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Anti-degradation gelatin films crosslinked by active ester based on cellulose†

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Functionalization of microcrystalline cellulose (MCC) with EDTA dianhydride (EDTAD) was first achieved using an esterification reaction. *N*-Hydroxysuccinimide-activated MCC-EDTAD ester (MEN), a novel macromolecule crosslinker based on MCC, was synthesized for the modification of gelatin films. The reaction between gelatin and MEN was verified by the residual free amino test, FTIR and XRD spectra. The introduction of MEN into gelatin decreased the film degradation ratio and increased its thermal stability, flexibility, hydrophobicity, light barrier performance and water uptake ability. Additionally, SEM images proved the successful surface grafting reaction and degradation phenomenon. This unique gelatin film material with advanced properties broke the limitation of the blending method for modification of gelatin with macromolecules and broadened its application as a novel sustained-release material.

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1. Introduction

Gelatin is a peptide-based polymeric material, obtained *via* the hydrolytic treatment of collagen under acidic or alkaline conditions. The triple helix structure of collagen partially separates and ruptures, and a non-uniform mixture of polypeptides with different amino acids are formed.[1] Because of its good biological properties and low toxicity, gelatin is widely used for different kinds of materials, such as sponges, films, microballoons, scaffolds, nanoparticles, bandages, *etc.*[2–6] However, the relatively weak thermal stability, poor mechanical properties and easily-degradable quality limit the potential application of gelatin as a practical material.[7] Microcrystalline cellulose (MCC), a linear polysaccharide comprising β-glucoside units, is usually blended with gelatin to overcome the obstacles of the biopolymer matrix.[8–10] Its excellent properties, such as renewable origin, biodegradability of its components, and environment-friendly and non-toxic character, further broaden its usages.[10–13] Ethylenediamine tetraacetic dianhydride (EDTAD) is commonly used as a chelating reagent.[14] Its biodegradable behavior and special molecular structure, which consists of two anhydride groups that can react with hydroxyl or amine groups, ensure its function in the modification of biomaterials.[15,16]

In recent years, a great number of researchers worldwide have been devoted to the modification of gelatin with various crude macromolecules, such as cellulose, chitosan, starch, montmorillonite, polyvinyl alcohol, zeolite, *etc.*[17–22] Jridi *et al.*[23] investigated the physical, structural, antioxidant and antimicrobial properties of gelatin/chitosan composite films and chose the best proportion of the two components to be applied as a food packaging material; Li *et al.*[24] prepared active gelatin-based films incorporating five kinds of natural antioxidants and compared the effect of these extracts on the antioxidant, physical and mechanical properties of the films; Alves *et al.*[25] studied the effect of three components (gelatin, cellulose, starch) on the biodegradation, water vapor permeability and mechanical properties of the starch/cellulose/gelatin nanocrystal films using orthogonal experiments; Andrade *et al.*[26] reported a new edible coating material containing gelatin and cellulose nanofibers, and evaluated the wettability of the coating film on banana and eggplant epicarps. Unfortunately, the existing modification of gelatin-based composite films with natural polymers, especially cellulose, is mostly achieved using a blending method, in which the hydrogen bonding or electrostatic interactions are used to explain the reaction mechanism of the polymer matrix. No exact chemical reaction occurs between the gelatin and the original cellulose. Therefore, proper chemical modification of cellulose is needed to make the crosslinking reaction with gelatin possible. Cheng *et al.*[27] oxidized cellulose by periodate oxidation to obtain 2,3-dialdehyde cellulose (DARC), which then reacted with collagen *via* a Schiff base reaction between $-NH_2$ in the collagen and –CHO in the DARC backbone to obtain DARC/Col composite films; Li *et al.*[28] employed the same oxidation process to oxidize carboxymethyl cellulose, and the product, with two aldehyde groups, reacted with gelatin to prepare an edible film material.

Recently, a novel crosslinker, *N*-hydroxysuccinimide (NHS) active ester, which is synthesized *via* the reaction between

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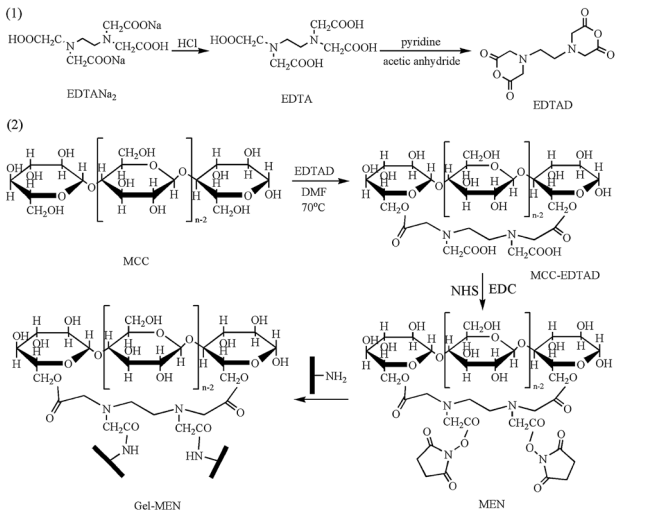
carboxylic acid and NHS in the presence of a carbodiimide,[29] has attracted a substantial amount of attention, mainly due to its cytocompatibility, biocompatibility and availability.[30,31] Furthermore, Gil's group[32] has concentrated on modifying sugarcane bagasse, which is a raw material of cellulose, with EDTAD to gain the ester group for its use as an absorbent material. In light of this research, the hydroxyl and/or carboxyl functional groups in these three biological polymers (gelatin, cellulose and EDTAD) further guaranteed the chemical reaction to produce materials with new properties.[33]

In this paper, microcrystalline cellulose was modified with EDTAD to obtain a new type of cellulose ester, MCC-EDTAD (ME). Then, a novel macromolecule crosslinker, *N*-hydroxysuccinimide activated MCC-EDTAD ester (MCC-EDTAD-NHS, MEN), was synthesized in the presence of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC) to react with gelatin (Scheme 1), and a biological polymer film with new qualities was found. FTIR, XRD, TGA-DSC, mechanical properties, contact angles and residual amino group testing were applied in our present study. Additionally, *in vitro* degradation studies, light barrier properties and water uptake measurements of the crosslinked gelatin films were investigated. On the basis of these results, the comparison of the thermal stability and light barrier properties between MEN-modified gelatin films (Gel–MEN) and cellulose blending films (Gel/MCC) were explored.

