

A New Approach to Chemically Modified Carboxymethyl Chitosan and Study of its Moisture-Absorption and Moisture-Retention Abilities

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ABSTRACT: A new approach to prepare chemically modified carboxymethyl chitosan (CM) derivatives was reported, from which initially CM was prepared from chitosan, then *N*-quaternary ammonium group was introduced by the reaction of CM with 2,3-epoxypropyl trimethylammonium. The structures of the derivatives were characterized by FTIR, XRD, ¹³C-NMR, and gel permeation chromatography. The capability of moisture-absorption and moisture-retention was investigated. It was found that the moisture-absorption and moisture-retention ability of the new derivatives quaternized carboxymethyl chitosan (CMQ) are higher than not only that of CM but also that of chitosan quaternary (Q) and carboxymethyl group and quaternary

ammonium group are in synergistic effect. Relationships between molecular structures (including degree of substitution of carboxymethylation group, degree of substitution of quaternary group, and molar mass) and functions of CMQ were also studied. The moisture absorption kinetics of CMQ was discussed and the diffusion of water molecules in it looks likely to be non-Fickian type. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 102: 1303–1309, 2006

Key words: quaternized carboxymethyl chitosan; structure characterization; moisture-absorption and -retention; structure–function relationship; moisture absorption kinetics

INTRODUCTION

Chitosan, originated from chitin, the second most abundant natural biopolymer,¹ has attracted more and more researchers because of its multiple bioactivities.^{2–4}

Hyaluronic acid (HA), which belongs to glycosaminoglycans, is unique for its moisture-retention ability and plays a key role in cosmetics and clinical medicine. Although HA is ubiquitous in plants and animals, the total amount is limited, and the price is very high.⁵

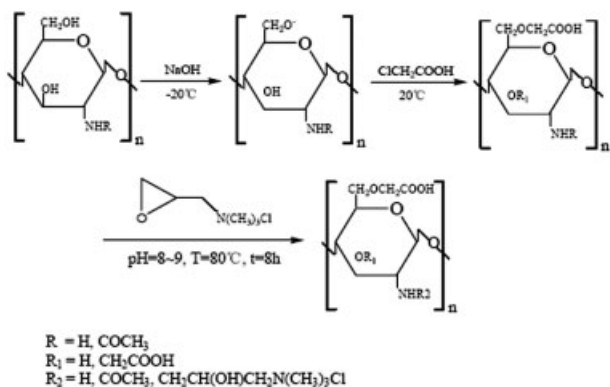
As the fundamental chemical structure of chitin is close to that of HA, the moisture-absorption and retention properties of several kinds of chitin derivatives were examined and compared with those of HA by

Shuichi et al. in 1989.⁶ Carboxymethyl chitosan (CM) appears to be more suitable than other derivatives such as hydroxyethyl chitin and carboxypropenoyl chitin for preparing HA-like substances. Muzzarelli⁷ also pointed out that 0.25% aqueous solution of *N*-carboxymethylated chitosan and carboxymethylated chitin were comparable to a 20% aqueous solution of propylene glycol in terms of moisture-retention ability and the viscosities of the solutions containing minor amounts of *N*-carboxymethylated chitosan and carboxymethylated chitin were higher than those of other moisture-retention agents and almost equal to that of HA. Our previous research reported that CM with DD value of 50%, DS value 0.6–1.0 and molar mass higher than 2.48×10^5 Da has the potential to substitute for HA to use as moisture-retention agent.^{8,9} In this paper, our interest has been focused on the interaction and effect of carboxymethyl group and quaternary ammonium group contemporaneously introduced onto chitosan molecular chain. Amphoteric copolymers, containing both anionic and cationic groups along the macromolecular backbone, have a similar structure as biopolymer (protein, nucleic acid, etc.)¹⁰ and superior biocompatibility. It has attracted much attention in the biomedical, cosmetic, pharmacological, agricultural

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Scheme 1 Synthesis of CM and CMQ.

and biotechnological fields during the past decades.^{11–14} Novel amphoteric polymer based on chitosan by introducing carboxymethyl group and quaternary ammonium group along chitosan molecular chain has been scarcely studied so far. This report deals with the preparation of chemically modified CM and their moisture-absorption and -retention abilities and the relationships between molecular structures and functions. The moisture absorption kinetics was also simply discussed. The preparation of CM and its quaternized derivatives is presented as Scheme 1.

