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Synthesis and characterization of chondroitin sulfate–methacrylate hydrogels

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Abstract

The various degree of methacrylate (MA) substitution on chondroitin sulfate (CS) was prepared by reacting chondroitin sulfate with methacrylic anhydride (MAA) in the presence of sodium hydroxide (NaOH) as a base. The effects of reaction time, reaction temperature, MAA concentration, and NaOH amount on the substitution degree of CS-MA were tested. The confirmation of the CS-MA chemical structure was carried out by ^{1}H-NMR, ^{13}C-NMR, FTIR and the degree of MA substituent on CS was calculated from the ratios of two peak intensities corresponding to methyl groups on methacrylate and chondroitin sulfate, respectively. Hydrogels were prepared by free radical polymerization of CS-MA precursors with or without acrylic acid (AA). CS-MA hydrogels were easily broken into small pieces during swelling study. However, CS-MA-AA hydrogels remained completely and showed a range of swelling ratio from 200 to 390% and exhibited an increase in swelling ratio with a decreasing degree of MA substitution. The thermal degradability observed with a TGA explained the unstableness of these hydrogels in comparison with the pure CS. The surface morphology conducted by SEM exhibited a porous structure after swelling.

Keywords: Chondroitin sulfate; Methacrylic anhydride; Acrylic acid; Hydrogels

1. Introduction

Chondroitin sulfate (CS) is an important structural component in connective tissues and cartilage. It belongs to the glycosaminoglycans (GAGs), which are primarily located on the surface of cells or in the extracellular matrix. The high viscosity of GAGs is also associated with low compressibility, making these molecules ideal as a fluid for lubricating the joints. CS is the result of the copolymerization of sulfated D-glucuronic acid with *N*-acetyl-D-galactosamine in C_4 or C_6. It forms a network with collagen in the connective tissues to allow the transport of macromolecule such as globular proteins. Moreover, many clinical studies demonstrate the therapeutic effects of orally administered CS on osteoarthritis patients with improvement of articular functions and reduction of pain (Morreale & Manopulo, 1996; Ronca & Palmieri, 1998).

CS is a soluble mucopolysaccharide, which is utilized as a substrate by the bacteroid inhabitants of the large intestine, mainly by *Bacteroides thetaiotaomicron* and *B. ovatus* (Saylers, 1979). Periplasmic enzymes are probably responsible for CS breakdown: apparently, an outer membrane receptor binds CS and brings it to contact with enzymes like chondroitinase ABC (Saylers & O'Brien, 1980). In human colon, the natural sources of CS are sloughed epithelial cells or dietary meat. Therefore, CS could be used as a colonic drug carrier. Since natural CS is readily water-soluble, it might not successfully sustain a drug release. Rubinstein et al. developed a method, which reduces the hydrophilicity of CS by cross-linking it with diaminododecane (Rubinstein, Nakar, & Sintov, 1992; Sintov, Di-capua, & Rubinstein, 1995). They reported that cross-linked CS-based matrices containing idomethacin retained the drug efficiently for more than 10 h at pH7 and released it massively through the biodegradation of CS in colonic medium. Bourie and Paillard (1998) repeated Rubinstein's experiment by cross-linking CS with different diaminoalkanes. Instead of using indomethacin, the authors used more water-soluble theophylline as a model drug. However, they concluded that cross-linking with diaminoalkane-type bifunctional compounds was not as promising as suggested in the work of Rubinstein.

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In previous study, we have tried to insolubilize CS by forming a semi-interpenetrating polymer network with AA (Wang & Wang 2002). Although the hydrogels with high swelling ratios were obtained, the sol factions of these hydrogels were as high as ~40 wt%, owing to the dissolution of CS. Therefore, we plan to incorporate vinyl monomers on CS, giving hydrogels through the chemical reaction of vinyl groups. By controlling the degree of substitution of vinyl monomers on CS, we can manipulate different physical properties of hydrogels. Methacrylate functional groups have been linked to polysaccharide by reacting methacrylic anhydride with the hydroxyl groups of polysaccharide (Kim & Chu, 2000; Smeds & Grinstaff, 2001). The similar method will be adapted to synthesize methacrylate substitution on chondroitin sulfate (CS-MA) precursor and then pH-sensitive hydrogels will be synthesized by copolymerization of CS-MA precursor with AA. The hydrogel structure is characterized with a FTIR. Swelling properties and thermal stability of the hydrogels will be studied.

2. Experimental

2.1. Materials

Sodium chondroitin sulfate (CS, oral grade, Mn = 58,000 by GPC), Lot No. OC-97112, was obtained from Tohoku Miyagi Pharmaceutical Co. Ltd. Methacrylic anhydride (MAA) and potassium persulfate were purchased from Aldrich, Milwaukee, Wisconsin; Acrylic acid (AA) from Janssen Chimica, Tokyo, Japan, was distilled before use. Dimethyl sulfoxide and dimethyl formamide were obtained from E. Merck, Darmstadt, Germany. All other stated chemicals are from Aldrich Chemical Co. (Milwaukee, WI). Four dextran standards from Polysciences, Inc. were used for GPC calibration.

