ELECTROPHORETIC, SEDIMENTATION AND DIFFUSION PROPERTIES OF CARRAGEENIN*

by

W. H. COOK, R. C. ROSE, AND J. R. COLVIN

Division of Applied Biology, National Research Laboratories, Ottawa (Canada)

INTRODUCTION

Structural studies indicate that carrageenin, the polysaccharide extracted from the marine algae *Chondrus crispus*[1], is composed largely of D-galactose residues linked 1:3 with an ethereal sulphate on carbon four[2-4]. This apparently linear structure does not account for the entire molecule since the number of hexose residues exceeds the number of sulphate groups[5]. Carrageenin has been claimed to contain more than one polysaccharide[6-9] and although differences based on solubility can now be discounted[2,5,10], the possibility still remains that this extract is a mixture. Recently JOHNSTON AND PERCIVAL[4] reported the presence of L-galactose, poor in sulphate groups, and evidence favouring branching. They interpret this as indicating that carrageenin is a complex molecule but admit the possibility that it may contain two polysaccharides.

In such a molecule ionization occurs only at the sulphate groups and neutral extracts are therefore the salt of this organic acid with the available cations. Since electrophoretic mobility is determined primarily by the charge density or the number of ionizing groups per unit weight of the material, it should be possible to resolve components if they exist as separate polysaccharides differing in sulphate content. Electrophoretic analyses were undertaken to determine whether this procedure would reveal the existence of more than one polysaccharide.

Viscosity and fractionation studies[11,12] show that carrageenin is polydisperse but these measurements provide little information on the range of molecular size or shape that occurs in such dispersions. If the molecule has a complex branched structure instead of a linear form, as indicated by the simple formula, it might be possible to distinguish between these possibilities from the information obtained by sedimentation and diffusion studies. The estimation of the molecular weight and axial ratio by these procedures was therefore undertaken.

MATERIAL

The marked variation in viscosity of different carrageenin samples reflects variations in the mean molecular size and shape, and presumably depends on the raw seaweed, the extractive procedure and the extent of fractionation. To provide representative material, four samples were selected on the basis of their intrinsic viscosity. One of these samples was prepared in the laboratory by hot water extraction. The others were selected from a series of commercial samples prepared by the same general method. A total nitrogen content, both as ammonium content and protein, of less than 1%, and a practical absence of insoluble material indicated an acceptable purity. Although carrageenin

* N.R.C. No. 2652

References p. 606.

is comparatively stable, effective purification steps tended to reduce the viscosity, and a low content of impurities was considered less objectionable than the degradation of the polymer. Fractionation of this polydisperse material was also avoided until further information was available.

The intrinsic viscosity was determined from specific viscosity measurements made on 0.01 to 0.1% carrageenin concentrations dissolved in 0.05 *M* sodium chloride at 40° C. Since the carrageenin was not purified, the samples may have had a small but differential content of cations other than sodium that might affect the intrinsic viscosity. Previous experience indicates that differences due to the cation are small at 40° C, and at this temperature the gross differences between the viscosities of the selected samples are due primarily to the carrageenin. The four samples, designated L1 (laboratory preparation), C1, C2 and C3, had intrinsic viscosities of 3.4, 4.3, 6.1 and 11.2.

Carrageenin solutions were prepared for all measurements by mixing weighed quantities of material of known moisture content in the appropriate salt or buffer solution and heating to 60° C for twenty minutes. This procedure was unlikely to alter the material as 20 minutes heating at even higher temperatures had no significant effect on viscosity[12]. The solutions were cooled immediately, made to volume, examined visually for optical clarity, and if not clear, centrifuged for 30 minutes at 15,000 gravities. For the electrophoretic, sedimentation and diffusion studies the carrageenin solutions were dialyzed at 2° C against 15–20 volumes of solvent for 40 hr or more. The final concentrations were checked when necessary by differential refractometer readings. For calibration of this instrument, partial specific volume determinations, and viscosity measurements, the solutions were prepared on a weight basis and were not dialyzed.

Early measurements indicated that carrageenin concentrations of 0.3% or higher in solvents having an ionic strength in excess of 0.15, showed an anomalous behaviour electrophoretically. In consequence the majority of the electrophoretic, sedimentation and diffusion measurements were made at concentrations of 0.2 or 0.3%, except where measurements of useful accuracy could be made at other concentrations. The solvent was 0.1 *M* sodium chloride, plus a sodium phosphate buffer at pH 6.6, giving a total ionic strength of 0.15.

