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Acid hydrolysis of sulphated polysaccharides. Desulphation and the effect on molecular mass

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Abstract

The functionality of sulphated polysaccharides is directly related to the degree of sulphation of these polymers. Acid hydrolysis is often carried out to study the kinetics of hydrolysis/chain scission of these polymers and the effect on molecular mass. However, measurement of molecular mass by size-exclusion chromatography (SEC) can be artefactual if the hydrolysis step also leads to desulphation of the polymer. A new and sensitive method based on a combination of size-exclusion and ion-repulsion principles has therefore been developed to enable the rapid measurement of free sulphate (and chloride if needed) levels in polymer solutions without any sample separation/preparation step. This and an SEC–refractive index method have been used to follow the desulphation and depolymerization of five sulphated polysaccharides (κ-, ι-, λ-carrageenan, dextran sulphate, heparin) during acid (pH 2) hydrolysis at 35 and 55°C. Dextran sulphate was found to be the most sensitive to desulphation, probably due to its high chain flexibility. Heparin and the carrageenans were stable to desulphation during the time period examined here, confirming the suitability and applicability of SEC-based methods for molecular mass determination of these polymers. The molecular mass of all but heparin was, however, found to be rapidly lowered by the (chain scission) hydrolysis. Kinetic parameters for depolymerization have been recovered for these polymers.

Keywords: Sulphated polysaccharides; Desulphation; Acid hydrolysis; Depolymerization; Molecular weight; Free sulphate

1. Introduction

Carrageenans are water soluble linear polysaccharides composed of alternating $\alpha(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linked D-galactose residues. Three primary forms (κ-, λ-, ι-) of carrageenan are identified based on the modification of the disaccharide repeating unit resulting from the occurrence of ester sulphate, or anhydride formation in the 4-linked residue. κ- and ι-carrageenans form thermoreversible gels in solution while λ- (and other minor forms) yield highly viscous solutions that do not gel (Clark and Ross-Murphy, 1987). Due to the frequent use of the carrageenans within, for example, the food and pharmaceutical industry, extensive toxicological evaluation has been carried out. The major problem that has been identified involves low molecular mass ($< 20\,000$ g mol^{-1}) fractions which can cause lesions. Degraded carrageenan causes ulcerative colitis in rats and guinea pigs (Delahunty et al., 1987), and is used in experimental models to study the effects of pharmacological and therapeutic agents (Marcus et al., 1989).

The degradation of carrageenans can be of thermal, oxidative and hydrolytic nature. Degraded carrageenan can be present in raw material due to long-term storage, or may be created during production processes, thus impacting on its functionality as a gel-forming or viscosity-enhancing polymer in foods. Since carrageenan in solution is susceptible to acid hydrolysis, molecular mass reduction may even occur in vivo in the acidic gastric environment after ingestion (Capron et al., 1996).

Previous studies on acidic hydrolysis of carrageenans have focused primarily on the characterization of the polymer chain itself by studying the depolymerization, either by viscosity (Hjerde et al., 1996) or by size-exclusion chromatography (SEC). In conjunction with SEC, various detector systems have been used. These include a refractive index (RI) concentration detector using narrow molecular mass standards (Ekström and Kuivinen, 1983), a dual detector system with light scattering (LS) and RI for absolute molecular mass distributions (Singh and Jacobsson, 1994), or a triple detector system (LS–viscometer–RI) (Myslabodski et

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al., 1996). However, in order to obtain reliable molecular mass distribution (MMD) results by SEC, it is of great importance to check whether the degraded polymer truly and solely depolymerizes or whether changes occur in its chemical structure in the repeating units of the polymer. For sulphated polysaccharides like carrageenans, there is a potential risk of hydrolysis of the sulphate groups which will change the repeating unit composition. The size of the polymer molecule in solution at a certain ionic strength will change depending upon the degree of desulphation (random coil to rigid rod) since the sulphate groups determine the total charge and charge distribution on the polymer. Loss of sulphate groups will result in the polymer assuming a less extended and more flexible conformation due to lower electrostatic intramolecular repulsion. A desulphated polysaccharide molecule would therefore effectively behave as a smaller molecule in solution compared to another molecule of the same degree of polymerization but unchanged degree of sulphation. To some extent, the impact of this factor on elution from an SEC column could be minimised by using a mobile phase with high ionic strength which will shield the electrostatic charges. However, use of SEC in combination with multiangle laser light scattering (MALLS) or the triple detector system, where a true size exclusion from the column is not necessary in order to obtain absolute MMD, would still give erroneous results as the refractive index increment, d*n*/d*c*, for the polymer in solution will change due to the change in repeating unit composition.

The objective for this study was therefore to measure the amount of liberated sulphate during acid hydrolysis and establish the reliability of the SEC measurements (and MMD results) on carrageenans after hydrolysis. A new method has been developed for this purpose to rapidly determine the amount of free sulphate in the polysaccharide solutions. To the best of our knowledge, this aspect of hydrolysis of carrageenans has not been examined before, although the kinetics of acid hydrolysis is still of interest as evidenced from the recent studies on this topic (Capron et al., 1996; Myslabodski et al., 1996). This issue was raised in our earlier work on kinetics of *κ*-carrageenan hydrolysis (Singh and Jacobsson, 1994).

