Supporting information

Carboxymethyl chitosan and its hydrophobically modified derivative as pH-switchable emulsifiers

Simo Kalliola\textsuperscript{a}\textsuperscript{*}, Eveliina Repo\textsuperscript{a}, Varsha Srivastava\textsuperscript{a}, Feiping Zhao\textsuperscript{a}, Juha P. Heiskanen\textsuperscript{b}, Juho Antti Sirviö\textsuperscript{c}, Henrikki Liimatainen\textsuperscript{c}, Mika Sillanpää\textsuperscript{a,d}

\textsuperscript{a} Lappeenranta University of Technology, Sammonkatu 12, Mikkeli FI-50130, FINLAND
\textsuperscript{b} Research Unit of Sustainable Chemistry, University of Oulu, P.O. Box 3000, FI-90014, Finland
\textsuperscript{c} Fibre and Particle Engineering Research Unit, University of Oulu, P.O. Box 4300, FI-90014, Finland
\textsuperscript{d} Department of Civil and Environmental Engineering, Florida International University, Miami, FL-33174, USA

\textsuperscript{*}Corresponding author. Tel.: +358 50 348 1188.

E-mail address: simo.kalliola@lut.fi (S. Kalliola)

6 Pages
3 Figures

\textsuperscript{1} CMC-Na = N,O-carboxymethyl chitosan
h-CMC-Na = Hydrophobically modified N,O-carboxymethyl chitosan
Materials

Low molecular weight (50-192 kDa) chitosan (deacetylation degree 75-85%), NaOH (≥98%), chloroacetic acid (≥99%), NaCNBH$_3$ (≥95%), and dodecanal (≥99%) were supplied by Sigma-Aldrich. 2-propanol (≥99.8%) was supplied by Merck. Deuterium chloride solution (35 wt. % in D$_2$O, 99 atom-% D) was obtained from Sigma Aldrich. D$_2$O was obtained from Euriso-top (99.96% atom-% D).

Synthesis of carboxymethyl chitosan in sodium salt form (CMC-Na)

One gram of chitosan, 1.35 g of NaOH, 6 ml of water, and 24 ml of 2-propanol were mixed and stirred in a round bottom flask (100ml) at 50 °C for one hour. Then, 1.5 g of chloroacetic acid dissolved in 2 ml of 2-propanol was added dropwise into the mixture while stirring. After 4 hours, the solid product was separated by centrifuging and washed thoroughly with ethanol (70v-% and 100v-%) and dried in room temperature on a watch glass over-night. The dried CMC-Na was purified by dissolving in 50 ml of water and separating the insoluble fraction by centrifugation. The CMC-Na was precipitated by slowly pouring the liquid phase into 400 ml of ethanol (100v-%). The precipitate was washed thoroughly with ethanol (70v-% and 100v-%) and separated by centrifuging. The product was dried as previously described and ground into a fine powder. The product was in sodium salt form (CMC-Na) due to the alkaline reaction medium.

Synthesis of hydrophobically modified carboxymethyl chitosan in sodium salt form (h-CMC-Na)

Portion of the purified and dried CMC-Na (750 mg) was dissolved in 42 ml of water. After the dissolution, 30 ml of ethanol (100v-%) was added slowly into the polymer solution while stirring. Dodecanal (30 µl) was added corresponding to approximately 5% substitution degree. The mixture was stirred for 20 minutes, NaCNBH$_3$ (30 mg) was added, and the stirring was continued for 20 h in room temperature. The reaction mixture was poured slowly into 400 ml of ethanol (100v-%) to precipitate the h-CMC-Na. The precipitate was separated by centrifuging and washed thoroughly with ethanol (90v-% and 100v-%). The product (h-CMC-Na) was separated by centrifuging, dried, and ground as previously described.
Characterizations of CMC-Na and h-CMC-Na

FTIR analysis

The FTIR spectra of native chitosan, CMC-Na, and h-CMC-Na are presented in Figure S1.

Figure S1. FTIR spectrum of native chitosan, CMC-Na, and h-CMC-Na.

Figure S1. shows the FTIR spectrum of the chitosan used in the synthesis of CMC-Na and h-CMC-Na, and their FTIR spectra, respectively. In the spectra of CMC-Na and h-CMC-Na, a broad band appears at 1600 cm\(^{-1}\) which is due to overlapping of \(-\text{COONa} (1598 \text{ cm}^{-1})\) and \(-\text{NH}_2 (1592 \text{ cm}^{-1})\) bands [1, 2]. In CMC-Na and h-CMC-Na the carboxyl groups are in sodium salt form due to the alkaline pH during the synthesis. Therefore, the band of \(-\text{COOH}\) group is not detected at around 1720 cm\(^{-1}\) [3]. The spectra of CMC-Na and h-CMC-Na are almost identical due to the low substitution degree of hydrophobic modification, and show successful carboxymethylation of chitosan.

\(^1\text{H}\) NMR analysis

The \(^1\text{H}\) NMR spectra of CMC-Na and h-CMC-Na are presented in Figures S2 and S3, respectively. The spectrum of h-CMC-Na shows the similar characteristic proton signals as can be seen in CMC-Na spectrum which was assigned according to our previous studies [4]. In the spectrum of h-CMC-Na, a new triplet with a typical coupling constant (\(J = 7.0 \text{ Hz}\)) can be found at 1.26 ppm. The peak is assigned to \(\text{CH}_3\)-group of dodecanyl substituent (marked with a in Fig. S3). Also, a clear signal can be seen at 1.68
ppm which is assigned to nine CH2-groups of dodecanyl chain (marked with b in Fig. S3). The signals of rest two CH2-groups can be hardly observed at 2.13 and 3.32 ppm. These peaks are assigned to protons c and d next to the nitrogen atom. In summary, the performed 1H NMR analysis clearly indicates the successful attachment of dodecanyl substituent to CMC-Na.

Figure S2. 1H NMR spectrum of CMC-Na.
Figure S3. $^1$H NMR spectrum of h-CMC-Na.
References

[1] X. Chen and H. Park, "Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions," *Carbohydrate Polymers*, vol. 53, pp. 355-259, 2003.

[2] A. Zhu, M. Chan-Park, S. Dai and L. Li, "The aggregation behavior of O-carboxymethylchitosan in dilute aqueous solution," *Colloids and Surfaces B: Biointerfaces*, vol. 43, p. 143–149, 2005.

[3] Y. Chen, V. Javvaji, I. MacIntire and S. Raghavan, "Gelation of Vesicles and Nanoparticles Using Water-Soluble Hydrophobically Modified Chitosan," *Langmuir*, vol. 29, p. 15302−15308, 2013.

[4] S. Kalliola, E. Repo, V. Srivastava, J. P. Heiskanen, J. A. Sirviö, H. Liimatainen and M. Sillanpää, "The pH sensitive properties of carboxymethyl chitosan nanoparticles cross-linked with calcium ions," *Colloids and Surfaces B: Biointerfaces*, vol. 153, p. 229–236, 2017.