ELECTROPHORETIC PROPERTIES

Electrophoretic measurements were made using Tiselius apparatus as modified and described by LONGSWORTH[13]. All measurements, including conductivity, were made at 0.10 ± 0.05° C. The patterns showed considerable spreading and the boundary position was taken as the ordinate dividing the area of the pattern into equal parts. The spreading of the pattern was estimated by locating a line, parallel to the base line, that divided the pattern area into two equal parts. Mobilities were computed for the points at which this line cut the leading and trailing edges of the pattern. The difference between these quantities gave a relative estimate of the spreading in terms of mobility units. The portion of the spreading attributable to electrophoretic heterogeneity was assumed to be that observed at the maximum excursion less that remaining after the boundary was returned to its original position by current reversal.

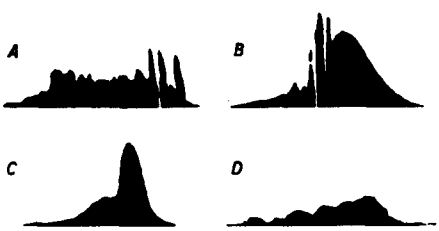

Fig. 1. Irregular descending patterns obtained with carrageenin.
A. 0.5% sample L1 in presence of potassium ions.
B. Sample C2 after reversal. Concentration 0.3%, ionic strength 0.15, time 365 min, field strength 2.17 volts/cm.
C and D. Sample C3 after 121 min (C), and 281 min, (D), other conditions as in B.

Initial work, undertaken on sample L1 in potassium phosphate buffer, always gave an irregular pattern as illustrated in Fig. 1A. Subsequent work with the two samples of lowest viscosity showed that this anomaly could be avoided by eliminating potassium ions from the buffer solution and limiting the carrageenin concentration to 0.3% when an ionic strength of 0.15 was used. These conditions proved to be of borderline character for the samples of higher intrinsic viscosity. Thus sample C2 occasionally gave irregular patterns when the current was reversed, as shown in Fig. 1B. It was fre-

References p. 606.

quently evident in C3 on the outward excursion as shown in Fig. 1C after 121 min and in Fig. 1D after 281 min. At lower carrageenin concentration or ionic strengths, this sample yielded regular patterns, as shown in Fig. 2C and D. The conditions favouring the appearance of these irregularities are those favouring gel formation and there is no evidence to indicate that they represent different components.

Patterns obtained under suitable conditions showed no definite separation into components but all showed considerable electrophoretic heterogeneity. Typical patterns are shown in Fig. 2. Pattern A was obtained with sample L1 and C1 was similar; pattern B shows ascending and descending boundaries for C2 at the same concentration and ionic strength. The determinations on C3 had to be obtained at lower carrageenin concentration or ionic strength to avoid irregularities and the patterns under these conditions are shown in Fig. C and D. While all patterns had a somewhat characteristic shape, all efforts to resolve clear-cut components failed. The reproducibility of form reflects a consistent asymmetric mobility distribution that may indicate, but is not considered sufficient evidence to establish, the existence of components differing only slightly in mean mobility or present only in small proportion.

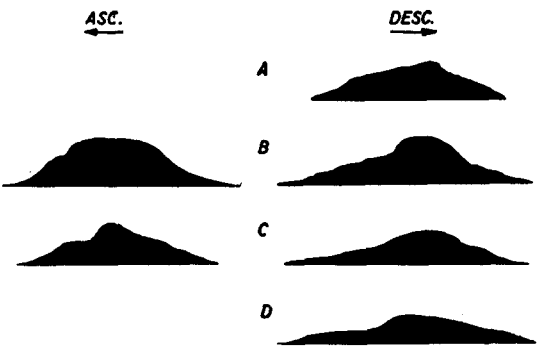

Fig. 2. Regular patterns obtained with carrageenin.
A. Sample L1, descending, 0.3% concentration, 0.15 μ, 121 min, field strength 3.10 volts/cm.
B. Sample C2, ascending and descending, 0.3%, 0.15 μ, 195 min, field strength 2.17 volts/cm.
C. Sample C2, ascending and descending, 0.2%, 0.15 μ, 192 min, field strength 2.14 volts/cm.
D. Sample C3, descending, as C, but 0.3% and 0.05 μ.

