Carbohydrate Research, 108 (1982) 181–195
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

CHIROPTICAL CHARACTERISATION OF POLYSACCHARIDE SECONDARY STRUCTURES IN THE PRESENCE OF INTERFERING CHROMOPHORES: CHAIN CONFORMATION OF INTER-JUNCTION SEQUENCES IN CALCIUM ALGINATE GELS

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(Received February 8th, 1982; accepted for publication, March 15th, 1982)

ABSTRACT

The changes in chain conformation which accompany Ca^{2+}-induced gelation of alginate have been investigated by a combined circular dichroism (c.d.) and optical rotatory dispersion (o.r.d.) approach. C.d. changes in the carboxyl $n \rightarrow \pi^*$ spectral region, arising predominantly from formation of calcium poly-L-guluronate junctions, were monitored for three alginates of widely differing block composition. The corresponding o.r.d. changes, calculated by Kronig–Kramers transform, were subtracted from the observed changes in o.r.d. on gelation, to "unmask" the changes in optical activity of the conformation-sensitive electronic transitions of the polysaccharide backbone. Contributions to the "residual" o.r.d. difference spectra from poly-L-guluronate, poly-D-mannuronate, and heteropolymeric chain-sequences were calculated by solution of simultaneous equations at each wavelength. Results for polyguluronate sequences are in agreement with previous studies of alginate films by vacuum ultraviolet c.d., and with observed c.d. and o.r.d. changes on addition of calcium ions to homopolyguluronate segments in solution. The much greater changes in backbone optical activity calculated for polymannuronate and heteropolymeric chain-sequences, however, have no counterpart in the behaviour of these sequences in isolation. An explanation is proposed in terms of stretching of interconnecting sequences between calcium polyguluronate junctions in alginate gels, to give a more-extended chain conformation than in free solution.

INTRODUCTION

Optical rotatory dispersion (o.r.d.), which was widely used in early chiroptical studies of biopolymer conformation, has now been largely supplanted by circular dichroism (c.d.) as a probe of secondary and tertiary structure in proteins and polynucleotides. For polysaccharides, however, the conformationally sensitive transitions of the polymer backbone[1] are inaccessible to normal c.d. equipment, and the indirect o.r.d. approach is still of considerable value.

In particular, the co-operative formation of ordered, inter-chain junction zones

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in polysaccharide gels[2] is often accompanied by an abrupt change in optical activity[3,4], which thus provides a convenient method for monitoring and characterising the disorder–order process. By extending the known correlations between D-line optical rotation and the geometry of the sugar ring[5,6], a direct, quantitative relationship has been established[7] between measured optical rotation values and the relative orientation of adjacent residues in the polymer chain.

Such substituent chromophores as acetamido or carboxyl groups, which absorb at higher wavelengths[4], may obscure the conformation-sensitive optical activity of the polysaccharide backbone. In favourable cases, however, these chromophores may themselves furnish structural and conformational information. In particular, the uronate carboxyl groups of alginate give c.d. bands in a readily accessible spectral region, and these have proved extremely informative[8–11].

Alginate occurs as the principal polysaccharide component of marine brown algae (*Phaeophyceae*), and is a (1→4)-linked linear copolymer of α-L-guluronate and β-D-mannuronate[12] with residues arranged[13,14] in homopolymeric sequences of both types, and in heteropolymeric sequences that were formerly referred to as "alternating blocks", but are now known[15] to show appreciable deviations from the idealised, alternating disaccharide repeating-structure. The two constituent sugars give c.d. bands of opposite sign[11], and this provides a simple, direct index of overall composition. The observed c.d. behaviour also shows a more subtle sensitivity to residue sequence, which may be utilised[10] to determine the relative proportions of each block-type present. Thirdly, the Ca^{2+}-induced gelation of alginate is accompanied[8] by large changes in c.d. which have been used[9] to monitor and characterise inter-chain association.

Previous studies of alginate gelation by c.d. and other techniques[8,9,16] have shown that the primary event in network formation is dimerisation of poly-L-guluronate chain sequences, in a regular 2_1 conformation[17,18], with specific chelation of Ca^{2+} ions between the participating chains[19]. In the present work, we have used the known quantitative relationships[20,4] between c.d. and o.r.d. to calculate the contribution of the carboxyl chromophores to overall optical activity, and hence, by difference, to "unmask" the behaviour of the polymer backbone.

Initially, it was expected that, as with other gelling-polysaccharide systems, the major change in optical rotation would come from the "locking" of chain conformation within the ordered, inter-chain junction zones[2]. In practice, however, we have found evidence of large, and unexpected, conformational changes in the "interconnecting" polymannuronate and heteropolymeric sequences.

