MOLECULAR WEIGHT AND HYDRODYNAMIC PROPERTIES OF LAMINARIN *

by

M. H. G. FRIEDLAENDER, W. H. COOK AND W. G. MARTIN

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

INTRODUCTION

Laminarin, a polysaccharide found in the brown algae, occurs in two forms that differ in solubility in cold water. The soluble form is an extract of *Laminaria digitata* and the insoluble of *Laminaria cloustoni*. Both forms are linear glucosans containing about 20 β-D-glucopyranose units linked 1:3[1,2] but there is considerable variation in the reported molecular weights (2,600–5,000)[3]. This variation suggested a possible explanation of the differences in solubility behaviour. Particle size is also of interest in connection with the possible use of sodium laminarin sulphate as a blood anticoagulant and of hydroxyethyl laminarin as a plasma substitute. Molecular weight estimates were therefore undertaken by means of velocity sedimentation, diffusion, and viscosity procedures.

EXPERIMENTAL

Material

Samples of soluble and insoluble laminarin prepared by the method of BLACK *et al.*[4] were obtained through the courtesy of the Institute of Seaweed Research, Inveresk, Scotland. Mild methylation of the less soluble form of laminarin renders it soluble in water. Dr. A. G. Ross at the Chemistry Department, University of Edinburgh, kindly made available a small sample of this form of laminarin methylated to the extent of 2.7% and another containing 6.9% methoxyl groups.

Solvents

The water-soluble form (referred to as Ld, since it is obtained from *L. digitata*) was dissolved at room temperature in phosphate buffers of high (1.0 *M* NaCl, 0.5 *M* Na_2HPO_4 and 0.5 *M* KH_2PO_4) and low (0.5 *M* NaCl, 0.025 *M* Na_2HPO_4 and 0.025 *M* KH_2PO_4) ionic strengths, at pH 6.7. Preliminary work showed that the solubility of the less soluble form (Lc after *L. cloustoni*) was favoured by alkaline conditions, high ionic strengths, and the effect of specific ions. A borate buffer of ionic strength $\mu = 0.5$ (0.1 *M* Na_2CO_3, 0.1 *M* KCl and 0.1 *M* H_3BO_3) at pH 9.5 was found to retain laminarin Lc in solution for the temperature-time sequence required for experimental purposes. Several other solvents tried were ineffective including the same buffer at lower concentration and veronal buffers at pH 9.2. As the methylated form of Lc laminarin dissolved readily in molar sodium chloride, this solvent was used. Possible formation of complexes in borate buffer[5] was checked independently by making measurements on the soluble form Ld and the methylated Lc form in both borate and non-borate solvents.

Solvents of unusually high salt concentration were used since preliminary results suggested some aggregation in the more dilute solvents. While the ionic strength principle does not hold at these high concentrations, the solutions are conveniently referred to as $\mu = 0.15$ and 3.0 for phosphate, and 0.5 and 1.0 for borate buffers.

* Issued as N.R.C. No. 3245.

References p. 144.

Preparation of solutions

The experimental solutions were prepared by dissolving the material at temperatures below 35° C followed by centrifuging and dialysis for 43 h at 5° C. During dialysis, laminarin loss (up to 25%) was found to follow a linear relation with time but the procedure was considered necessary because of the high ionic strength of the solvents. For measurements of sedimentation and diffusion, which were found to be independent of concentration, the final concentration of laminarin was not always determined. To facilitate comparison, the concentrations (weight percent) are expressed in terms of the initial values as $^{I}2.0$, $^{I}1.5$ and $^{I}1.75$. For viscosity measurements, however, final concentration was determined by freeze-drying the solution and dialysate or solvent and drying to equilibrium over P_2O_5 at 45° C. This procedure gave quite reproducible results but it is doubtful if all of the water was removed[6]. Drying at a higher temperature usually caused a further loss in weight and in consequence the concentration so determined may overestimate the concentration of water-free laminarin.

Physical methods

Sedimentation rates were determined in a Spinco ultracentrifuge using an equivalent mean force of 250,000 gravities and the usual viscosity and density corrections were made to permit computation of S^0_{20} values.

