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CALORIMETRIC DETERMINATION OF THE CONFORMATIONAL TRANSITION OF KAPPA CARRAGEENAN

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ABSTRACT

This paper deals with measurements of the heat of the helix–coil transition by microcalorimetry for kappa carrageenans; data are given in the absence of gel in various solvents (H_2O, Me_2SO, and formamide) with two counterions (K^+, Rb^+). In water, the influence of gel formation on ΔH is pointed out and the influence of the time of ageing is demonstrated. The ΔH values measured in the absence of gel are interpreted in terms of the electrostatic model proposed by Manning; the agreement is quite good if a double helix is formed in water, but a monochain, ordered conformation is suggested for solutions in Me_2SO and formamide.

INTRODUCTION

The kappa carrageenans are natural polysaccharides well known for their gel-forming property[1,2]. The mechanism of crosslinking is still under discussion. The sol–gel transition is thermoreversible, and has been investigated by optical rotation[3], ^{13}C-n.m.r.[4], and by mechanical properties. The crosslinking is proposed to involve formation of a double helix or a type of crystallization of helical segments[5,6]. Some calorimetric measurements have been published previously by Snoeren[7,8] and by Morris *et al.*[9] for kappa carrageenan, but the values show large discrepancies and never separate conformational-transition and crosslinking contributions. They[7,8] gave values of 5.5–8.4 and[9] 9.4–12.6 kJ per dimer unit, determined under various experimental conditions.

This work concerns determination of the heat of melting of the kappa carrageenan conformation in the presence and absence of gel. In the absence of gel, it was proposed to investigate separately the conformational helix–coil transition as a function of the nature and concentration of the counterions and of the solvent. The values are interpreted in terms of the electrostatic treatment proposed by Manning[10]. In the presence of gel, in water solution, the heat corresponding to the crosslinking of the gel is to be characterized as a function of the ionic content and time of ageing. As proposed by Ferry *et al.*[11], it is of interest to relate the microcalorimetric data to the intermolecular crosslinks. This point will be discussed later in connection with rheological properties of the gels.

EXPERIMENTAL

Purification of the kappa carrageenan sample has been described previously[3]. The conformational transition is characterized by a melting temperature T_m; T_m has been determined by the temperature dependence of the specific rotation and of the conductivity[3]. Fig. 1 shows examples of these experimental data in free salt solution. Whatever the technique, the same value of T_m was found. Conductivity measurements were performed with a Tacussel CD 78 conductivity bridge; in addition, it allowed determination of the derivative of the conductivity ($d\chi/dT$), by use of the DT 70 Setaram accessory. A programmer (Haake PG 10) was used to vary the temperature.

A Calvet[12] calorimeter (Setaram) equipped with a 12-cm^3 stainless-steel cell was used. The heating rate was 4 or 8 °/h. The calorimeter was calibrated by the Joule effect; a control experiment was performed with the melting heat of a standard sample of benzoic acid (Dupont). The experimental value was 18.2 J/mol (lit.[13] 18.08 J/mol).

RESULTS AND DISCUSSION

(*A*) *Helix–coil transition.* — A theoretical treatment for polyelectrolytes has been proposed by Record[14] and Manning[10,15] to predict the heat of melting for helix–coil conformational transitions. The calculations take into account condensation of counterions and the electrostatic screening due to ionic strength. The heat of melting may be expressed by:

$$\Delta H = -\frac{R}{2}(\phi_c - \phi_h)\frac{d\ln[c]}{d(1/T_m)} \qquad (1)$$

where R is the gas constant, ϕ_c and ϕ_h are the osmotic coefficients for the coil and helix, respectively, and c is the ionic concentration.

It may be noted that, from relation *1*, ΔH may be expressed as a function of the charge parameter λ as generally given in the literature[15]; the relation depends on the values of λ_{coil} and λ_{helix} compared to 1:

$$\lambda_c < \lambda_h < 1 \qquad \Delta H = \frac{R}{2}(\lambda_h - \lambda_c)\,d\ln c/d(1/T_m) \qquad (2)$$

$$\lambda_c < 1 < \lambda_h \qquad \Delta H = \frac{R}{2}(2 - \lambda_c - \lambda_h^{-1})\,d\ln c/d(1/T_m) \qquad (3)$$

$$1 < \lambda_c < \lambda_h \qquad \Delta H = \frac{R}{2}(\lambda_c^{-1} - \lambda_h^{-1})\,d\ln c/d(1/T_m) \qquad (4)$$

