The stability of green nanoparticles in increased pH and salinity for applications in oil spill-treatment

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HIGHLIGHTS

• Only the size of the nanoparticles increased as a function of NaCl concentration.
• Nanoparticles adsorbed at the dodecane/water interface as detected by Cryo-SEM.
• Nanoparticles enhanced the stability of dodecane/water emulsion.

ABSTRACT

The melting of the permanent ice cover in the arctic region due to the climate change is increasing industrial activity in the region and also consequently there is the enhanced risk for oil-spills. Therefore, there is a need for the development of green chemicals for oil-spill treatment in the arctic region. Carboxymethylated chitosan nanoparticles were cross-linked with Mg, Ca or Sr and the stability of nanoparticles in increased pH and salinity was studied. The carboxymethylation of chitosan was investigated with FTIR and the nanoparticles were studied with dynamic light scattering and SEM. The nanoparticles cross-linked with Ca ions were found to be most stable in increased salinity and pH. The interaction of Ca cross-linked nanoparticles with dodecane in water was also demonstrated. The nanoparticles showed promising potential for applications in oil-spill treatment.

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1. Introduction

Industrial activity, including oil production and transport, is increasing in the arctic region due to the climate change and melting of the permanent ice coverage. Increased oil related industrial activity has also increased the probability of oil-spills in the arctic region, which possesses vulnerable and slowly recovering eco-system [1].
Treatment of oil-spills usually involves mechanically removing and recovering oil from the ocean. However, mechanical methods are slow for large oil-spills in severe weather conditions of the arctic region where oil starts to spread quickly. In these cases, chemical surfactants that can be applied from aircrafts may be the most feasible solution for oil-spill treatment [2].

Surfactants can be used to treat oil-spills in two different ways: first, surfactants can be used to disperse the oil into smaller droplets. The surfactant adsorbs on the oil-water interface and reduces the interfacial tension between the two phases stabilizing smaller oil droplets. The reduced size of the oil droplets enhances the biological degradations of the oil by making it more available to natural microbes [3]. Second, surfactants can be used to herd the oil into thicker slicks that can be burned on site [4]. Both of these techniques have been applied to oil-spill treatment using synthetic surfactants. However, there have been concerns about the toxicity of synthetic surfactants for humans and marine ecosystems [5,6]. Therefore, there is a need for more environmentally friendly, green and nontoxic surfactants for oil-spill treatment that could replace the currently used hazardous chemicals and offer a feasible, efficient and sustainable oil-spill treatment method for the arctic environment.

Naturally occurring biopolymer chitosan derived from the shells of crustaceans has been studied for variety of applications since it is abundant, biocompatible, biodegradable, and nontoxic [7,8]. There are a number of applications for chitosan in biomaterials for tissue engineering and drug delivery in nanoparticles. Recently, there have been studies of chitosan for environmental applications in bulk and nanoparticle form [9–13]. For surfactant applications, the disadvantage of chitosan is its insolvency in neutral pH. It is soluble in acidic solutions when pH ≤ 6. The exact pH value depends on the properties of the polymer, such as deacetylation degree of amino groups. The solubility in acidic solutions is due to the protonation of amino groups in chitosan and it can be exploited in the synthesis of chitosan nanoparticles cross-linked with the negatively charged sodium tripolyphosphate (TPP) ion [14]. The synthesis of chitosan nanoparticles cross-linked with TPP in acidic solutions is well studied and also been evaluated for scale-up [15,16]. The disadvantage of chitosan nanoparticles is that they aggregate in neutral and alkaline solutions as a result of the neutralization of amino groups.

Aggregation of the chitosan nanoparticles limits their potential for surfactant applications in many natural waters and especially in sea water where pH varies between 7 and 8.5, approximately. Aggregation results in reduced surface area of the nanoparticles and large aggregates have a higher tendency to sink due to gravitation. Also, in sea water the salinity effects the behavior of surfactants. The salinity in sea water can be nearly 4.0m-% and constitutes mostly of sodium and chloride ions. There is also smaller amounts of other ions contributing to salinity such as K⁺, Mg²⁺, Ca²⁺, Sr²⁺ and SO₄²⁻, for example.