2. Experimental

2.1 Materials

Gelatin (type A, obtained from pigskin, with an approximate molecular weight of 50 000 and isoelectric point at pH = 8, determined using fluorescence measurements) was obtained from Sinopharm Chemical Reagent Co., Ltd. MCC (extra pure, average particle size 90 μm), NHS (AR, 98%) and EDC (AR, 99%) were purchased from Energy Chemical Technology Co., Ltd (Shanghai). Glycerol (AR, 99%), DMF (AR, 99.5%), EDTA disodium salt (AR, 99%), acetic anhydride (AR, 98.5%) and other agents were obtained from Tianjin Fu Yu Fine Chemical Co., Ltd. All chemicals and reagents were used as received without further purification.



Scheme 1 The formation process of crosslinked gelatin with MEN. (1) The synthetic route of EDTA anhydride (EDTAD); (2) the preparation path of gelatin modified with MEN.

2.2 Preparation of MEN

2.2.1 Synthesis of EDTA dianhydride (EDTAD). The EDTA dianhydride was prepared using the method described by Gil[34] with EDTA disodium salt and acetic anhydride as ingredients. 25 g EDTA disodium salt was dissolved in 250 ml distilled water to obtain a clear solution, and then HCl was added dropwise until precipitation of EDTA occurred. The precipitate was vacuum filtered and rinsed with 99% EtOH and 99% diethyl ether, subsequently dried in an oven at 70 °C, and cooled in a desiccator prior to use.

For the preparation of EDTA dianhydride, 18 g EDTA was suspended in 50 ml pyridine and 25 ml acetic anhydride was added. Then, the mixture was heated under reflux and kept stirring at 65 °C for 24 h. After reaction, the solid obtained was vacuum filtered, rinsed with diethyl ether and dried under vacuum at 50 °C. The prepared EDTAD was characterized using ^{1}H-NMR spectroscopy (Bruker Advance 400 spectrometer) and FTIR spectroscopy (Nicolet NEXUS 470 FT-IR spectrometer).

2.2.2 Synthesis of MCC-EDTAD (ME). The functionalization of MCC with EDTAD was carried out according to the method of Gil's group,[35] with slight modification. 9 g MCC and 3 g EDTAD were suspended in 100 ml DMF, and then the mixture was shaken and heated under reflux at 75 °C for 24 h. The modified material was isolated by filtration under reduced pressure, washed sequentially with DMF, distilled water, saturated $NaHCO_3$ solution (in order to release carboxylate and amine functions), distilled water, and then ethanol. After drying under vacuum at 50 °C, the percent mass gain was calculated using eqn (1).

$$\text{Weight gain } (\%) = \frac{m_{\text{modified}} - m_{\text{unmodified}}}{m_{\text{unmodified}}} \times 100 \qquad (1)$$

2.2.3 Synthesis of NHS MCC-EDTAD active ester (MEN). MEN was synthesized using the method of Li,[36] with a bit of improvement. A mixed solution was prepared by dissolving 12.5 mmol ME, 50.0 mmol NHS, and 50.0 mmol EDC together in 200 ml distilled water and was gently stirred at 40 °C for 1 h. After the reaction, the solid was vacuum filtered, washed with distilled water several times, then dried under vacuum at 50 °C to get purified MEN. The percent mass gain was once again calculated using eqn (1). The obtained ME and MEN were characterized using FTIR spectroscopy (Nicolet NEXUS 470 FT-IR spectrometer), an Elemental Analyzer (Vario EL III, Elementar Analysensysteme, Germany) and TGA-DSC (Q600SDT, TA, USA).

2.3 Modification process and film formation

A gelatin solution (3%, w/v) was prepared by dissolving gelatin powder in distilled water and was then heated at 45 °C for 2 h

under continuous stirring. Glycerol was added as a plasticizer at a certain concentration (15% of dry gelatin weight). The dosage of MEN was determined by the mass ratio with gelatin, *i.e.*, $m_{MEN}/m_{gelatin} = 0\%$, 5%, 10%, 15%, 20%, 25%, 30%. Therefore, the corresponding modified gelatin samples were named as Gel, Gel–5%MEN, Gel–10%MEN, Gel–15%MEN, Gel–20%MEN, Gel–25%MEN and Gel–30%MEN, respectively. The appropriate weight of MEN powder was dissolved in distilled water under stirring for 12 h at room temperature to produce a liquid suspension. Then, the solution was added dropwise to the gelatin liquid, and acetic acid (3% of water volume) was added dropwise into the whole system to promote the start of the interfacial reaction. These mixtures were gently stirred for 12 h at 45 °C.

To cast the films, 30 g of each gelatin reaction solution was transferred into a teflon dish and placed at room temperature for 2 h, then put in an oven at 40 °C until the films dried. The dried films were peeled off and stored in a desiccator with relative humidity ≤20%. In addition, one part of gelatin reaction solution was freeze dried at −55 °C, 70 Pa with a vacuum freeze drier (FD-1A-50, Beijing, China) and the lyophilized powder was characterized using FTIR spectroscopy (Nicolet NEXUS 470 FT-IR spectrometer).

2.4 XRD analysis

XRD analysis of the samples was performed on an X-ray diffractometer (D8-ADVANCER, Bruker AXE, Germany) with a thin film attachment using Cu-Kα radiation ($\lambda = 0.1541$ nm) at a current of 40 mA and an accelerating voltage of 40 kV. The patterns were recorded from 10° to 60°.

2.5 Determination of residual amino groups in gelatin

The residual $-NH_2$ groups in the modified gelatin solution were determined using the improved Van Slyke method at 45 °C.[37,38] Sample solutions were mixed with acetic acid and sodium nitrite and stirred for 45 min. The residual primary amine (mol g^{-1}) was calculated according to the volume of N_2. All samples were tested in triplicate.

