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Effect of Cellulose Nanocrystals Content and pH on Swelling Behaviour of Gelatin Based Hydrogel

(Kesan Kandungan Selulosa Nanohablur dan pH terhadap Sifat Pembengkakan Hidrogel yang Berasaskan Gelatin)

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ABSTRACT

In this research, a novel method was performed to obtain hydrogel with superior thermal stability by incorporation of cellulose nanocrystals (CNC) into gelatin based hydrogel. Glutaraldehyde was used as cross-linker due to its high chemical reactivity towards NH_2 group on gelatin. Different ratio of gelatin/CNC hydrogel was produced in order to study the effects of CNC towards the swelling behaviour and thermal stability of gelatin based hydrogel. The obtained hydrogel was subjected to Fourier transform infrared (FTIR) to verify that gelatin had been cross-linked, swelling test with different pH for swelling behaviour and thermogravimetric analysis (TGA) for thermal stability. The presence of C=N stretching group in the FTIR spectrum for gelatin/CNC hydrogel indicated that the cross-linking reaction between gelatin monomer had been successfully carried out. The hydrogel showed impressive pH sensitivity and maximum swelling was obtained at pH3. The TGA results clearly showed that the incorporation of CNC into gelatin was able to produce hydrogel with higher thermal stability compare to neat gelatin.

Keywords: Cellulose nanocrystals; cross-linking; gelatin; hydrogel; swelling behavior

ABSTRAK

Dalam kajian ini, kaedah baru telah digunakan untuk menghasilkan hidrogel yang mempunyai kestabilan terma yang lebih tinggi dengan penambahan selulosa nanohablur (CNC) ke dalam hidrogel yang berasaskan gelatin. Glutaraldehid telah dipilih sebagai agen taut silang bagi mentaut silangkan gelatin disebabkan ia mempunyai kereaktifan kimia yang tinggi terhadap kumpulan NH_2 pada gelatin. Hidrogel gelatin/CNC dengan nisbah yang berbeza telah dihasilkan untuk mengkaji kesan penambahan CNC terhadap sifat pembengkakan dan kestabilan terma hidrogel. Hidrogel yang dihasilkan telah dicirikan dengan menggunakan transformasi Fourier inframerah (FTIR) untuk mengesahkan terdapat tindak balas taut silang antara monomer gelatin. Ujian pembengkakan pula dijalankan untuk mengkaji sifat pembengkakan hidrogel pada pH yang berbeza manakala analisis termogravimetri (TGA) pula digunakan untuk mengkaji kestabilan terma hidrogel yang dihasilkan. Kewujudan puncak regangan kumpulan C=N pada spektrum FTIR menunjukkan bahawa monomer gelatin telah berjaya ditaut silangkan. Hasil kajian menunjukkan bahawa hidrogel yang dihasilkan mempunyai sensitiviti yang baik terhadap pH dan hidrogel mencapai nisbah pembengkakan maksimum pada pH3. Analisis TGA pula menunjukkan penambahan CNC ke dalam hidrogel telah meningkatkan kestabilan terma hidrogel.

Kata kunci: Gelatin; hidrogel; selulosa nanohablur; sifat pembengkakan; taut silang

INTRODUCTION

Hydrogels are hydrophilic three dimensional polymer networks which may absorb up to thousands times of their dry weight in water or biological fluids due to the presence of hydrophilic groups such as -OH, -CONH-, -$CONH_2$, -COOH and -SO_3H (Prestwich et al. 1998). They are able to retain large amount of water yet remain insoluble due to physical or chemical cross-links (Bell & Peppas 1996). Stimuli-responsive hydrogels are hydrogels that undergo large volume changes in response to slight environmental changes. The environment sensitive hydrogels are also called 'smart' hydrogels. The physical stimuli include temperature (Jagadeeshbabu et al. 2011; Zhang et al. 2009), electric fields (Li et al. 2004; Murdan 2003), light (Qiu & Park 2012), pressure (Qiu & Park 2012) and magnetic fields (Liu et al. 2006; Paulino et al. 2012), while the chemical stimuli include pH (Frutos et al. 2010; Karlsson & Gatenholm 1999) and ions (Rodrı´guez et al. 2003). A number of studies had focused to broaden the applications of these kind of hydrogel in various field (Lindblad et al. 2007; Wu et al. 2008), including controlled drug delivery (Zhang et al. 2013), tissue engineering (Hou et al. 2010), gene transfection (Gojgini et al. 2011) and sensors (Frisk et al. 2007).

