Preparation and Properties of Sulfopropyl Chitosan Derivatives with Various Sulfonation Degree

Huang-Shian Tsai,[1] Yen-Zen Wang,[2] Jong-Jing Lin,[3] Wan-Fu Lien[4]

[1]Research and Development Section, Yunlin Branch, Taiwan Textile Research Institute, 640, Douliou, Taiwan
[2]Department of Chemical Engineering and Material Science, National Yunlin University of Science & Technology, Yunlin 640, Taiwan
[3]General Education Division, Nan Jeon Institute of Technology, Tainan 746, Taiwan
[4]Department of Cultural Heritage Conservation, National Yunlin University of Science & Technology, Yunlin 640, Taiwan

ABSTRACT: In this investigation, chitosan (CS) is modified by propane sultone via a sulfonation reaction to create various degrees of sulfonation. The sulfonated chitosan (SCS) has a pendant alkyl sulfonic group dangling at the side chain, which can improve its hydrophilicity and water solubility. Elemental analysis, Fourier transform infrared spectroscopy (FTIR) and ^{13}C nuclear magnetic resonance (^{13}C NMR) were applied to identify the structure by determining the distribution of the substituents in the product. The degree of sulfonation in the SCS can be controlled and the hydrogen bonding interaction can be reduced by varying the degree of sulfonation. A solubility test proved that solubility increased with degree of substitution at over a wide range of pH values. X-ray diffraction patterns of SCS samples demonstrated that the crystallinity declined as the degree of sulfonation increased. Thermogravimetric analysis and modulated differential scanning calorimetry (MDSC) results indicated that thermal stability fell but water absorbance increased with the degree of sulfonation. More water-soluble SCS is thus obtained. The controllable different sulfonation degree of the CS polymer suggests new possibilities for the application of CS-based materials. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 1686–1693, 2010

Key words: Chitosan; water soluble; sulfonation

INTRODUCTION

Nowadays, interest is growing in the production and use of new materials from renewable sources. Natural polymers have excellent characteristics, such as biocompatibility, biodegradability, nontoxicity, and adsorption properties. Over the past few years, interest has been rapidly increasing in the class of naturally available polymers known as polysaccharides, because of their abundance in the nature.[1] Chitosan, a poly(β-1,4-D-glucosamine), is a basic polysaccharide obtained by partial or full alkaline N-deacetylation of chitin. It is also a multifunctional polymer that has three types of reactive functional groups: an amino group at the C2 position and primary and secondary hydroxyl groups at the C3 and C6 positions, respectively. Numerous amino groups and hydroxyl groups in chitosan's main chain, which make chitosan hydrophilic can participate in chemical reactions at pH < 6.5.[2–4] Chitosan and its derivatives have recently been increasing attention. Recently, research has been conducted in the application of chitosan and its derivatives to treat water and wastewater, by using them, for example, as coagulants,[5] to extract heavy metal ions from aqueous solutions,[6,7] biomaterials[8,9] and membrane materials.[10,11]

Industrial applications of chitosan raise numerous problems. For example, they cannot be easily dissolved in water at pH $\geq$ 7. Therefore, improving the hydrophilicity of chitosan is crucial if this abundant resource is to be used over a wide range of pH values. Various investigations were therefore performed to yield water-soluble derivatives of chitin and chitosan by modification. Chemical modification of chitosan to generate new materials is a particular interest because such a procedure does not alter the fundamental skeleton of chitosan but would keep the original physicochemical and biochemical properties, and enables a water-soluble polymer to be formed[1] depending on the characteristics of the group introduced such as hydrophilicity groups.[12]