EXPERIMENTAL

Materials

Chitin was supplied by Yuhuan Aoxing Biochemistry Co. Ltd. in Zhejiang province in China, with a deacetylation degree of 87% (determined by elemental analysis¹⁵) and the molar mass calculated from the GPC method was about 4.12×10^5 Da. Standard pullulans for GPC were purchased from Showa Denko, Tokyo, Japan; 2,3-epoxypropyl trimethylammonium chloride was prepared in the lab; All other chemicals were of reagent grade and were used without further purification as received.

Methods

FTIR spectra were recorded with KBr pellets on a Nicolet FT-IR 360 spectrophotometer. Sixteen scans at a resolution of 4 cm^{-1} were averaged and referenced against air.

X-ray diffraction patterns of the degraded chitosan fractions were measured by a Shimadzu Lab XRD-6000 diffractometer, which used a Cu K α target at 40 kV and 50 mA at 20°C. The relative intensity was recorded in the scattering range (2θ) of 5–40°.

¹³C NMR spectra were recorded on a varian mercury Vx300 spectrometer and chemical shifts were

given by taking methanol as reference for ¹³C-NMR in D₂O at 323 K.

Weight-average molar mass (M_w) of samples were measured by GPC. The GPC equipment consisted of connected columns (TSK G5000-PW and TSK G3000-PW), TSP P100 pump and RI 150 differential refractometer and Jiangshen Workstation. Each sample was dissolved in 0.1 mol/L aq NaCl which was the eluent, then filtered through 0.45 μm Millipore filters. The flow rate was maintained at 1.0 mL/min. The sample concentration was 0.4 mg/mL. The M_w was calculated by the following equation:

$$\log(M_w) = -0.4383V_e + 8.9236 \quad (1)$$

DS of carboxymethyl group of each sample was estimated from potentiometric titration.¹³ Samples were dissolved in 0.1 mol/L hydrochloric acid (50 mL) in the presence of 0.1 mol/L sodium chloride and titrated with 0.1 mol/L sodium hydroxide. The alkali-metric curves were recorded on a DELTA-320-S pH meter.

The degree of substitution of the quaternization group was determined by the potentiometry.¹⁶ Potentiometric titration of the chloride ion on Q and CMQ was carried out with the aqueous silver nitrate, using a calomel electrode as the reference, and a silver electrode for the measurement. DS is calculated as follows:

$$DS = \frac{\frac{C_i \dot{A}V}{1000}}{\frac{C_i \dot{A}V}{1000} + \frac{WE - \frac{C_i \dot{A}V \dot{A}M_2}{1000}}{M_1}}$$

where C (mol L⁻¹) is the concentration of AgNO₃ solution, V (mL) is the volume of AgNO₃ solution, W (g) is the weight of quaternary chitosan, M_1 (mol g⁻¹) is the molar mass of glucosamine and M_2 (mol g⁻¹) is the molar mass of quaternary chitosan.

Preparation of chitosan derivatives

Carboxymethyl chitosan

Chitosan (10 g) suspended in NaOH (15 mL) was kept –20°C overnight. The frozen alkali chitosan was transferred to 2-propanol (100 mL) and ClCH₂CO₂H was added in portions. Stirred at room temperature for 4 h and HOAC was added to the mixture to adjust the pH to 7.0. The carboxymethyl chitosan (CM) salt was filtered and washed with EtOH. After dialyzing against deionized water for 4 days, the product was vacuum-dried at room temperature. By changing the alkali concentration, a series of CM with various degrees of substitution was prepared.

TABLE I
Carboxymethyl Chitosan (CM), Quaternary Chitosan (Q), CMQ of Different Degrees of Substitution

Sample code	NaOH Concentration (%)	Mole ratio of functional reagent to glucosamine units	DS of CM	DS of Q
CM ₃	50		0.73	
CM ₁	30		0.45	
CM ₂	40		0.56	
CM ₄	60		0.86	
Q ₁		3 : 1		0.78
Q ₂		2 : 1		0.59
Q ₃		1 : 1		0.32
CM ₃ Q ₁	50	3 : 1	0.73	0.78
CM ₃ Q ₂	50	2 : 1	0.73	0.59
CM ₃ Q ₃	50	1 : 1	0.73	0.32
CM ₁ Q ₂	30	2 : 1	0.45	0.59
CM ₂ Q ₂	40	2 : 1	0.56	0.59
CM ₄ Q ₂	60	2 : 1	0.86	0.59