The water solubility of chitosan in near neutral pH can be enhanced with chemical modifications [17]. N,O-carboxymethyl chitosan (CMC) is a water soluble derivative of chitosan and it is also biodegradable and nontoxic [18]. CMC can be synthesized by reacting chitosan with mono-chloroacetic acid in alkaline and heterogeneous conditions [17,19]. In alkaline solutions the reaction favors hydroxyl groups over amino groups and the carboxylic groups are mainly attached to the hydroxyl groups of the chitosan. Deprotonation of the carboxylic groups makes CMC soluble in neutral and alkaline solutions. Carboxylic groups can be also exploited for nanoparticles synthesis with multivalent positive ions such as Ca²⁺ and Cr³⁺ to produce nanoscale particles [20–22]. The negatively charged deprotonated carboxylic groups on the surface of these nanoparticles may provide stable nanoparticles in alkaline solutions due to the electrostatic repulsion between nanoparticles. Ion cross-linked CMC nanoparticles and their stability in varying pH is not as well studied in literature as chitosan nanoparticles and their properties. According to our knowledge, there is no publications on the application of CMC nanoparticles to oil-spill treatment.

In this work, we studied the nanoparticles of CMC cross-linked with Ca²⁺ ions for use as green materials for environmental applications in alkaline and saline solutions. Also, nanoparticles cross-linked with Mg²⁺ and Sr²⁺ ions were studied. These ions were selected since they are naturally occurring in sea water and feasible for large scale production. The ability of CMC-Ca nanoparticles to stabilize dodecane in water emulsions was also studied to evaluate their possible use in oil-spill treatment applications.

2. Materials and methods

2.1. Materials

Chitosan (50–190 kDa, deacetylation degree 75–85%), chloroacetic acid (≥ 99%), MgCl₂·6H₂O (≥ 99%), SrCl₂·6H₂O (≥ 99%), NaOH (≥ 98%) and dodecane (≥ 99%) were purchased from Sigma–Aldrich. NaCl (≥ 99.5%) was purchased from AnaLAR. CaCl₂·2H₂O (≥ 99%) was purchased from Riedel-de Haén. 2-propanol (≥ 99.8%) was purchased from Merck. All chemicals were used without further purification.

2.2. Synthesis of CMC nanoparticles

As-delivered chitosan was carboxymethylated in alkaline conditions with mono-chloroacetic acid to produce a water soluble CMC. The CMC was dissolved in deionized water and nanoparticles were synthesized from CMC solution with ionic cross-linking with Mg²⁺, Ca²⁺ and Sr²⁺ ions. The reactions and cross-linking of CMC by Ca²⁺ ions is illustrated in Fig. 1.

Fig. 1 shows the reaction conditions to carboxymethylated chitosan to form CMC. The reaction occurs mainly at the hydroxyl groups shown in Fig. 1, but also some side reactions occur at the other hydroxyl groups and the amino groups in these reaction conditions [17]. The CMC can be cross-linked by using Ca²⁺ ions that interacts with the carboxylic groups of CMC to form nanoparticles.

2.2.1. Carboxymethylation of chitosan

Chitosan was carboxymethylated based on a previously reported method [17] with a few alterations. One gram of chitosan was added into a flask with 1.35 g of NaOH, 6 mL of water and 24 mL of 2-propanol. The mixture was stirred at 50 °C for 1 h. Oil bath was used for heating. After 1 h, 1.5 g of mono-chloroacetic acid dissolved in 2 mL of 2-propanol was added into the flask dropwise. The reaction was allowed to proceed for 4 h at 50 °C. After 4 h, 25 mL of 70% ethanol solution was added to stop the reaction. The product was washed with ethanol solution with gradually increasing the concentration from 70 to 90%. The product was dried over-night in room temperature.

The product was converted to its acid form by suspending 1 g of the product into a mixture consisting of 100 mL of 80% ethanol and 10 mL of 37% HCl. The mixture was stirred for 30 min in room temperature and then washed with ethanol with gradually increasing the concentration from 70 to 90%. The product was dried over-night in room temperature and characterized with Fourier transformation infrared spectroscopy (FTIR).