The water solubility of chitosan can be improved by chemical modification by introducing a hydrophilic functional group.[13] Many chitosan derivates—including CM-chitosan (carboxymethyl chitosan), N-sulfuryl chitosan, dicarboxymethyl, and quaternized chitosan—have been developed.[14–17] These chitosan derivates aforementioned site the specific
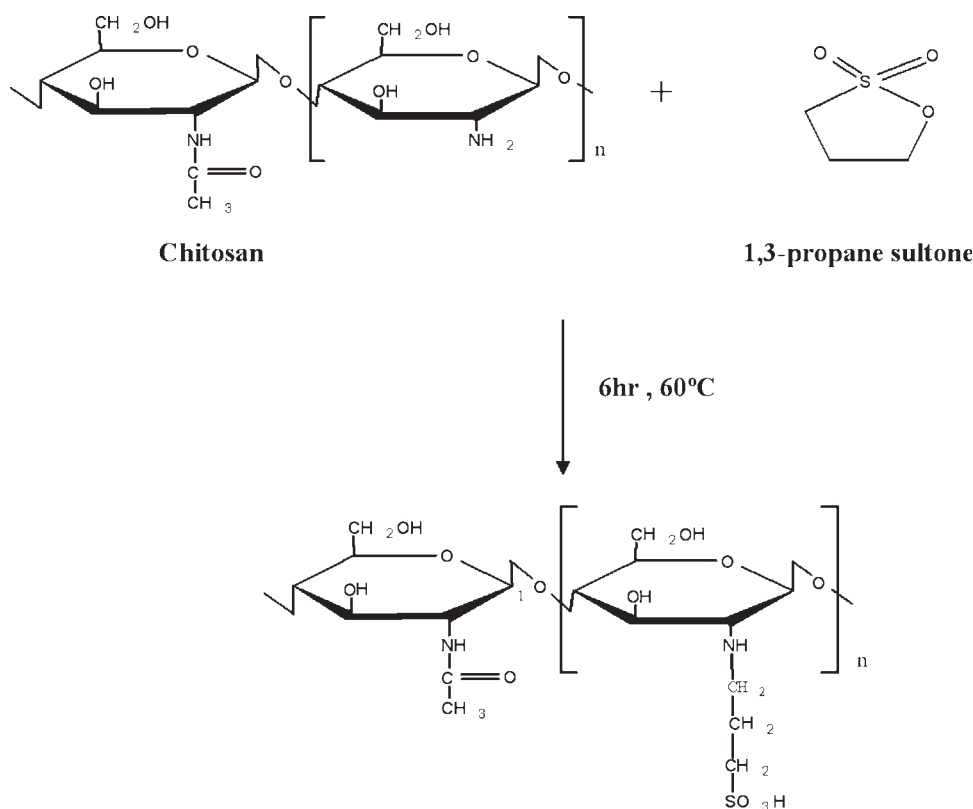
Scheme 1 Synthetic scheme of sulfate chitosan

chemical modification of the amino and hydroxyl groups in chitosan with sulfonation, which can increase the water solubility. As chitosan is insoluble or only slightly soluble in the organic solvents that are used as a reaction medium, it must usually undergo a preparative treatment in a complex solvent system. Therefore, the constitution of the product is assumed to be heterogeneous, but it is inconvenient and difficult to control.[18,19] This study aims to develop a chitosan-based water-soluble material containing various alkyl sulfonic substitution degrees for use in a more conveniently controllable reacting system. Various characterizations by EA, Fourier transform infrared spectroscopy (FTIR), ^{13}C NMR, were performed and the crystallinity in various degree of sulfonation was studied via the X-ray diffraction patterns. Solubility in different organic solution and in buffer solutions with different pH values was also measured and finally the thermal properties were carried out by Thermogravimetric analysis (TGA) and Modulated differential scanning calorimetry (MDSC).

EXPERIMENTAL

Materials

Chitosan was purchased from Fluka Chemical Co. The degree of deacetylation was around 85% and molecular weight was about 400,000 Da. Acetic acid (J. T. Baker), 1,3-propane sultone (Fluka), acetone (Mallinckrodt), methanol (Riedel-deHaë), ethanol (PANREAC QUIMICA SAU), sodium acetate (Riedel-deHaën), toluene (Alps Chem Co.), dimethylformamide (DMF, J.T.Baker), tetrahydrofuran (THF, Riedel-deHaën), and N-Methyl-2-pyrrolidone (NMP, Mallinckrodt) were used. All other reagents and solvents were reagent grade.

Preparation of sulfonated chitosan

Chitosan (1% w/w) solution was prepared by adding chitosan powder to 2% (w/w) acetic acid, stirring for 60 min, and then adding 1,3 propane sultone to the solution according to the molar ration described later. The mixture was allowed to react at 60°C for 6 h. The resulting solution was poured into acetone to precipitate. The precipitated product was washed sufficiently with acetone and methanol sequentially, and dried in a thermostat oven at 50°C for one day. The obtained products were the sulfonated chitosans (SCS).[20] The molar ratio of propane sultone unit reacted with glucosamine unit was 0.6, 0.8, and 1.0. The sulfonated chitosan thus obtained were denoted SCS60, SCS80, and SCS100, respectively, where the numbers 60, 80, and 100 refer to the degree of substitution of alkyl sulfonic groups for amino groups in chitosan. Scheme 1 shows the synthetic scheme of sulfate chitosan.

TABLE I
Elemental Analysis Result of Chitosan and Sulfonated Chitosan with Different Degree of Sulfonation

Theoretical values		Experimental values					
Sample codes	Degree of sulfonation[a] (molar ratio)	N (%)	C (%)	S (%)	H (%)	S/N (molar ratio)	Degree of sulfonation[a] (molar ratio)
CS	0.00	6.00	38.30	0.00	7.58	0.00	0.00
SCS60	0.60	5.04	36.10	5.93	7.43	0.51	0.61
SCS80	0.80	4.26	34.80	6.81	7.35	0.70	0.82
SCS100	1.00	4.13	33.70	8.10	7.18	0.86	1.01

[a] The degree of sulfonation calculated according to the molar ratio.

Elemental analysis

The percentages of carbon, nitrogen, hydrogen, and sulfur elements of sulfonated chitosans were evaluated using an elemental analysis (EA) instrument (Elementar Vario EL III).