Quaternization of carboxymethyl chitosan

The quaternization of CM was conducted as follows. Concentrated hydrochloric acid (0.1 mol) was dropped into the solution of trimethylamine (0.1 mol) at 4°C to avoid gasification of trimethylamine in the latter reaction. When it was stirred for about 10 min, the resulting solution was added to epoxy chloropropane (0.086 mol) at 31°C. After homogenization, the mixing solution was heated to 51°C, and was then trickled slowly by aqueous NaOH (0.1 mol) solution to maintain trimethylamine hydrochloride to be slowly decomposing into trimethylamine, which easily react with epoxy chloropropane. The addition of NaOH was performed within 1–1.5 h, after another 2 h of stirring, the reaction mixture was refined by vacuum distillation at 50°C.

CM (5 g) was dissolved in 20 mL distilled water and 2,3-epoxypropyl trimethylammonium chloride was added with different mole ratio to glucosamine unit. The mixture was reacted at 80°C for 8 h with stirring then dialyzed for 4 days and finally lyophilized to give CMQ as a yellow powder.

Degradation of quaternized carboxymethyl chitosan (CMQ)

CMQ powder (5 g) was suspended in 250 mL deionized water, after stirring at 40°C for 2 h, hydroperoxide of a desired volume was added for predetermined time to yield CMQ of various molar mass according to the method provided by Muzzarelli and Giacomelli¹⁷

Moisture absorption and retention test¹⁸

Prior to the moisture-absorption testing, the samples were dried over P₂O₅ *in vacuo* for 24 h. The water-

absorption ability was evaluated by the percentage of weight increase of dry sample (R_a):

$$R_a(\%) = 100 \times (W_n - W_0) / W_0 \quad (3)$$

W_0 and W_n are the weights of sample before and after putting it into a saturated (NH₄)₂SO₄ desiccator (81% relative humidity) and in a saturated K₂CO₃ desiccator (RH 43%) at 20°C after 48 h of the test.

In the moisture-retention test, wet samples were prepared by adding 10% water to each sample. The moisture-retention ability was evaluated by the percentage of residual water of wet sample (R_h):

$$R_h(\%) = 100 \times (H_n / H_0) \quad (4)$$

H_n and H_0 are the weights water in sample before and after putting in the silica gel at 20°C after 48 h of the test.

RESULTS AND DISCUSSION

DS and molar mass

A series of carboxymethylation and quaternization reactions were conducted to get samples with various degrees of substitution of carboxymethyl group and quaternary group. As shown in Table I, alkali concentration was the most important factors to regulate carboxymethylation of chitosan. Work by Tokura and coworkers demonstrated that the DS value of CM-chitosan increased with NaOH concentration changing from 20 to 40%.¹⁹ And in the present work, when NaOH concentration increased from 40 to 60%, the DS value increased from 0.56 to 0.86. At lower NaOH concentration, the rigid crystalline structure of chitosan was difficult to disrupt to ensure penetration of

TABLE II
Reaction Conditions and Molar Mass of Degraded CMQ

Sample code	Reagent	Conc. (%)	Volume (mL)	Time (h)	T (°C)	M_w ($\times 10^5$)
CM ₃ Q ₂ -1			Untreated			4.72
CM ₃ Q ₂ -2	H ₂ O ₂	30	1	0.5	40	2.28
CM ₃ Q ₂ -3	H ₂ O ₂	30	2	1.5	50	0.45
CM ₃ Q ₂ -4	H ₂ O ₂	30	3	6	50	0.11

the ClCH₂CO₂H into the interlocking polymer chains.²⁰

To obtain quaternary chitosan derivatives with different DS, several conditions were tried. It was found that the DS was affected by the ratio of 2,3-epoxypropyltrimethyl ammonium chloride to chitosan. This result was consistent with Xu et al.²¹ Under optimal conditions, Q and CMQ with different DS were obtained. The characteristics of them are listed in Table I.

M_w of degraded samples determined by GPC are shown in Table II, together with reaction conditions. The molar mass of samples studied varies from 4.72×10^5 Da to 1.1×10^4 Da. Degradation products are white, free-flowing powders.

