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Effect of chitosan characteristics and solution conditions on gelation temperatures of chitosan/2-glycerophosphate/nanosilver hydrogels

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ABSTRACT

In this study, we explored the effect of nanosilver, chitosan characteristics, and solution conditions on gelation temperatures of chitosan/2-glycerophosphate thermosensitive hydrogel. The gelation temperatures of hydrogels with or without 12 ppm nanosilver were insignificantly different regardless of the molecular weight (MW) of chitosan (degree of deacetylation, DD, 80% or 88%) and glycerophosphate concentration. The gelation temperature of hydrogel decreased with increasing DD (65–88%) of chitosan, increasing concentration of glycerophosphate (2–8%), and increasing pH value (6.5–6.8). It also decreased with decreasing chitosan's MW (DD88: 113–345 kDa, DD80: 145–900 kDa), but increased with increasing concentrations of NaCl (0–2%). By adjusting above conditions, the hydrogel gelation can be induced with a temperature range from 24.3 to 91.7 °C. Chitosan chains with higher flexibility and/or smaller hydrodynamic volume resulted in lower gelation temperature. Hydrogel became white turbidity in 2% NaCl below 38 °C because of its lower solubility at higher ionic strength.

1. Introduction

Chitosan/glycerophosphate hydrogel showing sol–gel transition at a physiological pH and temperature has gained attention. Applications of the hydrogel had reported as follows: Chenite et al. (2000) reported that chitosan/glycerophosphate hydrogel was used to deliver growth factors *in vivo* and encapsulated living chondrocytes for tissue engineering applications. Ruel-Gariépy, Chenite, Chaput, Guirguis, and Leroux (2000) encapsulated chlorpheniramine maleate, dextran, methylene blue, and albumin with chitosan/glycerophosphate hydrogels, respectively, and evaluated the *in vitro* release profiles of the above model compounds. Crompton et al. (2007) reported that chitosan/glycerophosphate hydrogel provided a suitable 3D scaffolding environment for neural tissue engineering. Richardson, Hughes, Hunt, Freemont, and Hoyland (2008) seeded human mesenchymal stem cells in chitosan/glycerophosphate hydrogels; the stem cells could be differentiated from nucleus pulposus-like cells. Zhou et al. (2008) found that the chitosan/glycerophosphate hydrogel was an ideal sustained release system especially for hydrophilic drugs, due to the release rate of adriamycin (hydrophilic) being slower than 6-mercaptopurine (hydrophobic) from chitosan/glycerophosphate hydrogels. Zhao et al. (2009) reported the chitosan/glycerophosphate hydrogel was suitable for the delivery of drugs, proteins and enzymes due to its high porosity, high capacity of protein adsorption and good biocompatibility; in addition, hydrogel films were a promising candidate for biomaterial for pharmaceutical and tissue-engineering applications. Kim et al. (2010) investigated the *in vitro* ellagic acid release rate from chitosan/glycerophosphate hydrogel and the chitosan degradation rate for cancer treatment. Moreover, a chitosan/glycerophosphate system containing collagen in different proportions also showed sol–gel transition at a physiological pH and temperature. The presence of collagen in chitosan-collagen materials was associated with increased cell spreading and proliferation, as well as increased gel compaction and a resulting stiffer matrix (Wang & Stegemann, 2010). Chitosan/glycerophosphate salt formulations were combined with inorganic nanoparticles in order to prepare novel injectable thermo-responsive hydrogels for orthopaedic applications (Muzzarelli, 2010). Molinaro, Leroux, Damas, and Adam (2002) reported that a chitosan/glycerophosphate hydrogel formed with higher degree of deacetylation (DD) chitosan was desirable in regard to superior biocompatibility.

The addition of polyol- or sugar-phosphate to the chitosan solution transformed pH-gelling solutions into thermal-sensitive pH-dependent gel-forming aqueous solutions (Chenite et al., 2000). The gelation of the chitosan/glycerophosphate hydrogel system might involve several interactions as follows: At low temperature, glycerophosphate can increase the pH of chitosan solutions to around neutrality. It might screen the electrostatic repulsion between chitosan molecules and theoretically lead to gelation behaviour because it is pH-induced. However, due to an attraction between the phosphate moieties of glycerophosphate and $-NH_3^+$ groups of chitosan, hydroxyl groups of glycerophosphate could

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increase stability and hydrophilicity in the chitosan chain, as well as maintain its solubility at low temperatures for a period of time. When the temperature increased, (1) it reduced the polarity of the chitosan chain and glycerol moiety of glycerophosphate increased the hydrophobicity. (2) This caused chitosan chain dehydration and increased an interchain hydrophobic attraction. (3) H^+ was removed from $-NH_3^+$ and was accepted by $-PO_4^{2-}$, thereby further reducing both the chitosan chain charge density and the attraction of chitosan and glycerophosphate; this allowed the hydrophobic and hydrogen-bonding between chains to predominate, and upon heating of the chitosan/glycerophosphate solution a hydrogel was formed (Chenite et al., 2000; Chenite, Buschmann, Wang, Chaput, & Kandani, 2001; Cho, Heuzey, Bégin, & Carreau, 2006; Kim et al., 2010; Ruel-Gariépy et al., 2000).