Infrared spectroscopy

FTIR spectra of the neat chitosan and sulfonated chitosan were obtained using a Perkin Elmer FTIR spectrum one spectrometer. A homogeneous polymer solution was cast directly onto the CaF surface, and then dried in a thermostat oven at 50°C, 16 scans from 1000 to 4000 cm^{-1} were performed on a sample with a resolution of 2 cm^{-1}.

^{13}C CP/MAS NMR spectra

Solid state carbon 13 nuclear magnetic resonance was performed using a 400 MHz Bruker NMR spectrometer. The ^{13}C CP/MAS NMR spectra were recorded at 100.62 MHz with a spinning rate of 6.2 kHz, and a CP contact time of 2 min.

Determination of solubility

To estimate solubility, 1.0 g of sulfonated chitosan was mixed with 100 mL test solution (shown in Table III) and 1.0 g, 2.0 g, 3.0 g, and 4.0 g of sulfonated chitosan was mixed with 100 mL of acetic acid/sodium acetate buffer solution at various pH values. The mixture was stirred for 6 h and whether the sulfonated chitosan was soluble (+), insoluble (−), or slightly soluble (+−) in the mixed polymer solution was determined. After 6 h, the solubility of mixtures was no longer changed. The solubility S, is defined as: $S(\%) = (W_0 - W_1)/W_0 \times 100$, where W_0 and W_1 are the weight of dry sample before test and the weight of dry sample after solubility test resided, respectively. The S-value between 3 and 15% was defined as slight solubility, that less than 3% defined as insoluble.

Wide-angle X-ray diffractometry

A Rigaku 18 kW Rotating Anode X-ray Generator X-ray Diffractometer was adopted to investigate the solid state morphology of sulfonated chitosan in film form. X-rays with a wavelength of 1.5406Å were generated using a CuKα source. The angle of diffraction was varied from 5 to 45°.

Thermal gravimetric analysis

The thermal stability of the polymer films was studied using a DuPont Q500 TGA instrument as they were heated from 25 to 600°C at a rate of 20°C/min, in flushing nitrogen at 40 mL/min.

Modulated differential scanning calorimetry

MDSC was performed using a DuPont Q100 DSC system with a refrigerated cooling accessory (RCS) and modulated capability. Samples were analyzed under continuously flowing dry nitrogen gas (40 mL/min) at a heating rate of 3°C /min from −50 to 200°C. The amplitude and period of the MDSC were 0.8°C and 60 s, respectively.

RESULT AND DISCUSSION

Elemental analysis

Table I shows the analyzed carbon(C), hydrogen(H), nitrogen(N), and sulfur(S) contents in chitosan and all modified polymers, and elucidates the relationship between the molar ratio of sulfur over nitrogen (S/N) and degree of sulfonation. The percentages of sulfur increase with the degree of sulfonation of the chitosan. The S/N and the degree of sulfonation (S/0.85 N) were also strengthened and the experimental degree of sulfonation agreed well with the theoretical value. This consistency arises from the reactions between chitosan and 1,3 propane sultone and supports the conclusion that the extent of sulfonation was controllable and can be very close to the predesignated one. Accordingly, a sulfonated chitosan could be formed and the percentage of the free
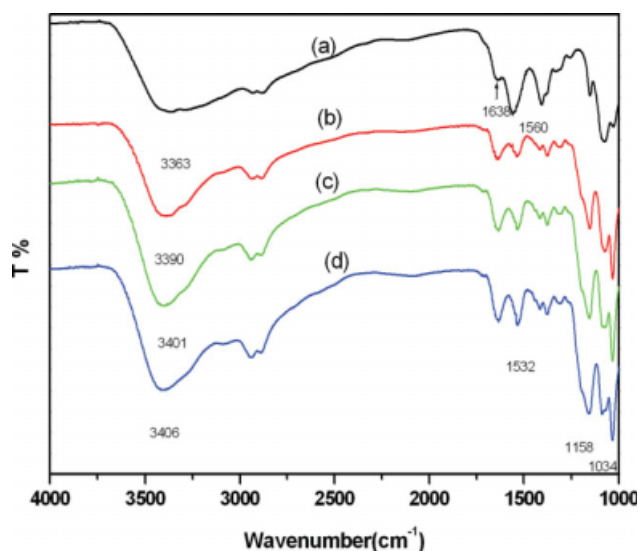
Figure 1 FTIR spectra of chitosan and sulfonated chitosan (a) CS (b) SCS60 (c) SCS80 (d) SCS100. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pendant amino groups in the chitosan polymer was controllable.

FTIR spectra of sulfonated chitosan

Figure 1 shows the FTIR spectra of chitosan and sulfonated chitosan films with various degrees of sulfonation. Figure 1(a) shows the characteristic bands of the chitosan in the spectrum, including a characteristic band at 1638 cm^{-1}, which corresponds to the stretching of amide C=O (amide I) and at 1560 cm^{-1}, which corresponds to the N—H deformations of a primary amine (amide II). The band at 1380 cm^{-1} is associated with the C—H bond in the CH$_3$ group of the acetamide group, and indicates that chitosan is not completely deacetylated. The band at 1078 cm^{-1} corresponds to the C—O stretching of a primary alcohol.