The results of the mobility and heterogeneity measurements are given in Table I. Tests on sample L1 showed that the mobility was not affected by pH between 4.0 and 8.0, and differences were not to be expected with this type of polyelectrolyte. The remaining tests made at pH 6.6 showed that ionic strengths of 0.1 and 0.15 yielded the same values, but the mobility increased about 2.4 units at $\mu = 0.05$. On the average the mobility was 1.8 units higher when measured by reversal than by the descending boundary alone; and the mobility increased about 1.2 units for a 0.1% increase in carrageenin concentration. Differences characteristic of the samples are evident from the average values reported in Table I. Considering the extreme spreading of these patterns and the consequent error involved in estimating the boundary position, little significance can be attached to the small differences. It is evident that in spite of the three-fold range of intrinsic viscosity, the mobilities of the different samples do not differ substantially from the average value of 12.0 cm^2/volt sec $\times 10^{-5}$.

In spite of the similarity of the mobilities, it was felt that the portion of the spreading attributable to electrophoretic heterogeneity might reflect some difference between the samples. The values computed by the method already outlined are also given in Table I. The difference between the spreading before and after reversal, given in the last column, provides a relative measure of heterogeneity in terms of mobility units. In Table I, although the spreading increases with increasing viscosity, the differences cannot be considered significant.

References p. 606.

TABLE I

AVERAGE MOBILITY AND SPREADING IN TERMS OF MOBILITY, cm^2/volt sec $\cdot 10^5$

Sample	*Mobility*	*Spreading*			*Remarks*
		Maximum	*After reversal*	*Difference (Heterogeneity)*	
L1	12.8	—	—	—	pH range = 4.0 to 8.0 μ = 0.1 to 0.15
C1	10.8	4.8	2.4	2.4	pH 6.6 throughout
C2	14.4	4.3	1.8	2.5	μ = 0.05 to 0.15
C3	13.8	4.6	1.6	3.0	Conc. 0.2 to 0.3%

SEDIMENTATION

Sedimentation rates were determined in a Spinco motordriven ultracentrifuge at an equivalent mean force of 270,000 gravities. The initial temperatures of the samples were usually between 22 and 25° C and the final temperatures 27 to 31° C. The distances of the apices of the peak from a reference mark were read from the original plate with a micro-comparator to $\pm$ 0.001 mm. Appropriate measurements were made to permit corrections for: the magnification of the optical system; the effect of temperature and viscosity, and to permit the results to be expressed as s^0_{20}.

The partial specific volume of carrageenin was determined by the method of intercepts[14] from the necessary density determinations using the solvent employed for sedimentation. Owing to the high viscosity, which made manipulation difficult, the concentrations used were limited to 1% or less. The values obtained with different samples at temperatures of 19.6° C and 29.6° C were the same within experimental error. A least squares fit to the results of 12 triplicate determinations yielded a linear relation between the apparent specific volume of the solution and the weight fraction of solvent with an intercept of 0.496 $\pm$ 0.002. As this value was lower than that reported for other polysaccharide materials[15,16], it was rechecked using water as the solvent, and the value 0.503 obtained. The value given to the expression $(1-\bar{v}\rho)$ in the sedimentation equation was therefore 0.50.

The sedimentation rate of carrageenin was found to be dependent on electrolyte concentration, as shown in Table II. As already indicated, an ionic strength of 0.15 was used.

TABLE II

EFFECT OF IONIC STRENGTH ON SEDIMENTATION COEFFICIENT

Ionic strength	*Sedimentation coefficient* $\times 10^{13}$	
	Of sample C2	*Of sample C3*
0.05	2.17	2.33
0.10	2.30	2.36
0.15	2.43	2.52
0.20	2.47	—

References p. 606.

Typical sedimenting boundaries for all four samples at 0.5% concentration are shown in Fig. 3. The spreading of the peaks during sedimentation exceeded that to be expected from diffusion, and indicates polydispersity, since the type of concentration dependence observed favours sharpening rather than spreading. The two samples of highest viscosity show separation of a second, more rapidly sedimenting component or aggregate, and it can also be detected in sample C1. Area measurements indicate that this minor component accounts for about 12% of sample C3 and 7% of C2. Its sedimentation rate was about 20% higher than that of the main component at a total concentration of 0.3%, the lowest concentration at which measurements could be made. As separation of these components proved difficult and is still under investigation, it has been impossible to obtain separate diffusion and sedimentation coefficients for them. All reported values are those obtained with the mixture.

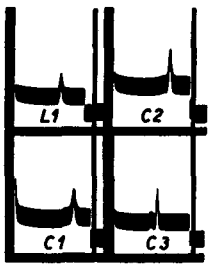

Fig. 3. Sedimentation boundaries of carrageenin dispersions at 0.5% concentration, chosen to show second component.

