

Cationization of kappa- and iota-carrageenan – Characterization and properties of amphoteric polysaccharides



Tamara Barahona^a, Héctor J. Prado^{b,c}, Pablo R. Bonelli^b, Ana L. Cukierman^{b,c},
Eliana L. Fissore^d, Lia N. Gerschenson^d, María C. Matulewicz^{a,*}

^a Departamento de Química Orgánica – CIHIDECAR (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428EGA Buenos Aires, Argentina

^b PINMATE – Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428EGA Buenos Aires, Argentina

^c Cátedra de Tecnología Farmacéutica II, Departamento de Tecnología Farmacéutica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113AAD Buenos Aires, Argentina

^d Departamento de Industrias – CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428EGA Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 11 June 2014

Received in revised form 10 February 2015

Accepted 16 February 2015

Available online 5 March 2015

Keywords:

Kappa-carrageenan

Iota-carrageenan

Cationization

Amphoteric polysaccharides

3-Chloro-2-

hydroxypropyltrimethylammonium

chloride

ABSTRACT

Commercial kappa- and iota carrageenans were cationized with 3-chloro-2-hydroxypropyltrimethylammonium chloride in aqueous sodium hydroxide solution. For kappa-carrageenan three derivatives with different degrees of substitution were obtained. Native and amphoteric kappa-carrageenans were characterized by NMR and infrared spectroscopy, scanning electron and atomic force microscopy; methanolysis products were studied by electrospray ionization mass spectrometry. Young moduli and the strain at break of films, differential scanning calorimetry, rheological and flocculation behavior were also evaluated; the native and the amphoteric derivatives showed different and interesting properties. Cationization of iota-carrageenan was more difficult, indicating as it was previously observed for agarose, that substitution starts preferentially on the 2-position of 3,6-anhydrogalactose residues; in iota-carrageenan this latter unit is sulfated.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Cationic polysaccharides, mainly starch and cellulose derivatives, have found very wide applications. Among others, they are employed as effective flocculants over a wide range of pH being very useful for sustained organic and inorganic matter in wastewater carrying negative charges. They are also used as wet-end additives in paper making, controlling flocculation, retention and paper strength. Introducing a cationic group in starch gives good mineral binding properties; this is required for anchoring the mineral to the fibers. Cellulose derivatives are employed in industries concerned with oilfield treatments, medical products, cosmetics, etc. (Kavaliauskaitė, Klimaviciute, & Zemaitaitis, 2008; Radosta et al., 2004; Song, Sun, Zhang, Zou, & Zhang, 2008). In these investigations several cationic groups have been linked to a polysaccharide

backbone (amino, imino, ammonium, sulfonium, or phosphonium groups) (Auzély-Velty & Rinaudo, 2003; Rinaudo, 2008).

One of the most common reactions used for modification involves the etherification with 2-hydroxy-3-(*N,N,N*-trimethylammonium)propyl groups (Prado & Matulewicz, 2014). This reaction can be carried out with 2,3-epoxy-trimethylammonium chloride (EPTAC). As EPTAC is unstable, usually this reagent is prepared *in situ* from 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC). Even though the latter reagent has been widely used for the cationization of starches and celluloses, there are only a few reports on its utilization with seaweed polysaccharides.

In a previous paper we reported the cationization of commercial agarose with CHPTAC and the influence of experimental parameters on different properties of the obtained derivatives (Prado, Matulewicz, Bonelli, & Cukierman, 2011a). Flocculation performances of two derivatives of different degree of substitution (*DS*) were evaluated showing a comparable behavior to that of commercial polyacrylamides used for water treatment (Prado, Matulewicz, Bonelli, & Cukierman, 2011b).

* Corresponding author. Tel.: +54 11 4576 3346; fax: +54 11 4576 3346.
E-mail address: cristina@qo.fcen.uba.ar (M.C. Matulewicz).

In recent years, there has been an increase in interest in the preparation of cellulose and starch derivatives containing both acidic and basic groups of different strengths in the chain. Thus amphoteric polysaccharides were obtained by etherification of anionic carboxymethylcellulose (CMC) with CHPTAC (Hebeish, Higazy, Shafei, & Sharaf, 2010; Zhang, 2001) or by reaction of starch with EPTAC and then with succinic anhydride (Lekniute, Peciulyte, Klimaviciute, Bendoraitiene, & Zemaitaitis, 2013) or with CHPTAC/EPTAC and sodium phosphates (Lin et al., 2012; Zhang, Zhang, Ju, & Yang, 2005). Recently, the cationization of heparin with CHPTAC has been reported (Šimkovic, Mendichi, Kelnar, Filip, & Hricovíni, 2015). The grafting of cationic monomers onto CMC with various initiation systems has been also investigated. For example CMC was used to graft with 2-(dimethylamino)ethyl methacrylate in aqueous medium using ammonium persulfate and *N,N,N,N'*-tetramethylethylenediamine as the initiating system (Zhang & Tan, 2000). Amphoteric polysaccharides have found various and different applications in: papermaking, flocculation, wastewater and oilfield drilling treatments, adsorption of dyes and heavy metal ions, cosmetics, etc. (Zhang, 2001; Zhang et al., 2005).

Herein, we report the cationization of native sulfated polysaccharides (kappa- and iota-carrageenan) with CHPTAC and characterization of the amphoteric derivatives.

2. Materials and methods

2.1. Materials

Kappa-carrageenan (Gelcarin PC-911, batch No. 30824049, water viscosity at 1.5%, 75 °C and 30 rpm on a Brookfield Viscosimeter 29 mPa s) and iota-carrageenan (Gelcarin GP-379, batch No. 30522040, water viscosity in the same conditions 26 mPa s) were provided by Productos Destilados S.A.I.C/FMC Biopolymer Corp. Number-average molecular weight of both polysaccharides was determined using the colorimetric method of Park and Johnson (1949): molecular weight of kappa-carrageenan 124,100 Da, molecular weight of iota-carrageenan 270,900 Da. The concentration of counterions in both carrageenans was determined by flame atomic absorption spectrometry using a Shimadzu AA 6800 equipment (Tokyo, Japan). Kappa-carrageenan: (%) Na⁺ 0.41, K⁺ 2.40, Ca²⁺ 3.40, Mg²⁺ 0.21; iota-carrageenan: (%) Na⁺ 0.93, K⁺ 2.50, Ca²⁺ 2.40, Mg²⁺ 0.20.