Figure 1(b–d) present the FTIR spectrum of chitosan that is derived from 1,3-propane sultone. The FTIR spectrum includes the stretching vibration of OH group in sulfonic acid at 2900, characteristic absorption bands of the group at 1200–1000 cm^{-1}, stretching vibration of sulfoxide in sulfonic acid at both 1165–1150 and 1350–1340.[20,21] revealing the presence of sulfonic groups in the modified chitosan.

A comparison of the FTIR spectrum of chitosan in Figure 1(a) with that of sulfonated chitosan in Figure 1(b–d) reveals differences between their absorbance bands at 1638 cm^{-1} (amide I) and 1560 cm^{-1} (amide II), respectively. The absorbance of amide II in sulfonated chitosan (SCS100) shifted from 1560 cm^{-1} to 1532 cm^{-1}, indicating that the sulfonation reaction involved amino groups in chitosan and amino groups to form secondary amide groups. Chitosan is known to include such hydrophilic groups as —OH and —NH$_2$, which exhibit strong hydrogen interactions, demonstrating broad peaks at 3300–3400 cm^{-1}. Following sulfonation, these peaks were sharpened because the strength of the hydrogen interaction decreased with the degree of sulfonation and the overlapping between —OH and —NH$_2$ absorption decreased following sulfonation with 1,3 propane sultone. The —OH position became clear and sharp and shifted from 3363 cm^{-1} to 3406 cm^{-1}. The solid state ^{13}C CP/MAS NMR spectra provide further evidence of completed sulfonation reaction.

Solid state ^{13}C CP/MAS NMR of sulfonated chitosan

The solid state ^{13}C CP/MAS NMR spectra were obtained to compare the structures of chitosan and sulfonated chitosan. Figure 2(a, chitosan; b–d, sulfonated chitosan) display the respective spectra and confirm the presence of sulfonation of chitosan. The spectrum of neat chitosan has carbon peaks (C=O: δ = 179.86 ppm; C-1: δ = 105.183 ppm; C-4: δ = 82.015 ppm; C-3/C-5: δ = 75.574 ppm; C-2/C-6: δ = 58.07 ppm, and CH3: δ = 23.754 ppm), matched with the report from Kittur,[22] Hirai,[23] and Jang et al.[24] The expected series of peaks from natural chitosan at 23.94 and 173.20 ppm were attributed to the methyl and carbonyl groups that were associated with the monomer unit form of chitosan and sulfonated chitosan. Comparing the spectra of chitosan and sulfonated chitosan revealed that the latter owned additional peaks at C8: δ = 48.10 ppm, C7: δ = 27.45 ppm and C9: δ = 29.79 ppm.

The ^{13}C CP/MAS NMR spectrum of neat chitosan was compared with that of sulfonated chitosan, and the peaks of C2/C6 and C3/C5 were all significantly split. Table II shows the ^{13}C NMR data for chitosan and its sulfonated derivatives. The signals at 61 ppm indicate that the C6 hydroxyl was not sulfated. The sulfated signal (δ = 74 ppm) from C3 hydroxyl groups, could not be detected clearly, and the unsulfated signal (δ = 71 ppm) was more intense than any other ones.[25,26] Hence, the sulfonated chitosan was not sulfated in position/s 3 and/or 6. In the methylene region, a signal absent chitosan at 48 ppm was attributed to C8; an analogous signal was present in the spectrum of *N*-alkyl-O-sulfonated chitosan,[27] indicating the 1,3 propane sultone reacted with the pendant amino groups of the chitosan polymer.

