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Comparative analysis of enzymatically digested κ-carrageenans, using liquid chromatography on ion-exchange and porous graphitic carbon columns coupled to an evaporative light scattering detector

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Abstract

Enzymatically digested κ (A-G4S)-carrageenans, apart from their biological activities in plants, could be used as 'model' molecules to elucidate potential problems in nuclear magnetic resonance spectroscopy of carrageenans. Thus, oligosaccharides obtained from κ-carrageenan by enzymatic digestion using κ-carrageenase have been separated on silica and polymeric based ion-exchange and porous graphitic carbon (PGC) columns, coupled to an evaporative light scattering detector. Oligomers were separated on ion-exchange columns using a gradient of ammonium acetate as a developing ion, while analysis on PGC column presented an additional adjacent peak next to each main one, using a gradient of ammonium acetate in water/acetonitrile as a mobile phase. The phenomenon can be attributed to different retention mechanisms that govern the PGC surface. Furthermore, it has been demonstrated that acetonitrile can regulate the selectivity between the peaks raising hopes for preparative chromatography.

Keywords: Porous graphitized carbon; Carrageenans; Carbohydrates

1. Introduction

The consumption of food additives, also known as gums, has increased [1,2] due to their use in foods designed to help maintain a good gastrointestinal environment and act to increase intestinal bifidobacteria [2]. Furthermore, they have been widely incorporated as a thickeners, emulsifiers, and stabilizers in a variety of food products [3].

Carrageenan, a well known food additive, is a generic name for a family of natural, water-soluble, sulfated galactans that are isolated from red seaweeds [4]. Their ideal backbone structure constitutes of an alternating β(1 → 3) linked D-galactose (unit G) and an α(1 → 4) linked D-galactose (unit D). In these units, various hydroxy groups may be substituted by ester sulfate [5]. Carrageenans that can create gels have a 3,6-anhydrogalactose unit (unit A) in place of unit D. The most well known of them are kappa (κ)-carrageenans (A-G4S) and iota (ι)-carrageenans (A2S-G4S). κ-Carrageenan is sulfated only in position C_4 in the galactose unit, while ι has an additional sulfate unit in position C_2 of the 3,6-anhydrogalactose unit (Fig. 1).

Both the number and the position of sulfate [1] and the 3,6-anhydrogalactose [6], have a dramatic effect on the tertiary structure and the possible interactions for the different types of carrageenans. κ-Carrageenan forms gels that are hard, strong and brittle, whereas ι-carrageenan forms soft and weak gels [7]. Therefore, rapid analysis of the sulfate ester and 3,6-anhydrogalactose content is important for studying the rheological properties of these polymers [4]. Hence, depolymerization and chromatographic separation are necessary for the detection of residues and of minority sequences [8].

Present chromatographic methods for carrageenan composition analysis rely on the quantitative estimation of galactose, 3,6-anhydrogalactose and sulfate as marker analytes, through the depolymerization of the

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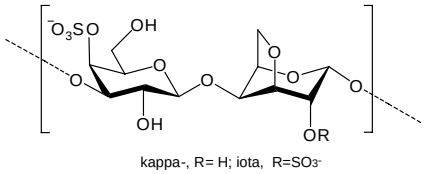

Fig. 1. Carrabiose, the principal disaccharide constituting the κ- and ι-carrageenans.

polysaccharide molecule [1,9]. Early hydrolysis methods were based on acid hydrolysis prior to derivatization and analysis by high-performance liquid chromatography (HPLC) or gas–liquid chromatography (GLC). However, these methods presented the drawback of degradation of the 3,6-anhydrogalactose residues to 5-hydroxymethyl furfural [10]. Nowadays, the presence of an acid-stable reducing agent such as the 4-methylmorpholine–borane complex, prevents the degradation of acid-sensitive monosaccharides [11,12]. Recently, Jol et al. have developed a novel method avoiding the derivatization step needed prior to GLC analysis [13].

Besides composition analysis, sequence analysis offers the possibility of studying in general the degraded forms of these polymers. Enzymes are a helpful tool in this respect. κ-Carrageenase is a specific enzyme [14] that cleaves the internal β(1 → 4) linkages of κ-carrageenans, yielding oligosaccharides of the neocarrabiose series [15]. Enzymatically digested κ-carrageenans have been shown to elicit biological activities in plants [16,17]. Other reports [8,18] have used κ-carrageenase in order to produce oligosaccharides of κ-carrageenans and study their structural heterogeneity by size-exclusion chromatography and electrospray ionization/mass spectrometry (ESI-MS). In addition, semi-preparative chromatography based on a strong anion-exchange (SAX)-HPLC column [19] has been used in order to separate the oligosaccharides and study their structure. However, it has proved difficult to desalt low-molecular weight oligosaccharides (monosaccharides to tetrasaccharides).