The cationizing reagent was (3-chloro-2-hydroxypropyl)trimethylammonium chloride 60% w/w aqueous solution (Sigma–Aldrich, Inc., USA). For the flocculation tests, USP type kaolin from Sigma–Aldrich Inc. was employed. All the other reagents were of analytical grade.

2.2. Methods

2.2.1. Synthesis of cationized carrageenans

The cationization of the polysaccharides was performed according to the method described by Prado, Matulewicz, Bonelli, and Cukierman (2011a). In brief, 1.333 g of kappa-carrageenan (molecular weight of the average unit, 204 Da) and 1.667 g of iota-carrageenan (molecular weight of the average unit, 255 Da), corresponding to 6.53 mmol of the average unit were dissolved in water and the final volume of reaction was 100 mL. The reaction time was 2 h at 60 °C. The concentrations of CHPTAC and NaOH were varied (Table 1). After the solution was neutralized with 1 M HCl, the mixture was dialyzed (molecular weight cutoff of 6000–8000 Da) against 0.5% NaCl and then distilled water, and freeze dried.

Table 1

Molar concentrations of reagents added and degree of substitution determined by elemental analysis.

Samples	NaOH (M)	CHPTAC (M)	DS
K1	0.30	0.15	0.21
K2	0.60	0.30	0.31
K3	0.90	0.60	0.41
I1	0.60	0.30	0.11

2.2.2. Elemental analysis

Elemental analyses were performed on an EAI Exeter Analytical, Inc. CE-440 apparatus (USA). These were carried out by triplicate and the mean values are reported.

Sulfate content was also analyzed by the turbidimetric method of Dodgson and Price (1962).

The degree of substitution was determined from the nitrogen content (%N). The formula used adapted to kappa-carrageenan, molecular weight of the average unit 204 Da, was:

$$DS = \frac{204 \times \%N}{1400 - (152 \times \%N)}$$

For iota-carrageenan, the molecular weight of its average unit (255 Da) was employed.

2.2.3. FT-IR infrared spectroscopy

FT-IR spectroscopy was performed in a PerkinElmer Spectrum BX II FT-IR Spectrophotometer (PerkinElmer Inc., USA) employing the KBr disc method; the range measured was 4000–650 cm⁻¹, and 32–64 scans were taken with a resolution of 2–4 cm⁻¹.

2.2.4. NMR spectroscopy

The spectra of the polysaccharides (20 mg) were recorded in D₂O (0.7 mL), after isotopic exchange (3 × 0.5 mL). Spectra were recorded at room temperature on a Bruker Avance II 500 MHz spectrometer (Karlsruhe, Germany). For 500 MHz ¹H NMR experiments, the parameters were: a spectral width of 7.5 kHz, a 30° pulse, and acquisition time of 4.37 s; for 8 scans. For 125 MHz ¹³C NMR experiments, the parameters were: a spectral width 30.9 kHz, a 45° pulse for K1 and 60° for K2, an acquisition time of 0.55 s, and a relaxation delay of 0.30 s; for 64,000 scans. In all cases, signals were referenced to internal acetone at 2.21 ppm for ¹H NMR and 31.1 ppm for ¹³C NMR experiments, respectively.

The degree of substitution was also determined by means of ¹H NMR (*DS*_{NMR}). *A* is the area at the indicated chemical shift:

$$DS_{NMR} = \frac{A_{3.21}}{(A_{5.08} + A_{5.11}) \times 18}$$

2.2.5. ESI mass spectrometry

Derivatization of the analyte prior to its analysis by electrospray ionization (ESI) mass spectrometry was performed by methanolysis: samples (20 mg) were treated with 3 mL of methanol–HCl (1.5 M) at 100 °C for 3 h. The solvent was removed with dry air and the products were left in a vacuum desiccator overnight in order to assure its dryness.

ESI mass spectra were obtained in the positive-ion mode using a Bruker Daltonics micrOTOF-Q II ESI-Qq-TOF mass spectrometer (Bremen, Germany). N₂ was used as both drying and nebulizing gas and the electrospray capillary was set at 4.5 kV. The mass scan was in the range of *m/z* 50–950.

2.2.6. Scanning electron microscopy

Samples were previously platinum sputtered under argon atmosphere. Scanning electronic microscopy (SEM) was performed in a Zeiss DSM 982 Gemini microscope (Carl Zeiss, Munich, Germany) equipped with a field emission gun (FEG) and an in-lens secondary

electrons detector (SE). Acceleration voltage was 4 kV. Magnification ranges were between 10 \times and 15,000 \times .

2.2.7. Atomic force microscopy of native and modified carrageenan films

For the preparation of the films, 10 mL samples of 1.5% polysaccharide solution were placed in polycarbonate Petri dishes (5 cm internal diameter) and evaporated by air convection at room temperature.

The surface topography of the films was studied with an atomic force microscope-AFM (NanoScope IIIa, Quadrex, Digital Instrument, Veeco (Bruker), Melville, New York, USA) provided with a silicon-cantilever of 40 N/m-elastic constant operating at 300 kHz of resonance frequency. Scan size was set to obtain 2.0 nm \times 2.0 nm-images. Three areas of each film were scanned using the tapping mode under nitrogen. 3D images were obtained with the software WSxM 4.0 Develop 11.3-Package (2007, Nanotec Electronica, Madrid, Spain) which was also used for image analysis (Horcas et al., 2007).

2.2.8. Mechanical properties of native and modified carrageenan films

The tensile stress of films prepared from aqueous solutions of the native and modified polysaccharides were measured, at 25 °C and at relative humidity of 40%, with a universal testing machine (Instron 3345-5KN, Instron Co., Ltd. USA) with a 100 N load cell by using a rectangular shaped specimen of: 25 \pm 2 μ m thickness, 70 mm length and 10 mm width. The specimens were tested with an initial grip distance of 30 mm and a crosshead speed of 1 mm min⁻¹ at room temperature. Twenty specimens were analyzed for each sample, and then their values were averaged.

2.2.9. Thermal analysis

Before thermal analysis the samples were dried overnight under vacuum in a desiccator with CaCl₂. A TG-DSC SDT Q600 (TA instrument, Delaware, USA) simultaneous thermal analyzer was used. Nitrogen was employed as purge at a flow rate of 100 mL min⁻¹ and indium was used to calibrate enthalpy and temperature values. Experiments were carried out with 5 mg of sample in an open aluminum oxide crucible. The heating rate was 10 °C min⁻¹ from room temperature to 600 °C.