Solubility

Table III shows the results concerning the solubility of sulfonated chitosan in various organic solvents. The results reveal that sulfonated chitosans were
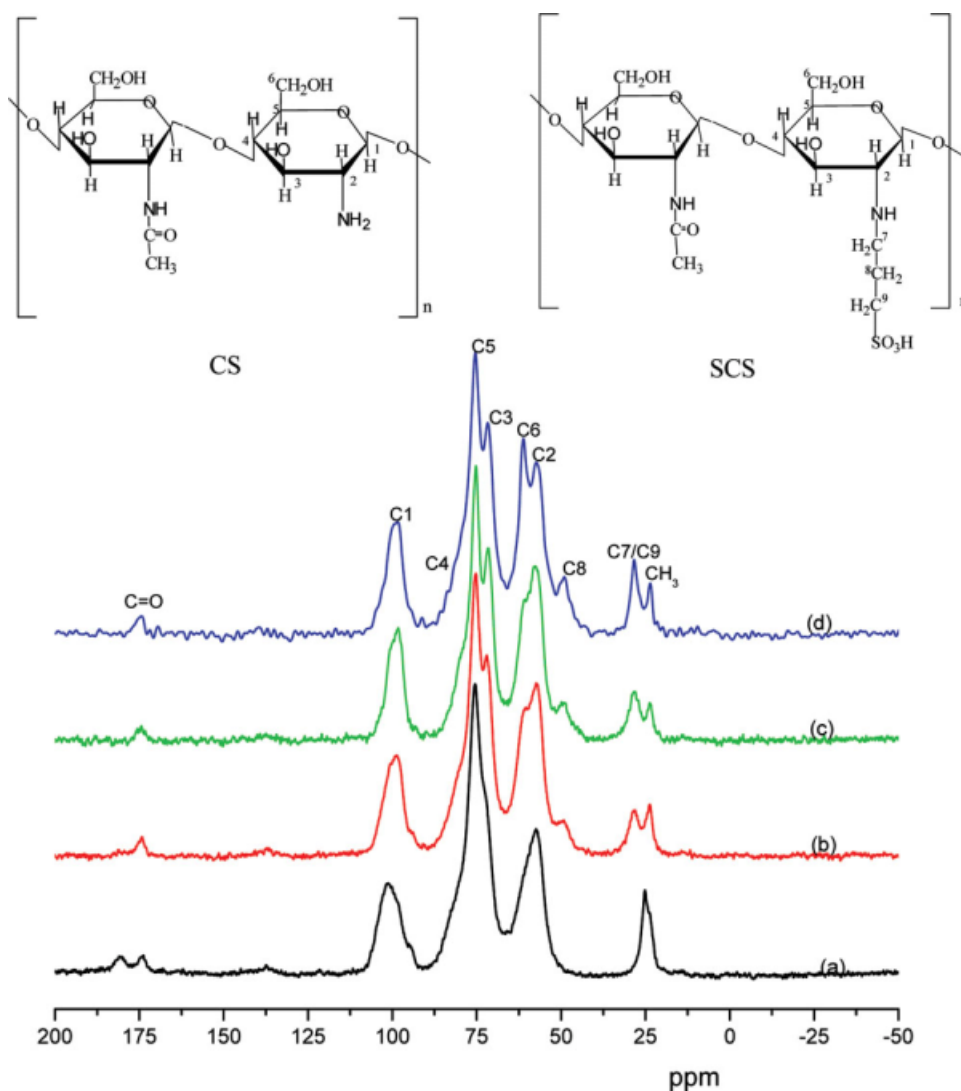
Figure 2 Solid state 13C CP/MAS NMR spectra of sulfonated chitosan (a) CS (b) SCS60 (c) SCS80 (d) SCS100. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

soluble not only in 2 wt % aqueous acetic acid solution but also in deionized water, and insoluble in any organic solvent, suggesting that the preparation of sulfonated chitosan via a sulfonation reaction was complete to improve the water absorption without losing the organic solvent resistance.

TABLE II
Solid State ^{13}C CP/MAS NMR Data for Chitosan and Sulfonated Chitosans

Compound	Chemical shifts (ppm)					
	C1	C2/C6		C3/C5		C4
CS	100.32	56.29		74.60		–
SCS60	97.99	56.29	60.19	71.10	74.60	–
SCS80	97.99	56.29	60.19	71.10	74.60	–
SCS100	98.77	57.68	60.57	71.10	74.21	–

TABLE III
Solubility Result of Chitosan and Sulfonated Chitosan in Different Organic Solution (Controlled Solubility = 1.0 g/100 mL)

Test solutions	Samples			
	CS	SCS60	SCS80	SCS100
Deionized water	–	+	+	+
2 wt % aqueous acetic acid solution	+	+	+	+
Methanol	–	–	–	–
Ethanol	–	–	–	–
Isopropanol	–	–	–	–
Toluene	–	–	–	–
NMP (*n*-Methyl-2-Pyrrolidone)	–	–	–	–
MEK (Methyl ethyl ketone)	–	–	–	–
THF (Tetrahydrofuran)	–	–	–	–
DMF (Dimethyl formamide)	–	–	–	–
DMSO	–	–	–	–

Soluble (+), Insoluble (−).

TABLE IV
The Solubility of Sulfonated Chitosan in Different pH Values Acetic Acid/Sodium Acetate Buffer Solution

Samples	Controlled solubility (g/100 mL)	pH				
		3	4	5	6	7
CS	1.0	+	+	+	−	−
	2.0	+	+	+	−	−
	3.0	+−	+−	+−	−	−
	4.0	+−	+−	+−	−	−
SCS60	1.0	+	+	+	+	+
	2.0	+	+	+	+−	+−
	3.0	+	+	+−	+−	+−
	4.0	+−	+−	+−	+−	+−
SCS80	1.0	+	+	+	+	+
	2.0	+	+	+	+	+−
	3.0	+	+	+	+−	+−
	4.0	+	+	+	+−	+−
SCS100	1.0	+	+	+	+	+
	2.0	+	+	+	+	+
	3.0	+	+	+	+	+
	4.0	+	+	+	+	+

Soluble (+), Insoluble (−), Slightly soluble (+−).