Sample L1 after 131 min, bar angle 65°
Sample C1 after 141 min, bar angle 60°
Sample C2 after 139 min, bar angle 55°
Sample C3 after 202 min, bar angle 65°

The effect of carrageenin concentration (c) on sedimentation rate (s) had to be estimated over a limited concentration range. Concentrations up to 0.5% could be used for the two samples of lowest viscosity. In the most concentration dependent samples of highest viscosity, peak sharpening enabled concentrations as low as 0.08% to be measured, but here measurements at concentrations above 0.2% were of little value.

The sedimentation coefficient at infinite dilution was obtained by plotting s vs. sc and $1/s$ vs. c to obtain a linear relation suitable for extrapolation. These linear relations were fitted by least squares to obtain the slope, the intercept and its standard error. For sample C3 the departures from linearity of the $1/s$ plot exceeded the random errors and the results at concentrations above 0.18% had to be discarded to obtain a linear relation and the intercept was therefore subject to a greater error. The results, given in Table III,

TABLE III

SEDIMENTATION PROPERTIES OF CARRAGEENIN

Sample	*Conc. range wt. %*	*Slope-linear portion* ($s \times 10^{13}$)		*Intercept* $S^0_{20} \times 10^{13}$ $c \to o$	
		$\frac{ds}{ds.c}$	$\frac{d^1/s}{dc}$	*From s vs. s.c*	*From* $^1/s$ *vs. c* ± *standard error**
L1	0.089–0.27	— 2.44	0.69	3.63	3.67 ± 0.17
C1	0.090–0.45	— 1.91	0.59	3.24	3.23 ± 0.08
C2	0.092–0.46	— 2.70	0.67	3.94	3.89 ± 0.15
C3	0.065–0.18	— 9.44	1.37	6.83	6.72 ± 0.50

* Difference between S^0_{20} ($c \to o$) for L1 and C2 not statistically significant.
All other differences highly significant.

References p. 606.

show that the extrapolation of both plots yields essentially the same sedimentation coefficients at zero concentration. The standard error was computed for the $1/s$ plot only, as a valid estimate of the error for the s vs. sc plot was made difficult by the presence of the variable s in both quantities.

The results in Table III show, except for L1 and C2 which do not differ significantly, that the sedimentation coefficients increase with the intrinsic viscosity of the sample. The standard error represents about $\pm$ 4% of the extrapolated value except for the sample of highest viscosity, where it is $\pm$ 8.0%. Since the molecular weights in a polydisperse material are at best mean values, this level of error was considered satisfactory.

DIFFUSION

Diffusion measurements were made at 20° C $\pm$ 0.05° using the equipment described for the electrophoretic measurements, except that a NEURATH[17] shearing cell was used instead of the electrophoresis cell. This gave a sharp boundary that could not be photographed effectively for quantitative study until some hours after its formation. The usual statistical procedure was used to compute the mean diffusion coefficient by the method of moments. Completely independent replications were made on all samples.

Although the material was concentration dependent, and early experiments revealed certain discrepancies and errors attributable to low concentration, it seemed desirable to avoid the behaviour observed in the electrophoretic determinations at higher concentrations. In certain experiments the area of the schlieren pattern decreased after an experimental period of about 70 hr and the D_m values were correspondingly smaller. GRALÉN[15] reports the same behaviour with cellulose and corrected the areas (and dependent quantities) to a constant value. While these corrections to constant area gave consistent D_m values in the present study, the effect on other quantities used for estimating concentration dependence was unknown, and such observations were discarded.

Plots of σ^2 against t were linear within experimental error in all acceptable experiments, and when extrapolated to zero time gave either a negligible or positive intercept on the σ^2 axis. The magnitude of this intercept varied between experiments and was apparently due to disturbance when the boundary was formed. Although spreading was not measurable at the time of boundary formation, a small degree of mixing has an appreciable effect when the concentration is low and the diffusion coefficient small. This plot of σ^2 vs. t for each experiment was fitted by least squares to obtain the slope and its standard error. The D_m values so obtained from different experiments were then in satisfactory agreement considering the magnitude of the random errors. The average values for each sample appear in Table IV.

TABLE IV

DIFFUSION COEFFICIENTS OF CARRAGEENIN

Sample	D_m $cm^2/sec \times 10^7$	$D^*_{c\to 0} \pm SE$ $cm^2/sec \times 10^7$	$\frac{Do}{D_m}$
L1	1.85	1.41	0.76
C1	1.97	1.39 $\pm$ 0.09	0.71
C2	1.29	0.95 $\pm$ 0.11	0.74
C3	0.99	0.61 $\pm$ 0.09	0.62

* Corrected for solvent viscosity

References p. 606.