FTIR spectra of the CM and CMQ

Figure 1 presents the FTIR spectra of CS, CM, and CMQ. The absorption bands at 1655, 1593, 1323, 1381 cm^{-1} in the spectrum of CS assigned to amides I, II, III, and —CH₃ vibration bands respectively.²² Two strong peaks at 1605 cm^{-1} and 1419 cm^{-1} (in CM spectrum) and 1603 cm^{-1} and 1415 cm^{-1} (in CMQ spectrum) were observed because of the asymmetrical and symmetrical stretching of COO— group.²³ In the spectrum of CM, the C—O stretching band at 1030 cm^{-1} corresponding to the primary hydroxyl group disappears,

corroborating a high carboxymethylation of OH-6. The characteristic peak of second hydroxyl group at 1080 cm^{-1} was not changed. Compared with CS, CMQ showed the disappearance of the NH₂-associate band at 1593 cm^{-1} , which can be ascribed to the characteristic peak of primary amine N—H vibration deformation and appearance of a new peak at 1480 cm^{-1} , which was attributed to the methyl groups of the ammonium. It was indicative of the attachment of N-(2-hydroxy, 3-trimethylammonio)-propyl at chitosan—NH₂ group. The IR spectrum was in agreement with previously reported spectra.^{21,24}

X-ray analysis of the CM and CMQ

The X-ray diffractograms of CS, CM, and CMQ are shown in Figure 2. It could be seen that there were some differences of peak height, width, and position between them. CS consisted of two major peaks at $2\theta = 12$ and 21 , while CM exhibited two characteristic peaks at $2\theta = 11$ and 20 . The X-ray diffraction pattern of CS and CM coincided with the pattern of the L-2 polymorph of shrimp chitosan reported by Sato and Kim.^{25,26} Compared with CS, which showed relatively narrow peak at $2\theta = 20$ with height 490, CM had a relatively broader peak at $2\theta = 20$ with height 330 and the peak at $2\theta = 12$ significantly weakened. In diffraction spectrum of CMQ the peak at $2\theta = 12$ even disap-

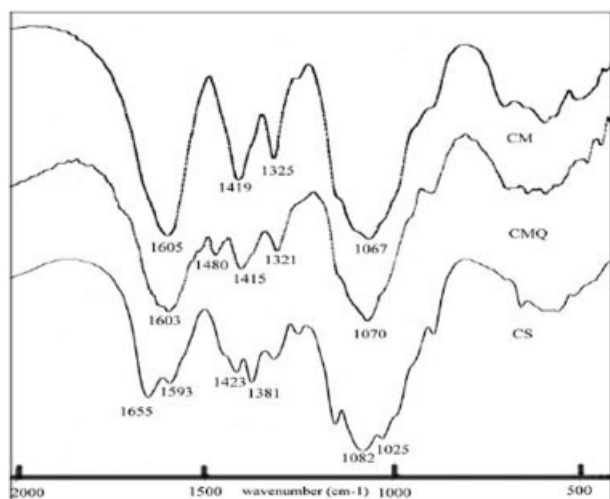


Figure 1 FTIR spectra of CS, CM, and CMQ.

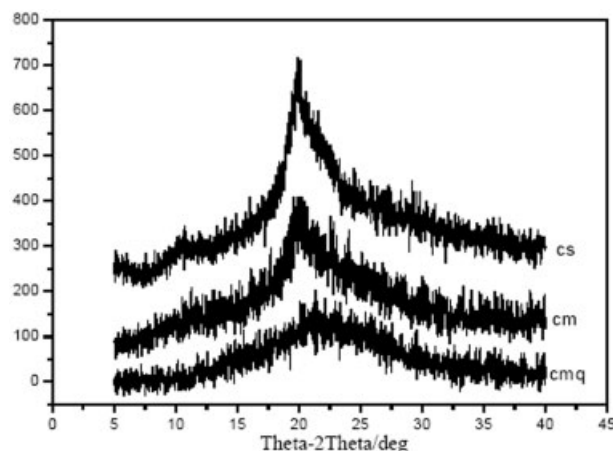


Figure 2 X-ray diffraction patterns of CS, CM, and CMQ.