Table IV shows the results concerning the solubility of sulfonated chitosan in acetic acid/sodium acetate buffer solution at various pH values. The results reveal that SCS100 had the best solubility over a wide range of pH values. The solubility of CS and SCSX increased with the degree of sulfonation at a given pH. The CS is soluble only at pH ≤ 5 and became insoluble, forming a gel at a concentration of 3.0 g/mL, suggesting that a high degree of sulfonated substitution of chitosan corresponded to a more amorphous structure because the alkyl sulfonic group in the chitosan chain had a less regular structure and caused higher water adsorption, resulting in the losing crystallinity, and increasing solubility. This result reveals that increasing the degree of sulfonation of chitosan can increase chitosan solubility over a wide range of pH values and the scope of applications.

X-ray diffraction

Figure 3 displays the X-ray diffraction patterns of the chitosan and sulfonated chitosan. Chitosan has two reflections at $2\theta = 11°$, $2\theta = 20°$. Samuels found that the reflection at $2\theta = 11°$ was associated with crystal form I. The strongest reflection is at $2\theta = 20°$, and corresponds to crystal form II.[28] Sulfonated chitosan [Fig. 3(b–d)] has a broader peak at about $2\theta = 20°$ and the peak at $2\theta = 11°$ was decreased with degree of sulfonation, suggesting that chemical modification reduced its capability to form a hydrogen bond. The structure of the modified chitosan is more amorphous from sulfonation, which makes the chitosan membrane more amorphous as well. This result was consistent with most of related literatures,[29] indicating that the sulfonation by propane sultone
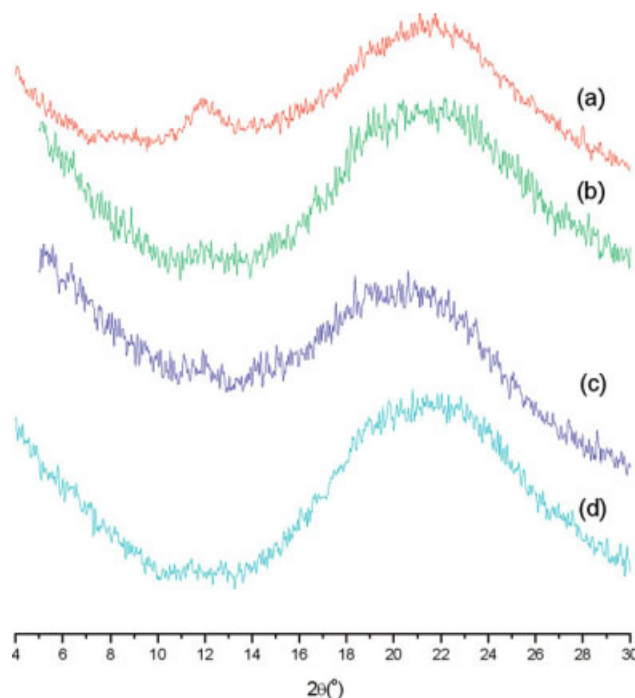

Figure 3 X-ray spectra for neat chitosan and sulfonated chitosan (a) neat CS (b) SCS60 (c) SCS80 (d) SCS100. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

proceeds randomly along the chitosan chain in solution, efficiently destroying the regular packing of the original chitosan units. Finally, a totally amorphous sulfonated chitosan is available.[30]

Thermal gravimetric analysis

Figure 4 shows TGA thermograms of neat chitosan (a) and sulfonated chitosan (b–d) membranes. The
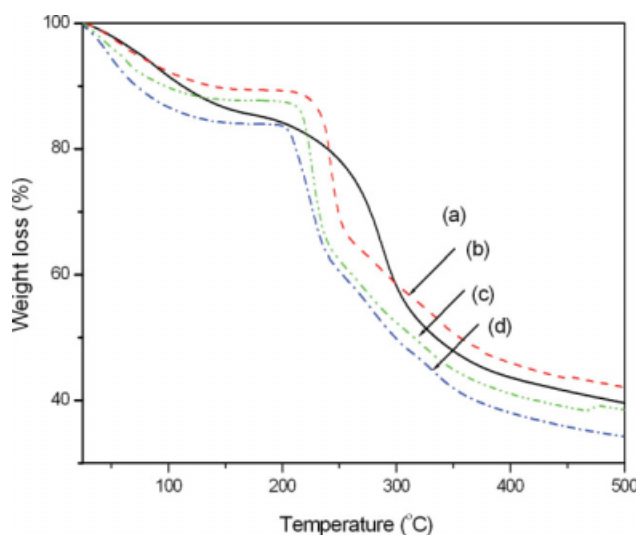

Figure 4 TGA thermograms of neat chitosan and sulfonated chitosan (a) neat CS (b) SCS60 (c) SCS80 (d) SCS100. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE V
The Degradation Temperatures (T_d) and Percent of Weight Resided for Neat Chitosan and Chitosan with Different Degree of Sulfonation

Sample name	T_{d1}[a] (°C)	T_{d2}[b] (°C)	Weight resided (%) (at 100°C)	Weight resided (%)
Neat CS	–	287.18	91.45	64.54
SCS60	242.52	283.68	92.25	61.41
SCS80	225.72	271.68	89.75	57.92
SCS100	224.44	278.93	86.61	54.44

