



## Synthesis and moisture absorption and retention activities of a carboxymethyl and a quaternary ammonium derivative of $\alpha,\alpha$ -trehalose

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### ABSTRACT

Carboxymethyl  $\alpha,\alpha$ -trehalose (CMT) and a quaternary ammonium derivative of  $\alpha,\alpha$ -trehalose (QT) were successfully prepared, and their moisture absorption and retention activities were assessed. Results showed that both CMT and QT had better moisture absorption abilities at 43% and 81% relative humidity (RH) than  $\alpha,\alpha$ -trehalose. In addition, the two  $\alpha,\alpha$ -trehalose derivatives had better moisture retention abilities than  $\alpha,\alpha$ -trehalose under three humidity conditions: 81% RH, 43% RH, and under dry conditions. Therefore, carboxymethylation and quaternarization could improve the moisture absorption and retention abilities of  $\alpha,\alpha$ -trehalose. CMT and QT showed better moisture absorption ability and moisture retention ability than that of hyaluronan (HA), and could potentially find a use as moisture retention ingredient, for example, in cosmetics.

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## 1. Introduction

Hyaluronan (HA), an important functional ingredient in cosmetics, is unique because of its excellent moisture retention ability, but the total amount is limited, and the cost of HA is high.<sup>1,2</sup>  $\alpha,\alpha$ -Trehalose ( $\alpha$ -D-glucopyranosyl  $\alpha$ -D-glucopyranoside) is a stable, colorless, odor-free and non-reducing disaccharide that is widespread in nature. It functions in many organisms as an energy source or a protectant against the effects of freezing or dehydration. It also possesses physical and/or chemical properties that are different than other sugars, which may make  $\alpha,\alpha$ -trehalose an attractive ingredient in food, health, and in pharmaceutical products.<sup>3–5</sup>  $\alpha,\alpha$ -Trehalose is much cheaper than HA, and it has the potential for use as a moisturizer in cosmetic preparations. However, it cannot replace HA as the moisture absorption and retention ingredient in cosmetics because of its lower moisture absorption–retention properties.

Modification of a carbohydrate, for example, carboxymethylation and quaternization, can improve its moisture absorption and retention ability.<sup>6,7</sup> Therefore, we prepared carboxymethyl  $\alpha,\alpha$ -trehalose (CMT) and the quaternary ammonium salt of  $\alpha,\alpha$ -trehalose (QT) in this study, aiming to improve the moisture absorption and retention abilities of the disaccharide.

## 2. Experimental

### 2.1. Materials

$\alpha,\alpha$ -Trehalose (99% purity) was purchased from Guangxi Nanning Javelly Biological Products Co., Ltd (China). HA (95% purity) was purchased from Shandong Freda Biochem. Co., Ltd (China). The other reagents were of analytical grade.

### 2.2. Synthesis of CMT and QT

CMT was prepared as follows:  $\alpha,\alpha$ -trehalose (3 g, 7.9 mmol) was dispersed into 30 mL of 2-propanol at room temperature for 20 min. A gross amount of 60 mmol NaOH was then added. After 40 min, a total amount of 3.6 g (38.1 mmol) chloroacetic acid was added in five portions at intervals of 5 min. The reaction was carried out with stirring at 40 °C for 3 h. The product was filtered and washed three times with MeOH, and then it was dried in vacuum.

QT was prepared as follows (Scheme 1):  $\alpha,\alpha$ -trehalose (3 g, 7.9 mmol) was dispersed into 30 mL of 2-propanol at room temperature, and 0.75 g NaCl (5%, 12.8 mmol) was added. NaOH (10 mol/L) was then added with stirring to pH 11. 2,3-Epoxypropyltrimethyl ammonium chloride (EPTMAC) (23.88 mL) was used as the etherification reagent. The mass fraction of EPTMAC used was 69%, which was dissolved in water. The reaction was carried out with stirring at 40 °C for 7 h. The mixture was

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**Table 1**

Yields, elemental analyses, results, and degrees of substitution (DS) of  $\alpha,\alpha$ -trehalose, CMT, and QT

Compounds	Yield (%)	Elemental analysis			DS
		C	H	N	
Trehalose	—	42.1	6.43	—	—
CMT	94.8	38.71	4.98	—	83.3
QT	96	44.5	7.68	4.09	89.7

then neutralized, and the product was filtered and washed three times with MeOH, and dried in vacuum. For synthesis pathway of CMT and QT see Scheme 1. For yields, analytical data and degree of substitution see Table 1.