EXPERIMENTAL

Materials. — Three alginate samples of low, moderate, and high contents of polyguluronate were used, and are identified as samples L, M, and H, respectively. Sample L (reference number R3616) was kindly donated by Alginate Industries Ltd. Alginates M and H, respectively, from *Ascophyllum nodosum* and *Laminaria hyper-*

TABLE I

ALGINATE BLOCK COMPOSITION AND GEL-STRENGTH

Sample	*Block content (%)*			*Yield stress (N)*
	Polyguluronate	*Heteropolymeric*	*Polymannuronate*	
H	58.6	22.7	18.7	5.5
M	20.7	41.0	38.4	2.2
L	13.4	32.3	54.4	1.2

borea stipes, were commercial materials from the same manufacturer (reference numbers F387 and SS/DJ). Block composition (Table I) was determined by the method of Penman and Sanderson[21]. Chain segments approximating to each structural type were prepared by partial hydrolysis with acid[14], and characterised[21] by n.m.r. spectroscopy. A solution of each sample was dialysed extensively against deionised water, accurately neutralised, filtered, and freeze-dried before use. Absolute concentrations were calculated from elemental analysis of the freeze-dried materials.

Spectroscopy. — C.d. spectra were recorded with a Cary 61 spectropolarimeter, using a 10-s integration period, 1-cm pathlength, and a sample concentration of 0.8 $mg.mL^{-1}$. O.r.d. measurements on the same samples were made with a Jasco J20 spectropolarimeter. Both instruments were accurately calibrated by using D-camphor-10-sulphonic acid (Cambrian Chemicals) as standard. Gelation studies of the intact alginates were carried out by stretching a dialysis membrane across the neck of the cell, immersing in a large excess (5 L) of 6mM calcium chloride, and allowing diffusion to proceed until no further spectral change was observed (typically, 7–10 days). The effect of calcium ions on the isolated chain segments was monitored by direct addition of calcium chloride in dilute, aqueous solution, to give exact stoichiometric equivalence of uronate and Ca^{2+}. ^{1}H-N.m.r. spectra were recorded at 100 MHz with a Varian XL-100 spectrometer, operating in the Fourier-transform mode. Computer programs used for curve fitting and Kronig–Kramers transform are reported in detail elsewhere[4].

Gel strength. — Alginate gels (2% w/v) for mechanical studies were prepared by the slow release of calcium ions by the action of acid on an insoluble calcium salt, using citrate as sequestrant to control the level of free Ca^{2+}. Sodium alginate (2 g) and sodium citrate (1 g) were dissolved in deionised water (86 mL), and dicalcium phosphate (0.5 g) was then dispersed finely through the solution. A solution of citric acid (1 g) in deionised water (10 mL) was added immediately, with vigorous mechanical stirring (10 s), and the mixture was rapidly transferred to cylindrical moulds (12.5-mm diameter, 12-mm height). The samples were then aged for 24 h, and gel strength (yield stress) was measured by compression between parallel plates on an Instron Universal Materials Tester, model 1122, using a 20 N load cell, and a crosshead speed of 10 $mm.min^{-1}$. The average yield-stress of 10 samples was taken in each case.

POLYSACCHARIDE C.D. AND O.R.D.

Dissymmetric molecules generally show differences in their interaction with circularly polarised light-beams of opposite rotational sense. This may be detected either as differential absorption (c.d.) or differential refraction (o.r.d.). Measured c.d. or o.r.d. values may be converted into molar quantities by the following equations:

$$[\theta] = \theta M/cl \qquad (1)$$

and

$$[\phi] = \phi M/cl. \qquad (2)$$

where c is concentration ($g.mL^{-1}$), l is pathlength (mm), M is residue molecular weight (198 for alginate). θ and ϕ are, respectively, c.d. ellipticity and optical rotation (deg), and $[\theta]$ and $[\phi]$ are molar ellipticity and molar rotation ($deg.cm^2.dmol^{-1}$).

Since c.d. and o.r.d. have a common origin in electronic excitation of the molecule by the incident light, they are quantitatively related in a predictable way[20]. A single, optically active, electronic transition may be characterised completely by three parameters: position (*i.e.*, wavelength at the band centre, λ_0), intensity ($[\theta]_0$) and band-width (w). C.d. molar ellipticity and o.r.d. molar rotation at any wavelength are then related to these parameters by the Gaussian (*3*) and Kronig–Kramers (*4*) equations, respectively.