2.2.2. Nanoparticle formation

A 0.5 mg/mL solution of CMC was made by dissolving appropriate amount of CMC in water and then adjusting the pH to 7.5 with 0.1 M NaOH. Nanoparticles were formed by adding MgCl₂, CaCl₂ or SrCl₂ solution (1.5 m-%) to 5 mL of prepared CMC solution while stirring in room temperature. Stirring was continued for 30 min.
Three different samples were prepared with each chloride salt by varying the volume of the salt solution from 0.10 mL to 0.30 mL. Samples synthesized with different salt solutions were denoted as CMC-Mg, CMC-Ca and CMC-Sr.

2.3. Characterizations

Carboxymethylation of chitosan was analyzed with FTIR-spectroscopy type Nicolet Nexus 8700 (USA). CMC nanoparticles were characterized with the ZetaSizer Nano ZS apparatus (Malvern Instruments Ltd.). Light scattering and zeta-potential measurements were done in triplicates. The CMC-Ca nanoparticles synthesized with 0.20 mL of CaCl₂ solution were also studied with scanning electron microscopy (SEM) on a Hitachi S-4800 FEG Cryo-SEM operated at 3.0 kV. As-synthesized and diluted (1:1 with water) samples were imaged. Droplets from the samples were placed on the sample holder and allowed to dry in air over night. The samples were carbon coated twice for 9.9 s with Cressington 208 Turbo Carbon Coater before imaging. The carbon coating was done at 4 kV in vacuum (10⁻⁴ mbar). The CMC-Ca nanoparticles synthesized with 0.20 mL of CaCl₂ solution were studied with transmission electron microscopy (TEM) on a FEI Tecnai G² F30 FEG TEM operated at 200 kV. A small droplet of the sample was placed on the grid and dried in air at room temperature before imaging. Also, the elements present in the CMC-Ca nanoparticles were analyzed with X-ray energy dispersive spectroscopy (EDS) on Bruker AXS Microanalysis GmbH operated at 200 kV. The dodecane in water emulsions stabilized by CMC-Ca and native CMC were studied with Cryo-scanning electron microscopy (Cryo-SEM) imaging on a Hitachi S-4800 FEG Cryo-SEM operated at 3.0 kV at −120 °C. One droplet of the emulsion sample was frozen in slushed liquid nitrogen. The frozen droplet was fractured at −130 °C and the solvent was sublimated at −95 °C for 5 min. The sample was sputtered with Pt-Pd composite at 10 mA for 88 s.

2.4. Stability of nanoparticles in increased pH and salinity

The stability of nanoparticles in increased pH was studied by increasing the pH gradually with 0.01 M NaOH and measuring the size and zeta-potential of the nanoparticles. The starting pH was approximately 7.0 for all nanoparticles solutions and the pH was increased to 9.0 or until complete dissolution or aggregation of nanoparticles. The pH was measured with the pH meter and the intensity based size and z-potential were measured with ZetaSizer.

The stability of nanoparticles in increased salinity was studied by measuring the size of the particles as a function of the salinity. The NaCl concentration was increased by dissolving solid NaCl into the sample solution. The sample solution was stirred gently and appropriate amount of NaCl was added to increase the concentration from 0m-% to 4m-%. The sample solution was stirred for 10 min and then the size of the nanoparticles were measured.

CaCl₂ nanoparticles synthesized with 0.20 mL of CaCl₂ solution were studied for their stability as a function of salinity in varying pH. The studied pH values were 7.0, 7.5, 8.1 and 8.5. The pH of the solution was adjusted with 0.01 M NaOH solution. Solid NaCl was dissolved into the sample to adjust the salinity and the size of the nanoparticles were measured.