TABLE I

CALORIMETRIC DATA FOR KAPPA CARRAGEENAN IN WATER IN THE ABSENCE OF EXTERNAL SALT

C_p *(g/L)*	*Salt form*	ΔH *(kJ/mol)*	*Ageing (storage at 4°) (h)*
4	K^+	12.1 ±0.5	2
		12.1 ±0.5	24
	Rb^+	12.5 ±0.5	2
		12 ±0.5	24
8	K^+	12.8 ±0.3	2
		13.4 ±0.3	16
		13.6 ±0.3	53
	Rb^+	12.7 ±0.3	2
16	K^+	15.5 ±0.2	2
		15.9 ±0.2	24
		16.7 ±0.2	48
	Rb^+	15.0 ±0.2	2
		15.9 ±0.2	24
32	K^+	15.9 ±0.1	2
		16.8 ±0.1	20

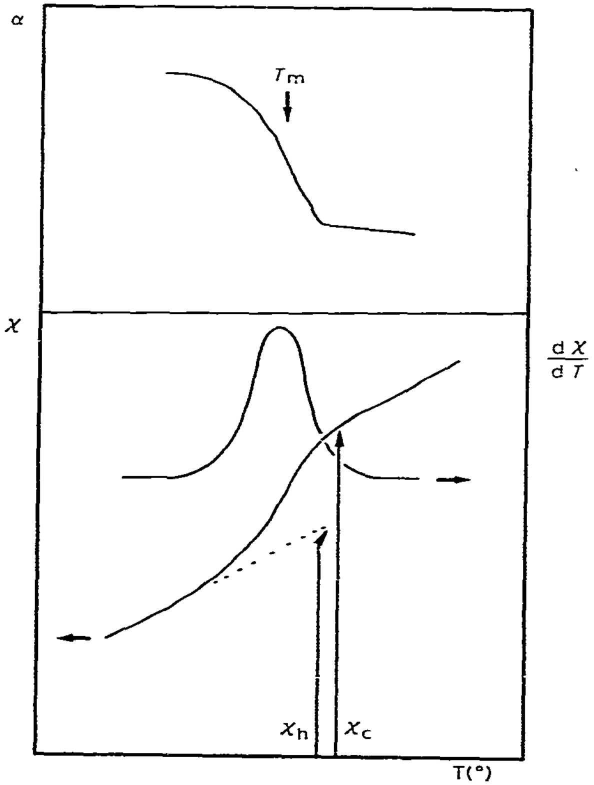

Fig. 1. Temperature dependence of optical rotation and of conductivity measurements for potassium kappa carrageenan in absence of external salt.

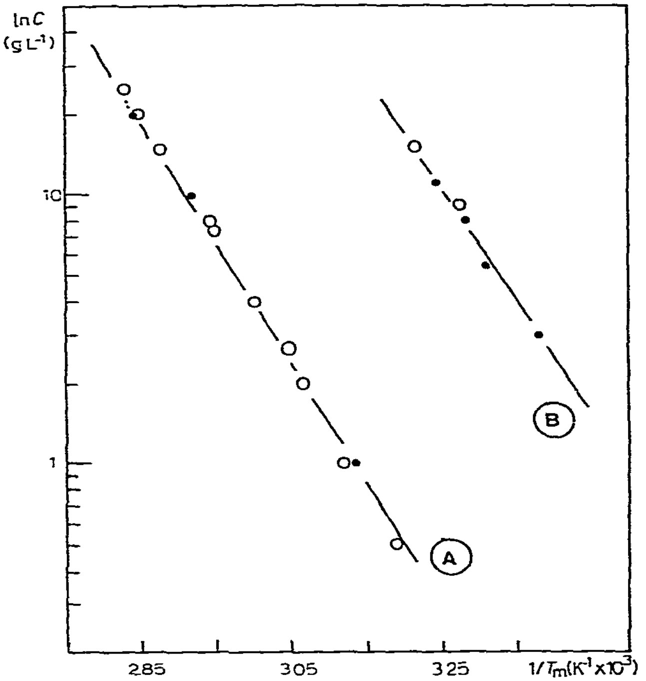

Fig. 2. Variation of the melting temperature T_m with the logarithm of the concentration, C: ○ ○ ○, ● ● ◐ conductivity and optical rotation; A, Me$_2$SO; B formamide.

and considering the relation between ϕ and λ in Manning's theory[10]. In this work, it is proposed to compare the experimental values of ΔH with those calculated by relation *1*.