Hydrogels can be produced from natural polymer (Shang et al. 2008) as well as synthetic polymer (Moriyama et al. 2013). Hydrogels based on natural polymer, such as gelatin, had been widely used as a raw material in hydrogel preparation due to their prominent characteristic such as high water absorption, low cost, good biocompatibility

and biodegradability (Chen et al. 2011a; Wan et al. 2000). Gelatin is a type of protein derived from partial hydrolysis of native collagens that can be found in the animals' skins, tendons, cartilages and bones (Ward & Courts 1977). Gelatin can forms physical thermo-reversible gels where they are in gels state at room temperature and melt at temperature above 37°C (Pezron et al. 1991; Ross-Murphy 1992). In order to stabilize these gels and improve its thermal and the mechanical properties, gelatin need to be cross-linked (Draye et al. 1998). The presence of large amount of amino acids' side chain groups in gelatin, make it readily undergoes chemical crosslinking (Spizzirri et al. 2009). Various chemical reagents including carbodiimide (Kuijpers et al. 1999), formaldehyde (Carvalho & Grosso 2004), glutaraldehyde (Farris et al. 2009) and genipin (Bigi et al. 2002) or enzymes such as transglutaminase (Zhu et al. 2012) had been used to chemically cross-link gelatin chains in order to increase the gel's performance. So far, glutaraldehyde is most widely used in gelatin cross-linking because it's inexpensive, easily available and has high efficiency in stabilizing collagenous materials (Khor 1997).

The main disadvantage of gelatin is due to its poor mechanical properties (Lee & Mooney 2001; Lee et al. 2004), which limit its possible applications as a biomaterial. In order to improve its mechanical and thermal stability, CNC have been incorporated into gelatin hydrogels. CNC consist of rod like cellulose crystals with widths of 5-70 nm and lengths between 100 nm and several micrometers. CNCs are obtained by acid hydrolysis where the amorphous regions of a purified cellulose were removed and the crystalline domains were kept intact (Klemm et al. 2011). Recently, CNCs has received a great deal of attention as the reinforcement of nano composites and polymeric hydrogel matrices because of their excellent properties, such as high mechanical strength, high aspect ratio (Eichhorn 2011), low density (about 1.57 g/cm^3), non-toxic, biocompatibility and biodegradability. Moreover, CNCs are produced from renewable natural sources which are abundant and readily available (Peng et al. 2011). Zhang et al. (2010) has prepared hydrogels using cyclodextrin and CNC and the results showed that the addition of CNC had successfully enhanced the gelation of hydrogel, which lead to improvement in mechanical properties and facilitates sustained release of drugs.

The aim of this study was to increase the thermal stability and to study the swelling behaviour of gelatin hydrogel by incorporation of CNC into gelatin hydrogel. The swelling test of hydrogel is carried out at different pH range from pH3 to 11 to investigate the optimum swelling behaviour.

Materials and Methods

Materials

Pharmaceutical grade gelatin was purchased from Halagel (M) Sdn.Bhd. Rice husk was acquired from Bernas Malaysia Sdn. Bhd. Acetic acid, sodium chlorite, sodium hydroxide, sulphuric acid, glutaraldehyde and acetone were purchased from System ChemAR and Sigma-Aldrich.

Preparation of CNC from rice husk fibers

Three chemical treatments which involved in the preparation of CNC from rice husk were alkaline treatment, bleaching and acid hydrolysis. For alkaline treatment, 50 g of grinded rice husk were treated with 4 wt. % NaOH at 90°C for 2 h under reflux condition for four times. After alkaline treatment, the fibers were subjected to bleaching process. Three solutions were involved in bleaching process, which were buffer solution of acetic acid, aqueous chlorite (1.7 wt. %) and distilled water. The fibers were mixed with these three solutions at certain portions at 100°C for 4 h under reflux condition and were carried out for six times. Acid hydrolysis was performed using 65 wt. % H_2SO_4. 5 g of cellulose and 65 wt. % H_2SO_4 were placed into a beaker and stirred using magnetic bar at 45°C. After 30 min, the mixture was then placed into an ice bath to stop the reaction. The suspension was then washed by centrifugation at 4500 rpm at 10°C for 10 min. This washing process was repeated 10 times before it is placed into dialysis tube for dialysis against distilled water until pH was neutral. The resulting suspension was then kept refrigerated for further used.

