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Kinetics of acid hydrolysis of κ-carrageenan as determined by molecular weight (SEC-MALLS-RI), gel breaking strength, and viscosity measurements

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The degradation of kappa-carrageenan by acid hydrolysis in a LiCl/HCl pH 2 buffer has been studied at various temperatures. The reduction in weight-average molecular weight has been measured over time by size exclusion chromatography with multi-angle laser light scattering and refractive index detection. The hydrolysis process has also been followed by gel breaking strength and low-shear viscosity measurements. These parameters have been correlated to the molecular weights, thus allowing for the possibility of replacing complicated molecular weight determinations by parameters which can be measured more simply. The results can be of use in process design and optimization as well as control. Kinetic constants and activation energies for kappa-carrageenan hydrolysis have also been obtained from each of the parameters measured.

INTRODUCTION

Carrageenans are water soluble cell-wall linear polysaccharides extracted from certain members of the class of red seaweeds (*Rhodophyceae*). They are 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. Kappa (κ)-carrageenan is composed of alternating $\alpha(1 \rightarrow 3)$-D-galactose-4-sulphate and $\beta(1 \rightarrow 4)$-3,6-anhydro-D-galactose. The κ- and ι-carrageenans form thermoreversible gels in solution while λ- (and other minor forms) yield highly viscous solutions that do not gel. The gelling behaviour is dependent on the nature and concentration of cations present in the solution (see, for example, Clark & Ross-Murphy, 1987). Significant interactions also occur with proteins (e.g. in milk) and hydrocolloids such as starch, locust bean gum, guar gum, etc. (Descamps *et al.*, 1986). On the basis of these properties, carrageenans are used extensively in the food industry as viscosity/gel or texture enhancers, stabilizers, etc. (Christensen, 1964). The same properties are utilized by the pharmaceutical/cosmetic industry, in lotions, creams, toothpaste, cough preparations, etc. Due to its widespread use, extensive toxicological evaluation of carrageenan has been carried out. The major problem that has been identified involves low molecular weight ($< 20\,000$ g/mole) fractions which can cause lesions. Degraded carrageenan is in fact used to induce ulcerative colitis in the colon in guinea-pigs as an experimental model to study the effects of pharmacological and therapeutic agents

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(Marcus *et al.*, 1989). Low molecular weight fractions may be present in the raw material or may be created during production processes, especially since carrageenan in solution is susceptible to acid hydrolysis; it is most stable at pH 9 and the hydrolysis rate increases rapidly with the lowering of pH or increased temperature (Ekström, 1985; Andersen, 1988).

In the present work, the kinetics of acid hydrolysis of κ-carrageenan has been studied in an LiCl/HCl buffer solution in a batch mode, and at various temperatures. An LiCl/HCl buffer was chosen for this study since the presence of Li^+ ions reduces the tendency of κ-carrageenan molecules to form gels or aggregates via the (double) helix stabilization mechanism (see, for example, Morris *et al.*, 1980, Smidsrød & Grasdalen, 1982), thereby allowing non-specific bond cleavage to occur. The hydrolysis process has been followed by measuring the weight-average molecular weight (M_w), low-shear viscosity (η) of the polymer solution, as well as relative gel strength (GS_r) (defined below) of the polymer solution (at room temperature) over time. The parameters, η and GS_r, were also examined to see if they correlated well with M_w in order to serve as simple measures of molecular weight/extent of hydrolysis.

'Gel strength' in this report is defined as the pressure required to break through the surface of a gel with a cylindrical probe and is also called 'gel breaking strength' in the literature. This quantity is extensively used in the literature to characterize gels (see, for example, Mitchell, 1976; Belton *et al.*, 1984; Ozawa *et al.*, 1985), although doubts have been raised over its suitability (Smidsrød *et al.*, 1972). However, it is a quantity that can be easily and rapidly measured and therefore suitable for routine testing. It is dependent on the experimental system employed to measure the force, the shape and surface finish of the probe, and its rate of movement, etc. Since there does not exist any defined standard equipment or procedure for this purpose, values obtained from different measuring systems should not be compared except in qualitative terms. However, values obtained for different gels from a particular well defined measuring system can be compared.

The (viscosity-average) molecular weight is related to the intrinsic viscosity of the polymer via the Mark–Houwink constants. However, these constants are specific to the polymer–solvent system in question. Measurement of intrinsic viscosity is, while not difficult, certainly tedious. Therefore, instead of intrinsic viscosity, the low-shear viscosity was utilized as a measure of molecular weight during hydrolysis in this study.

M_w is an obvious measure of hydrolysis of the polymer since it directly reflects the result of macromolecular chain cleavage. GS_r and η are less direct but equally relevant measures since gels (of carrageenans) are formed as a result of association of (double) helices which serve as crosslinks. The length of these helices depends in turn on the lengths of the chains, as does the viscosity. However, it must be noted that none of these parameters is a fundamental measure of the rate of hydrolysis of the chemical bond between monomer units making up the polymer; this rate is measured, most appropriately, by the rate of change of 'number of intact bonds' in the polymer mixture. It is only at short hydrolysis reaction times that the number- or weight-average molecular weights can be substituted instead of the above quantity (Tanford, 1961). Changes in GS_r, η, or even M_w are non-linear functions of the individual bond hydrolysis rate(s).

Data such as those obtained above can be useful in the design and scale-up of processes, and assist in determining the limits of operating conditions during process optimization.

MATERIALS AND METHODS

Materials

κ-carrageenan was obtained from Sigma Chemicals C-1263, batch 19F0637. Lithium chloride and hydrochloric acid 37% were p.a. grade from Merck. Milli-Q HPLC grade water was used after being filtered through a 0·1 μm filter and subsequently deaerated. The composition of the pH 2 buffer was LiCl 0·088 mol/litre, HCl 0·012 mol/litre.

Hydrolysis of κ-carrageenan in buffer

The procedure essentially involves dissolving the polymer in the buffer and holding the mixture at the reaction (hydrolysis) temperature while removing samples periodically to measure the M_w and room temperature gel strength. Due to difficulties with the apparatus involved, measurements of both these parameters could not be carried out on the same samples; separate reaction mixtures had to be prepared for each. The procedures for the individual techniques involved are summarized below. Three (at 35°C, 45°C) or four (at 55°C) independent experiments were performed for 'hydrolysis as measured by M_w', two experiments at each temperature were performed for 'hydrolysis as measured by gel strength', while 'hydrolysis–low shear viscosity measurements' at each reaction temperature (35°C, 45°C) were run in triplicate.

The gel strengths were measured on gels at room temperature, irrespective of the hydrolysis temperature, in order to eliminate the influence of (hydrolysis) temperature on the measured value of this parameter (as well as to obtain good gels with measurable gel strengths). The values obtained for gel strengths within a hydrolysis experiment have been normalized with respect to the gel strength of the 'zero-time' sample from the same experiment, thus giving their 'relative gel

strengths'. These relative gel strengths (GS_r) are independent of the starting material and can thus serve as independent measures of the hydrolysis process.

Similarly, all M_w values were also measured at the same temperature (= detector cell temperature), irrespective of hydrolysis temperature, in order to eliminate any influence attributable to differences in possible aggregation behaviour at the different temperatures.

Low-shear viscosity (η) was measured continuously in a rheometer at the chosen reaction temperature.

Measurement of weight-average molecular weights (M_w)

κ-carrageenan was dissolved in (10 ml) buffer to obtain a 4–5 mg/ml solution. The powder was poured into the buffer while stirring simultaneously. Particles of κ-carrageenan have a tendency to form a gel layer on their surfaces at room temperature, thus slowing their dissolution; this process was therefore speeded-up by heating the mixture to approximately 70°C for 2–3 min and stirring rapidly with a magnetic bar. The first aliquot for analysis (0·50–0·75 ml) was taken immediately afterwards and cooled in an ice-bath. The rest of the reaction mixture was then placed in a water-bath maintained at the selected reaction temperature (35/45/55°C). This initial 'heat-to-dissolve' step introduces a source of error in the measurements since rapid hydrolysis can occur due to the high temperature during the dissolution process itself; however, it was considered preferable to the uncertainties caused by a slow dissolution process at room temperature. (An alternative procedure to be used in future work is to dissolve the polymer in water and dilute with concentrated buffer solution.) The true 'zero-time' or unhydrolysed polymer M_w values were obtained independently by dissolving the polymer in a non-hydrolysing 0·1 mol/litre LiCl solution to a concentration of approximately 3 mg/ml.

Small aliquots (0·50–0·75 ml) of the reaction mixture were removed periodically for determination of M_w. Each sample (100 μl) was injected into the chromatography system, described below. Samples were placed in an ice-bath while waiting to be analysed. Each sample was injected only once. The peak eluting from the size exclusion chromatography (SEC) columns consists of the sample distribution according to hydrodynamic volume, which is in turn assumed to represent molecular weights.