2.2.10. Rheological characterization

Studies were performed in a rheometer (Paar Physica MCR 300, Anton Paar GmbH, Austria) equipped with 2.5 cm diameter serrated parallel plate geometry (PP25/S). Temperature was maintained constant with a Peltier system. A gap size of 500 μ m was set and data points were recorded at steady state. All measurements were performed in duplicate. For the flow and dynamic assays 1.5% (w/v) aqueous systems of the native and derivatized carrageenans were used.

2.2.10.1. Flow behavior. The flow properties were obtained at 25 °C at shear rates of 0.01–300 s⁻¹. The power law and Herschel Bulkley equations were considered to fit data (Launay, Doublier, & Cuvelier, 1985; Wang & Cui, 2005).

2.2.10.2. Dynamic assays. All samples were rheologically characterized after setting for 30 min. Sample aliquots were placed onto the rheometer's plate and measurements were performed at 25 °C after 20 min of equilibration.

Amplitude (stress versus strain) sweeps were performed at constant frequency (1 Hz) in order to determine the linear viscoelastic region for each system. Frequency sweeps were performed at 25 °C in the region of $\omega = 0.1$ –500 rad/s⁻¹ at a strain amplitude within the

linear viscoelastic region (Fissore, Rojas, Gershenson, & Williams, 2013).

2.2.11. Melting and gelling temperature determination

Gelling temperatures were determined in duplicate by cooling 1% (w/v) hot sample solution in 0.1 M KCl with a temperature drop of about 0.5 °C min⁻¹. Melting temperatures were determined in duplicate by heating the same samples with a temperature increase of about 0.5 °C min⁻¹ (Murano et al., 1992).

2.2.12. Flocculation assay

Flocculation efficiency was obtained from absorbance measurements, according to the method described by Prado, Matulewicz, Bonelli, and Cukierman (2011b). In brief, 10 flasks of 50 mg of kaolin in 50 mL of Tris/HCl buffer (0.05 M Tris(hydroxymethyl)aminomethane, 0.03 M HCl) of pH 8.0 were prepared. The test was performed with one flask at a time. The solution was agitated for 5 min in a vortex stirrer to suspend the kaolin. Different concentrations of polysaccharides were used (0–10 mg of polysaccharide per gram of kaolin). Then, stirring was repeated for another 5 min. A portion of the sample was immediately poured in a rectangular spectrophotometer cell (1 cm optical path) and its turbidity at 500 nm was registered at time 0 and once a minute, for 15 min (Jenway 6505 UV-Vis Spectrophotometer; Staffordshire, UK). Additionally, control flasks were prepared and treated in the same way that the other samples except for the addition of the flocculating agent. The residual turbidity (RT) was calculated as follows:

$$RT = \frac{A_t}{A_0} \times 100$$

where A_t is the absorbance of the sample at 500 nm at different times, and A_0 , the absorbance of the sample at 500 nm at the beginning of the test. This test was performed at 25 °C by duplicate.

3. Results and discussion

Kappa-carrageenan and iota-carrageenan are sulfated polysaccharides extracted from red seaweeds. Kappa-carrageenan is constituted by alternating 3-linked β -D-galactose 4-sulfate and 4-linked 3,6-anhydro- α -D-galactose; in iota-carrageenan the α -unit is sulfated in the 2-position (Usov, 2011).

Commercial kappa- and iota-carrageenans were treated with NaOH-CHPTAC and Table 1 shows the different molar concentrations of CHPTAC and NaOH in each of the experiments. All the experiments were carried out at 60 °C and the reaction time was 2 h. In all of them, an excess of NaOH respect to CHPTAC was used; this is because an equivalent of NaOH is necessary for generating the epoxide EPTAC. In addition, NaOH is also required in order to ionize the hydroxyl groups of the polysaccharide.

In Table 1 it is clearly observed that even though the conditions of the experiments carried out with iota-carrageenan were the same as those employed for K2, a very low degree of substitution (DS) of 0.11 was obtained for I1. This result is consistent with our previous observation (Prado, Matulewicz, Bonelli, & Cukierman, 2011a) that in the cationization reaction of agarose, substitution starts preferentially on the 2-position of 3,6-anhydrogalactose residues and then the proportion of substituted galactose begins to increase. In iota-carrageenan the 2-position of the α -unit is sulfated and cationization of the β -unit is more difficult, leading to a lower DS. Fig. 1 shows a general structure for substituted kappa- and iota-carrageenans.

In order to confirm that the 2-hydroxy-3-(*N,N,N*-trimethylammonium)propyl groups were linked to the polysaccharide chain, native and modified carrageenans were subjected to methanolysis. The ESI mass spectra, in the positive-ion mode showed for all

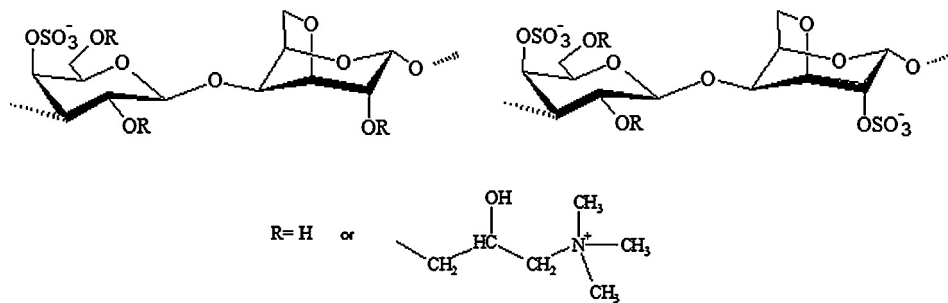


Fig. 1. Cationization reaction of kappa- and iota-carrageenan with CHPTAC.

the kappa derivatives important peaks at m/z 310 [monosubstituted methylgalactoside]⁺ and m/z 292 [monosubstituted methyl 3,6-anhydrogalactoside]⁺ or [monosubstituted methylgalactoside - H₂O]⁺, and a small peak at m/z 324 [monosubstituted dimethylacetal of 3,6-anhydrogalactose]⁺. In the iota derivative I1, as expected, an intense signal was detected at m/z 310 and a very small signal at m/z 292. This strongly suggests that, for kappa-carrageenan, the signal at m/z 292 is mainly due to [monosubstituted methyl 3,6-anhydrogalactoside]⁺.

