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Effect of natural cross-linker on swelling and structural stability of kappa-carrageenan/hydroxyethyl cellulose pH-sensitive hydrogels

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Abstract−Genipin cross-linked kappa-carrageenan/hydroxyethyl cellulose hydrogels were prepared and the effect of cross-linking on hydrogels characteristics was investigated. Swelling and transform mechanisms of both native and cross-linked gels in different pH were also studied. We found that the concentration of genipin affects the physical stability of gels. The optimum concentration that a cross-linked hydrogel molecular structure can be in its most stable form is also discussed. Native hydrogels exhibited more swelling in alkaline medium than acidic and neutral; however, by increasing the cross-linker concentration, the swelling ability in neutral medium increased so that genipin cross-linked hydrogels could swell in pH 7 more than in pH 1.2 and 12. Fourier transform infrared spectroscopy (FTIR) was applied to study the formation of new bonding due to genipin reactions and explain hydrogels' stability in various concentrations. Differential scanning calorimetry (DSC) measurements reveal that by increasing genipin, the gel ability to hold water increases to some point and then decreases due to less structural stability. X-ray diffraction (XRD) and field emission scanning electron microscope (FESEM) tests were performed to study the crystallinity changes and microstructure of hydrogels. Finally, the power law model was applied to study the transform mechanism of hydrogels.

Key words: Swelling, Hydrogels Structural Stability, Genipin Cross-linking, Kappa-carrageenan/hydroxyethyl Cellulose, Characterization

INTRODUCTION

Colon-targeted delivery of bioactives has attained great value in addressing specific needs in the therapy of colon-based diseases. Many approaches into the utilization of the metabolic activity and the colonic microenvironment in the lower gastrointestinal tract have recently gained importance in the design of novel colon-targeted delivery systems based on natural biodegradable polymers. Hydrogels are polymeric networks capable of absorbing large amounts of water while remaining insoluble in water regarding chemical or physical cross-linking of individual polymer chains [1]. Hydrogels are of special interest in controlled release applications due to their soft tissue biocompatibility, easy dispersion of drugs within their network, and the high degree of control by selecting suitable physical and chemical properties of the hydrogel [2,3].

κ-carrageenan is a hydrophilic polysaccharide found as a matrix material in numerous species of seaweed [4]. It is a linear, sulfated polysaccharide, composed of repeating D-galactose and 3, 6-anhydro-D-galactose units [5]. The thermo-sensitive nature of κ-carrageenan hydrogels as well as their ability to form microparticles using in situ ionic gelation [6] makes these biomaterials interesting candidates for drug delivery applications. Moreover, applying κ-carrageenan as a supporting material to immobilize protein in controlled drug delivery systems has also received wide attention [7,8]. Improving the chemical and mechanical properties of these biomaterials for their various applications in medicine has gained much attention. Aqueous κ-carrageenan gels are known to possibly show an undesirable syneresis when under mechanical deformation or aging that can affect the mechanical and rheological properties of the hydrogels [9]. In response to that, some modifications have been reported such as hydroxyalkyl κ-carrageenan derivatives which exhibit a decrease in syneresis; hence they can be used in a wider range of industrial applications [9]. Daniel-da-Silva and coresearchers [10] reported the modification of carrageenans by co-precipitation of calcium phosphates into a κ-carrageenan matrix for applications in bone tissue engineering.

Cellulose and its various derivatives exhibit unique advantages due to their low cost, abundance as a natural polysaccharide, biocompatibility and better biodegradability. Hydroxyethyl cellulose (HEC) is known as one of the most important derivatives of cellulose, exhibiting excellent water solubility and biocompatibility. Its unique properties make it applicable in many industrial, biotechnological and biophysical fields [11]. On the HEC chains, there are high numbers of reactive -OH groups. HEC can also be modified by using grafting polymerization with hydrophilic vinyl monomers to formulate new materials [12].

To increase the time frame and stability of hydrogel carriers for drug delivery, the polymeric material needs to be cross-linked. Several cross-linking reagents have been used such as glutaraldehyde, tripolyphosphate, ethylene glycol, diglycidyl ether and diisocyanate. Genipin, however, as a natural cross-linker is found to be 10,000 times less toxic compared to other cross linking agents [13]. It has been widely used in herbal medicine due to its anti-inflammatory, diuretic, choleretic and hemostatic properties. Recently, it has been used as a cross-linking agent in polymers [14]. Touyama et al. [15] reported that cross-linked product with 5% of genipin offers a promising scaffold for disk tissue engineering. A dark blue pigment as a

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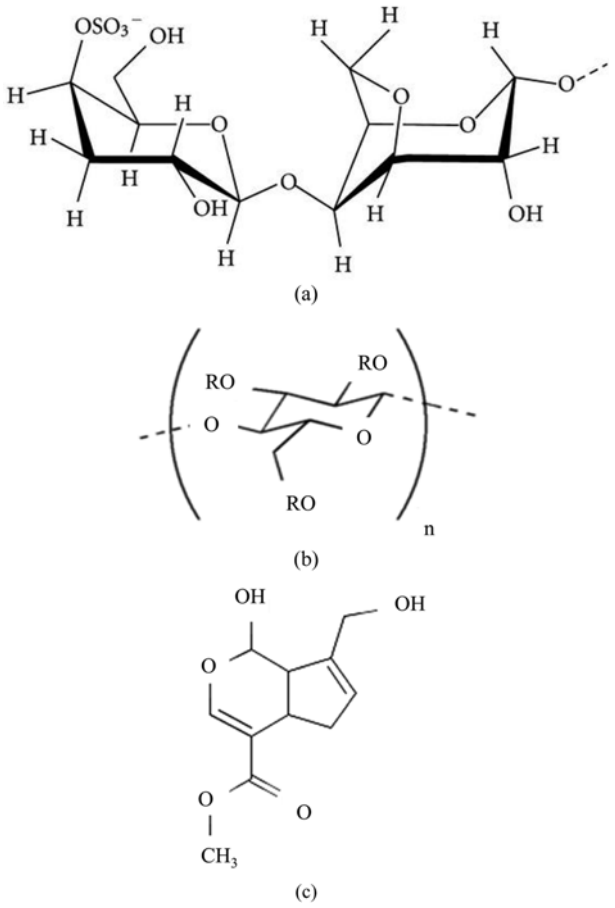

Fig. 1. Structure of (a) kappa-carrageenan (b) hydroxyethyl cellulose (c) genipin.

result of spontaneous reaction of genipin with amino acids or proteins can also be used in the fabrication of food dyes [15].