The peak formed by this low molecular weight material in the standard ultracentrifuge cell left the meniscus slowly and tended to be quite flat before adequate separation occurred (Fig. 1, S.U.C., No. 1–3). From suitable exposures it was possible to estimate the mean position of the peak with reasonable consistency but the experimental error was large. A synthetic boundary cell[7] was found to give sharper peaks (Fig. 1, S.B.C., No. 1–3), smaller errors of measurement, shorter running times, and smaller temperature corrections.

In seven comparative trials using different combinations of the solutes and solvents, the synthetic boundary cell gave significantly higher sedimentation coefficients than the standard cell in four trials, and similar results for the other three. These differences could be attributed to the greater fractionation of this polydisperse material (see later) during the longer running times necessary with the standard cell. The results subsequently reported were all obtained with the synthetic boundary cell.

Diffusion experiments were conducted in a double Claesson cell[8], using the scanning procedure of LONGSWORTH at a temperature of 29.5 ± 0.05° C. The diffusion coefficients, D_a[11], were estimated from height and area measurements made on photographic enlargements either directly or by using an Amsler mechanical integrator. All results were corrected to D^0_{20} values.

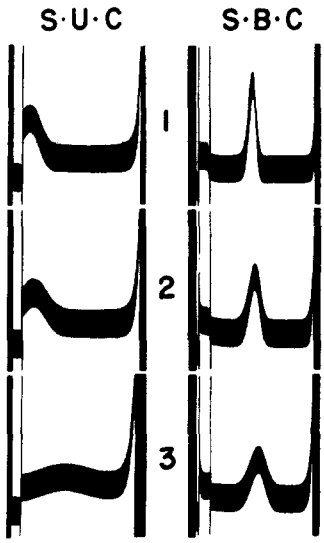

Fig. 1. Typical sedimentation patterns of Ld-laminarin produced in the standard ultracentrifuge (SUC) and synthetic boundary cell (SBC).

The intrinsic viscosity was measured on 0.5, 1.5 and 3%[6] solutions at 20° C using Fenske viscometers having a water-flow time of approximately 225 seconds.

RESULTS

Sedimentation

The observed sedimentation coefficients are summarized in Table I together with their standard errors. The effects on the sedimentation coefficient of ionic strength and solute concentration need to be examined individually. Thus, for the Ld form in phosphate buffer, the increase in the coefficient with a lowering of ionic strength is interpreted as indicating a higher degree of aggregation than occurs in the stronger buffer. The sedimentation coefficients obtained for the Ld and Lc forms in borate buffer of different concentration showed that whereas the higher concentrations appeared to be slightly better solvents, thus indicating less aggregation, the effect of varying the ionic strength did not result in significantly different values.

The effect of solute concentration was a limiting factor in establishing an accurate sedimentation coefficient. In concentrated buffers the effects were irregular and small;

in the phosphate buffer the reverse of normal concentration behaviour[9] was encountered, which can only be attributed to residual aggregation at the higher solute concentration. In the borate buffer both forms of laminarin showed slight but normal concentration effects. Since the sedimentation coefficient at infinite dilution would differ from that observed at the lowest experimental concentration by an amount that scarcely exceeds

TABLE I

SEDIMENTATION COEFFICIENTS

Sample	*Conc.*	$S^0_{20} \times 10^{13}$		
		Phosphate 0.15	*Phosphate 3.0*	*Accepted value and S. E.*
Ld	$I_{2.0}$	(2) 1.02	(2) 0.94	
	$I_{0.75}$	(2) 1.10	(4) 0.85	0.85 ± 0.023
		Borate 0.5	*Borate 1.0*	
Ld	$I_{2.0}$	(2) 0.91	(4) 0.92	
	$I_{0.75}$	(4) 0.97	(2) 1.00	1.00 ± 0.019
		Borate 0.5	*Borate 1.0*	
Lc No methylation	$I_{1.5}$	(2) 0.86	(2) 0.77	
	$I_{0.75}$	(6) 0.80	(3) 0.83	0.83 ± 0.027
		Borate 0.5	*1.0 M NaCl*	
2.7% methylation	$I_{0.75}$	0.72	0.57	0.57 ± 0.04
6.9% methylation	$I_{0.75}$	0.71	0.41	0.41 ± 0.06

