of Schiff base, however, the increased intensity of the band at 1656 cm^{-1} indicated its existence. This band confirms the formation of coupling reaction between —CHO groups of DAK and NH2 of gelatin.

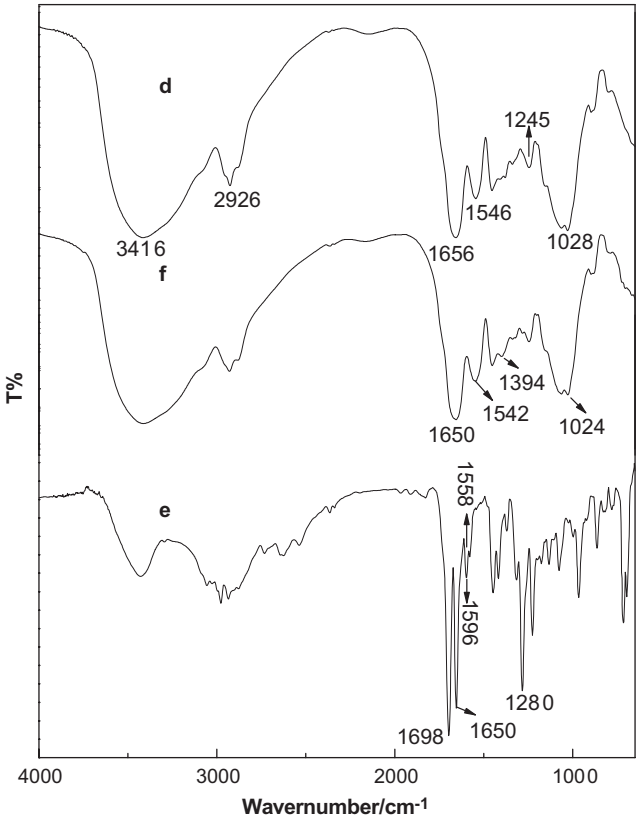


Fig. 2. FTIR spectra of blank composite gel DKG (d), ketoprofen (e) and drug-loaded composite gel DKG (f).

For ketoprofen (Fig. 2e), the characteristic bands appeared at 1698 cm^{-1} due to the C=O stretching vibration of the carboxylic group, the carbonyl C=O stretching vibrations were represented by a band observed at 1650 cm^{-1}, and the characteristic absorption bands appeared at 1596 cm^{-1} and 1558 cm^{-1} of ketoprofen due to the stretching vibration of phenyl framework. In case of FT-IR spectra of drug-loaded composite gel DKG, all the peaks present in the blank composite gel DKG appeared, but the peaks observed for ketoprofen were hard to detect. However, we can see that the characteristic absorption bands at 1656, 1546 and 1028 cm^{-1} of blank composite gel DKG shifted to lower wave number at 1650, 1542 and 1024 cm^{-1}, respectively. The results indicated that ketoprofen had intermolecular interactions with the composite gels. At the same time, there were no new characteristic absorption bands of drug-loaded composite gels, allowing it to be concluded that there was no obvious chemical reaction between the drug and the composite hydrogels. Thus an important result is that ketoprofen did not lose its activity in the drug-loaded composite gels.

3.2.2. X-ray diffraction studies

X-ray diffractograms of the gelatin, DAK, and blank composite gel DKG are presented in Fig. 3, whereas Fig. 4 shows the X-ray diffraction patterns of blank composite gel DKG, ketoprofen and drug-loaded composite gel DKG.

As observed, the pattern of gelatin (Fig. 3a) exhibited two distinct diffraction peaks at $2\theta = 7.6°$ and 18°, which is consistent with the reported result (Payne, Mccormick, & Francis, 1999). As to DAK (Fig. 3b), it exhibited amorphous structure, as a diffraction peak was not observed. However, the crystalline peaks of gelatin entirely disappeared, and an amorphous state was observed in composite gel DKG (Fig. 3c). This suggested that the formation of the Schiff's cross-linking structure between amino groups of gelatin and aldehyde groups of DAK destroyed the close packing of the gelatin molecules for the formation of regular crystallites.