The molecular structures of κC, HEC and genipin are shown in Fig. 1. In this article, swelling behavior and characterization of kappa-carrageenan/hydroxyethyl cellulose hydrogels cross-linked with genipin is studied. The effect of genipin cross-linker on the stability of hydrogel networks as well as its transform mechanism is also investigated.

EXPERIMENTAL

1. Materials

Genipin was purchased from Challenge Bioproducts Co., Ltd. (Taiwan). κ-carrageenan (κC) was provided by Sigma-Aldrich. Hydroxy ethyl cellulose (HEC) was also provided by Sigma-Aldrich. All chemicals were used as received. Distilled water was used for the hydrogel synthesis.

2. Preparation of Hydrogels

Control blends of κ-carrageenan/HEC films were prepared by mixing the hot κ-carrageenan solution blend with HEC solution in different ratios. To formulate the blends, 30 ml of distilled water was added to required proportions of κ-carrageenan and HEC at 80 °C. This was done under reflux to make sure that water content does not change during the experiment for different samples. Applying gentle stirring ensures that no bubbles are formed in the final gel solutions. Samples were stirred for approximately one hour to obtain the desired solutions. To make samples in the shape of film, the resultant solutions were poured into ceramic moulds and samples with a diameter of 5.5 cm and a thickness of 1.5 cm were made. The heated polymer solutions in the moulds were then allowed to reach equilibrium for almost 24 hours at ambient temperature (25 °C) so the gels could form. In the final stage of the process, samples were dried at 37 °C in an oven over night.

3. Genipin Cross-linking

The most suitable κC: HEC blend ratio of hydrogel was selected for cross-linking. Genipin and a hot solution blend of selected κC: HEC hydrogel were added to make different concentrations of genipin stock solutions (0.1, 0.2, 0.3, 0.5 and 1.0 mM) by dissolving the genipin in 10 percent of water. This was done under continuous stirring to ensure no air bubbles will form in the solutions at 80 °C. The homogeneous viscous solutions were kept in the moulds at room temperature (27 °C) over night. Finally, the formulated films were dried in an oven at 37 °C for about 24 hours.

4. Measuring the Swelling Ratio

To study the swelling properties of synthesized hydrogels, buffer solutions of pH 1.2, 7 and 12 were prepared. Hydrogel samples were placed in separated Petri dishes filled with 30 ml of the buffer solutions. At different intervals, samples were removed from Petri dishes and weighed. Using filter paper, samples were wiped free of surface water before weighing. After weighing, samples were immersed in fresh buffer solution. The swelling ratios were then calculated using the following equation:

$$\text{Swelling ratio } (\%) = \left[\frac{W_t - W_0}{W_0}\right] \times 100 \quad (1)$$

where W_t is the weight of swollen gels at time intervals t, and W_0 is the initial weight of samples. Tests were performed in triplicate to increase the accuracy and results were reported as mean values.

5. Characterization of Hydrogel

5-1. Fourier Transform Infrared Spectroscopy (FTIR) of Hydrogel

FTIR analysis was performed for both native and cross-linked samples with KBr pellets. Measurements were carried out within the range of 370-4,000 cm^{-1} on Nicolet 670 FTIR. The number of scans was 16.

5-2. Differential Scanning Calorimetry (DSC) Measurements

The dried gels differential scanning calorimetry (DSC) was recorded using DSC822 METTLER TOLEDO (Switzerland) thermal analysis system. To do this, 4.8 mg of each sample was sealed in an aluminum pan and heated in the temperature range of 30 to 230 °C with an incremental heating rate of 5 °C/min. The flow rate of nitrogen was set at 50 ml/min.

5-3. X-ray Diffraction (XRD)

X-ray diffraction measurements of cross-linked κC/HEC were carried out at room temperature using a Siemens Diffraktometer D5000 X-ray diffractometer with Cu Kα at 40 keV and 40 mA with a step length of 0.05° and a step time of 1 s. The diffraction angle (2θ) was between 20 and 80. Hydrogel films were dried in an oven before experiment to reach a constant weight. The film thickness was approximately 0.6±0.05 mm.

5-4. Hydrogel Microstructure

Field emission scanning electron microscope (FESEM) test was performed to study the surface morphology of the hydrogels and

the effect of cross-linking on native hydrogels using a Gemini Supra 35 VP field emission scanning electron microscope operating at 10 kV accelerating voltage. Before observation, the dried hydrogels were carefully placed on metal grids and coated with gold using a gold sputter coater (Bio Rad Polaran Division, E6700, USA) under vacuum condition. Photomicrographs were then taken at 100× magnification.

RESULTS AND ANALYSES

1. Native Hydrogel Swelling Studies

Different blends of κC: HEC hydrogel were prepared and the swelling tests were performed in triplicate. The swelling results for native films in acidic (pH 1.2), neutral (pH 7) and alkaline (pH 12) medium are shown in Figs. 2-4, respectively. The blend ratio of 50 : 50 could not stand more than 8 hours in both medium solutions and then began to lose weight. The swelling ratio of hydrogels increases as carrageenan content increases due to increasing the hydrophilicity of hydrogel network. This has also been reported by other researchers [13,14]. As can be seen from the figures, 100 : 0 ratio displays the most swelling in both neutral and alkaline mediums (15.43% and 22.3%, respectively) followed by 90 : 10, 70 : 30 and 60 : 40.

