# Preparation of *O*-Carboxymethyl-*N*-Trimethyl Chitosan Chloride and Flocculation of the Wastewater in Sugar Refinery

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**ABSTRACT:** A novel water soluble amphiphilic O-carboxymethyl-N-trimethyl Chitosan chloride (CMTMC) was synthesized. The structure of this material was characterized by Fourier transform infrared (FTIR) spectroscopy, $^{13}C$ nuclear magnetic resonance ($^{13}C$-NMR) spectroscopy and X-ray diffraction (XRD) techniques. The results showed that CMTMC had been successfully prepared. To determine the flocculation performance of the synthesized amphiphilic polymer, a comparison was made among Chitosan (CS), N-trimethyl chitosan chloride (TMC), O-carboxymethyl chitosan (CMC), and CMTMC on the turbidity and COD removal efficiency of 1% (v/v) wastewater in sugar refinery suspensions at pH 5.0, 7.0 and 9.0 at a dosage range of 0–8 mg/L. The results showed that the water soluble amphiphilic polymer CMTMC, which contains longer polymer anion and polymer cation, had the best performance not only in turbidity removal but also in COD removal on sugar refinery wastewater. The using of CMTMC as a flocculant to treat wastewater in sugar refinery was actually more effective than CS, CMC, and TMC. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2742–2748, 2010

**Key words:** O-carboxymethyl-N-trimethyl chitosan chloride; preparation; sugar refinery wastewater; flocculation

## INTRODUCTION

In recent years, growing concern for wastewater treatment has promoted different technologies to meet up-to-date strict wastewater quality discharged in various industrial fields. China is a large cane sugar producer, where sugar refinery will discharge 12–15 ton of wastewater to produce 1 ton of alcohol.[1] The components of the wastewater are very complex, such as proteins, vitamins, sugar residuals, short fibers and also a large quantity of Mycelium, acid and alkali, and other ingredients.[2] At present, polyacrylamides are in groups of polymers frequently used as flocculants in treatment of wastewater from sugar refinery.[3] Though the polyacrylamides themselves are nontoxic, the nonbiodegradable property presents another major drawback of the polymeric flocculants, which will result in "secondary pollution" for environment.[4] The kind of material as flocculants in water treatment undoubtedly could not reach satisfactory effects, so environmentalists have set their sights on water soluble polymers which also can be used as flocculants, sludge dewatering agents, adsorbents and metal ion chelating agents in water treatment.[5] Since chitosan is biodegradable and nontoxic, it is also one of the most widely used organic polymer flocculants in industrial wastewater treatment.[6,7] Research of chitosan and its derivatives is one of the most popular hot aspects. However, because chitosan has poor solubility above pH 6.5, it shows its unique properties only in acidic medium, and it has relatively low charge density.[8,9] To improve its aqueous solubility and charge density, many derivatives of chitosan have been synthesized, such as carboxymethyl chitosan (CMC) and N,N,N-trimethyl chitosan chloride (TMC).[10,11] TMC is obtained by the reaction of chitosan and $CH_3I$ in N-methy-2-pyrrolidone in the presence of sodium iodide. Unfortunately, TMC only has strengthened chitosan cation, but not appearing amphoteric. Since there are plenty of hydroxy groups in the TMC molecule, which may undergo further carboxymethylation in the presence of sodium hydroxide different derivates of chitosan with improved flocculation performance have been prepared to treat different kinds of wastewater.[12,13] In this article, we report a new water-soluble chitosan derivative (structure shown in Fig. 1) and its ability in the sugar refinery wastewater treatment. A water-soluble chitosan derivative,

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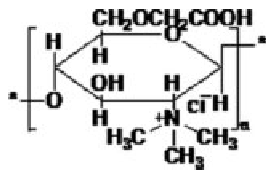


**Figure 1** Chemical structure of CMTMC.

N,N,N-trimethyl chitosan chloride (referred to here as TMC), was prepared by reacting chitosan with $CH_3I$. The TMC was further modified by reacting with monochloroacetic acid to prepare an amphiphilic chitosan derivative (CMTMC). In this article, self-made high-charge density of amphoteric polymeric flocculant CMTMC was then studied as the objective to treat the sucrose wastewater, having carried out the first step on flocculation mechanism. Amphoteric chitosan has shown good performance of the flocculation when treating wastewater of sugar refinery. Compared with chitosan, TMC, and CMC, the amphiphilic polymer showed better flocculation ability under all conditions, especially under both neutral and alkaline conditions.