The skewed patterns indicated concentration dependence and the diffusion coefficient at infinite dilution ($D_{c\to o}$) had to be obtained. Since the acceptable concentration range was too limited for direct determination of $D_{c\to o}$ by extrapolation, this quantity was evaluated from an analysis of the pattern. Application of the Boltzmann equation[18] showed no departures from linearity in the diffusion coefficient-concentration relation, that exceeded the rather large random errors. In consequence a modification of the following simplified equation given by GRALÉN[15] was used:

$$D_{c\to o} = D_m + \frac{AMo}{4Ht}$$

where A = area
Mo = mode – a negative displacement from the arithmetic mean
H = height at maximum point
t = time in sec.

It is evident from this equation that $D_{c\to o}$ will be affected by any spreading that occurs when the boundary is formed, as H will be reduced by mixing while A remains constant. This was confirmed by the experimental results and necessitated some modification of the equation. An empirical procedure was to extrapolate the σ^2 vs. t plot used to compute the D_m value back to the time axis and add this time increment to the observed t in GRALÉN's equation. This is equivalent to adding the time required for the observed spreading to occur by diffusion. This type of correction yielded values of $D_{c\to o}$ from replicate experiments that were constant within the random error. The average $D_{c\to o}$ values reported in Table IV were computed in this way.

This correction neglects the fact that skewing (Mo) does not occur during the added arbitrary time increment required to correct other terms in the equation. A rigorous treatment, that will not be detailed here, permitted the proper evaluation of Mo and the correction of the other terms in the equation for the effects of the initial mixing. The application of this procedure established the validity of the simpler empirical method for the magnitude of the intercepts observed in these experiments.

The results in Table IV show that wherever the differences are significant the diffusion coefficient decreases as the intrinsic viscosity increases. The ratio D_o/D_m indicates the degree of concentration dependence is about the same for all samples except C3, where concentration effects are greater.

SIZE AND SHAPE ESTIMATES

The weight average molecular weight of these polydisperse solutions was obtained using the usual Svedberg equation[18]. The s and D values employed were those at infinite dilution. Except for sample L1, for which the data were inadequate, the standard error of these molecular weights was estimated from the standard errors of the s and D values at infinite dilution by the following equation[19]:

$$\sigma^2_{s/D} = \frac{\sigma_s^2}{D^2} + \sigma_D^2\left(\frac{S^2}{D^4}\right)$$

where σ^2 = square of standard error for quantity indicated by subscript.
s = sedimentation coefficient at infinite dilution.
D = diffusion coefficient at infinite dilution.

References p. 606.

The other quantities in the SVEDBERG equation are subject to comparative negligible random errors and they were regarded as a constant.

The molecular weights and their standard errors appear in Table V. It is evident that the three-fold increase in intrinsic viscosity represented by the samples results in a five-fold increase in molecular weight. The standard errors are of the order of ± 8 to 10% except for the sample of highest intrinsic viscosity, where it is about twice as great.

TABLE V

ESTIMATES OF SIZE AND SHAPE OF CARRAGEENIN MOLECULE

Sample	*Size* $M \pm$ *standard error*	*No hydration*			*50% hydration*			*Axial ratio from* (η) (21)
		Frictional ratio	*Axial ratio*	*Diam. A*	*Frictional ratio*	*Axial ratio*	*Diam. A*	
L1	120,000 —	5.1	160	10.7	4.0	95	12.7	110
C1	110,000 ± 8,500	5.4	180	9.9	4.3	110	11.6	120
C2	200,000 ± 22,000	6.7	270	10.5	5.2	170	12.4	150
C3	530,000 ± 96,000	7.3	340	13.5	5.8	200	16.0	210

Table V also contains the quantities used for estimating molecular shape. The observed frictional coefficient, and the calculated frictional coefficient for a spherical molecule of the same average weight and volume, were used to obtain the frictional ratio in the usual manner[18]. This value was used in the PERRIN[18] equation to obtain the axial ratio of the molecule assuming a prolate ellipsoid of rotation. The diameter of this ellipsoid was then computed by the prismoidal formula. The theoretical and experimental limitations of these computed quantities must be recognized, and while they cannot be taken as valid absolute values, they provide the best means for distinguishing between linear or branched structure with the procedures used.

If the observed quantities are used in these calculations, the results are in terms of an unhydrated molecule. Since carrageenin is almost certainly hydrated, although the degree of hydration is unknown, the quantities calculated for an unhydrated molecule must represent unlikely extremes. Lacking definite information, a 50% hydration was assumed and the frictional ratio and other quantities recomputed on this basis[20]. The reported values serve to show the effect of hydration on the computed quantities.