As a complementary method, nuclear magnetic resonance (NMR) spectroscopy (both ^{1}H and ^{13}C NMR) is one of the standard tools for the determination of the chemical structure of carrageenan samples [1,9,20]. These samples, in their natural state, are mixtures of different sulfated polysaccharides, thus their identification is complex without 'model' molecules [20] since ^{1}H NMR spectra of complex polysaccharides usually show broad resonances, sometimes overlapping and difficult to integrate. Moreover, ^{13}C NMR spectroscopy cannot detect a low amount of minor components owing to its low sensitivity, or if detected the results will have a limited accuracy [20].

To overcome these problems, elaborate process extraction and fractionation are needed. A chromatographic method could be used to obtain purified components, but the steric exclusion chromatography used is time-consuming. By degrading natural carrageenans with structure-specific enzymes, a series of sulfated oligosaccharides of known structure is formed. However, some larger fragments, resistant to enzymolysis remain present [8,20], which samples are suitable for NMR analysis as they concentrate these unusual minor details [20].

Thus far, evaporative light scattering detection (ELSD) has never been used for the detection of these important compounds. This detection mode is universal since the analyte has a lower volatility than the mobile phase; it is compatible with a gradient elution mode and requires the complete volatilization of the mobile phase [21]. The further analysis of oligosaccharides is often dependent on salt-free samples, and thus the use of volatile buffers for purification is an important consideration [22].

This paper therefore describes the analysis of oligosaccharides of κ-carrageenan (enzymatically digested by κ-carrageenase) with ion-exchange and porous graphitic carbon (PGC) columns, coupled with ELSD.

2. Experimental

2.1. Apparatus

The liquid chromatographic apparatus consisted of a Thermoseparation (Les Ulis, France) model P4000 inert quaternary gradient pump, a Rheodyne (Berkeley, CA, USA) model 7125 injector with a 20 μl sample loop and an ELSD system (Sedere, Alfortville, France) model Sedex 75. The ELSD settings were as follows: photomultiplier range, 10; evaporative drift tube temperature, 47 °C; nebulization gas pressure, 2.0 bar.

The columns used were as follows: Nucleosil Anion II (250 × 4 mm i.d., particle size 10 μm) from Macherey–Nagel (Düren, Germany); IC-A1S (100 × 4.6 mm i.d., particle size 10 μm) from Shimadzu (Kyoto, Japan); Biobasic-AX (50 × 2.0 mm i.d., particle size 5 μm) from Hypersil (Runcorn, UK); Hypercarb (100 × 2.1 mm i.d., particle size 5 μm) from Hypersil (Runcorn, UK). Dead volume on each column was determined by injecting xylose.

The heater oven was a block type heater Gecko-Cil oven (Cil Cluzeau, Saint-Foy-La-Grande, France). The system was left 2 h to equilibrate before injecting the oligo-κ-carrageenans.

Data acquisition of the chromatograms has been performed using EZChrom Elite Client/Server software, version 2.5, Scientific Software (Pleasanton, CA, USA).

2.2. Reagents

Eluent constituents were purchased as follows: water from an Elgastat UHQ II system from Elga (Antony, France), acetonitrile (CH_3CN) and methanol (CH_3OH) from J.T. Baker (Noisy le Sec, France).

Solutes were purchased as follows: oligo-κ-carrageenans were produced according to Rochas and Heyraud [23]

using recombinant κ-carraggenase from *Alteromonas carrageenovora* [24]. They were prepared by enzymatic digestion with κ-carrageenases. All samples of hydrolysates of κ-carrageenans were injected at a concentration of 1220 ppm. Ammonium acetate and ammonium formate were from Fluka (St. Quentin Fallavier, France), ammonium hydrogen carbonate from Prolabo (Paris, France). All reagents were of analytical grade.

The theoretical values of pH and ionic strength of each eluent were calculated from PHoEBuS [25], an application program help for buffer studies and then for each mobile phase, the pH value was checked with a Beckman pH meter (model F10, Gagny, France).

3. Results and discussion

The degradation of carrageenans with structure specific carrageennase forms a series of sulfated oligosaccharides (OLIG) [8]. The highly charged character of these hydrolysates would entail strong-ion-exchange interactions with an anion-exchanger. Consequently, we have first investigated the chromatographic behavior of carrageenans on a low capacity exchanger.