2.5. CMC-Ca in stabilizing dodecane in water emulsions

CaCl₂ nanoparticles synthesized with 0.20 mL of CaCl₂ solution were studied for their ability to stabilize dodecane in water emulsions. Dodecane was added in 1:3 volume ratio to CMC-Ca sample and the mixture was vortex mixed (Thermolyne Maxi Mix II 37600 Mixer) at 3000 rpm for 10 s to form a dodecane in water emulsion. The average droplet sizes were determined from optical microscope images by manually measuring the diameter of at least 800 droplets, approximately. Nikon Eclipse LV100 microscope with NIS-Elements F 3.0 software was used for imaging. The droplets were further characterized by Cryo-SEM. The stability of emulsions was studied by centrifuging the emulsion at 1000 rpm for 2 min. The separated dodecane was removed and weighed. The amount of separated dodecane correlates with the stability of the emulsion.

3. Results and discussion

3.1. Characterizations of CMC and nanoparticles

3.1.1. FTIR analysis

The FTIR spectra of the untreated chitosan and CMC were analyzed to evaluate the success of the carboxymethylation reaction. The FTIR spectrum of CMC and untreated chitosan used in the synthesis of CMC is shown in Fig. 2.

Fig. 2 shows the characteristic bands of chitosan: 1589 cm⁻¹ (N–H bending), 1149 cm⁻¹ (O–C–O stretching) and 1064 cm⁻¹ (C–O–C stretching). After carboxymethylation, new bands are observed at 1718 cm⁻¹ (−COOH) and a broad band centered around 1614 cm⁻¹ (−NH₂ and −COOH overlapping). Also, another broad band can be
seen centered around 1054 cm\(^{-1}\) (C=O stretching)\(^{[17,23]}\). The FTIR spectra of the untreated chitosan and the reaction product suggest that the carboxymethylation of chitosan was successful. Also, the solubility of the product in neutral and alkaline solutions further indicates the successful carboxymethylation of chitosan.

### 3.1.2 Dynamic light scattering measurements

CMC nanoparticles form in a narrow range of CMC to cross-linking ion ratios, influenced by the molecular weight of CMC and the degree of substitution\(^{[24]}\). nanoparticle formation requires a sufficient concentration of the cross-linking ion, at lower concentrations nanoparticles do not form. If the concentration is increased over the nanoparticle formation range, large aggregates are formed and they sink to the bottom of the reaction vessel. The nanoparticle formation region is narrower at high molecular masses and high amounts of carboxylic groups. Furthermore, the size of the nanoparticles has been found to increase with increasing molecular weight of CMC and slightly decrease with increasing degree of substitution\(^{[24]}\).

The number of carboxylic groups is influenced by the synthesis conditions in the preparation of CMC, such as temperature, water to 2-propanol ratio, and NaOH concentration\(^{[17]}\). High NaOH concentrations in water increase the reaction extent and more carboxylic groups are grafted into the chitosan polymer\(^{[17,24]}\). Since relatively large molecular mass chitosan was used in this study, the amount of carboxylic groups was adjusted to be low by using relatively low NaOH concentration of approximately 18mM in water.

With MgCl\(_2\) and SrCl\(_2\) solutions all resultant CMC nanoparticle solutions were transparent when the volume of the chloride solution was varied between 0.10, 0.20 and 0.30 mL. When CaCl\(_2\) was used, only the volume of 0.10 mL resulted in a transparent solution. Volume of 0.20 mL resulted in an opalescent solution indicating the formation of small aggregates. Volume of 0.30 mL precipitated the CMC in large aggregates. These observations suggest that the interaction of calcium ions with carboxylic groups is stronger than the interaction of magnesium and strontium ions.

The sizes of the synthesized particles were determined by light scattering measurements. The sizes of CMC-Mg and CMC-Sr were nearly constant, approximately 200 nm, and independent of the volume of chloride solution used. CMC-Ca nanoparticles size increased from 200 to 300 nm when the volume of CaCl\(_2\) solution was increased from 0.10 mL to 0.20 mL, respectively. The polydispersity index (PDI) values for CMC-Mg and CMC-Sr were circa 0.3 and only minor variations of the PDI value was observed when the volume of the chloride solution was varied. The PDI values are scaled such that values smaller than 0.05 are highly monodisperse and values higher than 0.7 indicate that the particles have a very broad size distribution. The PDI value for CMC-Ca nanoparticles synthesized with 0.10 mL of CaCl\(_2\) solution was circa 0.15.