The characteristics of chitosan such as DD and molecular weight (MW) and the preparation conditions include concentration of chitosan, type and concentration of glycerophosphate, pH value of the solution, concentration of urea, and so on, affect the gelation temperature and gelation rate of chitosan/glycerophosphate hydrogel (Chenite et al., 2000, 2001; Cho et al., 2006; Kim et al., 2010; Ruel-Gariépy et al., 2000; Wang & Stegemann, 2010; Wu, Su, & Ma, 2006; Zhou et al., 2008). Factors, such as the DD and MW of chitosan, pH value of solution, concentration of urea, and so forth, influence the hydrodynamic behaviour and chain flexibility of chitosan (Chen & Tsaih, 1998, 2000; Chen, Lin, & Lin, 1994; Chen, Tsaih, & Lin, 1996; Chen, Chen, Wang, Hsu, & Tsai, 2009; Tsaih & Chen, 1997, 1999). Therefore, studying the relationships between gelation temperature or gelation rate of chitosan/glycerophosphate hydrogel and hydrodynamic behaviour and chain flexibility of chitosan is worthwhile.

The antimicrobial activity of nanosilver has been well established and is great interest. Nanosilver has several biotechnological applications including wound dressings, nanosilver coated or impregnated ceramic water filters, activated carbon air filters, and catheters (Kora, Sashidhar, & Arunachalam, 2010; Maneerung, Tokura, & Rujiravanit, 2008; Sharma, Yngard, & Lin, 2009). Adding nanosilver to chitosan/glycerophosphate hydrogel enhances the antimicrobial activity of the hydrogel and expands the hydrogel's applications.

The goal of this study was to investigate the effect of nanosilver, MW and DD of chitosan, concentration of glycerophosphate, and pH value and ionic strength of solutions on gelation temperatures of chitosan/2-glycerophosphate hydrogel. The relationships between the gelation temperature of hydrogel and the hydrodynamic behaviour as well as the chain flexibility of chitosan were also discussed.

2. Experimental

2.1. Materials

Squid (*Illex argentinus*) pens were donated as a gift from Shin Dar Bio-Tech. Co. Ltd. (Taoyuan, Taiwan). 2-Glycerophosphate, acetic acid, sodium acetate, sodium azide, sodium chloride, silver nitrate, sodium borohydride and potassium bromide were purchased from the Sigma–Aldrich Co. (MO, USA). Hydrochloric acid and sodium hydroxide were purchased from Merck & Co., Inc. (Darmstadt, Germany). Cellulase was purchased from the Challenge Bioproducts Co., Ltd. (Yunlin, Taiwan). Pullulan standards (for SE-HPLC calibration) were purchased from Showa Denko (Tokyo, Japan).

2.2. Preparation of chitosan

β-Chitin was prepared from squid pens. In brief the squid pens were ground to a 40–60 mesh size. Each 100 g batch of powder was immersed in 500 ml of 1 M of hydrochloric acid solution overnight. The sample was washed to neutrality and drained. Then, the sample was soaked in 500 ml of 2 M of sodium hydroxide at an ambient temperature overnight, washed and drained. Subsequently, sample was reacted in 500 ml of 2 M of sodium hydroxide solution at 100 °C for 4 h, washed to neutrality and dried. β-Chitin was added to a 50% (w/w) sodium hydroxide solution at a ratio of 1 (g solid):10 (ml solution). The deacetylation reaction took place at 100 °C for 1 h, 100 °C for 4 h and 140 °C for 4 h, respectively. Then the chitosan was washed to neutrality and freeze-dried (Tsai, Bai, & Chen, 2008).

2.3. Preparation of same DD but different MW chitosans

The chitosans were prepared by a modified method proposed by Feng, Gong, Du, and Huang (2009). Chitosan was dissolved in 1% (v/v) aqueous acetic acid. The pH value of the final solution was 5.5, which was regulated with 2 N NaOH at the optimum pH value for cellulase. The cellulase (100 U/g) was used to hydrolyze chitosan for 3, 6, 12, 18, 24, 36 and 48 h at 50 °C. At the end of the reaction, cellulase was denatured quickly, by heating for 10 min at 100 °C. The supernatant was isolated by 10,000 rpm centrifugation for 30 min at 4 °C. Then the supernatant was precipitated by adding 2 N NaOH, was washed with de-ionized water until neutrality and freeze-dried.

2.4. Measurement of DD of chitosan

Infrared spectrometry was used to determine the DD of the chitosan (Baxter, Dillon, Taylor, & Roberts, 1992). Chitosan powder was sieved through a 200 mesh and then mixed with KBr (1:100), dried at 60 °C for 3 days to prevent interference of the –OH group in FTIR measurements, and pressed into a pellet. The absorbance of amide 1 (1655 cm^{-1}) and the hydroxyl band (3450 cm^{-1}) were measured using a Bio-Rad FTS-155 infrared spectrophotometer (Hercules, CA, USA). The band of the hydroxyl group at 3450 cm^{-1} was used as an internal standard to correct for disc thickness and for differences in chitosan concentration when making the KBr disc. The percentage of the amine group's acetylation in a sample was given by $(A_{1655}/A_{3450}) \times 115$. Here, A_{1655} and A_{3450} were the absorbances at 1650 cm^{-1} and 3450 cm^{-1}, respectively. Every sample measurement was repeated three times.