The SEC-MALLS-RI system consisted of an HPLC pump, LKB model 2248, connected to an on-line pulse dampener and an on-line high pressure effluent filter (Milipore) with a 0·1 μm membrane compatible with aqueous solutions. A Shodex OH-Pak B-800P precolumn followed by Shodex OH-Pak columns B-806/S, B-805/S and B-804/S in series (8 × 250 mm each) were used for the chromatography. Light scattering detection was by a DAWN-F MALLS photometer equipped with a K5 flow cell ($n = 1{\cdot}52064$) and a high temperature read-head. The photometer light source is a He–Ne laser, 633 nm, 5 mW (Wyatt Technology, Santa Barbara, California). A Tecator Optilab 5922 RI chromatography module comprising an interference refractometer (RI detector), and a Valco injection valve compartment was used. The measuring wavelength of the refractometer is 633 nm. The path length of the RI cell is 1·0 mm. The whole module is thermostatable (Tecator, Stockholm, Sweden). A schematic of the whole system is given in Fig. 1. The SEC, RI systems and the MALLS detector are connected by a controlled-temperature dual line tubing (Wyatt Technology).

The multi-angle laser light scattering (MALLS) detector, operating on the principles of classical light scattering from macromolecules, calculates the molecular weight of thin 'slices' of this chromatographic peak while the refractive index (RI) detector measures the concentration of polymer in these thin 'slices'. Combining the molecular weight of the slices with the corresponding concentration allows the M_w of the whole sample and its molecular weight distribution to be calculated (Wyatt *et al.*, 1988; Jackson *et al.*, 1989).

In order to obtain a chromatographic separation of κ-carrageenan based on the size of the molecules in the sample (size exclusion), it is important to suppress other possible interactions of the sample and the column such as ionic exclusion/inclusion, dipolar interactions, hydrogen bonding, etc. Based on the report by Lecacheux *et al.* (1985), 0·1 mol/litre LiCl was used as the eluent in this work; apart from the electrolyte serving to screen the electrostatic interactions between column and

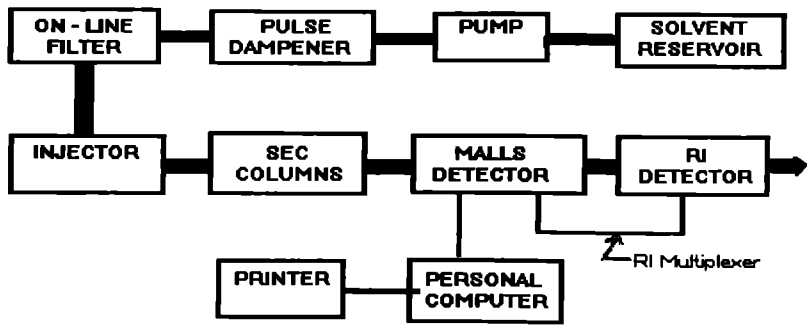


Fig. 1. Schematic representation of the SEC-MALLS-RI experimental system.

sample, Li^+ also prevents or reduces the tendency of κ-carrageenan to aggregate, as discussed earlier. The experimental system consisting of the chromatography module, the MALLS detector and the connecting tubing were operated at 60°C to further preclude any aggregate formation. An eluent flow rate of 1·0 ml/min (25°C) was used; the eluent is passed through a long loop in the compartment containing the columns, thus raising its temperature to 60°C, before it actually enters the columns.

Molecular weight determination by light-scattering requires the measurement of the refractive index (n) of the eluent as well as the refractive index increment (dn/dC) of the polymer in the solvent/eluent. For this purpose the refractive index increment of the LiCl–water solution was measured (using the RI detector in isolation), and the refractive index (n) of 0·1 mol/litre LiCl solution calculated by extrapolation. (The absolute calibration constant of the RI detector in isolation was determined earlier by injecting increasing concentrations of NaCl solutions into a 0·2 mm path length sample cell.)

$(dn/dC)_{LiCl-H_2O} = 0{\cdot}1879$ RIU ml/g at 60°C
$(n)_{0{\cdot}1\ mol/litre\ LiCl} = 1{\cdot}3287$ RIU at 60°C
$(n_o)_{H_2O} = 1{\cdot}327$ RIU at 60°C

The refractive index increment of κ-carrageenan solution in 0·1 mol/litre LiCl was also measured. A stock solution of κ-carrageenan in 0·1 mol/litre LiCl was dialysed against an excess of 0·1 mol/litre LiCl for 3 days. The stock solution was then diluted with the dialysate to produce a series of solutions with increasing κ-carrageenan concentration. These were then injected into the sample cell of the RI detector as above. A value of 0·111 RIU ml/g at 25°C was obtained. Lecacheux *et al.* (1985) reported refractive index increments for κ-carrageenan in 0·1 mol/litre LiCl between 0·113 and 0·118 at 60°C and used a constant value of 0·115 in all their measurements. Considering the inaccuracies involved (in measurement of concentration after dialysis, absolute RI calibration factor, etc.), a value of

$(dn/dC)_{\kappa\text{-Carr. in } 0{\cdot}1\ mol/litre\ LiCl} = 0{\cdot}115$ RIU ml/g at 60°C

was used in all the experiments reported here.

The other material constant of interest is the second virial coefficient (A_2) of κ-carrageenan in 0·1 mol/litre LiCl. The MALLS detector was used for this purpose, in isolation, i.e. as a photometer. Measurements were made in the micro-batch mode (Wyatt *et al.*, 1988) on κ-carrageenan (samples prepared as above). A value of $1{\cdot}71 \times 10^{-3} \pm 1{\cdot}1 \times 10^{-4}$ ml mole/g^2 was obtained for a solution at 25°C. The literature value at 60°C is $2{\cdot}62 \times 10^{-3}$ ml mole/g^2 (Lecacheux *et al.*, 1985). The value used in the subsequent analyses was

$(A_2) = 2{\cdot}0 \times 10^{-3}$ ml mole/g^2 at 60°C

This value was chosen since it was found that the term containing (A_2) did not significantly influence the calculated value of M_w.

Measurement of gel breaking strength

κ-carrageenan was dissolved in the (warm) buffer to obtain 300 g of 1·5 w/w solution. As mentioned above, the dissolution process was speeded up by heating the mixture to approximately 70°C for 2–4 min while stirring with a magnetic bar. The stock solution was immediately divided in two parts into two ehrlenmeyer flasks and an aliquot of 10 ml removed from each to serve as the reference or 'zero-time' sample for calculation of GS_r of subsequent samples (≡ ratio of gel strength of sample to gel strength of 'zero-time' sample). These reference aliquots were placed in cylindrical glass bottles (29 mm ID), stoppered and placed in an ice-bath to cool the samples. The ehrlenmeyer flasks were placed in a water-bath with shaker, at the selected reaction temperature (35/45/55°C). Aliquots of the reaction mixture (10 ml each) were then periodically removed, placed in identical glass bottles, and cooled in the ice-bath. Care was taken to keep the bottles level in order to obtain a flat gel surface after cooling. Gel strength measurements were taken the day after; samples were stored at 5°C overnight.

Gel strength was measured on a home-built apparatus, the schematic of which is shown in Fig. 2. It basically consists of a step-motor which drives a polished stainless-steel plunger (diameter 10 mm, thickness 0·8 mm) at a known rate (1·0 mm/s), downwards on and into the gel. The bottle containing the gel is placed on a balance (Mettler PM2000, resolution 0·01 g) which registers the resistance offered by the gel to the plunger. Data from the balance is transferred to the controlling computer six times per second while the internal clock in the computer keeps track of the time; a time resolution of approximately 0·16 s is obtained. Software for control of the step-motor was supplied by the manufacturer (Berger Lahr, Germany); the programs for overall control and operation of the apparatus as well as data acquisition and analysis were written in-house.