The results in Table V show the axial ratio about doubles for a five-fold increase in molecular weight, varying from 160 to 340 on the basis of an unhydrated molecule and from 95 to 200 at 50% hydration. While the intrinsic viscosity measurements, used for sample selection, were made under somewhat different conditions, the axial ratios computed from these measurements by SIMHA's[21] equation are in good agreement with those reported for the hydrated molecule.

Theoretically, the figures reported for the diameter represent that of the assumed ellipsoid of rotation. This value will overestimate the width of the chain if it is flexible, and for the same reason will increase with the length of the molecule. This effect is shown by the results: the reported diameter increasing for the hydrated molecule from 11.6 to 16.0 A with increasing mean molecular weight. The significance of these results in relation to a branched and linear structure will be discussed later.

References p. 606.

OSMOTIC PRESSURE

Some osmotic pressure measurements undertaken earlier on sample C3 provide additional information on the molecular weight and polydispersity of this sample. These measurements were made in 0.1 *M* sodium chloride solutions at 25° C. using osmometers and procedures described by DONNAN AND ROSE[22]. A well-established equilibrium was obtained in two days.

TABLE VI

OSMOTIC PRESSURE, IN cm WATER AT 4° C, OF CARRAGEENIN (SAMPLE C3) IN 0.1 *M* SODIUM CHLORIDE

Conc. %	$\frac{O.P.}{C}$
0.486	2.49
0.389	2.24
0.292	1.92
0.194	1.65
0.097	1.34
Limit c→o	1.03
Mn = 250,000	

When the results given in Table VI are extrapolated to infinite dilution, π/c had a value of 1.0 cm of water at 4° C. This corresponds to a number average molecular weight (Mn) of 250,000. This value is about half the weight average molecular weight (Mw) obtained by sedimentation and diffusion. These values are only equal in monodisperse systems and the ratio of these two averages gives a relative measure of the polydispersity. By assuming a logarithmic distribution of the molecular weights, the following relation is valid:

$$\frac{Mw}{Mn} = \exp \frac{B^2}{2}$$

where B is termed a non-uniformity coefficient. For sample C3 this coefficient had a value of 1.20.

GRALÉN reports values for this coefficient varying from a minimum of 0.44 for unbleached linters to a maximum of 1.94 for over-aged cellulose. The value found for carrageenin is similar to that given for sulphite cellulose, a preparation of intermediate polydispersity.

DISCUSSION

When experimental conditions were chosen to avoid anomalies, the electrophoretic analysis revealed considerable heterogeneity, but no definite evidence of separable components. If the "galactose chains" and the "resistant fragment" of the composition reported by JOHNSTON AND PERCIVAL[4] came from separate polysaccharides, the difference in charge density should have been sufficient to permit clear-cut electrophoretic separation. Since mobility is relatively insensitive to particle size and shape, the hetero-

References p. 606.

geneity indicates a continuous variation in charge density among the particles. This could be explained by random variations in the distribution of sulphate groups among the hexose units, since on the average there is less than one sulphate group per hexose residue[5].

The possibility exists that the marked spreading masked a second electrophoretic component, present in small amount and differing only slightly in mobility. Since all samples had similar mean mobilities, such a component, if present, must have either formed too small a proportion to affect the mean values, or been present in the same proportion in all samples. The second component, demonstrated by sedimentation, was present in varying amounts in the several samples, and since this had no detectable effect on the mean mobility, it appears that this fraction must be similar to the bulk of the material electrophoretically. The electrophoretic spreading did increase slightly with increasing particle size, but the differences, while suggestive, cannot be considered as evidence of a second electrophoretic component. It therefore appears that all of the material has a similar mean charge density, *i.e.*, content of ionizing sulphate groups.

Available information indicates that dispersions of carrageenin are polydisperse in the sense that there is a continuous variation in particle size. This was confirmed qualitatively from sedimentation, diffusion and osmotic pressure studies. In contrast to the electrophoretic studies, however, sedimentation revealed two components that sedimented at different rates. The more rapidly sedimenting component was not evident in sample L1, but in the other samples it increased with intrinsic viscosity to about 12% in the sample of highest viscosity. Subsequent studies have shown it to be present in all available samples of high viscosity. This minor component is not affected by sedimenting at 50° C, and it can still be resolved in similar amounts from mixtures of high and low viscosity material (C1 and C3), although the major components cannot be separated. These and other preliminary experiments suggest that it is not a non-polysaccharide impurity or a simple reversible aggregate. Further studies now under way will be reported later.