2.5. Determination of MW of chitosan

The size exclusion high performance liquid chromatography (SE-HPLC) method of Tsai et al. (2008) was followed. A column (7.8 mm × 30 cm) packed with TSK gel G4000 PW_{XL} and G5000 PW_{XL} (Tosoh Co. Ltd., Japan) was used. The mobile phase consisted of 0.2 M of acetic acid/0.1 M sodium acetate and 0.008 M of sodium azide. A sample concentration of 0.1% (w/v) was loaded and eluted with a flow rate of 0.6 ml/min by an LDC Analytical ConstaMetric 3500 pump. The elute peak was detected by an RI detector (Gilson model M132, USA). The data were analyzed by Chem-Lab software (Scientific Information Service, Taipei, Taiwan). Pullulan standards (Shodex, Kawasaki, Japan) with different MW values were used as markers. The MW values of the samples were calculated from the pullulan calibration curve with Chem-Lab software.

2.6. Intrinsic viscosity measurement

A capillary viscometer (Cannon-Fenske, No 75) was used to measure the passage time of solutions flowing through the capillary. Solutions of chitosan (80% DD, 225 kDa) in 1% acetic acid containing 0.1%, 0.5%, 1.0%, and 2.0% glycerophosphate, were prepared. The capillary viscometer was filled with 5 ml of the sample and equilibrated in a water bath (Tamson TMV 40, Holland) at 30 ± 0.1 °C for

10 min. The sample was passed through the capillary once before the running time was measured. The running time was used to calculate the relative viscosity, specific viscosity and reduced viscosity. Then, the reduced viscosity was plotted against the concentration, with the intercept being the intrinsic viscosity (Chen & Tsaih, 1998; Tsaih & Chen, 1997).

2.7. Preparation of thermosensitive hydrogel solutions

The chitosan/glycerophosphate hydrogel solution was prepared by a modified method proposed by Chenite et al. (2001). Chitosan with different DDs and MWs (200 mg) was dissolved in 1% (v/v) aqueous acetic acid (9 ml) and stirred for 12 h until complete dissolution was achieved. Glycerophosphate (200–800 mg) and NaCl (0–200 mg) were dissolved in distilled water and stirred for 15 min into a clear solution. Both the chitosan solution and glycerophosphate/NaCl solution were chilled at 4 °C for 1 h, respectively, then these solutions were mixed and stirred for 2 min under an ice bath. Next, the hydrogel solution was shaken for 40 s by a vortex shaker. Finally, the hydrogel solution was obtained and the pH value of the solution was adjusted within the range of 6.5–6.8. The hydrogel solution eventually was made up of 2% chitosan, 2–8% glycerophosphate, and 0–2% NaCl.

2.8. Preparation of thermosensitive hydrogel containing silver nanoparticles

The nanosilver solution was prepared by a modified method proposed by Lee and Meisel (1982). The 30 ml of 0.002 M sodium borohydride solution was chilled in an ice bath for 20 min, and 6 ml of 0.001 M silver nitrate solution was slowly dropped into the sodium borohydride solution. Then the silver nanoparticle was formed and easily observed thanks to a visible colour change of the solution to pale yellow. The size of the nanoparticles was determined by scanning electron microscopy (SEM) (Hitachi, S-4800, Tokyo, Japan). Then the nanosilver solution was added to the chitosan/glycerophosphate solution. The concentration of nanosilver in the hydrogel solution was determined by an atomic absorption spectrophotometer (PerkinElmer 510 OPC, Norwalk, CT, USA).

2.9. Determination of hydrogel's gelation temperature

A chitosan/glycerophosphate hydrogel solution (2 ml) was poured into a 10 ml tube to determine the gelation temperature in a water bath at 25 °C. We determined the temperature in the test tube using a temperature detector (SUNTEX, SP-701, Taipei, Taiwan), and kept the temperature constant for 1 min after the tube temperature was the same as the set temperature. The sol–gel transition temperature was determined by a flow or no-flow criterion over 30 s with the test tube inverted (Chung, Simmons, Gutowska, & Jeong, 2002).

3. Results and discussion

3.1. Effect of nanosilver on gelation temperature

The particle size of the nanosilver was measured by SEM and found to be 21.8 nm. The nanosilver concentration of chitosan/glycerophosphate hydrogel solution was determined by atomic absorption spectrophotometer and found to be 12 ppm. Fig. 1 shows the gelation temperature of chitosan/glycerophosphate hydrogel with or without 12 ppm nanosilver. Results show that the gelation temperatures of hydrogels with or without nanosilver were insignificantly different regardless of the MW of chitosan (DD 80% or 88%) and concentration of glycerophosphate. This indicates that adding nanosilver will insignificantly affect the gelation temperature of chitosan/glycerophosphate hydrogel.