2.2. Synthesis of chondroitin sulfate-methacrylate (CS-MA)

In general, chondroitin sulfate (1 g) was dissolved in the 50 ml double-distilled water. After complete dissolution, the various amount of methacrylic anhydride (MAA) was added dropwise into the CS solution. Then, the 5N NaOH solution was carefully added to control the reaction mixture to ~pH 8.0 (mol ratio of MAA/NaOH is 1/1.12). The reaction solution was stirred at room temperature for 2 h and then moved to refrigerator at 6 °C for another 24 h. The reaction mixture was precipitated in alcohol and the precipitate was filtered and washed with a large amount of alcohol several times until no methacrylic anhydride residue was traced by NMR. The result CS-MA precursor was dried in a vacuum oven at room temperature. In the synthesis of CS-MA, four parameters were tested: the amount of base, the amount of MAA, the reaction temperature, and the reaction time.

2.3. Preparation of CS-MA and CS-MA-AA hydrogels

Four different degrees of methacrylate substituted CS-MA precursors (1 g) were dissolved in 5 ml of double distilled water, respectively. After complete dissolution, 0.025 g of potassium persulfate was added as a redox initiator. The viscous solution was poured into two pieces of glass plates with a silicon spacer of 3 mm thickness. The plates were irradiated with a long wave UV lamp (UVP Model B-100A, Upland, CA, USA) for 30 min. The resulting hydrogels were dried under a vacuum hood for 1 d and a vacuum oven for another day at room temperature.

The synthesis of CS-MA-AA hydrogels was similar to that of CS-MA hydrogels except 1 g of acrylic acid was added simultaneously with potassium persulfate.

2.4. Characterization

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian, Germini-200 VT spectrometer in D_2O with tetramethylsilane (TMS) as the internal standard. FT-IR spectra were performed using a Perkin–Elmer System 2000 apparatus. Six–four scans were single averaged at a resolution of 4 cm^{-1}. Dried CS-MA precursors and their hydrogels were ground with KBr powder, and pressed into pellets for FT-IR measurement.

2.5. Solubility and swelling measurements

CS as well as CS-MA precursor (20 mg) was placed in various solvents (5 ml) and stirred for 24 h at room temperature for the solubility tests. Solvents included doubled distilled water, acetone, chloroform, dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), and *N*-methyl pyrrolidone (NMP).

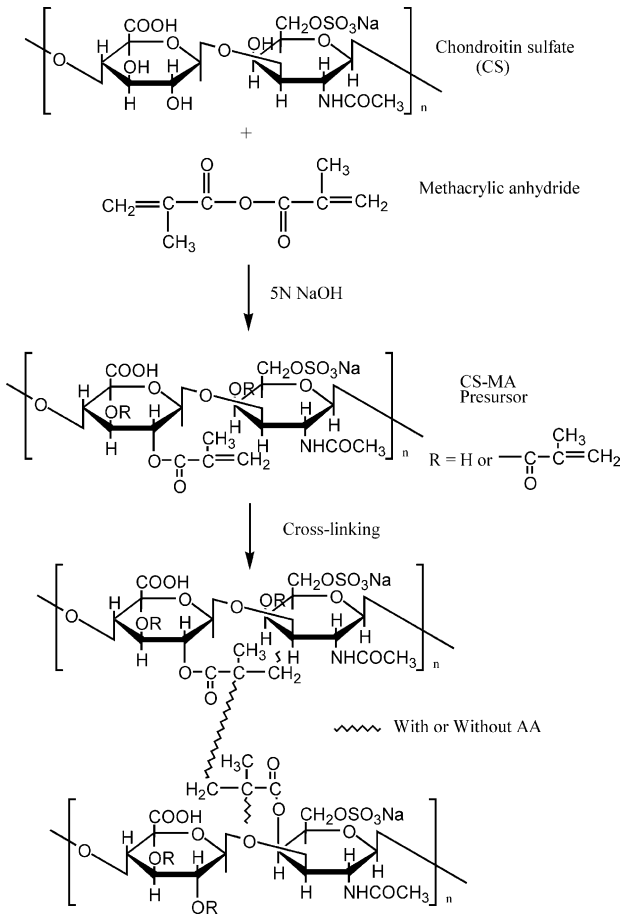
A piece of 10 × 10 mm square film (~200 mg) was swollen in doubled distilled water. At predetermined time intervals, the films were weighed after removal of excess surface liquid by light blotting with a laboratory tissue and returned to the buffer media until no additional weight gain was observed. The swelling percentage was expressed as follows.

$$\text{Swelling}\ (\%) = (W_s/W_d) \times 100 \quad (1)$$

Where W_s is the weight of the swollen sample and W_d is the weight of the dry unswollen sample. Each experiment was done in triplicate.