In Fig. 4d, many sharp peaks are observed in the diffraction patterns of ketoprofen, and they located at $2\theta = 6.3°$,

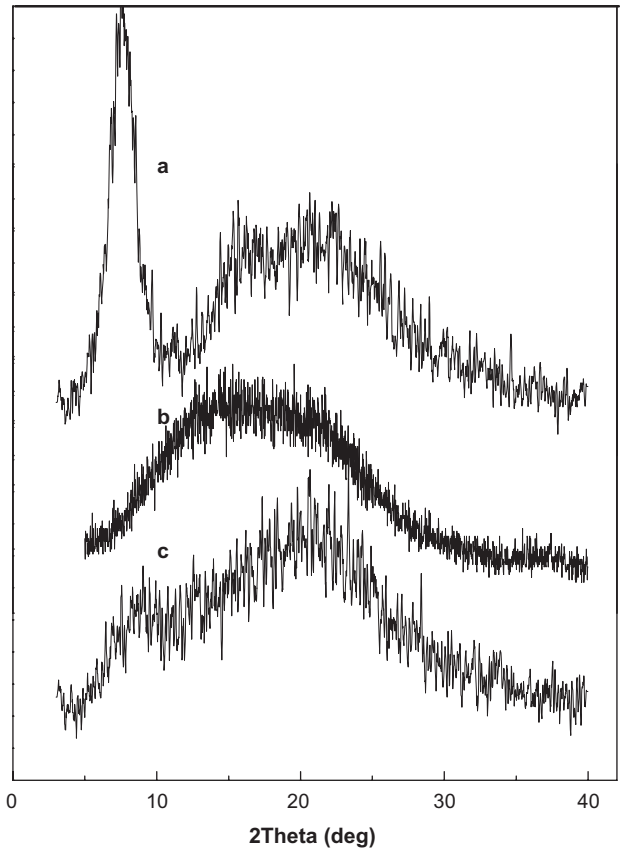


Fig. 3. X-ray diffractograms of the gelatin (a), DAK (b), and blank composite gel DKG (c).

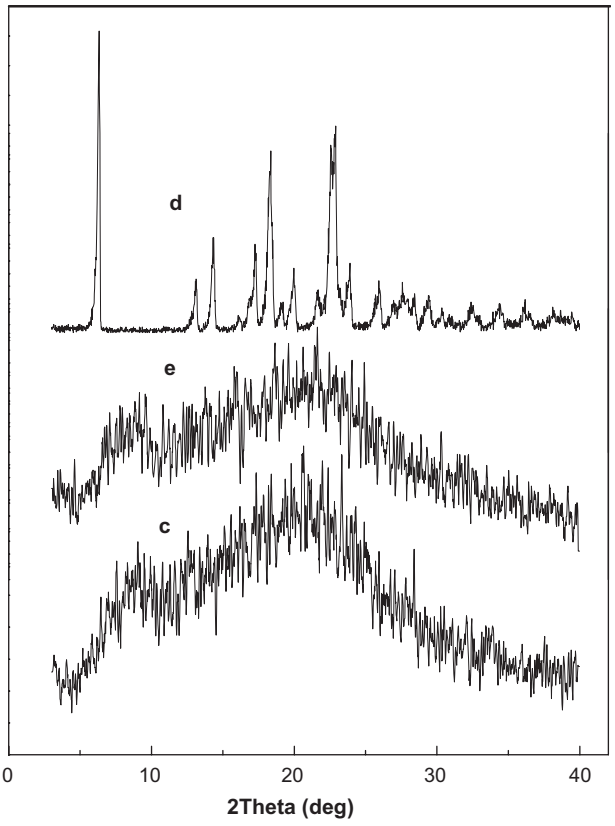


Fig. 4. The X-ray diffraction patterns of blank composite gel DKG (c), ketoprofen (d) and drug-loaded composite gel DKG (e).

14.3°, 18.4° and 22.9°; this indicates that ketoprofen is crystalline; but the characteristic peaks of ketoprofen are very difficult to detect in drug-loaded composite gels. Namely, the loaded drug is amorphous, which indicates that drug is dispersed at the molecular level in the composite gel matrix. However, when comparing the X-ray diffraction patterns of the blank matrix with drug-loaded matrix (Fig. 4c and e), we found the diffraction intensities of composite gels decreased at 20.6° after the addition of the drug. This confirms the existence of interaction between drug and the composite gel DKG.

3.2.3. Morphology observations

The SEM images were obtained to characterize the microstructure of the freeze-dried DKG composite gels and are presented in Fig. 5.