3.1. Ion-exchange columns

Nucleosil Anion II is a low capacity silica based column with quaternary ammonium anion-exchangers. A volatile salt of ammonium acetate was selected as a developing ion owing to its elution strength and compatibility with ELSD [26]. Fig. 2 presents the retention factors (*k*) of oligomers of κ-carrageenans as a function of the organic modifier added

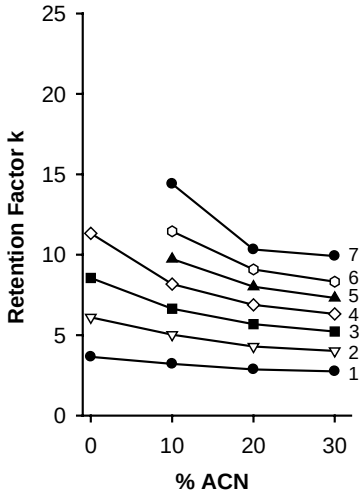

Fig. 2. Retention factors (*k*) of the oligomers of κ-carrageenan as a function of CH_3CN percentage (*x*% CH_3CN) added in the mobile phase on the Nucleosil Anion II column. Mobile phase: eluent A, water; eluent B, 1 M CH_3COONH_4; eluent C, *x*% CH_3CN. Gradient program: 0–4 min, 0–27% of B; 4–20 min, 27–70% of B. Flow-rate: 1 ml min^{-1}.

in the mobile phase in the same gradient mode. The use of CH_3CN improved the elution process, resulting in lower *k* values and smaller selectivities among oligomers. Nevertheless, once the organic modifier was added, different concentrations in the range 10–30% had no great influence on retention time and selectivity. Fig. 3a and b depicts the separation of oligomers of κ-carageenan in different concentrations of CH_3CN in the same gradient mode of ammonium acetate. However, despite the enhancement of the response with the addition of CH_3CN, a high baseline drift was observed, as shown in Fig. 3c. This drift is caused by a bleeding

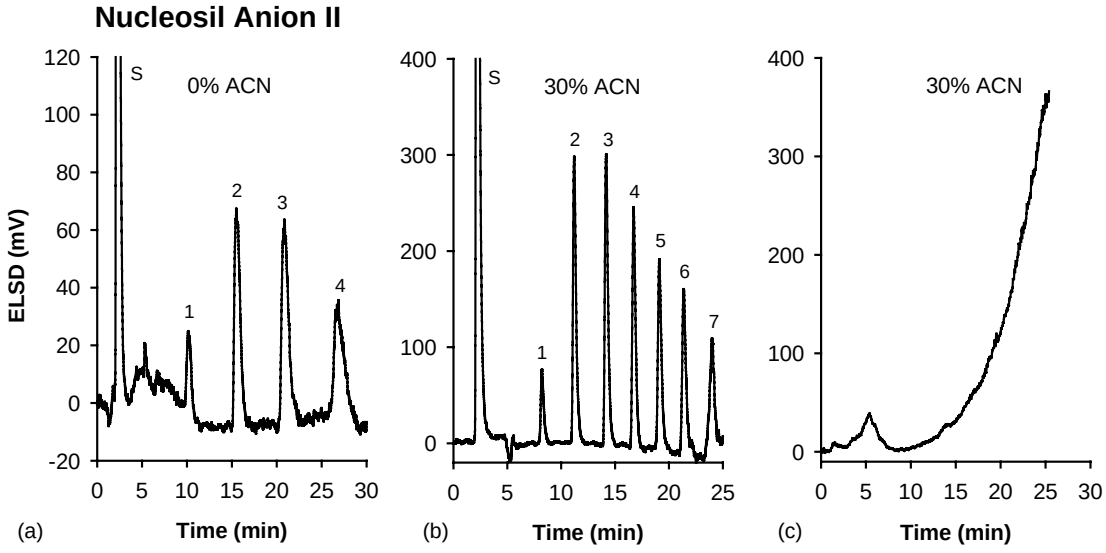

Fig. 3. Chromatograms of hydrolysates of κ-carrageenan in aqueous mobile phase; (a) in 30% of CH_3CN added in the mobile phase; (b) resulting from substraction of the baseline; (c) on the Nucleosil Anion II column. Experimental conditions: eluent A, water; eluent B, 1 M CH_3COONH_4. Gradient program: 0–4 min, 0–27% of B; 4–20 min, 27–70% of B. Flow-rate: 1 ml min^{-1}; S designates salt.