Preparation of gelatin /CNC hydrogels

Adequate quantities of CNC were dispersed in 50 mL of water. The CNC suspension was then homogenized using a homogenizer to ensure the CNC suspended uniformly. Certain amount of gelatin was then added into CNC suspension. The mixture was then stirred at 55°C, 2 wt. % of glutaraldehyde which act as cross-linking agent was then added dropwise once a homogeneous viscous mixture was obtained. The mixture was poured into a petri dish after 4 h and placed in oven at 45°C until the mixture was dried. Hydrogels in the form of thin films were removed from the petri dish and washed with distilled water to remove unreacted chemicals. Acetone in a Soxhlet apparatus was then used to extract aldehyde and water traces. The film was then dried in an oven at 45°C. The gelatin/CNC composition was tabulated in Table 1.

Characterization

Dimensions of the CNC extracted from rice husk fiber were determined by transmission electron microscopy (TEM) (model Philips CM 12). A drop of a diluted CNC suspension was deposited on the surface of a clean copper grid covered with a thin carbon film. The CNC were negatively stained with 2 wt. % solution of uranyl acetate for 10 s to enhance the contrast and then washed using 50 wt. % of filtered alcohol. Before the TEM analysis was carried out, the sample was allowed to dry at room temperature.

The changes in the functional groups in CNC after various chemical treatment and gelatin/CNC hydrogel

TABLE 1. Composition of Gelatin/CNC hydrogel

Weight % of CNC	Weight of gelatin (g)	Weight of CNC (g)
0	5.00	0.00
1	4.95	0.05
2	4.90	0.10
3	4.85	0.15
4	4.80	0.20
5	4.75	0.25

was determined using FTIR spectroscopy (Perkin-Elmer, GX Model). The samples were grounded and mixed with potassium bromide, KBr in the ratio of 1:1. The mixture was then compressed into pellets and analyzed in transmittance mode within the range of 4000-400 cm^{-1}.

5 buffers solution of pH 3, 5, 7, 9 and 11 were prepared. The swelling behaviour of neat gelatin and gelatin/CNC hydrogel were measured by immersing the dried hydrogel in buffer solutions at different pH. The hydrogel samples were taken out from the buffer solution at predetermined time intervals, the excess buffer solution on the surface of hydrogel were then removed using filter paper before weighing them. The swelling percentage of the hydrogel can be calculated as:

$$\text{Swelling } (\%) = \frac{W_t - W_0}{W_0} \times 100\%,$$

where W_t is the weight of swollen hydrogel and W_0 is the initial weight of hydrogel.

Mettler Toledo thermogravimetric analyser (TGA/SDTA 851^e) was used to determine the thermal stability of gelatin/CNC hydrogel. The materials were heated from 50 to 800°C at a heating rate of 10°C min^{-1} under a nitrogen atmosphere with a gas flow of 10 mL min^{-1}.

RESULTS AND DISCUSSION

MICROSCOPY

Figure 1 shows the transmission electron micrographs of CNC extracted from the rice husk. Acid hydrolysis treatment was carried out in order to remove the amorphous part of cellulose and leaving the crystalline part intact. After acid hydrolysis, the size of fibres will reduced from micrometer to nanometer (Azizi Samir et al. 2005). From the TEM image, rods like shape of CNC can be clearly observed and they were characterized by their diameter (D), length (L) and aspect ratio (L/D). The dimensions of CNC were shown in Figure 1. CNC displayed a diameter of 5-10 nm with aspect ratio around 10 - 50.

