

Short Communication

Solubility, viscosity and rheological properties of water-soluble chitosan derivatives

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Received: 10 June 2011 / Accepted: 6 August 2012 / Published: 17 August 2012

Abstract: An investigation and comparison of solubility, viscosity and rheological properties under neutral, acidic and alkaline conditions of water-soluble chitosan derivatives, viz. *O*-carboxymethyl chitosan, *N,O*-carboxymethyl chitosan, *N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride and *O*-carboxymethyl-*N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride, was undertaken.

Keywords: chitosan, chitosan derivatives, *O*-carboxymethyl chitosan, *N,O*-carboxymethyl chitosan, *N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride, quaternised carboxymethyl chitosan, *O*-carboxymethyl-*N*-[(2-hydroxy-3-trimethyl ammonium) propyl] chitosan chloride, rheological properties

INTRODUCTION

Chitosan (CS) is a polysaccharide similar in structure to cellulose. It is a polycationic copolymer consisting of glucosamine and *N*-acetylglucosamine units. CS, commonly obtained by partial deacetylation of chitin derived from the exoskeleton of crustaceans [1], exhibits various useful physico-chemical properties such as film forming ability [2], gelation characteristics [3] and bioadhesion properties [4]. Moreover, it is biodegradable and biocompatible with low toxicity [5]. For these reasons, it has received considerable attention as a potential pharmaceutical and cosmetic excipient.

However, although CS is a very useful polymer with low toxicity, its limited solubility particularly at a physiological pH is a major obstacle for pharmaceutical and cosmetic applications. CS is a weak base with a pK_a value of 6.2-7.0 [6] due to the D-glucosamine residue, which leads to its insolubility at neutral and alkaline pH. CS dissolves in water at pH lower than 6.5, at which a substantial fraction of its amino groups are ionised. It is generally soluble in acidic solutions such as those of acetic acid, lactic acid and dilute hydrochloric acid [7]. Various chemical modifications have been used to improve its solubility at neutral and alkaline pH. CS has three reactive groups, i.e. a primary hydroxyl group at C-6 and a secondary hydroxyl group at C-3 on each repeated unit, and an amino group at C-2 on each deacetylated unit (Figure1). These reactive groups have been subjected to chemical modifications, for example reaction with glycidyl trimethylammonium chloride [8-10], carboxymethylation [11-12] and sulphonation [13-14]. The results of many studies have demonstrated that these chemical modifications can improve the solubility of CS in water over a wide range of pH. However, the rheological properties of the chemically modified CSs have not yet been fully reported. Thus, in this study we undertook to prepare four kinds of water-soluble CS derivatives, namely *O*-carboxymethyl chitosan (*O*-CMC), *N,O*-carboxymethyl chitosan (*N,O*-CMC), *N*-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (HTPC) and a carboxymethyl derivative of HTPC (*O*-CM-HTPC), using a specific kind of CS with a specified molecular weight and degree of deacetylation. Subsequently, the rheological properties of the water-soluble CS derivatives in aqueous solutions at different pH were investigated.

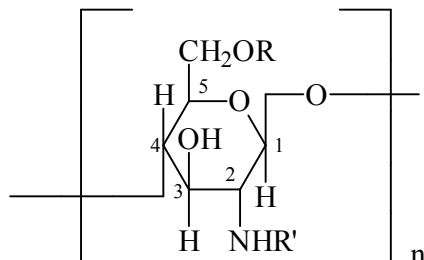


Figure 1. Structures of CS and CS derivatives: CS (R, R' = H); *O*-CMC (R = CH₂COOH, R' = H); *N,O*-CMC (R, R' = CH₂COOH); HTPC (R=H, R' = CH₂CH(OH)CH₂N⁺(CH₃)₃ Cl⁻); *O*-CM-HTPC (R = CH₂COOH, R' = CH₂CH(OH)CH₂N⁺(CH₃)₃ Cl⁻)

PREPARATION OF CS DERIVATIVES AND DETERMINATION OF DEGREE OF SUBSTITUTION

Shrimp-shell-derived CS, purchased from Seafreshchitosan Co. Ltd (Thailand), was used as starting material for the preparation of CS derivatives. Its molecular weight was about 350 kDa and the degree of deacetylation was about 84% according to the company's product information. All other chemicals used were of analytical grade.