The PDI value of decreased to circa 0.1 when the volume of CaCl\(_2\) was increased to 0.20 mL. This value suggests that the particle size distribution is very narrow and this observation is supported by the detected opalescence of the solution. The zeta-potential of all synthesized nanoparticles were \(-10\) mV, approximately. The zeta-potentials were low but on the stable region and no significant changes in the size or PDI value were observed in the synthesized particles over several days at room temperature. For example, after 4 days the average diameter of CMC-Ca increased from 250 to 285 nm and the PDI value decreased from 0.07 to 0.04, and the solution remained opalescent. The stability is probably due to the electrostatic repulsion between nanoparticles induced by the deprotonated carboxylic groups on the surface of the nanoparticles.

### 3.1.3. SEM imaging of the CMC-Ca nanoparticles

A drop of CMC-Ca sample synthesized with 0.20 mL of CaCl\(_2\) solution was placed on the sample holder and the drop was allowed to dry in air at room temperature. The dried sample was coated with carbon and imaged with SEM. The CMC-Ca nanoparticles in dried state are shown in Fig. 3.

Fig. 3A shows the nanoparticles of as-synthesized CMC-Ca separated from the solution by evaporation in room temperature. Fig. 3B shows the nanoparticles of CMC-Ca separated from a diluted sample by evaporation in room temperature. The nanoparticles appear to be closely packed when water is evaporated as shown in Fig. 3A.

In the dried diluted sample, the nanoparticles were less densely packed. The nanoparticles are to some extent spherical and the diameter ranges from 150 nm (Fig. 3A) to over 300 nm (Fig. 3B), approximately. These observations are in agreement with the light scattering measurements since the light scattering measurements are more sensitive to larger particles and thus affect the detected average diameter of the particles. Also, in light scattering measurements, the hydrodynamic radius of the particle is measured which may deviate from the radius in dry state.

### 3.1.4 TEM imaging and EDS analysis of the CMC-Ca nanoparticles

CMC-Ca nanoparticles synthesized with 0.20 mL of CaCl\(_2\) solution were imaged on TEM. A small drop of the sample was placed on the grid and allowed to dry in room temperature. TEM image of CMC-Ca nanoparticles and EDS analysis results are presented in Fig. 4.

Fig. 4A shows CMC-Ca nanoparticles of approximately 100–200 nm in size and the shape is almost spherical. Similarly shaped and approximately same sized nanoparticles were detected with SEM. Fig. 4B shows the EDS analysis that indicates the presence of calcium in the nanoparticles. Chloride is detected due to the use of CaCl\(_2\) in the synthesis of nanoparticles. It is worth noting that the atomic ratio Ca/Cl is about 1.4 in the nanoparticle based on the EDS analysis and in the CaCl\(_2\) compound the ratio Ca/Cl is 0.5. Therefore, the presence of Ca cannot be explained only with the crystallization of CaCl\(_2\) inside the nanoparticle. Carbon and oxygen are detected due to the CMC. Copper is detected due to the grid of the sample holder.

### 3.2 Nanoparticles stability in increased pH and salinity

The CMC-Mg and CMC-Sr nanoparticles were not stable in increased pH nor salinity. The nanoparticles aggregated and or dissolved as pH or salinity was increased (Figs. A.1 and A.2, Appendix). The stability of the CMC-Ca nanoparticles synthesized with 0.10 mL of CaCl\(_2\) solution where studied in varying pH and salinity. The measured size, zeta-potential, and PDI value are presented in Fig. 5.