The figures in parentheses indicate the number of independent determinations from which the averages were computed. In general, differences of less than $0.04 \cdot 10^{-13}$ (ca. 5%) are not significant.

the experimental error, the results were not extrapolated. In the borate buffer the sedimentation coefficient and molecular weight would have been reduced by about 5% if extrapolated to zero concentration, but it will be shown later that there is evidence of complex formation in these borate buffers. In consequence the results at the highest ionic strength and lowest salt concentration were accepted for subsequent calculations. Obviously the sedimentation coefficients differ significantly for the different samples in different solvents (*cf.* Table I) but this will be dealt with later in connection with the molecular weight estimates.

Diffusion

The diffusion coefficients are given in Table II. These measurements were made only at the lowest salt concentration and as the different ionic strengths had no significant effect, the results have been averaged. Clearly the diffusion coefficients are less sensitive to the differences in aggregation or size distribution that are responsible for the small differences in the sedimentation coefficients. Because of the standard error (about 5%), in determining the diffusion coefficient, no difference was found between the diffusion rate of the Ld form in either phosphate or borate buffers. Representative

References p. 144.

diffusion diagrams are given in Fig. 2. Similarly, the diffusion coefficient for the Lc form proved the same regardless of solvent or degree of methylation but was significantly higher than the values obtained with the Ld form. The $D_m : D_a$ ratio of about 1.1 for the methylated samples provides strong evidence of their polydispersity[11]. A similar ratio would doubtless apply to the unmethylated samples.

TABLE II

DIFFUSION AND INTRINSIC VISCOSITY COEFFICIENTS

Sample	*Ld*			*Lc*	
Methylation	—	—	—	2.7%	6.9%
Conc.	$I_0.75$	$I_0.75$	$I_0.75$	0.75%	0.75%
Buffer	Phos. 0.15 Phos. 3.0	Bor. 0.5 Bor. 1.0	Bor. 0.5 Bor. 1.0	1.0 *M* NaCl	1.0 *M* NaCl
Av. $D^0_{20} \times 10^7$					
D_a	(4) 10.2 ± 0.45	(3) 10.6 ± 0.52	(5) 11.7 ± 0.40	11.72 ± 0.07	11.78 ± 0.02
D_m	—	—	—	13.08	12.99
D_m/D_a	—	—	—	1.12	1.10
$[\eta]$	—	0.101	0.096	—	—

The numbers in parentheses indicate the number of independent determinations from which the average and standard errors were computed.

Intrinsic viscosity

Intrinsic viscosity measurements (Table II) were made only in borate buffer. As the specific viscosity was independent of concentration, results at three concentrations were averaged. Since the methods employed for estimating concentration may have failed to remove all of the water, these intrinsic viscosities may be somewhat low. However, the results are consistent with the sedimentation and diffusion measurements in that the less soluble form has the lower intrinsic viscosity.

Molecular weight

A knowledge of the partial specific volume is necessary in order to estimate the molecular weights by the SVEDBERG equation[9]. Difficulties associated with the determination of moisture in laminarin precluded the accurate determination of the partial specific volume with the size of sample and facilities available. All forms of laminarin in all solvents were therefore assumed to have a partial specific volume of 0.60, the value reported for starch[10], and close to the accepted value for several polysaccharides, amylose and amylopectin[11]. This assumption may be open to some doubt. A determination made in triplicate on the Lc form in borate yielded a value of 0.81 when the concentration was determined by drying over P_2O_5 at 40° C in vacuo but was reduced to 0.75 after drying to equilibrium at 100° C. These limited observations suggest that the partial specific volume of laminarin in the solvents used may be higher than the assumed value.

References p. 144.