[a] T_{d1} is the first stage degradation temperature (related to sulfonic degradation).
[b] T_{d2} is the second stage degradation temperature (related to chitosan main chain).

thermograms were similar to each other. The neat chitosan membrane degrades at two stages. The first stage begins at 50°C and involves a weight loss of ∼ 10%. The second stage begins at 225°C and continued to 425°C with a weight loss of 40%. This result is similar to those presented by Nieto et al.[31] and Tirkistani.[32] The first stage involves loss of water. The second corresponds to the decomposition of chitosan, and the vaporization and elimination of volatile fragments.[33] The sulfonated chitosan membrane is heated in nitrogen [Fig. 4(b–d)] and undergoes a three-stage decomposition. The first stage is between 50°C and 120°C with a loss of almost 10% of the initial weight. It is followed by a further 40% weight loss just above 200°C, ending at approximately 250°C, which involves the decomposition of the pendant sulfonate groups of the chitosan polymer. The third stage of decomposition, which ends at around 400°C, involves a weight loss of around 15%.

Table V shows data on the second stage of weight loss. Table V reveals that the weight loss in the second stage increased with degree of sulfonation. This result is reasonable as the numbers of pendant alkyl sulfonic groups increased with the degree of sulfonation, and the decomposition temperature at 200–250°C is dominated by the decomposition of pendant alkyl sulfonic groups.

Table V indicates that as the degree of sulfonation increases, the second stage degradation begins sooner, which explains why the sulfonated chitosans are thermally unstable. That is increasing the sulfonationity that reduce the decomposition temperature of the sulfonated chitosan. This change might render the material more hydrophilic following sulfonation and destroy the crystalline structure. Eventually after sulfonation, the structure of the polymer became irregular, deteriorating the thermal properties.

Modulated differential scanning calorimetry

Figure 5 shows the MDSC thermograms of neat chitosan and chitosans with various sulfonationity. The results of thermal analysis of the chitosan, shown in Figure 5(a), reveal a broad endothermic peak

between 40 and 150°C. According to Rueda et al.,[34] this endothermic peak, ranging over a large range of temperatures, is related to the evaporation of water from inside.

Different positions of the endothermic peaks correspond to the different water binding capacities of these macromolecules and different strengths of the water–polymer interactions.[35,36] The endothermic peaks from chitosan and sulfonated chitosan, shifted to higher temperature as the degree of sulfonation increased, indicating a possible correlation between the water binding capacity and the chemical and supra-molecular structures of these polymers.[33,37] This result is consistent with that observed by TGA. Increasing the degree of sulfonation increases the hydrophilicity of the polymer; most of the bound water resulted from the interaction of the hydroxyl group/sulfonic group with water molecules. Therefore, at a relatively low degree of sulfonation, and even in neat chitosan, the water absorbed by the
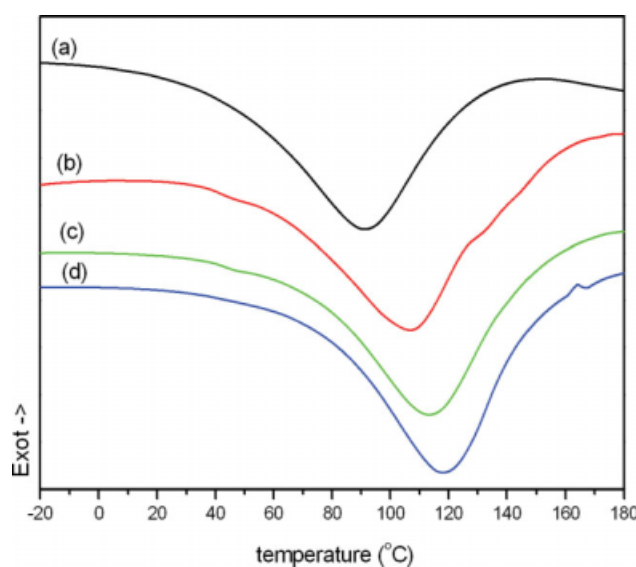

Figure 5 MDSC heat flow curves of chitosan and neat chitosan and chitosan with different degree of sulfonation (a) neat CS (b) SCS60 (c) SCS80 (d) SC100. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

hydroxyl groups is more difficult to be removed and evaporated.[33,34]

CONCLUSION

Chitosan is a highly reactive polymer with its active side chain amino group. This characteristic is taken advantage in modifying the chitosan using 1,3-propane sultone. Controlling the mole ratio to produce different degrees of sulfonation makes available water-soluble chitosan with different numbers of amino groups.

EA, FTIR, and ^{13}C NMR demonstrate that the sulfonation was successful and various degrees of sulfonate substitution can be created. The solubility and the chemical resistance increased over a wide range of pH values of sulfonated chitosan. The SCS100 had the highest solubility of higher than 4.0 g/100 mL at pH values from 3 to 7. The crystallinity of the polymer declines as the degree of sulfonation increases, as revealed by the X-ray diffraction patterns, because the disordered structure prevents the polymer from absorbing more water and/or the loss of hydrogen bonding following sulfonation.