The bottles containing the gels were removed from cold storage and kept at room temperature for approximately 2 h before the measurements were taken. For measurement of gel strength, the bottle was mounted on a teflon holder to provide a stable and level base. It was then placed on the balance under the plunger with the plunger centred on and just above the gel. The balance was tared, and the motor was started to lower the plunger; the internal clock started simultaneously. The plunger was allowed to break through the gel surface before stopping and reversing the motor. The 'force on plunger' recorded from the balance was plotted against time to obtain curves, an example of which is shown in Fig. 3. The 'gel breaking strength' of the sample was taken to be the force at the point at which

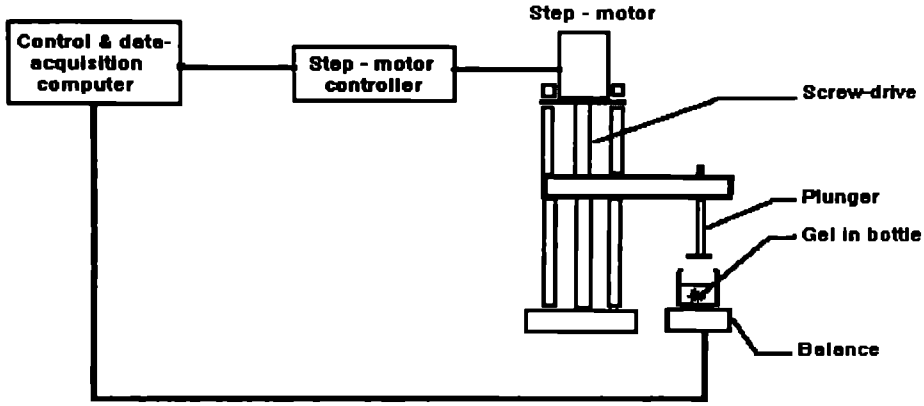


Fig. 2. Schematic of the experimental system to measure gel breaking strength.

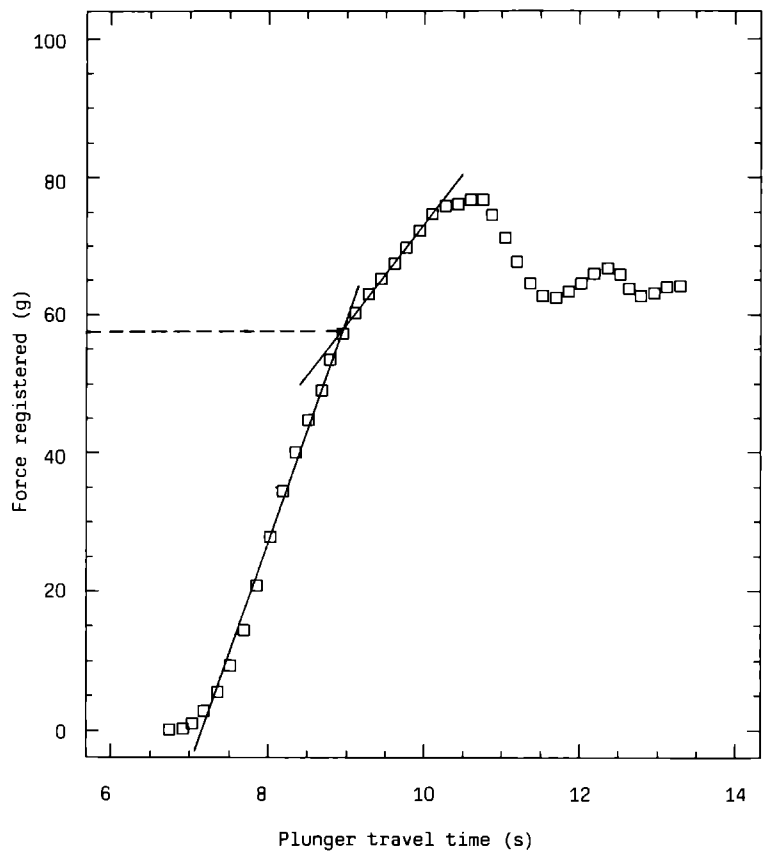


Fig. 3. An example of force on gel versus plunger travel time curve for measuring gel breaking strength. The intersection of the two lines drawn through two distinct sections of the curve gives the gel breaking strength for the sample.

the slope of the curve changes, representing the breaking of the plunger through the gel surface (see Fig. 3). (Note that the peak in the force versus time curve occurs as a result of compression of the gel before the gel flows back onto the upper part of the plunger relieving the pressure, and is not representative of the gel strength; the plunger has already penetrated the gel by this time.)

Measurement of low-shear viscosity (η)

A 1·5 w/w solution of κ-carrageenan in the (warm) buffer was prepared as above, and introduced into the C25 measuring system (concentric cylinders) of a Bohlin VOR Rheometer (Bohlin Rheology, Lund, Sweden). The surface of the solution was covered with light paraffin oil to prevent evaporation during the course of the measurements. Shear viscosity was measured at constant (hydrolysis reaction) temperature using a shear rate of 9·207 s^{-1}. Constant shear was utilized in order to keep the contents of the cup mixed. A 1·68 g cm or 0·29 g cm torque element was used as appropriate and measurements were made every 120 s, over an integration time of 10 s. Three or four separate runs were made at each temperature.

It was found that reproducible viscosity measurements could not be taken on this system for the solution at 55°C. The smallest available torque bar lacked sensitivity at this temperature for the shear rate chosen. Thus results are presented for 35 and 45°C only.

Regression analyses on experimental data have been performed using STATGRAPHICS® for PCs (Ver. 2.6e) and SYSTAT® for Macintosh (Ver. 5.2; Systat Inc., Illinois, USA). Data were fit to equations in their original form and not to linearized versions of the equations.

RESULTS

The true weight-average molecular weight $[M_w(\phi)]$, of the κ-carrageenan used in this work as found to be 504 300 g/mole when measured under non-degrading conditions as mentioned above.

The SEC-MALLS-RI technique used in this work also provides the molecular weight distribution (MWD) of each sample analysed. The MWD is in fact more important for characterising the samples than just their M_w only. A follow-up report is being prepared where the change in MWD with reaction time has been modelled as a stochastic process. In such a process, the rate of change of number of molecules in a particular state *'i'* (molecular length) is the summation of rates at which molecules enter the state *'i'* from a higher state $(i+1, i+2 \ldots)$ by degradation and the rate at which they leave the state *'i'* (Singh *et al.*, 1993; see, for example, Nassar *et al.*, 1991).

The weight-average molecular weight of the polymer samples undergoing hydrolysis are presented in Figs 4a and 4b as a function of time for the various reaction temperatures studied. Each data point represents an independent measurement of a sample from a particular hydrolysis experiment; three (at 35°C and 45°C) or four (at 55°C) hydrolysis experiments were performed. As indicated earlier, the number of intact bonds for random hydrolysis reaction follows the first-order exponential rate equation. For short reaction times, while the fraction of bonds broken is small, such a rate equation can be simplified to (Tanford, 1961)

$$1/M_w(t) = 1/M_{w1}(0) + (k_{M1} \times t/m) \quad (1)$$

where

$M_w(t) = M_w$ after t min of hydrolysis (g/mole),
$M_{w1}(0)$ = Initial M_w (g/mole); obtained from regression,
k_{M1} = First-order rate constant for change of weight-average molecular weight (1/min),
t = Reaction time (min),
m = Molecular weight of the monomer (= 192 g/mole).

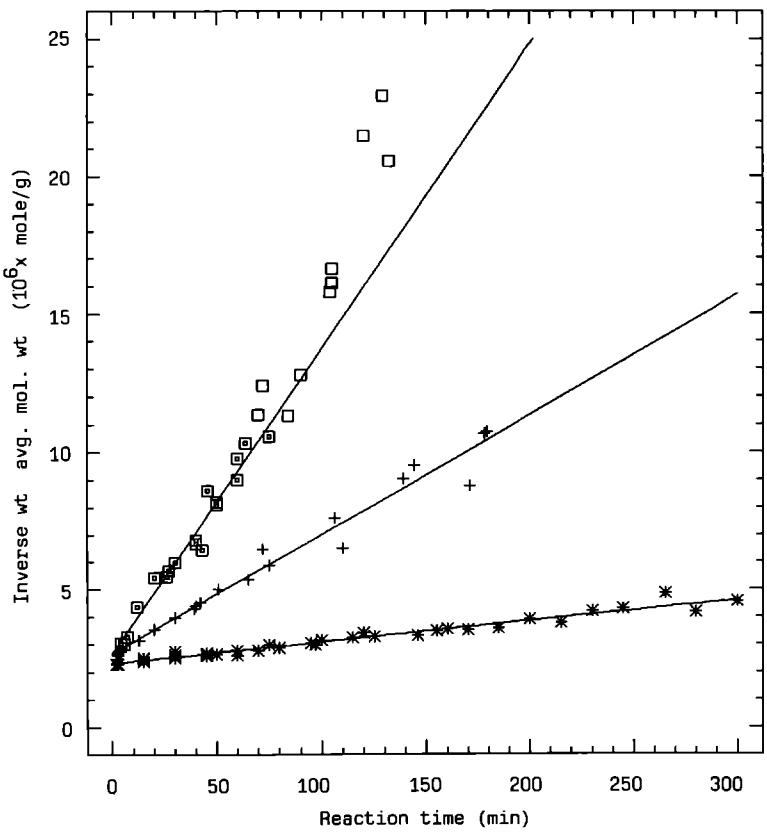


Fig. 4a. Inverse weight-average molecular weight plotted against reaction time for various hydrolysis temperatures: *, 35°C; +, 45°C; □, 55°C. The lines have been obtained from regression based on eqn (1); the regression, in case of the 55°C data, has only been performed on the points represented by ⊡.