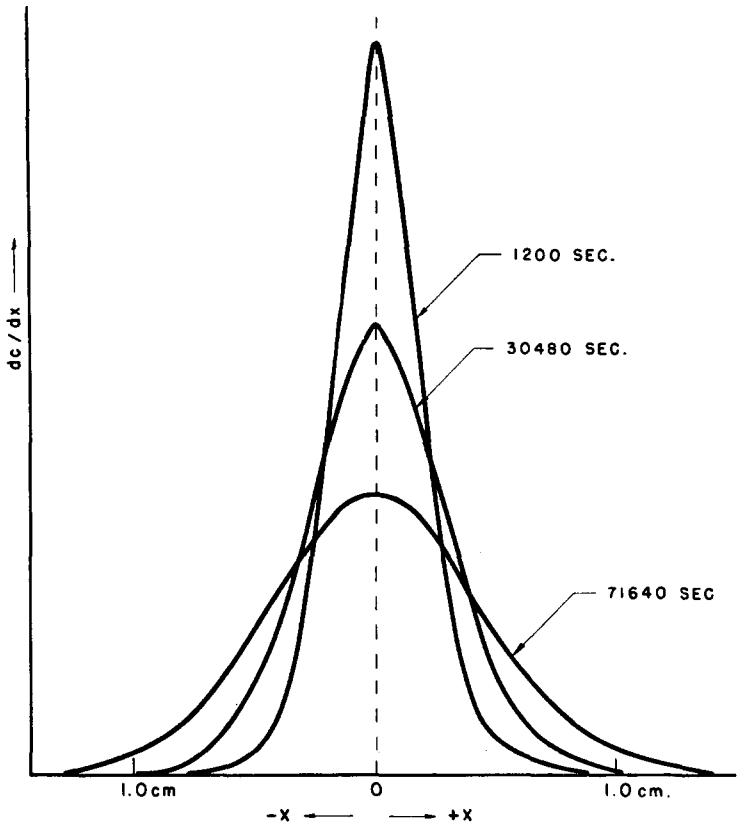

Fig. 2a

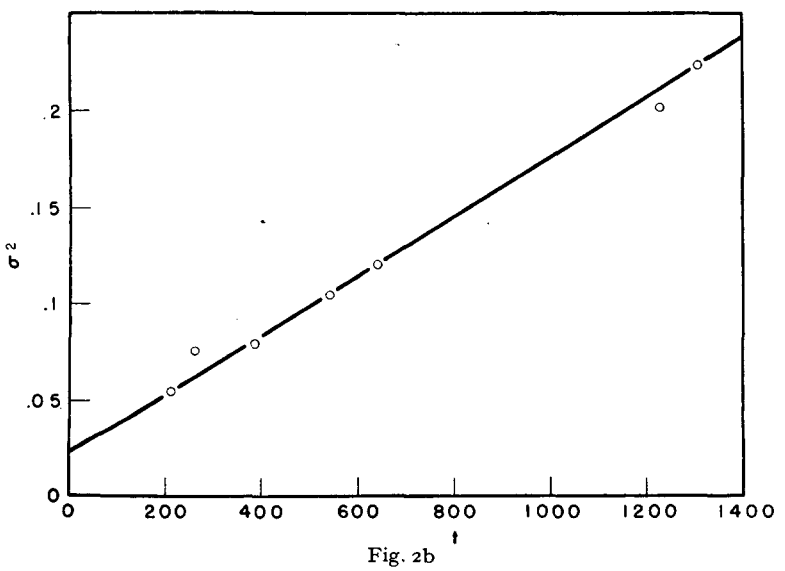

Fig. 2b

Fig. 2. (a) Typical diffusion patterns of the soluble form of laminarin in phosphate or borate buffers, (b) Square of the standard deviation of a typical diffusion curve plotted as a function of time, t. in minutes.

The molecular weights computed by the Svedberg equation from the sedimentation and diffusion coefficients appear in Table III. The standard errors of these weights were computed from the errors applicable to the sedimentation and diffusion coefficients[12], the remaining quantities in the equation, including the partial specific volume, being treated as constants. Molecular weights were also computed from the sedimentation and viscosity coefficients, where the latter were available, using two formulae, the first assuming the validity of the SIMHA-PERRIN equations for ellipsoidal molecules[13,14], and the second assuming $\varnothing^{1/3}/P = 2.5 \cdot 10^6$ in the MANDELKERN AND FLORY equation[15] for a random coil molecule.

