Sodium salt of oleoyl carboxymethyl chitosan: A sustainable adsorbent in the oil spill treatment

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Spills due to the floating oil on the surface of sea water shows a significant alteration in the natural phenomenon of the marine ecosystem. Hence, amphiphilic sodium salt of oleoyl carboxymethyl chitosan (NaO-CMCS) was synthesized, characterized and studied as a sustainable adsorbent for the removal of floating oil in the oil spill treatment. Successful chemical modification of chitosan was confirmed by chemical analysis using Fourier Transform Infrared (FTIR), 1H Nuclear Magnetic Resonance (1H NMR), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDS) and Elemental Analyzer. The effect of pH and salinity on the surface charge of NaO-CMCS was studied. Marine diesel was chosen as an oil phase for the emulsion studies. Hydrophobically modified NaO-CMCS shows the oil-in-water (o/w) emulsion stability for more than 6 weeks with droplet size ≤ 30 μm which increases with decreasing temperature. The behavior of emulsion with different salinity shows phase separation at pH 5, bridging flocculation at pH 6–7 and o/w emulsion at pH 8. Calcium ions act as a cross-linker between two carboxylic acid groups of NaO-CMCS, enhancing the removal of oil from the creamy emulsion. The recovery of oil was 75–85% and 19–49% from deionized water and sea water, respectively. Oil was not chemically altered during the recovery, demonstrating the effectiveness of this derivative in the oil spill response for the removal and recovery of floating oil from the sea water. © 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Oceans are the world’s largest water resource getting polluted by oil either in the form of routine shipping or industrial run-offs or oil spills, which become a severe environmental threat. Out of these, about 12% of oil entering the oceans is due to oil spill. In past 37 years, Exxon Valdez in 1987 and Deep Horizon in 2010 were among the largest oil spill disasters in the history. Not only the water surface, but also the marine creatures (U.S. Fish and Wildlife Service, 2004) and seashores were trapped through this spilled oil (Hong and Yanjie, 2009; Jernelöv, 2010). Scientists studying the fate of the oil estimated that 20% evaporated, 50% biodegraded, 14% was cleaned up, 13% remained in subtidal sediments, 2% remained on shorelines, and less than 1% remained in the water (Wolfe et al., 1994). Cleanup is the first step towards the recovery process and therefore, various spill contaminant methods such as use of booms, skimmers, vacuum trucks, in-situ burning, dispersants, and chemical cleaners were used for the cleaning up of the spilled oil. Amongst them, use of oil dispersants such as Corexit 9500A and Corexit 9527A were commonly used for the Deep Horizon oil spill. However, these materials turned out to be toxic and had adverse effect on the aquatic life (Graham et al., 2016), which has led the researchers to develop more and more non-toxic and biodegradable biomaterials for the oil spill response (Zhang et al., 2013b; Zhang et al., 2014).

Chitosan, being a deacetylated form of chitin obtained from the crustaceans of crabs and shrimp shells, is an effective biomacromolecule consisting both reactive amino and hydroxyl groups. In addition, chitosan is a charged biopolymer that is capable of adsorbing oppositely charged emulsion droplets via electrostatic attraction. This behavior of chitosan is based on the presence of both the main polysaccharide backbone and the N-acetyl groups at...
C2 position. Therefore, fully protonated chitosan tends to form aggregates through hydrogen of amino groups and hydrophobic interactions in acidic conditions (Arana et al., 2010), and hence, acts as a good emulsifier (Liu et al., 2012). The water solubility of chitosan was improved through chemical modifications of these functional groups either by Michael addition reaction (Jiang et al., 2010), or by enzymatic and chemical grafting, and similar property of chitosan was also reviewed (Pillai et al., 2009; Shukla et al., 2013). Physically and chemically modified chitosan either in the form of beads (Mubarak et al., 2017), coated (Nawi et al., 2010), or cross-linked (Repo et al., 2011; Jawad and Nawi, 2012; Jawad et al., 2017) has been widely studied and reviewed (Bhatnagar and Sillanpää, 2009; Pintor et al., 2016; Kyzas and Bikiaris, 2015) for the removal of pollutants from water, in food industry (No et al., 2007; Ngo et al., 2015), in biomedical (Sonía et al., 2010; Dash et al., 2011), and in cosmetics (Rinaudo, 2006).

Many factors such as type of oil, thickness of the oil deposition, geographical parameters, biological communities and type of cleanup have been considered for the determination of oil spills impacts and recovery rates (Dicks, 1999). Some oils with volatile chemicals can easily be evaporated, some oil components can be broken by sunlight through photochemical reaction and some can be biodegraded by microbes (Farrington and McDowell, 2004). However, viscous and heavy oils like petroleum crude oil and heavy fuel oil, which cannot be evaporated easily, may form the blanket covering areas on the water surface causing a significant threat to the birds and mammals. So, spilled oil spread out on the water surface forming a thin layer, from which some lighter components evaporate whereas some water soluble components dissolve, and some transform to droplets through wave action by forming emulsion. The formation and stability of emulsion also depends on the nature of oil used, e.g., its interfacial tension, polarity, water solubility, and rheology (Jumaa and Müller, 1998; Langevin et al., 2004). A mixture of oil and water is generally unstable for emulsion and hence addition of surfactants, which reduce the interfacial tension between the oil and water to form a stable emulsion by preventing coalescence of dispersed oil droplets is mandatory. A surfactant used in oil spill treatment can either be adsorbed at the oil-water interfaces or form thicker slicks with oil. However, prolonged use of synthetic surfactants is not feasible as it reflects toxic effects on the marine ecosystems (Smit et al., 2009). Thus, nowadays, bio-surfactants or biopolymers, due to their biocompatibility, non-toxicity and sustainability, are focused more in the oil-spill response rather than synthetic surfactants.