## EXPERIMENTAL

### Materials

#### Reagents

Chitosan was supplied by Golden-shell Biochemical Co., China (Zhejiang, China). The degree of deacetylation was 95% (determined by potential titration),[14] and its average molecular weight was $2.46 \times 10^{-5}$ (determined by GPC method).[15] CMC,[16] ethanol, chloroacetic acid, N-methyl-2-pyrrolidone, isopropyl, methyl iodide, sodium iodide were purchased from Shanghai Chemical Co, China. All other reagents used were of analytical grade.

#### Wastewater samples

Wastewater used in the experiments was obtained from Nan-ning sugar refinery (Guangxi, China), the wastewater sample was diluted by 100 times with water, and the main characteristics of which were presented in Table I. It had high COD, turbidity, and pH values.

### Preparation of CMTMC

#### Preparation of TMC

TMC was synthesized by reductive methylation of chitosan that was accomplished by a chemical reaction between chitosan and iodomethane in the presence of sodium hydroxide based on the methods reported in the literature.[10,11]

A 5 g of chitosan was dispersed in 125 mL of N-methyl-2-pyrrolidone (NMP) for 12 h at room temperature. To the dispersion, appropriate amount of sodium iodide was added to the reaction medium to achieve a final concentration of 0.2*M* 15 mL of a 1*M* NaOH solution and 27 mL of methyl iodide (5-fold excess to amine of chitosan) were added. The mixture was stirred at 60°C for 2 h. The solution was collected by extraction with acetone, which was then dried to obtain the TMC.

Ion exchange: After washing with acetone and diethylether, the final product was dissolved in 80 mL of 5% (w/w) aqueous NaCl solution to exchange the iodide ion with a chloride ion. The polymer was precipitated from solution using acetone and was isolated by centrifugation. The product was finally dissolved in 80 mL of water to remove the remaining NaCl from the material and precipitated from solution with acetone. This product was dried in a vacuum oven at 40°C for 12 h.

#### Preparation of CMTMC

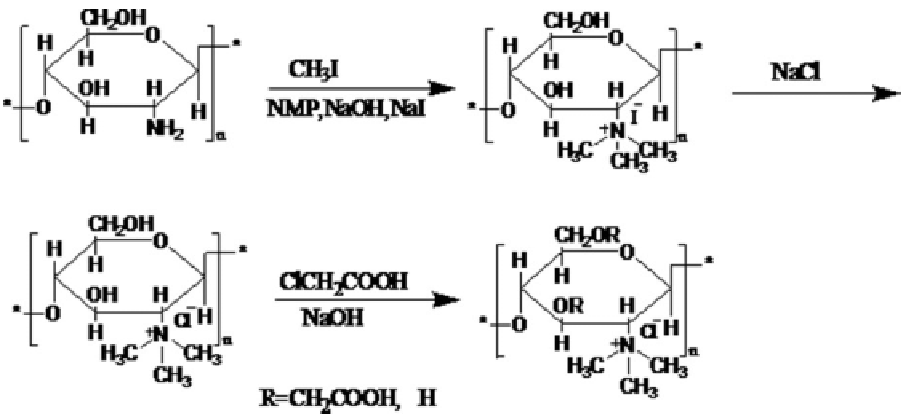
CMTMC was synthesized by reductive carboxymethylation of TMC that was accomplished by a chemical reaction between TMC and monochloroacetic acid in the presence of sodium hydroxide based on the methods described.[17]

A 2 g TMC was immersed in 25 mL NaOH solution (50 wt %) to swell and alkalize for 24 h. The alkalized chitosan was crushed into a filtration cake and then transferred into a flask. About 6 g of monochloroacetic acid was dissolved in 30 mL of isopropanol, and then added to the flask drop-wise in 20 min. The reaction mixture was stirred for 8 h at room temperature, and then the mixture was filtered to remove the solvent. The filtrate obtained was dissolved in 300 mL of water. After this solution was centrifuged to remove the precipitation, 400 mL of anhydrous ethanol was added to it to precipitate the product. Finally, the product was filtered, rinsed thrice with anhydrous ethanol, and the CMTMC was obtained after being vacuum dried at room

**TABLE I**
**The Properties of Wastewater from Nan-ning Sugar Refinery**

| Parameter | Characteristic value[a] |
|---|---|
| Colour | Blackish brown |
| $COD_{Cr}$ | 86020 |
| Alkalinity | 890 |
| Dissolved solids | 2458 |
| pH | 6.7 |
| Turbidity (NTU) | 3450 |

[a] All values are in mg/L except turbidity, pH and colour.