Acid hydrolysis has been performed on (*κ*-, *ι*-, λ-) carrageenans and on two other sulphated polysaccharides (dextran sulphate, heparin) to test the suitability of the free sulphate determination method developed here. SEC-RI has been used to determine the corresponding changes in mass average molecular mass ($< M_m >$) and MMD, and judge the impact of desulphation if any. The mildly acidic conditions used here also partly simulate human gastric conditions, thus providing some idea as to the fate of these polysaccharides in an in vivo situation. Idealized structures of these polysaccharides are shown in Fig. 1.

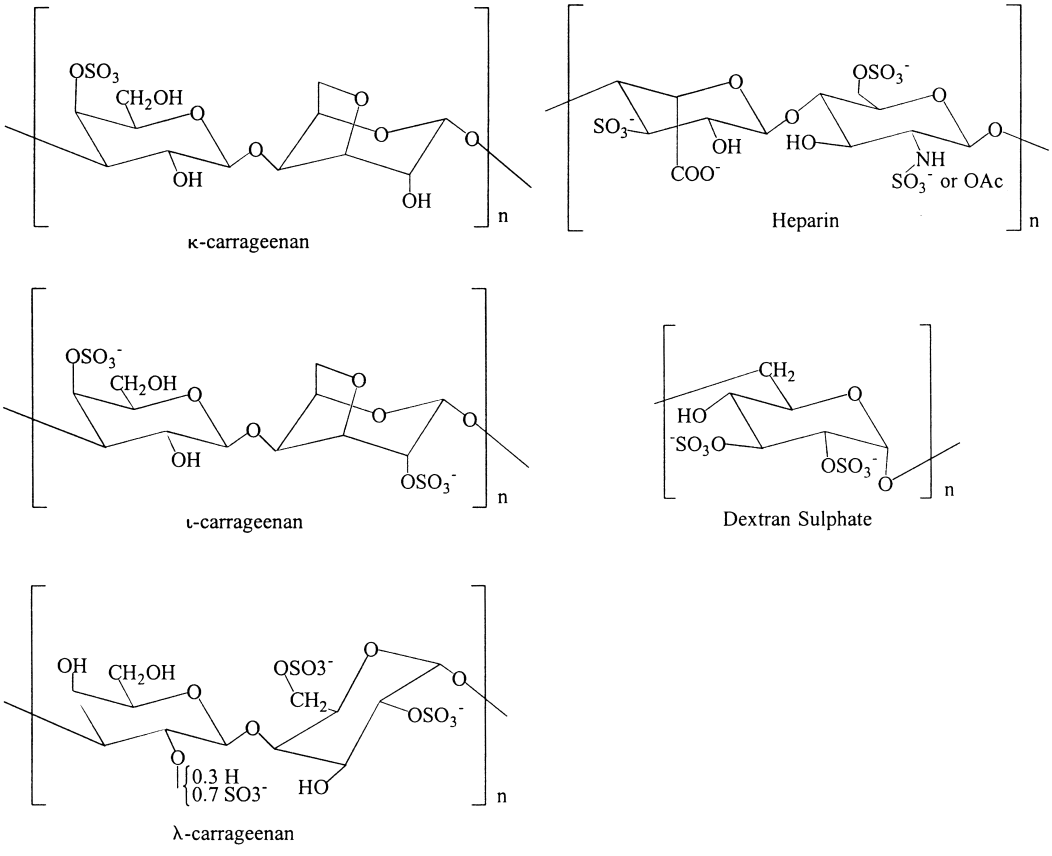

Fig. 1. Idealized structures of the polysaccharides studied.

2. Materials and methods

2.1. Materials

κ-Carrageenan (Lot No. 321365/1), ι-carrageenan (Lot No. 334125/1) and λ-carrageenan (Lot No. 319938/1 1093) were obtained from Fluka. Dextran sulphate (Batch QF 97474) was obtained from Pharmacia Biotech AB, Uppsala, Sweden, while Heparin (Lot No. 440007-01) was obtained in-house. Lithium chloride, hydrochloric acid and sodium sulphate were of p.a. grade from Merck. Lithium nitrate was obtained from Alfa. Milli-Q HPLC grade water was used in the preparation of all solutions. The composition of the pH 2 (at 25°C) hydrolysis buffer was 0.008 M LiCl and 0.012 M HCl.

2.2. Acid hydrolysis of the polysaccharides

The procedure essentially involves dissolving the polymer in the buffer and holding the solution at the desired hydrolysis temperature while removing samples periodically for analysis. The polysaccharide was first dissolved in water to obtain a 20 mg ml^{-1} solution. Preparation of this solution required the water to be heated up especially for the carrageenans. This solution was then mixed by weight with an equal amount of warm 2 × concentrated buffer to give a 10 mg ml^{-1} solution in the correct concentration of hydrolysis buffer. This solution was then rapidly divided into two parts and placed in a water bath at the appropriate temperature with a magnetic stirrer. A zero-time aliquot (2 ml) was removed from both solutions at this point and diluted into a vial containing 170 μl 0.1 M NaOH solution to neutralise the hydrochloric acid. The vial was then cooled in an ice bath to stop any further reactions. This procedure was repeated at the chosen time intervals. The vials were then stored frozen, and thawed just prior to analysis.