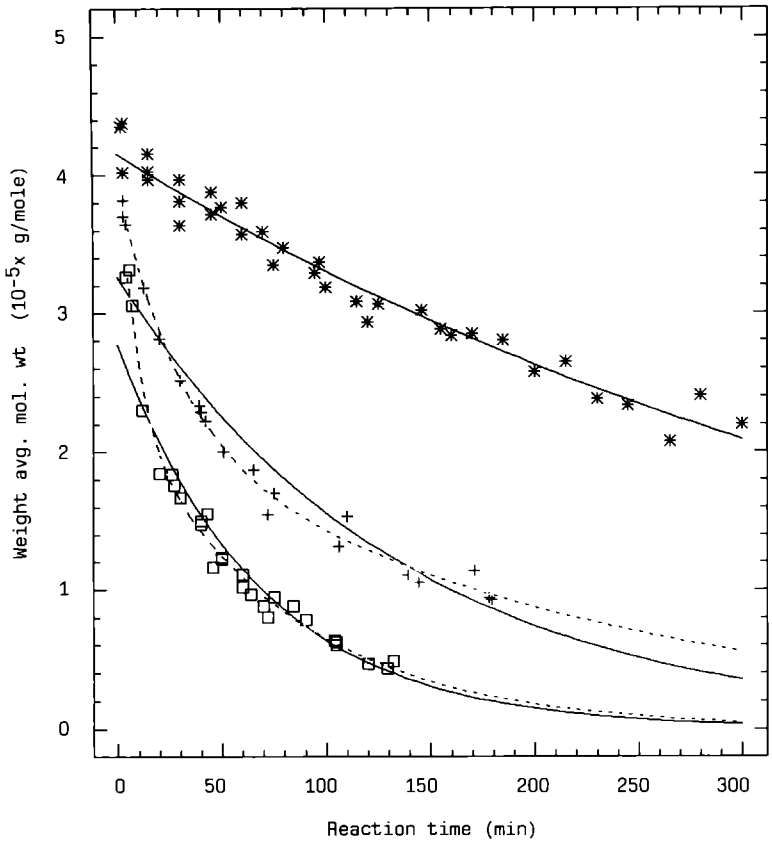


Fig. 4b. Weight-average molecular weight versus reaction time of κ-carrageenan samples undergoing acid hydrolysis at various temperatures: *, 35°C; +, 45°C; □, 55°C. The solid curves represent regression based on eqn (2), while the broken curves represent regression based on the bi-exponential model, eqn (3).

(The actual molecular weight of the repeating unit in κ-carrageenan is 395; however, since both the $\alpha(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ links are likely to hydrolyse, an average of the molecular weights of the two residues making up the repeating unit is used here).

Equation (1) has also been used by other authors to analyse data for carrageenan hydrolysis under various conditions (see, for example, Masson, 1955; Desai & Hansen, 1986; Bradley & Mitchell, 1988). The data from all experiments at a particular temperature were pooled together [not including $M_w(\phi)$] and fit to the above equation (Fig. 4a); the resulting parameters are summarized in Table 1a along with the 'molecular weight-hydrolysis reaction half-times' (t_{M1}) calculated on the basis of $M_{w1}(0)$ obtained from regression. For the 55°C data, the equation is applied to data up to approximately 80 min only (i.e. for $M_w > 90\,000$ g/mole only). The data from all molecular weight experiments at a particular temperature [not including $M_w(\phi)$] were also fit to the first-order exponential rate equation

$$M_w(t) = M_{w2}(0) \times \exp(-k_{M2}t) \qquad (2)$$

(This equation has no theoretical basis; it is being used solely in order to facilitate comparison with the other data obtained in this study.) The solid curves in Fig. 4b represent this regression model fit to the data at each of the temperatures studied. The results of the analysis are summarized in Table 1b along with the corresponding half-time t_{M2}.

Note that $M_{w1}(0)$ and $M_{w2}(0)$ for the various temperatures obtained above by regression (i.e. extra-

Table 1a. Analysis of weight-average molecular weight data by eqn (1)

Rxn temp. (°C)	$M_{w1}(0)$ (g/mole)	k_{M1} (1/min)	t_{M1} (min)	R^2
35	429 100	$1{\cdot}451 \times 10^{-6}$	308	0·960
45	378 400	$8{\cdot}352 \times 10^{-6}$	61	0·971
55[a]	378 700	$2{\cdot}130 \times 10^{-5}$	24	0·965

[a]Molecular weights > 90 000 g/mole only.

Table 1b. Analysis of weight-average molecular weight data by eqn (2)

Rxn temp. (°C)	$M_{w2}(0)$ (g/mole)	k_{M2} (1/min)	t_{M2} (min)	R^2
35	415 400	$2{\cdot}29 \times 10^{-3}$	303	0·963
45	326 700	$7{\cdot}42 \times 10^{-3}$	93	0·940
55[a]	277 500	$1{\cdot}47 \times 10^{-2}$	47	0·958

[a]All data.

Table 1c. Analysis of weight-average molecular weight data by eqn (3)

Rxn temp. (°C)	A_{31} (g/mole)	A_{32} (g/mole)	k_{M31} (1/min)	k_{M32} (1/min)	R^2
35[a]	—	—	—	—	—
45[b]	215 100	179 300	$4{\cdot}50 \times 10^{-3}$	$3{\cdot}40 \times 10^{-2}$	0·993
55[c]	236 400	196 500	$1{\cdot}30 \times 10^{-2}$	$1{\cdot}27 \times 10^{-1}$	0·986

[a]Model not applicable.
[b]The parameters are not independent.
[c]All data.

polation back to zero reaction time) are not equal to each other or to $M_w(\phi)$. This is probably due to errors introduced by the 'heat-to-dissolve' step mentioned earlier, accumulated errors in measurements, as well as limitations of the regression model(s) employed.

From the residuals (not shown) it appears that the hydrolysis data for 35°C are fit equally well by eqns (1) and (2); the data for 45°C are better described by (1) especially between 0 and 90 min; data for 55°C are better described by eqn (2). The data points in Fig. 4b, especially for 45 and 55°C, suggest a stronger curvature than provided by a single exponential. This, and the presence of two types of bonds between the monomer units of κ-carrageenan prompted a trial of a bi-exponential equation

$$M_w(t) = A_{31} \times \exp(-k_{M31} \times t) + A_{32} \times \exp(-k_{M32} \times t) \quad (3)$$

to fit the M_w versus time data. The parameters and their asymptotic correlation matrix (not shown) obtained for the 35°C data imply that a bi-exponential model is not justified for this temperature. On the other hand, the 55°C is clearly best modelled by the bi-exponential as compared to eqns (1) or (2), also apparent from the residuals (not shown). The case for the 45°C data is not as clear; the data are well fit by the bi-exponential but the asymptotic correlation matrix of the parameters shows that the two exponentials are not independent. The parameters obtained from this regression analysis are given in Table 1c and represented by the broken curves in Fig. 4b.

The data obtained from the gel strength measurements at room temperature for each hydrolysis experiment are presented in Fig. 5. Relative gel strength has been plotted against reaction time for various hydrolysis temperatures. Each data point represents an indepen-

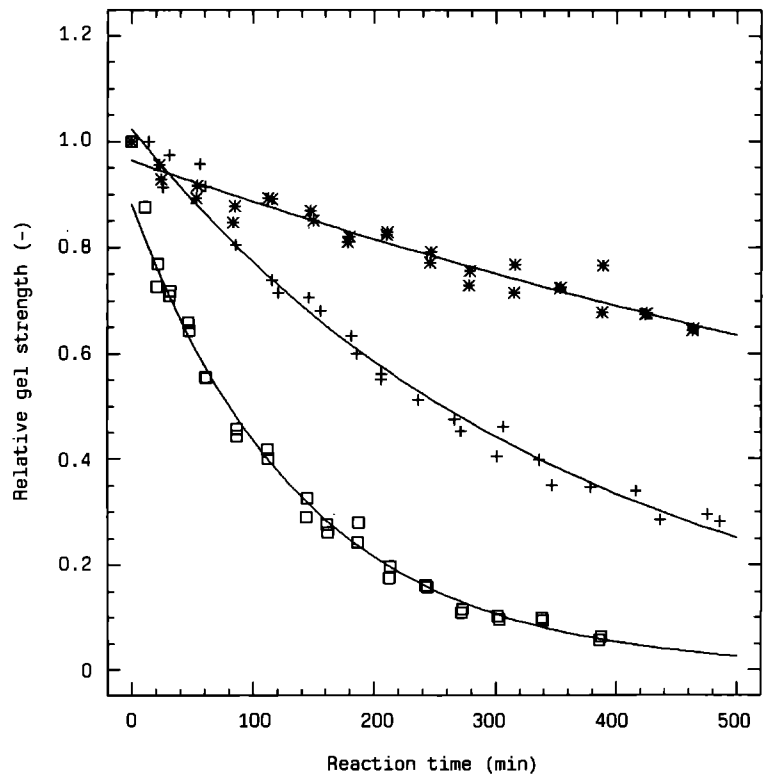


Fig. 5. Relative gel breaking strength versus reaction time of κ-carrageenan samples undergoing acid hydrolysis at various temperatures: *, 35°C; +, 45°C; □, 55°C. The solid curves have been obtained from regression based on eqn (4).