**Scheme 1** Synthetic route of CMTMC.

temperature for 12 h. The preparation process is shown in Scheme 1.

### Characterization of the CMTMC

Infrared spectra were obtained by FTIR spectroscopy using a Nicolet 470 FTIR spectrophotometer and potassium bromide pellets at room temperature.

FTIR spectroscopy was conducted on an FTIR spectrometer (Nicolet 5 DXB, Nicolet Biomedical Madison, WI) in the range between 4000 and 400 $cm^{-1}$, with a resolution of 4 $cm^{-1}$. Thirty-two scans at a resolution of 4 $cm^{-1}$ were averaged and referenced against air. All powder samples were compressed into potassium bromide disks for the FTIR measurement.

$^{13}C$-NMR spectra of the CMTMC polymers were obtained with a Bruker AVANCEAV 500 MHz spectrophotometer (Switzerland) by dissolving samples of the polymers in $D_2O$ at 60°C with suppression of the water peak.

XRD was performed using a diffractometer type D/max-rA (Tokyo, Japan) with Ni target and Kα radiation ($\lambda = 0.154$ nm) at 50 kV and 100 mA. The scanning rate was 0.2°/min and the scanning scope of 2θ was 3–40° at room temperature.

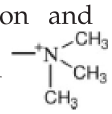
### Flocculation test

Flocculation performance of the polymers was examined using jar tests.[18] The turbidity measurement was carried out with the Digital Nephelometer HACH 2100N IS, purchased from autocontrol instrument (Liaoning, China). The COD value was determined in accordance with the dichromate method of the standard methods for the examination of water and wastewater (China National Standard, GB11914-89). Ten beakers of 1% (v/v) sugar refinery wastewater suspension (prepared by sugar refinery raw wastewater 5 mL in 500 mL of distilled water) were used for the flocculation study. The following procedure was uniformly applied to the suspension. Immediately after the addition of the polymer flocculant, the suspension was stirred at a constant speed of 120 rpm for 5 min, followed by a slow agitation at 60 rpm for 10 min. The flocs were then allowed to settle down for 15 min. At the end of the settling period, the turbidity of the supernatant liquid was measured. The dose of flocculants was varied from 0 to 8 mg/L, calculated with respect to the total weight of the solution.

## RESULTS AND DISCUSSIONS

### Preparation of CMTMC

A new water soluble amphiphilic CMTMC was prepared. The degree of the quaternization of the TMC was determined by the potentiometry.[19]

Potentiometric titration of the halide form was carried out with the aqueous silver nitrate, using a calomel electrode as reference, and a silver electrode for the measurement.[20] The degree of the carboxymethylation of the CMTMC was determined.[21] The results show that the degree of the quaternization of the TMC was 90.5%, the degree of the carboxymethylation of the CMTMC was 70%, with the yield of the CMTMC being 69% (Yield is the ratio of CMTMC and added TMC). The chemical structure of CMTMC is shown in Figure 1, which may be explained as follows: after quaternization and carboxymethylation of chitosan, the number of free amino and hydroxyl bonds in the obtained O-carboxymethyl-N-trimethyl chitosan chloride decreases, the formation of hydrogen bonds in chitosan molecular chains are also reduced, the H of $-NH_2$ and $-OH$ in chitisan is substituted by $-CH_3$, $-CH_2COOH$, the size of substituents increases; the inter and intramolecular hydrogen bonds of the chitosan are weakened, meanwhile, after modification and substitution, the hydrophilic $-COOH$ and  appeared in substituents.[22]