This suggests that the DKG hydrogel matrices are porous, with a three-dimensional interconnected microstructure by virtue of the freeze-drying step (Noble, Gray, Sadiq, & Uchegbu, 1999) with the pores being the result of ice crystal formation, resembling other natural macromolecular hydrogel system structure. The apertures in the matrices are around 2–8 μm wide estimated from the micrographs. Additionally, the stronger wall appeared due to the rather orderly aggregates of the polymer chain segments in the interior of the DKG composite hydrogel.

The interconnection between pores could be assigned to the cross-linking network formation in gels. Clearly, the internal morphology of the cross-linked DKG composite hydrogels was dependent on the content of DAK. Sample 3DKG had the largest pore sizes. Whereas sample 1DKG and 2DKG had smaller pore size and there was no discernible difference in the internal pore structure of these both matrices. This is mainly explained by the number of cross-linking node between gelatin and DAK being enhanced with the increasing of DAK content.

The effect of buffer media on the morphology of DKG matrices is also shown in Fig. 5. After swelling completely in two buffer solutions of pH 4.0 and 9.0, 2DKG-A and 2DKG-B display the different cross sectional morphologies and diameter of the pores. By comparison with the morphologies of 2DKG (relaxed state) hydrogels, we can see that 2DKG-A shows the smaller three-dimensional pores after swelling in a buffer solution of pH 4.0. 2DKG-B shows the larger three-dimensional pore after swelling in buffer solution of pH 9.0. From these we would easily understand why the composite hydrogel DKG has different swelling capacities in the two solutions.

3.3. Mechanical properties study of gel films

From the engineering aspect, an ideal material should have good mechanical properties that keep their shape dur-

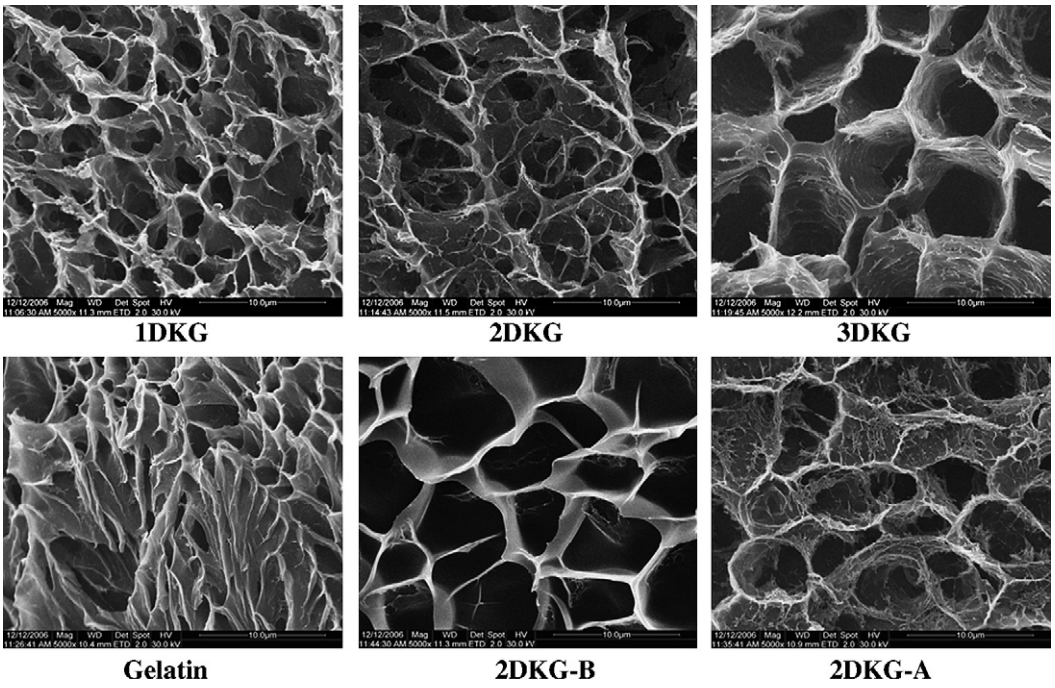


Fig. 5. The microstructure of the freeze-dried DKG composite gels, gelatin and swollen state of 2DKG at pH 9.0 (2DKG-B) and at pH 4.0 (2DKG-A).