Amphiphilic chitosan derivative comprise of both polar and non-polar groups along with the glucosamine backbone, so when adsorbed to the oil droplet surface, the polar part extends towards the polar part of the oil phase (McClennan and Gumus, 2016). Various methods such as acylation (Auzely and Rinaudo, 2003; Huo et al., 2011), acylation (Sun et al., 2008), and grafting (Sun et al., 2003; Yang et al., 2012; Jena and Sangamwar, 2016) have been used to prepare amphiphilic chitosan by introducing hydrophobic moieties onto the carboxymethylated chitosan backbone. For an amphiphilic copolymers, longer hydrophobic chains along with larger hydrophilic groups helps to stabilize the micelle structure (Li et al., 2011). Therefore, from past few years naturally occurring bio-polymers such as chitosan present in sea food waste (Ummadisingu and Gupta, 2012) or fungal biomass (Srivastava and Viraghavan, 2010) along with their derivatives either in the form of hydrogel/ aerogel (Sokkera et al., 2011; Chavithary et al., 2015) or microspheres (Gumus et al., 2013) or grafted (Zhang et al., 2013a; Liu et al., 2015) have been studied more for oil spill. But as per our knowledge, the sodium form of amphiphilic chitosan for oil spill has not yet been studied.

The aim of this study was to investigate effectiveness of sodium form of amphiphilic chitosan obtained by carboxymethylation and acylation as an emulsion stabilizer for floating oil onto the water surface through minimization of one synthesis step. Sodium salt of oleoyl carboxymethyl chitosan (NaO-CMCS) was synthesized by introducing hydrophobic groups on amino groups of hydrophilic sodium salt of carboxymethylchitosan (Na-CMCS). After the modifications, amphiphilic NaO-CMCS was hydrophilic in nature. The solubility of this synthesized polymer was reversed than that of chitosan, i.e. it was insoluble at pH < 6. The molecular structure was analyzed by Fourier Transform Infrared (FTIR), 1H Nuclear Magnetic Resonance (1H NMR), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDS) and Elemental Analyzer. Binks et al. (2006) had explained that the particle wettability at the oil-water interface alters by changing the pH or salt concentration. Therefore, surface charge of NaO-CMCS was investigated at different pH with different ionic strength.

Oil-in-water (o/w) emulsion was obtained using marine diesel as a dispersed phase. Interfacial tension (IFT) between the aqueous phase and dispersed phase was investigated by the addition of NaO-CMCS. The formation of o/w emulsion and its stability was studied at room temperature (about 22 °C) for six weeks by measuring the average oil droplet size periodically as well as at lower temperature (2 °C) to extent its applicability for arctic oil spill also. Addition of hydrophobic moiety to Na-CMCS alters the average oil droplet size in comparison to our previous work (Doshi et al., 2017) by making the o/w emulsion more stable. Furthermore, the stability of o/w emulsions was studied with the optimized dosage of NaO-CMCS at pH range (5–8) with different salinity (0–3.5%) to build equivalent condition as seawater oil spill. Recovery of oil from the stabilized o/w emulsions was investigated by using calcium chloride as a cross-linker to show the effectiveness of NaO-CMCS as a biopolymer cleaner for the floating oil. Recovered oil was further studied by FTIR and Gas Chromatography using Flame ionization detector (GC-FID). The chitosan derivative after cross-linking was investigated by SEM and EDS analysis.

2. Experimental

2.1. Materials

Chitosan from shrimp shells (practical grade, MW 190000–375000 Da; >75% deacetylated; viscosity >200 cP, CAS No. 9012-76-4, Lot # SLB3564V, PubChem CID: 71853), chloroacetic acid (>99%, CAS No. 79-11-8, Lot #SHBD8969V, PubChem CID: 300), oleoyl chloride (>89%, CAS No. 112-77-6, Lot #SHBF3232V, PubChem CID: 5364783), sodium chloride (CAS No. 7647-14-5, Lot #SZBB0170V, PubChem CID: 300), acetone (>99.5%, CAS No. 67-64-1, Lot #STBG1776V, PubChem CID: 180), and calcium chloride dihydrate (>99%, CAS No. 10035-04-8, Lot #SLBB013V, PubChem CID: 329775159) were purchased from Sigma-Aldrich Finland Oy. Sodium hydroxide (NaOH) (CAS No. 1310-73-2, Lot #SBE1130V, PubChem CID: 14798) was purchased from Fluka. Isopropanol (IPA) (CAS No. 67-63-0, Lot #K4044834, PubChem CID: 3776) was purchased from Merck. Ethanol (99.5%) was purchased from Altia Oy. Deuterium chloride solution (35 wt. % in D2O, 99 atom-% D, CAS No 7698-05-7, Lot #MBB1157, PubChem CID: 122198780) was obtained from Sigma Aldrich. D2O was obtained from Euro-top (99.96% atom-% D, CAS No 7789-20-0, Lot #D215FL0701, PubChem CID: 24602). All chemicals were used without further purification. The marine diesel (sulfur-free oil with a density of 828 kg/m3 at 15 °C and viscosity of 1.846 mm2/s at 40 °C) was received from Fibre and Particle Engineering Research Unit, University of Oulu. Deionized water (Siemens Ultra Clear RO with Conductivity 2.1 μS/cm at 23.8 °C) was used throughout the experiments. Sea water (Conductivity 8.46 mS/cm and pH 7.844 at
22.3 °C) collected from the area near to Matinkylä Espoo, Finland was used only for the recovery of oil experiments.