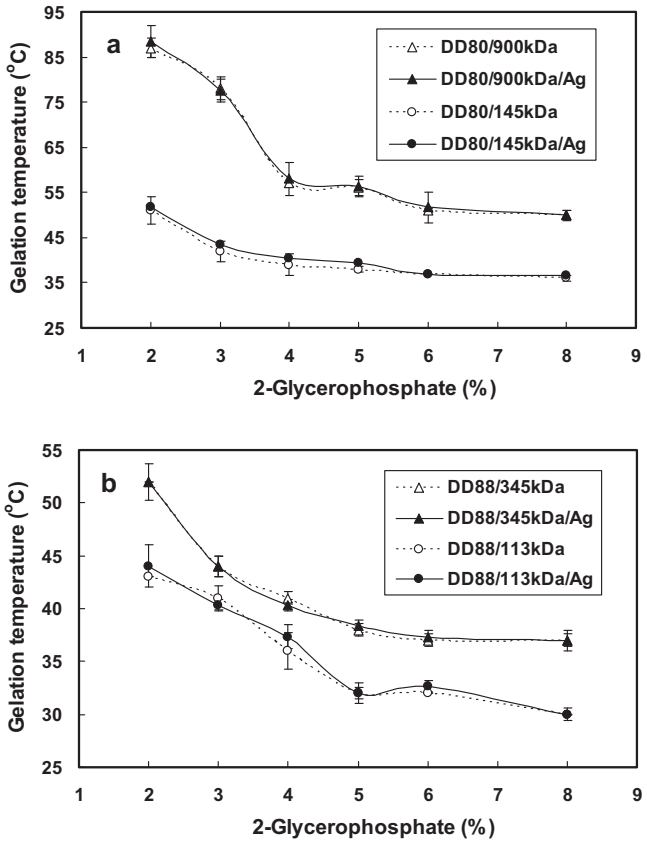

Fig. 1. Change of gelation temperature of chitosan/2-glycerophosphate hydrogels formed with or without silver nanoparticles at different 2-glycerophosphate concentrations in pH 6.5 solution.

3.2. Effect of DD on gelation temperature

Table 1 shows the effect of the DD and MW of chitosan and the concentration of glycerophosphate on the gelation temperature of chitosan/glycerophosphate/nanosilver hydrogels formed in a pH 6.5 solution. Results show that the gelation temperatures of hydrogels which were prepared with chitosan that had different DDs but similar MWs, decreased with the increase in DD. These results are similar to reports by Chenite et al. (2000) and Ruel-Gariépy et al. (2000). They considered that a higher DD of chitosan, which had more amine groups, could form more cross-links with the phosphate group of glycerophosphate. This led to an increase in the gelation rate and consequently decreased the gelation temperature. However, Zhou et al. (2008) reported that the viscosity of chitosan/glycerophosphate hydrogel, prepared with a chitosan DD of 75.4%, increased quickly at 37 °C, while others increased either slowly or variedly. Therefore, the optimal DD for prepared hydrogel was 75.4%.

A lower DD of chitosan has more $-NCOCH_3$ on the molecular chain. It forms a large three-dimensional spatial barrier for rotation of the β-1,4 glycoside, so the chitosan chain is more rigid. Conversely, a higher DD of chitosan has less $-NCOCH_3$ on the molecular chain. It induces easy turning of the glycosidic bond, so the chitosan chain is more flexible (Chen et al., 1996). In the gelling course of chitosan/glycerophosphate hydrogel, the hydrated chitosan molecular chains were gradually dehydrated; the chitosan chains interacted with each other and then rearranged chains to form crystalline regions and gel formation. Conformation and chain flexibility in

Table 1
Effect of degree of deacetylation and molecular weight of chitosan and concentration of 2-glycerophosphate on gelation temperature (°C) of chitosan/2-glycerophosphate/nanosilver hydrogels formed in pH 6.5 solution.