O-CMC was prepared according to the method of Zhu et al [12]. The preparation of *N,O*-CMC was carried out according to the procedure reported by Hayes [15]. HTPC was prepared by a simple method as described by Spinelli et al [9], and *O*-CM-HTPC was prepared by the method as described by Sun et al [10]. All products were characterised by Fourier-transform infrared (FTIR)

spectra and ^1H -nuclear magnetic resonance (^1H -NMR) spectra. The FTIR spectra of *O*-CMC, *N*, *O*-CMC, HTPC and *O*-CM-HTPC matched those reported by Sun et al [10]. The ^1H -NMR spectra of all products were identical to those found in the literature [8, 10, 16].

The degree of carboxymethyl group substitution (DS_{CM}) in *O*-CMC and *N*,*O*-CMC was determined by potentiometric titration using a calomel electrode as reference electrode and a glass electrode for the measurement, as described by Ge and Luo [11]. A sample (0.2 g) was dissolved in distilled water (40 ml) and the solution was adjusted to $\text{pH} < 2$ by adding 0.1N HCl. Then the solution was titrated with 0.1N NaOH and the pH value of the solution was simultaneously recorded. The amount of aqueous NaOH consumed was determined by second-order differential method. The degree of substitution was calculated as follows:

$$\text{DS}_{\text{CM}} = \frac{161 \times A}{W - 58 \times A}$$

$$A = V_{\text{NaOH}} \times C_{\text{NaOH}}$$

where V_{NaOH} and C_{NaOH} are the volume and molarity of NaOH solution respectively, W is the weight of sample (g), and 161 and 58 are the molecular weights of glucosamine (CS skeletal unit) and carboxymethyl group respectively.

The degree of substitution of (2-hydroxy-3-trimethylammonium)propyl group (DS_{HTP}) was determined by potentiometry. The chloride ions of HTPC and *O*-CM-HTPC were potentiometrically titrated with aqueous silver nitrate using a calomel electrode as reference electrode and a silver electrode for the measurement. The method was described by Sun et al [10]. A sample (0.05 g) was dissolved in distilled water (100 ml) and the solution was titrated with 0.03N AgNO_3 , the mV value of the solution being simultaneously recorded. The amount of aqueous AgNO_3 consumed was determined by second-order differential method. The degree of substitution was calculated as follows:

$$\text{DS}_{\text{HTP}} = \frac{A}{A + \frac{W - A \times 314.5}{161}}$$

$$A = \frac{V_{\text{AgNO}_3} \times C_{\text{AgNO}_3}}{1000}$$

where V_{AgNO_3} and C_{AgNO_3} are the volume and molarity of AgNO_3 solution respectively, W is the weight of sample (g), and 161 and 314.5 are the molecular weights of glucosamine (CS skeletal unit) and (2-hydroxy-3-trimethylammonium)propyl chloride group respectively. The degrees of substitution (DS_{CM} and DS_{HTP}) and appearance of CS and CS derivatives are summarised in Table 1.

PHYSICAL PROPERTIES

Solubility

To estimate the solubility of a CS derivative, 100.0 mg of sample were suspended in 10.0 mL of a solvent (distilled water, 0.1N HCl or 0.1N NaOH) and the suspension was stirred at 25°C for 5 hr [17]. Then the mixture was filtered through filter paper to retain the undissolved portion, which was then washed with acetone and dried at 50°C overnight [18]. The total weight of sample was

subtracted by the weight of the insoluble portion to obtain the weight of the soluble portion. The solubility of samples was expressed as g/100 mL.

Table 1. DS_{CM} , DS_{HTP} and appearance of CS and CS derivatives

Sample	$DS_{CM}^{a,b}$	$DS_{HTP}^{a,c}$	Colour
CS	-	-	yellow
<i>O</i> -CMC	0.88 ± 0.16	-	light yellow
<i>N,O</i> -CMC	1.53 ± 0.22	-	dark yellow
HTPC	-	0.40 ± 0.24	light yellow
<i>O</i> -CM-HTPC	0.87 ± 0.22	0.37 ± 0.17	white

^a average \pm SD; ^b DS_{CM} for complete reaction is 1 for *O*-CMC and *O*-CM-HTPC, and 2 for *N,O*-CMC; ^c DS_{HTP} for complete reaction is 1 for HTPC and *O*-CM-HTPC.