2.3. Molecular mass distribution

Polymer degradation by acidic hydrolysis was followed by size-exclusion chromatography (SEC) on a Waters liquid chromatography system (Waters Corp., Milford, MA, USA). The system consisted of an M615 pump, a WISP 712 autosampler, an M410 RI detector and a CHM column heater. The instruments were controlled and the data were processed by the Waters Millennium 2020 chromatography manager. The column set consisted of two TSK-GEL GMPWXL columns 7.8 × 300 mm (nominal range 10^3–10^7 poly(ethylene oxide)) with a guard column PWXL 6.0 × 40 mm (TosoHaas, Stuttgart, Germany). The column set was kept at 60°C in order to reduce the viscosity and thereby the back-pressure of the column set. The mobile phase was 0.1 M $LiNO_3$ at a flow rate of 1.0 ml min^{-1}. Narrow molecular mass distribution poly(ethylene oxide) standards used in the calibration were from Polymer Laboratories, Shropshire, UK. All molecular masses reported in this work are therefore nominally based on PEO standards and are not absolute.

2.4. Free sulphate

There are several methods available to quantify sulphate in an aqueous solution, e.g. titration using ion selective electrodes or indicators or precipitation with barium or lead, turbidimetry, photometric complexes, sulphur elementary analysis, X-ray fluorescence (XRF), atomic emission spectroscopy (ICP), etc. However, these methods are not suitable for use in the presence of sulphated polysaccharides in solution, as the polymer molecules will interfere with the determination of free sulphate. Therefore, it is normally necessary to apply a primary separation technique, such as precipitation of the polymer, dialysis or preparatory-SEC chromatography, before using any of the above techniques.

Direct analysis could be made with ion chromatography (IC) in order to separate the free sulphate from the matrix, and quantified using conductometric detection (McPhee et al., 1990; Miyazaki and Yamamoto, 1996), or a highly selective and sensitive detection with inductively coupled plasma mass spectrometry, ICP-MS (Menegario and Gine, 1997). However, it is not recommended to apply this technique to an analytical IC separation column as the sulphated polysaccharides will stick to the column and will barely elute from the column even with strong eluents. Capillary electrophoresis (Lurie, 1996) and ion-pair reversed phase liquid chromatography with the use of indirect UV detection are two other techniques that could be used without sample pretreatment.

However, we chose to develop a method in which it was possible to analyse for free sulphate directly without any pre-treatment of the sample solution. It has been reported that it was possible to separate anions on a Sephadex G-15 column (Deguchi et al., 1977) which is normally used as a size-exclusion gel for biopolymers. The elution order of the anions was according to the lyotropic series. We applied this method using a similar but modern separation gel, Superdex Peptide HR 10/30 (nominal molecular mass range 100–7000 for peptides, Amersham Pharmacia Biotech AB, Uppsala, Sweden). An RI detector was employed for the detection of the anions. The instrument and instrumental conditions were the same as in the molecular mass distribution determination above. Two eluents with different ionic strengths were tested, 0.01 and 0.1 M $LiNO_3$. Li^+ was chosen as the cation in the eluent because of its tendency to reduce the intermolecular interactions in sulphated polysaccharides and thereby prevent gelation (Smidsrød and Grasdalen, 1982). Nitrate was chosen as the anion in the eluent instead of chloride as the latter is not compatible with the HPLC instrumentation and its stainless steel components. Examples of chromatograms are shown in Fig. 2.

The separation mechanism in the above method is probably a combination of size-exclusion and ion-repulsion