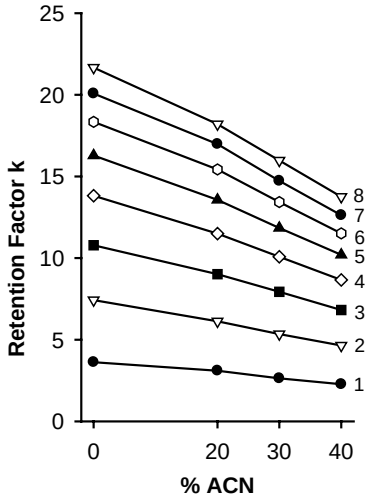

Fig. 4. Retention factors (k) of the oligomers of κ-carrageenan as a function of CH_3CN percentage (x% CH_3CN) added in the mobile phase in the IC-A1S column. Mobile phase: eluent A, water; eluent B, 500 mM CH_3COONH_4; eluent C, x% CH_3CN. Gradient program: 0–35 min, 2–32% of B. Flow-rate: 1 ml min^{-1}.

of the column as has been previously observed on amino-propyl column with hydro-organic mobile phases [27] and although it can be subtracted (Fig. 3b), it is not compatible with ESI/MS.

The use of CH_3CN was also investigated on the Shimadzu column and it was found that its use had an effect in the retention mechanism. The k values of the oligomers of κ-carrageenan presented in Fig. 4 as a function of CH_3CN added in the mobile phase, in the same gradient mode of ammonium acetate, revealed a decrease in retention time (t_R) as the concentration of organic modifier increased. It appeared that the ion-exchange interactions were reinforced by considerable adsorption of these anionic compounds onto the polymeric support; moreover, the use of CH_3CN resulted in changing the coefficient of distribution (K). In addition, a decrease in selectivity among the compounds was noticeable, a phenomenon caused by a modification in Donnan equilibrium [28]. Fig. 5 presents the separation of hydrolysates of κ-carrageenans on the Shimadzu column using the same gradient mode of ammonium acetate without and with organic modifier. Efficiencies and background noise were improved by the addition of CH_3CN as shown, although a baseline drift was observed. An enhancement of the ELSD response can be noted when CH_3CN was added in the gradient elution due to a modification of the microparticle size of solutes in the nebulisation/vaporization process of the mobile phase [27]. It should also be mentioned that as in the Nucleosil Anion II Column, the use CH_3CN resulted in greater signal responses.

Carrageenans are negatively charged polymers through the entire pH range. Our primary aim was a reasonable retention time without lose of resolution. Thus, a buffer of HCO_3NH_4/CH_3COOH was selected at a pH value of 7, because this volatile buffer has high elution strength with regard to the high capacity of Biobasic-AX relative to the Macherey–Nagel exchanger (Table 1). Indeed, the oligosaccharides were separated in the Biobasic-AX column at a concentration of 150 mM of the later developing ion in a gradient mode.

However, at this pH, the buffer was found to be unstable (about 16% of HCO_3 ion existed in H_2CO_3 form). At a value of pH 8, a buffer of HCO_3NH_4/NH_3 was more stable (percentage of H_2CO_3, 1.67%), but on the other hand, there was a risk of column stability due to the silica type stationary phase. For this reason, we turned to a developing ion that is more stable than the HCO_3^- for batch-to-batch

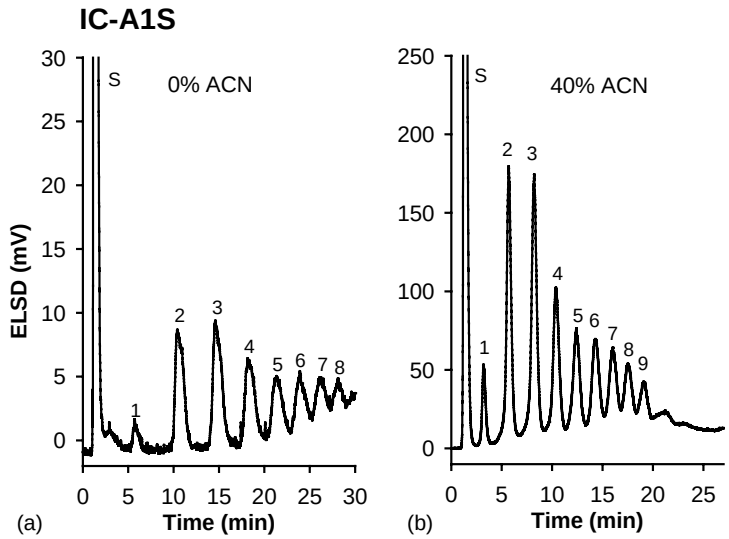

Fig. 5. Chromatograms of hydrolysates of κ-carrageenan in aqueous mobile phase; (a) in 40% of CH_3CN added in the mobile phase; (b) on the IC-A1S column. Experimental conditions: eluent A, water; eluent B, 500 mM CH_3COONH_4. Gradient program: 0–35 min, 2–32% of B. Flow-rate: 1 ml min^{-1}; S designates salt.