2.6 *In vitro* degradation studies

The degradation study of gelatin films was carried out *in vitro* by incubating in phosphate buffer (pH 7.40) at 37 °C for different intervals (1, 3, 5, 7, 9, 12 and 24 h), which was developed from the method of Haroun.[39] The gelatin films were dried at 60 °C to a constant weight prior to use and marked as m_0. After different degradation times, the samples were washed with distilled water after filtering under vacuum and dried at 60 °C to a constant weight (m_t). The degradability performance was examined from the weight remaining using eqn (2).

$$\text{Weight remaining } (\%) = \frac{m_t}{m_0} \times 100 \quad (2)$$

2.7 Scanning electron microscopy (SEM) of gelatin films

The microstructures of the prepared films were investigated using a Quanta 200 environmental scanning electron microscope (FEI Company, Holland). Before observation, the film surfaces were coated with Au using a SEM coating device. More than ten micrographs were taken from different zones of each surface film.

2.8 Thermogravimetric analysis

The thermal stability of the gelatin films was determined using thermogravimetric analysis and differential thermal scanning calorimetry synchronous apparatus (TGA-DSC, Q600SDT, TA, USA). The gelatin film samples (approximately 2.5 mg) were weighed accurately into aluminium pans and sealed. The endothermal curve of the crushed film was recorded from 20 °C to 500 °C at a scanning rate of 10 °C min^{-1} under nitrogen atmosphere. Additionally, the thermal stability of the Gel/MCC blend film was also studied and compared with the Gel–MEN films.

2.9 Mechanical testing

Prior to investigating the mechanical properties, the films were conditioned for 48 h at 20 °C and 50 ± 5% RH. Tensile strength (T_s), elasticity modulus (E_m) and elongation at break (E_{ab}) were determined as described by Benjakul[40] with a slight modification, using a Microcomputer Controlled Electronic Tensile Testing Machine (WDL-005, Jinan, China) equipped with a tensile load cell of 300 N. Samples with initial grip length of 25 mm were used for testing and the cross-head speed was set at 10 mm min^{-1}. The thickness of each film was measured using a Vernier Caliper (0.02 mm/150 mm, Shanghai, China).

2.10 Contact angle measurement

The water contact angles (CAs) of all films were measured using the Sessile drop method with a DSA100 contact angle measuring system from Krüss. The gelatin reaction solution was coated on the surface of a glass sheet to obtain films with thickness of about 0.1 mm, and then stored in a desiccator with relative humidity ≤20%.

2.11 Light barrier properties and transparency

The ultraviolet and visible light barrier properties of the films (1 cm × 2 cm) were measured using an ultraviolet-visible spectrophotometer (UV-7504C, Shanghai, China) at selected wavelengths from 200 to 800 nm following Fang's method.[41] The transparency values of the films were calculated using eqn (3), where T is the transmission (%) at each wavelength and x is the film thickness (mm). According to the equation, high transparency values indicate good light barrier performance.

$$\text{Transparency value} = -\log T/x \quad (3)$$

2.12 Water uptake measurement

The water uptake of the films was determined similarly to Kavoosi[42] and Tang,[43] with a little development. Rectangular specimens sized 15 mm × 10 mm with a thickness of 0.1 mm were prepared. The samples were conditioned at 20 °C in a desiccator containing silica gel (RH 20% ± 5%) for three days, to constant weight (W_i). Then, the film samples were transferred into desiccators at 100% relative humidity (super-saturated salt solution of $CuSO_4 \cdot 5H_2O$) at 20 °C for eleven days to absorb water until the weight reached equilibrium. The weights of the samples at the adsorption time t were noted as W_t. The amount of water adsorbed at different intervals and at equilibrium were calculated using eqn (4). All tests are the means of at least three measurements.

$$\text{Water absorption } (\%) = \frac{W_t}{W_i} \times 100 \quad (4)$$

3. Results and discussion

3.1 Characterization of MEN

3.1.1 Spectra of EDTAD. ^{1}H NMR (400 MHz, DMSO, Fig. S1†): δ 3.691 (s, 8H), 2.657 (s, 4H), 3.080 (s, DMSO), 2.496–2.488 (m, DMSO), which is in accord with the characteristic peaks of H in the ideal product. The FTIR spectrum (Fig. S2†) further proved the dianhydride structure, with two groups of splitting peaks. The peaks in the high frequency region split at 1813, 1759 and 1689 cm^{-1}, with a gap of 60 cm^{-1} between adjacent peaks. The low frequency groups split at 1139, 1074, and 991 cm^{-1}, with the same interval. Additionally, the bands at 1245 and 1400 cm^{-1}, related to C–O and C–N stretching, respectively, were also evidence of the EDTAD structure.

3.1.2 Spectra of MCC, ME and MEN. The FTIR spectra (Fig. S3†) fully depicted the functional groups of MCC, ME and MEN. Compared with MCC, the appearance of a strong band at 1741 cm^{-1} in ME can be attributed to axial deformation of the ester bond, and bands at 1633 and 1406 cm^{-1} are attributed to asymmetric and symmetric axial deformations of the carboxylate. These bands confirmed the successful functionalization of MCC with EDTAD *via* the formation of ester linkages. For MEN, absorption peaks at 1706, 1210 and 811 cm^{-1} represented γ-dicarbonyl stretching vibration, C–N stretching and C–C vibration respectively. In particular, the reinforcement of the ester carbonyl band at 1742 cm^{-1} and the weakening of the carboxy carbonyl band at 1600 cm^{-1} further proved the structure of the active ester.

3.1.3 Elemental analysis and thermal properties of MCC, ME and MEN. As can be seen in Table 1, there was a considerable increase in nitrogen content of 1.92% after functionalization of MCC with EDTAD. Accompanied by the significant weight gain of 72.50%, the modified material (ME) with EDTAD incorporated was obtained. Similarly, the content of N increased to 2.48% in MEN, 0.50% higher than that of ME, which shows that the esterification reaction occurred between ME and NHS, with linkage of a five-membered nitrogenous ring. Also, the weight gain of 30.80% further proved this.