dent measurement of a sample withdrawn from each of the two hydrolysis experiments at each temperature. These data after pooling were also fit to the first-order rate equation

$$GS_r(t) = GS_r(0) \times \exp(-k_G \times t) \quad (4)$$

Note that the $GS_r(0)$ above is obtained from regression; the theoretical value for this term is 1·0. The results of the regression analysis are also plotted in Fig. 5. The results of the above data analysis have been summarized in Table 2, along with the 'relative gel strength—hydrolysis reaction half-time, t_G'.

In a similar fashion, the viscosity data can also be modelled by a first-order equation

$$\eta(t) = \eta(0) \times \exp(-k_\eta \times t) \quad (5)$$

The results are shown in Table 3 with the original data and fitted curves at 35 and 45°C plotted in Fig. 6; t_η is the 'low shear viscosity–hydrolysis reaction half time'.

The rate constants, k_{M1}, k_{M2}, k_G and k_η, at the different temperatures (T) can be fit to Arrhenius equations of the form

$$k\_(T) = k\_0 \times \exp(-E/RT) \quad (6)$$

to obtain an estimate of the activation or psuedo-activation energy E of the hydrolysis process. E obtained using rate constant k_{M1} versus temperature can be termed as an activation energy, since the rate eqn (1) has a sound theoretical basis for modelling the hydrolysis process. E obtained from the other data (k_{M2}, k_G, k_η versus temperature) must be considered as peuedo-activation energies in that rate eqn (2) has no theoretical basis, while GS_r and η are not fundamental measures of the hydrolysis reaction; these E may include the activation energies of junction zone disengagement, and unfolding of helices, along with that of the individual hydrolysis reactions lumped together in a complicated way. k_0 in the above equation is the pre-exponential factor, R is the universal gas constant (8·314 J/gmole/K), and T is absolute temperature in Kelvin. The semi-logarithmic plot is shown in Fig. 7, while the estimates of the parameters are given in Tables 4a and 4b.

Table 2. Analysis of (room temperature) relative gel strength data by eqn (4)

Rxn temp. (°C)	$GS_r(0)$ (–)	k_G (1/min)	t_G (min)	R^2
35	0·964	$8{\cdot}36 \times 10^{-4}$	829	0·942
45	1·023	$2{\cdot}80 \times 10^{-3}$	247	0·986
55	0·880	$7{\cdot}03 \times 10^{-3}$	98·5	0·958

Table 3. Analysis of low-shear viscosity data by eqn (5)

Rxn temp. (°C)	(0) (Pa s)	k_η (1/min)	t_η (min)	R^2
35	0·041	$2{\cdot}82 \times 10^{-3}$	246	0·931
45	0·016	$6{\cdot}38 \times 10^{-3}$	108	0·956

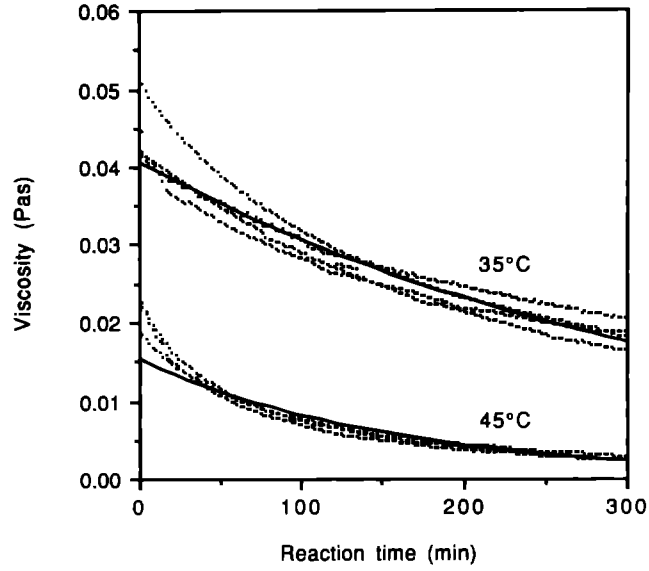


Fig. 6. Low-shear viscosity versus reaction time of κ-carrageenan samples undergoing acid hydrolysis at various temperatures. The broken curves represent the data points obtained every 120 s from the rheometer for each run, while the solid curve is the regression model based on eqn (5) applied to the pooled runs. The shear rate used in the experiments is $9{\cdot}207\,s^{-1}$.

DISCUSSION

In the discussion presented here, rate eqn (1) will be employed to compare the results (M_w) of this study to those in the literature, while the rest of the discussion will be based on the analogous rate eqns (2), (4) and (5).

It is instructive to compare the rate constants obtained in this work for molecular weight reduction by hydrolysis, to those in the literature. Masson (1955) and Masson *et al.* (1955) studied the heat degradation of a low (number-average) molecular weight (approx. 70 000 g/mole) carrageenan in a 0·03 mol/litre sodium phosphate buffer (pH 7·0) between 60 and 101°C, using intrinsic viscosity and end-group titration. Badui *et al.* (1978) used sedimentation equilibrium to measure the rate of degradation of carrageenan in a 'synthetic milk salt' system containing the cations Na^+, K^+ with smaller amounts of Ca^{2+}, Mg^{2+}, and the anions of P and Cl^-, and citrate with smaller amounts of SO_4^{2-} and CO_3^{2-}, the pH of the system drops from 6·7 on heating at 122°C. In a follow-up study, Desai and Hansen (1986) studied the hydrolysis of carrageenan over 100–130°C in a deaerated buffer system with higher ionic strength (0·05 mol/litre sodium cacodylate buffer with 0·25 mol/litre NaCl, pH 7·0). Bradley and Mitchell (1988) used viscometric measurements to examine the

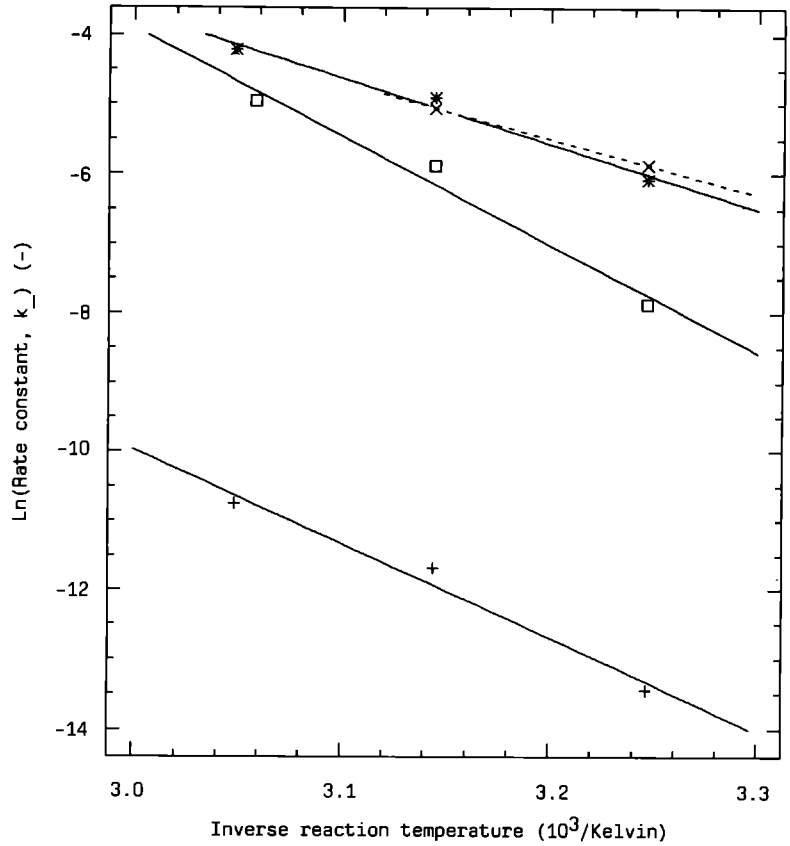


Fig. 7. Arrhenius plot for the various rate constants obtained in this study: +, k_{M1}; *, k_{M2}; □, k_G; ×, k_η.

hydrolysis of κ-carrageenan between 80 and 120°C at pH 7 in a solution containing 0·005 mol/litre EDTA and 0·2 mol/litre NaCl. Rate constants in all these cases were obtained using the reaction rate equation (1). The rate parameters are summarized in Table 5 along with those obtained in this work using the same rate equation.