Table 1
Characteristics of columns used for ion-exchange chromatography

	Column		
	Biobasic-AX	IC-A1S	Nucleosil Anion II
Dimensions (mm × mm)	50 × 2.0	100 × 4.6	250 × 4.0
Particle diameter (μm)	5	10	10
Type of matrix	Silica-resin	PMA[a]	Silica
Capacity (μequiv/g)	220	[b]	50
Pore diameter (Å)	300	–	300
Functional group	Amine I, II, III	Amine IV	Amine IV
Manufacturer	Hypersil	Shimadzu	Macherey–Nagel

[a] Polymethyl acrylate.
[b] Non-available.

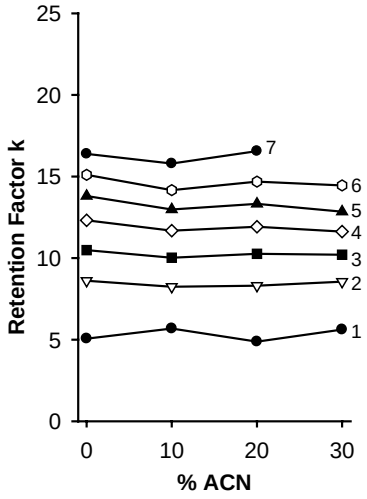

Fig. 6. Retention factors (k) of the oligomers of κ-carrageenan as a function of CH_3CN percentage (x% CH_3CN) added in the mobile phase in the Biobasic-AX column. Mobile phase: eluent A, water; eluent B, 1 M CH_3COONH_4; eluent C, x% CH_3CN. Gradient program: 0–2 min, 0–2% of B; 2–14 min, 2–63% of B; 14–25 min, 63% of B. Flow-rate: 0.2 ml min^{-1}.

injections, but on the contrary less eluent. Ammonium formate ($HCOONH_4$) and ammonium acetate (CH_3COONH_4) currently used in anion-exchange chromatography [28] and for which the compatibility with the ELSD has already been demonstrated [29] were evaluated in aqueous mobile phases. Between these two salts, no difference in the elution strength was found.

Due to the fact that Biobasic-AX is a specific anion-exchanger, polyethyleneimine (PEI) bound to high quality base deactivated silica [30]; we speculated that a secondary hydrophobic interaction occurs between the solutes and the stationary phase. Hence, CH_3CN was added in the mobile phase. Fig. 6 presents the retention factors (k) of oligomers of κ-carrageenan as a function of CH_3CN percentage in the mobile phase. Compared with the previous plots, the CH_3CN content had no effect on retention. Fig. 7a shows the separation of oligomers of κ-carrageenans in an aqueous mobile phase using CH_3COONH_4 as a salt buffer. This time, 630 mM were necessary to elute the hydrolysates of carrageenan. Efficiencies were similar and no baseline drift or background noise were observed despite the high salt concentration required to elute the oligomers. This shows a good stability of the packing. Moreover, it can be noticed that the response is enhanced when CH_3CN is used in the mobile phase.

Fig. 7b depicts the separation of the same compounds in the presence of 20% of CH_3CN (same gradient as Fig. 7a). Between Fig. 7a and b two contradictionary phenomena can be noticed with respect to the presence of CH_3CN; the latter