DD (%)	MW (kDa)	2-Glycerophosphate (%)						SL[a]	SH	SL/SH
		2%	3%	4%	5%	6%	8%			
88	345	52.0 ± 0.0[b]	44.0 ± 1.0	40.3 ± 0.6	38.3 ± 0.6	37.3 ± 0.6	36.0 ± 1.0	−4.5	−0.6	7.5
	326	51.0 ± 0.0	44.3 ± 0.6	40.0 ± 0.0	38.0 ± 0.0	37.3 ± 0.6	36.0 ± 1.0	−4.3	−0.5	8.6
	270	51.0 ± 0.0	43.3 ± 0.6	39.7 ± 0.6	38.0 ± 0.0	37.0 ± 0.0	36.0 ± 1.0	−4.3	−0.6	7.2
	225	49.7 ± 1.2	42.7 ± 0.6	39.0 ± 1.0	37.3 ± 0.6	37.3 ± 0.6	36.0 ± 1.0	−4.1	−0.4	10.3
	204	52.7 ± 2.3	43.0 ± 1.0	37.3 ± 1.5	32.3 ± 0.6	31.7 ± 0.6	30.3 ± 0.6	−6.7	−0.6	11.2
	160	49.0 ± 1.0	41.3 ± 2.9	37.3 ± 1.5	32.3 ± 1.5	32.0 ± 0.0	30.3 ± 0.6	−5.4	−0.6	9.0
	146	48.3 ± 2.3	41.3 ± 0.6	37.3 ± 1.2	32.3 ± 2.1	31.7 ± 0.6	30.0 ± 0.0	−5.2	−0.7	7.4
	113	47.0 ± 4.0	40.3 ± 0.6	37.3 ± 1.2	32.0 ± 1.0	32.7 ± 0.6	30.0 ± 0.0	−4.8	−0.7	6.9
80	900	88.3 ± 3.5	77.7 ± 2.5	58.0 ± 3.6	56.3 ± 2.3	51.7 ± 3.5	50.0 ± 1.0	−11.6	−1.8	6.4
	497	60.7 ± 1.2	55.7 ± 2.5	46.3 ± 8.4	43.0 ± 5.3	39.0 ± 1.0	39.0 ± 1.0	−6.3	−1.3	4.8
	407	55.0 ± 0.0	46.3 ± 2.3	45.3 ± 3.8	38.7 ± 0.6	37.3 ± 2.1	36.3 ± 1.5	−5.0	−0.8	6.3
	335	55.0 ± 2.0	44.7 ± 1.5	41.7 ± 2.1	40.0 ± 1.0	39.0 ± 1.0	36.5 ± 0.6	−4.8	−1.1	4.4
	198	54.7 ± 2.1	44.7 ± 1.5	41.3 ± 1.2	39.3 ± 1.2	38.3 ± 0.6	36.3 ± 2.9	−5.0	−1.0	5.0
	161	55.0 ± 2.0	44.0 ± 2.0	41.0 ± 1.0	39.3 ± 1.2	38.3 ± 0.6	36.3 ± 0.6	−5.0	−1.0	5.0
	145	51.7 ± 0.6	43.3 ± 1.2	40.3 ± 1.2	39.3 ± 1.2	37.0 ± 0.0	36.7 ± 1.2	−4.0	−0.8	5.0
65	530	91.7 ± 2.3	81.0 ± 1.7	83.7 ± 1.2	76.7 ± 7.5	75.0 ± 6.9	65.7 ± 3.1	−4.8	−3.8	1.3

[a] SL and SH express the slopes of plots in 2-glycerophosphate concentration of 2–5% and 5–8%, respectively.
[b] Value of gelation temperature represents mean ± S.D (n = 3).

the gelling process play an important role. Chitosan chain flexibility increased as DD increased (Chen et al., 1996). Molecular chains were more flexible, creating easier turning of the glycosidic bond, and it became easier to change the original molecular conformation in the gelation process. Then chitosan chains approached, entangled and interacted with each other; the result was a rearrangement of chains and crystalline regions formed, resulting in decreased gelation temperature.

3.3. Effect of MW and glycerophosphate concentration on gelation temperature

Table 1 also indicates that the gelation temperature of chitosan/glycerophosphate/nanosilver hydrogel decreased with the increased concentration of glycerophosphate. This result was similar to the reports by Chenite et al. (2001), Wang and Stegemann (2010) and Wu et al. (2006). Fig. 2 shows that the concentration of glycerophosphate affected the intrinsic viscosities of chitosan (80% DD, 225 kDa) at 30 °C. Fig. 2 indicates that the intrinsic viscosities of chitosan decreased from 11.5 to 4.7 dl/g with increasing concentrations of glycerophosphate (0.1–2.0%). Intrinsic viscosity is an indicator of hydrodynamic volume: the greater the intrinsic viscosity, the more extended the conformation of the molecular chain. The phosphate group of glycerophosphate is the counter-ion for chitosan. When the concentration of glycerophosphate is increased, it enhances the neutralization charge on the $-NH_3^+$groups of chitosan chains (Chen et al., 1996; Tsaih & Chen, 1999). Therefore, reducing both the chitosan chain charge density and the electrostatic repulsion between the $-NH_3^+$groups (Chen et al., 1996) increases the chain flexibility. Then the chitosan chains are easier to close, entangle, interact and gel.

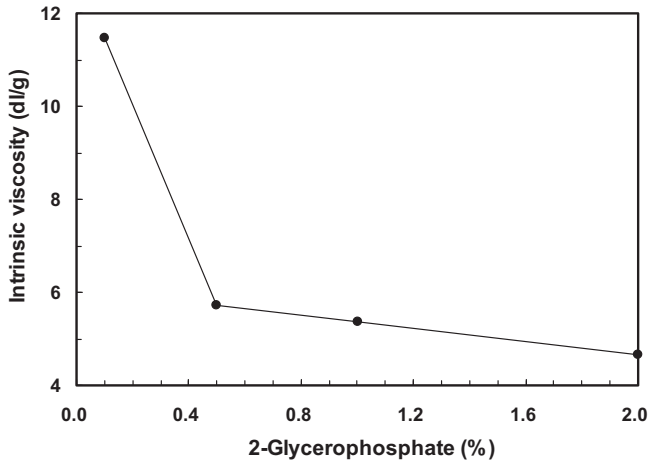

Fig. 2. The 2-glycerophosphate concentration affected the intrinsic viscosities of chitosan (80% DD, 225 kDa) in 1% acetic acid solution at 30 °C.