$$[\theta]_\lambda = [\theta]_0 e^{-(\lambda-\lambda_0)^2/w^2} \qquad (3)$$

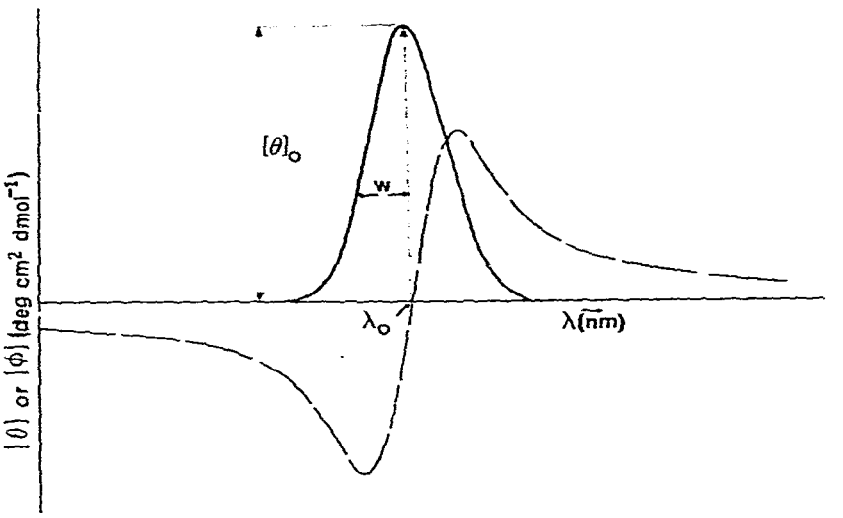

Fig. 1. Quantitative inter-relation and fundamental spectral form of c.d. (——) and o.r.d. (– – – –). For a single, optically active, electronic transition, both spectra may be defined completely by the same three parameters: position (λ_0), intensity ($[\theta]_0$), and width (w; defined as the half-width of the c.d. band at 1/e of the maximum height).

$$[\phi]_\lambda = \frac{2[\theta]_0}{\sqrt{\pi}} \left\{ e^{-(\lambda-\lambda_0)^2/w^2} \int_0^{(\lambda-\lambda_0)/w} e^{x^2} \cdot dx - \frac{w}{2(\lambda + \lambda_0)} \right\} \quad (4)$$

In principle both techniques yield the same structural and conformational information, but, as shown in Fig. 1, the fundamental band-form in c.d. is much simpler than in o.r.d., thus facilitating spectral resolution and assignment. Whereas o.r.d. remains finite at wavelengths far from the band centre, c.d. drops off rapidly to zero, and its use is therefore confined to chromophores absorbing in an accessible spectral region. In the absence of such substituent chromophores as carboxyl groups, carbohydrates[4] show no c.d. activity down to the lower wavelength limit of current commercial instruments (~190 mm).

Recent work[22–25] using specialist c.d. equipment operating in the far-vacuum ultraviolet region, however, has shown that polysaccharide optical activity arises predominantly from two intense bands of opposite sign, centred at ~150 and ~170 nm. Both appear to be sensitive to changes in chain conformation[22,23], such as those which accompany polysaccharide gelation. In general, the deeper-lying (150 nm) band is the more intense, and determines the sign of optical rotation at higher wavelengths (*e.g.*, the sodium D-line). In the particular case of alginate, studies of solid films[25] by vacuum ultraviolet c.d. showed the transitions to be centred at 149 and 169 nm, and of width 10.2 nm. The intensities of both bands showed a marked, systematic dependence on the level of Ca^{2+} incorporated in the film.

RESULTS AND DISCUSSION

The gel strength (yield stress) of the three alginate samples studied (Table I) shows an approximately linear dependence on polyguluronate content, consistent with previous evidence[26,27] that it is these sequences which are principally responsible for interchain association on Ca^{2+}-induced gelation. In earlier investigations[8,9], c.d. changes in the carboxyl $n \rightarrow \pi^*$ spectral region were used to monitor and characterise the formation of ordered calcium polyguluronate junctions. Fig. 2 illustrates the spectral changes observed on Ca^{2+}-induced gelation of the three alginates. The overall magnitude of c.d. change increases with increasing content of polyguluronate, but the form of the "difference spectra" (gel c.d. minus solution c.d.) varies from sample to sample. This is consistent with previous evidence[9] of a small contribution to overall spectral change from heteropolymeric chain-sequences. The observed solution and gel spectra, and the resulting difference spectra, are listed in Table II.