The initial decomposition temperature at 5% weight loss (T_i), the maximum weight loss temperature (T_m), the glass transition temperature (T_g) and the char residue at 500 °C of MCC, ME and MEN are recorded in Table 1 (Fig. S4†). The T_i values of MCC and ME were 309.10 °C and 271.45 °C, respectively, while that of MEN was 222.54 °C, which suggested a reduction in thermal stability. This can be related to the reactivity of the three materials with –NH_2 in gelatin, which was in accord with the results of the residual amino groups, below. To summarize, the difference of each item further verified the introduction of EDTA and NHS into MCC, which agreed with the FTIR and elemental analysis.

3.2 Confirmation of MEN crosslinking with gelatin

3.2.1 FTIR spectra analysis of MEN, gelatin and Gel–MEN film. FTIR spectra of the pristine gelatin (curve a), pristine MEN (curve b), and Gel–25%MEN film (curve c) are compared in Fig. 1. In the case of pristine gelatin, the C=O stretching vibration appearing at 1664 cm^{-1} demonstrated the amide I band, while the amide band II indicating the N–H bending vibration was observed at 1535 cm^{-1}. In addition, aliphatic C–H bending vibrations were observed at 1450 cm^{-1} and bands at 1331 and 1230 cm^{-1} showed the C–N bond stretching vibrations. Gel–MEN showed all the characteristic peaks of gelatin and MEN, such as those at 1643 and 1546 cm^{-1}, which indicated the successful reaction between gelatin and crosslinker MEN, along with a representative peak at 1741 cm^{-1}, which clearly indicated the amidation reaction between –NH_2 in gelatin and the active ester base in MEN.

3.2.2 X-ray diffraction studies of MEN, gelatin and Gel–MEN film. In order to examine the effect of MEN on the crystal structure and crystallinity of gelatin, the XRD patterns of the freeze-dried gelatin films are investigated. Data on the 25% MEN formulation are presented as a representative example. As shown in Fig. 2, curve (a) was the XRD pattern of MEN, which displayed the typical XRD pattern of the native cellulose with the main diffraction signals at around 14.9°, 16.2°, 22.5° and 34.3°.[44] Curve (b) only showed an extensively broadened peak in the 2θ range of 15–25°, which was a typical XRD pattern of pure gelatin, originating from the α-helix and triple-helical structure.[45,46] The XRD pattern of the Gel–25%MEN film is given in Fig. 4(c), in which the characteristic peaks of MEN (22.6°) and the characteristic broad diffraction peak of gelatin were each observed. This suggests that the gelatin was modified with MEN after the crosslinking reaction, which is consistent with the FTIR results.

3.2.3 Free –NH_2 in Gel–MEN film formation solution. Fig. 3 shows the changing curve (a) of residual primary amino groups in the Gel–MEN film formation solution and gelatin liquid blend with ME (Gel/ME, curve (b)) against the ratio δ ($\delta = m_{(MEN/ME)}/m_{(dry\ gelatin)}$). For Gel–MEN, the dosage of crosslinker played an important role in the content of free –NH_2, while the free –NH_2 changed little no matter how much ME was added. This suggested the stability of the ester group in ME, which was not active enough to react with –NH_2 in gelatin. This means that the amine groups in gelatin did not act as nucleophiles to break the ester bonds in ME but could break the ester bonds in MEN. All of these results confirmed that the reaction process (Scheme 1)

Table 1 Elemental analysis and thermal property values of MCC, ME and MEN

Materials	C (%)	H (%)	N (%)	Weight gain (%)	T_i (°C)	T_m (°C)	T_g (°C)	Residue (%)
MCC	42.21	6.40	0.11	—	309.10	364.08	340.68	15.28
ME	42.29	6.45	1.92	72.50	271.45	331.50	342.70	33.01
MEN	42.97	6.65	2.48	30.80	222.54	377.31	364.09	10.45

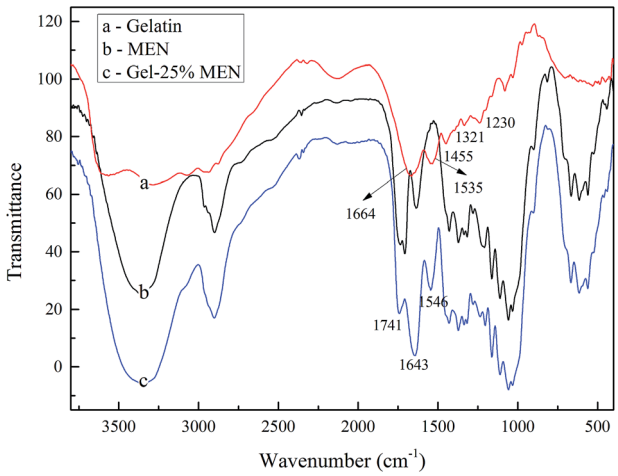


Fig. 1 The FTIR spectra of MEN, gelatin and Gel–MEN.