In the FT-IR spectrum of native kappa-carrageenan characteristic bands appeared at 1240 cm⁻¹ (sulfate), at 930 cm⁻¹ (3,6-anhydrogalactose), and 840 cm⁻¹ (sulfate on C-4 of galactose) (Matsuhira, 1996). After cationization, an additional band at 1490 cm⁻¹ (asymmetric bending mode of the N-CH₃ of the ammonium group) and not present in the native kappa carrageenan was detected in K2 and K3 (Viana, da Silva, & Pimentel, 2012).

Cationized kappa-carrageenans K1 and K2 were analyzed by ¹H and ¹³C NMR spectroscopy. In the ¹H NMR spectra of K1 and K2 (Fig. 2) a strong signal at 3.21 ppm, corresponding to the methyl groups linked to the quaternary nitrogen in the substituent, was observed. Taking into account the area at 3.21 ppm of the methyl groups, the area at 5.08 ppm of the anomeric proton of 3,6-anhydrogalactose and the area at 5.11 ppm of the anomeric proton of 3,6-anhydrogalactose substituted at C-2, it was possible to calculate, using the formula shown in the experimental, the DS of the derivatives K1 (0.25) and K2 (0.33) (Prado, Matulewicz, Bonelli, & Cukierman, 2011a; Usov, 1984).

Cationized kappa-carrageenans K1 and K2 were also analyzed by ¹³C NMR spectroscopy. Fig. 2 shows for K1 signals that are in agreement with those described previously for kappa-carrageenan (Usov, 1984), together with those corresponding to the cationic substituent (C-7, 73.4 and 73.6 ppm; C-8, 66.2 ppm; C-9, 68.4 ppm; C-10, 55.0 ppm) (Prado, Matulewicz, Bonelli, & Cukierman, 2011a). As it was previously reported, the signal of C-7 seems to be split due to different environments/positions of substitution. In K2, with higher DS than K1, the signals corresponding to the substituent are also increased, and those belonging to the repeating unit of kappa-carrageenan decreased. In addition in the spectrum of K2, a new resonance at 96.3 ppm next to the signal at 95.1 ppm of 3,6-anhydrogalactose is detected; this may be assigned to substitution on C-2 of this unit. No spectral analysis of I1 was carried out due to its high viscosity in water; regarding K3, its solubility in water was low.

Fig. 3 shows scanning electron microscopy (SEM) images of native kappa- and iota-carrageenans and of the corresponding cationized derivatives. It can be observed that kappa-carrageenan shows a fibrillar-like structure. K1 and K2 have similar characteristics with smooth surface appearance. In K3, a reticular arrangement is observed. Native iota-carrageenan and its derivative I1, afforded different images, despite the low DS of I1; the native carrageenan gave particles of irregular shape while for I1 a more laminar surface was observed.

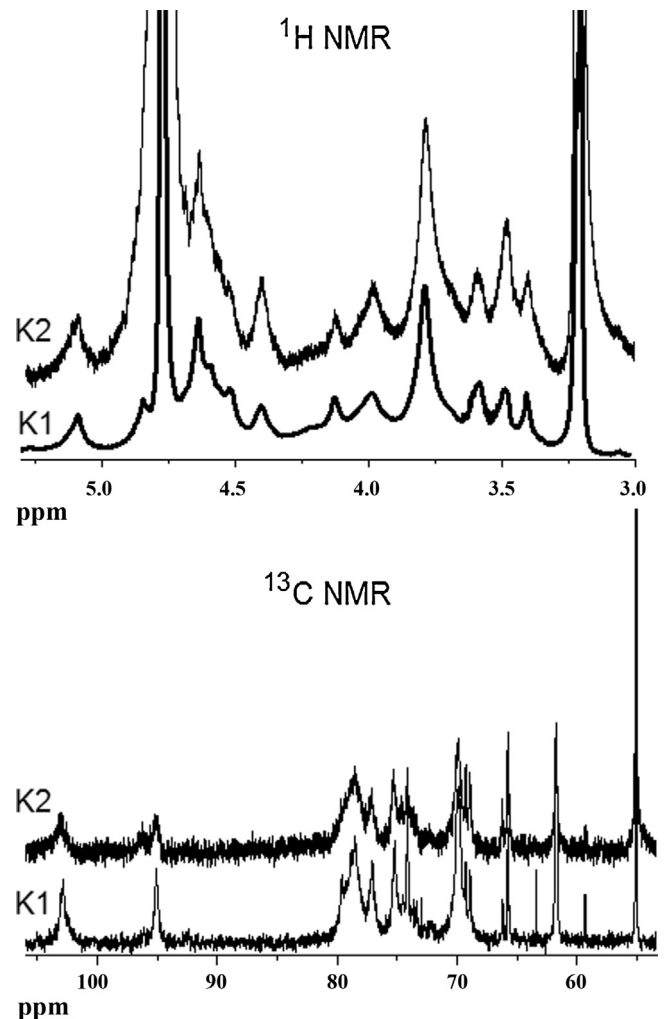


Fig. 2. ¹H and ¹³C NMR spectra of K1 and K2.

Atomic force microscopy (AFM) is now being used to obtain images of dispersed molecules and molecular assemblies of polysaccharides (Funami, 2010). Fig. 4 shows the images of the native and modified polysaccharides. For native kappa-carrageenan, fibers constituted by aggregated chains together with single chains were observed. For K1, the images were similar but lower packing was found, suggesting that the cationic substituent precluded association between the different strands. In K2, chains exhibited a new order probably due to electrostatic crosslinking. For K3, AFM images showed bundles of folded chains possibly as a result not only of intermolecular but also intramolecular interactions. Native and cationized iota-carrageenans (images not shown)