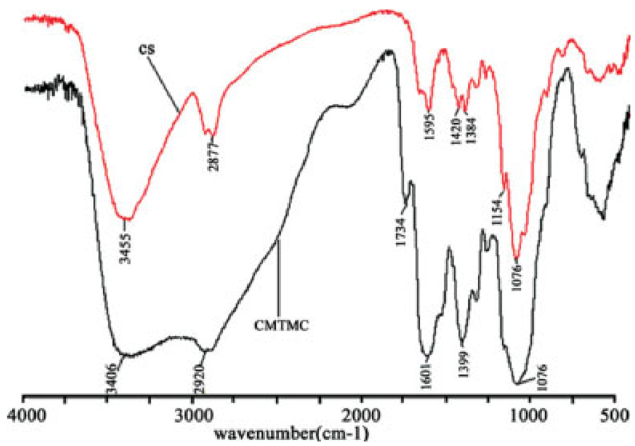



**Figure 2** FTIR spectra of CS and CMTMC. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

## Structure of CMTMC

### FTIR

The IR spectra of CMTMC and CS are shown in Figure 2. Chitosan shows the basic characteristics peaks at: 3455 cm$^{-1}$ (O—H stretch), 2877 cm$^{-1}$ (C—H stretch), 1154 cm$^{-1}$ (bridge-O-stretching), and 1076 cm$^{-1}$ (C—O stretching).[15,16] Characteristic peaks of amine (N—H) vibration deformation appeared at 1595 cm$^{-1}$ (N—H bending) for chitosan. Three new absorption peaks were observed in the spectrum of the polymer. The new peak at 1601 cm$^{-1}$ came from the vibrating absorption of carbonyl groups in the grafted CMTMC. The two peaks at 1399 cm$^{-1}$ and 1734 cm$^{-1}$ were attributed to the methyl groups of ammonium and quaternary ammonium in CMTMC, respectively. The appearance of the new peaks in the spectrum of CMTMC clearly demonstrated that carboxymethyl and N-trimethyl had been successfully grafted onto chitosan. The appearance of all these peaks demonstrates that CMTMC had been successfully prepared.

### $^{13}$C-NMR

Figure 3 shows the $^{13}$C-NMR spectra of CMTMC. The peak at 56.5 ppm was attributed to the carbons of the trimethylammonium group (C-7). The signal for —COOH substituted on —OH is obvious at 178 ppm. The peaks at 99.0, 62.5, 70.2, 77.3, 75.0, and 60.8 ppm were attributed to the C-1, C-2, C-3, C-4, C-5, and C-6, respectively. The results were in agreement with L. Sun et al.[23] The analytical results of the $^{13}$C-NMR spectrum provide further support for the formation of the CMTMC.

### XRD

In the X-ray diffractograms of CS and CMTMC shown in Figure 4, the XRD pattern of chitosan shows two reflections fall at 2θ 10.8°and 20.1°. The reflection fall at 2θ 10.8° was attributed to the hydrated crystals of low crystallinity and corresponded to the form I,[24,25] while the reflection appeared at 2θ 20.1° was identified as representative of the crystallinity of the form II.[26] For the grafted polymer (CMTMC), the peak at 2θ 10.8° disappeared, whereas the peak at 2θ 20.1° decreased drastically, which attributed to the decrease in crystallinity. This suggests that the hydrogen bonding ability of CS was reduced after the grafting of Carboxymethyl and N-trimethyl onto the chitosan backbone. The analytical results of the XRD spectrum provide further support for the formation of the CMTMC.

## Application of CMTMC

### CMTMC removal to turbidity of wastewater in sugar refinery

The performance of the CS, TMC, CMC, and amphiphilic polymer (CMTMC) in turbidity removal of 1% (v/v) wastewater in sugar refinery suspensions was studied at pH 5.0, 7.0, and 9.0, the results are shown in Figure 5. From the figure we see, at any pH value,