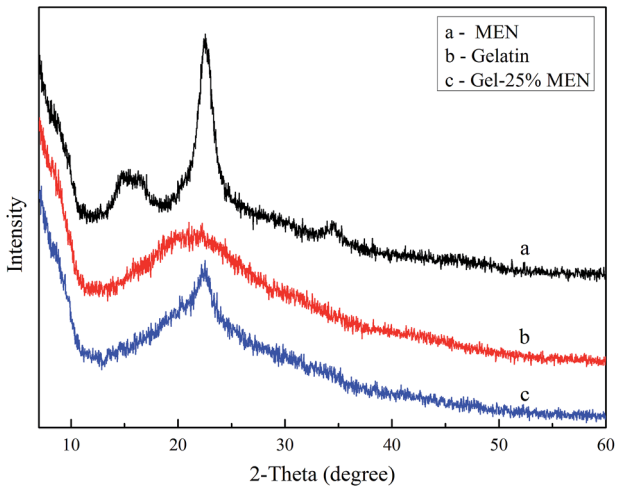


Fig. 2 The XRD patterns of MEN, gelatin and Gel–MEN.

we proposed was correct. Interestingly, after activation with NHS, the active ester MEN could consume $-NH_2$ in gelatin and was dose dependent. The amount of free $-NH_2$ decreased sharply when the ratio δ increased from 0 to 25%, and then decreased slightly when the ratio further increased. In particular, the amount of free $-NH_2$ decreased down to a minimum value of about 1.74×10^{-4} mol g^{-1} when $\delta = 30\%$. All of these results proved that the whole system overcame the inhibition of the interfacial reaction. Compared with the former interface reaction study by Xu,[47] in which gelatin was modified with glycidol and the maximum $-NH_2$ conversion rate was 42%, the $-NH_2$ conversion rate in this work was 28% higher than that reported.

3.3 Performance of Gel–MEN films

3.3.1 Degradation properties *in vitro*. As sustained-release materials, the composite films are expected to degrade at a proper rate to match particular needs and maintain activity within their service life. The degradation behavior of the films in a physiological environment plays an important role in their application as sustained-release materials. The *in vitro* degradation performance of the Gel and Gel–MEN films in phosphate buffered saline (PBS, pH 7.40) at different intervals was investigated. As shown in Fig. 4, the blank gelatin degraded rapidly because of the large number of hydrophilic amino and carboxyl groups in the gelatin backbone. In addition, the physical structure of gelatin, which possesses higher porosity and a thinner pore-wall, contributed to the minimum remaining weight of 15% at 24 h. The composite polymer Gel–MEN degraded proportionally slowly because of the incorporation of the cellulose-based crosslinker, MEN. The remaining weights of Gel–25%MEN and Gel–30%MEN at 24 h were 57% and 58%, respectively, 40% greater than that of the original gelatin films. The amido bond formed between MEN and gelatin was stable enough to resist adverse external factors. It was reasonable to consider that the strong hydrogen bonding and electrostatic interaction between the gelatin polypeptide and the hydrophilic hydroxyl or carboxylic groups in MEN also depressed the

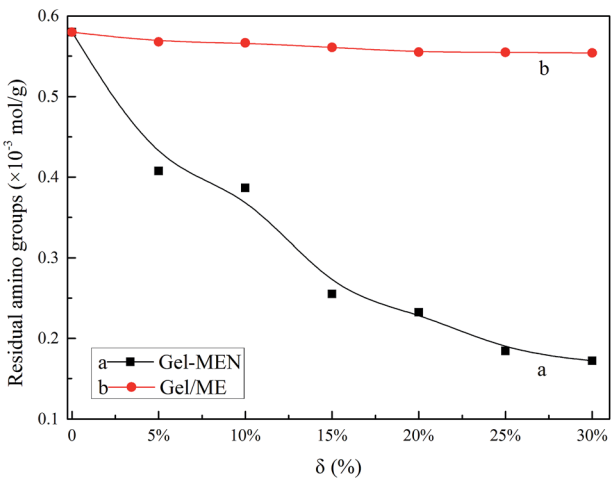


Fig. 3 Residual amino group content of gelatin solution modified with MEN (a) and ME (b) at different dosages.

diffusion of the PBS medium and protected the gelatin polypeptide chains from degradation. Meanwhile, the presence of MEN, a macromolecule crosslinker based on cellulose, also served as physical crosslinking sites, which enhanced the stability of the network. To conclude, MEN improved the anti-degradation performance of the gelatin films and this guarantees its potential usage as a sustained-release material in many fields, such as food packaging, medical engineering, controlled-release fertilizer in agriculture, and so on.

3.3.2 Morphology evaluation. SEM photographs of the blank films revealed a dense, smooth and compact structure without any embossing or holes, as shown in Fig. 5(a). The magnification of the first row (a_1, b_1, c_1) is lower than that of second row (a_2, b_2, c_2). The introduction of the cellulose-based crosslinker MEN destroyed the homogeneous film surface, with slice-like or rod-like macromolecules grafted on the covering of gelatin (Fig. 5(b)). The inset of Fig. 5(b_2) clearly displays the features of the MEN.

In addition, the SEM images provided very good evidence in favor of the *in vitro* degradation of the test sample (Gel–25% MEN). It can be seen from Fig. 5(b) and (c) that the film surface was almost planar and even, though combined with some sags and crests, before the degradation started. After one hour of degradation, a porous structure with irregularities and apertures can be observed on the surface of the composite film, which confirmed that the internal structure of the Gel–MEN polymeric film had started to degrade in the liquid medium. It can be assumed that the degradation of the films was gradually penetrating deeper from the surface.[48]

3.3.3 Thermal stability. The thermogravimetric analysis (TGA) and differential thermogravimetric curves (DTG) of the composite films, as shown in Fig. 6, are used to investigate the thermal stability. Curve a (gel) and curve b (gel/glycerol) were almost similar, with two representative peaks at 190.00–220.00 °C and 321.44 °C, which corresponded to the initial decomposition temperature at 5% weight loss (T_i) and maximum weight loss temperature (T_m) of gelatin. The special peak at 250.07 °C in curve b was due to the blending of glycerol

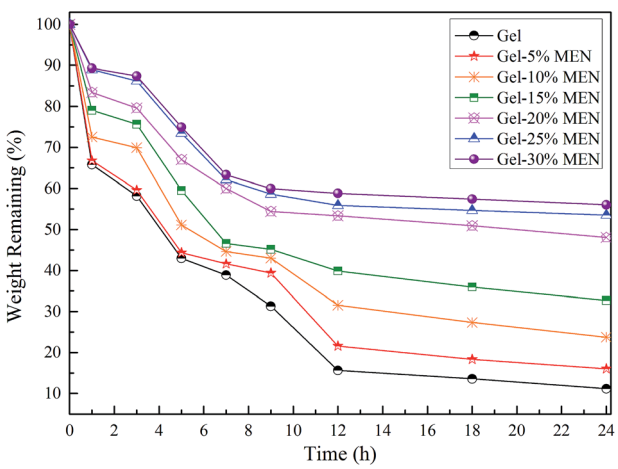


Fig. 4 Effect of macromolecule crosslinker on *in vitro* degradation of the Gel–MEN composite films.