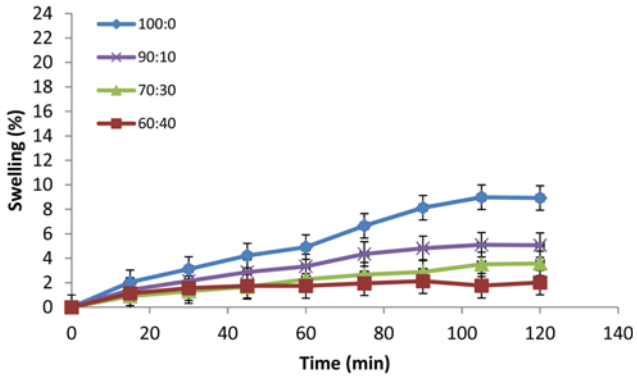

Fig. 2. Swelling of native hydrogels of different concentration of kappa-carrageenan: hydrohyethyl cellulose in pH 1.2 at room temperature.

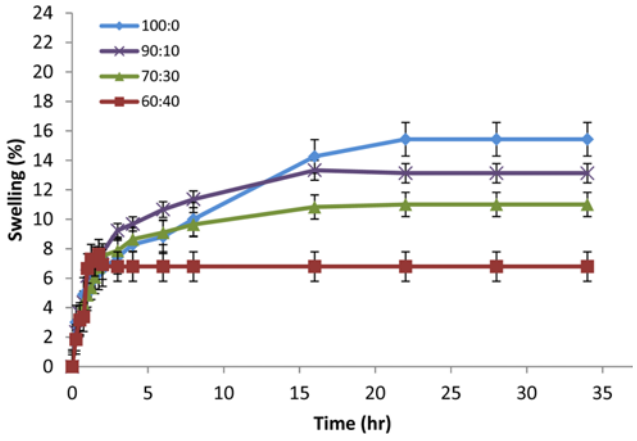

Fig. 3. Swelling of native hydrogels of different concentration of kappa-carrageenan: hydrohyethyl cellulose in pH 7 at room temperature.

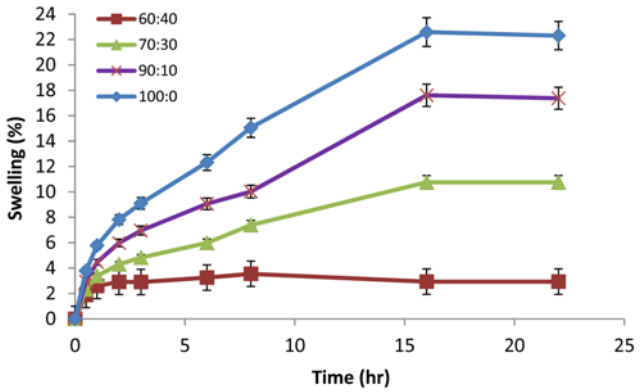

Fig. 4. Swelling of native hydrogels of different concentration of kappa-carrageenan: hydrohyethyl cellulose in pH 12 at room temperature.

Clearly, the swelling ratio increased with time up to 16 hours and then continued to increase with time, but at lower rate. This phenomenon can be explained by the chemical potential difference:

$$\mu_1-\mu_{1.0}=\Delta\mu_{mixing}+\Delta\mu_{ionic}+\Delta\mu_{elastic} \quad (2)$$

Here, $\mu_1-\mu_{1.0}$ is the chemical potential difference between solvent and polymer, $\Delta\mu_{mixing}$ is chemical potential difference, defined as interactions between polymer and solvent, $\Delta\mu_{ionic}$ is ionic chemical potential difference and $\Delta\mu_{elastic}$ is chemical potential difference for elastic, always acting against the swelling. Both $\Delta\mu_{ionic}$ and $\Delta\mu_{mixing}$ facilitate the swelling process.

At the beginning of the swelling process, $\Delta\mu_{ionic}$ controls the swelling, but after equilibrating between hydrogel internal and external ions, $\Delta\mu_{mixing}$ dominates the swelling process with a value less than $\Delta\mu_{ionic}$. As a result, the swelling trend initially increased and then decreased [16-18].

After 2 hours in acidic medium, the hydrogels reached equilibrium. Hydrogels with the blending ratio of 100 : 0 and 90 : 10 swelled up to 8.92 and 5.06%, respectively. The protonation of carboxylic groups and the creation of more hydrogen bonds in κC hydroxyl groups results in more compact networks and therefore less swelling.

Both 100 : 0 and 90 : 10 blend ratios showed more swelling in alkaline environment than acidic and natural. This can be due to electrostatic repulsion of polymer chains as a result of ionization of carboxylic acid groups that breaks the hydrogen bonds. This repulsion force will then push the hydrogel network to expand; thus more water can penetrate into the network [2,19].

Conversely, 70 : 30 and 60 : 40 blend ratios did not show the same behavior and swelled at pH 7 more than pH 1.2 and 12. This can be due to the presence of less carrageenan and therefore less repulsion force in the network. Fig. 5 illustrates maximum swelling of native hydrogels in all medium solutions. According to Fig. 5, maximum hydrogel swelling is strongly dependent on κC concentration.