### 2.3. Characterization of CMT and QT

IR spectra were measured on a Nicolet Magne-Avatar 360 instrument using KBr disks. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Apx 500 (500-MHz) NMR spectrometer in  $\text{D}_2\text{O}$  solvent. The elemental analyses (C, H, and N) were performed on a Carlo-Erba 1106 elemental analyzer.

### 2.4. Measurement of moisture absorption properties

Each sample was ground into a powder and dried in an oven at  $100^\circ\text{C}$  for 4 h. Samples (0.5 g) were put into desiccators that contained water saturated with  $(\text{NH}_4)_2\text{SO}_4$  (81% relative humidity, RH) and  $\text{Na}_2\text{CO}_3$  (43% RH) at room temperature, respectively. The moisture absorption ability ( $R_a$ ) was evaluated by the percentage increase of weight:

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

$W_0$  and  $W_n$  were the weights of sample before and after they were put into the desiccators, respectively. Samples were consecutively tested at different time points.

### 2.5. Measurement of moisture retention properties

Samples (0.5 g) were ground into powders in a mortar and pestle, and dried in an oven at  $100^\circ\text{C}$  for 4 h. Water (0.05 g) was added to each sample. Samples were then put into desiccators that contained water saturated with silica gel,  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{Na}_2\text{CO}_3$ , respectively. The moisture retention ability ( $R_h$ ) was evaluated by the percentage of residual water in the wet sample:

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

$H_0$  and  $H_n$  were the weights of water in the sample before and after they were put into the desiccators, respectively. Samples were consecutively tested at different time points.

## 3. Results and discussion

The IR spectra of  $\alpha,\alpha$ -trehalose, CMT and QT are shown in Figure 1. As shown in Figure 1, the IR spectrum of CMT shows new

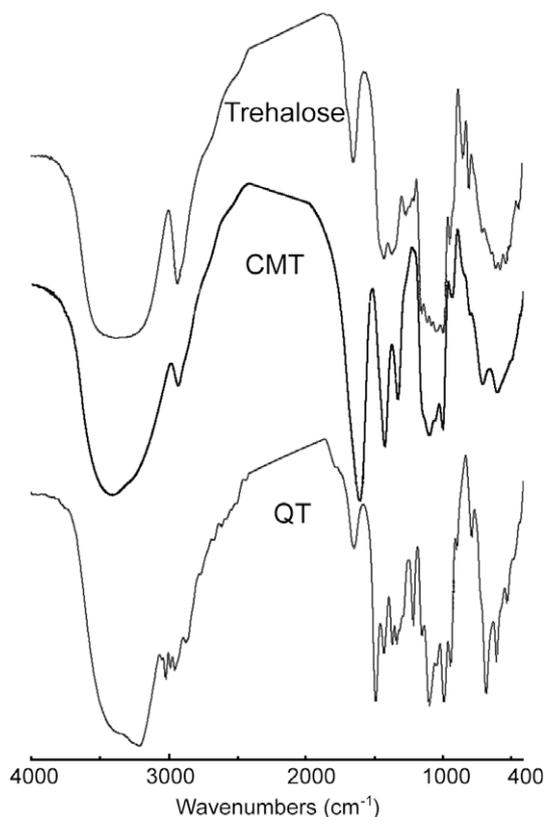
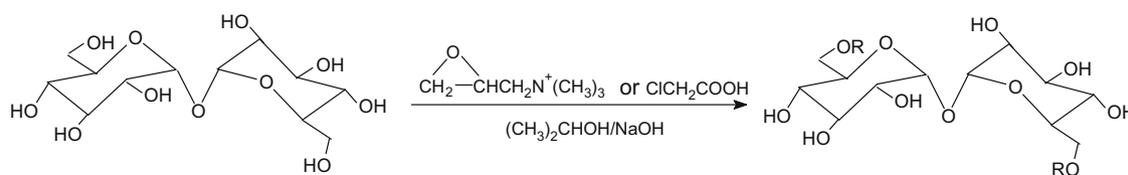


Figure 1. IR spectra of  $\alpha,\alpha$ -trehalose, CMT, and QT.