2.6. Gel permeation chromatography

The molecular weights of CS and CS-MA precursor were measured using a Water Model 501 pump equipped with a Shodex sugar KS-G, a KS-804 column, and a HP 1047 refractive index detector. An aqueous solution of 0.05 M NaCl was used as a mobile phase at a flow rate of 1 ml/min



Scheme 1. Synthesis CS-MA Precursor and its hydrogels.

at 50 °C The column setting was calibrated using four monodisperse dextran standards.

2.7. Thermogravimetric analysis (TGA)

The thermal stability of CS-MA and CS-MA-AA hydrogels was carried out with a Perkin–Elmer TGA-7 thermogravimetric analysis (TGA), scanning from 50 to 800 °C with a heating rate of 10 °C/min. The sample mass was in the range of 20–30 mg.

2.8. Scanning electron microscopy (SEM)

The film specimen before the hydration was washed with double distilled water and dehydrated by gradually increasing the concentration of absolute alcohol to 100% followed by critical drying (Hitachi HCP-2). A sample equilibrated in double distilled water for 24 h at room temperature, was taken off and frozen in liquid nitrogen immediately. The frozen specimen was freeze-dried to remove the imbibed water completely. The surface of the sample was observed using a JEOL-JSM5300 scanning electron microscope after coating with gold.

3. Results and discussion

3.1. Synthesis of CS-MA precursor

The synthesis of CS-MA precursor was illustrated in Scheme 1. The three hydroxyl groups on CS could nucleophilicly attack the carbonyl groups on methacrylic anhydride to form an ester bonds, giving off the methacrylic acid by-product. The reaction was driven to the right direction based on Le Châtelier's principle by neutralizing methacrylic acid with 5N NaOH. The products could be purified either by precipitation into alcohol as a non-solvent or by membrane dialysis against a large amount of double distilled water for several days to remove the methacrylic acid by-product. If the methacrylic acid residue was not removed cleanly, a peak corresponding to methyl groups of MAA would be seen at 1.6 ppm by NMR. The successful incorporation of MA on CS was confirmed by NMR and FTIR. As shown in Fig. 1, the characteristic ester absorption band at

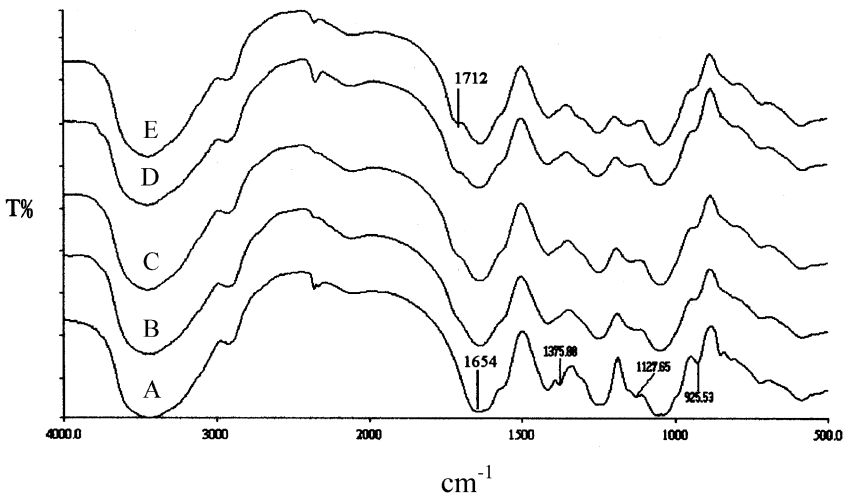


Fig. 1. FTIR spectra of (A) CS; (B) CS-MA-66; (C) CS-MA-81; (D) CS-MA-108 and (F) CS-MA-186.

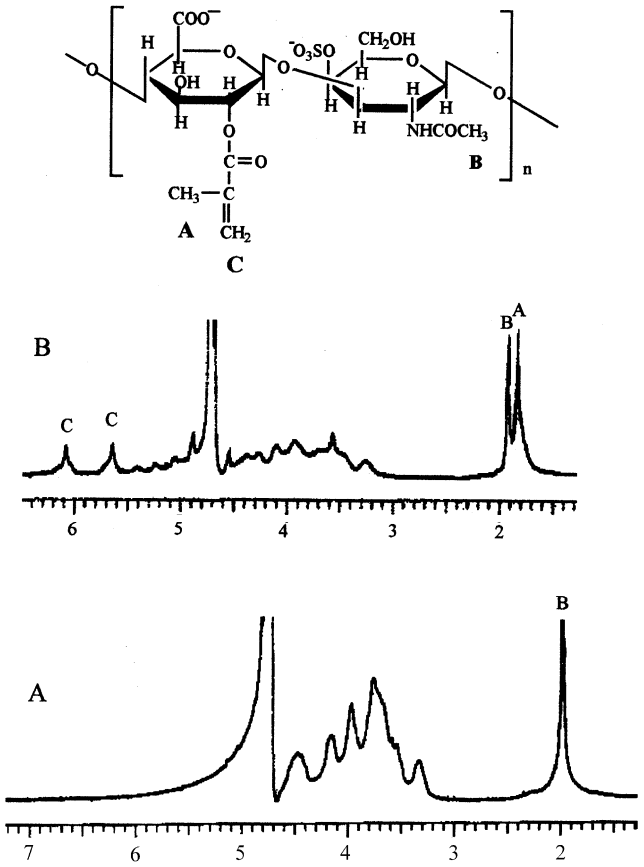


Fig. 2. ^{1}H-NMR spectra of (A) CS and (B) CS-MA-108.