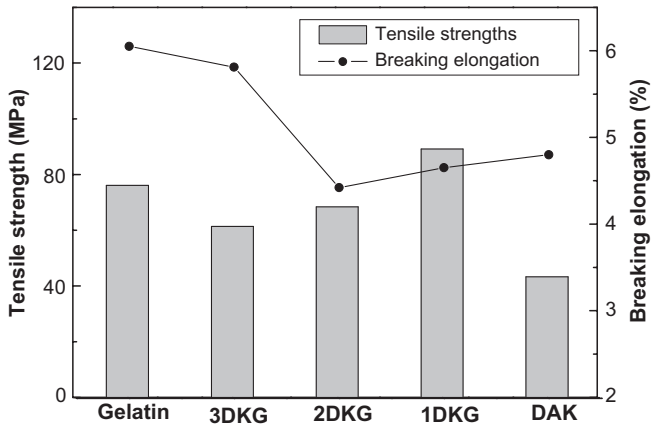


Fig. 6. The mechanical properties of three DKG composite hydrogel films, pure gelatin and DAK.

ing application. In this regard, we conducted mechanical properties study of DKG gel films and the results are shown in Fig. 6. There are two factors which affect the tensile properties of DKG gel films. One is the nature of the material used, and the other is the structure of the film. In the present study, DAK is not only basic macromolecular material but also a chemical cross-linking agent which can react with amino groups of gelatin. So, the mechanical profile of the gel films showed a different ranking order with respect to the gel film composition. As we can see, the breaking elongations of gelatin and DAK are 6.06% and 4.81%, respectively. In the cross-linked gel films, 3DKG with the lowest cross-linked density has the highest breaking elongation of 5.82%. But the breaking elongations of 1DKG and 2DKG are 4.66% and 4.43%, respectively, although the former possesses a higher cross-linked density than the latter.

The change of the tensile strengths showed a different tendency from that of breaking elongation, and the maximum value was 89.25 MPa when the DAK content is 66.7% (1DKG), while the minimum value of 61.48 MPa was found in the 3DKG gel film (the DAK content is 33.3%). As far as 2DKG is concerned the tensile strength is 68.47 MPa. It was obvious that the Schiff base cross-linking structure exerts an influence on mechanical properties of gel films. Since the enhancement of DAK content in gel films will increase the number of junction zones, a tighter and less elastic structure is formed, and chain-relaxation ability of the polymeric network also significantly decreased, which led to the increasing tensile strengths of the films and the reducing breaking elongation of the films.

3.4. Studies of equilibrium swelling

The photographs of dry state and swollen state DKG hydrogel are shown in Fig. 7. From the photos it is clear that the hydrogels remain in the cylindrical form after swelling.

Compared with dry state hydrogels, the swollen state hydrogel volume displays huge increases. The diameter of swollen hydrogel is about 4.0 cm, while the diameter of the dry state hydrogel is only about 1.5 cm.

Equilibrium swelling ratio (SR) of hydrogels exerts an influence on their release rates (Ritger & Peppas, 1987). Fig. 8 indicates the equilibrium swelling ratio (SR) of composite hydrogels at different pH media. At the same pH, the SR value rapidly decreased with the increase of the DAK content. In case of the pH 9.0 medium, the SR value was