peaks at  $1602\text{ cm}^{-1}$  and  $1422\text{ cm}^{-1}$ , which are assigned to  $-\text{COONa}$  and  $-\text{CH}_2-\text{COONa}$  groups, respectively. The strong absorption at  $1095\text{ cm}^{-1}$  was assigned to the C–O–C group. There were new peaks of QT at  $1482\text{ cm}^{-1}$  and  $1360\text{ cm}^{-1}$ , which were assigned to the characteristic absorption of the methyl groups ( $\text{CH}_3-\text{N}$ ) of the ammonium groups. The strong absorptions at  $1094\text{ cm}^{-1}$  was assigned to the C–O–C group. In the molecule of CMT and QT, the absorption of the primary hydroxyl at  $1038\text{ cm}^{-1}$  disappeared, indicating that the carboxymethyl and ammonium groups were mainly substituted at the C-6 position. The  $^{13}\text{C}$  NMR spectra of  $\alpha,\alpha$ -trehalose, CMT and QT in  $\text{D}_2\text{O}$  are shown in Figure 2. In the molecule of CMT, a new peak observed at  $49.0\text{ ppm}$  was assigned to  $-\text{OCH}_2$ , and that at  $178.5$  and  $179.9\text{ ppm}$  were assigned to the carbonyl group ( $\text{C}=\text{O}$ ). The two chemical shifts at  $93.5$  and  $60.2\text{ ppm}$  were assigned to  $-\text{CH}_2\text{COOH}$  groups substituted on C-1 and C-6, indicating that there were two possible sites for the carboxymethylation of  $\alpha,\alpha$ -trehalose. The signal intensity also suggested that C-6 was the major site for formation of CMT. There was a new peak at  $54.4\text{ ppm}$  in the  $^{13}\text{C}$  NMR spectrum of QT, which was assigned to the carbons of the trimethylammonium group ( $-\text{N}(\text{CH}_3)_3$ ). The peaks at  $46.9$  and  $65.5\text{ ppm}$  were attributed to  $-\text{OCH}_2$  and  $\text{N}-\text{CH}_2$ . At the same time, two chemical shifts at  $93.2$



Scheme 1. Synthetic pathway for CMT and QT.

and 60.6 ppm were assigned to  $-\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{N}^+(\text{CH}_3)_3$  groups substituted on C-1 and C-6, and the signal intensity suggested that C-6 was the major site for the formation of QT. These IR and  $^{13}\text{C}$  NMR results serve to establish the structure of CMT and QT.

The moisture absorption properties of CMT and QT are shown in Figure 3. At both 43% and 81% RH, the  $R_a$  (%) of all the samples increased gradually at first, and then leveled off at 240 h. At both 43% and 81% RH,  $R_a$  of  $\alpha,\alpha$ -trehalose was 20.3% and 31.6% at 408 h, and  $R_a$  of HA was 30.7% and 50.5%. After modification, CMT showed better moisture absorption ability than that of either  $\alpha,\alpha$ -trehalose or HA, which were 95.9% and 127.6%, respectively. QT had the same tendency. When the RH was 43%, the moisture absorption index was 82.6% at 408 h, and when the RH was 81%, the  $R_a$  was 99.3%.

The moisture retention properties of CMT and QT are shown in Figure 4. At both 43% and 81% RH, the  $R_h$  (%) of the derivatives increased rapidly before 64.5 h, and then leveled off. At 43% RH, the  $R_h$  of  $\alpha,\alpha$ -trehalose was 80% and 95.8% at the time point of 16 h and 76.5 h, respectively, while the  $R_h$  of CMT was 282% and 777.2%, respectively. The moisture indexes of QT were 306.4% and 552.2%, respectively, at the two time points. At 81% RH, the  $R_h$  of  $\alpha,\alpha$ -trehalose and HA were in the 89.5–103.3% and 188.2–273.6% ranges, respectively, at 16–76.5 h, respectively. The maximum  $R_h$  value of CMT and QT were 835.1% and 583.3%, respectively, at 76.5 h, and the minimum  $R_h$  was 310.2% and 320.6%, respectively, at 16 h, which was better than that of both  $\alpha,\alpha$ -trehalose and HA. Under a silica gel environment, CMT and QT showed the same