1712 cm^{-1} appeared, and became clearly with an increase in the degree of substitution (DS) of the methacrylate groups on CS. The evidence of MA substitution on CS was observed by ^{1}H-NMR as shown in Fig. 2. Two distinctive peaks at 5.65 and 6.10 ppm were attributed to the two protons attached to the double bond (C=CH_2) and the peak shown at 1.84 ppm, ascribed to methyl groups adjacent to double bond (CH_3–C=CH_2), which are not present in the virgin chondroitin sulfate. The successful synthesis of CS-MA was further confirmed by ^{13}C-NMR. An additional carbonyl carbon of the ester bond and the carbon of the methyl substituent in methacrylate group are seen at 169 and 18 ppm. The carbons in the double bond (C=CH$_2$) are found at 127 and 135 ppm, respectively. The same distinctive peaks of CS and CS-MA are observed at 22.2 (N–CO–CH$_3$), 51, 61, 62, 67, 72, 73, 76, 80, 101, 103, 174 and 175 ppm (N–CO–CH_3 and COO$^-$), respectively.

The ^{1}H-NMR region from 1.6 to 2.1 ppm was expanded and the two methyl groups were deconvoluted and integrated. The peak intensity at 1.84 ppm to that at 1.93 ppm, corresponding to the methyl groups on original CS, was used to calculate the degree of MA substitution on CS Since there are three hydroxyl groups on CS, which could be substituted by methacrylate, thus, the maximum degree of substitution will be equal to 3.

Table 1
Effect of reaction time on the degree of substitution (DS) for the synthesis of methacrylate-substituted chondroitin sulfate

Hydroxyl group (mol)	Methacrylic anhydride (mol)	Reaction time (h)	Yield (%)	Degree of substitution (DS)
6.18×10^{-3}	0.114	5	68	0.35
6.18×10^{-3}	0.114	10	71	0.76
6.18×10^{-3}	0.114	20	73	0.98
6.18×10^{-3}	0.114	30	76	1.02

The reaction was stirred for 2 h at room temperature and stored at 6 °C for the time studied. The mol ratio of MAA/NaOH was controlled at 1/1.12.

Effect of reaction time: The reaction mixture was stirred for 2 h at room temperature and stored at 6 °C for several studied time. The mol ratio of MAA/NaOH was controlled at 1/1.12. The degree of substitution (DS) of the methacrylate on chondroitin sulfate was increased with the prolonged reaction time but the increase in the DS was much slower from 20 to 30 h (DS 0.98–1.02), as indicated in Table 1. Therefore, the 24 h was chosen for the future studies. The yield was slightly increases with the prolonged reaction time from 68 to 76%.

Effect of temperature: The effect of temperature on DS of methacrylate group on chondroitin sulfate by fixing the mol ratio of CS/MAA as 1/55.34 and MAA/NaOH as 1/1.12 for 24 h is represented in Table 2. As seen from this table, the highest DS value of 1.14 was obtained at 27 °C but considering the smell of MAA, we still chose 6 °C (DS = 1.08) for the future study. Because no refluxed condenser was used in this study, the vaporization of MAA might result in the lowest DS value at 75 °C. Kim and Chu (2000) have studied the effects of temperature and time on DS in dextran by methacrylate. They found that the DS of hydroxyl groups in dextran by methacrylate increased with an increase in the initial 20 h and not further increase DS for 30 h reaction time. The increase in the reaction time on increasing DS value in our aqueous reaction system was agree with their studies in organic solvent using triethylamine as a catalyst,

Table 2
Effect of reaction temperature on the degree of substitution (DS) for the synthesis of methacrylate-substituted chondroitin sulfate

Hydroxyl group (mol)	Methacrylic anhydride (mol)	Temperature (°C)	Yield (%)	Degree of substitution (DS)
6.18×10^{-3}	0.114	6	75	1.08
6.18×10^{-3}	0.114	27	78	1.14
6.18×10^{-3}	0.114	50	74	1.04
6.18×10^{-3}	0.114	75	69	0.77

The reaction was stirred for 2 h at room temperature and stored for 24 h at the temperature studied. The mol ratio of MAA/NaOH was controlled at 1/1.12.