Table 1 also shows that the gelation temperatures of chitosan/glycerophosphate/nanosilver hydrogels prepared with the same DD but different MW chitosans, decreased with the decreasing MW of chitosan. However, this trend, with an increasing concentration of glycerophosphate, becomes imperceptible; for example, the gelation temperature of chitosan/glycerophosphate/nanosilver hydrogels which were prepared with a DD of 88% and MWs of 113–204 kDa chitosans mixed 4% glycerophosphate was 37.3 °C. Zhou et al. (2008) reported that the viscosity of chitosan/5% glycerophosphate hydrogels prepared with different MW chitosans (75.5% DD) increased as the MW increased from 88 to 1360 kDa when incubated at 37 °C. Therefore, the increase of MW was favourable for sol-to-gel transition, and a high MW chitosan was optimal for hydrogel preparation. However, Chenite et al. (2000) reported that the effect of the chitosan's MW on the gelation temperature of chitosan/5.6% glycerophosphate hydrogel (pH 7.15) prepared with 81% DD and 124–846 kDa chitosan was insignificant.

The effect of the chitosan's MW on the gelation temperature of chitosan/glycerophosphate hydrogel became less obvious with increasing concentration of glycerophosphate, which may be related to the hydrodynamic behaviour and chain flexibility of chitosan. When the concentration of glycerophosphate was lower, the electric shielding effect of glycerophosphate on the chitosan was weaker, and the chitosan chain had more $-NH_3^+$ groups. Consequently, the intrinsic viscosity was greater (Fig. 2), i.e. the hydrodynamic volume was larger. Therefore, the effect of the chitosan's MW on the hydrodynamic volume was more evident and caused the gelation temperature to differ more. However, when the concentration of glycerophosphate was higher, the electric shielding effect of the phosphate group on the $-NH_3^+$ group of a chitosan chain was stronger, and the chitosan chain had fewer $-NH_3^+$ groups.

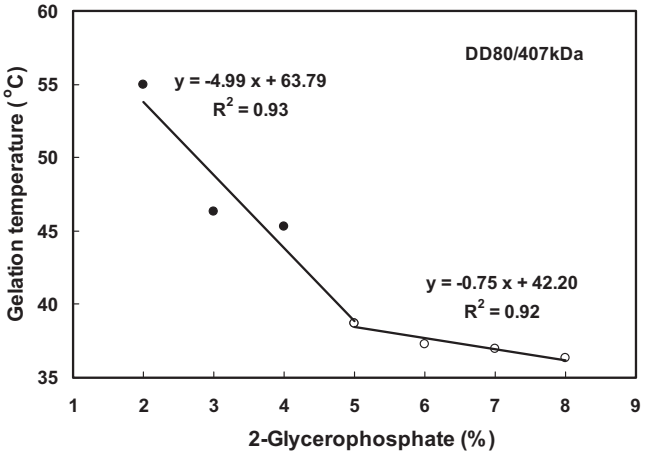

Fig. 3. The 2-glycerophosphate concentration affected the gelation temperature of hydrogel formed with 80% DD, 407 kDa chitosan.

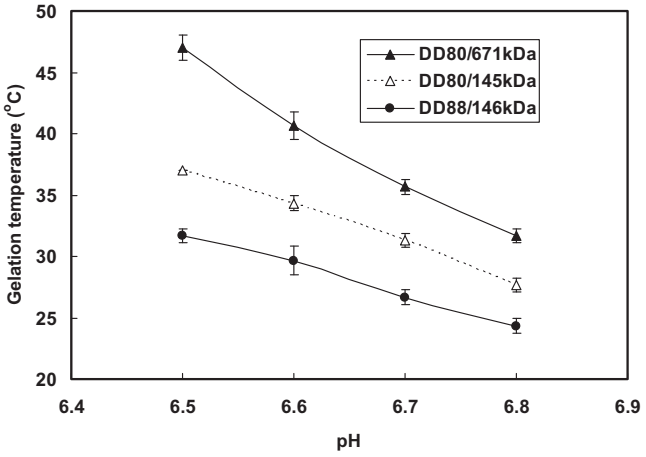

Fig. 4. The pH of the solution affected the gelation temperature of chitosan/6% 2-glycerophosphate hydrogel. The pH differences were manipulated by 2 N NaOH or 1 N HCl solution.

Furthermore, the chain flexibility of chitosan with a higher MW was greater and conformation was more contracted. In contrast, the chain flexibility of a lower MW was more rigid and had more extended conformation (Chen et al., 2009; Tsai et al., 2008; Tsaih & Chen, 1997). For these reasons, the higher and lower MWs of chitosan had little effect on the hydrodynamic volume with higher concentrations of glycerophosphate, and caused little difference to the gelation temperature. Chenite et al. (2000) indicated that the effect of the chitosan's MW on the gelation temperature of chitosan/glycerophosphate hydrogel was insignificant. This may be attributed to the hydrogel solution having a higher glycerophosphate concentration and higher pH value.