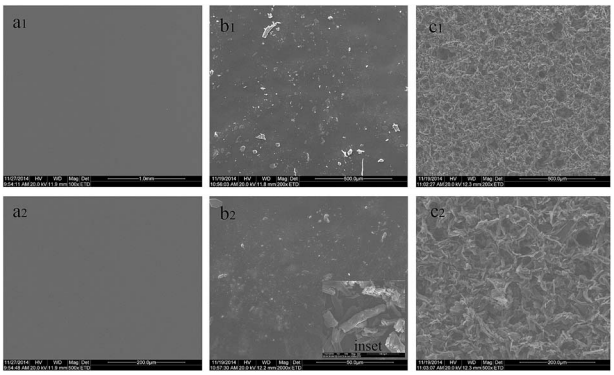


Fig. 5 Film surface morphology of Gel (a_1, a_2), Gel–25%MEN (b_1, b_2) and Gel–25%MEN after 1 h degradation (c_1, c_2).

as a plasticizer in the blank gelatin film. The DTG patterns of Gel–MEN presented three steps for weight loss at temperatures of around 100 °C, 250.07 °C and 320–350 °C, involving one strong and two weak endothermic peaks. The first weight loss at the temperature around 100 °C and the second weight loss at about 250.07 °C were similar to those of curve b. The third weight loss, with a strong endothermic peak at 320–350 °C, was due to the incorporation of the active ester MEN into gelatin, and exhibited a positive correlation with the dosage of crosslinker. This demonstrated that the crosslinking effect of the cellulose-based crosslinker improved the thermal stability of the material to some degree, as found in the literature. On the one hand, the crosslinking reaction between gelatin and MEN with formation of amido bonds made the macromolecule structure more stable and impregnable. On the other hand, the hydrogen bonding and electrostatic interaction of the functional groups in gelatin and MEN further strengthened the structure. All of these factors provided an effective reinforcement layer to endure thermal degradation. As Fig. 6-1 and 6-2 show, T_m reached a maximum of 349 0.26 °C when δ ($\delta = m_{(MEN)}/m_{(dry\ gelatin)}$) was 25%.

The thermal properties of Gel–25%MEN and Gel/25%MCC in the presence of glycerol were compared in Fig. 6-3 and 6-4, in which curve b consisted of four decomposition stages. The four peaks at 104.42, 192.23, 250.72 and 359.12 °C were resolved into four different components of water, gelatin, glycerol and MCC, respectively. The obvious peak at 192.23 °C that almost disappeared in curve a indicated the severe phase separation in the Gel/MCC system. However, compared with the typical T_m (309.10 °C) of MCC,[49] the increased decomposition temperature of 359.12 °C in curve b may caused by the hydrogen bonds formed between gelatin and MCC, which increased the thermal stability of the Gel/MCC films.

3.3.4 Mechanical properties. Mechanical properties, especially elasticity modulus (E_m) and elongation at break (E_{ab}), are particularly crucial for sustained-release materials in many fields. Table 2 shows the thickness, tensile strength (T_s), E_m and E_{ab} of the Gel–MEN composite films. The decreased T_s indicated that the modified films yielded at lower stress than the pure gelatin film. The tensile strength increased with added