Table 3
Effect of methacrylic anhydride concentration to the hydroxyl group of chondroitin sulfate on the degree of substitution (DS) for the synthesis of methacrylate-substituted CS

Hydroxyl group (mol)	Methacrylic anhydride (mol)	Yield (%)	Degree of substitution (DS)	Abbreviation
6.18×10^{-3}	0.038	80	0.66	CS-MA-66
6.18×10^{-3}	0.076	81	0.81	CS-MA-81
6.18×10^{-3}	0.114	75	1.08	CS-MA-108
6.18×10^{-3}	0.152	71	1.86	CS-MA-186

The reaction was stirred for 2 h at room temperature and stored for 24 h. at 6 °C. The mol ratio of MAA/NaOH was controlled at 1/1.12.

However, the temperature effect on DS in the aqueous solution was not as promising as the organic system, where the DS of the hydroxyl groups in dextran by methacrylate increased with the increasing temperature profoundly.

Effect of the amount of methacrylic anhydride: At constant condition of MAA/NaOH of 1/1.12 for 24 h reaction time at 6 °C, the effect of the amount of MAA on DS in chondroitin sulfate by methacrylate is shown in Table 3. The DS was increased from 0.66 to 1.86 when the amount of MAA was increased four times at constant concentration of CS. The increase in DS goes slightly linear with the increase in the MAA amount.

Effect of the amount of NaOH base: The effect of base on DS value of methacrylate on CS was summarized in Table 4, when the other parameters were fixed at mol ratio of CS/MAA = 1/32 for 2 h at room temperature and 24 h at 6 °C. The DS value increased with the increasing amount of NaOH and the highest DS value of 0.88 was obtained at NaOH/MAA mol ratio of 1.12/1. As the mol ratio of NaOH to MAA increased to 1.45/1, the DS value was decreased to 0.76. The failure of the substitution of hydroxyl groups by methacrylate was obtained when an additional increasing the NaOH amount to 0.15 mol. Since the NaOH solution was added after the complete addition of MAA in the reaction, the role of NaOH was to neutralize the methacrylic acid by-product and brought

Table 4
Effect of sodium hydroxide concentration on the degree of substitution (DS) for the synthesis of methacrylate-substituted chondroitin sulfate

Hydroxyl Group (mol)	Methacrylic anhydride (mol)	NaOH (mol)	Degree of substitution (DS)
6.18×10^{-3}	0.067	0.025	0.46
6.18×10^{-3}	0.067	0.050	0.54
6.18×10^{-3}	0.067	0.075	0.88
6.18×10^{-3}	0.067	0.100	0.76
6.18×10^{-3}	0.067	0.150	–

The reaction was stirred for 2 h at room temperature and stored for 24 h at 6 °C.

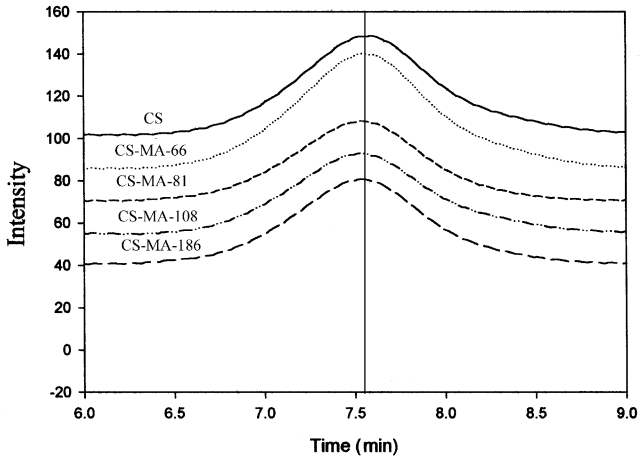


Fig. 3. GPC chromatograms of CS and CS-MA precursors.

the reaction to the right direction by Le Châtelier's principle. However, it is well known that the base can catalyze the cleavage the glucuronic ether linkage as well as the ester bonds in the chemical structure. It seems that both catalytic phenomena occurred in this system when too much amount of NaOH was used. The cleavage of the glucuronic ether linkage was confirmed by GPC. As shown in Fig. 3, the different degree of substation of methacrylate on CS altered the molecular weight insignificantly (The number-averaged molecular weight of virgin CS is 58,000). Fig. 4 indicated that the GPC chromatograms of CS and the product collected from the reaction of CS and MAA with 0.15 mol of NaOH as the fixed parameters as stated in Table 4. It is clearly seen that the GPC chromatogram moved to the longer elution time. The number-averaged molecular weight of CS under this condition decreased to 44,000 calculated by using four dextran standards. Therefore, the optimum condition to obtain the highest DS of methacrylate on CS was using the mol ratio of NaOH/MAA around 1.12/1.