The osmotic coefficients ϕ are deduced from the activity coefficients γ determined previously in water[3] (Table I), taking into account the relation proposed by Manning[10]:

$\phi = 1 + \ln\gamma$ when $\lambda < 1$

and $\phi = \gamma/1.21$ when $\lambda > 1$

in which λ is the charge parameter defined by $\lambda = e^2/\varepsilon kTb$, with e being the electronic charge, ε the bulk dielectric constant, k the Boltzman constant, T the absolute temperature, and b the average spacing between two ionic sites. It may be recalled that the structural data[8,16]:

$b_{\text{doublehelix}} = 4.3$ Å and $b_{\text{coil}} = 10.3$ Å

allow predictions with good agreement[3], of the experimental γ values extrapolated to infinite dilution from the electrostatic model of Manning.

The term $\mathrm{d}\ln[c]/\mathrm{d}(1/T_m)$ in relation (*1*) corresponds to the slope of $\ln[c]$ versus $1/T_m$ plots obtained by experiment. In this work, it was found that $1/T_m$ is linearly dependent on the logarithm of the ionic concentration in the different solvents investigated: water[3], Me$_2$SO, and formamide (Fig. 2).

TABLE II

CALORIMETRIC DATA IN WATER FOR KAPPA CARRAGEENAN IN THE ABSENCE OF GEL

	ϕ_c (3)	ϕ_h (3)	ΔH (*kJ/mol disaccharide*)		$d\ln c/d(1/T_m)$ (K^{-1})
			calc.	*exp.*	
$C \to 0$	0.72	0.37	24	—	8080
$C = 4$ g.L^{-1}	0.61	0.37	13.0	12.1	
$C = 8$ g.L^{-1}	0.575	0.325	12.0	12.8	

TABLE III

CALORIMETRIC DATA FOR THE POTASSIUM SALT OF KAPPA CARRAGEENAN IN DIMETHYL SULFOXIDE IN THE ABSENCE OF EXTERNAL SALT

C_p *(g/L)*	ΔH *(kJ/mol)*	*Ageing*
3	6.7 $\pm$0.6	No effect
6	6.9 $\pm$0.3	No effect
12	6.7 $\pm$0.4	No effect

TABLE IV

CALORIMETRIC DATA FOR KAPPA CARRAGEENAN IN FORMAMIDE IN THE ABSENCE OF EXTERNAL SALT

C_p *(g/L)*	*Salt form*	ΔH *(kJ/mol)*	*Ageing*
5	K^+	5.6 $\pm$0.6	No effect
10	K^+	5.4 $\pm$0.3	No effect
15	K^+	5.6 $\pm$0.2	No effect
6	Rb^+	5.9 $\pm$0.5	No effect
12	Rb^-	5.9 $\pm$0.3	No effect

The experimental values obtained in water are given in Table I; they are compared in Table II with the predicted ΔH values from equation (*1*) by using the experimental values[3] of γ and assuming a double helix–coil transition.

The ΔH value is expressed per unit charge, namely, per disaccharide residue of the kappa carrageenan molecule. There is good agreement between both sets of values; Table II points out the importance of the dependence of γ and ΔH with the concentration of polymer.

The experimental results obtained in Me_2SO and formamide are given in Tables III and IV and are compared with those calculated by relation (*1*) in Table V. A large discrepancy is observed if a coil–double helix transition is considered. A possible way to interpret the calorimetric data is to assume a monohelix–coil transition; under such conditions the structural-charge parameter of the helical form is one half that of the double helix. Evidence from this process is deduced from conductimetric data (Fig. 1). The conductivities of the helix and the coil forms (χ_h and χ_c) at a given temperature may be written:

$$\chi_h = K f_h \Lambda_p \quad \text{and} \quad \chi_c = K f_c \Lambda_p \tag{5}$$

where K is a constant including the polymer concentration, the properties of the solvent and units adopted; f is the coefficient of conductivity[17], and Λ_p is the equivalent conductivity of the polyelectrolyte. The coefficient f has been calculated by Manning's relations:

$$f = 1 - \frac{0.55\,\lambda^2}{\lambda + 3.14} \quad \lambda \leqslant 1 \quad \text{and} \quad f = \frac{0.87}{\lambda} \quad \lambda \geqslant 1 \tag{6}$$

It follows that $(\chi_c/\chi_h) = (f_c/f_h)$ if Λ_p is assumed to be constant.