As shown in Table 2, all CS derivatives showed sufficient solubility in all solvents. Interestingly, the difference in solubility of all CS derivatives in each solvent was slight, ranging between 90.0-95.5 % in 0.1N HCl, 82.6-84.2 % in distilled water and 31.6-37.6 % in 0.1N NaOH, which shows that the type and number of modification group does not seem to affect the solubility of CS derivatives.

Table 2. Solubility and apparent viscosity of CS and water-soluble CS derivatives under acidic, neutral and alkaline conditions at 25°C

Sample	0.1N HCl		Distilled water		0.1N NaOH	
	solubility (%w/v)	viscosity (mPas)	solubility (%w/v)	viscosity (mPas)	solubility (%w/v)	viscosity (mPas)
CS	72.5 ± 0.5	5.37 ± 2.10	-	-	-	-
<i>O</i> -CMC	90.3 ± 0.5	12.10 ± 3.32	82.6 ± 0.4	25.83 ± 4.93	35.8 ± 1.0	18.54 ± 4.05
<i>N,O</i> -CMC	92.4 ± 0.4	10.57 ± 2.28	84.2 ± 0.3	22.83 ± 4.44	33.8 ± 0.4	14.28 ± 2.65
HTPC	90.0 ± 0.1	3.23 ± 1.18	84.1 ± 0.7	3.01 ± 1.18	31.6 ± 0.5	1.30 ± 0.64
<i>O</i> -CM-HTPC	95.5 ± 0.5	4.08 ± 1.31	83.9 ± 0.3	4.05 ± 1.75	37.6 ± 0.6	2.64 ± 1.31

Note: Values are average \pm SD.

Viscosity and Rheological Property

The rheological property of a 1% (w/v) CS derivative in distilled water, 0.1N NaOH or 0.1N HCl was investigated using a Bob-and-Cup format rotational rheometer (R/S Rheometer, Brookfield Viscometer LTD., England). The measuring system was CC48 and the mode used was CSR (controlled shear rate). The measurement was done in two stages: (1) during an increase of shear rate from 0 to 1,000 s^{-1} in 3 min., and (2) during a decrease of shear rate from 1,000 to 0 s^{-1} in 3 min. All measurements were performed in triplicate at a controlled temperature of $25 \pm 1^\circ\text{C}$. The data were analysed with Brookfield Rheo 2000 software. Apparent viscosity (Table 2), expressed in mPas, was an average of the ratio of shear stress to shear rate calculated from both the upward and downward curves of the rheogram.

Rheograms of the CS derivatives are shown in Figure 2. *O*-CMC and *N,O*-CMC showed higher apparent viscosity in 0.1N HCl than that of CS, and the apparent viscosity of *O*-CMC was slightly higher than that of *N,O*-CMC in all pH conditions studied. These observations were most likely due to the increasing hydrogen bonding and steric factor and the interplay between the two phenomena. In contrast, the cationic HTPC and *O*-CM-HTPC showed a remarkably lower viscosity than CS in 0.1N HCl and hence were less useful as a viscosity inducing agent. It should be noted that the apparent viscosity of *O*-CMC and *N,O*-CMC in water was significantly higher than in 0.1N HCl and 0.1N NaOH. The decrease in viscosity under alkaline condition seemed to be due to a screening effect of the counter ions (Na^+), which limits swelling at pH 9-13 [19].

O-CMC and *N,O*-CMC under acidic condition showed a Newtonian rheological property while under neutral and alkaline conditions they showed a distinct pseudoplastic property and a thixotropic hysteresis loop respectively. CS, HTPC and *O*-CM-HTPC on the other hand exhibited a Newtonian fluid property in all conditions. The shear stress of *O*-CMC in water at low shear rate ($<100 \text{ sec}^{-1}$) was remarkably greater than that of *N,O*-CMC, The smaller thixotropic hysteresis area in alkaline condition compared to that in neutral condition again may be explained by the screening effect of the counter ions (Na^+) [19].

A polymer solution with pseudoplastic property and high apparent viscosity is suitable for certain pharmaceutical formulations such as suspensions and gel formulations. Thus, *O*-CMC and *N,O*-CMC may be preferably used as a viscosity inducing agent.