2.2. Synthesis of NaO-CMCS

Carboxymethylated chitosan was crosslinked with long chained hydrocarbon, to form amphiphilic chitosan having hydrophilic and hydrophobic groups. The two steps reaction is shown in Fig. 1.

2.2.1. Synthesis of sodium salt of carboxymethyl chitosan (Na-CMCS)

Na-CMCS was synthesized based on previously reported method (Doshi et al., 2017; Chen and Park, 2003) with some modification. Briefly, chitosan from shrimp shells (4 g) was alkalized at 50–55 °C for 1 h with the mixture of NaOH (5.6 g), IPA (96 ml), and water (24 ml) using water bath (IKA C-MAG HS 7 Digital). Chloroacetic acid (6 g) dissolved in IPA (8 ml) was added to the reaction mixture over a period of 30 min, and the reaction was continued for 3 h at the same temperature. Then, 70% ethanol (50 ml) was added to stop the reaction. The solution was centrifuged at 1500 rpm for 2 min (using Centrifuge 5810R from Eppendorf). The supernatant was removed and the solid was washed with increasing concentration of ethanol (70–90%) for three times using same centrifugation method, and dried overnight at room temperature. The obtained product was Na-CMCS as shown in step-1 of Fig. 1.

2.2.2. Synthesis of sodium salt of oleoyl carboxymethyl chitosan (NaO-CMCS)

NaO-CMCS was synthesized based on previously reported method (Sun et al., 2008) with some modifications. Na-CMCS (2.3 g) was soaked in acetone (10 ml) for 1 h at 5 °C. Dissolved oleoyl chloride (2.6 ml) in acetone (2.6 ml) was added along with 4M NaOH solution (10 ml) to the reaction mixture dropwise over a period of 30 min. Acetone (20 ml) was added to the reaction mixture and the reaction was continued for 3 h at same temperature. To stop the reaction, 70% ethanol (60 ml) was added. The workup was carried out as mentioned in section 2.2.1. The product NaO-CMCS shown in step-2 of Fig. 1 was grounded in the tube mill to remove lumps of particles formed during the drying process.

2.3. Characterizations

FTIR images of chitosan, Na-CMCS and NaO-CMCS were recorded in the range of 400–4000 cm⁻¹ with a resolution of 4 cm⁻¹ for 60 scans (ATR-FTIR, Bruker Vertex 70 model) to investigate the functional groups changes in chitosan due to modifications with chloroacetic acid and oleoyl chloride. Samples were measured without any further treatment. For ¹H NMR measurements, samples of Na-CMCS and NaO-CMCS were dissolved in D₂O containing 0.7% of DCl and placed in 5 mm NMR tubes. The ¹H NMR spectra were recorded by using Bruker Ascend 400 MHz spectrometer and standard proton parameters with the delay time (d1) of 6 s at 70 °C. Surface morphology of chitosan and its derivatives were studied by SEM analysis using Hitachi SU3500 at accelerating voltage of 10 kV. Elemental distribution and composition were examined by EDS (Thermo Scientific UltraDry SDD EDS, dual detector with Software NSS at 15.0 kV accelerating voltage using Aluminium sample holder) and CHNS-Elemental Analyzer (Thermo Flash 2000 using tin sample holder). The degree of substitution was calculated from the CHNS elemental analysis as follows:

\[
DS_1 = \frac{\left(N_a\right)Na - CMCS - \left(N_C\right)Chitosan}{estimated\ no.\ of\ carbon\ atoms\ in\ Na - CMCS} (1)
\]

\[
DS_2 = \frac{\left(N_a\right)NaO - CMCS - \left(N_C\right)Na - CMCS}{estimated\ no.\ of\ carbon\ atoms\ in\ NaO - CMCS} (2)
\]

where DS₁ and DS₂ are the degree of substitution of Na-CMCS and NaO-CMCS, respectively. (C/N) are the mol. ratio of carbon to nitrogen obtained from CHNS analysis. The BET surface area of chitosan and NaO-CMCS was determined by nitrogen (N₂) adsorption-desorption method at 77.35 K using Micromeritics Tristar II plus instrument. The samples were degassed using Vac Prep 061 degassing unit with nitrogen at 30 °C for 18 h prior to the measurements. The total pore volume and average pore diameter were determined by the amount of nitrogen adsorbed at the relative pressure of 0.95 and BET (4V/A), respectively.

2.4. Effect of pH and salinity on surface charge

The amount of charge on the particle surface is the important characteristic of the particles as it determines the behavior of the emulsion. Therefore, the effect on surface charge of NaO-CMCS was studied as a function of pH with increasing salinity. Firstly, solid NaCl was added to NaO-CMCS solution (1 g/L) to increase the salinity from 0% to 3.5%. This solution was mixed for 5 min and pH was adjusted with 0.01 M HCl using pH meter (WTW Inolab calibrated before use). The studied pH range was in between 4 and 10. All the measurements were recorded in triplicate using Malvern Zetasizer (Nano ZS, Malvern UK) at 25.0 ± 0.1 °C.