The quantitative estimates of molecular size and shape are necessarily mean values. Lacking information on the size distribution, they are more useful for comparative purposes than as absolute values. The sample of highest viscosity was comparable in molecular weight, degree of polydispersity, axial ratio and diameter, to those reported[15] for sulphite and sulphate celluloses by similar investigational procedures. In spite of the polydispersity and the presence of two components in sample C3, the maximum diameter obtained for the unhydrated molecule was 13.5 A as against values of 15 A or more, reported by GRALÉN, for cellulose preparations in the same molecular weight range. Since cellulose is generally regarded as having a linear structure, these findings favour a predominantly linear structure in carrageenin. The reported diameters estimated from the mean values are too uncertain and insensitive to provide a direct indication of branching in the minor component. This is not precluded, however, by the present measurements, since apart from the error of estimation, the increase in apparent diameter with molecular weight may not be due entirely to flexibility in the chain.

The presence of two components in carrageenin differing sufficiently in size or shape to permit their being resolved from the general polydispersity has been demonstrated. Since the average values indicate a predominantly linear structure, it seems reasonable to consider the major component as being of this type. There is also structural evidence for branching[4] in a portion of the material roughly similar in amount to that of the minor

References p. 606.

component found by sedimentation. This indicates that the linear and branched forms may indeed be separate entities. The decrease in the amount of the minor sedimenting component with decreasing viscosity also suggests that it can be degraded to something similar to the predominantly linear form, since it cannot be distinguished either by sedimentation or electrophoresis.

ACKNOWLEDGEMENTS

The authors wish to thank the numerous firms and individuals who provided seaweeds and extract for this investigation, and particularly to acknowledge the large series of special samples provided by the Krim-Ko Corporation, Scituate, Mass., and Kraft Foods Company, Chicago. It is a pleasure to acknowledge Mr C. REIMER's assistance in calculating the diffusion coefficients, curve-fitting and related statistical problems. The exacting technical work performed by Messrs. G. DOWD, D. R. MUIRHEAD, R. H. WHITEHEAD and G. A. YOUNG is also gratefully acknowledged.

SUMMARY

An electrophoretic analysis of four samples of carrageenin ranging in intrinsic viscosity from 3.4 to 11.2 revealed considerable spreading but no evidence of separable components. All samples had similar mean mobilities averaging $12.0 \cdot 10^{-5}$ cm²/volt sec. The sedimentation and diffusion studies showed that the molecular weight, axial ratio and estimated diameter of the ellipsoid of rotation all increased with the intrinsic viscosity of the sample. Over the threefold viscosity range the molecular weight increased from 110,000 to 530,000, the axial ratio from 160 to 340, and the diameter from 9.9 to 13.5 A assuming no solvation. These are mean values since all samples were polydisperse and in addition two components were revealed by sedimentation. The amount of the more rapidly sedimenting minor component increased with the viscosity from nil to about 12% of the most viscous sample. Nevertheless the mean values given above are comparable with those reported for certain cellulose preparations from which it appears that the major component has a linear structure. The higher rate of sedimentation of the minor component indicates a different size or shape, and it may be branched, but this cannot be established or disproved from the present measurements. The electrophoretic analysis indicates that both components are similar in attributes of composition affecting charge density.

RÉSUMÉ

L'analyse électrophorétique de quatre échantillons de carraghène dont les viscosités intrinsèques ont des valeurs entre 3.4 et 11.2 révèlent un écart considérable sans mettre en évidence des constituants distincts. Tous les échantillons ont des mobilités semblables, la moyenne étant de $12.0 \cdot 10^{-5}$ cm² par volt sec. L'étude de la diffusion et de la sédimentation montre que le poids moléculaire, le rapport axial, et le diamètre évalué de l'ellipsoide de rotation augmentent tous avec la viscosité intrinsèque de l'échantillon. Pour un accroissement de la viscosité du simple au triple, le poids moléculaire augmente de 110,000 à 530,000, le rapport axial de 160 à 340, et le diamètre de 9.9 à 13.5 A sans tenir compte de solvation. Ce sont là des valeurs moyennes, puisque tous les échantillons sont polydisperses et que, de plus, la sédimentation révèle deux constituants. La fraction du constituant se déposant le plus vite augmente avec la viscosité de 0 à 12% dans l'échantillon le plus visqueux. Néanmoins, les valeurs moyennes mentionnées sont comparable à celles rapportées pour certaines préparations de cellulose; il en ressort que la majeure partie possède une structure linéare. La sédimentation plus rapide du constituant le moins abondant indique une grandeur ou une forme différente, peut-être une ramification, mais les mesures faites ne ne prouvent ni ne réfutent ce point. L'analyse électrophorétique indiquent que l'un et l'autre des constituants sont semblables en ce qui concerne les attributs de la composition régissant la densité ionique.