Hydrogels reached equilibrium after 16 hours, and for the next 18 and 6 hours in the neutral and alkaline mediums, respectively, no changes in hydrogel weight were observed. In acidic medium, however, hydrogels swelling time was much faster than that of neutral and alkaline. In this medium, the maximum swelling occurred after 120 minutes. After that, degradation of polymeric network stopped the swelling. Hydrogels in neutral environment showed more sta-

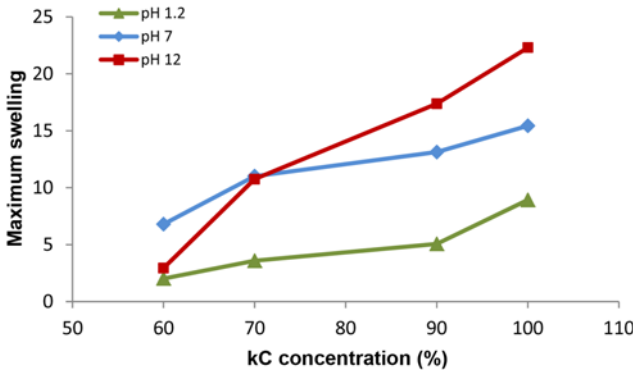

Fig. 5. Maximum swelling of native hydrogels vs. κC concentration in different pH mediums.

bility compared to acidic and alkaline environment. Note that a 60 : 40 blend ratio in all mediums reached equilibrium after 2 hours. The swelling ability decreases as kappa carrageenan decreases because of the less hydrophilic kappa-carrageenan present in the matrix.

2. Cross-linking the Hydrogels

Considering more stability of 90 : 10 blend ratio compared to 100 : 0 in all mediums especially in alkaline medium, this blend was chosen to be cross-linked with genipin. 90 : 10 blend was cross-linked with different genipin concentrations: 0.1, 0.2, 0.3, 0.5 and 1.0 mM to study the effect of cross-linker concentration on swelling behavior of hydrogels. Figs. 6-8 show that the hydrogel swelling ratio decreases when cross-linked with genipin in all mediums, which has been also reported in some other researches [13,14]. It is clear that by increasing the concentration of genipin, the hydrogel, swelling decreases noticeably.

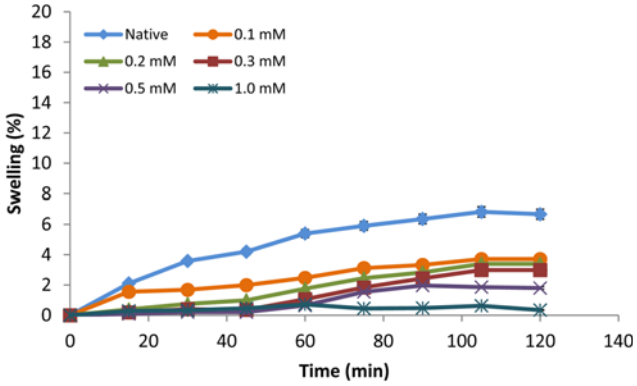

Fig. 6. Swelling of crosslinked and native hydrogels with different genipin concentrations at room temperature in pH 1.2.

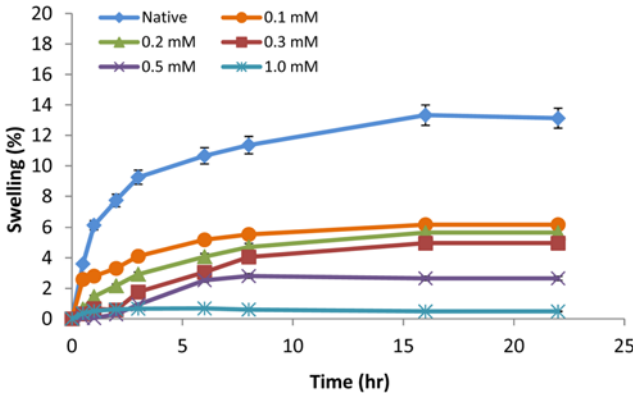

Fig. 7. Swelling of crosslinked and native hydrogels with different genipin concentrations at room temperature in pH 7.

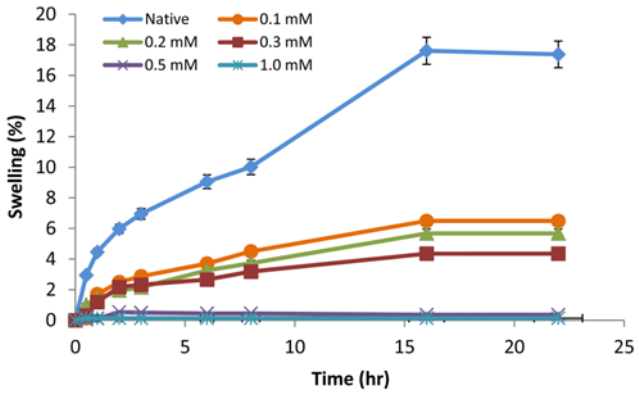

Fig. 8. Swelling of crosslinked and native hydrogels with different genipin concentrations at room temperature in pH 12.

Cross-linked hydrogels showed less swelling ability in acidic environment as a result of carboxylic group protonation and degradation of hydrogels in this pH. In pH 1.2, 0.1 mM cross-linked hydrogel swelled 3.7% which was much less than that of pH 7 and 12. In neutral medium, 0.1 mM concentration of genipin caused 6.18% swelling at the equilibrium; however, 1.0 mM hydrogel swelled only 0.48%. In pH 12 this amount was even less (0.14%), which means that hydrogel swelling almost stopped.