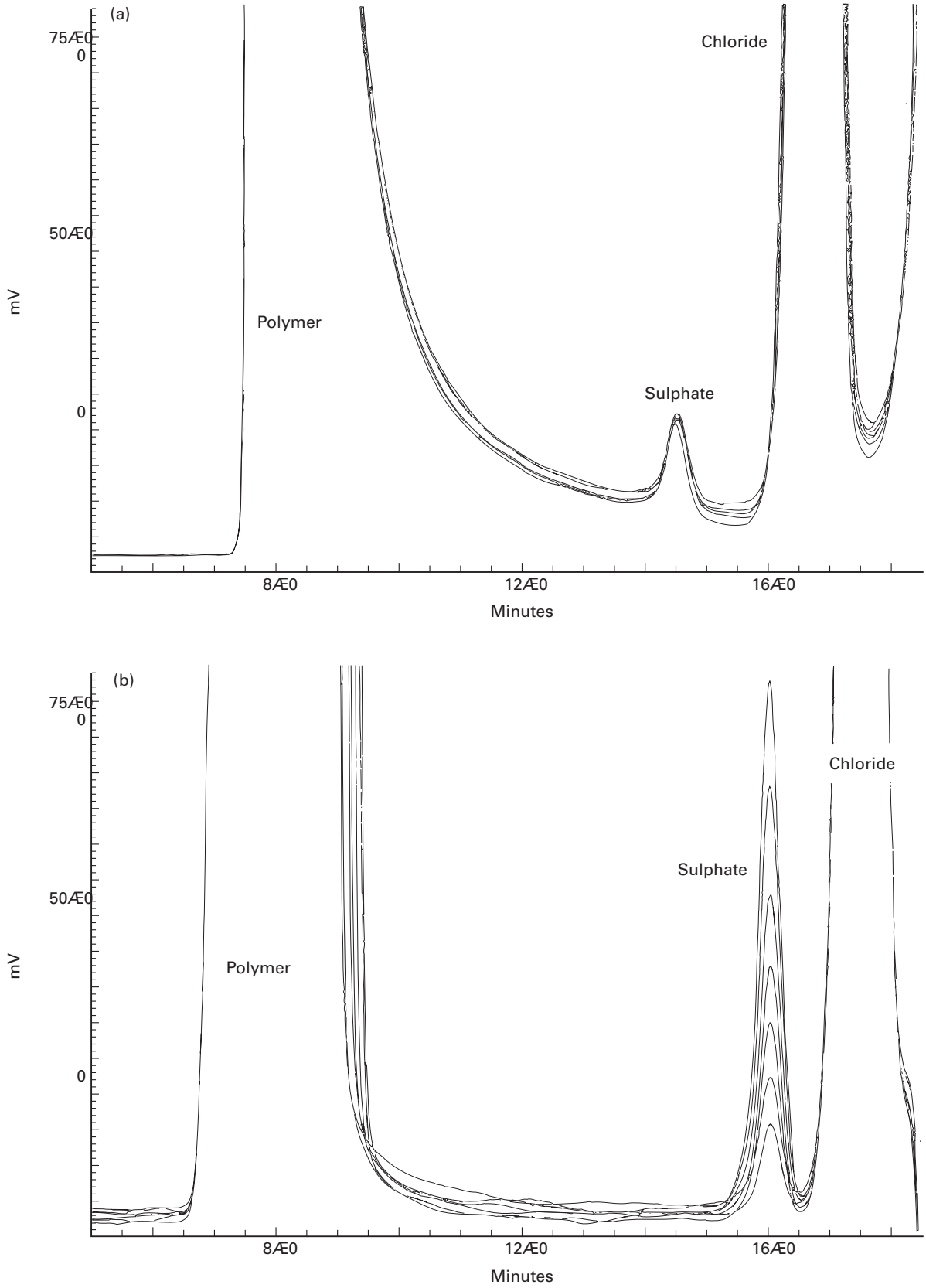

Fig. 2. Chromatograms from the method for determination of free sulphate in (a) heparin and (b) dextran sulphate, after acidic hydrolysis at 55°C for increasing amounts of time.

effects. Deguchi et al. (1977) proposed hydrophobic interaction as the separation principle in their Sephadex G-15 column. The small amount of charged carboxylic groups in the gel repels the sulphate and chloride ions (from hydrochloric acid), while the polysaccharides elute in the void volume, primarily according to the size-exclusion mechanism. It was found that 0.01 M $LiNO_3$, i.e. the lowest ionic strength tested of the eluent, gave the best separation between polymer, free sulphate and the large amount of chloride from the buffer. Detection with a differential refractive index detector provided sensitivity enough to quantify the sulphate concentrations in our samples. Limit of detection for sulphate in the 0.01 M $LiNO_3$ mobile phase was approximately 2 μg ml (three times the noise). This concentration corresponds to a sulphate content of 0.02% of the dry polymer in a 10 mg ml^{-1} polymer solution. If there is a need for detecting lower concentrations of the anion, other detection techniques could be used; a conductometric detector would be very sensitive but the system would require conductivity suppression of the eluent as in ion chromatography. The linearity range of the calibration curve was very large, exceeding 1000 μg ml^{-1}. Furthermore, it is possible to perform a simultaneous quantitative analysis of free sulphate and the polysaccharide itself with this method, since the polymer and the anions are well separated.

3. Results and discussion

The possibility of desulphation of the carrageenans during acid hydrolysis has been given little attention in the literature. Most studies have focused on the depolymerization process and the decrease in molecular mass of the polymers studied. On the other hand, there are a number of studies discussing the desulphation of heparin, because heparin has important pharmacological properties that are dependent on its degree of sulphation (Ehrlich and Stivala, 1974).

As the studied polysaccharides are hygroscopic it was necessary to perform a moisture content determination in the samples (approximately 10–11% w/w loss on drying at 105°C for 2 h for each polymer). After correction for moisture, the measured sulphur content in the different polysaccharides correlates well to the ideal structures drawn in Fig. 1. These values are shown in Table 1.

The rate of hydrolysis of sulphate groups from the polysaccharide samples show large differences. Fig. 2 shows some examples of overlaid chromatograms from the determination of free sulphate where the samples was treated at 55°C up to 120 min. The increased liberation of sulphate for dextran sulphate is obvious, while heparin seems almost unaffected in this time period. The difference in sulphate retention times for the two polysaccharides provides the possibility to optimize separation by changing the mobile phase composition, in this case 0.01 and 0.1 M $LiNO_3$, respectively. Furthermore, the first peak in the chromatograms represents the polymer fraction of the sample, which shows that it is possible to perform a simultaneous quantification of the polymer, free sulphate and chloride using this method.