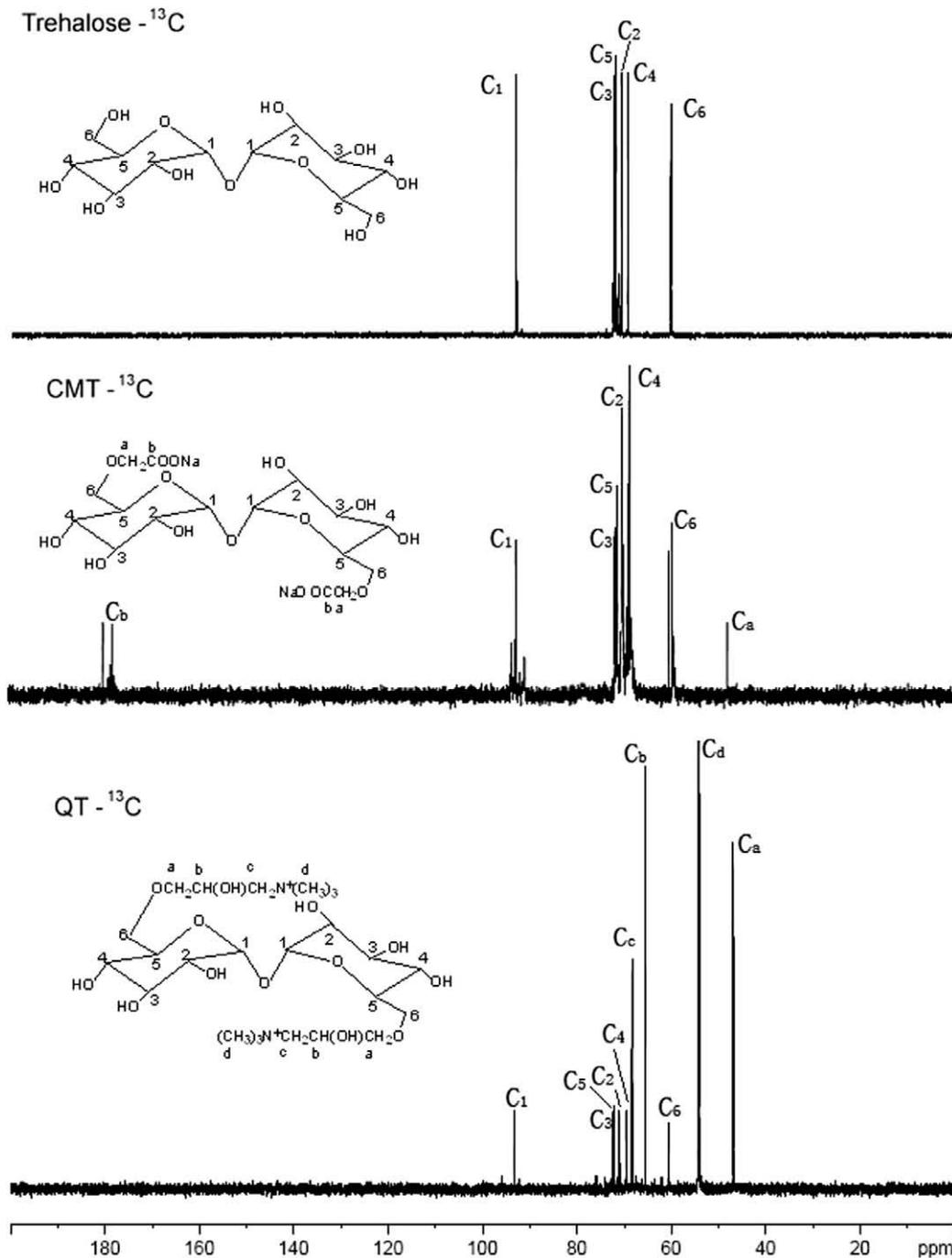


Figure 2.  $^{13}\text{C}$  NMR spectra of  $\alpha,\alpha$ -trehalose, CMT, and QT.

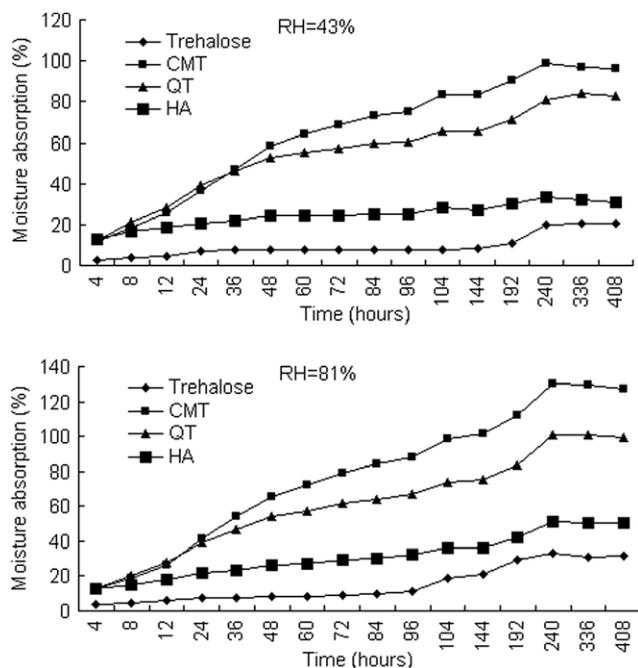


Figure 3. Moisture absorption abilities of CMT and QT.