In Table V, the values of the ratio f_c/f_h are computed for both possibilities and are compared with experimental values; in formamide and Me_2SO, the results are in agreement with a coil–monohelix transition. Similarly, the ΔH values support this hypothesis. Furthermore, it should be mentioned that no gel is ever observed in either solvent. This new ordered conformation is characterized by a broadening of the transition[4] and a symmetrical melting-curve. At least it is shown that, in the three solvents, the counterions K^+ and Rb^+ give nearly the same value of ΔH in the absence of gel; from polyelectrolyte theory, it is clear that no effect from the nature of the monovalent counterions would be predicted.

(*B*) *Conformational transition in presence of gel.* — Gels are not obtained[4] in

TABLE V

CONDUCTIMETRIC AND CALORIMETRIC DATA IN ORGANIC SOLVENTS

Solvent	ε *(25°)*	$d\ln c/d(1/T_m)$ *(K^{-1})*		f_c/f_h		ΔH *(kJ)*	
				Calc.	*Found*	*Calc.*	*Found*
Formamide	111	10 000	double helix	1.17	1.07	27	5.4 to 5.9
			single helix	1.02		4.4	
Me_2SO	46.7	10 700	double helix	2.43	1.25	23	6.7
			single helix	1.22		6.9	

TABLE VI

CALORIMETRIC DATA FOR KAPPA CARRAGEENAN (POTASSIUM SALT) IN THE PRESENCE OF POTASSIUM NITRATE

C_p *(g/L)*	C_s *(eq/L)* × 10^3	ΔH *(kJ/mol)*	*Ageing*	
			Time of storage (h)	*Temperature of storage (°)*
20	100	16.9 ±0.2	2	+4
10	100	17.8 ±0.3	1	+20
10	100	17.6 ±0.3	14	+4
13.3	67	15.5 ±0.3	2	+4
13.3	67	15.9 ±0.3	12	+4
6.8	33	15.0 ±0.3	2	+4
6.8	33	15.0 ±0.3	14	+4
5.0	50	17.9 ±0.4	2	+4
5.0	50	17.9 ±0.4	720	+4

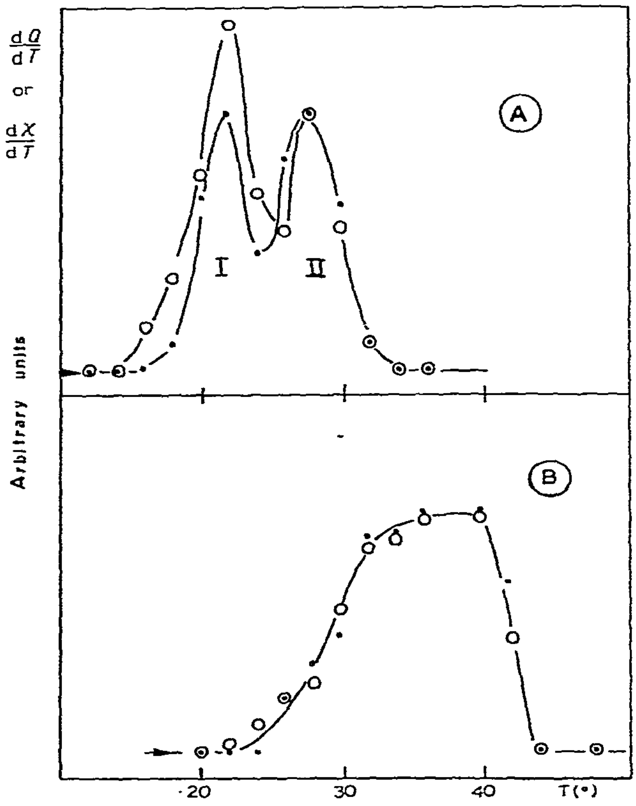

Fig. 3. Conformational transition of kappa carrageenan (potassium salt) followed by conductivity and calorimetry: ○ ○ ○: dX/dT, scan rate 24°.h^{-1}; ● ● ●: dQ/dT, scan rate 4°.h^{-1}; A: 8 g/L solution aged 2 h at +4°; B: 16 g/L solutions aged 53 h at +4°.