Fig. 5A shows the behavior of CMC-Ca in varying pH. Some aggregation to larger particles was observed since the PDI value increased slightly as the pH of the solution increased. Also, the average size of the particles increased to 300–400 nm when pH of the solution
reached the value of 7.4 and thereafter the size remained within this range. Fig. 5B shows the zeta-potential of CMC-Ca as a function of pH and the zeta-potential is observed to decrease linearly until saturation at pH 8.5. Fig. 5C shows the size and PDI value of the nanoparticles as a function of salinity. The size was observed to increase slightly and the PDI value increased moderately but remained almost unchanged after the NaCl concentration was set to 1.0m-%. The CMC-Ca nanoparticles showed less changes in their size and PDI value but some aggregation to larger particles occurs when NaCl concentration is increased. This may be explained by the screening of the negatively charged carboxylic ions on the surface of the nanoparticle, which decreases the electrostatic repulsion.
between nanoparticles. The reduction in the electrostatic repulsion increases the hydrophobic interactions and may result in the aggregation of nanoparticles. The CMC-Ca sample showed less changes in size and PDI value compared to the CMC-Mg and CMC-Sr samples. This may be explained by the stronger interaction of calcium ions with the carboxylic groups that results in only partial dissolution of CMC in increased pH. The free CMC polymers may cross-link nanoparticles together via free Ca\(^{2+}\) ions resulting in larger observed particle size. The zeta-potential was observed to decrease linearly which may be explained by the deprotonation of the surface carboxylic groups due to the increased pH. Also, it is possible that CMC dissolves from the nanoparticles and the decreased zeta-potential is partially attributed to the free CMC polymers.

CMC-Ca nanoparticles synthesized with 0.20 mL of CaCl\(_2\) solution were studied for their stability in saline solutions in varying pH, since CMC-Ca proved to be most stable from the studied nanoparticles and the 0.20 mL addition of CaCl\(_2\) formed highly monodisperse particles. The stability of CMC-Ca nanoparticles in varying pH is presented in Fig. 6.

Fig. 6A shows the behavior of CMC-Ca nanoparticles in varying pH. The size and PDI values increase until pH 7.5 and remain nearly constant in higher pH values. The results suggest that the nanoparticles are aggregating probably due to dissolution of the CMC at increased pH. The Fig. 5B shows the zeta-potential of the sample as a function of pH. The zeta-potential is observed to decrease linearly as pH is increased but no saturation of the zeta-potential is observed unlike the zeta-potential of CMC-Ca sample with 0.1 mL of CaCl\(_2\) presented in Fig. 6B, which saturated at pH 8.5. This observation suggests that the CMC-Ca sample with 0.2 mL CaCl\(_2\) is more stable than the sample with 0.1 mL of CaCl\(_2\) and that the decrease is mostly due to the deprotonation of surface carboxylic groups since the size and PDI value remain nearly constant above pH 7.5. Also, part of the decreased zeta-potential may be explained by the dissociation of CMC and with free negatively charged CMC polymers in the solution. The behavior of CMC-Ca nanoparticles synthesized with 0.20 mL of CaCl\(_2\) solution as a function of salinity in varying pH are presented in Fig. 7.

Fig. 7A shows the size and PDI of the CMC-Ca sample with 0.20 mL CaCl\(_2\) solution in pH 7.0. The average size of the nanoparticles increased sharply when the salinity increased from 0 to 4.0m-\%. The PDI value increased nearly constant as the salinity increased up to 4.0m-\%. The PDI value remained nearly constant as the salinity increased from 0 to 4.0m-\%. Fig. 7B shows the behavior of the CMC-Ca with 0.20 mL of chloride solution in pH 7.5. When the salinity increased to 0.5m-\% the size and PDI increased. As the salinity increased, the size of the nanoparticles was observed to increase while the PDI remained nearly constant. Fig. 7C shows the behavior of CMC-Ca in pH 8.1. The PDI value remained nearly constant but the observed size varied considerably. Fig. 7D shows the behavior of CMC-Ca in pH 8.5. This sample also shows nearly constant PDI value but the observed size of the nanoparticles is varying significantly similar to the sample in pH 8.1.