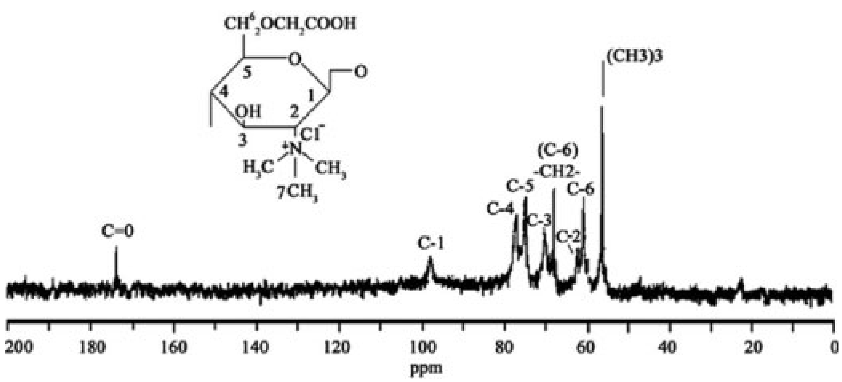



**Figure 3** $^{13}$C —NMR spectra of CMTMC.

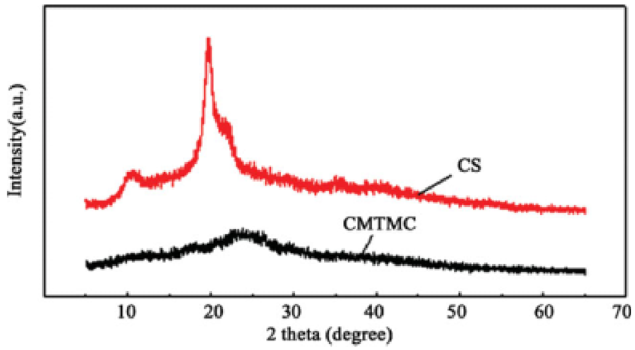



**Figure 4** XRD spectra of CS and CMTMC. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the amphiphilic polymer (CMTMC) showed better turbidity removal ability than the other three polymers under all conditions, especially at pH 7.0 and 9.0. Under acidic condition (pH 5.0), the turbidity of the wastewater was 5.0 NTU after being flocculated by the amphiphilic polymer at a dosage of 4 mg/L, but 12.8, 9.8, 6.1 NTU by CS, TMC, CMC, respectively with initial turbidity of 34.5 NTU. With the increase of the dosage (0–8 mg/L) of the CS and TMC, the residual turbidity decreased first and then increased, but to CMTMC, the residual turbidity kept decreasing until reaching to 3.9 NTU (at 8 mg/L) (Fig. 5.). Under neutral condition (pH 7.0), at the optimal dosage of 5 mg/L, the turbidity of the wastewater was 4.3 NTU after being flocculated by CMTMC, but 11.0, 8.5, 7.3 NTU by CS, CMC, TMC, respectively (Fig. 5). In addition, the amphiphilic polymer had a greater flocculation capacity than chitosan under alkaline condition, at pH 9.0, CS almost had no flocculation ability, but the chitosan derivatives still exhibited a remarkable flocculation capacity. The turbidity of the wastewater was about 3.5 NTU after the flocculation with the CMTMC at a dosage range of 4.0–8.0 mg/L (Fig. 5), but more than 4.2 NTU, 7.2 NTU with TMC and CMC at the same dosage (4.0–8.0 mg/L). From the figure, we can see that CMTMC is the most effective one in turbidity removal among these polymers. This is the obvious advantage of the amphiphilic polymer synthesized in this work. According the structure