In each case, the difference spectra could be fitted accurately to two Gaussian bands (Eq. *3*). Using the values of λ_0, $[\theta]_0$, and w for these fitted components, the $n \rightarrow \pi^*$ contribution to the overall o.r.d. change on gelation could then be calculated by Kronig–Kramers transform (Eq. *4*). It should be noted that, since the c.d. difference spectra contain contributions from both polyguluronate (predominantly) and hetero-

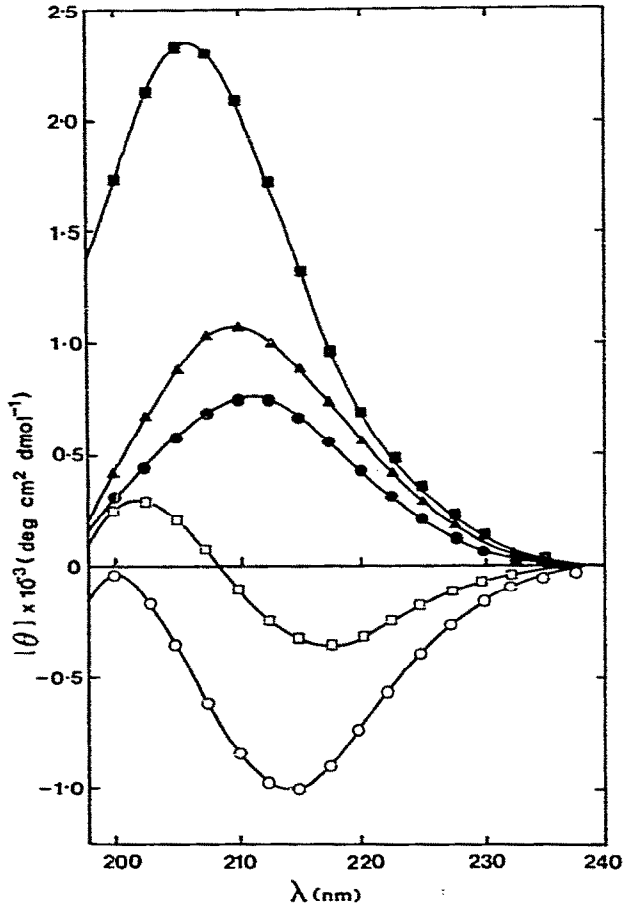

Fig. 2. C.d. change (—●—) between Na^+ solution (—○—) and Ca^{2+} gel (—□—), illustrated for alginate L. The corresponding c.d. difference spectra for alginates M (—▲—) and H (—■—) are also shown.

polymeric sequences, the fitted parameters have no fundamental significance. For the purposes of Kronig–Kramers transform, however, it is necessary only to match the observed c.d. envelope, irrespective of whether or not the fitted components correspond to discrete electronic transitions.

Table III lists the observed solution and gel o.r.d. spectra for the three alginates, and the corresponding o.r.d. difference spectra. By subtraction of the calculated $n \rightarrow \pi^*$ contribution to o.r.d. change from the observed difference spectra, the change in o.r.d. from all other optically active electronic transitions of the molecule could be "unmasked", as illustrated in Fig. 3. The resulting "residual" o.r.d. difference spectra are shown in Fig. 4. It is immediately obvious that, in contrast to the c.d. changes shown in Fig. 2, these o.r.d. changes bear no simple relationship to the proportion of polyguluronate present in each sample. The "residual" o.r.d. change

TABLE II

CHANGES IN CIRCULAR DICHROISM[a] ON GELATION OF ALGINATE

Wavelength (nm)	*Sample H*			*Sample M*			*Sample L*		
	Solution	*Gel*	*C.d. change*	*Solution*	*Gel*	*C.d. change*	*Solution*	*Gel*	*C.d. change*
200.0	−1.12	0.59	1.72	−0.30	0.12	0.41	−0.03	0.26	0.29
200.5	−1.16	0.97	2.13	−0.34	0.33	0.67	−0.18	0.27	0.45
205.0	−1.19	1.14	2.33	−0.51	0.36	0.87	−0.34	0.22	0.56
207.5	−1.24	1.07	2.30	−0.75	0.28	1.03	−0.61	0.08	0.69
210.0	−1.27	0.82	2.09	−0.94	0.12	1.06	−0.83	−0.10	0.73
212.5	−1.22	0.51	1.72	−1.05	−0.05	1.01	−0.98	−0.24	0.74
215.0	−1.14	0.19	1.33	−1.07	−0.20	0.88	−1.00	−0.33	0.67
217.5	−0.99	0.00	0.99	−0.97	−0.24	0.73	−0.90	−0.35	0.55
220.0	−0.81	−0.12	0.69	−0.81	−0.24	0.57	−0.75	−0.31	0.44
222.5	−0.61	−0.13	0.49	−0.61	−0.18	0.43	−0.57	−0.25	0.33
225.0	−0.45	−0.12	0.33	−0.43	−0.13	0.30	−0.39	−0.17	0.22
227.5	−0.32	−0.09	0.22	−0.28	−0.09	0.20	−0.26	−0.11	0.15
230.0	−0.20	−0.06	0.14	−0.17	−0.06	0.11	−0.15	−0.07	0.08
232.5	−0.11	−0.03	0.08	−0.10	−0.04	0.06	−0.09	−0.04	0.04
235.0	−0.06	−0.02	0.04	−0.06	−0.03	0.03	−0.05	−0.03	0.02
237.5	−0.03	0.00	0.03	−0.03	−0.02	0.01	−0.03	−0.02	0.01

[a]All c.d. values are expressed in terms of $[\theta] \times 10^{-3}$ (deg.cm^2.dmol^{-1}).