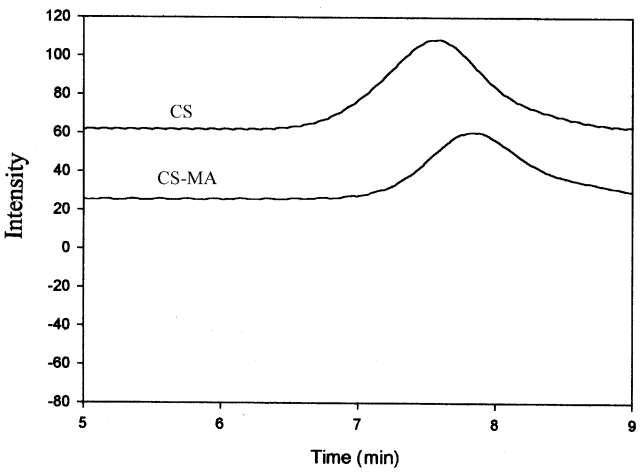


Fig. 4. GPC chromatograms of CS and CS-MA precursor prepared from 0.15 mol NaOH and 0.67 mol MAA and 2.06×10^{-3} mol CS.

Table 5
Solubility of CS-methacrylate in various solvents

Solvent	Sample				
	CS	CS-MA-66	CS-MA-81	CS-MA-108	CS-MA-186
Water	+	+	+	+	+
Acetone	−	−	−	−	−
Chloroform	−	−	−	−	−
Dimethyl formamide	−	+	+	+	+
Dimethyl sulfoxide	−	+	+	+	+
Tetrahydrofuran	−	+	+	+	+
N-methyl-2-pyrrolidone	+	−	−	−	−

+ : Dissolve at room temperature; − : do not dissolve at room temperature.

3.2. Solubility of CS-MA precursors

The natural CS is readily water-soluble and limits its application on drug-sustained release. Thus, the solubility was tested. The solubility of the CS-MA precursor in organic solvents was improved drastically in comparison with the natural CS, as shown in Table 5. Chondroitin sulfate only dissolved in water. CS-MA precursors did not dissolved in acetone, chloroform and NMP but dissolved in highly polar organic solvents like DMF, DMSO, THF and water at room temperature. The effect of degree of substitution on solubility was trivial.

3.3. Synthesis of CS-MA and CS-MA-AA hydrogels

The degrees of methacrylate substitution on CS equal to 0.66, 0.81, 1.08, and 1.86, were used to prepare CS-MA hydrogels with a use of potassium persulfate as a redox initiator. Although the gelation occurred rapidly upon a long-wave UV irradiation, however, the completely nice films were not obtainable after swelling the hydrogels. This might be due to the steric hindrance of bulk carboxylic and sulfate groups on polysaccharide, which hindered the chain polymerization between the double bonds in the substituted methacrylate groups and resulted in the low degree of the cross-linking density. The FTIR spectra of CS-MA hydrogels are similar to those of CS-MA precursors. An example of CS-MA-P 81, representing the hydrogel prepared from 0.81 DS of methacrylate on CS, and CS-MA-81 were shown in Fig. 5, where the characteristic peak of ester bands appeared at 1712 cm^{-1} and that of amide bonds showed at 1654 cm^{-1}.

In order to have the pH sensitive properties as well as the nice film property, the acrylic acid (AA) was introduced to copolymerize with methacrylate groups. The merits of this AA are: (1) to extend the distance as a bridge between two polysaccharides, and (2) to perform as a pH sensitive hydrogel due to the functional carboxylic acid groups. The synthesis of CS-MA-AA hydrogels was similar to that of CS-MA hydrogels except 1 g of acrylic acid was added simultaneously with potassium persulfate. All FTIR spectra are identical independent of the DS value of methacrylate on CS. The CS-MA-AA-81 was exhibited in Fig. 5, where the absorption peak at 1712 cm^{-1} of carboxyl groups in pure

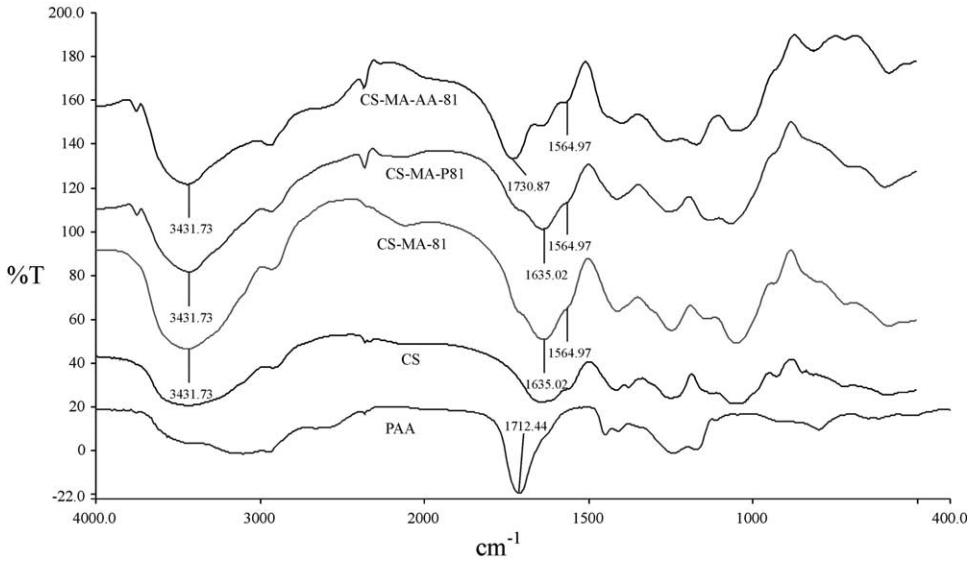


Fig. 5. FTIR spectra of PAA, CS, CS-MA-81, CS-MA-P81, and CS-MA-AA-81.