The results at pH 7.0 suggests that the nanoparticles did not aggregate despite the increase in detected average size since the PDI was nearly constant. It appears that the nanoparticles increase in size due to the addition of NaCl. The increase in size may be explained by the diffusion of sodium and chloride ions into the nanoparticles. Similar results of increased size of nanoparticles have been reported for untreated chitosan nanoparticles cross-linked with TPP that were loaded with various metal ions [25]. This result further indicates that the CMC-Ca nanoparticles with 0.20 mL of CaCl\(_2\) are more stable in saline solution than the CMC-Ca nanoparticles with 0.10 mL of CaCl\(_2\) presented in Fig. 5. The difference between the two samples could be explained with the size of the nanoparticles. The average size of CMC-Ca with 0.10 mL of CaCl\(_2\) is circa 200 nm and the average size of the CMC-Ca with 0.20 mL of CaCl\(_2\) is circa 250 nm. The screening of the surface charge of the nanoparticles could cause the aggregation of the smaller particles into larger particles resulting in increased size and PDI value. Also, in larger particles the amount of calcium ions is greater and therefore the nanoparticle is more strongly cross-linked resulting in more stable particles.

The results at pH 7.5–8.5 suggest that the increase in pH made the nanoparticles more susceptible to the salinity of the solution. The stability of the nanoparticles in saline solutions decreased considerably as the pH increased and aggregation was observed in pH 8.1 and 8.5 indicated by the large size and PDI values. This may be explained by the dissolution of CMC in increased pH and the resulting aggregation of nanoparticles. In alkaline solutions the CMC polymer is expected to be highly negatively charged and in linear configuration rather than in twisted configuration due to the intra molecular repulsion along the polymer chain. This favors the decomposition of the nanoparticles in saline solutions since the electric charges are screened by the sodium ions preventing the dissolved CMC to cross-link back into nanoparticles and bend into spherical configuration.

### 3.3 Emulsion stabilizing effect of CMC-Ca

The oil in water emulsion stabilizing ability of CMC-Ca synthesized with 0.20 mL of CaCl\(_2\) was studied by using dodecane as the oil phase. Dodecane was added onto the CMC-Ca solution and the sample was vortex mixed at 3000 rpm for 10 s. The produced emulsion is presented in Fig. 8.

Fig. 8A shows the dodecane and CMC-Ca solution (0.5 mg/mL) emulsion after vortex mixing at 3000rpm for 10 s. The average droplet size for dodecane/CMC-Ca emulsion is 84 μm and standard deviation is 55 μm determined from approximately 800 droplets. Fig. 8B shows the dodecane and CMC solution (0.5 mg/mL) emulsion after vortex mixing at 3000 rpm for 10 s. The average droplet size for dodecane/CMC emulsion is 59 μm and standard deviation is 36 μm.
determined from approximately 1000 droplets. Dodecane/CMC-Ca emulsions and dodecane/CMC emulsions with different concentrations of CMC were studied to compare the stabilizing effect of CMC-Ca and CMC. After the emulsification with dodecane the samples were centrifuged at 1000 rpm for 2 min and the separated dodecane was removed and weighed. The amount of the separated dodecane as a function of CMC concentration is presented in Fig. 9.

Fig. 7. CMC-Ca size and PDI value as a function of salinity in varying pH. (A) Size and PDI value in pH 7.0. (B) Size and PDI value in pH 7.5. (C) Size and PDI value in pH 8.1. (D) Size and PDI value in pH 8.5. (●) size or z-potential, (○) PDI value.

Fig. 8. Dodecane in water emulsion stabilized by CMC-Ca and CMC. (A) Dodecane/CMC-Ca solution (0.5 mg/mL) emulsion. (B) Dodecane/CMC solution (0.5 mg/mL) emulsion.

Fig. 9 shows that the amount of CMC required to stabilize dodecane in water emulsions is lower in the CMC-Ca samples than in CMC samples. In high concentrations the emulsions are stable and only circa 5m-% of dodecane is separated from both CMC-Ca and CMC samples. The stability of the emulsion starts to decrease significantly at concentrations lower than 0.25 mg/mL for CMC samples and for CMC-Ca samples the stability starts to decrease at concentrations lower than 0.05 mg/mL, approximately. Dodecane in pure water emulsion separates completely into two different phases after centrifugation.

These results indicate that the addition of calcium and the formation of particles enhances the emulsion stabilizing properties of the CMC polymer. In high concentrations the emulsion is very stable with or without the addition of calcium. The interaction of CMC-Ca and CMC polymer with dodecane was studied using Cryo-SEM technique. The Cryo-SEM images of dodecane droplets stabilized with CMC-Ca and pure CMC polymer are presented in Fig. 10.