As shown in Fig. 1, the influence trend of glycerophosphate concentrations on the gelation temperature with different chitosan MWs was not consistent. For instance, Fig. 1a shows that the gelation temperature of two different chitosan MWs decreased as glycerophosphate concentrations increased at a lower concentration of glycerophosphate and the decreasing range was not consistent. But in the higher concentrations of glycerophosphate, the decreasing range was almost the same. So gelation temperature versus glycerophosphate concentration was plotted for each chitosans, and the SL and SH expressed the slopes of the plots in glycerophosphate concentration of 2–5% and 5–8%, respectively (Fig. 3). The SL and SH indicated the effect of glycerophosphate concentration on the gelation temperature, and are listed in Table 1.

Results in Table 1 indicate that the SL of chitosan with a DD of 80% and 88% was more evident than the SH (SL/SH > 4.4), while the chitosan with 80% DD and 900 kDa was the greatest among these SL data. However, the SH of a chitosan with 65% DD was similar to the SL (SL/SH = 1.3), and the SH was greater than the other SHs of different DDs for chitosan. The influence trend of glycerophosphate concentration on gelation temperature for different MWs for chitosan was not consistent, which may relate to the hydrodynamic behaviour and chain flexibility of chitosan. As the DD of chitosan decreased (DD 65%), the molecular chain was more rigid; the chitosan did not decrease rapidly in hydrodynamic volume and had a contracted conformation by charge neutralization, so the SL/SH was small. The 80% DD and 900 kDa chitosan had a large hydrodynamic volume due to a large MW, but the molecular chain was more flexible (Chen et al., 2009; Tsai et al., 2008; Tsaih & Chen, 1997). The hydrodynamic volume of chitosan decreased rapidly, the electric shielding effect of glycerophosphate created a contracted conformation, and the SL/SH was large. Moreover, the SL/SH of 88%, 80%, and 65% DD for chitosan were 6.9–11.2, 4.4–6.4, and 1.3, respectively. This indicates that the SL/SH is related to the chitosan DD.

3.4. Effect of pH on gelation temperature

Fig. 4 shows the gelation temperature of chitosan/glycerophosphate hydrogel with different pH values. Results show that the gelation temperature of the hydrogel decreased with the increase in the solution's pH value (Fig. 4). These results were similar to the reports by Chenite et al. (2000, 2001) and Kim et al. (2010). These results may be due to the higher pH value of hydrogel causing a lower degree of chitosan protonation. Therefore, glycerophosphate had fewer opportunities to combine with $-NH_3^+$ groups, and the chitosan hydration was poor (Chenite et al., 2000, 2001). In addition, the pH value of the hydrogel was higher and the degree of chitosan protonation was lower, indicating decreased solubility of the chitosans. In an endothermic process, the protons are released more easily and hydrating water is more easily removed from the chitosan, with increased hydrophobic interactions between chitosan molecules (Kim et al., 2010). Furthermore, the chitosan molecular charge density was lower, which while reducing the charge repulsion between the $-NH_3^+$ groups of chitosan led to a more flexible chain (Chen et al., 1994, 1996; Tsaih & Chen, 1999). Consequently, it was easier for the chitosan interchain to approach, entangle, and interact, so the chitosan formed a gel easily in the endothermic process.

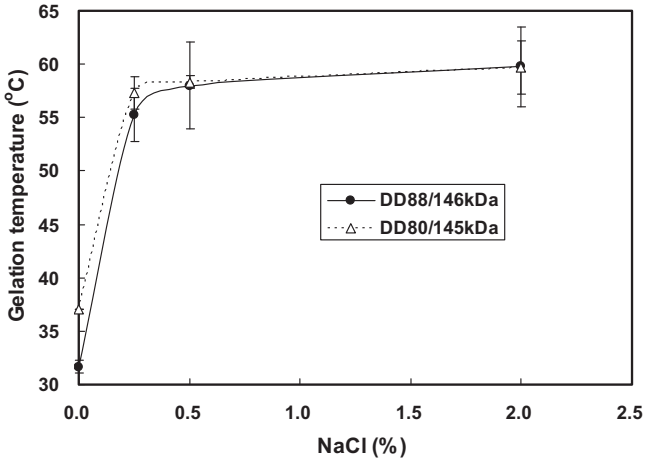

Fig. 5. Effect of sodium chloride concentration on gelation temperature of chitosan/6% 2-glycerophosphate hydrogel formed in pH 6.5 solution.