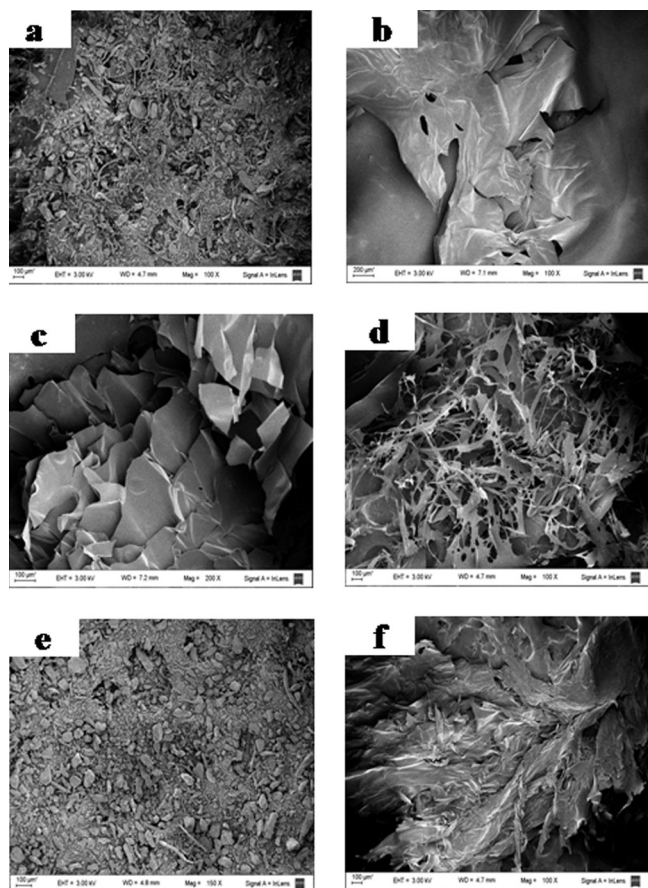


Fig. 3. Scanning electron micrographs of (a) kappa-carrageenan (100 \times), (b) K1 (100 \times), (c) K2 (200 \times), (d) K3 (100 \times), (e) iota-carrageenan (100 \times), and (f) I1 (100 \times).

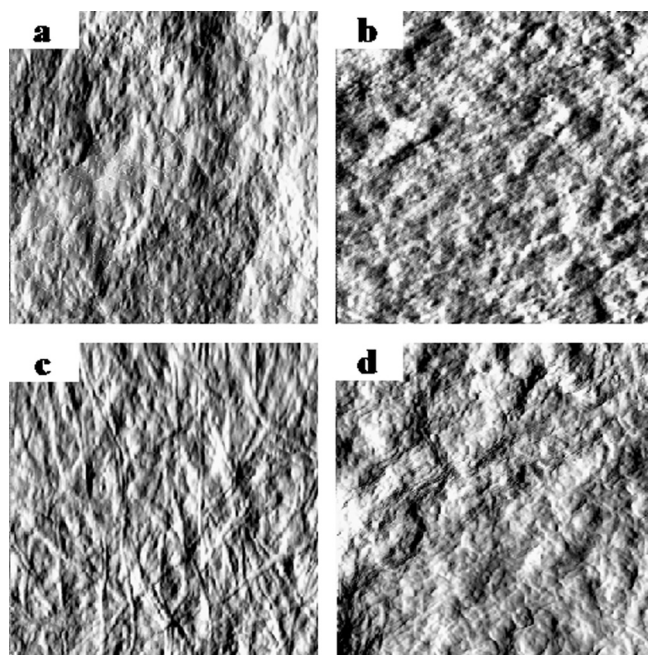


Fig. 4. Atomic force microscopy, scan size $2 \times 2 \mu\text{m}$, of (a) kappa-carrageenan, (b) K1, (c) K2, and (d) K3.

gave similar images due to the low substitution degree of the derivative; fibers and single chains adopted a less ordered packing but alike to that of kappa-carrageenan.

The mechanical tensile properties of carrageenans changed sensibly as a result of the cationization process. The strain at break values increased and the Young moduli decreased compared to those of native carrageenans. Results are presented in Table 2. In the case of kappa-carrageenan, strain at break values increased more than twofold for K1 and more than fivefold for K2; values for K3 increased more than threefold. Native iota-carrageenan showed a higher strain at break value than native kappa-carrageenan. Cationization reaction increased strain at break value of iota-carrageenan by approximately 64%. Young moduli values of K1 and K2 were almost a half of that of native kappa-carrageenan and Young modulus of K3 was even lower. Native iota-carrageenan presented lower Young modulus value than native kappa-carrageenan as reported earlier (Briones et al., 2004). Young modulus reduction of I1 compared to that of native iota-carrageenan was around 26%. Results from K3 do not fully follow the same trend than those of K1 and K2 as no homogeneous films were obtained with the K3 due to its DS and low solubility. This low solubility was evident during solution preparation and the macroscopic appearance of K3 films was granular and translucent instead of transparent as the other films.

Mechanical results could be rationalized conceiving the polysaccharide chains interacting through multiple hydrogen bonds in the native carrageenan films (Hennink & van Nostrum, 2002). In the cationized carrageenans, amphoteric products are obtained as a result of the original sulfate groups and the introduced quaternary ammonium groups. Cationic and anionic groups in the polysaccharide lead to ionic crosslinking among chains that increases with increasing DS. The presence of the cationic groups and thus of ionic interactions, would diminish hydrogen bonding with respect to native carrageenans. The resulting derivatives would have a more open matrix with less but stronger crosslinking, leading to higher deformation (higher strain at break values) and lower Young moduli than the native carrageenans.

The exothermic peak of kappa-carrageenan decomposition was detected at a temperature of 229 °C, K1 decomposition temperature was 218 °C, with increasing temperatures associated to increases in the cationic group DS for K2 (231 °C) and K3 (248 °C). A similar effect was observed in the thermal decomposition of iota-carrageenan (197 °C) and its derivative I1 (210 °C). The lower temperature of decomposition observed in K1 could be related to the partial disorganization of the hydrogen bonded crosslinked polymer structure and to the incomplete reorganization of a new ionic crosslinked structure, as this derivative has the lowest cationic group DS among the kappa carrageenan derivatives.

Rheological characterization of native and cationized carrageenans was carried out in aqueous solutions. All the derivatives showed pseudoplastic behavior and flow data for most of them fitted the power law model, except for kappa-carrageenan and K3 which showed a minimum shear stress (τ_0) and their flow behavior adjusted to the Herschel Bulkley model (Table 3) (Launay et al., 1985; Wang & Cui, 2005).

Table 3 also indicates that cationization of kappa-carrageenan led to the loss of τ_0 in K1 and K2 and a very small value was determined for K3. In addition, all the derivatives showed a lower consistency index (k) than the native polysaccharide and variations in the pseudoplasticity index (n), being the n value higher for K1 (0.857), the derivative with lower pseudoplastic behavior. These results may be explained taking into account that, for K1, introduction of the cationic group may affect packing of the chains by hydrogen bonding with respect to native carrageenan; in K2 order is reestablished due to intermolecular electrostatic interactions between the sulfate and ammonium groups. The behavior of K3 is different possibly due to an increase of intermolecular and

Table 2
Mechanical properties of films of native and modified carrageenans.