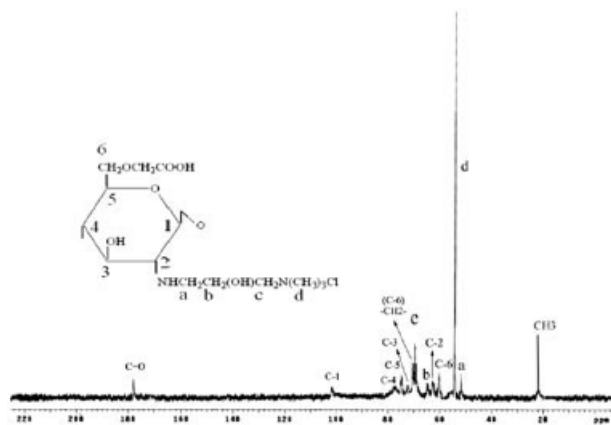


Figure 3 ^{13}C -NMR spectrum of CMQ.

peared and the peak at $2\theta = 20$ became even broader with height 201 and it became amorphous. It is well-known that the width of X-ray diffraction peak is related to the size of crystallite, the broadened peak usually results from small crystallites.²⁷ Hence in this reaction the chitosan in the amorphous region was carboxymethylated first and with further quaterization, the crystalline structure was destroyed and the crystallinity disappeared.

^{13}C NMR of the CMQ

NMR method is the most effective technique to determine the structure of chitosan derivatives.²⁸ Figure 3 depicts the ^{13}C -NMR spectrum of CMQ. The peak at $\delta = 54.3$ ppm was attributed to the carbons of the trimethylammonium group (C-d). The signal for $-\text{COOH}$ substituted on $-\text{OH}$ is obvious at 178 ppm. The peaks at $\delta = 101.5, 62.6, 72.4, 77.9, 74.9,$ and 60.8 ppm were attributed to the C-1, C-2, C-3, C-4, C-5, and C-6 respectively. The peaks at $\delta = 51.7$ and 68.7 ppm were attributed to C-a and C-c respectively. The peak appearing at 64.4 ppm was attributed to C-b. The peaks at $\delta = 22.5$ ppm was attributed to the carbons of the residual CH_3 acetyl. The results were in agreement with Loubaki et al.²⁴ and Rinaudo et al.²⁹

Moisture-absorption and -retention properties

Figure 4 and Figure 5 show that the moisture-absorption and -retention properties of CMQ, CM, and Q are quite similar to those of HA. The weight of moisture absorbed increased rapidly in the first stage, slowed down in the later stage, then became relatively constant 24 h later. The moisture-absorption and -retention ability of CMQ are higher than that of CM and Q, which indicated that during the water-absorption process, carboxymethyl group and quaternary ammonium group are in synergistic effect. The intermolecular hydrogen bonds of molecular chains, which affect

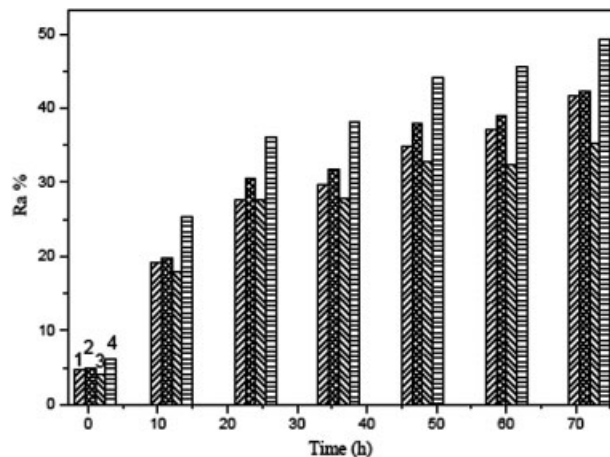


Figure 4 Moisture-absorption ability of dry samples at 81% relative humidity at 20°C : (1: HA, 2: CM, 3: Q, and 4: QCM).

the interaction between water and polymer chains directly, may be a very important factor to regulate moisture-absorption and -retention ability of CMQ. On the other hand, this ability of CMQ is also higher than that of HA. It indicated that amphoteric chitosan has the potential to substitute HA to use in cosmetics and clinical medicine.