During the experiment the physical stability of cross-linked hydrogels increased when genipin was added to the blend. This was observed in 0.1, 0.2 and 0.3 mM genipin concentrations. Less swelling in these ratios can be due to the fact that higher concentration of genipin could result in creating vast chains and therefore, limiting the mobility of the matrix causing less swelling [13]. More than these concentrations, the physical stability of gels decreases during swelling. This can be a result of breaking some bonds and the formation of weaker bindings that will be discussed in FTIR analysis. Also hydrogels in neutral medium had more swelling ratios in comparison with other mediums. This means that adding cross-linker to

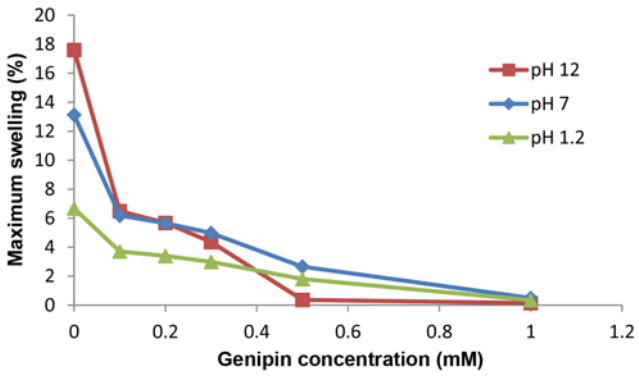

Fig. 9. Maximum swelling of crosslinked hydrogels vs genipin concentration at different pH mediums.

the gels altered the swelling ability in medium solutions. As previously discussed, native hydrogels exhibited more swelling in alkaline environment than neutral; however, by increasing the cross-linker concentration, the swelling capability in neutral medium increased so that genipin cross-linker hydrogels can swell in pH 7 more that pH 12. Obviously, swelling ability depends on molecu-

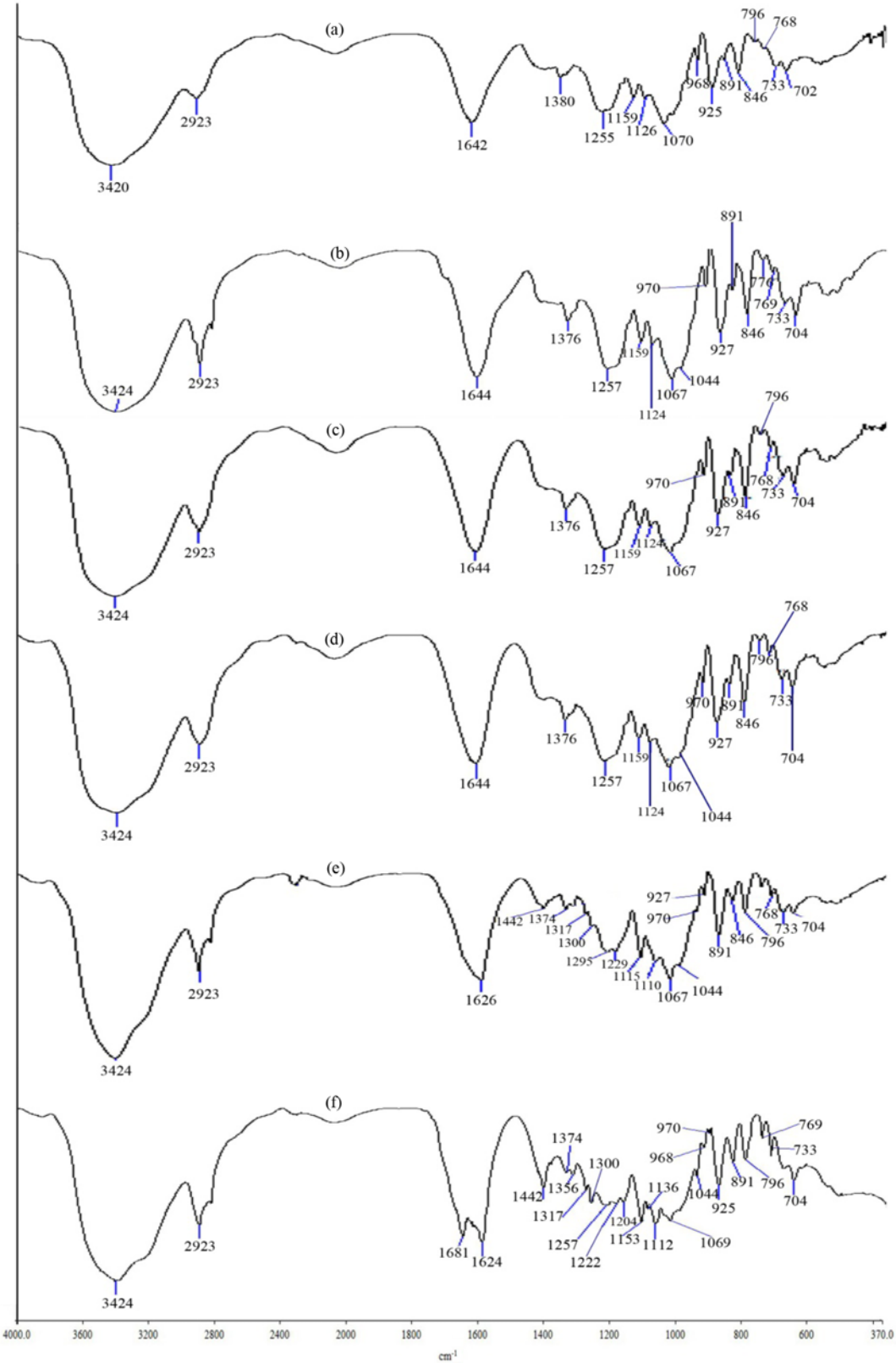

Fig. 10. FTIR results for (a) Native (b) 0.1 mM (c) 0.2 mM (d) 0.3 mM (e) 0.5 mM (f) 1.0 mM genipin cross-linked hydrogels.

lar structure and these results suggest that cross-linker has a noticeable impact on molecular structure. This will be discussed in more detail in the following section. Fig. 9 shows maximum swelling ratio *versus* genipin concentration. The trend clearly shows that the swelling ratio drops to zero by increasing the concentration of genipin in the matrix.