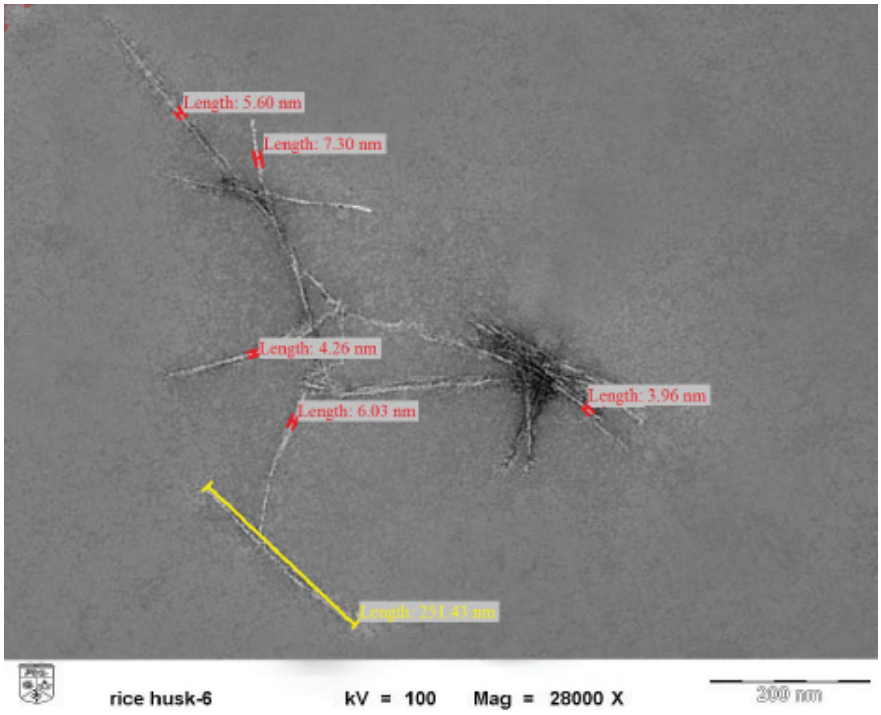

FIGURE 1. Transmission electron micrograph from diluted suspension of cellulose nanocrystals extracted from rice husk fibres

FOURIER TRANSFORMS INFRARED (FTIR) SPECTROSCOPY

It can be seen in Figure 2, the presence of OH stretching vibrations and C-H stretching groups in rice husks were indicated by the absorption peaks at 3368 and 2927 cm^{-1}, respectively. The smaller shoulder peak at 1734 cm^{-1} in raw rice husk was due to the presence of acetyl ester and carbonyl aldehyde groups of hemicellulose and lignin. The peak disappeared upon chemical treatment showing the removal of noncellulosic materials in rice husk. The absorbance peak at 1430 cm^{-1} assigned to $–CH_3$ asymmetric of lignin was also weakened upon chemical treatments indicating lignin removal. The absorbance peaks at 1376 and 1242 cm^{-1} can be assigned to C–H ester bands and C–O stretching vibrations, respectively. The presence of these peaks was due to partial acetylation of hydroxyl groups in both polysaccharides and residual lignin. The decrease in intensity of these peaks showed that lignin content is largely removed after various chemical treatments. The peak at 1059 cm^{-1} was assigned to C–O–C pyranose ring skeletal vibration. The peak at 898 cm^{-1} represented the glycosidic C–H deformation with ring vibration contribution and OH bending in the treated rice husk, indicating the typical structure of cellulose. Comparing both cellulose and CNC's spectra, no significant differences were observed indicated that the molecular structure of cellulose stays the same even after going through acid hydrolysis process (Chen et al. 2011b; Johar et al. 2012; Saha et al. 2010).

In the case of gelatin (Figure 3), peaks at 3289 and 1538 cm^{-1} were corresponded to N-H stretching and N-H bending vibration (amide II), respectively. The peak at 1627 cm^{-1} reflected the amide I (C=O) stretching frequency and peak at 1239 cm^{-1} was due to the amide III (C-N stretch plus N-H in phase bending). Peak at 1451 cm^{-1} was corresponded to the bending absorption of the methylene group, while the peak at 1337 cm^{-1} was assigned to the C-N bond stretching vibrations. The peaks at 1163 and 1081 cm^{-1} were due to the C-O stretching vibrations of gelatin. For gelatin/CNC hydrogel, the increase in intensity at 3289 cm^{-1} indicated the presence of CNC in the hydrogel and the peak 1627 cm^{-1} were due to C=N stretching vibration of the imine group of Schiff base which confirmed the formation of cross-link between gelatin chains by glutaraldehyde.

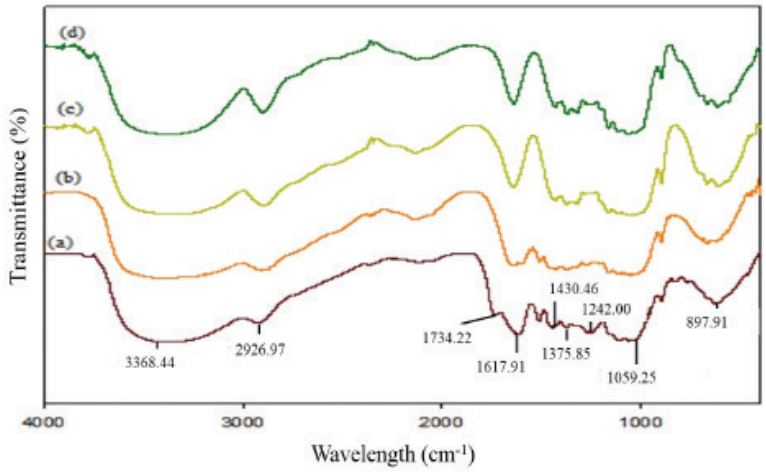

FIGURE 2. FTIR spectra for (a) raw rice husk, (b) alkali-treated rice husk, (c) bleached rice husk and (d) CNC obtained from rice husk after hydrolysis with sulphuric acid