Finally, the TGA and MDSC thermograms demonstrated that the evaporation temperature of water in the chitosan derivatives is 20–50°C higher than that in the neat chitosan, suggesting that modification strengthens the interaction between water and the chitosan chains.

References

1. Shahidi, F.; Arachchi, J. K. V.; Jeon, Y. J. Trends Food Sci Technol 1992, 10, 37.
2. Skjak-Braek, G.; Anthonsen, T.; Sandford, P. Chitin and Chitosan; Elsevier Applied Science: London, 1989.
3. Claesson, P. M.; Ninham, B. W. Langmuir 1992, 8, 1406.
4. Batista, M. K. S.; Pinto, L. F.; Gomes, C. A. R.; Gomes, P. Carbohydr Polym 2006, 64, 299.
5. Eikebrokk, B.; Saltnes, T. Water Sci Technol: Water Supply 2001, 1, 131.
6. Bassi, R.; Prasher, S. Q.; Simpson, B. K. J Environ Sci Health A: Tox Hazard Subst Environ Eng 1999, 34, 289.
7. Jeon, C.; Höll, W. H. Hydrometallurgy 2004, 71, 421.
8. Chen, H.; Tian, X.; Zou, H. Artif Cells Blood Substit Immobil Biotechnol 1998, 26, 431.
9. Sashiwa, H.; Aiba., S.-I. Prog Polym Sci 2004, 29, 887.
10. Qurashi, M. T.; Blair, H. S.; Allen, S. J. J Appl Polym Sci 1992, 46, 255.
11. Struszczyk, M. H. Polim/Polymers 2002, 47, 396.
12. Jayakumar, R. N.; New, T.; Tokura, S.; Hamura, H. Int J Biol Macromol 2007, 40, 175.
13. Holme, K. R.; Perlin, A. S. Carbohydr Res 1997, 302, 7.
14. Muzzarelli, R. A. A. Carbohydr Polym 1985, 5, 461.
15. Watanabe, K.; Saiki, I.; Matsumoto, Y.; Azuma, I. Carbohydr Polym 1992, 17, 29.
16. Dung, P. I.; Milas, M.; Rinaudo, M.; Desbrièies, J. Carbohydr Polym 1994, 24, 209.
17. Jia, Z.; Shen, D.; Xu, W. Carbohydr Res 2001, 333, 1.
18. Ilyina, V.; Tikhonov, V. E.; Albulov, A. I.; Varlamov, V. P. Process Biochem 2000, 35, 563.
19. Kubota, N.; Tatsumoto, N.; Sano, T.; Toya, K. Carbohydr Res 2000, 324, 268.
20. Kondo, K.; Nakagawa, S.-I.; Matsumoto, M.; Yamashita, T.; Furukawa, I. J Chem Eng Jpn 1997, 30, 846.
21. Miao, J.; Chem, G.-H.; Gao, C.-J. Desalination 2005, 181, 173.
22. Kittur, F. S.; Harish Prashanth, K. V.; Sankar, K. U. Carbohydr Polym 2002, 49, 185.
23. Hirai, A.; Odani, H.; Nakajima, A. Polym Bull 1991, 26, 87.
24. Jang, M. K.; Kong, B.; Jeong, Y. I. J Polym Sci part A: Polym Chem 2004, 42, 3423.
25. Baumann, H.; Faust, V. Carbohydr Res 2001, 331, 43.
26. Gamzazade, A.; Sklyar, A.; Nasibov, S.; Sushkov, I.; Shashkov, A.; Knirel, Y. Carbohydr Polym 1997, 34, 113.
27. Zhang, C.; Ping, Q.; Zhang, H.; Shen, J. Carbohydr Polym 2003, 54, 137.
28. Samuels, R. J. J Polym Sci Polym Phys Ed 1981, 19, 1081.
29. Qu, X.; Wirsen, A.; Albertsson, A.-C. Polymer 2000, 41, 4589.
30. Qu, X.; Wirsen, A.; Albertsson, A.-C. Polym Mater Sci Eng 1998, 79, 242.
31. Nieto, J. M.; Peniche-Covas, C.; Padron, G. Thermochim Acta 1991, 176, 63.
32. Tirkistani, F. A. A. Polym Degrad Stab 1998, 60, 67.
33. Neto, C. G. T.; Giacometti, J. A.; Job, A. E.; Ferreira, F. C.; Fonseca, J. F. E.; Pereira, M. R. Carbohydr Polym 2005, 62, 97.
34. Rueda, D. R.; Secall, T.; Bayer, R. K. Carbohydr Polym 1999, 40, 49.
35. Ratto, J.; Hatakeyyama, T.; Blumstein, R. B. Polymer 1995, 36, 2915.
36. Berthold, A.; Cremer, K.; Kreuter, J. J Control Release 1996, 39, 17.
37. Harish Prashanth, K. V.; Kittur, F. S.; Tharanathan, R. N. Carbohydr Polym 2002, 50, 27.