3. FTIR Analysis

Fig. 10 illustrates the FTIR spectra of 90 : 10 blend ratio and genipin cross-linked κC/HEC with different concentrations. A new absorption band at 1,044 cm^{-1} is attributed to carbonyl stretching that is formed when native hydrogels are cross-linked. It is observed when cross-linking occurs, bands at ~796, 768 and 733 cm^{-1} intensify. Also, at ~1,376 cm^{-1} absorption band (symmetrical C-H bending) for 0.2, 0.3, 0.5 mM is intensified after cross-linking. The bands observed at 844 and 927 cm^{-1} are attributed to d-galactose-4-sulfate and 3, 6 anhydrod- galactose, respectively.

The intensity of the absorption band at 1,257 cm^{-1} that is attrib-

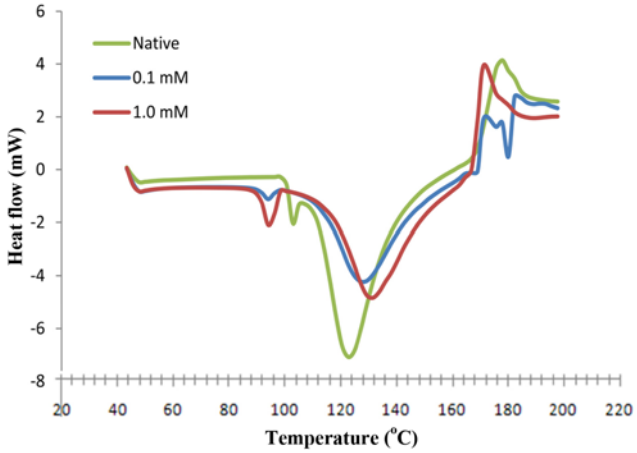

Fig. 11. DSC thermograms recorded during heating of native and genipin cross-linked hydrogels.

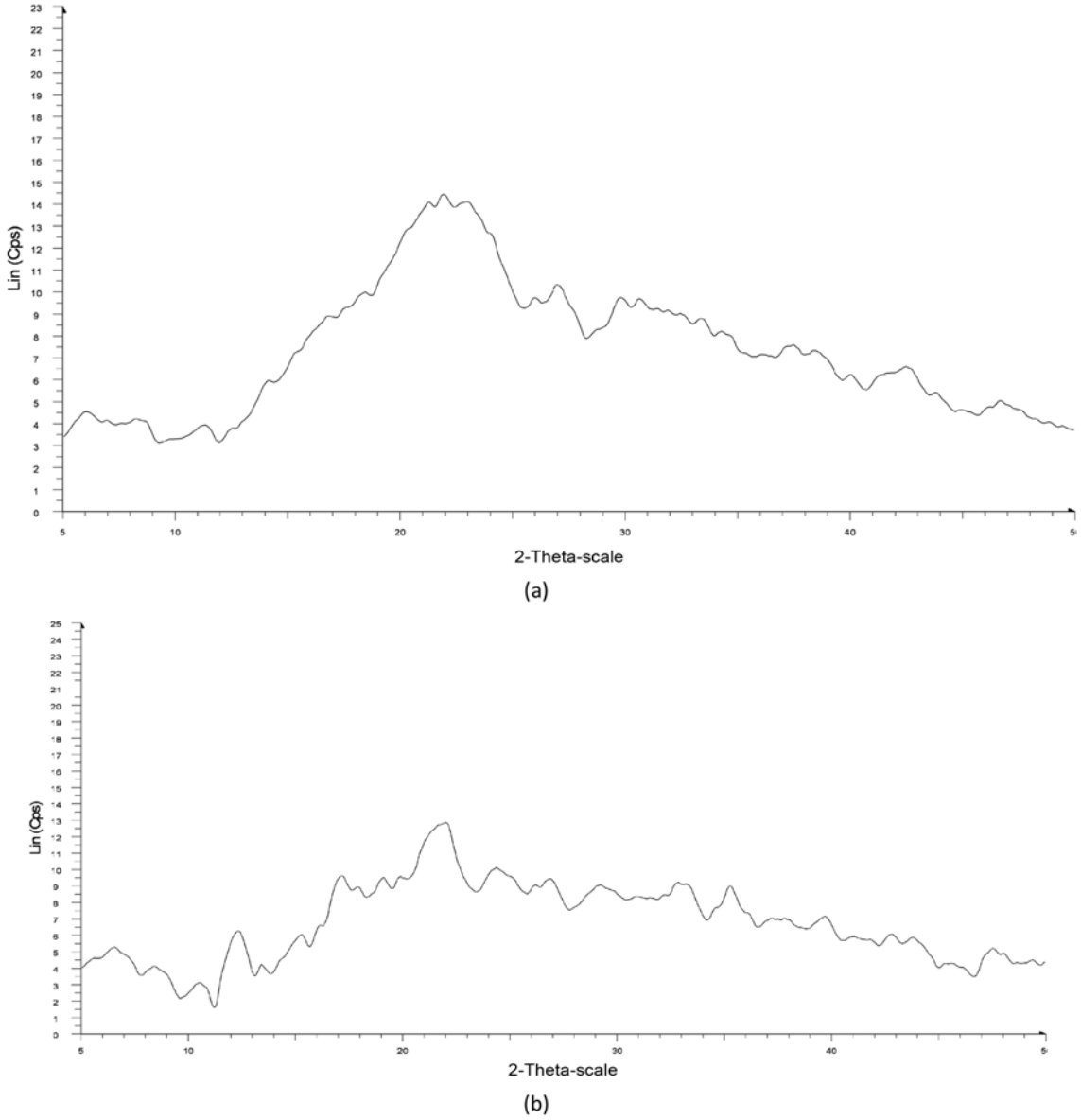

Fig. 12. XRD results for (a) 0.1 mM (b) 1.0 mM genipin crosslinked hydrogels.

uted to the strong C-O stretch bond of the main chain disappeared, and the less intense peaks of 1,229, 1,153 and 1,112 cm^{-1} in lower regions (consistent with lower energy) appeared. This indicates that the main chain of κC molecular structure is seriously affected. As a result, the strength of κC as main monomer in the gel is reduced. This was observed in previous studies by other researches [21] and explains less stability of 0.5 mM and particularly 1.0 mM genipin cross-linked hydrogels during the test.