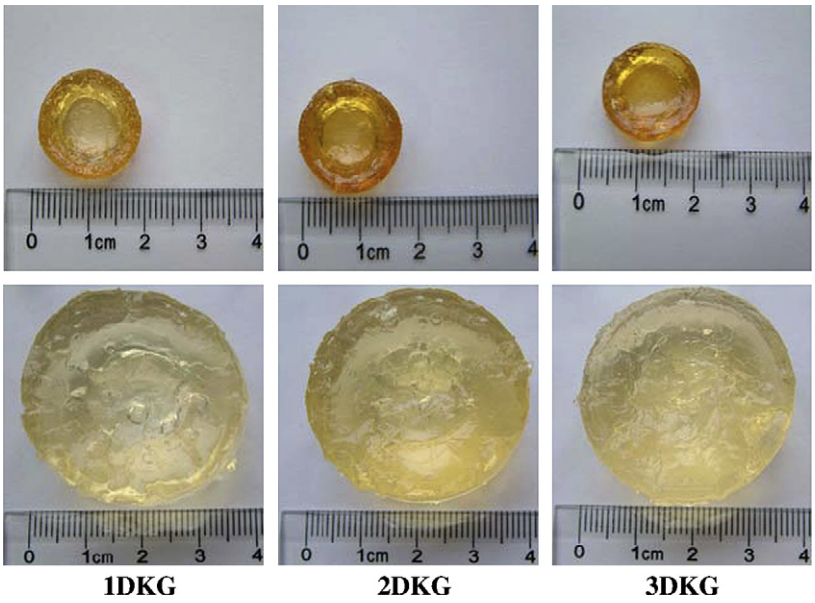


Fig. 7. Photos of DKG hydrogel obtained from dry state (above) and swollen state (below) immersion at pH 9.0. The scale is given in centimeters.

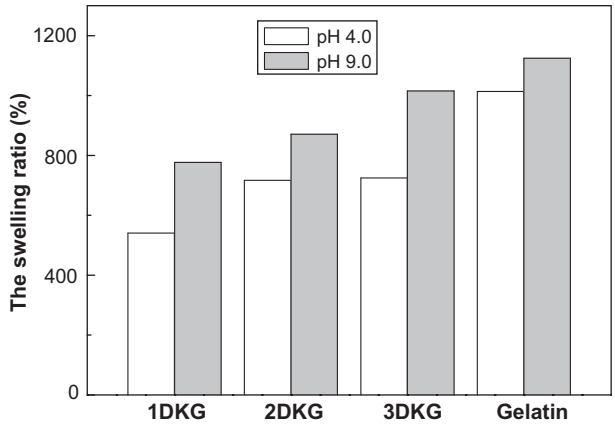


Fig. 8. The equilibrium swelling ratio of three DKG composite hydrogels at pH 9.0 and 4.0 conditions.

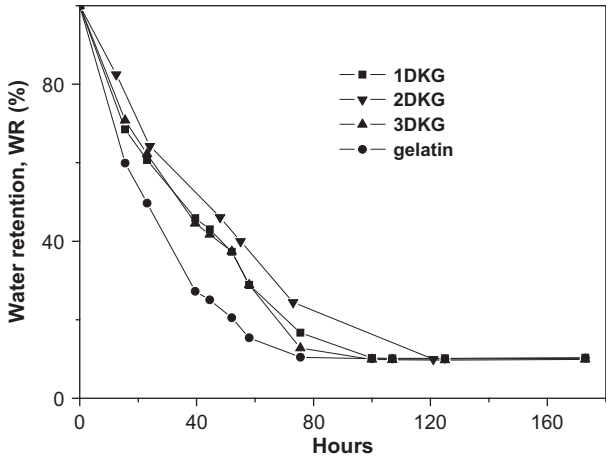


Fig. 9. The kinetics curves of water retention of the 1DKG, 2DKG, 3DKG and gelatin hydrogels.

1050% for 3DKG (33.3% w/w DAK content) hydrogels, while the SR values were 870% for 2DKG gels (50% w/w DAK content) and 770% for 1DKG gels (66.7% w/w DAK content).

The reduction in equilibrium swelling capacity is due to the formation of a tight network structure in high content DAK composite gels, which hinders the mobility of the polymer chains and minimizes their exposure to the water molecules. On the other hand, the nature of the polymer plays an important role in swelling capacity of the hydrogel matrix. The solubility of DAK was less than that of KGM as seen before. In DKG composite gels, the —C=N group is hydrophobic group, so the increase of DAK content will bring higher amount of hydrophobic group in hydrogels and lower content of gelatin. These effects are also reflected in the swelling capacity of the hydrogels, so the lower SR value was presented by the 1DKG hydrogels.

Environmental pH value has a large effect on the swelling behavior of these gels. From Fig. 8, it is clear that the SR value increases with the increase of pH. Such pH-dependent properties of the hydrogels come from the polyelectrolyte nature of gelatin segments in the hydrogel network. Namely, when the pH value of the buffer solution (pH 9.0) was far higher than the isoelectric point (PI) of GEL (PI 4.0–5.0), the carboxyl groups were de-protonized to carry negative charges, which made molecular chains repulsed to each other. The network became looser and it was easy for the water molecules to diffuse into the cross-linked network.