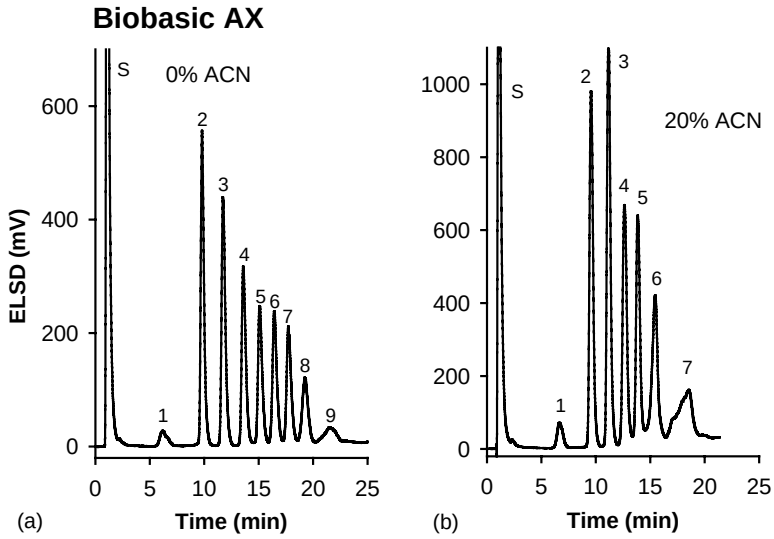

Fig. 7. Chromatograms of hydrolysates of κ-carrageenan in aqueous mobile phase (a) and hydro-organic mobile phase (b) on the Biobasic-AX column. Experimental conditions: eluent A, water; eluent B, 1 M CH_3COONH_4. Gradient program (a): 0–2 min, 0–2% of B; 2–14 min, 2–63% of B; 14–25 min, 63% of B. Flow-rate: 0.2 ml min^{-1}. Gradient program (b): same as (a) reformed with 20% CH_3CN content; S designates salt.

resulted in an increase in the signal response and at the same time its presence resulted in poor elution of the last peaks (peak numbers 7, 8 and 9).

Applying the same experimental conditions on the Shimadzu and Macherey–Nagel columns, it has been found that the hydrolysates of κ-carrageenan were less retained in the former but more retained in the latter.

To conclude, Biobasic-AX appeared to be the most suitable for the separation of the oligo-κ-carrageenans but the high content of salt at the end of the gradient could lead to difficulties in coupling with ESI/MS. Indeed, preliminary results verified this hypothesis. We were able to identify the first three peaks (peaks 1, 2 and 3 with *m*/*z* values 394, 391 and 390 which correspond to the tetra-, hexa- and octa-saccharide, respectively) but further identification was not possible due to high salt concentration.

Nevertheless, these oligomers also exist in structures others than the ideal one. This fact supported the idea of studying their separation on a PGC column that has shown capabilities of separating closely related compounds [31].

3.2. PGC column

Common inorganic anions were totally retained on PGC column with deionized water as mobile phase [32]. Davies and Hounsell [22] reported for monosaccharides, GlcNAc-3-SO_4 and GlcNAc-6-SO_4, that they were significantly retained on PGC column (100% aqueous TFA, trifluoroacetic acid), unless an increasing CH_3CN concentration was used. Elfakir and Dreux in their work [33] found that electronic modifier (TFA) was necessary in order to elute intact glucosinolates. In fact, they reported the need for both electronic and organic modifiers so as to have good efficiency.

The retention time on PGC column is governed by both hydrophobic and electronic interactions [31,33]. In detail, despite the absence of ion-exchange sites on PGC columns, the retention mechanism of charged compounds with H_2O as eluent can be attributed to specific electronic interactions on PGC column [33].

The oligomers of κ-carrageenans were totally retained on PGC using pure aqueous or a salt of ammonium acetate/formate as mobile phases. This shows a different behavior compared to classical anion-exchangers. Moreover, a mixture of organic modifier/water was unable to elute these hydrophilic compounds in comparison to a typical reversed phase column. In fact, a mixture of organic modifier/buffered aqueous mobile phase was required to elute these compounds.

Using 30% of CH_3OH, it was not possible to elute the oligosaccharides in comparison with 12.5% of CH_3CN. This is in agreement with experiments showing that intact glucosinolates were better separated with CH_3CN [33]. Therefore, CH_3CN was selected as the organic modifier because of a greater eluting strength. In order to study the effect of CH_3CN in eluting the oligomers, different percentages of CH_3CN (10, 12.5, 15, 17.5, 20%) were tested in the same gradient of ammonium acetate and ammonium formate. The results revealed that the lower the percentage of CH_3CN, the greater the selectivity between the two anomers.

Table 2
Resolution (R_s) and selectivity (α) for oligomers of κ-carrageenan using ammonium acetate and ammonium formate as developing ions, in the same gradient mode, in different concentrations of CH_3CN on the Hypercarb column

CH_3CN (%)	Salt	Peak number							
		1	1′	2	2′	3	3′	4	4′
R_s									
10.0	Acetate	13.25	2.82	7.97	3.07	9.38	2.70		
	Formate	12.63	2.90	10.57	3.65				
12.5	Acetate	11.02	1.36	7.23	2.12	5.11	1.75	4.27	1.54
	Formate	14.78	1.58	7.10	2.10	6.00	2.75	6.83	3.11
15.0	Acetate	12.02	0.90	4.02	1.29	2.4	1.39	3.19	1.62
	Formate	9.19	0.92	4.46	1.39	2.10	1.89	3.25	1.75
		1/1′		2/2′		3/3′	4/4′	5/5′	6/6′	7/7′
α									
10.0	Acetate	1.15	1.46	1.14	1.40	1.08	[a]		
	Formate	1.20	1.88	1.20	1.77				
12.5	Acetate	1.06	1.51	1.11	1.20	1.06	1.17	1.05	
	Formate	1.08	1.43	1.11	1.34	1.13	1.33	1.13	
15.0	Acetate	1.05	1.27	1.07	1.21	1.08	1.17	1.08	
	Formate	1.05	1.28	1.08	1.23	1.10	1.15	1.08	

[a] Not calculated.