TABLE III

MOLECULAR WEIGHT ESTIMATES

Method of computation	*Sample and Solvent*				
	Ld		*Lc*		
	Phosphate	*Borate*	*% Methylation*	*Borate*	*NaCl*
1. From S^0_{20} and D^0_{20} by SVEDBERG	5300 ± 270	6000 ± 310	—	4500 ± 210	—
	—	—	2.7	3700	2900 ± 210
	—	—	6.9	2900 ± 210	2100 ± 330
2. From $[\eta]$ and S^0_{20} by SIMHA-PERRIN equations	—	5300	—	4000	—
3. From $[\eta]$ and S^0_{20} by MANDELKERN AND FLORY $\varnothing^{1/3}/P = 2.5 \cdot 10^6$	—	5000	—	3700	—

The results by the SVEDBERG equation (Table III) indicate a molecular weight of 5,300 for the Ld form in phosphate and a value about 12% higher in borate buffer. The Lc form is actually a smaller molecule having a molecular weight of about 4,500 in the borate buffer. The estimates obtained by the two equations involving the viscosity coefficients are somewhat lower, possibly due to an underestimation of the intrinsic viscosity, but again confirm that the Lc form has the smaller molecule. Methylation evidently decreases the molecular weight of the Lc form in borate and molar sodium chloride, a finding which suggests degradation during methylation. The molecular weights of these methylated samples were from 28 to 38% lower in molar sodium chloride than in borate solvent. While the errors on the samples with extremely low sedimentation coefficients are doubtless high, the use of borate evidently causes aggregation or complex formation in the Lc as well as in the Ld form of laminarin. The best available information from the present data suggests a molecular weight of about 5,300 for the Ld form in phosphate and 3,500 for the unmethylated Lc form in a non-borate solvent. The latter value is subject to a large and uncertain error since it is estimated from results with the methylated samples.

Polydispersity

If the material is polydisperse the above values will approximate weight average molecular weights. Polydispersity was first suggested by the discrepancy between the

References p. 144.

results obtained with the ordinary and synthetic boundary ultracentrifuge cells and this impression received confirmation from the results of the diffusion measurements.

To examine this possibility more rigorously, the boundaries observed in the synthetic boundary cell were enlarged and the standard deviation or spreading estimated by means of an Amsler mechanical integrator. The results obtained for this extremely small molecule were not considered adequate for estimating molecular weight distribution[16] but were useful for an analysis of a qualitative type. The spreading ($\sigma^2_{obs.}$) observed in the ultracentrifuge results from both diffusion (σ^2_D) and size heterogeneity (σ^2_{SH}), and these quantities are related according to the equation:

$$\sigma^2_{obs.} = \sigma^2_D + \sigma^2_{SH}$$

Since σ^2_D is known from the diffusion coefficient, it can be compared with the observed spreading in the ultracentrifuge. In Fig. 3, $\sigma^2_{obs.}$ and σ^2_D (broken line) are plotted against the time in seconds. No corrections were applied for position etc., as they were small and in a direction which increased the already obvious separation from σ^2_D. Different sets of determinations gave somewhat different intercepts; these have been corrected to a common origin since only the slope is of interest here. As the spreading observed in the ultracentrifuge ranges from three to five times that attributable to diffusion, the heterogeneity in the sedimentation rates is obviously significant. The differences in the spreading rates observed for the different samples in the several solvents may not exceed experimental variability for all comparisons. However, the borate solvents appear to decrease spreading and polydispersity. The differential spreading of the solutes cannot be separated from the effect of solvent in these experiments, but it appears that polydispersity was maximal in the methylated samples. These observations appear to be reasonable since mild degradation during methylation could increase, and complex formation in the borate buffer might decrease, polydispersity, if the mechanism of complex formation resulted in preferential association of smaller laminarin units.

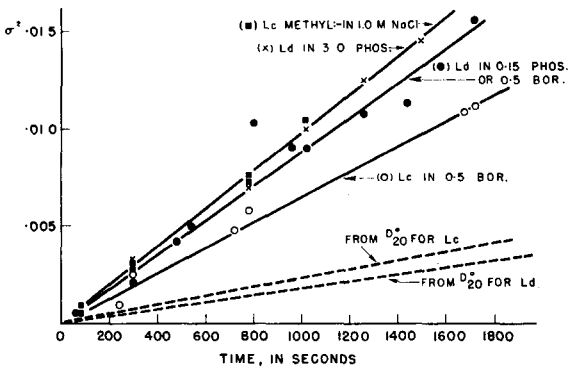

Fig. 3. Boundary spreading (σ^2) during sedimentation compared with that predicted from diffusion alone (broken line).