Fig. 3 shows the relative amounts of hydrolysed sulphate for the different polymers at 35 and 55°C. For dextran sulphate there is a linear increase of desulphation, especially at 55°C, while the sulphation of the other samples is seemingly unaffected by acid hydrolysis. While single samples were analysed for dextran sulphate and heparin, these samples show much clearer trends than the carrageenans. The larger variation in results for carrageenans is a consequence of the necessity to dilute these samples five times with the mobile phase prior to analysis, in order to reduce the viscosity of the solutions to levels suitable for injection. The levels of sulphate hydrolysis at 120 min for all samples are summarized in Table 2. The carrageenans and heparin show the same stability to acidic sulphate hydrolysis in the time and temperature range studied. (Note, however, that the susceptibility of heparin to desulphation under acidic conditions is well documented; Ehrlich and Stivala, 1974).

Table 1
Initial polymer characteristics: sulphate content (dry basis), mass average molecular mass, $< M_m >$, relative to poly(ethylene oxide) standards, and charge density

Polysaccharide (Na^+ form)	Sulphur, ideal structure (% w/w)	Actual sulphur content (corr. for moisture) (% w/(% w/w)	Actual sulphate content w)	$< M_m >$ (g mol^{-1})	Charge density[d]
κ-Carrageenan	7.5[a]	7.8	23.4	2.9×10^6	1 (1.03)[e]
ι-Carrageenan	12.2[a]	11.1	33.3	5.6×10^6	2 (1.49)[e]
λ-Carrageenan	14.0[a]	12.8	38.4	9.8×10^6	3 (2.09)[e]
Dextran sulphate	17.5[b]	18.2	54.6	3.2×10^6	0.86[f]
Heparin	12.5[c]	11.5	34.5	6.8×10^4	1.6[c]

[a]Yalpani (1988).
[b]Based on two sulphate groups per monosaccharide unit.
[c]Jandik et al. (1996).
[d]Number of ionizable groups per monosaccharide unit.
[e]Ideal (actual values in parentheses reported by Caram-Lelham and Sundelöf, 1995).
[f]Actual; manufacturer's information.

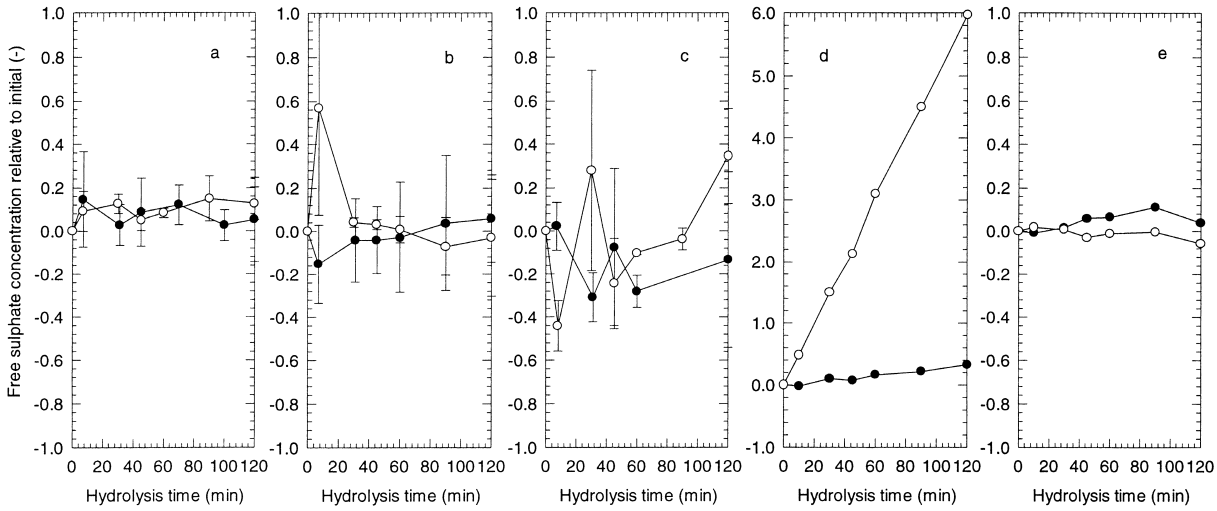

Fig. 3. Relative (to initial) concentration of free sulphate formed as a function of hydrolysis time at (●) 35°C and (○) 55°C, for (a) κ-carrageenan, (b) ι-carrageenan, (c) λ-carrageenan, (d) dextran sulphate, and (e) heparin. Initial free sulphate levels are given in Table 2.