Fig. 10A shows the CMC-Ca particles attached on the surface of a dodecane droplet. From Fig. 10B one can see that the CMC-Ca particles attach to each other forming a net-like structure on the surface of the droplet. Also, individual particles can be seen on the surface of the dodecane droplet. CMC-Ca nanoparticle cluster and individual nanoparticles on the droplet surface is presented in Fig. 10C. Fig. 10D shows dodecane droplets stabilized by CMC polymer. The surface of the droplets is smooth suggesting that the polymer is not attached to the droplets. The surface of the water phase surround-
ing a dodecane droplet is shown in Fig. 10E. The surface is rough suggesting that the polymer is concentrated in the water phase near the interface between the water phase and dodecane. Fig. 10F shows the polymers in the water phase near the dodecane-water interface.

The images in Fig. 10 suggest that the CMC-Ca nanoparticles stabilize the emulsion by attaching to the interface and to each other forming a net-like structure around the dodecane droplet and by providing a barrier between dodecane droplets preventing them from coalescing. The pure CMC is not detected on the surface of the dodecane droplets and this suggests very weak interaction of the CMC with the dodecane. The CMC seems to stay in the water phase and stabilize the emulsion by steric effects that prevent the dodecane droplets from coalescing when the concentration of the polymer is high enough. This is supported by the emulsion stability experiments where decreasing the CMC concentration decreased the stability of the emulsion.

The low zeta-potential of the CMC-Ca nanoparticles, circa −10 mV, may be one factor contributing to the emulsion stabilizing effect of the CMC-Ca nanoparticles, since the repulsion between nanoparticles is weak and the nanoparticles can attach to each other at the water/dodecane interface. Untreated chitosan nanoparticles with similar zeta-potential have been found to stabilize oil in water emulsions [11] and low surface charge of particles has been found to enhance the emulsion stabilizing effect of cellulose particles [26]. In CMC-Ca nanoparticles, there are both hydrophilic and hydrophobic functional groups present. Carboxyl groups, amino groups, and hydroxyl groups contribute to the hydrophilic character of the nanoparticles, while acetylated amino groups contribute to the hydrophobic character. The simultaneous presence of hydrophilic and hydrophobic characteristics on the surface of the nanoparticles may result in partial wetting of the nanoparticles by both the water and oil phase and explain the adsorption of the nanoparticles at the dodecane-water interface.

4. Conclusions

The stability of CMC nanoparticles formed with MgCl₂, CaCl₂ and SrCl₂ was studied at increased pH and salinity. When Mg²⁺ or Sr²⁺ were used to cross-link the CMC polymer into particles, the resultant nanoparticles were unstable in both alkaline and saline solutions. The measurements of the size of the synthesized particles indicated that the nanoparticles decomposed and aggregated when the pH or salinity was increased. Nanoparticles synthesized with CaCl₂ were more stable in the studied conditions and the nanoparticles showed less changes in their conformation. In saline conditions the average size of the CMC-Ca nanoparticles increased due to formation of larger particles due to the reduced electrostatic repulsion between particles. In alkaline solutions the average size of the nanoparticles increased when pH of the solution was increased from 7 to 9 and small amounts of larger aggregates were formed. The stability of CMC-Ca nanoparticles could be enhanced by increasing the concentration of Ca²⁺ ions in the reaction mixture resulting in more monodisperse and larger particles. These nanoparticles were observed to be most stable with an average size of circa 300 nm and PDI value circa 0.1. These nanoparticles were almost completely stable in pH 7 and salinity up to 4.0m-% of NaCl. The nanoparticles were found to increase in size but remained monodisperse, indicating that no aggregation occurred. The potential of CMC-Ca nanoparticles for oil-spill treatment was demonstrated with the ability of CMC-Ca nanoparticles to stabilize dodecane in water emulsions at pH 7. The emulsion stabilizing ability of CMC-Ca was greater compared to native CMC. The nanoparticles were attached on the surface of dodecane droplets while the polymer stays in the water phase as detected by Cryo-SEM technique.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfa.2016.01.011.

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