TABLE III

CHANGES IN OPTICAL ROTATORY DISPERSION[a] ON GELATION OF ALGINATE

λ (nm)	Sample H					Sample M					Sample L				
	Observed o.r.d.		O.r.d. change			Observed o.r.d.		O.r.d. change			Observed o.r.d.		O.r.d. change		
	Solution	Gel	Total	$n \rightarrow \pi^*$	Residual	Solution	Gel	Total	$n \rightarrow \pi^*$	Residual	Solution	Gel	Total	$n \rightarrow \pi^*$	Residual
205	−3.32	−5.51	−2.18	−0.38	−1.80	−2.76	−2.12	0.64	−0.56	1.20	−2.16	−2.13	0.03	−0.40	0.43
210	−3.24	−4.17	−0.93	0.70	−1.63	−2.94	−1.96	0.98	−0.06	1.04	−2.13	−1.69	0.44	−0.10	0.54
215	−3.38	−3.94	−0.56	1.21	−1.77	−3.19	−1.90	1.29	0.36	0.93	−2.46	−1.45	1.01	0.23	0.78
220	−3.41	−3.72	−0.30	1.19	−1.49	−3.14	−1.81	1.33	0.55	0.78	−2.73	−1.48	1.26	0.40	0.86
225	−3.27	−3.52	−0.26	1.00	−1.26	−3.03	−1.74	1.29	0.56	0.73	−2.70	−1.42	1.28	0.41	0.87
230	−3.03	−3.36	−0.34	0.80	−1.14	−2.68	−1.57	1.11	0.47	0.64	−2.49	−1.31	1.18	0.34	0.84
235	−2.69	−3.14	−0.45	0.63	−1.08	−2.49	−1.48	1.01	0.35	0.66	−2.31	−1.17	1.13	0.26	0.87
240	−2.47	−2.92	−0.45	0.50	−0.95	−2.35	−1.40	0.95	0.27	0.68	−2.05	−1.07	0.98	0.19	0.79
245	−2.31	−2.69	−0.38	0.42	−0.80	−2.18	−1.33	0.85	0.21	0.64	−1.91	−0.98	0.93	0.15	0.78
250	−2.13	−2.53	−0.40	0.35	−0.75	−2.05	−1.26	0.79	0.18	0.59	−1.78	−0.90	0.87	0.13	0.74
255	−1.95	−2.40	−0.42	0.31	−0.73	−1.90	−1.19	0.71	0.15	0.56	−1.64	−0.79	0.85	0.11	0.74
260	−1.86	−2.24	−0.38	0.27	−0.65	−1.76	−1.13	0.64	0.13	0.51	−1.56	−0.77	0.79	0.10	0.69
270	−1.63	−1.99	−0.35	0.22	−0.57	−1.60	−1.01	0.59	0.11	0.48	−1.37	−0.63	0.74	0.08	0.66
280	−1.46	−1.79	−0.34	0.19	−0.53	−1.42	−0.93	0.49	0.09	0.40	−1.23	−0.57	0.66	0.06	0.60
290	−1.30	−1.63	−0.34	0.16	−0.50	−1.28	−0.85	0.43	0.08	0.35	−1.13	−0.52	0.62	0.06	0.56
300	−1.17	−1.47	−0.30	0.14	−0.44	−1.17	−0.79	0.39	0.07	0.32	−1.04	−0.48	0.56	0.05	0.51
310	−1.07	−1.35	−0.27	0.12	−0.39	−1.07	−0.74	0.33	0.06	0.27	−0.96	−0.45	0.51	0.04	0.47
320	−0.99	−1.22	−0.22	0.11	−0.33	−0.99	−0.68	0.31	0.05	0.26	−0.87	−0.44	0.44	0.04	0.40
330	−0.91	−1.22	−0.21	0.10	−0.31	−0.93	−0.67	0.27	0.05	0.22	−0.81	−0.38	0.42	0.03	0.39

[a]All o.r.d. values are expressed in terms of $[\phi] \times 10^{-3}$ (deg.cm^2.dmol^{-1}).