When increasing the genipin content of gels, bands at 1,642 and 1,126 cm^{-1} shift to ~1,626 and 1,110 cm^{-1}, respectively. The broad band absorbed at 3,424 cm^{-1} is attributed to the stretching of -OH groups of κC. Meanwhile, at 1,644 cm^{-1} a typical amide I peak appears in the spectrum of the cross-linked hydrogel. The absorption bands at 2,923 cm^{-1} also result from the stretching frequency of $-CH_3$ groups. In 0.5 mM and 1.0 mM concentration a new absorption band appears at 1,354 cm^{-1} and 1,442 cm^{-1} that is assigned to C-OH and symmetrical stretching vibration of $-COO^-$ groups, respectively.

4. Differential Scanning Calorimetry (DSC) Measurements

Hydrogel swelling when immersed in water is related to the elasticity of polymer chains in the network, where glass-rubber transition temperature (T_g) becomes important. The thermal behavior of both native and cross-linked κC/HEC was characterized using differential scanning calorimetry (DSC). It is evident from Fig. 11 that the glass-rubber transition temperature increases when genipin concentration increases. For native gels the T_g is 118 °C and for 0.1 mM and 1.0 mM it is 120 and 124 °C, respectively.

The first endothermic peak at around 100 °C is due to water evaporation. When adding cross-linker to the system, this peak decreases as a result of the involvement of hydrophilic hydroxyl side-groups in the cross-linking reaction; consequently, the ability to retain water molecules increases, and a lower amount of water can evaporate from the hydrogel network [22]. On the other hand, at 1.0 mM genipin concentration, by decreasing the hydrogel stability and weakening the hydrogel network as discussed in FTIR section, this peak increases again. The exothermic peak which appears at around 180 °C (for native films) corresponds to the polymer's thermal decomposition [23]. When genipin is added, this temperature shifts to higher temperature (around 185 °C); however, at 1.0 mM concentration of cross-linker this temperature again decreases to 170 °C, indicating the reduced network strength as mentioned above.

5. X-ray Diffraction

X-ray diffraction (XRD) results are shown in Fig. 12. The broad peak indicates the amorphous characteristic of the hydrogel. It is clear that increasing the genipin in the network did not change the 2θ=22 value. However, the signal intensity of the κC/HEC cross-linked with 1.0 mM of genipin at a 2θ=22 was slightly decreased. Therefore, the crystallinity is decreased when genipin concentration is increased. Less crystallinity of gels in 1.0 mM indicates less structural stability of gels in this cross-linker concentration.

6. Hydrogel Microstructure

Fig. 13 illustrates the surface morphology of native and cross-linked hydrogels with different concentrations at 100× magnification. It can be seen that native hydrogel had an almost smooth surface; however, by increasing genipin concentration, new chains will be created, changing the morphology of the matrix noticeably. These changes in surface morphology of hydrogels are due to the creation of new bonds when genipin is added to the network as discussed in the FTIR analysis section. As seen in Fig. 13(b), new chains will appear that can cause changes in swelling properties. These chains formed in the hydrogel network increase so that in 1.0 mM concentration of genipin, (see Fig. 13(c)) the surface roughness increases.