Table 4a. Estimated activation energy for the hydrolysis process

Measure/ parameter	$k_{\_0}$ (1/min)	E (J/g mole)	R^2
Molecular weight k_{M1}, rate eqn (1)	$2{\cdot}52 \times 10^{13}$	113 140	0·976

Table 4b. Estimated psuedo-activation energies for the hydrolysis process

Measure/ parameter	$k_{\_0}$ (1/min)	E (J/g mole)	R^2
Molecular weight/ k_{M2} rate eqn (2)	$4{\cdot}65 \times 10^{10}$	78 290	0·982
Relative gel strength/k_G, rate eqn (4)	$7{\cdot}35 \times 10^{12}$	93 980	0·999
Low-shear viscosity/k_η rate eqn (5)	$5{\cdot}31 \times 10^{8}$	66 480	2 data points only

The activation energies in Table 5 can be divided into two groups, one with a high value (161 kJ/g mole; Ref. C), and the other three (Refs A, D, E) with lower values averaging 113 kJ/g mole. Comparing the various experimental systems leads to the conclusion that an increase in ionic strength, and deaeration of the solution causes a large absolute change in activation energy (approx. +42%; stabilizing effect). Increase in ionic strength promotes the formation and association of (double) helices in solution via the cations. The formation of these junction zones makes the bonds less accessible to hydrolysis. This shielding results in a higher activation energy. Deaeration also helps to reduce the rate of hydrolysis (Desai & Hansen, 1986), suggesting that oxygen must be taking part in the reaction. The lowering of pH does not influence the activation energy, i.e. the mechanism. The above conclusions are also supported by the calculated rates of hydrolysis at 35 and 122°C, which are approximately equal for various experimental systems at pH ≈7 (Refs A, B, D), somewhat lower for the deaerated system at pH 7 (Ref C), and much higher at pH 2 (Ref E).

If a higher ionic strength solution shields the bonds through the formation of junction zones, then the rate of hydrolysis will also be influenced by the type of cations present. Solutions with cations like K^+, Rb^+,

Table 5. Rate equations for hydrolysis of carrageenan under different conditions

Reaction conditions	Pre-expon. factor, k_{M0} (1/min)	Activation energy, E (J/g/mole)	Rxn, rate at 35°C[a] (1/min)	Rxn rate at 122°C[a] (1/min)	Ref.[b]
0·03 mol/l Na-Phosphate buffer, pH 7	4.6×10^{11}	122 180	8.7×10^{-10}	3.2×10^{-5}	A
Milk salt system, pH ≤ 6·7				7.2×10^{-5}	B
0·05 mol/litre Na-Cacodylate, buffer, 0·25 mol/litre NaCl Deaerated, pH 7	7.3×10^{15}	161 170	3.4×10^{-12}	3.6×10^{-6}	C
0·005 mol/litre EDTA 0·2 mol/litre NaCl, pH 7	1.1×10^{9}	104 700	6.0×10^{-10}	1.6×10^{-5}	D
0·09 mol/litre LiCl, 0·01 mol/litre HCl buffer, pH 2	2.5×10^{13}	113 140	1.6×10^{-6}	2.7×10^{-2}	E

[a]Calculated using $k_M(T) = k_{M0} \times \exp(-E/RT)$; T = temperature in Kelvin.
[b]A, Masson (1955); B, Badui *et al.* (1978); C, Desai and Hansen (1986); D, Bradley and Mitchell (1988); E, this work.

Cs^+, Ca^{2+}, are likely to have lower hydrolysis rates than those containing Li^+, Na^+, or $(CH_3)_4N^+$ (Rochas & Rinuado, 1980).

Acid hydrolysis performed in this study probably leads to a random cleavage of the bonds between the galactose residues. However, the two types of bonds between these residues may have different propensities for hydrolysis. A bi-exponential equation can be considered to represent the sum of the two rate processes in this case since the processes will not be competitive. As discussed earlier, the M_w versus time data for 55°C are bi-exponential, the 35°C data are not, while the 45°C data are borderline bi-exponential. The two types of bonds could hydrolyse simultaneously but with differing rates. It is probable that at 35°C, one hydrolysis process is more rapid than the other, while at 55°C both rates are significant; the transition occurring around 45°C. The data in this study are not enough to prove this hypothesis but experiments with NMR analysis of the hydrolysed samples are currently underway to follow the rate of degradation of the two type of bonds. Another possible hydrolysis pathway is the cleavage of the sulphate groups, which would influence the reduction of M_w in a complicated fashion.

The loss of (room temperature) gel strength by hydrolysis occurs at a slower rate than the corresponding reduction in M_w or viscosity (compare t_G with t_{M2} and t_η in Tables 1b, 2 and 3). This is due to the fact that gel strength reflects not only the length of the polysaccharide chains but also the junction zones. κ-carrageenan gels through the formation of (double) helices and the cation mediated association of these helices into junction zones. The junction zones serve as crosslinks leading to gelation (Clark & Ross-Murphy, 1987). The macromomolecular chains may be hydrolysed at a number of points before the length and number of these zones is influenced enough to reflect in the strength of the corresponding gel; however, the molecular weight of the chains is directly influenced by each hydrolysed bond. The t_G is approximately double t_{M2} or t_η. Low-shear viscosity and molecular weight have similar order of magnitudes of half-times (compare t_η with t_{M2}; Tables 1b, 3). Chain length, i.e. molecular weight is a primary factor that determines the coil dimensions, apart from their geometry. The coil geometry of the sample in the rheometer is expected to be similar over the temperature and concentration range studied here; therefore change in molecular weight directly influences the viscosity. We further note that the psuedo-activation energies for the different measures are of the same order of magnitude (Table 4b). This emphasizes that the underlying mechanism is the same in all cases.

The gel breaking strength of a polymeric gel depends on the number of crosslinks between the molecules. As discussed above, the crosslinks in a κ-carrageenan gel are composed of junction zones formed by association of helices. Short segments of κ-carrageenan molecules, while capable of forming helices, need not contribute to the gel formation (see, for example, Bryce *et al.*, 1982). On the other hand, the longer chains, due to their ability to be involved in a large number of helices throughout the bulk, contribute disproportionately to the gel strength. Since gel breaking is a 'large deformation' phenomenon, the measured value will be a result of cooperative effect over a portion of the system and not just a point effect. Thus gel breaking strength can be considered proportional to the weight-average molecular weight in order to reflect the influence of the larger molecules, i.e.

$$GS_r \propto M_w^{\,b}$$

with b as a scaling parameter. The above relation has been employed to correlate the relative gel strength of hydrolysed κ-carrageenan samples with their molecular weights at different degrees of hydrolysis. This was done by reading the molecular weight after a certain reaction

time (for a particular temperature) from Fig. 4b while obtaining the corresponding relative gel strength after the same amount of time from Fig. 5; interpolation between data points was performed where required. The M_w values were normalized by dividing by $M_w(\phi)$; the relative gel strengths were then plotted against the corresponding normalized molecular weights as shown in Fig. 8(a). The data points were correlated by fitting an equation of the form

$$GS_r = a[M_w/M_w(\phi)]^b \quad (7)$$

for each reaction temperature (Fig. 8(a)). Since the gel strengths in this study have been measured at room temperature irrespective of the hydrolysis temperature, the data points in Fig. 8(a) can also be pooled. Thus eqn (7) was also used after pooling, (i) all the points and (ii) the 35°C and 55°C points together (Fig. 8(b)). The values obtained for the parameters a and b are given in Table 6. The results in Table 6 show that GS_r and M_w are correlated as is to be expected. Qualitatively, the results in Figs 8(a) and (b) show that the gel strength approaches a maximum asymptotically with increasing molecular weight. An increase in the molecular weight results in an increase in the junction zone density, but the incremental contribution of each new such junction to the gel strength decreases. Quantitatively, allowing for experimental errors, the scaling parameters b are found to be approximately equal ($\cong$0·4), irrespective of the data included in the calculation. This confirms the utility of this technique as an indirect measure of molecular weight. Gelation in the presence of other counter-cations, e.g. K^+, Rb^+ or Cs^+, however, will lead to different (higher, but less than 1) values of b.