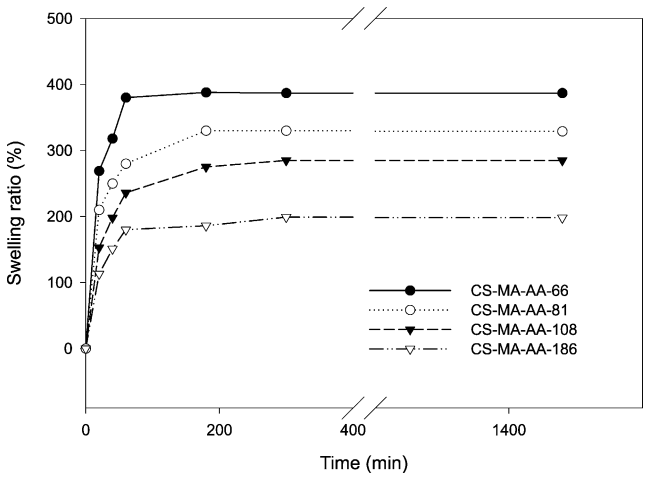


Fig. 6. Swelling profiles of CS-MA-AA hydrogels.

PAA was shifted to 1731 cm^{-1} and the amide peaks showed at 1654 cm^{-1} in CS previously, was shifted to 1635 cm^{-1}. This shifts implied that the intermolecular or intramolecular H-bonding among carboxylic groups in PAA would be disrupted by amide functional groups in CS. The new H-bonding was formed between carboxylic groups in PAA, as hydrogen donors, and the amide groups in CS, as hydrogen acceptors. The hydrogen bonding interaction between CS and PAA was stronger than that in PAA itself. This brought the amide absorption band to the lower frequency and carbonyl absorption frequency to the higher frequency (the more free carbonyl groups were released in PAA).

The swelling profiles of four different hydrogels, having DS from 0.66 to 1.86 in pure water were investigated. The swelling response of these four hydrogels was quick and similar (~1 h). Swelling ratio depended much on the DS value of methacrylate. The higher DS value resulted in the lower degree of swelling ratio as shown in Fig. 6. The equilibrium swelling ratios in 24 h were 3.9, 3.4, 2.8 and 2.0 corresponding to CS-MA-AA-66, CS-MA-AA-81, CS-MA-AA-108, and CS-MA-AA-186, respectively. The surface morphologies of CS-MA-AA-108 before and after swelling were observed by SEM. As shown in Fig. 7, the unswollen surface was quite smooth while the swollen hydrogel showed a porous structure. This porous structure implied the potential application of CS-MA-AA hydrogel in drug controlled release.

3.4. Thermal stability

To pursue whether the thermal stability improved after modification of CS, the thermogravimetric analysis (TGA) was carried out dynamically (weight loss vs. temperature). The weight of CS dramatically lost around 263 °C and gradually decreased thereafter, as indicated in Fig. 8. The weight loss of CS-MA hydrogels showed two degradation stages; one was around 230 °C and the other was around 550 °C. The first degradation temperatures of CS-MA

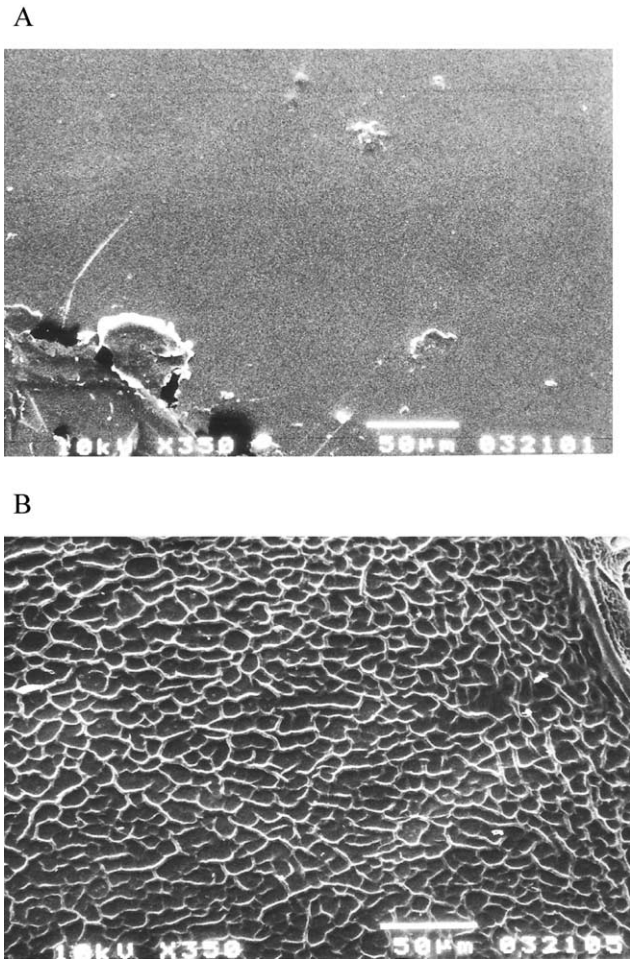


Fig. 7. SEM photographs of (A) unswollen and (B) swollen CS-MA-AA-108 hydrogel.