References p. 606.

ZUSAMMENFASSUNG

Die elektrophoretische Analyse von vier Carragheenproben, deren Eigenzähigkeit zwischen 3.4 und 11.2 liegt, zeigt beträchtliche Spreizung, ohne aber das Vorhandensein von besonderen Bestandteilen zu beweisen. Alle Proben haben ähnliche mittlere Beweglichkeiten, im Durchschnitt $12.0 \cdot 10^5$ cm^2/Volt Sek. Sedimentation und Diffusionsversuche ergeben, dass das Molekulargewicht, das Achsenverhältnis und die geschätzten Durchmesser des Rotationsellipsoides alle zunehmen, wenn die Eigenzähigkeit zunimmt. Wenn die Viskosität auf das Dreifache steigt, nimmt das Molekulargewicht von 110,000 auf 530,000 zu, das Achsenverhältnis von 160 auf 340, und der Durchmesser von 9.9 auf 13.5 A, ohne Solvation einzubeziehen. Dies sind mittlere Werte, da alle Proben polydispers sind, und die Sedimentationsversuche ausserdem zwei Bestandteile nachweisen. Der Bruchteil, der sich rascher niedersetzt, nimmt mit der Viskosität von 0 auf 12% der zähesten Probe zu. Die angegebenen Werte sind indessen mit denen, die für Cellulose Proben angegeben werden, vergleichbar, woraus hervorgeht, dass der überwiegende Teil lineare Struktur hat. Die grössere Geschwindigkeit mit der sich der geringere Anteil niederschlägt, weist auf andere Grösse oder Form hin, vielleicht auf eine Verzweigung, ohne dass aber die gegenwärtigen Messungen eine Entscheidung dafür oder dagegen ermöglichen. Die elektrophoretische Analyse zeigt, dass die Eigenschaften von denen die Ladungsdichte abhängt, für beide Bestandteile ähnlich sind.

REFERENCES

[1] C. K. TSENG, *Science*, 101 (1945) 597.
[2] J. BUCHANAN, E. E. PERCIVAL, AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1943) 51.
[3] E. T. DEWAR AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1947) 1622.
[4] R. JOHNSTON AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1950) 1994.
[5] M. R. BUTLER, *Biochem. J.*, 28 (1934) 759.
[6] P. HAAS, *Biochem. J.*, 15 (1921) 469.
[7] P. HAAS AND T. G. HILL, *Ann. Applied Biol.*, 7 (1921) 352.
[8] P. HAAS AND B. RUSSELL-WELLS, *Biochem. J.*, 23 (1929) 425.
[9] B. RUSSELL-WELLS, *Biochem. J.*, 16 (1922) 578.
[10] E. J. REEDMAN AND L. BUCKBY, *Can. J. Research* D, 21 (1943) 348.
[11] R. C. ROSE, *Can. J. Research F*, 28 (1950) 202.
[12] R. C. ROSE AND W. H. COOK, *Can. J. Research, F*, 27 (1949) 323.
[13] L. G. LONGSWORTH, *Ind. Eng. Chem. Anal. Ed.*, 18 (1946) 219.
[14] G. N. LEWIS AND M. RANDALL, *Thermodynamics and the free energy of chemical substances*, 1923, McGraw-Hill Book Co.
[15] N. GRALÉN, Sedimentation and Diffusion Measurements on Cellulose and Cellulose Derivatives, Inaugural Dissertation, Upsala, 1944.
[16] C. O. BECKMANN AND Q. LANDIS, *J. Am. Chem. Soc.*, 61 (1939) 1495.
[17] N. NEURATH, *Chem. Rev.*, 30 (1942) 357.
[18] A. E. ALEXANDER AND P. JOHNSON, *Colloid Science*, (1949), Oxford.
[19] OWEN L. DAVIES, *Statistical Methods in Research and Production*, 1947, Oliver and Boyd.
[20] J. L. ONCLEY, *Ann. New York Acad. Sci.*, 41 (1941) 121.
[21] R. SIMHA, *J. Phys. Chem.*, 44 (1940) 25.
[22] F. G. DONNAN AND R. C. ROSE, *Can. J. Research* B, 28 (1950) 105.

Received July 31st, 1951