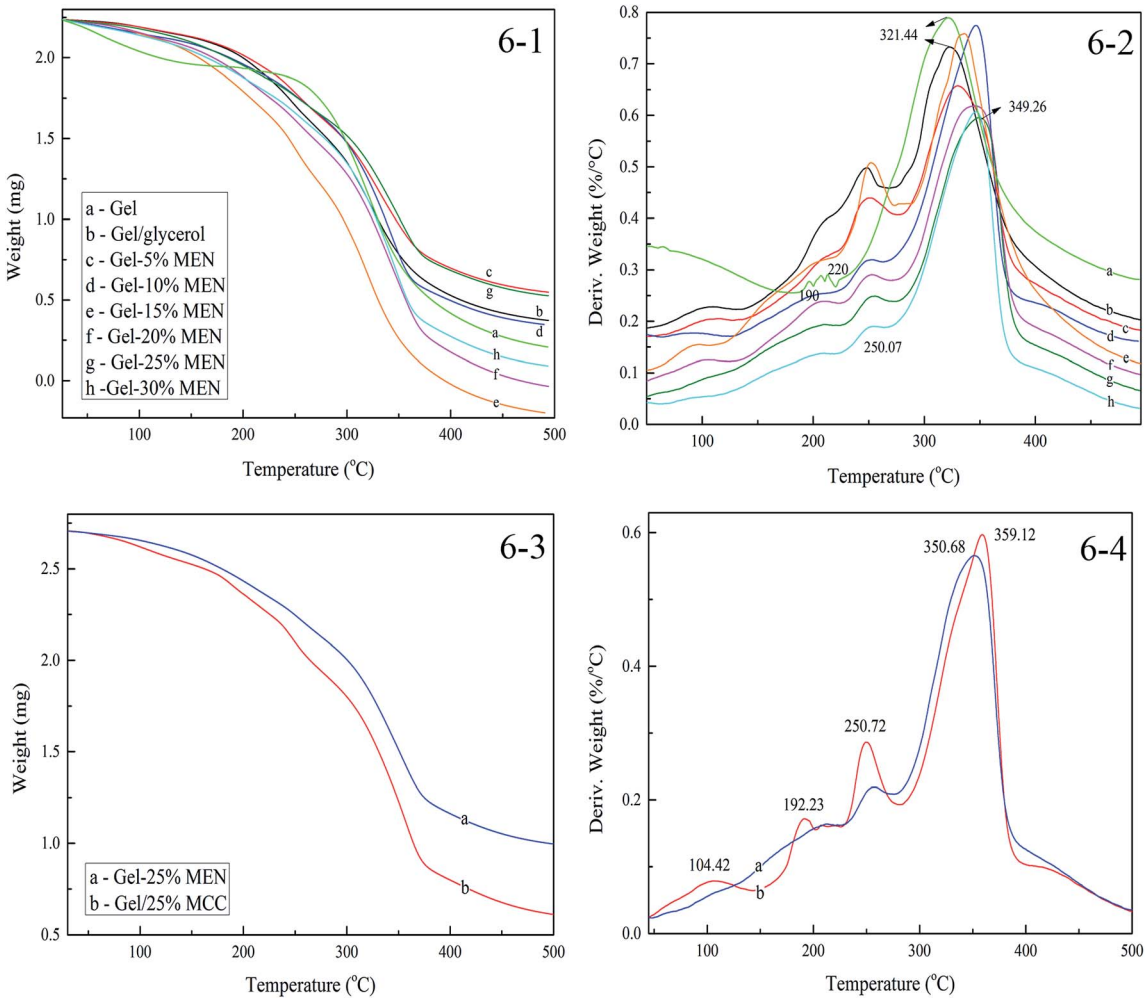


Fig. 6 TGA and DTG curves of modified gelatin films with different dosages of crosslinker (6-1 and 6-2) and comparison curves between Gel–25%MEN and Gel/25%MCC blend films (6-3 and 6-4).

crosslinker from Gel–5%MEN to Gel–20%MEN, but decreased in Gel–25%MEN and Gel–30%MEN. The results were in accord with the work reported by Azeredo.[50] This may caused by the fact that when the amount of the macromolecule crosslinker was high, adding it to the film may induce the development of a heterogeneous structure with the presence of discontinuous areas, which produced lower tensile strength. Similarly, Martucci[51] reported that the addition of dialdehyde starch in gelatin resulted in lower T_s values than the control film and explained this apparently anomalous behavior. The polymeric nature of the dialdehyde starch did not introduce severe restrictions within the gelatin matrix, as usually occurrs with short chain dialdehydes such as formaldehyde or glutaraldehyde, and some degree of phase separation in the gelatin–dialdehyde starch films could also reduce the T_s. Fortunately, E_m and E_{ab} tended to predict better elasticity and flexibility, indicating that the new material was not fragile anymore. The E_{ab} of Gel–25%MEN was 31.96%, thirty times that of the blank gelatin film, while the E_m of Gel–25%MEN was 448.72 MPa, a quarter that of the blank film, which was 1736.11 MPa. The reason was that the active ester group in MEN could form covalent bonds with the amino groups in the gelatin polypeptide, and the hydrophilic groups could form hydrogen bonds. All the newly formed bonds weakened the protein–protein interactions, which was effective for stabilizing the gelatin network. In addition, MCC, the base of the crosslinker MEN, has been demonstrated to be an effective nano-reinforcement for biopolymer films that can drastically influence the mechanical properties of biomaterials.[50] All of these factors contributed to better flexibility of the new biological polymer matrix.

3.3.5 Hydrophobicity analysis using contact angles. Gelatin is a kind of hydrophilic material because of its functional groups, amino, carboxyl, hydroxyl, and so on. Its water-sensitive properties have limited its application in many fields and hydrophobization was needed. Ninan[21] reported a new material based on a gelatin/zeolite porous scaffold and the contact angle was found to increase from 88.6 °C to 108.0 °C with increasing concentration of zeolites in the gelatin. The hydrophobic effect of the crosslinker MEN on gelatin was confirmed by the results of the water contact angle measurements (Fig. S5†). The pure gelatin film (Fig. S5a†) had a typical contact angle of 77.8° because of the hydrophilic groups

Table 2 Mechanical performance of different Gel–MEN films

Films	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Elasticity modulus (MPa)
Gel	0.10	24.17	1.84	1736.11
Gel–5%MEN	0.18	15.28	7.64	435.73
Gel–10%MEN	0.20	16.17	11.52	468.75
Gel–15%MEN	0.20	18.25	12.44	595.24
Gel–20%MEN	0.28	18.10	28.64	525.21
Gel–25%MEN	0.26	13.97	31.96	448.72
Gel–30%MEN	0.20	14.08	83.08	476.19

exposed on the gelatin chains. After being crosslinked by MEN, the Gel–MEN films (Fig. S5b and c†) presented a sharp increase to 125.1° and 135.5°, respectively. This was due to the replacement of some surface amino groups in the gelatin polypeptide with active ester groups in MEN. In addition, the hydrogen bonds formed between the hydrophilic groups of the gelatin backbone and the MCC-based crosslinker also contributed to good hydrophobicity of the modified gelatin films. The modified films, with perfect hydrophobicity, overcame the permanent weakness of water-sensitivity in the application of gelatin. The advanced properties broaden its usage as different kinds of material.

3.3.6 Water uptake studies. The hydrophilic property of gelatin can be controlled in two ways. One is the hydrophobization referred to above, and the other is the control of the expected absorption of water molecules. This occurs because of two beneficial structural features of gelatin. One advantage of gelatin-based materials is its highly hygroscopic nature, due to which it swells and transforms into any shape easily in a humid environment. The other is the porosity in the network that allows more water to enter, because of which the porous gelatin films show higher swelling capacity. The water uptake (%) values of the pure gelatin films and Gel–MEN composites tested over 11 days are shown in Fig. 7. The water uptake (%) can be controlled by incorporating different dosages of MEN in the polymer matrix. In the case of Gel–15%MEN, the water uptake (%) reached the maximum among all the MEN crosslinked gelatin films. This was attributed to the increase in pore size of the gelatin film with the presence of MEN. Additionally, the Gel–MEN composites showed an increase in the swelling capacity up to the 11th day, which indicated a better water absorption ability. By comparison, the original gelatin film acquired its maximum swelling capacity on the 10th day and thereafter the percentage of water uptake was found to be invariant, or even declining. This event suggested that introduction of MEN into gelatin provided an effective channel for water molecules to diffuse into the polymer matrix, and thereby the swelling ability increased. Uncontrolled swelling properties can badly affect the mechanical properties, so it is advantageous to tune the swelling capacity.[52]