The reason for the lower stability to desulphation for dextran sulphate compared to the other sulphated polysaccharides is not clearly understood, but some aspects of the polymer structure may have an effect on the hydrolytic stability. It is likely that the flexibility of conformation of the polymer chain has relevance towards both ease of desulphation and chain scission. Greater flexibility allows better access. The chain flexibility of glucans can be expressed in terms of a flexibility parameter (B) which is a measure of the ability of polyelectrolytes to respond to changes in salt concentration by altering their hydrodynamic volume (Smidsrød and Haug, 1971). This term can vary from 0.004 for rigid rods to over 0.24 for flexible random coil polyelectrolytes. The available B values are summarized in Table 2 and show that dextran sulphate at $B = 0.230$ is highly flexible while κ-carrageenan ($B = 0.100$) and λ-carrageenan ($B = 0.053$) are more rigid polymers (Yalpani, 1988). Chain flexibility in solution is determined by the monomer units and the linkage between them, as well as the charge density. It can be modulated by the ionic strength of the solution. We expect ι-carrageenan to have less flexibility than κ-carrageenan and more than λ-carrageenan when compared at the same ionic strengths. Greater flexibility does lead to larger susceptibility to desulphation, at least for dextran sulphate. However, since the (negligible) levels of desulphation in the other polymers do not correlate with their flexibility, other factors must be playing a role. In addition, as shown in Table 1, the relative amount of sulphate groups is the highest for dextran sulphate, which thereby gives it the highest probability of undergoing desulphation.

Measured $< M_m >$ values as a function of hydrolysis time for all samples are plotted in Fig. 4. The decrease in $< M_m >$ is relatively large for the carrageenans while the decrease for dextran sulphate is much less and heparin is almost unaffected under the conditions and time period studied here.

Assuming that desulphation is the only mechanism for molecular mass loss, a theoretical average molecular mass at each time point can be calculated. These values are presented in Table 3 along with the actual measured mass average molecular mass after 120 min of hydrolysis. The theoretical $< M_m >$ value after 120 min, if reduced solely by desulphation, is only slightly lower than the initial value for dextran sulphate, while it is unaffected for the other polymers tested. (This theoretical $< M_m >$ is plotted in Fig. 4d for dextran sulphate). Actual $< M_m >$ values show significant depolymerization for dextran sulphate and carrageenans while heparin has a very slight decrease in

Table 2
Amount of free sulphate detected in polymer solutions before and after hydrolysis, and flexibility parameter of the polymer

Polysaccharide	Flexibility parameter B	Free sulphate concentration (mg g^{-1} polymer)		
		Initial	After hydrolysis for 120 min at 35°C	55°C
κ-Carrageenan	0.110[a]	2.0	2.0	2.1
ι-Carrageenan	–	1.8	1.9	2.5
λ-Carrageenan	0.053[a]	9.3	9.0	10.9
Dextran sulphate	0.230[a]	2.5	3.4	17.5
Heparin	–	2.6	2.7	2.5

[a]Yalpani, 1988.

Table 3
$< M_m >$ for the various polysaccharides after acid hydrolysis for 120 min

Polysaccharide	Theoretical $< M_m >$ (g mol^{-1}) if solely due to measured sulphate loss		Actual measured $< M_m >$ (g mol^{-1})	
	35°C	55°C	35°C	55°C
κ-Carrageenan	2.9×10^6	2.9×10^6	4.0×10^6	5.1×10^5
ι-Carrageenan	5.6×10^6	5.6×10^6	3.3×10^6	1.1×10^5
λ-Carrageenan	9.8×10^6	9.8×10^6	5.8×10^6	6.6×10^5
Dextran sulphate	3.2×10^6	3.1×10^6	3.1×10^6	2.2×10^6
Heparin	6.8×10^4	6.8×10^4	6.6×10^4	6.4×10^4

$< M_m >$. Thus, sulphate loss alone cannot account for the final observed $< \frac{M}{m} >$ for dextran sulphate implying that chain scission is occurring. On the other hand, since about 3% of the sulphate groups are hydrolysed for this polysaccharide, results from size-exclusion chromatography become prone to error since the technique measures hydrodynamic volume of a sample. Changes in degree of sulphation will influence the charge density and thereby the hydrodynamic volume, at a constant ionic strength. Part of the observed decrease in $< M_m >$ might therefore simply be due to the polymer being less extended in solution due to smaller intramolecular sulphate group repulsions.

Kinetic parameters have been extracted from the data in Fig. 4 using the reciprocal molecular mass versus time relationship in Eq. (1) given by Tanford (1961) and used in earlier carrageenan studies by Singh and Jacobsson (1994) and Myslabodski et al. (1996).

$$\frac{1}{\langle M_m(t) \rangle} = \frac{1}{\langle M_m(0) \rangle} + \frac{kt}{m} \quad (1)$$

where $< M_m(t) >$ and $< M_m(0) >$ are the molecular masses at time t and time zero respectively, k is the first-order rate constant for change of mass average molecular mass, and m is the molecular weight of the repeating unit. The repeating unit in these calculations has been taken as that of an average monosaccharide for dextran sulphate ($m = 366$, no counterion) and heparin ($m = 183$, no counterion), while that of an average disaccharide for the carrageenans ($m = 392$ for κ-; 465 for ι-; 551 for λ-; no counterions) based on the very different responses towards acid attack of the two bridging bonds in κ-carrageenan as surveyed by Myslabodski et al. (1996). The calculated kinetic parameters are summarized in Table 4. Parameters for heparin could not be calculated due to the very small change in molecular mass detected in the time period of the experiment. Also given in this table are the Arrhenius parameters derived from the kinetic data.