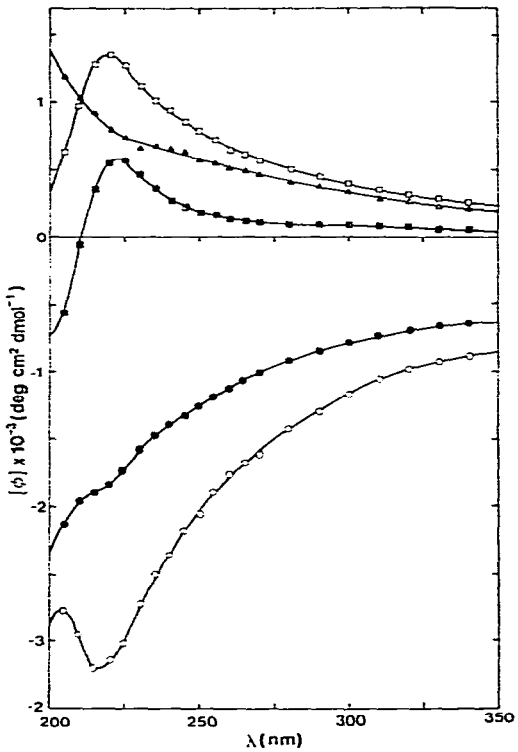

Fig. 3. O.r.d. change (—□—) between Na^+ solution (—○—) and Ca^{2+} gel (—●—), illustrated for alginate M. By substraction of the calculated n → π* contribution (—■—) to overall spectral change (derived by Kronig–Kramers transform of the c.d. difference spectrum in Fig. 2), the residual change in optical activity (—▲—) from all other electronic transitions of the molecule may be obtained.

for sample H is strongly negative, that for sample M is positive, and the spectrum for sample L is positive at long wavelength, but decreases sharply below ~230 nm, indicating a negative o.r.d. change below the lower wavelength limit of our equipment.

In each case, the residual o.r.d. difference spectra could be fitted (Fig. 4) with reasonable precision to two bands with the positions (λ_0 = 149 and 169 nm, respectively) and widths (w = 10.2 nm for both) observed[25] in the solid state by vacuum ultraviolet c.d. (The slight scatter of experimental points around the fitted curves, particularly in the region of the n→π* maxima, probably reflects the difficulties in measuring accurately the separation of two spectra both of which are changing steeply with wavelength.)

To investigate further the origin of these large, residual changes in o.r.d., the spectra were resolved into contributions from each of the three structural sequences present, on the assumption that the overall spectral change can be treated (Eq. 5) as the linear sum of the changes from each block type. (The validity of this assumption is discussed later.)

$$[\phi]_{obs} = f_G[\phi]_G + f_M[\phi]_M + f_H[\phi]_H, \qquad (5)$$

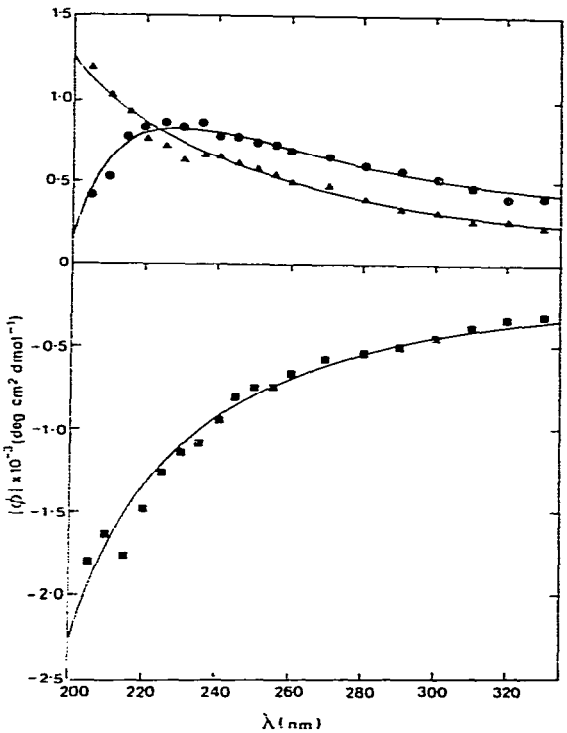

Fig. 4. "Residual" o.r.d. difference spectra for alginates H (—■—), M (—▲—), and L (—●—).

where $[\phi]_{obs}$ is the observed, residual o.r.d. change at a particular wavelength; f_G, f_M, and f_H are the fractions of polyguluronate, polymannuronate, and heteropolymeric chain-sequences present; and $[\phi]_G$, $[\phi]_M$, and $[\phi]_H$ are the changes in molar rotation of these sequences, from all transitions other than the carboxyl $n\rightarrow\pi^*$. Having investigated three alginates of very different block composition, we then had, at each wavelength, three simultaneous equations in which the only unknowns were $[\phi]_G$, $[\phi]_M$, and $[\phi]_H$. Solution of these equations gave the calculated o.r.d. difference spectra shown in Fig. 5 for polyguluronate, polymannuronate, and heteropolymeric chain-sequences. Once more, the spectra could be fitted, to within experimental error, to changes in the intensity of the two backbone transitions at 149 and 169 nm. The fitted parameters are listed in Table IV. Calculation of the expected changes in both bands for the three alginates, from the values derived for each sequence type and from the proportions in which they are present, gave values in excellent agreement with those obtained by direct fitting (Fig. 4) of the residual o.r.d. change for each sample.