Moisture absorption kinetics

The absorption mechanism of samples was determined using the following equation.

$$(W_t - W_0) / W_0 = Kt^n \quad (5)$$

In the above equation, W_t and W_0 are the weights of the wet and dry samples at time t , respectively, t is the time, K is the moisture-absorption constant, and n is

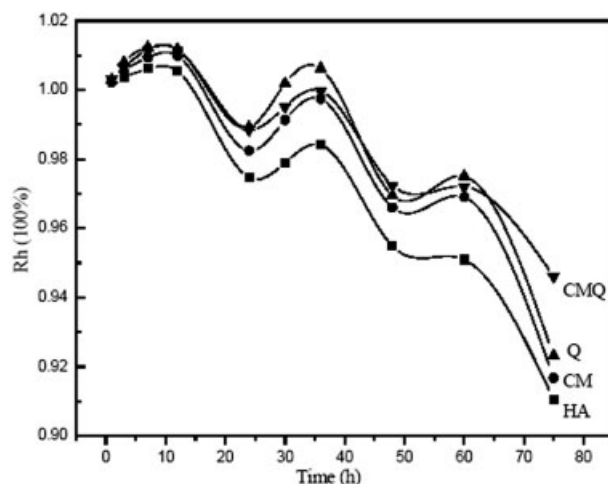


Figure 5 Moisture-retention ability of wet samples at 43% relative humidity at 20°C .

TABLE III
Moisture-Absorption Exponents, Constants of CMQ
and Comparisons

Sample	n	K	R
HA	0.5413	5.00	0.9981
CMQ	0.5609	6.31	0.9986
CM	0.5273	4.80	0.9995
Q	0.5338	4.14	0.9938

the moisture-absorption exponent. In Jannari's report,³⁰ $n = 0.45-0.50$ and corresponds to Fichian diffusion whereas $0.50 < n < 1.0$ indicates the diffusion is non-Fichian type. The moisture-absorption constant K and the moisture-absorption exponent n were calculated and listed in Table III, where it is shown that the moisture-absorption exponent range generally between 0.5 and 1.0. In the experiments, the number to determine type of diffusion (n) was found to be over 0.50. Hence the diffusion of water into samples systems had a non-Fickian character.

Structure–functional relationships

Figure 6 demonstrated the effect of DS of quaternary ammonium group on the moisture-absorption ability. It indicated that as the carboxymethyl group is given, the degree of substitute (DS) of quaternary ammonium group along the chitosan chain is of less contribution to the moisture-absorption ability.

Figure 7 showed the effect of DS of carboxymethyl group on the moisture-absorption ability. From Figure 7 we can see that as the quaternary ammonium group is given, with an increase in DS of carboxymethyl group, moisture-absorption ability of samples increased accordingly.

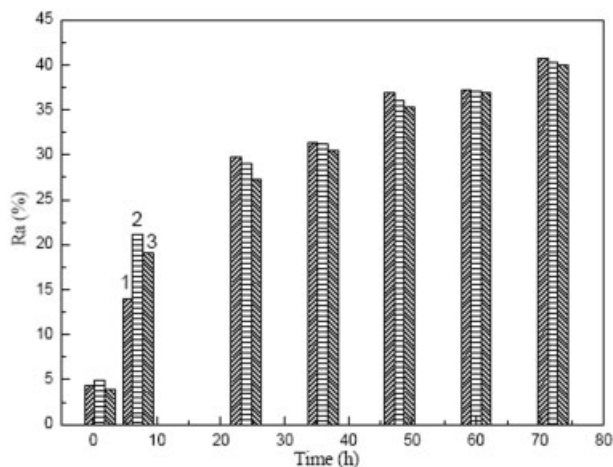


Figure 6 Effect of DS of quaternary ammonium group on the moisture-absorption ability at 81% relative humidity at 20°C: (1: CM_3Q_1 , 2: CM_3Q_2 , and 3: CM_3Q_3).

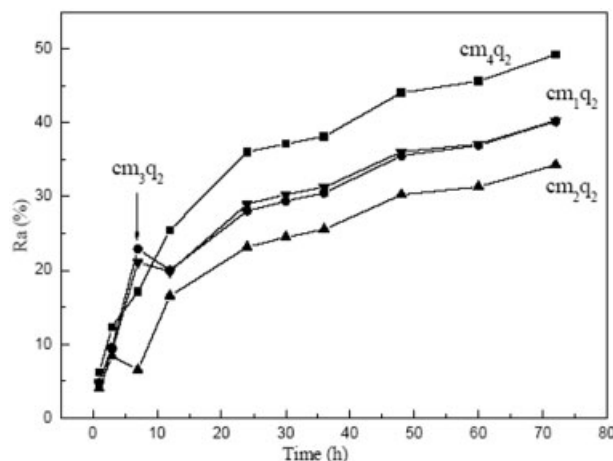


Figure 7 Effect of DS of carboxymethyl group on the moisture-absorption ability at 81% relative humidity at 20°C.