3.5. Studies of water retention

The water retention (WR) of hydrogels was measured as function of time. Kinetics curves of WR of the 1DKG,

2DKG, 3DKG and gelatin hydrogels are displayed in Fig. 9. The result showed that the decrease in weight was curvilinear with time up to 100 h and thereafter did not change significantly; evaporative loss was about 90% within 100 h.

It is interesting that the water retention of hydrogels is inconsistent with the swelling behavior of the hydrogels. Pure gelatin possesses excellent hydrophilic nature and the highest SR value, while it hold most rapid water loss rate. At the same time, the content of DAK did not exert an obvious influence on water retention of the hydrogels. We can see that there is only a slight difference in water retention between 1DKG and 3DKG gels, although the 3DKG composite gel possesses higher SR value than 1DKG gel. Even then, compared with pure gelatin, all composite hydrogels showed more excellent water retention. It is interesting in the sense that, when used as wound coverings, they will be able to provide a moist environment to the wound surface for prolonged periods leading to better healing of the wounds.

3.6. Network parameters of composite hydrogels

In this paper, the structural features of DKG hydrogels were investigated by equilibrium swelling measurement, which is a well-established method in polymer network studies. One important structural parameter characterizing crosslinked polymer is M_c, the magnitude of M_c significantly affects the physical and mechanical properties of crosslinked polymers. High values of M_c imply loosely cross-linked hydrogel. M_c was calculated according to the (Peppas, Hilt, Khademhosseini, & Langer, 2006) following equation:

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{(\upsilon/V_1)[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} - \chi_1\upsilon_{2,s}]}{\upsilon_{2,r}\left[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} - \left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)\right]} \quad (3)$$

where M_n is the number average molecular weight of the uncross-linked polymer, $M_{n\mathrm{GL}} = 8.7 \times 10^4$, $M_{n\mathrm{DAK}} = 1.06 \times 10^5$, and the M_n of each composite hydrogel was calculated as a weighted average of the $M_{n\mathrm{GL}}$ and $M_{n\mathrm{DAK}}$ values. υ is the specific volume of the polymer (i.e., the reciprocal of the amorphous density of the dry gels which was measured by the equilibrium sedimentation method). V_1 is the molar volume of the swelling medium (18.06 cm^3/mol for water), and χ_1 is the Flory–Huggins interaction parameter between solvent and polymer. The higher the value of χ_1, the weaker is the interaction between the polymer and water. Where X_1 is calculated as average value of DAK and gelatin, for DAK, similar with that of guar gum (Li, Wu, Wang, & Duan, 2006), $X_{1\mathrm{DAK}} = 0.4940$, and gelatin $X_{1\mathrm{GL}} = 0.49$ in water (Patil, Mark, Apostolov, Vassileva, & Fakirov, 2000), so $X_{1\mathrm{DKG}}$ is about 0.4920. $\upsilon_{2,s}$ and $\upsilon_{2,r}$ are the volume fraction of polymer of swollen and relaxed state gel, respectively, and calculated using the method of the literature (Spizzirri & Peppas, 2005). The swelling ratio (Q) is equal to $1/\upsilon_{2,s}$.

Table 2
The structure parameters of the DKG hydrogels in pH 4.0 and pH 9.0

Sample code	1DKG	2DKG	3DKG
Weight ratio of DAK/GL	2/1	1/1	1/2
Specific volume (cm^3/g), υ	0.686	0.705	0.707
$V_{2,r}$	0.0821	0.0747	0.0718
pH 4.0			
Equil. swelling ratio, Q	18.98	19.75	24.30
Crosslinks density (mol/cm^3), $\rho_x \times 10^4$	2.803	2.524	1.527
$V_{2,s}$	0.0526	0.0506	0.0412
Crosslinks molecular weight, M_c	5200	5620	9262
pH 9.0			
Equil. swelling ratio, Q	25.90	29.03	30.79
Crosslinks density (mol/cm^3), $\rho_x \times 10^5$	10.18	8.549	7.697
$V_{2,s}$	0.0386	0.0345	0.0325
Crosslinks molecular weight, M_c	14320	16590	18376

An additional parameter of importance in structural analysis of hydrogels is the cross-linking density, ρ_x, which is defined by the following equation:

$$\rho_x = \frac{1}{\upsilon \overline{M}_c} \quad (4)$$

Table 2 depicts the calculated values of various network parameters for each hydrogel at different pH. This clearly indicates that as the DAK content increases, the cross-linking density increases, and leads to a decrease in the equilibrium swelling ratio as mentioned earlier. This may be attributed to the fact that the increase in the cross-linking density will finally cause less space for accommodation of water molecules in the network, which incurred to low swelling ratio. Moreover, the increased cross-linking density of the hydrogels also restricts the movement of macromolecular chains in the matrix, thus contributing to higher mechanical properties and lower breaking elongation. So the results in Table 2 coincide with the result of mechanical and swelling properties analysis earlier.