For polyguluronate, the effect of introducing calcium ions is to make both bands more negative, which agrees well with vacuum ultraviolet c.d. studies[25] on solid films of alginate of high polyguluronate content, prepared with various levels of Ca^{2+}. The very large changes in backbone optical activity of polymannuronate

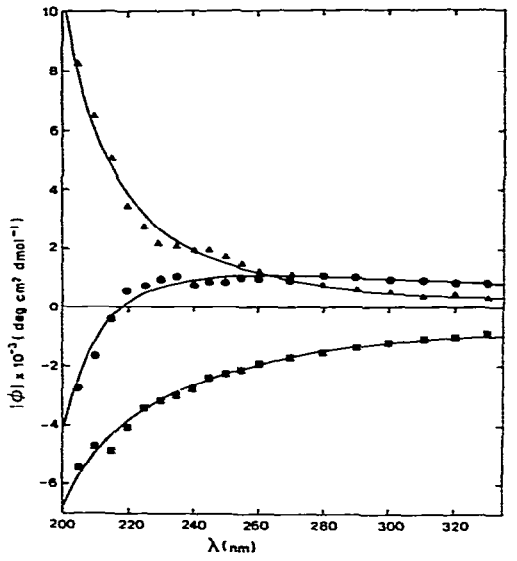

Fig. 5. Calculated contributions to the "residual" o.r.d. difference spectra (Fig. 4) from polyguluronate (—■—), polymannuronate (—●—), and heteropolymeric (—▲—) chain-sequences.

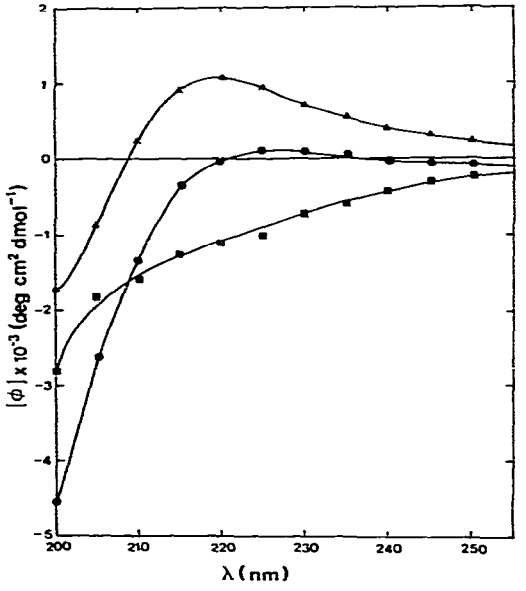

Fig. 6. Observed o.r.d. change (—●—) on addition of a stoichiometric equivalence of Ca^{2+} to isolated polyguluronate segments in aqueous solution. By subtraction of the n → π^* contribution (—▲—) to overall spectral change (obtained by Kronig–Kramers transform of the changes observed[9] in c.d.), the "residual" o.r.d. change (—■—) from all other optically active transitions may be calculated.

TABLE IV

CALCULATED CHANGES IN BACKBONE OPTICAL ACTIVITY ON GELATION OF ALGINATE

Sequence type	$\Delta[\theta]_0 \cdot 10^{-3}$ *(deg.cm².dmol⁻¹)*	
	149-nm Band	*169-nm Band*
Polyguluronate	−15 ±1	−27 ±1
Heteropolymeric	−160 ±4	143 ±3
Polymannuronate	205 ±8	−135 ±5

and heteropolymeric chain-sequences, however, are without precedent, and totally unexpected. In both cases, the changes are of opposite sign for the two transitions, with the magnitude of change being greater for the deeper-lying (149 nm) band. This behaviour is similar to that observed for the conformational changes which accompany gelation of agarose[23] and carrageenan[22]. It should be emphasised, however, that the exact quantitative values of the fitted parameters in Table IV must be treated with caution, for the following reasons: (*a*) o.r.d. curves, being comparatively featureless, tend to give "ill-conditioned" fits (*i.e.*, compensating changes of opposite sign in the two transitions may not greatly alter the form of the overall o.r.d.); (*b*) there may be some slight contribution to the residual o.r.d. difference spectra from changes in the carboxyl $\pi \rightarrow \pi^*$ transition[9,11]; (*c*) the measured proportions of each block type may be subject to considerable experimental error[28]; and (*d*) the extent of deviation from a regular, alternating arrangement of residues within heteropolymeric sequences[15,29–33] may vary appreciably between alginate samples, and thus the inherent assumption in Eq. 5 that heteropolymeric sequences in each of the alginates studied behave identically is unlikely to be strictly accurate.