Under high relative humidity conditions (RH 81 and 43%), water molecules are accessible to the surface of the sugar residues and readily develop hydrogen-bond interactions with the CMQ molecular chains. As a result, they intervene between chains or chain sheets in the network. We can conclude that introduction of carboxymethyl group is a convenient and effective method to entitle chitosan high water-retention ability. It is considered that 6-carboxymethyl group in the molecular structure of chitosan is a main active site. Quaternization at N position also contribute to the moisture-absorption ability. A high degree of substitution of carboxymethylation group helps to improve moisture-absorption ability.

Figure 8 and Figure 9 showed the effect of molar mass on the moisture-absorption and -retention ability. As shown in Figure 8, when RH was 81%, CMQ

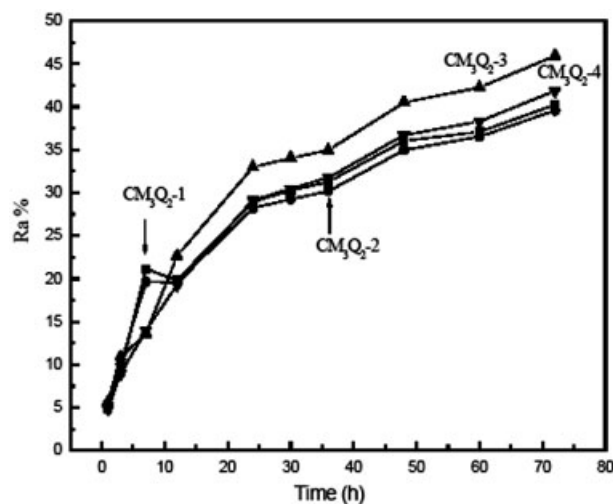


Figure 8 Effect of molar mass on the moisture-absorption ability at 81% relative humidity at 20°C.

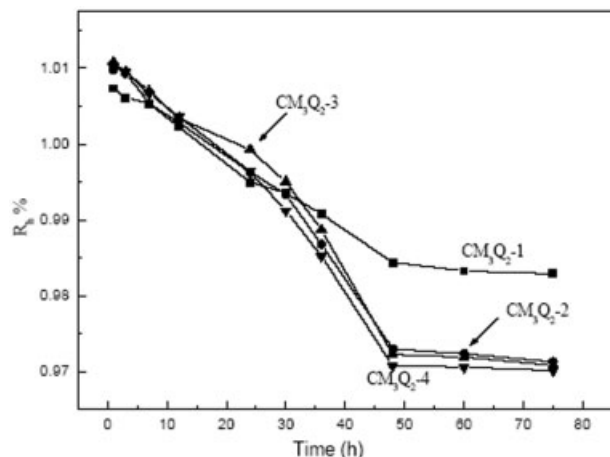


Figure 9 Effect of molar mass on the moisture-retention ability at 43% relative humidity at 20°C

with molar mass lower than 4.5×10^4 demonstrated very high moisture-absorption ability. The weight of water absorbed increased with time in the whole process of the test. All samples absorbed moisture rapidly in the first 12 h. And in Figure 9, when RH was 43%, they released water slowly in the latter hours in varying degrees and became constant after 48 h of the test. It is clear that molar mass plays an important role in moisture-absorption and -retention ability. CMQ with molar mass above 4.72×10^5 forms networks easily, which helps to improve moisture-retention ability, because they prevent water from going out. However, in high humidity environment (RH = 81%), CMQ with low molar mass dissolved in water so rapidly that they became solutions after 30 h of the test. In such processes, they absorbed a large amount of water. Thus they have very high moisture-absorption ability.

CONCLUSIONS

It is considered that CMQ has good moisture-absorption and -retention abilities. Carboxymethyl group is the major and active group and the quaternary ammonium group also contributes to the moisture-absorption and -retention abilities. Carboxymethyl group and quaternary ammonium group are in syn-

ergistic effect. The moisture absorption kinetics of CMQ demonstrated that the diffusion of water molecular in it looks likely to be non-Fickian type. Furthermore these novel amphoteric chitosan derivatives present wide outlook because of the antimicrobials activity of quaternary ammonium group to use in cosmetic and medical fields.

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