2.5. o/w emulsion stability

For this study, different dosage of NaO-CMCS (0.5–3 g/L) were used. Simultaneously, marine diesel was mixed with water at
2000 rpm using vortex mixture (VWR International) to form the o/w emulsion. The oil to water ratio was 1:1 (v/v). To this o/w emulsion, solution of NaO-CMCS was added. This was again mixed at 2000 rpm for 30 s. The oil droplet size of o/w emulsion was measured immediately after mixing using optical microscope (Zeiss Axio using 206 the camera unit Axio CamERC5s by ZEN software). Then the o/w emulsion was allowed to saturate and oil droplet size and stability of emulsion was studied periodically for six weeks. The oil droplets size after mixing, after 2 h stability and 6 weeks saturation was also measured by laser diffraction particle size analyzer (Malvern Mastersizer 3000 with Hydro SV unit, UK). The mean droplet diameter at D[4,3] was taken from five replicate measurements as the volume mean diameter, and the magnitude of the droplet size distribution is expressed by the span, defined as

$$\text{Span} = \frac{\text{Dx}(90) - \text{Dx}(10)}{\text{Dx}(50)}$$

where Dx(10), Dx(50), and Dx(90) denote the equivalent volume diameters at 10%, 50%, and 90% cumulative volume. The same procedure was repeated by storing all the solutions at lower temperature (2 °C).

2.6. Effect of pH and salinity on o/w emulsion

The 10 mL of optimized dosage of NaO-CMCS (1 g/L) was used through the study. To the optimized NaO-CMCS solution, solid NaCl (0–350 mg) was added. These solutions were mixed for 5 min with magnetic stirrer (2 mag MIX 15, Germany) and pH was adjusted with 0.01 M HCl using a pH meter (WTW InoLab, Germany). The studied pH were from 5 to 8. Simultaneously, marine diesel and water (1:1 v/v) were mixed at 2000 rpm using a vortex mixture (VWR International) to form the o/w emulsion. To this o/w emulsion, previously prepared NaO-CMCS solution was added separately. These were again mixed at 2000 rpm for 30 s. The emulsions were kept for about one month. All the experiments were carried out at room temperature.

2.7. Recovery of marine diesel

Recovery of oil from the emulsified water has a significance value for the oil spill response. Here both, deionized water and sea water were used for the o/w emulsion (oil:water is 1:1 v/v). The o/w creamy emulsions were prepared as per section 2.5 with different dosage of NaO-CMCS (0.5–5 g/L). The stabilized o/w creamy emulsion was collected and 10 ml calcium chloride dihydrate solution (0.1%) was added to it. The mixtures were kept for 1 h to stabilize naturally. All the experiments were carried out at room temperature in triplicate. The separated oil was collected (using micropipette from Sartorius), weighed and the recovery of the oil (R %) was calculated as follows:

$$R\% = 100 - \left( \frac{C_i - C_r}{C_i} \right) \times 100$$

where C_i and C_r are the initial and recovered amount of oil. Recovered oil was characterized using FTIR and GC-FID. GC-FID parameters are mentioned in Supplementary Material (SM8). The NaO-CMCS crosslinked with calcium was collected and dried, and SEM and EDS analysis were performed (using the same instruments as mentioned earlier in section 2.3).

3. Results and discussion

Chitosan is a multi-nucleophilic polymer due to the presence of the hydroxyl and amino functional groups. Introduction of carbamoylmethyl groups (–CH2COO–) to chitosan, makes Na-CMCS pH-sensitive, so that in acidic pH, this group exists in non-ionized form, whereas in alkaline pH it exists in ionized form, which makes the derivative hydrophilic in nature. Nucleophilic acyl substitution takes places between the free amino groups of the carbamoylmethylated polymer and oleoyl chloride through an addition/elimination type mechanism. The reaction driven towards amide formation by the low reaction temperature, had favors N-acylation over O-acylation (Chiandotti et al., 2010). The sizes of chitosan, Na-CMCS and NaO-CMCS particles are available in the SM1 (Supplementary Material).

3.1. Structural characterization of NaO-CMCS

3.1.1. FTIR analysis

Structural changes of chitosan and its derivatives were confirmed by FTIR spectra. In Fig. 2A, peaks at 3351 cm⁻¹ and 3286 cm⁻¹ express the N–H and O–H group, 2866 cm⁻¹ shows C–H stretching, 1650 cm⁻¹ and 1585 cm⁻¹ indicates C=O stretching of the secondary amide (amide I) and N–H bending of 2-aminoalcohol units, 1150 cm⁻¹, 1060 cm⁻¹ and 1026 cm⁻¹ attributed to asymmetric stretching of the C–O–C stretching and the skeletal vibration of C–O stretching, respectively in chitosan.

![Fig. 2. FTIR spectra of (A) Chitosan (B) Na-CMCS and (C) NaO-CMCS.](image-url)
Fig. 3. $^1$H NMR spectra of (A) Na-CMCS and (B) NaO-CMCS.
Fig. 2B, peaks at 3369 cm\(^{-1}\) and 3289 cm\(^{-1}\) express the N–H and O–H group, 2906 cm\(^{-1}\) and 2840 cm\(^{-1}\) attributes to the C–H stretching, 1724 cm\(^{-1}\) (acid C=O, stretch), which was absent in chitosan conforming carboxymethylation of chitosan. The characteristic bands of Na-CMCS found at 1589 cm\(^{-1}\) (C=O of –COONa asymmetric stretching) had overlapped the N–H peak of chitosan at 1585 cm\(^{-1}\) which corresponds to the previously observed results (Kong, 2012) and 1416 cm\(^{-1}\) (C=O of –COONa symmetric stretching). In Fig. 2C, the 3279 cm\(^{-1}\) indicates secondary N–H, intensified peaks at 2920 cm\(^{-1}\), 2850 cm\(^{-1}\) and 1442 cm\(^{-1}\) attributes to C–H stretching of oleoyl chains. After acylation, the 1585 cm\(^{-1}\) peak of primary amine disappeared, while the prominent band bands at 1560 cm\(^{-1}\) for N–H (amide II) was observed similar to earlier findings (Tien et al., 2003), which indicates the incorporation of oleoyl moiety onto the primary amine of Na-CMCS. The peaks, in the range of 1150–850 cm\(^{-1}\) are the results of vibrations of C–O and C–O–C of the polysaccharide chain also observed in chitosan and Na-CMCS.