Figs. 8 and 9 present the separation of oligosaccharides of κ-carrageenans for 15 and 10% of CH_3CN, respectively, in the same gradient mode, using ammonium acetate and formate as developing ions. Acetate was more eluting than formate, but on the other hand, the latter was more selective than the former (Table 2). However, as the concentration in organic modifier increased, the difference in selectivity decreased. In Fig. 9a and b the difference in ELSD signal for the same quantity injected can be observed. This is due to the modification of the scattered light from residual microparticules, after the nebulization–vaporization process, in which the size changes with the nature of the mobile phase [27].

As for glucosinolates, the presence of both electronic and organic modifier was found to be inevitable. However, CH_3CN seemed to play a key role in eluting the oligomers while the developing ion has a secondary one. A concentration of 20% CH_3CN resulted in eluting the hydrolysates in less than 25 min, but also at a lower resolution, especially between the adjacent peaks.

A difference between ion-exchange and PGC columns is the additional adjacent peak presented next to the primary one (quoted numbers, ex: 1 and 1′). Anomer split separation has been reported for both neutral [34] and charged [22] monosaccharides and neutral disaccharides [34]. It is possible therefore to have an anomer split separation of oligomers of κ-carrageenan due to the PGC surface.

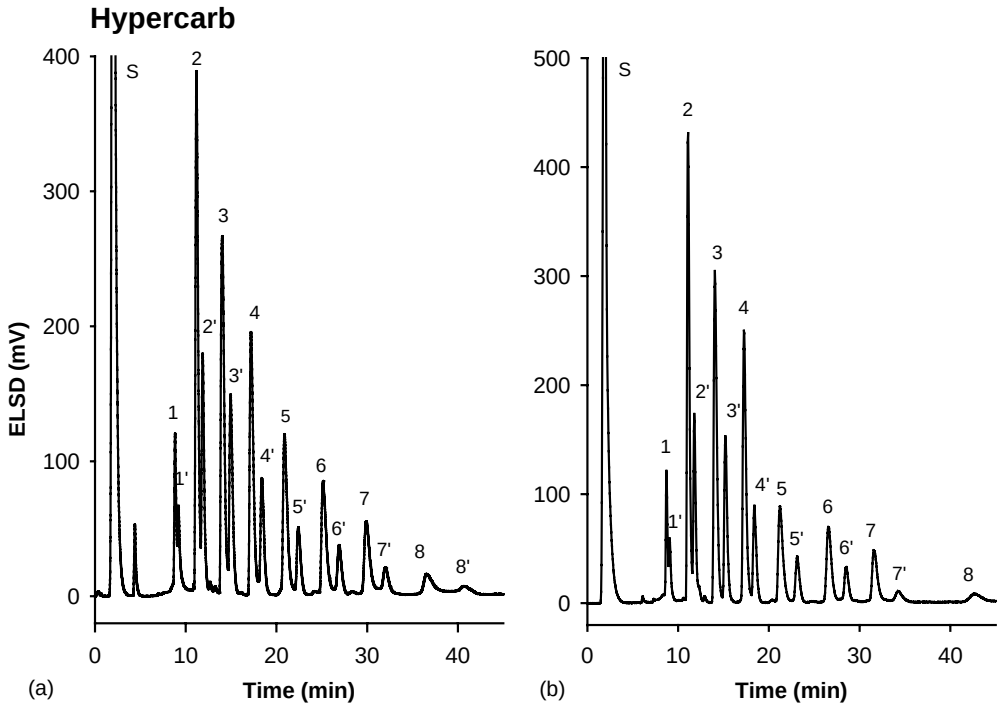

Fig. 8. Separation of oligosaccharides of κ-carrageenan in Hypercarb column using 15% of CH_3CN in the mobile phase and ammonium acetate (a) and ammonium formate (b) as developing ions. Eluent A, water; eluent B, 700 mM of salt; eluent C, 15% CH_3CN. Gradient program: 0–45 min, 0–70% of B. Flow-rate: 0.2 ml min^{-1}; S designates salt. Peak numbers designate same peak with peaks in Figs. 3, 5 and 7. Identification of peaks is based on relative heights.