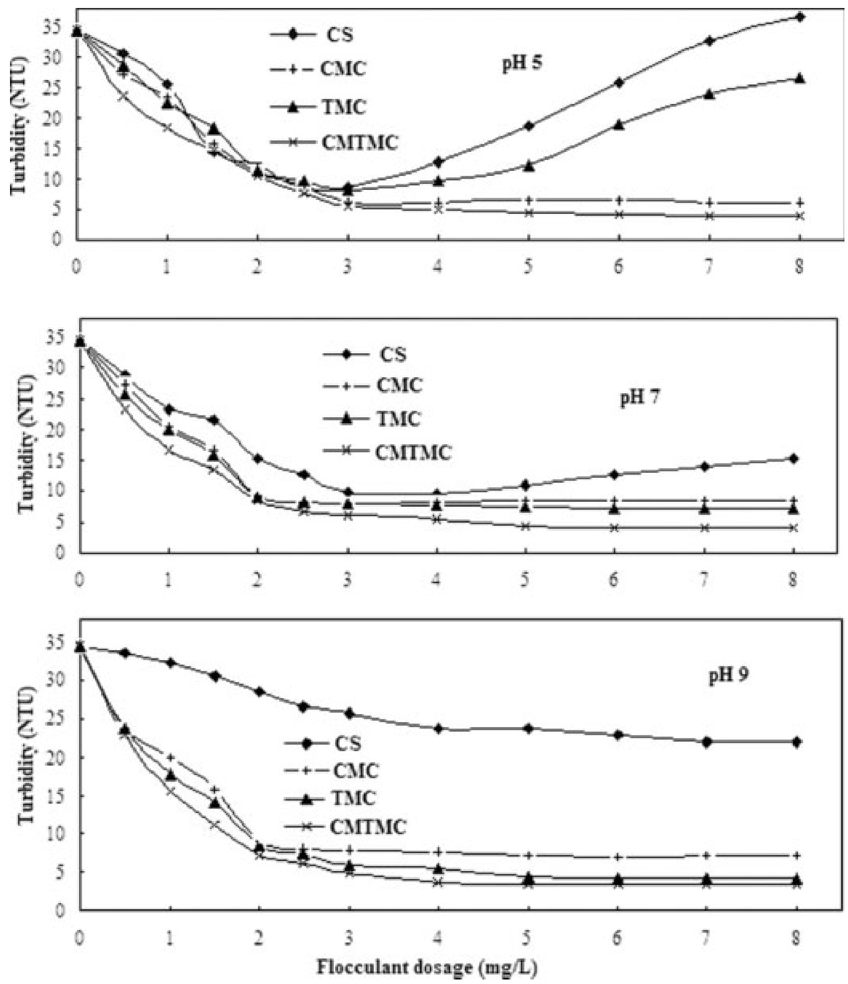



**Figure 5** Performance of the CS, TMC, CMC, and CMTMC on turbidity removal at pH 5.0, 7.0, 9.0.

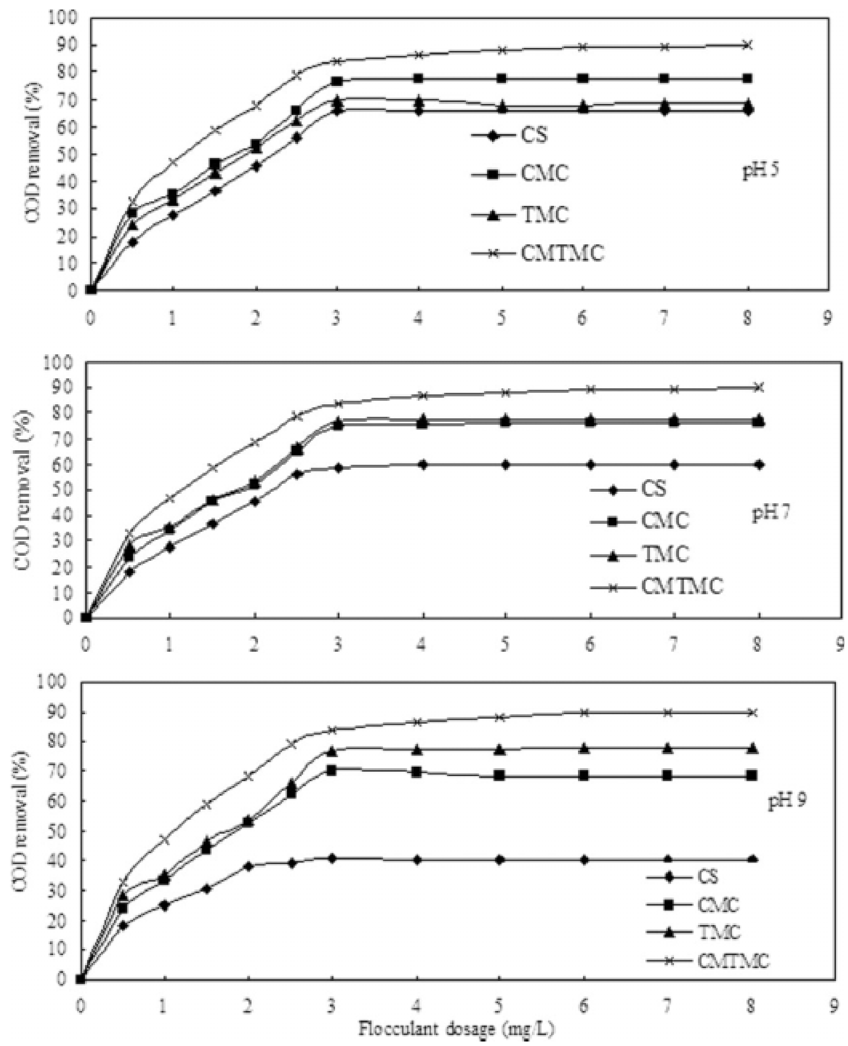



**Figure 6** Performance of the CS, TMC, CMC, and CMTMC on COD removal at pH 5.0, 7.0, 9.0.

characterization of CMTMC, this turbidity removal efficiency may be attributed to its long molecular chains, which promotes the association among polymer molecules and particles. In addition, CMTMC has relatively higher charge density,[27] which promotes the interaction between particles and affects the flocculation performance.