3.1.2. \(^1\)H NMR analysis

The \(^1\)H NMR spectra of Na-CMCS and NaO-CMCS are presented in Fig. 3A and 3B, respectively. The spectrum of NaO-CMCS shows the similar characteristic proton signals as can be seen in the spectrum of Na-CMCS which was assigned according to our previous studies (Kalliola et al., 2017). In addition in the spectrum of Na-CMCS, the successful attachment of oleoyl substituent to Na-CMCS can be clearly seen. The triplet (a) (\(J = 6.6 \text{ Hz}\)) at 1.28 ppm is assigned to CH\(_3\) unit of oleoyl chain. Signals of 20 protons of CH\(_2\) units (b) can be observed at around 1.69 ppm. The protons of CH\(_2\) unit (c) next to the carbonyl group appear as a clear triplet with a coupling constant of 7.4 Hz at 2.87 ppm. The protons of CH\(_2\) unit in a position c appear as a multiplet at around 2.01 ppm. The protons of double bond (f) show the peak at 5.72 ppm which corresponds a typical shift of alkene type protons. The protons of CH\(_3\) units (d) next to alkene protons appear at around 2.42 ppm. The assignments are consistent with the previous assignments of a similar type oleoyl amide structure (Niu et al., 2010).

3.1.3. SEM/EDS analysis

The SEM images of raw and treated chitosan are shown in Fig. 4. The surface morphology of chitosan (Fig. 4A and B) has been altered after carboxymethylation (Fig. 4C and D) followed by acylation (Fig. 4E and F). The surface roughness increases in Na-CMCS as compared to that of chitosan. The attachment of hydrocarbon long chain to Na-CMCS, makes the surface of NaO-CMCS rougher compared to Na-CMCS. These alterations on the surface topography were likely to result from the long hydrocarbon chain attached to the Na-CMCS. Thus, chitosan after carboxymethylation and acylation, appears to be rough and more porous. This was further confirmed by the BET analysis by considering the porosity.

Elemental analysis for chitosan, Na-CMCS and NaO-CMCS are shown in Table 1. The increasing C/N ratio from chitosan to its derivatives indicates the addition of carbon atoms to chitosan. The degree of substitution (DS) in Na-CMCS was 1.63, which confirms that the synthesized derivatives might have been substituted after carboxymethylation. Reduction of nitrogen (%) shows that addition of long carbon chain groups took place during the acylation and the DS of 0.51 was obtained. The presence of sodium in EDS results of Na-CMCS and NaO-CMCS, and not in chitosan, concludes that the synthesized derivatives were in the sodium form. The EDS results of Na-CMCS and NaO-CMCS were also taken at 1.0 kV accelerating voltage to confirm the presence of nitrogen as mentioned in Table T1. At 1.0 kV, heavier elements other than oxygen cannot be detected. From the results, it was concluded that the carbon (%) increases from Na-CMCS to NaO-CMCS indicating that the hydrocarbon long chain was attached to the backbone of chitosan after acylation. The C/N ratio of EDS (at 1.0 kV) is also in a good resemblance to the CHNS results.

3.1.4. BET analysis

\(N_2\) adsorption and desorption isotherms at 77.35 K plotted as a function of \(P/P^0\) is shown in Fig. S2 (Supplementary Material). The isothermal curve of NaO-CMCS can be classified as IUPAC type IV showing type H3 hysteresis loop between 0.45 and 0.95, which is associated with the capillary condensation taking places in mesopores. The type H3 hysteresis loop in NaO-CMCS, also reveals to the
and tended to zero (at isoelectric point) at intermediate pH and electrical charge in NaO-CMCS at higher pH was highly negative building up the positive charges. Hence, the magnitude of the NaO-CMCS particle was negative and addition of HCl started ~9.6 with increasing salinity (3.5%). Initially the zeta potential of which shows the presence of free ions in the solutions. Down, but did not reach zero even after the surface was neutralized, almost all Na ions there was a sudden drop in the zeta-potential, which shows that the carboxylate groups (–COONa), being the hydrophilic component of NaO-CMCS, was 19.53 ± 0.54 mN/m and a small addition of NaO-CMCS reduced the IFT by 50%. However, at the critical micelle concentration (CMC), the IFT reduction was up to 70%. The IFT was affected by the increasing concentration of NaO-CMCS (Fig. S3 in Supplementary Material SM3). Thus, the result confirms that the attachment of hydrophobic hydrocarbon chain made NaO-CMCS more amphiphilic, which drastically reduced the interfacial tension. At lower interfacial tensions, the free surface energy associated with the formation of droplets reduces, enhancing the formation of smaller emulsion droplets with greater stability.