While the gel strength increases and approaches a maximum asymptotically with increasing molecular weight, the elastic modulus has been found to become independent of M_w over approximately 180 000 (Rochas *et al.*, 1990). The yield stress of the gel on the other hand, increases linearly with molecular weight. An explanation for this difference in behaviour is not available at present. Unlike the gel breaking strength, the elastic modulus and yield strength are obtained by making small perturbations in the gel. Theoretically therefore, the initial slope of the force registered versus plunger penetration distance curve, obtained during a gel breaking strength experiment (see, for example, Fig. 3), should be proportional to the elastic gel modulus. Such an analysis was attempted with the present data but was not successful because the precise moment of contact of plunger and gel could not be estimated in the experiments. A faster force registration system and a slower plunger movement rate than used here, would be required for this purpose.

The low-shear viscosity of κ-carrageenan has also been correlated with molecular weight at different degrees of hydrolysis. As with the gel strength–molecular weight correlation, the molecular weight after a certain reaction time was obtained from Fig. 4b. The viscosity for the same reaction time and temperature was obtained from the raw data table after averaging over the different runs for the chosen reaction time. These data points are plotted in Fig. 9. Data in this experiment cannot be pooled since the viscosities were measured at the specific reaction temperatures.

The low-shear viscosity of many (synthetic) polymer solutions follow a characteristic power-law behaviour (Porter & Johnson, 1966)

$$\eta \propto M_w^f$$

where $f \approx 1$ for $M_w < M_c$ and $f \approx 3{\cdot}4$ for $M_w > M_c$. M_c is thereby defined as a characteristic entanglement molecular weight. The change in molecular weight dependence occurs smoothly but over a relatively narrow range. Concentrated melts (> 0·25 g/ml) show a similar behaviour with M_c increasing with concentration (Graessley, 1974). For polysaccharides, on the other hand, a somewhat similar relationship has been obtained between low-shear viscosity and the product of concentration, c, and the intrinsic viscosity, $[\eta]$ (Morris *et al.*, 1981)

$$\eta \propto (c[\eta])^g$$

The parameter, g, was found to be equal to 1·4 for dilute solutions and 3·3 for concentrated solutions, provided the chains conform to the random coil geometry. Substituting the Mark–Houwink relationship for $[\eta]$, the following expression can be obtained for constant concentration in the dilute regime

$$\eta \propto M_w^{(1{\cdot}4a)}$$

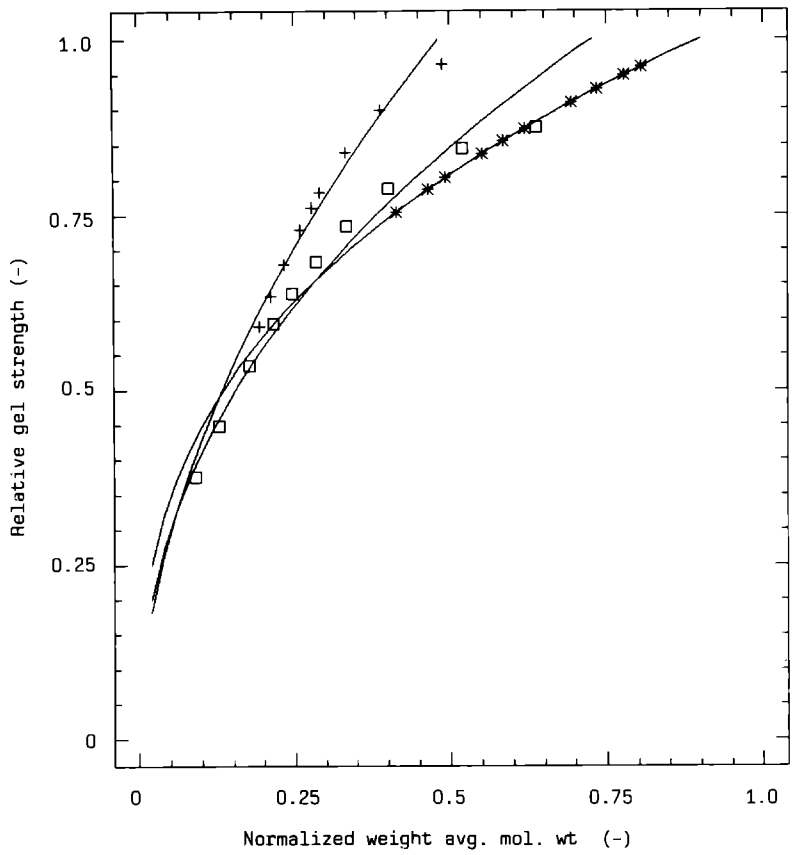
where a is the Mark–Houwink exponent for the solvent system employed here. This exponent ranges around or less than one for κ-carrageenan in solutions of different ionic strengths containing sodium ions ($a \approx 0{\cdot}67$–$0{\cdot}90$) or lithium ions ($a \leq 0{\cdot}98$) (Smidsrød, 1974; Vreeman *et al.*, 1980). Thus, an equation of the form

$$\eta = pM_w^q \quad (8)$$

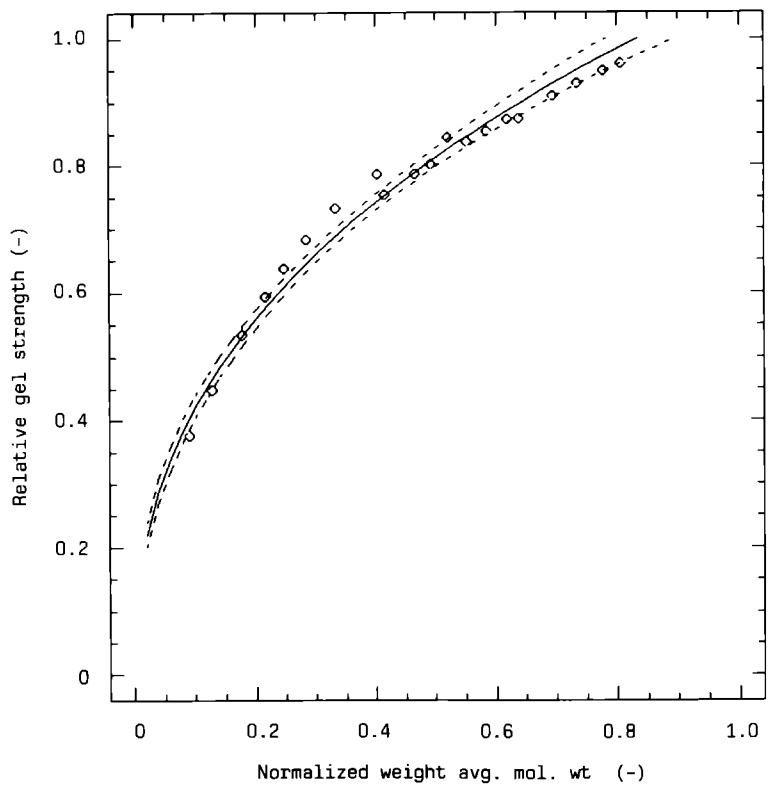
when fit to the data in Fig. 9, would be expected to have an exponent of between 1·0 and 1·4. The actual value obtained from regression is $q = 1{\cdot}2$ (see Table 7).

CONCLUSIONS

It can be concluded from this study that 'relative gel breaking strength' at room temperature can be employed as a measure of the extent of hydrolysis of κ-carrageenan. The results in Table 6 and Fig. 8(b) show that this quantity correlates well with the M_w. This allows for the possibility of replacing a relatively difficult experimental technique (for the measurement of M_w) with a simpler and faster technique (for GS_r), at least for those instances where only a relative measure is



(a)



(b)

Fig. 8. Plot showing the correlation between relative gel strength and normalized weight-average molecular weight for hydrolysis performed at various temperatures: (a) *, 35°C; +, 45°C; □, 55°C (the different reaction temperatures are plotted and modelled by eqn (7) individually) and; (b) ◇ 35°C and 55°C (the data for 35 and 55°C have been pooled and modelled by eqn (7)).