3.3.7 Light resistance performance. Much research has indicated that ultraviolet radiation is one of the main causes of skin damage, light-stimulated aging and skin cancer. Hence, the low light transmission also gives active gelatin films some health function.[24] Light transmission at selected wavelengths from 200 to 800 nm in the UV and visible ranges and the transparency values of the gelatin films are shown in Table 3 (& Fig. S6†). Comparison of the results with the control films revealed that lower light transmittance (T) was found in the Gel–MEN composite films and the films with 30%MEN displayed the lowest values among them. This revealed that the addition of MEN improved the UV barrier properties of the gelatin films,

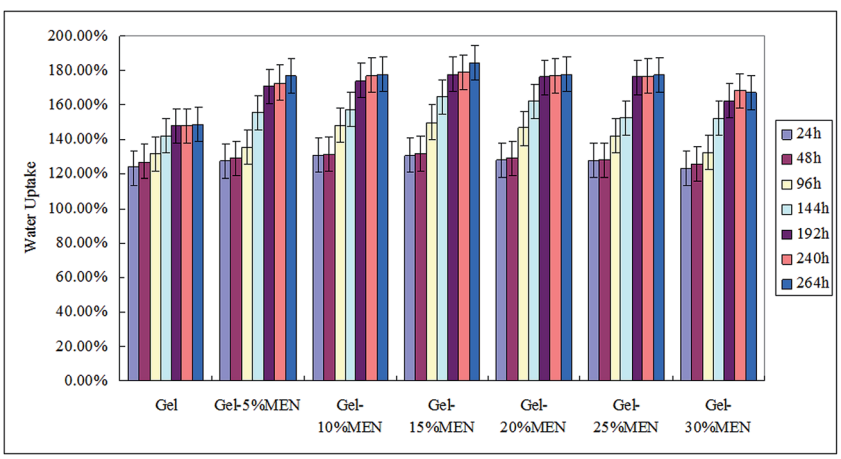


Fig. 7 Water uptake properties of gelatin films incorporated with different dosage of MEN.

Table 3 Light transmission and transparency values of different Gel–MEN films

	Wavelength (nm)										Transparency value	
Films	200	280	350	400	450	500	550	600	700	800	280	600
Gel	2.2	1.5	43.2	55.0	59.7	62.6	64.4	66.5	67.6	68.5	10.13	0.98
Gel–MEN5%	1.5	5.8	29.8	34.4	35.4	36.4	37.0	37.8	38.4	39.0	7.73	2.64
Gel–MEN10%	0.6	2.6	12.4	14.4	15.1	15.5	15.8	16.2	16.4	16.4	11.32	5.65
Gel–MEN15%	0.2	0.2	4.4	5.8	6.3	6.6	6.7	7.1	7.1	7.2	13.49	5.74
Gel–MEN20%	0.1	0.2	2.2	3.0	3.5	3.6	3.7	3.7	3.7	3.7	13.49	7.16
Gel–MEN25%	0	0	0.6	1.0	1.3	1.4	1.5	1.6	1.6	1.6	—	8.98
Gel–MEN30%	0	0	0.4	0.6	0.9	1.0	1.1	1.1	1.1	1.1	—	9.79
Gel/MCC15%	1.9	9.3	34.6	39.6	40.9	42.3	43.3	45.1	46.5	47.7	10.32	3.46
Gel/MCC25%	1.4	5.3	26.6	31.7	33.0	34.4	35.5	36.9	38.0	39.3	12.78	4.32

resulting from the amido bonds from the Schiff base formation between the active ester groups in MEN and the amino groups of the lysine or hydroxylysine side chains in gelatin. Based on the transparency values (T_v, Table 3), more crosslinker led to greater T_v, which represented the opacity of the resultant films. The opacity was highly influenced by the crystalline content of a sample: more compact polymer chains made it more difficult for light to pass through, and the opacity of the films was increased.[53] All of these results indicated that the protein-based films exhibit high UV barrier properties, owing to their high content of aromatic amino acids, which absorb UV light.

Table 3 also displays the light barrier properties of Gel/15% MCC and Gel/25%MCC blend films, as compared with the corresponding mass ratio of Gel–MEN films. The Gel/MCC blend films exhibited better transparency but worse light resistance performance. This fact may be an indication that MCC nanoparticles were homogeneously distributed in the matrix because they are white, and light incident on the film surface was reflected in a larger quantity due to the white particles.[25] The light barrier properties of the films are relatively important when used as sustained-release materials for food packaging or food coating. The polymer matrix in this work matches these needs.

4. Conclusion

In summary, the structure and conformation of gelatin were modified by the macromolecule crosslinker MEN. The FTIR spectra, elemental analysis and TGA values verified the structure of MEN. The reaction between $-NH_2$ in gelatin and the active ester in MEN was confirmed by the residual primary amino groups test, and the FTIR and XRD spectra, which broke the limitations of the blending method for modifying gelatin with macromolecules. The dose-dependent effect of the crosslinker was investigated through degradation *in vitro*, in which the weight remaining decreased with the increase in MEN dosage. The SEM images further proved the successful surface grafting reaction and the degradation phenomenon in PBS medium. The decomposition temperature obtained from TGA curves increased to 350 °C compared with that of the native film (320 °C). In addition, the TGA patterns of the Gel–MCC composites exhibited serious phase separation. The mechanical properties changed to some degree, with higher E_{ab} and lower E_m, which suggested better flexibility and shatter-proofing. The contact angles, with a highest value of 135.5°, indicated good hydrophobic properties. The swelling ability after absorbing water could be regulated by adding different weights of crosslinker. The light barrier performance was improved with the introduction of MEN compared with both pure gelatin film and Gel/MCC composites. Given the potential applications of gelatin, our study is an extension of the existing NHS crosslinking technique and will broaden the application of gelatin films as a sustained-released material in the food industry, medicine, agriculture, and so on.

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