hydrogels were lower than the natural CS, although the weigh loss amounts were smaller in CS-MA hydrogels. It still implied that the thermal stability was not improved after incorporation of the methacrylate substitution of hydroxyl groups on CS. The effect of DS of methacrylate on CS on the thermal stability was insignificant. Furthermore,

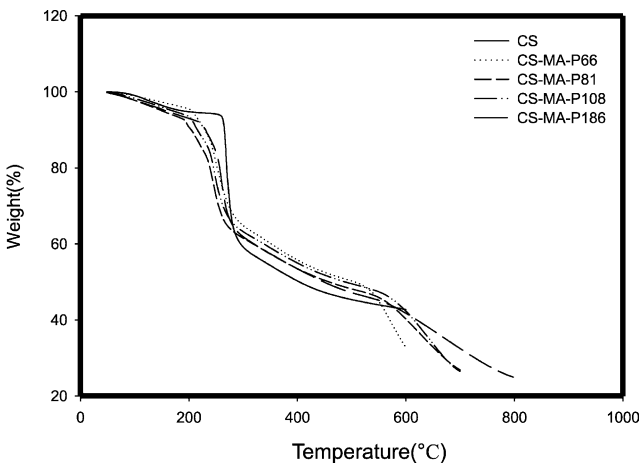


Fig. 8. TGA thermograms of CS-MA hydrogels.

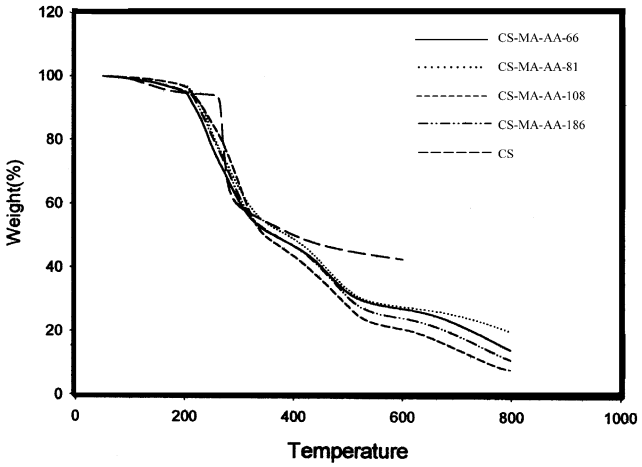


Fig. 9. TGA thermograms of CS-MA-AA hydrogels.

the TGA thermograms of CS-MA-AA hydrogels after incorporating AA as a bridge unit between two methacrylate groups, was shown in Fig. 9. The degradation pattern exhibited three stages, the first occurred around 219–245 °C; the second was in the range of 421–428 °C and the last was around 586–626 °C. It seems that the thermal stability was worse and the degradation mechanism became more complicated after introduction acrylic acid in the hydrogels. Blanco-Fuente, Anguiano-Igea, Otero-Espinar, and Blanco-Méndez (1996) stated that Caropol™ (polymers derived from polyacrylic acid) showed three endothermic peaks by DSC (differential scanning calorimeter) and DTG (derivative thermogravimetric analysis), which were ascribed to be water loss below 70 °C, anhydride formation within 75–170 °C, and decarboxylation above 170 °C, respectively. The unstableness of PAA might cause the easier degradation of CS-MA-AA hydrogels. Moreover, the increase in the DS value (suppose to be higher cross-linking density) did not improve the thermal stability as we expected.

4. Conclusions

The methacrylate (MA) substitution on chondroitin sulfate (CS) could be prepared by a simple reaction of chondroitin sulfate with methacrylic anhydride (MAA) in the presence of sodium hydroxide (NaOH) as a base. The various degree of substitution of MA on CS could be controlled by four parameters: reaction time, reaction temperature, MAA concentration, and NaOH amount. The solubility in the common organic solvents was enhanced while independent of the DS value of methacrylate. The better hydrogels were obtained by incorporating acrylic acid as a counter monomer. The degree of swelling showed a fast response and a large dependence on the DS of methacrylate on CS. CS-MA-AA hydrogels remained as a nice film shape in the swelling study and showed a range of swelling from 200 to 390%. The increase in degree of MA substitution resulted in the decrease in swelling ratio. The thermal stability of CS-MA hydrogels did not show any improvement and became worse for CS-MA-AA hydrogels, compared to the virgin CS. The surface morphology conducted by SEM exhibited a porous structure after hydration. This is a merit for an application in the drug-controlled release.

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