Sample	Young's modulus (mPa)		Strain at break (%)	
	Mean	Standard deviation	Mean	Standard deviation
Kappa-carrageenan	1777	204	1.87	0.53
K1	969	104	4.46	0.56
K2	958	137	9.62	0.93
K3	754	74	5.93	0.67
Iota-carrageenan	1086	128	11.28	1.33
I1	887	109	18.49	1.90

Table 3
Rheological behavior of native and modified carrageenans and melting and gelling temperatures in 0.1 M KCl.^a

Sample	τ_0 (Pa)	k (Pa s ⁻ⁿ)	n	R^2	Melting (°C)	Gelling (°C)
Kappa-carrageenan	77.59 ± 4.29	74.48 ± 5.23	0.43 ± 0.02	0.951	76	50
K1	–	0.136 ± 0.009	0.857 ± 0.014	0.991	52	37
K2	–	34.92 ± 0.66	0.212 ± 0.005	0.918	56	≈20
K3	0.819 ± 0.199	6.58 ± 0.25	0.38 ± 0.01	0.981	62	≈36
Iota-carrageenan	–	44.05 ± 0.42	0.135 ± 0.002	0.921	60	48
I1	–	73.14 ± 0.63	0.122 ± 0.002	0.921	58	43

^a τ_0 : minimum shear stress; k : consistency index; n : pseudoplasticity index; R^2 : determination coefficient.

intramolecular electrostatic interactions which determined a lower k than the one observed for K2, and a slight decrease in pseudoplasticity (higher n).

The modified iota-carrageenan has a very low DS (0.11), even lower than that of K1, but it has a consistency index considerably higher than that of native iota-carrageenan. In

iota-carrageenan, cationization may only take place on the β -D-galactose unit suggesting that the presence of the substituent on this residue confers stabilization to the system, in opposition to what is observed when the substituent is linked to the 3,6-anhydro- α -D-galactose, in the case of K1 (derivative of kappa-carrageenan).

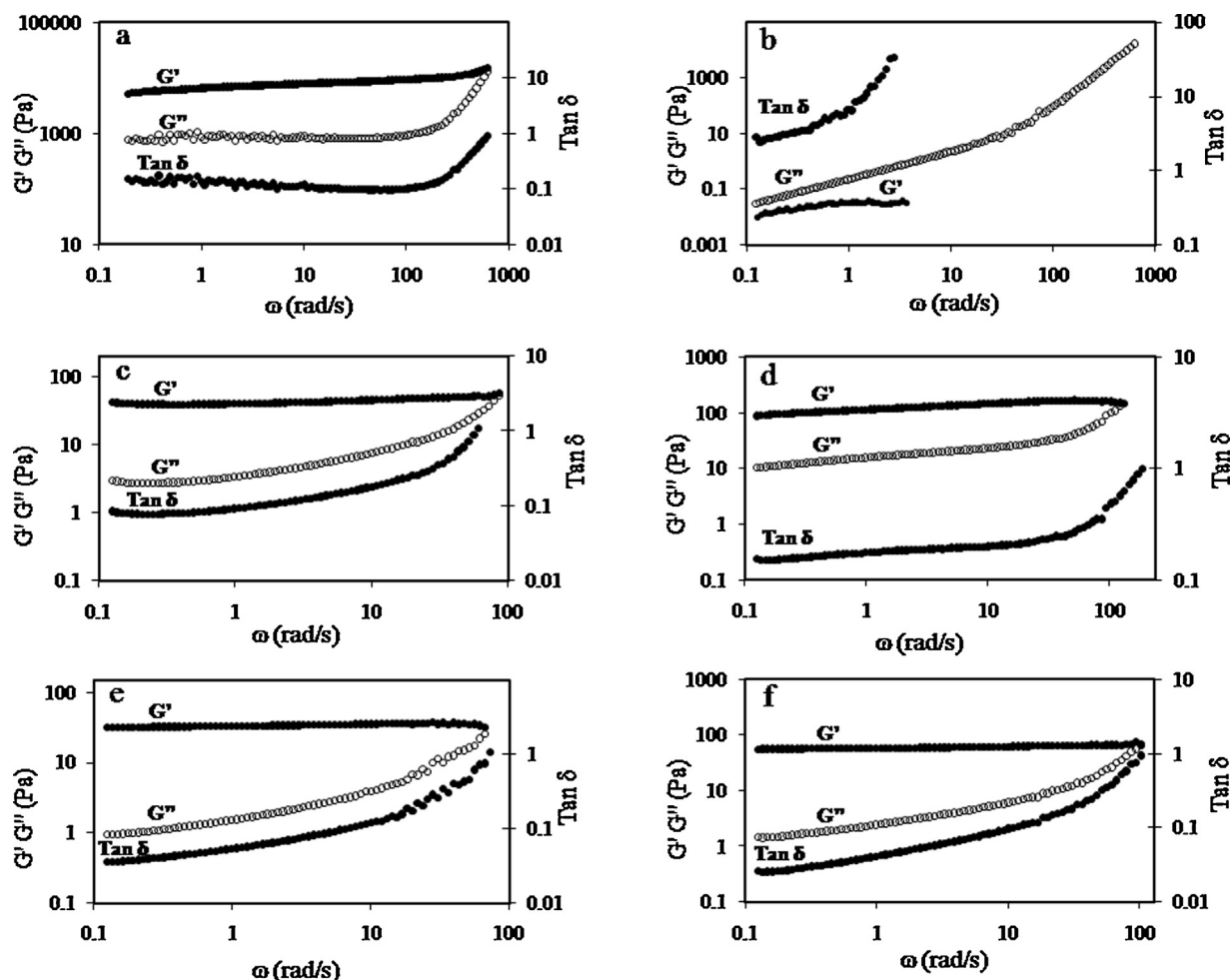


Fig. 5. Elastic (G') and loss (G'') moduli, and $\tan \delta$ of (a) kappa-carrageenan, (b) K1, (c) K2, (d) K3, (e) iota-carrageenan, and (f) I1.