tendencies. The maximum  $R_h$  values of  $\alpha,\alpha$ -trehalose and HA were 36% and 42%, respectively, at 16 h, and the minimum  $R_h$  values were 17% and 23% at 76.5 h, respectively. The maximum  $R_h$  values of CMT and QT were 59.9% and 54.1%, respectively, at 16 h, and the minimum  $R_h$  values were 38% and 33% at 76.5 h, respectively. Both CMT and QT showed better  $R_h$  than that of both  $\alpha,\alpha$ -trehalose and HA under the three conditions tested, and CMT had the best moisture retention properties.

CMT and QT showed better moisture absorption and retention abilities than  $\alpha,\alpha$ -trehalose at each combination of time and RH, indicating that the introduction of either  $-\text{COOH}$  ( $-\text{COONa}$ ) or  $-\text{N}^+(\text{CH}_3)_3$  groups is a convenient and effective method to enhance both moisture absorption and retention abilities for  $\alpha,\alpha$ -trehalose. Both groups have obvious charges, which are similar to those of HA, and the increased moisture absorption and retention abilities may be largely due to the charged groups. As we all know, most of the cheap natural carbohydrates are neutral, such as starch, cellulose, sucrose, and glucose. Chemical modification is an effective method to improve their activities. We measured the substitution degree (DS) of CMT and QT, and they were 83.3% and 89.7%, respectively. At the same time, we assessed the reaction yields, and they were 94.8% and 96.0% for CMT and QT, respectively (Table 1). Considering the multifunctional properties of  $\alpha,\alpha$ -trehalose, as well as its low cost and low toxicity, research on the moisture absorption and retention abilities of its carboxymethyl and quaternary ammonium derivatives will be helpful to expand its applications in the areas of cosmetics and biomedicine. CMT and QT showed better  $R_a$  and  $R_h$  than that of HA, and had the potential to be used as a moisture retention ingredient, for example, in cosmetics.

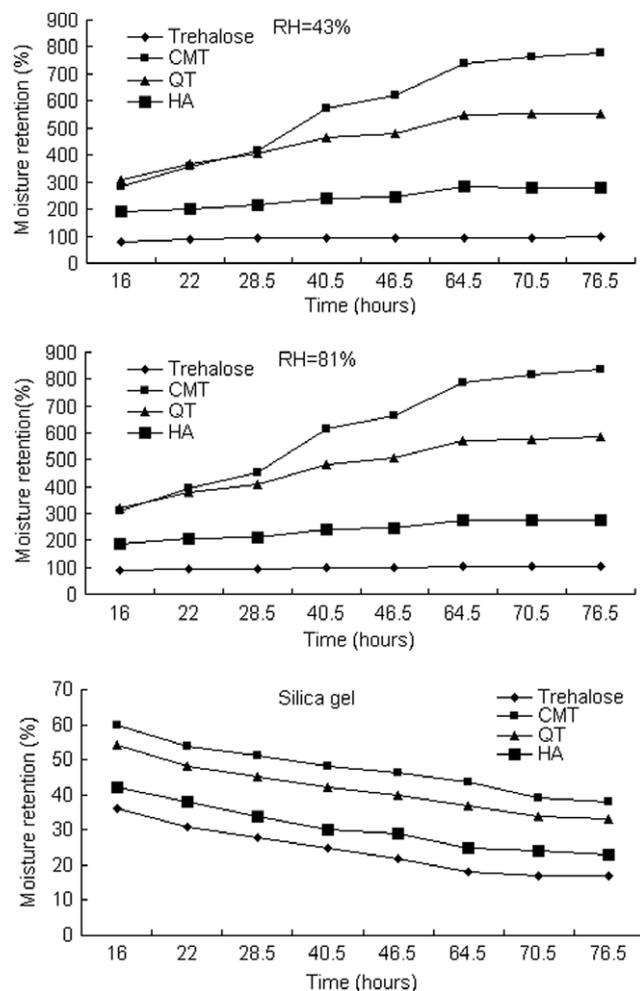


Figure 4. Moisture retention abilities of CMT and QT.

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