ι-Carrageenan shows the highest (pseudo)-activation energy (Singh and Jacobsson, 1994) for hydrolysis of the polysaccharides examined. Hjerde et al. (1996) also observed higher activation energies for ι-carrageenan compared to κ-carrageenan in the disordered state, and relate this to the steric hindrance afforded by the sulphate group at the 2-position in the 3,6-anhydrogalactose unit. A compilation of Arrhenius parameters for acid hydrolysis of these two carrageenans is given in Table 5, and shows that while there is a certain degree of variation in the data, the ranges

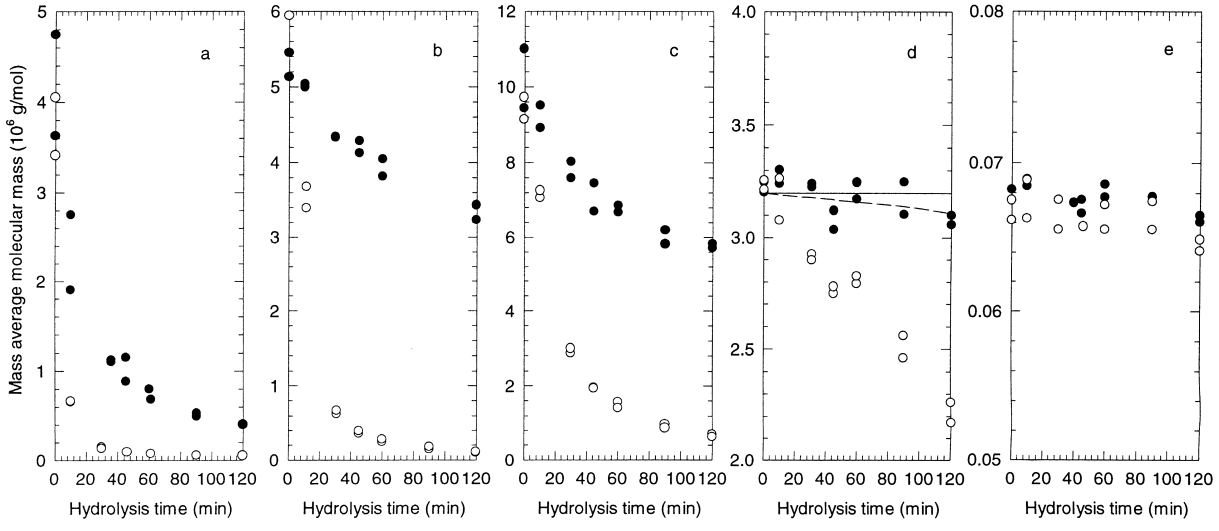

Fig. 4. Mass average molecular mass as a function of hydrolysis time at (●) 35°C and (○) 55°C, for (a) κ-carrageenan, (b) ι-carrageenan, (c) λ-carrageenan, (d) dextran sulphate, and (e) heparin. Initial values are also given in Table 1. Theoretical molecular mass as a function of hydrolysis time based solely on the desulphation effect as described in the text is plotted as (——) at 35°C and (– – –) at 55°C.

Table 4
First-order rate constants and Arrhenius parameters for the hydrolysis of sulphated polysaccharides at pH 2

Substance	k_{35} (min^{-1})	k_{55} (min^{-1})	Pre-exponential factor, A (min^{-1})	Activation energy, E ($kJ\ mol^{-1}$)
κ-Carrageenan	7.4×10^{-6}	9.0×10^{-5}	5.3×10^{12}	105
ι-Carrageenan	4.3×10^{-7}	2.0×10^{-5}	1.3×10^{21}	162
λ-Carrageenan	4.2×10^{-7}	6.6×10^{-6}	1.9×10^{13}	116
Dextran sulphate[a]	$3.1x10^{-8}$	3.6×10^{-7}	9.7×10^{10}	103
Heparin	–	–	–	–

[a]Desulphation occurs simultaneously with depolymerization.

are well in agreement. More comprehensive summaries for κ-carrageenan are given in Singh and Jacobsson (1994) and Myslabodski et al. (1996). Differences in values obtained reflect differences in starting materials, experimental conditions, and techniques. Our data do not seem to show a higher degree of stability against acid hydrolysis for the λ- form.

The molecular mass distribution for the carrageenans at different hydrolysis times at 55°C are shown in Fig. 5. They all show a more or less bimodal distribution for the non-hydrolysed samples, which tends towards a unimodal distribution as the hydrolysis proceeds. The reason lies in the fact that there are simply a larger number of bonds in the larger molecular chains to hydrolyse, thus giving a larger probability of chain cleavage in the higher molecular weights initially. This process of stochastic chain cleavage has been modelled earlier by Singh et al. (1994). Since the calibration standards for SEC used here are based on poly(-ethylene oxide), no conclusions about the fraction of polymer below a molecular mass of 20 000 g mol^{-1} can be drawn. However, the method shows that, in principle, after a single calibration run with an absolute mass detector such as an MALLS, RALLS or LALLS, a robust system can be set up for detecting the low molecular mass products.