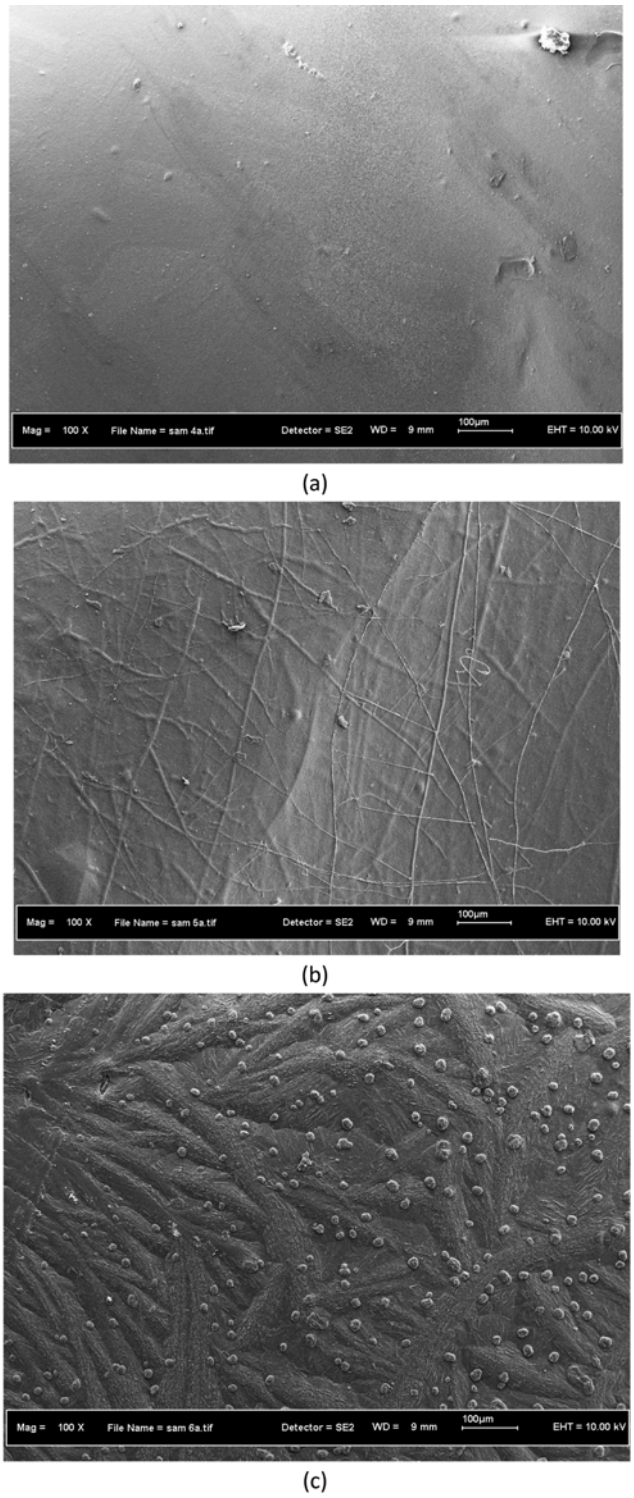

Fig. 13. FESEM images of (a) native (b) 0.1 mM genipin (c) 1.0 mM genipin crosslinked hydrogels.

7. Swelling Control Models

Palace et al. [23] reported that the estimated amino acid concen-

tration in the polymeric matrix of κC is 4,870 ng/mg (0.487%). It is believed that the amino groups in the hydrogel have enabled the cross-linking reaction between genipin and κC/HEC blend. Blue pigments formed as a result of the reaction between genipin and the amino acids in the matrices [24]. Hydrogels were characterized for drug-entrapment efficiency, particle size, and water transport into the polymer matrix, as well as for drug release kinetics [25]. The diffusion mechanism of water in the hydrogels has a great deal of relevance due to the unique applications of the hydrogels. The release of loaded materials depends upon the cross-linking of the network and also on the amount of material loading. The effect of cross-linking ratios on the swelling profiles of developed hydrogels (no material loaded) under different cross-linking ratios was remarkable. Both the rate and extent of swelling decreased as the cross-linking ratio increased. The result is attributed to more bonds between the polymer chains in highly cross-linked samples, which provide greater resistance to the gel swelling. The increase of the cross-linking ratio in hydrogel samples decreased the water uptake and hence the swelling rate because of the large degree of restriction in the hydrogel network [26].

To determine water diffusion into the hydrogels network power law equation presented by Peppas [27] is applied:

$$\frac{M_t}{M_\infty} = kt^n \quad (3)$$

where M and M_∞ are the absolute cumulative water penetrated into the network at time t, and infinite time, respectively. k is a constant that indicates structural and geometric characteristics of matrix, and n is the diffusion exponent that determines the mechanism of transport.

When n=0.5, the transport mechanism is diffusion-controlled. n=1 represents the non-Fickian or Case II mode of transport and values between 0.5 and 1 are known as anomalous mechanism of transport in which the superposition of both phenomena can be observed. Depending on the shape of hydrogels different values have been derived, which are listed in Table 1 [28,29].

Plotting ln (M_t/M_∞) vs. ln (t), for native and cross-linked hydrogels, n and k parameters were calculated from the slope and intercept of the line. Obtained values as well as the corresponding determination coefficients (R^2) are listed in Table 2. Value of n=0.63 for native hydrogel indicates the anomalous behavior of transportation. As can be seen from Table 2, increasing the genipin content of hydrogel results in increasing the n value (varying from 0.68 to 0.9), which means that the transport mechanism is affected by cross-linking. At 1.0 mM concentration of cross-linker, the n value approaches 1. It can be said that if genipin concentration continues to go up, a change in transport mechanism may be observed.

Table 1. Values of exponent n from polymeric controlled systems of different geometry

Exponent n			Transport mechanism
Film	Cylinder	Sphere	
0.5	0.45	0.43	Fickian diffusion
0.5<n<1.0	0.45<n<0.89	0.43<n<0.85	Anomalous transport
1.0	0.89	0.85	Case II transport

CONCLUSIONS

Genipin cross-linked κC/HEC hydrogels were formulated and their swelling behaviors in different pH were studied. The results show that depending on κC and genipin concentration, the swelling and stability of hydrogels in different mediums changes. Results have shown that the cross-linked gels possess lower swelling ability in all pH conditions (pH 12, 1.2 and 7), and swelling ratio decreases with increasing genipin concentration. It was observed that the physical stability of cross-linked hydrogels with 0.1-0.3 mM genipin concentrations increases as a result of bonds stretching, which limits the mobility of the network. Microstructure study also shows that cross-linking has enhanced the stability and structure of the hydrogels network. However, at higher genipin concentrations 1.0 mM, the physical stability of gels decreases perhaps due to the over-stretching of bonding resulting in main chain of κC molecular structure breakdown and formation of weaker bonding. DSC analysis also demonstrated a similar trend in the ability of gel in keeping water in its network and the polymer's thermal decomposition temperature shifts. The power law model signifies the water diffusion mechanism is not changed by adding genipin; however, by increasing the genipin concentration the n value of hydrogels increases up to 0.9, which may alter the transport mechanism.

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Table 2. Different n and k values of power law model for native gel and various concentrations of genipin

	Samples					
	Native hydrogel	0.1 mM genipin	0.2 mM genipin	0.3 mM genipin	0.5 mM genipin	1.0 mM genipin
n	0.63	0.68	0.7	0.73	0.8	0.9
k	0.4481	0.3214	0.2931	0.2472	0.2035	0.1956
R^2	0.98	0.98	0.96	0.95	0.98	0.96
Transport mechanism	Anomalous	Anomalous	Anomalous	Anomalous	Anomalous	Anomalous

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