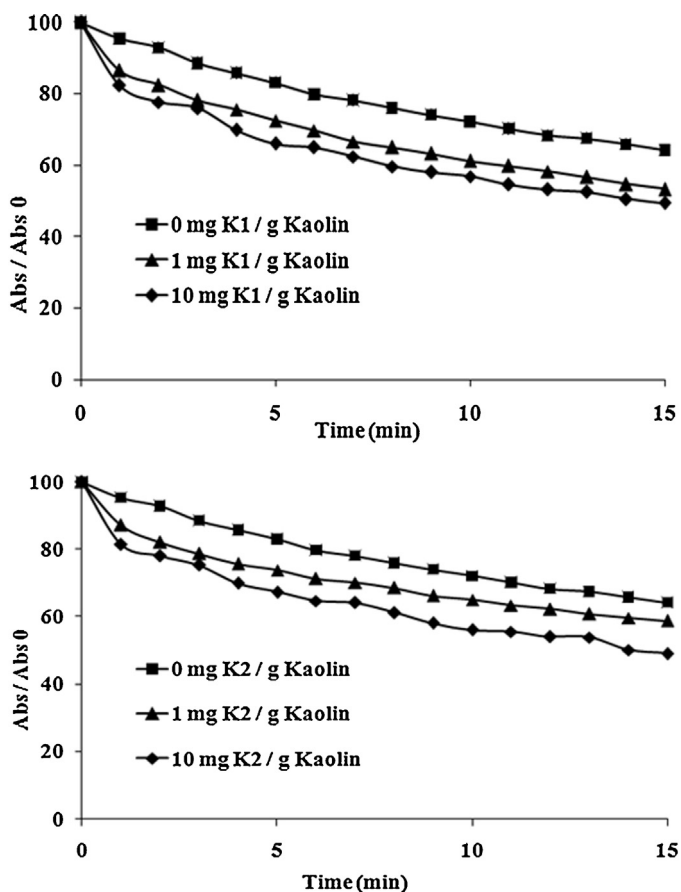


Fig. 6. Time evolution of kaolin flocculation for K1 and K2.

All the systems showed a storage modulus (G') higher than the loss modulus (G''), trend that indicates a gel behavior except for K1 (Fig. 5). For the latter, $G'' > G'$ at low frequencies indicating that the system behaved as a polymeric solution, but at higher frequencies it was impossible to record G' data. For kappa-carrageenan, K2, K3 and I1, it was observed a change in G'' with frequency determining a moduli cross at high frequencies, trend that reveals a weak gel behavior. The $\tan \delta$ (G''/G') values previous to the crossing frequency, took values around 10^{-1} . For K2, K3 and I1, crossing occurred at frequencies of $\approx 100 \text{ rad s}^{-1}$; at this frequency gel stability was lost. In kappa-carrageenan, intersection took place at 800 rad s^{-1} confirming a more solid character. It is important to state that this hydrocolloid showed a high τ_0 in the flow assays. According to Vu, Ovarlez, & Château (2010), the τ_0 in the flow assays is an expression of the solid behavior that is observed in the dynamic assays.

The melting and gelling temperatures of all the derivatives are lower than those of the corresponding native carrageenans. The gelling temperatures of K2 and K3 were difficult to measure: for K2 transparent lumps were observed at the gelling temperature ($\approx 20^\circ\text{C}$), for K3 translucent lumps were formed ($\approx 36^\circ\text{C}$). Both systems presented syneresis (Ramakrishnan, Gerardin, & Prud'homme, 2004).

The separation of solids from suspensions *via* flocculation is widely used in many fields of industry such as water treatment or sludge dewatering. Organic polymers have been used, for this purpose, for the last four decades but growing demand of environmentally friendly separation technologies is now promoting the interest in searching for non-toxic, biodegradable flocculants derived from natural renewable raw materials. A variety of cationic polysaccharide have been used, mainly starch and cellulose

derivatives. Recently, we also evaluated the flocculation performance of cationized agaroses of different degrees of substitution and found that two of them with *DS* of 0.19 and 0.58 showed a comparable behavior to that of commercial polyacrylamides (Prado, Matulewicz, Bonelli, & Cukierman, 2011b).

The main disadvantage of polyacrylamide based flocculants is the very narrow "flocculation window", that is at a small overdose the systems are restabilized. For natural flocculants a broader window is observed and this feature is associated with the differences of structure of adsorbed layers of the flexible cationic polyacrylamide chains and the semi-rigid cationic polysaccharide chains (Lekniute et al., 2013).

The flocculation performance of kappa-carrageenan, K1 and K2 was tested using kaolin particles (Fig. 6) and it was found that they were very similar and that the plot of the residual turbidity after 15 min showed a lower efficiency than that of those determined for cationized agaroses. It was found (Lekniute et al., 2013) for amphoteric starch that complete neutralization of negative kaolin particles charge corresponds to the end of the flocculation window. A further increase in the amount of modified starch reverses the kaolin particles charge. In addition, these authors reported that an amphoteric starch bearing an excess of anionic groups restabilized kaolin dispersion. This result is consistent with our observation of low flocculation performance which could be attributed to the excess of sulfate groups present in the native and modified carrageenans. Iota-carrageenan and I1 gave also similar flocculation performances.

4. Conclusions

This is the first time, to the best of our knowledge that the native sulfated polysaccharides, kappa- and iota-carrageenans extensively used as food hydrocolloids, were cationized and the new amphoteric materials characterized by several methods.

These new materials showed interesting properties for different purposes depending on the degree of substitution with the cationic group. The higher strain at break and lower Young modulus values of the modified polysaccharide films could eventually find application in packaging and/or coating. Even though the flocculation performance of these amphoteric derivatives is lower than that observed for cationized agaroses and commercial polyacrylamides, flocculation of kaolin is improved compared to controls. Their application is possible depending on the characteristics of the waste water streams to be purified and the availability of carrageenans from natural sources in a specific region. Anyhow, an evaluation of their toxicity must be assessed previous to all applications, especially in food, medical or cosmetic industries. However, the known low toxicity of native carrageenans and of starches and celluloses modified with the same cationic group is a promising antecedent.

Acknowledgements

This work was supported by grants of the National Research Council of Argentina (112-200801-00234/2009-2011, 112-200901-00531/2010-2012), the University of Buenos Aires (20020100100479/2011-2014, 20020100100726/2011-2014). All the authors are Research Members of the National Research Council of Argentina (CONICET) with the exception of T.B. who is a Research Fellow of the same Institution.