As shown in Table 2, the external pH imposes an effect on the cross-linking density. Comparing with pH 9.0, hydrogels possess higher cross-linking density at pH 4.0, which is consistent with a lower equilibrium swelling ratio at pH 4.0. The above result also indicated that there are other polymer chain entanglements or intermolecular interaction contributed to cross-linking density of network.

3.7. In vitro release study

Figs. 10 and 11 show the cumulative release profiles of different hydrogel formulations with varying amounts of gelatin and DAK in buffers of pH 4.0 and 9.0 at 37 °C as a function of time. In individual buffer conditions, we observed that all formulations present sustained release properties.

Fig. 10 suggests that the release rates of the three formulations of composite hydrogels are slightly different. About 73.6% of the incorporated ketoprofen was released from hydrogels 3DKG within 6.5 h at pH4.0. The relatively

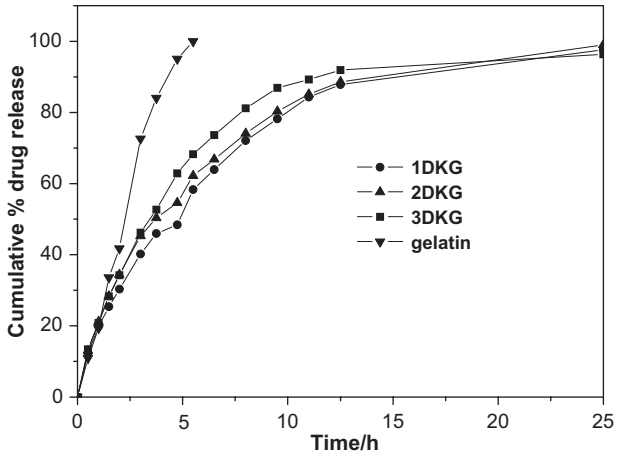


Fig. 10. Time dependence of ketoprofen release from the hydrogels at pH 4.0.

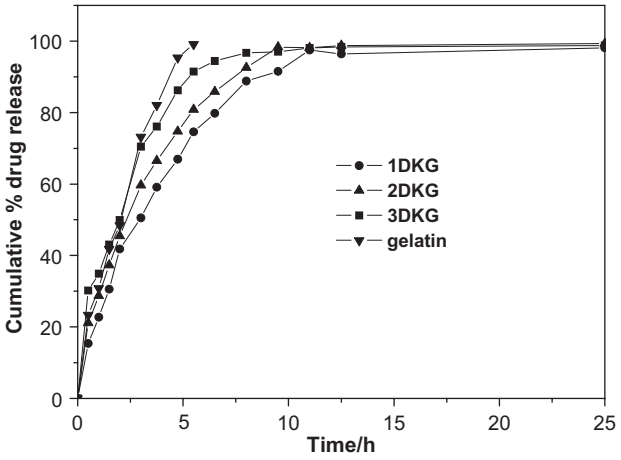


Fig. 11. Time dependence of ketoprofen release from the hydrogels at pH 9.0.

slower release of ketoprofen was recorded from 2DKG and 1DKG hydrogels, and the release percent decreased to 66.8% and 63.9% over the same time period, respectively. At the same time, the sole gelatin hydrogel showed most rapid release rate, almost all drug was released within 5 h. Obviously, the release rate is affected by the content of DAK in hydrogel matrix. The high content of DAK in the hydrogel matrix causes a delay in the kinetics of drug release. Furthermore, the similar in vitro release trend is shown at pH 9.0 medium (Fig. 11).