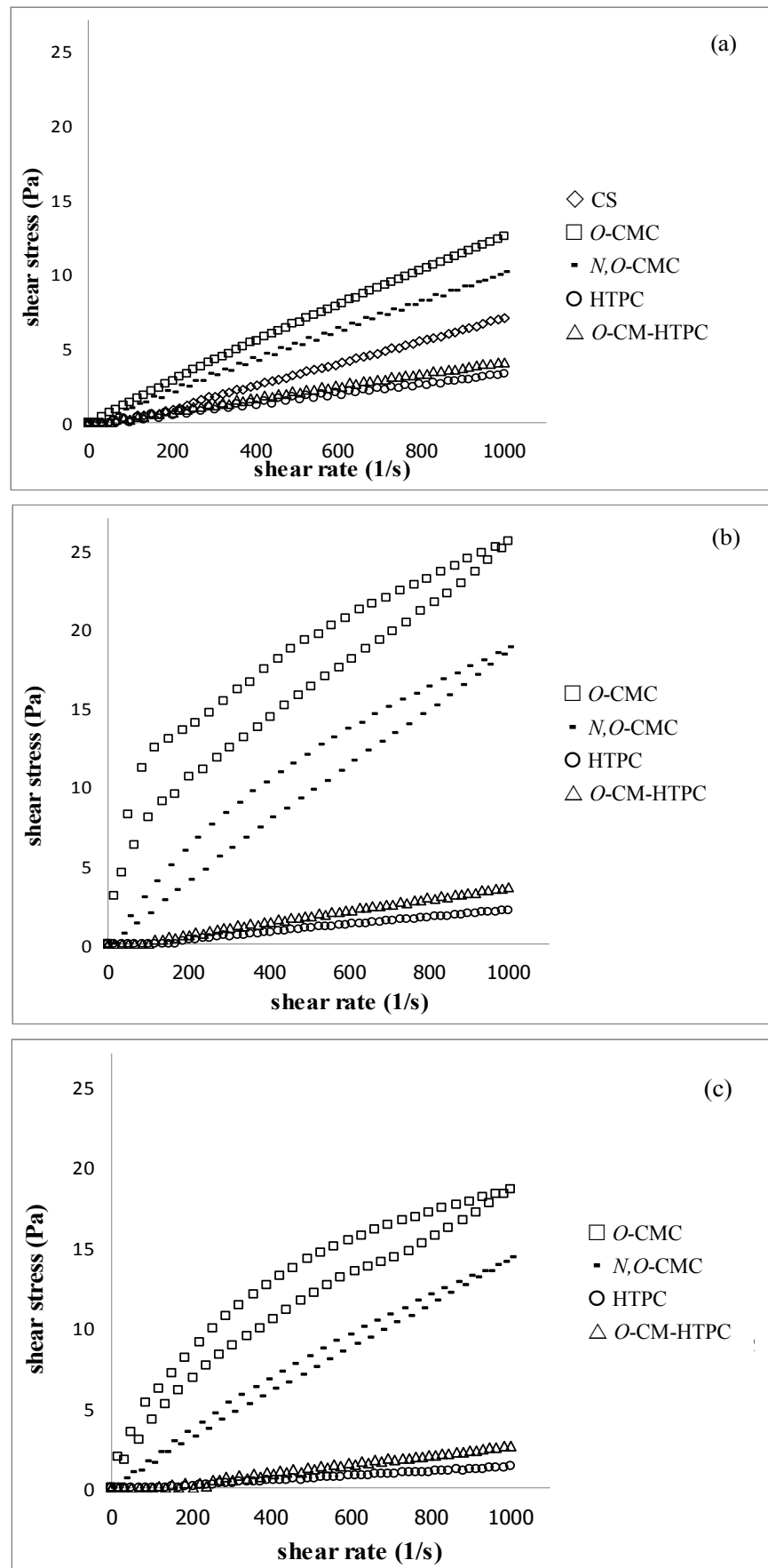


Figure 2. Rheograms of CS derivatives in: (a) 0.1N HCl; (b) distilled water; (c) 0.1N NaOH at 25°C

ACKNOWLEDGEMENTS

We thank the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University for providing all facilities and the Graduate School, Chiang Mai University for financial support. The Department of Chemistry, Maejo University was acknowledged for providing a potentiometer. We are grateful to Dr. Yohji Ezure for his valuable comments and proofreading of the manuscript.

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