Another possibility concerning the adjacent peaks is not to be anomers, but structures coming from the non ideal repeating unit of κ-carrageenan. Knutsen et al. have reported [8] the existence of oligosaccharides of κ-carrageenan, assigned as octa-, deca- and dodecasaccharides, and separated by size-exclusion chromatography, that also contained small amounts of oligosaccharides with a shorter chain length in addition to the major ones. In fact, they proved to be sulfated neocarrabiose oligosaccharides consisting of one less neocarrabiose unit but with an extra sulfate group that co-eluted with the regular ones above. They attributed the phenomenon to inclusions of ι-carrageenan.

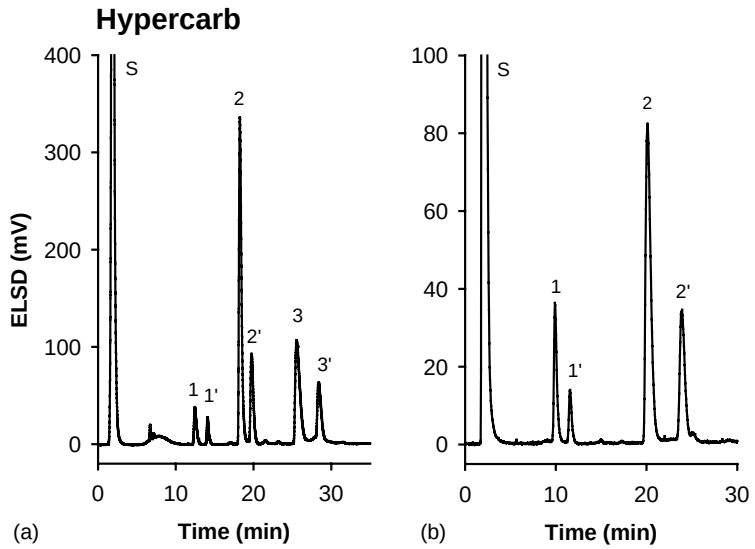

Fig. 9. Separation of oligosaccharides of κ-carrageenan in Hypercarb column using 10% of CH_3CN in the mobile phase and ammonium acetate (a) and ammonium formate (b) as developing ions. Eluent A, water; eluent B, 700 mM of salt; eluent C, 10% CH_3CN. Gradient program: 0–45 min, 0–70% of B. Flow-rate: 0.2 ml min^{-1}; S designates salt. Peak numbers designate same peak with peaks in Figs. 3, 5 and 7.

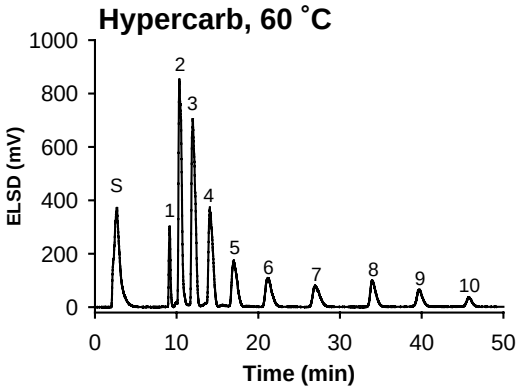

Fig. 10. Separation of oligosaccharides of κ-carrageenan in Hypercarb column at 60 °C (other experimental conditions same as Fig. 8a). Eluent A, water; eluent B, 700 mM of ammonium acetate; eluent C, 15% CH_3CN. Gradient program: 0–45 min, 0–70% of B. Flow-rate: 0.2 ml min^{-1}.

The separation of the oligomers into two fractions, possibly due to anomerization (adjacent peaks) makes the PGC column not suitable for the off-line LC/ESI-MS and sample preparations for the NMR. This problem could be overcome imposing the column under a controlled temperature environment. The disappearing of the doublets (anomers) for monosaccharides with increasing temperature has been reported previously [34]. Fig. 10 depicts the separation of oligo-κ-carrageenans at 60 °C where the doublets no longer exist supporting the hypothesis of anomers. The phenomenon can be attributed to the acceleration of the rate of interconversion between α and β anomers. Therefore a further increase in selectivity is achieved.

4. Conclusion

The present report has shown that the separation of oligomers of κ-carrageenan is possible using ion-exchange and PGC columns. In ion-exchange chromatography the retention mechanism is charge-dependent whereas in PGC column it is due to simultaneous hydrophobic and electronic interactions which enable better regulation of the resolution between peaks, especially by the use of an organic modifier. In addition, high temperature in PGC column gives one peak for each oligomer owing to the acceleration of the interconversion rate between the anomers resulting in a greater selectivity. This fact raises hopes in a new preparative chromatography method, given that the mobile phase is completely volatile.

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