4. Conclusions

We have examined the effect of acid hydrolysis on the sulphate content of various sulphated polysaccharides, as well as their mass average molecular mass, as a function of hydrolysis time and temperature. A new and sensitive size- and ion-exclusion based chromatographic method has been developed for this purpose, enabling rapid determination of free sulphate levels in a polymer solution without a sample preparation/polymer separation step. The data have been used to study polymer desulphation and depolymerization, and kinetic parameters have been extracted for the depolymerization of four of the polysaccharides. Dextran sulphate was found to be sensitive to desulphation, while the carrageenans and heparin were stable under the conditions examined. Dextran sulphate and carrageenans were, however, sensitive to depolymerization by chain scission during the same acid hydrolysis process.

In general, when studying depolymerization by size-exclusion chromatography, it is important to ensure that there is no change in the repeating unit of the polymer. This is especially important for polyelectrolytes as their coil volume in solution may vary depending upon the nature of the counterions and the ionic strength in the mobile phase. We have found in this work that under mild acidic hydrolysis (over at least 2 h), the carrageenans and heparin do not desulphate, and are therefore not likely to change their hydrodynamic volume as a result of loss of charge. Therefore, the earlier studies on mild acidic hydrolysis of carrageenans are correctly concerned with true depolymerization. On the other hand, for polysaccharides like dextran sulphate where there is a significant sensitivity to sulphate hydrolysis, the degree of depolymerization cannot be accurately determined by conventional size-exclusion chromatography. To overcome this problem, one possibility would be to collect fractions of the degraded polymer and to determine the refractive index increment and concentration

Table 5
Comparision of Arrhenius parameters for acidic hydrolysis of some sulphated polysaccharides at pH 2

κ-Carrageenan			ι-Carrageenan		
A (min^{-1})	E ($kJ\ mol^{-1}$)	Reference	A (min^{-1})	E ($kJ\ mol^{-1}$)	Reference
2.5×10^{13}	113	1	–	–	–
4.6×10^{15}	126	2	–	–	–
3.2×10^{16}	120	3	9.0×10^{17}	133	3
5.3×10^{12}	105	4	1.3×10^{21}	162	4

1: Singh and Jacobsson, 1994.
2: Myslabodski et al., 1996.
3: Hjerde et al., 1996.
4: This work.

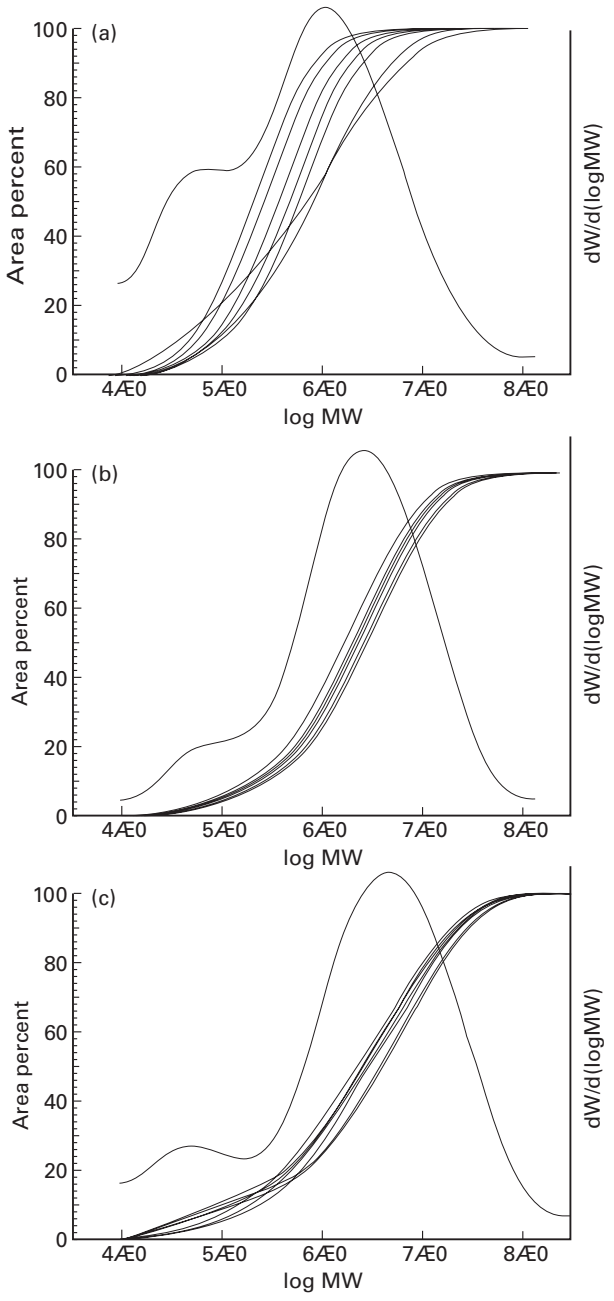

Fig. 5. Molecular mass distributions with increasing hydrolysis time at 55°C for (a) κ-carrageenan, (b) ι-carrageenan, and (c) λ-carrageenan.

for the fractions and then determine the $< M_m >$ by a static-light scattering experiment.

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