3.2. Effect of salinity and pH on surface charge of NaO-CMCS

The influence of salinity and pH on the electrical characteristics and solubility of NaO-CMCS were studied. In order to investigate this effect, the pH of NaO-CMCS solution (1 g/L) was studied with different salinity (0–3.5%) in the pH range (4–10). The initial conductivity of NaO-CMCS solution was 0.670 mS/cm, which shows that the carboxylate groups (–COONa) of NaO-CMCS were ionized easily in water. Increase in conductivity to 54.80 mS/cm at 3.5% salinity (Fig. 5A) confirms that the ionized particles in the water were significantly affected by the addition of the salt. So, there was a formation of a net charge at the surface of NaO-CMCS, which affected the distribution of ions in the surrounding of its interfacial region. This resulted in an increased concentration of Na ions close to the surface forming an electrical double layer around NaO-CMCS. Thus, increasing salt concentration reduces the charged density of NaO-CMCS by altering the interactions between the NaO-CMCS and negatively charged components (Klinkesorn, 2013). On the other hand, the conductivity significantly lowered down, but did not reach zero even after the surface was neutralized, which shows the presence of free ions in the solutions.

The prime and important factor affecting zeta potential is pH. The natural pH of NaO-CMCS solution was ~10.2, and declined to ~9.6 with increasing salinity (3.5%). Initially the zeta potential of NaO-CMCS particle was negative and addition of HCl started building up the positive charges. Hence, the magnitude of the electrical charge in NaO-CMCS at higher pH was highly negative and tended to zero (at isoelectric point) at intermediate pH and then to positive values at lower pH as shown in Fig. 5B. At pH 9, there was a sudden drop in the zeta-potential, which shows that almost all Na ions from the sodium carboxylate group of NaO-CMCS were dissociated by making the surface more negative. The addition of H ions, firstly protonates the free amino groups followed by the protonation of some carboxylate groups causing the increase of zeta-potential up to zero where the amount on positive and negative charges on the surface of NaO-CMCS were either equal or neutralized. The acylated amino groups remained unaffected. Further addition of H ions made the surface more and more positive aligning the deprotonated carboxylate groups to get protonated. The zeta-potential of NaO-CMCS in the presence of electrolyte was observed to increase linearly which states that the process of protonation and deprotonation on the NaO-CMCS surface was not interfered significantly by the salinity variance except the change in the isoelectric point. Therefore, the optimal pH for the formation of emulsion can be in the range of 6–8, were the zeta potential is negative irrespective of the salinity.

3.3. Interfacial tension

IFT between the water phase and oil phase is the key parameter for the formation of oil droplets followed by the stability of oil/w emulsion. IFT was measured by the pendant drop method (Video-based contact angle instrument, Dataphysics OCA 15 EC Series). The initial IFT between these two (water and oil) phases without NaO-CMCS, was 19.53 ± 0.54 mN/m and a small addition of NaO-CMCS reduced the IFT by 50%. However, at the critical micelle concentration (CMC), the IFT reduction was up to 70%. The IFT was affected by the increasing concentration of NaO-CMCS (Fig. S3 in Supplementary Material SM3). Thus, the result confirms that the attachment of hydrophobic hydrocarbon chain made NaO-CMCS more amphiphilic, which drastically reduced the interfacial tension. At lower interfacial tensions, the free surface energy associated with the formation of droplets reduces, enhancing the formation of smaller emulsion droplets with greater stability.

3.4. Emulsion stability

Amphiphilic NaO-CMCS was mixed within the aqueous phase, and when the oil and aqueous phase were combined and mixed, there was an instant formation of oil/w emulsion. Firstly, the oil phase deformed and disrupted into small droplets forming the new interface, which reduced the IFT followed by the adsorption of NaO-CMCS on that interface forming creamy oil/w emulsion. After standing, this creamy emulsion formed a layer at the top, by removing the extra water from the emulsion at the bottom. The stability of this emulsion retained for more than 6 weeks, due to the strong interaction between the non-polar carbon chains (attached to the amino groups) in NaO-CMCS and oil through Van der Waals attractive forces, which had enhanced the oil/w emulsion stability. On the other hand, emulsion was stable when the repulsive interactions dominates, and when the attractive forces dominate, the oil droplets in an emulsion tend to aggregate (McClements and Gurus, 2016). Increasing concentration of NaO-CMCS creates more and more hydrophobic atmosphere surrounding the oil surface and lowers the IFT by creating denser creamy emulsion. Moreover, sodium carboxylate (–COONa), being the hydrophilic part of the polymer, is attached strongly towards the aqueous phase and inhibits the oil/w emulsion to coalescence. Metastable emulsion (8% salinity) formed immediately after mixing (Fig. 6A), and after saturation, creamy emulsion of dispersed phase (Fig. 6C) was obtained. Whereas, in the absence of NaO-CMCS, higher IFT between the water phase and oil phase showed faster phase separation in an hour. This was because the intermolecular forces among the oil phase and aqueous phase were significantly lower than the
intramolecular forces, which makes these phases to be liable to interact themselves but not with each other. Imaging of droplets was conducted periodically using optical microscope. Fig. 6B and 6D are the images of the emulsion after mixing and saturation. The oil droplets sizes were measured weekly (Fig. S4 in Supplementary Material SM4). The average oil droplets size was \( \mu m \) for the room temperature emulsions.