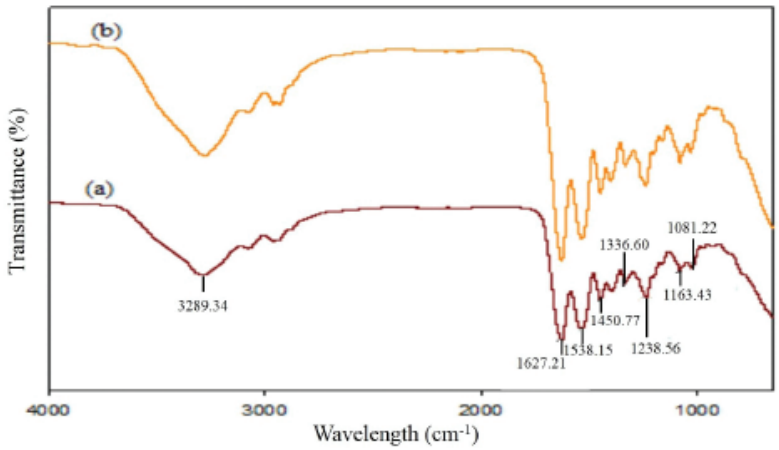

FIGURE 3. FTIR spectra for (a) gelatin and (b) gelatin/CNC hydrogel

SWELLING TEST

The swelling tests were conducted to study swelling behaviour of gelatin/CNC hydrogel as a function of time and the results were summarized in Figure 4. Hydrogels were immersed in buffer solutions of pH range of 3-11 for 48 h.

Gelatin is an amphoteric polymer which can reacts as acid or base because of the presence of carboxyl and amino side chains group. The isoelectric point (pI) of gelatin is 4.9. At this point, the numbers of positive and negative charges are equal, therefore the total charge of the network is zero. The hydrogel network will collapsed due to the electrical attractions between opposite charges. By changing the pH of medium to increase the degree of ionization, the hydrogels swelled significantly. Below pI, the gelatin network bore a net positively charged yielding a cationic gel. Above pI, the network was negatively charged forming an anionic gel as shown in (1) and (2). The swelling degree was unsymmetric with respect to the isoelectric point, hydrogels with net positive charges had higher swelling ratio compare with hydrogels with net negative charges (Curcio et al. 2010; Jain et al. 2006; Liu et al. 2005). Gelatin/CNC hydrogel achieved highest swelling at pH3, which was below the pI. At pH5, which pH ≈ pI, the swelling ratio of hydrogel is minimum. At pH7, 9 and 11, the swelling ratio increased with increasing counter ions in gelatin hydrogel.

$$\text{Gelatin-NH}_2 \xrightarrow{H^+} \text{Gelatin-NH}_3^+. \quad (1)$$

$$\text{Gelatin-COOH} \xrightarrow{OH^+} \text{Gelatin- COO}^- + \text{H}_2\text{O}. \quad (2)$$

THERMOGRAVIMETRIC ANALYSIS (TGA)

Thermogravimetric analysis (TGA) is used to measure weight loss of material as a function of temperature for a given heating rate. Figure 5 shows the thermal stability of CNC, neat gelatin and gelatin/CNC hydrogel. Usually, thermal chemical degradation of CNC started between 200-300°C (Moon et al. 2011). Figure 5(a) shows that in the temperature range of 0-100°C, an initial weight loss was observed which can be linked to water loss due to evaporation. Weight loss appeared at 250-450°C was attributed to the degradation of gelatin chain. The higher temperature step (T>450°C) was attributed to the decomposition of remaining materials, which were more thermally stable structures due to crosslinking reaction. From the DTG analysis (Figure 5(b)) the maximum degradation temperature of gelatin hydrogel was increased with the incorporation of CNC. The maximum degradation temperature for neat gelatin occurred at 310°C, whereas for gelatin/CNC hydrogel the degradation occurred at 325°C. This significant improvement of the thermal resistance may be due to the formation of a more rigid hydrogel structure. The results also indicated good dispersion and well interactions between gelatin and CNC, which imparted stability to the entire system. From the thermogram, it can be seen that the incorporation of CNC had increased the thermal stability of the hydrogel as the hydrogel degraded at a higher temperature compared with neat gelatin (Li et al. 2013; Mu et al. 2012).