References

- Auzély-Velty, R., & Rinaudo, M. (2003). Synthesis of starch derivatives with labile cationic groups. *International Journal of Biological Macromolecules*, 31, 123–129.
- Briónes, A. V., Ambal, W. O., Estrella, R. R., Pangilinan, R., De Vera, C. J., Pacis, R. L., Rodríguez, N., & Villanueva, M. A. (2004). Tensile and tear strength of

- carrageenan film from Philippine *Eucheuma* species. *Marine Biotechnology*, 6, 148–151.
- Dodgson, K. S., & Price, R. G. (1962). A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochemical Journal*, 84, 106–110.
- Fissore, E. N., Rojas, A. M., Gershenson, L. N., & Williams, P. A. (2013). Butternut and beetroot pectins: Characterization and functional properties. *Food Hydrocolloids*, 31, 172–182.
- Funami, T. (2010). Atomic force microscopy imaging of food polysaccharides. *Food Science Technology Research*, 16, 1–12.
- Hebeish, A., Higazy, A., El-Shafei, A., & Sharaf, S. (2010). Synthesis of carboxymethyl cellulose (CMC) and starch-based hybrids and their applications in flocculation and sizing. *Carbohydrate Polymers*, 79, 60–69.
- Hennink, W. E., & van Nostrum, C. F. (2002). Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews*, 54, 13–36.
- Horcas, I., Fernández, R., Gómez-Rodríguez, J. M., Colchero, J., Gómez-Herrero, J., & Baró, A. M. (2007). WSM: A software for scanning probe microscopy and a tool for nanotechnology. *Review of Scientific Instruments*, 78, 013705.
- Kavaliauskaitė, R., Klimavičiūtė, R., & Zemaitaitis, A. (2008). Factors influencing production of cationic starches. *Carbohydrate Polymers*, 73, 665–675.
- Launay, B., Doublier, J. L., & Cuvelier, G. (1985). Flow properties of aqueous solutions and dispersions of polysaccharides. In J. R. Mitchell, & D. A. Ledward (Eds.), *Functional properties of food macromolecules* (pp. 1–78). London/New York: Elsevier Science Applied Publishers.
- Lekniute, E., Peciulyte, L., Klimavičiūtė, R., Bendoraitienė, J., & Zemaitaitis, A. (2013). Structural characteristics and flocculation properties of amphoteric starch. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 430, 95–102.
- Lin, Q., Qian, S., Li, C., Pan, H., Wu, Z., & Liu, G. (2012). Synthesis, flocculation and adsorption performance of amphoteric starch. *Carbohydrate Polymers*, 90, 275–283.
- Matsushiro, B. (1996). Vibrational spectroscopy of seaweed galactans. *Hydrobiologia*, 326/327, 481–489.
- Murano, E., Toffanin, R., Zanetti, F., Knutsen, S. H., Paoletti, S., & Rizzo, R. (1992). Chemical and macromolecular characterization of agar polymers from *Gracilaria dura* (C. Agardh) J. Agardh (Gracilariaceae, Rhodophyta).
- Park, J. T., & Johnson, M. J. (1949). A submicrodetermination of glucose. *Journal of Biological Chemistry*, 181, 149–151.
- Prado, H. J., Matulewicz, M. C., Bonelli, P. R., & Cukierman, A. L. (2011a). Studies on the cationization of agarose. *Carbohydrate Research*, 346, 311–321.
- Prado, H. J., Matulewicz, M. C., Bonelli, P. R., & Cukierman, A. L. (2011b). Potential use of a novel modified seaweed polysaccharide as flocculating agent. *Desalination*, 281, 100–104.
- Prado, H. J., & Matulewicz, M. C. (2014). Cationization of polysaccharides: A path to greener derivatives with many industrial applications. *European Polymer Journal*, 52, 53–55.
- Radosta, S., Vorwerg, W., Ebert, A., Begli, A. H., Grülç, D., & Wastyn, M. (2004). Properties of low-substituted cationic starch derivatives prepared by different derivatisation processes. *Starch/Stärke*, 56, 277–287.
- Ramakrishnan, S., Gerardin, C., & Prud'homme, R. K. (2004). Syneresis of carrageenan gels: NMR and rheology. *Soft Materials*, 2, 145–153.
- Rinaudo, M. (2008). Polyelectrolytes derived from natural polysaccharides. In M. N. Belgacem, & A. Gandini (Eds.), *Monomers, polymers and composites from renewable resources* (pp. 495–516). Amsterdam: Elsevier.
- Šimković, I., Mendichi, R., Kelnar, I., Filip, J., & Hricovíni, M. (2015). Cationization of heparin for film applications. *Carbohydrate Polymers*, 115, 551–558.
- Song, Y., Sun, Y., Zhang, X., Zhou, J., & Zhang, L. (2008). Homogeneous quaternization of cellulose in NaOH/urea aqueous solutions as gene carriers. *Biomacromolecules*, 9, 2259–2264.
- Usov, A. I. (1984). NMR spectroscopy of red seaweed polysaccharide: Agars, carrageenans, and xylans. *Botanica Marina*, 27, 189–202.
- Usov, A. I. (2011). Polysaccharides of the red algae. *Advances in Carbohydrate Chemistry and Biochemistry*, 65, 115–217.
- Viana, R. B., da Silva, A. B. F., & Pimentel, A. S. (2012). Infrared spectroscopy of anionic, cationic, and zwitterionic surfactants. *Advances in Physical Chemistry*, 1–14.
- Vu, T., Ovarlez, G., & Château, C. (2010). Macroscopic behavior of bidisperse suspensions of noncolloidal particles in yield stress fluids. *Journal of Rheology*, 54, 815–833.
- Wang, Q., & Cui, S. W. (2005). Understanding the physical properties of food polysaccharides. In S. W. Cui (Ed.), *Food carbohydrates chemistry, physical properties, and applications* (pp. 161–218). Boca Raton: Taylor & Francis Group.
- Zhang, L. M. (2001). New water-soluble cellulosic polymers: A review. *Macromolecular Materials and Engineering*, 286, 267–275.
- Zhang, L. M., & Tan, Y. B. (2000). Graft copolymerization of 2-(dimethylamino)ethyl methacrylate on to carboxymethylated cellulose. *Macromolecular Materials and Engineering*, 280/281, 59–65.
- Zhang, M., Zhang, S. F., Ju, B. Z., & Yang, J. Z. (2005). A review of amphoteric starches in preparation and applications. In *The proceedings of the 3rd international conference on functional molecules China*, (pp. 31–35).