Despite these reservations, however, it is clear that there are large changes in the optical activity of polymannuronate and heteropolymeric chain-sequences in alginate on Ca^{2+}-induced gelation, and that these changes arise predominantly from the conformation-sensitive electronic transitions of the polymer backbone. At the simplest level of interpretation, the observed o.r.d. changes (Table III) are negative for alginate H, but positive for alginates M and L, and therefore cannot all have their origin in a single process, such as the formation of calcium polyguluronate junctions.

On direct addition of calcium ions to isolated polyguluronate chain-segments in dilute, aqueous solution (Fig. 6), the residual o.r.d. change after substraction of the $n \rightarrow \pi^*$ contribution (determined, as before, by Kronig–Kramers transform of the observed c.d. change[9]) is similar in form to that obtained (Fig. 5) by analysis of our results for intact alginates. (The somewhat lower magnitude is due to practical restrictions on the level of Ca^{2+} which may be added before the chain segments, in the absence of a supporting gel-network, are precipitated from solution.) Addition of calcium chloride to heteropolymeric chain-segments, by contrast, produced only very small changes in o.r.d., which, within experimental error, were identical to those

calculated from the small c.d. changes in the $n \rightarrow \pi^*$ spectral region, and no detectable change in either c.d. or o.r.d. was observed for polymannuronate.

It is clear from the above results that the large changes in optical rotation on Ca^{2+}-induced gelation of alginate are a property of the gel network as a whole, rather than of the component sequences in isolation. A possible explanation is that the o.r.d. changes are optical artefacts induced by, for example, stress birefringence in the gel. This, however, seems unlikely, since (*a*) the results were highly reproducible, (*b*) the calculated o.r.d. behaviour for polyguluronate chain-sequences is in good agreement with results for isolated polyguluronate segments in solution, and (*c*) no such artefacts have been encountered in previous extensive studies of other gelling polysaccharides.

The mechanisms that may be proposed for these effects are (*a*) that the strong associations of polyguluronate chain-sequences serve to stabilise weaker interactions between polymannuronate and heteropolymeric regions, which would not survive in the absence of the supporting network; or (*b*) that formation of junction zones within the gel causes stretching of connecting sequences between junctions, to give a more extended chain-conformation than in free solution. Although the present results cannot distinguish between these two interpretations, we favour the latter proposal.

In contrast to polyguluronate which, in the solid state, shows 2_1 chain geometry, both as the undissociated acid[18] and in all the salt forms so far investigated[17], polymannuronate can adopt both 2_1 (acid form[34]) and 3_1 (salt form[17]) geometry, indicating considerably greater conformational freedom. This is reflected in the relative hydrodynamic volumes of alginate chain-sequences in solution, where polyguluronate adopts a highly extended, stiff chain-conformation, while polymannuronate, despite the greater residue length, shows less extended coil dimensions, and heteropolymeric chain-sequences are even more flexible and compact[35,36]. We therefore suggest that the most probable origin of the large changes in optical activity of polymannuronate and alternating chain-sequences on Ca^{2+}-induced gelation of alginate is from conformational adjustment to a more extended chain-profile than in free solution, as the polyguluronate sequences which are contiguous on either side become tied down in junctions.

No such effects have been observed in previous chiroptical studies of the gelation of agar[37] and carrageenan[38] polysaccharides, where the changes in optical rotation which accompany gel formation are closely similar to those observed on conformational ordering of structurally regular, short chain-segments in solution, and are in good quantitative agreement with values calculated[7] from the known chain-geometry in the ordered conformation. In both the agar and carrageenan series, inter-chain association through double-helical junction zones is terminated by the occurrence in the primary structure of shorter "kinking" sequences that are sterically incompatible with incorporation in the ordered structure[2,3], and these solubilising features represent a smaller proportion of the chain length than the polymannuronate

or heteropolymeric chain-sequences of alginate. In the gel state, therefore most of the polymer chain is involved in ordered, inter-chain association.

However, for other block copolysaccharides where an appreciable fraction of the component residues occur in chain sequences which are incapable of ordered association, it now seems likely that the overall changes in optical activity on gelation will contain a substantial contribution from these sequences. Thus, the importance of parallel studies, using structurally regular, chain segments in solution[3], is once more emphasised.

ACKNOWLEDGMENTS

We thank Mr. D. Cooke, Mr. S. M. Bociek, Mrs. S. A. Frangou, Mrs. L. A. Linger, and Mr. E. J. Murray for expert technical assistance, and Alginate Industries Ltd. for kindly providing a research sample of alginate of low polyguluronate content.

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