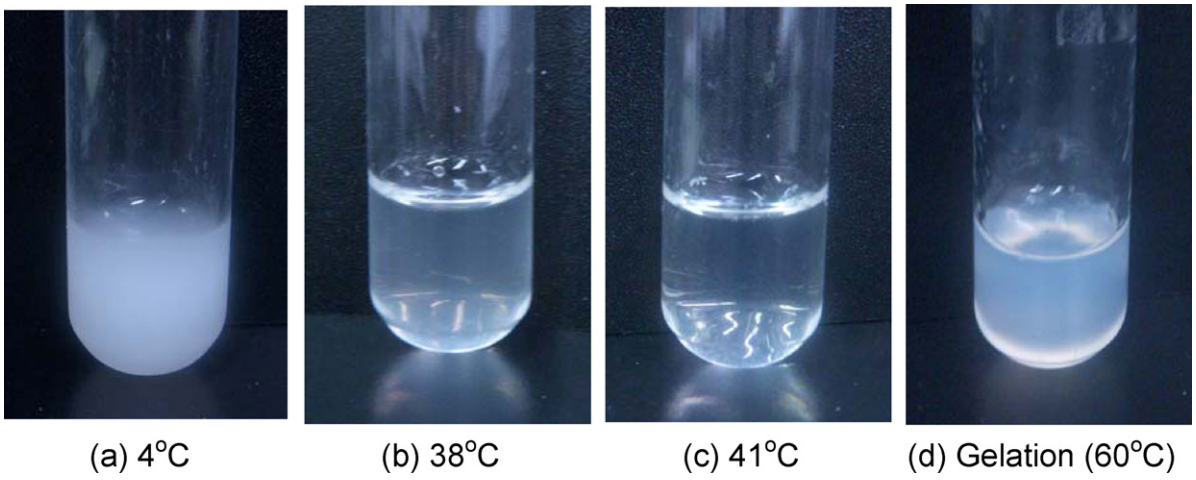

Fig. 6. Changes of appearance of 88% DD, 146 kDa chitosan/6% 2-glycerophosphate hydrogel containing 2% sodium chloride formed in pH 6.5 solution.

3.5. Effect of NaCl concentration on gelation temperature

Fig. 5 shows the gelation temperature of chitosan/glycerophosphate hydrogel when different concentrations of NaCl were added. Results show that the gelation temperature of the hydrogel increased when the concentration of NaCl increased (Fig. 5). This may be due to the fact that adding NaCl to hydrogel causes the chloride ions to compete with the phosphate group at the glycerophosphate to neutralize the chitosan $-NH_3^+$ group charge. Size and steric hindrance of the chloride ions were less than for glycerophosphate, causing the chloride ions to interact with a $-NH_3^+$ group more predominantly than with the glycerophosphate, thereby resulting in a more contracted chitosan chain. However, these small ions formed diffuse double layers on the surface of chitosan chains, limited the glycoside of a chitosan rotation, caused the molecular chains to become more rigid (Chen et al., 1994; Tsaih & Chen, 1999), and hindered the chitosan chains from closing with one another. Therefore, for a chitosan interchain it was more difficult to form a crystalline region and gel while the temperature was rising. Consequently, a greater quantity of NaCl was added to the hydrogel, and it was more difficult to form a gel, resulting in a higher gelation temperature (Fig. 5).

At lower concentrations of NaCl (0.25%, 0.5%), the hydrogel solution's appearances were transparent below gelation temperatures. However, when the concentration of NaCl was 2%, the hydrogel solution appearance was white turbidity below 38 °C (Fig. 6a), and changed into more transparent at higher temperature (Fig. 6b and c), then converted into white turbidity again due to gelation (Fig. 6d). This may be due to the number of chloride ions being about three times the number of amine groups. The concentration of chloride ions was high and the charge neutralization was strong, leading to a strongly contracted chitosan chain. The interaction of the intrachain and interchain was strong, leading to decreasing solubility. Therefore, the appearance of hydrogel solution showed white turbidity below 38 °C. However, the conformation of chitosan became more extended as the temperature rose (Chen & Tsaih, 1998). The molecular movement was more intense than it had been at low temperature, so the interaction between an intrachain and interchain was weaker. Sequentially the solubility of chitosan was increasing and the appearance of solution was changed into more transparent.

4. Conclusions

The gelation temperatures of chitosan/glycerophosphate hydrogels with or without 12 ppm nanosilver were insignificantly different no matter of the MW of chitosan (DD 80% or 88%) and concentration of glycerophosphate. The gelation temperature of the hydrogel decreased with increasing DD of chitosan, concentration of glycerophosphate and pH value. It decreased as the MW of chitosan decreased and increased with an increased concentration of NaCl. By adjusting above factors, the hydrogel gelation can be induced with a temperature range from 24.3 to 91.7 °C. Effect of chitosan's MW on gelation temperature of hydrogel become less obvious with increasing concentration of glycerophosphate. The hydrodynamic behaviour and chain flexibility of chitosan were affected by the DD and MW of chitosan, concentration of glycerophosphate, pH value of the solution, and concentration of NaCl; then the gelation temperature was affected. Briefly, the chitosan chains with higher flexibility and/or smaller hydrodynamic volume leaded to lower gelation temperature. The appearance of hydrogel solution containing 2% NaCl was white turbidity below 38 °C, was different from hydrogel in lower NaCl concentration, due to its lower solubility at higher ionic strength.

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