The measured size of NaO-CMCS along with the oil droplets of emulsion by laser diffraction is shown in Fig. 6E. The particle size of NaO-CMCS is 113 \( \mu m \) which was in a good resemblance of the microscopic measured values. The o/w emulsion after mixing shows variety of size range due to the presence of water along with NaO-CMCS and oil. However, by increasing the stability time of emulsion the measured droplet diameter became 19.2 \( \mu m \) after 2 h stability and to 13.4 \( \mu m \) after 6 weeks stability, respectively, which were in a good resemblance with the droplet diameter measured by optical microscope (which was \( \leq 30 \mu m \)). The oil droplet size at Dx (10), Dx (50) and Dx (90) are shown in Table T1 (in Supplementary Material SM4). The particle size >100 \( \mu m \) at Dx (90) for the emulsion after mixing, reveals that the NaO-CMCS was not completely soluble in water. However, insoluble NaO-CMCS being at the interface between aqueous phase and emulsion, shows partial formation of oil droplets on its surface (Fig. S5 in Supplementary Material SM4). The span value measured for the o/w emulsion after mixing is 18.44 showing a wide range of size distribution, as the size of NaO-CMCS were in \( \mu m \). However, the decrease in the span value measured for o/w emulsion after 2 h of mixing and after 6 weeks to 2.05 and 1.70, respectively also favors the stability of creamy o/w emulsion.

The o/w emulsion stability was also studied at lower temperature (2 °C) with different dosages of NaO-CMCS. At lower temperature, the interaction between water and hydrophilic moiety of NaO-CMCS increased, which was seen from the increase of the

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**Fig. 5.** Effect of salinity and pH on (A) Conductivity and (B) Zeta-potential of NaO-CMCS.

**Fig. 6.** o/w emulsion at room temperature (A) after mixing without and with NaO-CMCS (B) Microscopic images of o/w emulsion with NaO-CMCS after mixing (C) after 6 weeks of stabilization without and with NaO-CMCS (D) Microscopic images of o/w emulsion with NaO-CMCS after 6 weeks of stabilization (E) Size distributions of NaO-CMCS, oil droplet from o/w emulsions after mixing, after 2 h and after 6 weeks. Scale bar for microscopic images is 10 \( \mu m \).
mean droplet diameter of the o/w emulsion and similar behavior was also observed earlier (Shinoda and Saito, 1969). Hence, there was a size expansion of droplets in the o/w emulsion at lower temperature compared to that at room temperature (Fig. 7). However, at lower concentration of NaO-CMCS, the coalescence rate showed up a slight separation of oil from the emulsion, but increasing the dosage had slower coalescence rate and made a stable o/w emulsion. Therefore, the mean volume diameter in the emulsion was ≤60 μm for higher concentration of NaO-CMCS (>1.5 g/L), and was extended up to 100 μm for the lower concentration of NaO-CMCS. The peak with >100 μm mean volume obtained for lower dosage might be due to the size of NaO-CMCS particles (Fig. S6 in Supplementary Material SM4). The results conclude that the o/w emulsions were stable at lower temperature with higher concentration of NaO-CMCS.

The changes in emulsified layer, turbid layer, and clear layer were studied with respect to time after mixing (Fig. S7 in Supplementary Material SM4). The height of the emulsified layer almost remained uniform, whereas the turbid layer and clear layer competed with each other. With time span, the intensity of the turbid layer decreased with increase in the clear layer. In the case of an oil spill, this type of the stable creamy emulsion can be skimmed away from the water surface, demonstrating a presence of very small amount of NaO-CMCS in the aqueous phase. Additionally, the stability of the o/w emulsion was verified by shaking it periodically, with the same reformation of creamy layer after few hours as before. Hence, NaO-CMCS was robust to remain stable even in the wave action.

3.5. Emulsion behavior with pH and salinity variance

Ionic strength along with pH, also plays an important role in the enhancement of the stability of o/w emulsion. Hence, the o/w emulsion behavior was studied for the salinity (0–3.5%) in the pH range of 5–8, i.e. before and after the isoelectric point. Increasing concentration of the electrolyte makes the electrically charged double layer near the hydrophilic part of NaO-CMCS narrower. Addition of HCl shows the ion exchange effect among the amino groups and the Na⁺ and H⁺ ions present in the NaO-CMCS and aqueous phase, respectively, by making the hydrophilic part (–COONa) attracted more strongly towards the aqueous phase. The repulsive forces among the hydrophobic long carbon chain attached to the amino groups in NaO-CMCS, increase the area in between the hydrophobic long carbon chain of NaO-CMCS by making its way to interact with the oil. Furthermore, the oil droplets have same electrical charge in the emulsion as they were stabilized by the same stabilizer, showing repulsive electrostatic interaction between the oil droplets in an emulsion (Klinkesorn, 2013) by inhibiting the coalescence. After saturation, it was observed that, NaO-CMCS adsors onto the surface of more than one droplet and links them together by forming bridging flocculation between the NaO-CMCS and emulsion droplets. Decreasing pH with variable salinity tended towards the instability of o/w emulsion. About pH 6–7 with salinity (0–3.5%), the formation rate of bridging flocculation was maximum (Fig. S8 in Supplementary Material SM5) due to slightly negatively charged NaO-CMCS, whereas, at pH 8 only o/w emulsion was observed, and at pH 5 the stability of o/w emulsion suppressed the coalescence because of the positively charged NaO-CMCS. Increasing salinity accelerates the turbidity of the NaO-CS solution and this builds the bridging flocculation between the NaO-CMCS making the emulsion droplets denser.