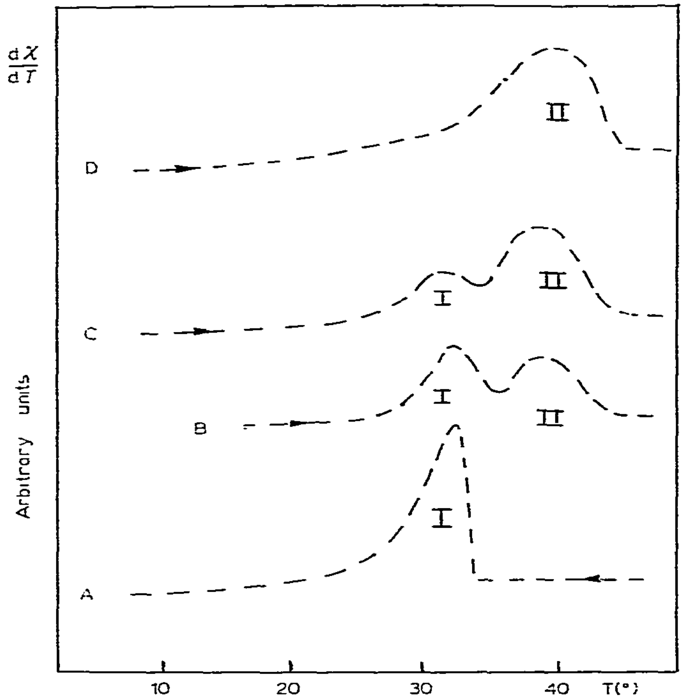

Fig. 4. Evolution of I and II peaks with ageing for the rubidium salt of kappa carrageenan (polymer concentration 8 g.L^{-1})

	Temperature range (°)	*Scan rate* (°.h^{-1}) *Cooling*	*Heating*	*Ageing* *(h)*
A	55–5	18		0
B	17–55	18	54	0
C	5–55	18	18	0
D	5–55	18	18	53

Me_2SO or formamide with salt-free solutions. This absence is proved by a thermal transition without hysteresis and by the physical state of the solution. Consequently, the measured ΔH values are independent of the time of ageing (Tables III, IV). In aqueous, salt-free solution there is no gel when the polymer concentration is <4 g.L^{-1}. For higher concentrations, in the presence or absence of external salt, a gel is formed. The experimental ΔH values increase with salt and polymer concentrations, and on ageing rise up to +4 kJ/mol above the ΔH value of the helix-coil transition (Tables V and VI). This heat content must be related to the degree of intermolecular cross-links when the conditions are those of gel formation.

An intermediate state exists for a solution (8 g.L^{-1}) in the absence of external salt. At low temperature, there is no coherent gel, but a very viscous solution. The melting curve obtained by optical rotation[6], calorimetric, or conductimetric determination (Fig. 3A) shows a two-step mechanism. The first one (peak I) decreases and the second (peak II) increases with the time of ageing (Fig. 4). We had proposed[6] that the first corresponds to the melting of isolated segments of the helix and the second step to the melting of aggregates of helical segments. Recently, Morris[5] has given the same arguments for iota carrageenans. For this polymer concentration, the sol–gel transition implies a hysteresis between the melting and cooling curves (Fig. 4). For a 16-g.L^{-1} solution and at higher concentrations, the fraction of species [I] is negligible, because of aggregation of all the material (Fig. 3B).

CONCLUSION

This report concerns determination of the heat of melting of the ordered conformation of kappa carrageenan in the absence of gel in different solvents (water, Me_2SO, and formamide) to investigate the influence of the dielectric constant on ΔH. The values are interpreted by the electrostatic treatment proposed by Manning. In water, experimental results agree with the value predicted considering a double helix $\leftrightarrows$ coil conformational transition. In Me_2SO and formamide, this mechanism is not consistent with the experimental data. By use of conductivity measurements, it is suggested that a monochain, ordered conformation is stabilized in both solvents. According to this hypothesis, the electrostatic model predicts the ΔH values very well. In addition, it is established that there is no dependence of the counterions (K^+ or Rb^+) on the ΔH values, in agreement with a pure electrostatic model.

In the presence of gel in water and for large polymer concentration (>8 g.L^{-1}), or in the presence of an excess of neutral salt, the ΔH value increases; the increase of 4 kJ/mol of disaccharide must be related to the intermolecular crosslinks. Much data have been obtained with an 8-g.L^{-1} solution, where the two-step mechanism is shown by different techniques (calorimetry, conductivity, and optical rotation), even if no physical gel exists. The influence of ageing is demonstrated from the increase of the content of the species formed in both steps (isolated helix or aggregates of helix).

In conclusion, this work allows the clear separation of both contributions in the enthalpy of melting of carrageenans: the conformational contribution (in agreement with the values predicted by an electrostatic treatment) and the contribution of crosslinking in stabilizing the tridimensional, network structure.

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