CONCLUSION

In this study, gelatin/CNC hydrogels were successfully prepared by casting techniques. The FT-IR analysis showed that cross-linking reaction between gelatin monomer had been sucessfully carried out. From the TGA thermogram, it showed that the incorporation of CNC had effectively increased the thermal stability of the hydrogel. Then, the results of the swelling studies suggested that the hydrogels

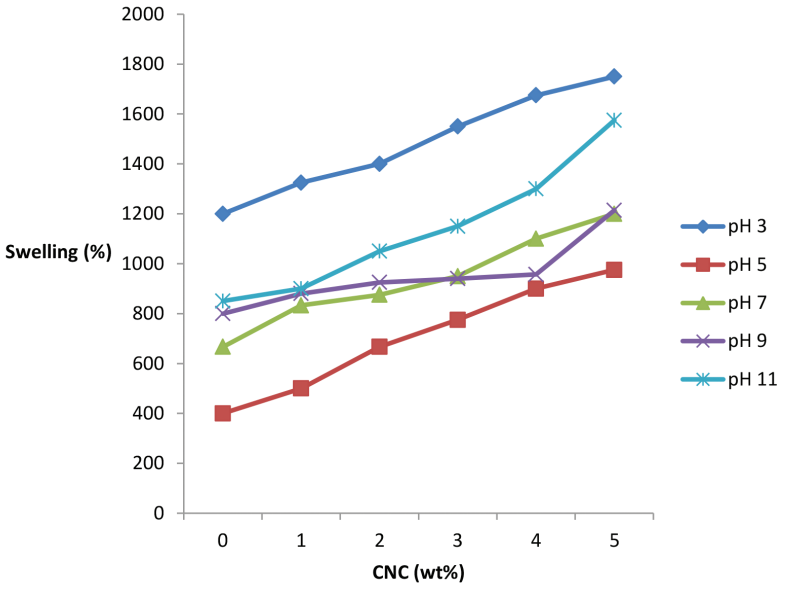

FIGURE 4. Swelling behaviour of gelatin/CNC hydrogels at different pH

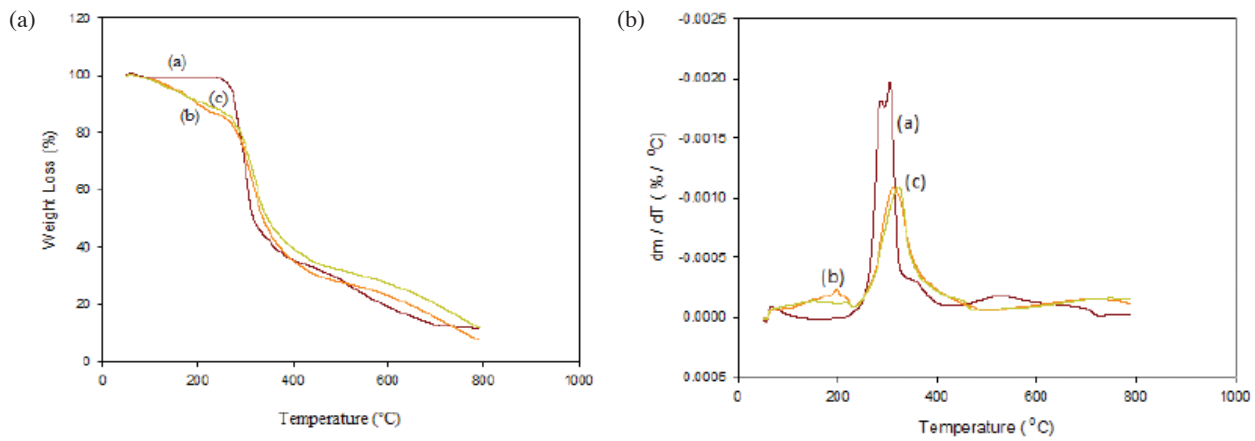

FIGURE 5(a) & (b). TGA and DTG thermogram for (a) CNC, (b) neat gelatin, (c) gelatin/CNC hydrogel

were pH-sensitive. The gelatin/CNC hydrogels had the highest swelling ratio at pH less than pI and lowest swelling ratio at pH ≈ pI.

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