## CMTMC removal to COD of wastewater in sugar refinery

The flocculation performance can be explained in terms of the COD removal efficiency of the supernatant liquid. The higher the COD removal efficiency, the better the flocculating agent is. So COD removal efficiency was chosen as the control index to determine the optimal dosage for flocculants. In this article, the performance of the CS, TMC, CMC, and CMTMC in COD removal of wastewater in sugar refinery suspensions of 1% (v/v) was studied at pH of 5.0, 7.0, and 9.0. The results are depicted in Figure 6, which showed that the COD removal efficiency of CS was the lowest under three conditions, the performance of TMC and CMC was better than CS, while CMTMC showed the best COD removal ability under the three conditions. At pH 9 and the flocculant dosage of 5 mg/L, CMTMC removal to COD reached to 95%, compared with 40%, 68%, 78% of CS, CMC, TMC, respectively. So it is obvious from these results that the amphiphilic polymer CMTMC is a better flocculant for sugar refinery wastewater suspensions than the other three polymers (CS, CMC, and TMC). According to study, cationic chitosan derivatives can be easily adsorbed to the colloid surface of anionic in sugar refinery wastewater suspensions because of electrostatic attraction. Adsorbed polymers tend to form loops and extend some distance from the particle surface into the aqueous phase. Their ends also dangle and get adsorbed on the surface of another particle forming a bridge between particles. For effective bridging to occur, the length of polymer chains should be long

**TABLE II**
**Characteristic Values of Purified Water**

| Parameter | Characteristic value[a] (Dilution × 100) | Purified water[a] |
|---|---|---|
| Colour | Fuscous brown | Buff yellow |
| $COD_{Cr}$ | 860.2 | 43.01 |
| Dissolved solids | 24.58 | 3.58 |
| pH | 6.6 | 6.7 |
| Turbidity (NTU) | 34.5 | 3.5 |

[a] All values are in mg/L except turbidity, pH, and colour

enough to extent from one particle surface to another. Amphiphilic polymer CMTMC, which contains more polymer anion and polymer cation, has longest chains among the four polymers. Hence, it would be more effective in COD removal than the ones with the shorter chains.

### Effects of CMTMC in treatment of sugar refinery wastewater

Wastewater obtained from Guangxi Nanning sugar refinery is first diluted by 100 times, then treated by adding amphiphilic flocculants (CMTMC) according to experimental methods, the results are shown in Table II.

From Table II, we can see amphiphilic flocculants have good treatment effects on turbidity, COD, etc. After flocculation and purification, the treated water could reach the national first level discharge standards. (GB8978-88, China).

## CONCLUSIONS

The new water soluble amphiphilic polymer CMTMC has been successfully synthesized as possible alternative to chitosan and its derivatives (CMC, TMC) to promote flocculation performance in sugar refinery wastewater treatment, the optimum reaction conditions are first to process radical quaternarization on main chain of the chitosan, and then get gender carboxymethyl chitosan quaternary ammonium salt by processing a carboxymethylation reaction in strong alkaline condition.

According to the structure characterization of CMTMC showed by FTIR, $^{13}$C-NMR, and XRD, this new amphiphilic polymer has bigger charge density (than CS, CMC, TMC), which could enhance its flocculation ability in wastewater treatment.

Besides, according to the comparison made with CS, CMC, and TMC on the turbidity and COD removal efficiency of 1% (v/v) wastewater in sugar refinery suspensions at different pH (5.0, 7.0, 9.0), the synthesized novel amphiphilic polymer showed better flocculation performance in turbidity removal and COD removal under all conditions, especially under both neutral and alkaline conditions. So amphiphilic polymer CMTMC is a better flocculant in treatment of sugar refinery wastewater.

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