Table 6. Correlation of relative gel strength (at room temperature) with weight-average molecular weight using eqn (7)

Rxn temp. (°C)	a (–)	b (–)	R^2
35	1·09	0·36	0·999
45	2·47	0·54	0·975
55	1·39	0·45	0·979
Pooled all temps	1·25	0·38	0·871
Pooled 35 and 55°C	1·19	0·41	0·980

Table 7. Correlation of low-shear viscosity with weight-average molecular weight using eqn (8)

Rxn temp. (°C)	p	q (–)	R^2
35	$5{\cdot}0 \times 10^{-9}$	1·23	0·992
45	$3{\cdot}4 \times 10^{-9}$	1·23	0·994

needed. There is also the possibility of recovering approximate values for M_w from GS_r if a good calibration curve or correlation [e.g. eqn (7)] is obtained first. However, this would have to be obtained for well defined experimental conditions, especially pH, buffer cation and temperature. Low-shear viscosity can also be used for the above purpose, with the limitation that the method may lack sensitivity at high temperatures. However, a distinct advantage of viscosity is the possibility of in -process measurement.

REFERENCES

Andersen, A.G. (1988). Carrageenan in toothpaste. *Manufact. Chemist.*, **59**(9), 33.

Badui, S., Desai, N. & Hansen, P.M.T. (1978). Heat degradation of carrageenan in a milk salt system. *J. Agric. Food Chem.*, **26**, 675–9.

Belton, P.S., Chilvers, G.R., Morris, V.J. & Tanner, S.F. (1984). Effect of group I cations on the gelation of iota carrageenan. *Int. J. Biol. Macromol.*, **6**, 303–8.

Bradley, T.D. & Mitchell, J.R. (1988). The determination of the kinetics of polysaccharide thermal degradation using high temperature viscosity measurements. *Carbohydr. Polym.*, **9**, 257–67.

Bryce, T.A., Clark, A.H., Rees, D.A. & Reid, D.S. (1982). Concentration dependence of the order–disorder transition of carrageenans. Further confirmatory evidence for the double helix in solution. *Eur. J. Biochem.*, **122**, 63–9.

Christensen, O. (1964). Carrageenan. A useful food-additive. *Food Manuf.*, **39**, 49–52.

Clark, A.H. & Ross-Murphy, S.B. (1987). Structural and mechanical properties of biopolymer gels. *Adv. Polym. Sci.*, **83**, 57–192.

Desai, N. & Hansen, P.M.T. (1986). Heat stability of carrageenan. In *Gums and Stabilizers for the Food Industry, 3*, eds G.O. Phillips, D.J. Wedlock & P.A. Williams. Elsevier Applied Science, London, pp. 341–9.

Descamps, O., Langevin, P. & Combs, D.H. (1986). Physical

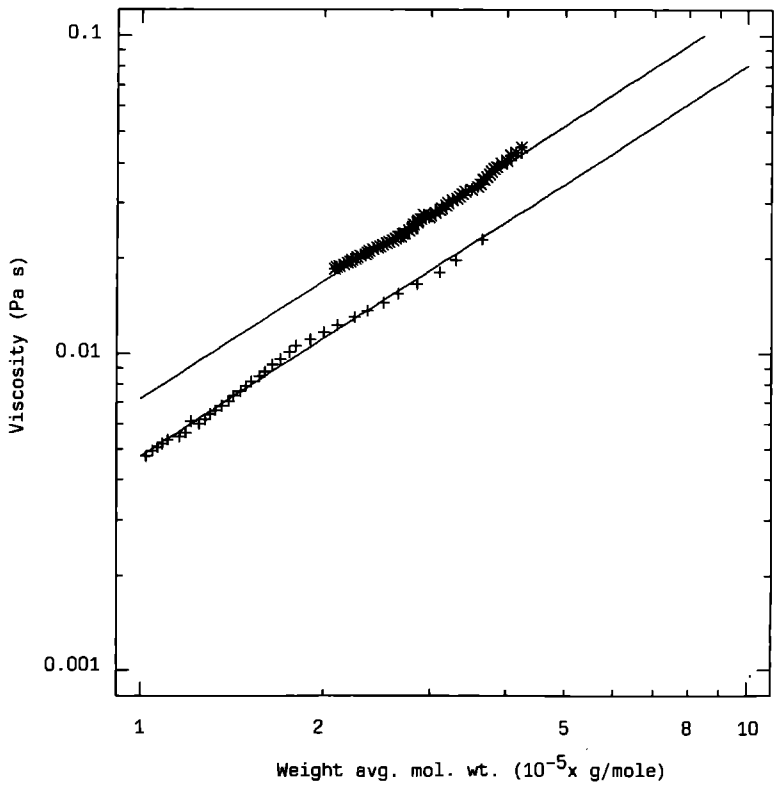


Fig. 9. Correlation between low-shear viscosity and weight-average molecular weight for hydrolysis performed at various temperatures: *, 35°C; +, 45°C. The solid lines have been obtained from modelling the data by eqn (8).

effect of starch/carrageenan interactions in water and milk. *Food Technol.*, April, 81–8.

Ekström, L-G. (1985). Molecular-weight-distribution and the behaviour of kappa-carrageenan on hydrolysis. *Carbohydr. Res.*, **135,** 283–9.

Graessley, W.W. (1974). The entanglement concept in polymer rheology. *Adv. Polym. Sci.*, **16,** 3–179.

Jackson, C., Nilsson, L.M. & Wyatt, P.J. (1989). Characterization of biopolymers using a multi-angle light scattering detector with size exclusion chromatography. *J. Appl. Polym. Sci.*, **43,** 99–106.

Lecacheux, D., Panaras, R., Brigand, G. & Martin, G. (1985). Molecular weight distribution of carrageenans by size exclusion chromatography and low angle laser light scattering. *Carbohydr. Polym.*, **5,** 423–40.

Marcus, A.J., Marcus, S.N., Marcus, R. & Watt, J. (1989). Rapid production of ulcerative disease of the colon in the newly-weaned guinea-pigs by degraded carrageenan. *J. Pharm. Pharmacol.*, **41,** 423–6.

Masson, C.R. (1955). The degradation of carrageenin. I. Kinetics in aqueous solution at pH 7. *Can. J. Chem.*, **33,** 597–603.

Masson, C.R., Santry, D. & Caines, G.W. (1955). The degradation of carrageenin. II. Influence of further variables. *Can. J. Chem.*, **33,** 1088–96.

Mitchell, J.R. (1976). Rheology of gels. *J. Text. Stud.*, **7,** 313–39.

Morris, E.R., Rees, D.A. & Robinson, G. (1980). Cation specific aggregation of carrageenan helices. Domain model of polymer gel structure. *J. Mol. Biol.*, **138,** 349–62.

Morris, E.R., Cutler, A.N., Ross-Murphy, S.B., Rees, D.A. & Price, J. (1981). Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydr. Polym.*, **1,** 5–21.

Nassar, R., Chou, S.T. & Fan, L.T. (1991). Stochastic analysis of stepwise cellulose degradation. *Chem. Engng. Sci.*, **46,** 1651–7.

Ozawa, K., Niki, R. & Arima, S. (1985). Effects of coexistence of β-casein on gelation of κ-carrageenan. *Agric. Biol. Chem.*, **49,** 3123–9.

Porter, R.S. & Johnson, J.F. (1966). The entanglement concept in polymer systems. *Chem. Rev.*, **66,** 1–27.

Rochas, C. & Rinuado, M. (1980). Activity coefficients of counterions and conformation in kappa-carrageenan systems. *Biopolymers*, **19,** 1675–87.

Rochas, C., Rinuado, M. & Landry, S. (1990). Role of the molecular weight on the mechanical properties of kappa carrageenan gels. *Carbohydr. Polym.*, **12,** 255–66.

Singh, S.K., Shen, B.C., Chou, S.T. & Fan, L.T. (1993). Acid hydrolysis of κ-carrageenan in a batch reactor. Stochastic analysis of the change of molecular weight distribution with time. Presented at the Session on Chemical Reaction Engineering and Catalysis, AIChE Summer Meeting, Seattle, Washington, USA, 15–19 August 1993. *Biotech Progr.* (in press).

Smidsrød, O. (1974). Molecular basis for some physical properties of alginates in the gel state. *Faraday Discuss. Chem. Soc.*, **57,** 263–80.

Smidsrød, O. & Grasdalen, H. (1982). Some physical properties of carrageenan in solution and gel state. *Carbohydr. Polym.*, **2,** 270–2.

Smidsrød, O., Haug, A. & Lian, B. (1972). Properties of poly(1,4-hexuronates) in the gel state. I. Evaluation of a method for the determination of stiffness. *Acta Chem. Scand.*, **26,** 71–8.

Tanford, C. (1961). *Physical Chemistry of Macromolecules*, John Wiley, New York, pp. 611–18.

Vreeman, H.J., Snoeren, T.H.M. & Payens, T.A. (1980). Physicochemical investigation of κ-carrageenan in the random state. *Biopolymers*, **19,** 1357–74.

Wyatt, P.J., Jackson, C. & Wyatt, G.K. (1988). Absolute GPC determination of molecular weights and sizes from light scattering I, II. *Am. Lab.*, May, June.