As illustrated in Figs. 10 and 11, the release of ketoprofen from hydrogels also depends on the pH value of the release medium. Compared with the release profile at pH 4.0, ketoprofen was released more rapidly at pH 9.0. The release half time t_{50} (time required for releasing 50wt% of drug) for 3DKG, 2DKG, and 1DKG is 2.0, 2.5, 3.0 and 3.5, 3.75, 4.75 h at pH 9.0 and 4.0, respectively. It suggests that the drug release profiles of DKG hydrogels are pH-sensitive. The lower solubility of the ketoprofen at pH 4.0 may contribute to hinder the release process directly; otherwise, this result is also in good agreement with the effect of the pH values on swelling of hydrogels as mentioned earlier. Namely, at pH 9.0, the relatively high swelling degrees of DKG hydrogels result in higher release rates.

To study ketoprofen transport mechanism from different designed DKG hydrogels, the experimental data have been further analyzed according to the following Ritger–Peppas (Ritger & Peppas, 1987) model (Eq. (5)) and Peppas–Sahlin (Peppas & Sahlin, 1989) model (Eq. (6)).

$$M_t/M_\infty = k_t^n \tag{5}$$

$$M_t/M_\infty = A_t^{1/2} + B_t \tag{6}$$

In the above equations, M_t/M_∞ is the fraction of drug released at time t, k is a constant related to the properties of the drug delivery system, and n is the diffusion exponent, which characterizes the drug release mechanism. A value of n of 0.5 indicates the drug release follows the Fickian diffusion; when $n = 1$, case II transport occurs; when $0.5 < n < 1$, anomalous transport is observed. In Eq. (6), A, B are diffusion and erosion terms, respectively. When $A > B$, the diffusion factor prevails in release system, when $A < B$, erosion predominates. If $A = B$, then the release mechanism includes both diffusion and erosion equally (Toti & Aminabhavi, 2004).

The characteristics of a drug delivery system are evaluated using the first 70% release data in this paper. Values of the various parameters in pH 4.0 and pH 9.4 media are shown in Table 3.

From Table 3, in pH 4.0 medium, the release rate is in good accord with the Ritger–Peppas model (Eq. (5)) and the Peppas–Sahlin model (Eq. (6)) with high correlation coefficient r^2 value (>0.98). The n values of 3DKG, 2DKG and 1DKG were 0.695, 0.607 and 0.624, respectively. It is believed that the ketoprofen release mechanism followed anomalous transport process and is mainly governed by the swelling of the hydrogel. Moreover, in all cases, the value of A is greater than B value, again maintaining dominant role of diffusion release mechanism. At pH 9.0, release rate is also in good accord with the Ritger–Peppas model and the Peppas–Sahlin model. Accord-

Table 3
Comparison of drug release kinetic rate from DKG composite hydrogels derived using various modeling equation

Samples	Equation					
	Ritger–Peppas			Peppas–Sahlin		
	k	n	r^2	A	B	r^2
pH 4.0						
Gelatin	0.232	0.941	0.981	0.193	0.049	0.986
3DKG	0.212	0.695	0.999	0.164	0.057	0.999
2DKG	0.221	0.607	0.993	0.232	0.015	0.995
1DKG	0.197	0.624	0.993	0.177	0.029	0.996
pH 9.0						
Gelatin	0.321	0.704	0.990	0.191	0.120	0.993
3DKG	0.368	0.541	0.983	0.309	0.041	0.986
2DKG	0.303	0.583	0.997	0.266	0.039	0.997
1DKG	0.255	0.613	0.994	0.227	0.039	0.995

ing to the diffusion exponent n, diffusion and erosion terms *A* and *B*, drug release is expressed by anomalous transport process too, and mainly controlled by the cross-linking density of the hydrogel.

According to above results, we believed that the ketoprofen release mechanism can result from the superposition of various effects, such as swelling property of hydrogels, the solubility of the drug and erosion property of matrix; it is not necessarily based on a single factor.

4. Conclusion

Novel, non-toxic, in situ forming hydrogel was prepared by employing DAK as macromolecular crosslink agent of gelatin. The structure–function relationship associated with the mechanical, swelling, morphology, and drug release properties could be modulated and correlated by varying the amount of DAK. The composite hydrogels were sensitive to the pH value of the medium. The results of in vitro drug release experiments showed that all the hydrogels showed sustained release properties, and the dependence of release rate on the equilibrium swelling ratio of hydrogels and pH value of medium. Taken together our results suggest that DAK could be an interesting alternative method to cross-link gelatin. This specialized delivery system could be potentially useful for localized drug delivery in in vivo or in vitro environment.

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