3.6. Recovery of oil from emulsified oil

The significant step in the oil spill treatment is the recovery of oil. The stable creamy emulsion with deionized water was gained at lower concentration of NaO-CMCS (below CMC), whereas with sea water, this stability was acquired at higher concentration of NaO-CMCS (above CMC) as shown in Fig. S9 (Supplementary Material SM6). One reason for this might be the presence of multiple ions along with sodium ions in the seawater, which has altered the stability of emulsion below CMC of NaO-CMCS by suppressing the coalescence rate. Addition of Ca²⁺ (CaCl₂ solution) to the emulsified oil causes the crosslinking of Ca²⁺ ions with two carboxylic acid groups of the NaO-CMCS. This cross-linking reduces the water solubility of NaO-CMCS through precipitation, which depresses the interaction between the long carbon chain of NaO-CMCS and oil in the o/w emulsion, and pulls out calcium cross-linked NaO-CMCS from the stable emulsion. Hence, stable o/w emulsion breaks, and increasing coalescence rate of the oil droplets concludes that the calcium cross-linked NaO-CMCS lost its property as a stabilizer.

![Fig. 7. Droplet size of o/w emulsion at 2 °C with different dosage of NaO-CMCS measured weekly by optical microscope.](image-url)
Therefore, the recovery of oil was feasible at lower concentrations of NaO-CMCS with deionized water, whereas with sea water it was above CMC of NaO-CMCS due to the instability of o/w emulsion below CMC. The collected oil was weighed and its characteristics compared with those of the initial oil. From the results (Fig. 8A), it was concluded that there was about 75–85% and 19–49% recovery of oil from the emulsified oil using deionized water and sea water, respectively. The recovered oil was characterized using FTIR (Fig. 8B) which shows that the peak intensities at 2854 cm$^{-1}$, 2922 cm$^{-1}$ and 2954 cm$^{-1}$ of C–H stretching, and at 1377 cm$^{-1}$ and 1457 cm$^{-1}$ of C–H bending remained unaltered, concluding that the recovered diesel was identical to the original one. The detailed of FTIR spectra for each concentration are available in Figs. S10 and S11 (Supplementary Material SM7). The recovered oil was also characterized by GC-FID (Supplementary Material SM8). The SEM images (Fig. 8C and D) show the morphological change in the surface of NaO-CMCS after the recovery of the oil. The presence of calcium in the EDS results confirms the crosslinking of Ca$^{2+}$ ions with the two carboxylic acid groups of NaO-CMCS, which in turn reduces the amount of sodium.

Performance comparison between NaO-CMCS and other adsorbents for the removal and recovery of oil is shown in Table 2.

4. Conclusions

The amphiphilic sodium salt of oleoyl carboxymethyl chitosan (NaO-CMCS) was successfully used as a stabilizer for o/w emulsion. NaO-CMCS forms emulsion when mixed with oil, because of the hydrophobic groups in NaO-CMCS, which reached the surface of the solution and decreased the interfacial tension. The o/w emulsion obtained using NaO-CMCS was stable over 6 weeks. The average oil droplets size was <30 μm at room temperature and increased with decreasing temperature. The oil droplets were surrounded by the hydrophobic part of NaO-CMCS making the oil intact by reducing the coalescence of oil droplets. Although the surface area for NaO-CMCS was lower than that of unmodified chitosan, the oil affinity of NaO-CMCS was rather impressive due to

![Fig. 8.](A) Recovery of oil from the emulsified oil using deionized water and sea water with different dosage of NaO-CMCS. (B) FTIR images of oil before emulsion, after recovery of diesel using deionized water with 1 g/L of NaO-CMCS and sea water with 2.5 g/L of NaO-CMCS. (C) and (D) are the SEM images of NaO-CMCS cross-linked with CaCl$_2$ after removal of oil along with the EDS results.

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<tr>
<th>Table 2</th>
<th>Comparison between NaO-CMCS and other adsorbents for the removal or separation of different types of oil.</th>
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<tbody>
<tr>
<td>Adsorbents</td>
<td>Different type of Oils</td>
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<tr>
<td>M. rouxii biomass (Chitosan content – 32.7%)</td>
<td>Mineral oil</td>
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<tr>
<td>Chitosan based polyacrylamide hydrogel</td>
<td>Vegetable oil Cutting oil</td>
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<td>Chitosan microspheres</td>
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<td>Chitosan-coated mesh</td>
<td>Total oil and grease (TOG) in oily water</td>
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<td>Polyurethane foam</td>
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<td>Crude oil in water emulsion</td>
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<td>NaO-CMCS</td>
<td>Marine Diesel</td>
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its porosity. The salinity and pH of water played a significant role in the deformation of oil to oil droplets. NaO-CMCs being charged showed a strong dependence of O/W emulsion on the pH. At lower pH (<5.5), there was a loss of electrostatic repulsion between the charged groups, which decreased the solubility of NaO-CMCs by forming insoluble NaO-CMCs aggregates. Whereas at higher pH (>5.5), the negative charged groups of NaO-CMCs dominated the rate of the formation of bridging flocculation causing o/w creamy emulsion at pH 8. Oil was recovered from creamy o/w emulsion using calcium ions (Ca$^{2+}$), which acts as a cross-linker between the two carboxylic acid groups of NaO-CMCs. The recovery of oil was 76% from deionized water with 0.5 g/L concentration of NaO-CMCs and 20% from sea water with 2 g/L concentration of NaO-CMCs. This derivative of chitosan proves to be an effective material for the oil spill removal from the sea water. Increasing the chain of hydrophobic part through carboxy-acetylation and its effectiveness in O/W emulsion stability should be studied in the future.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jclepro.2017.09.016.

References