Shape estimates

Although the reported measurements permit the computation of such shape parameters as frictional ratio, β values[17], and axial ratios, the significance of these estimates is limited by the evident aggregation in the borate solvents, polydispersity, and the experimental errors applicable to measurements on such a small molecule. Such computations also assume an ellipsoidal model instead of a random coil and laminarin probably conforms more closely to the latter. For these short chains, however, the ellipsoidal model is not an unreasonable approximation and it is of interest to examine briefly the effect of aggregation on shape.

The frictional ratios obtained from sedimentation and diffusion were 1.90 for the

References p. 144.

Ld form in phosphate and 1.75 in borate. These correspond to axial ratios of 10.2 and 8 respectively assuming 50% hydration. Although the absolute value of the figures is doubtful, their relative magnitude indicates that the aggregation occurring in the borate solvent produces a more centro-symmetric shape.

Frictional ratios from sedimentation and diffusion were the same within experimental error for both the Ld and Lc forms in borate solvents. The axial ratios of the two forms computed from the intrinsic viscosity (SIMHA) were also the same for the two forms but somewhat lower than those indicated by sedimentation-diffusion. The β coefficients of SCHERAGA AND MANDELKERN for the Ld and Lc forms were 2.22 and 2.19 respectively, corresponding to axial ratios of 4.6 and 3.8. These small differences may be indicative but do not establish a more centro-symmetric shape for the less soluble form.

DISCUSSION

These results show that the difference in solubility of the two forms of laminarin cannot be explained by polymer size. For the samples tested the more soluble form actually had a larger molecule, *i.e.* about 32 glucose units, while the less soluble form had about 21. Should the partial specific volume of either or both forms differ significantly from the 0.60 value assumed, these size estimates would be altered accordingly. The differential solubility must therefore arise from structural differences and some recent evidence[18] indicates that the less soluble form may be branched. This is of interest in view of a markedly similar situation in amylose and amylopectin[19].

It has also been shown that in spite of the small size of the molecule, both forms of laminarin are polydisperse, although no information was obtained on the molecular weight distribution. In consequence the values reported are approximate weight average molecular weights. In such polydisperse materials, the average size of each form may depend on the source of the material and the method of preparation. Thus it is possible that the lower molecular weight observed for the less soluble form is an artefact depending on the preparative procedure. Since the soluble form is usually precipitated by the addition of a non-solvent (alcohol), it is more likely that light fractions would be lost from such a precipitation than from the less soluble form. If fractionation of this sort occurred, it would raise the apparent molecular weight of the soluble form.

The presence of borate in the solvent appears to cause some form of aggregation in laminarin since the molecular weights observed in such solvents were always higher than in non-borate solvents. Some residual aggregation may have remained in all the solvents used but this is believed to be inconsequential in the better solvents. Methylation increases the solubility of the less soluble form but the results indicate that increasing levels of methylation decrease polymer size. While this seems reasonable, the obvious polydispersity precludes a definite conclusion. The methylated samples were obtained from preparations that may have differed in their original molecular weight from those used to represent non-methylated material in this investigation. Since the small mean size of the molecule must limit the molecular weight distribution, the consistency of the results suggests that methylation does cause some degradation.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. W. A. P. BLACK of the Institute of Seaweed Research, Inveresk, Scotland, who kindly provided samples of laminarin

References p. 144.

and criticized the manuscript. They are also grateful to Dr. A. G. Ross, Chemistry Department, University of Edinburgh, for samples of methylated laminarin. The technical assistance of Mr. D. R. MUIRHEAD is gratefully acknowledged.

SUMMARY

From sedimentation, diffusion and viscosity measurements, the molecular weight of soluble laminarin from *L. digitata* was estimated as approximately 5,300 while that of the insoluble form from *L. cloustoni* was about 3,500. Both forms of laminarin are polydisperse. Mild methylation of the laminarin from *L. cloustoni* decreased the molecular weight and increased polydispersity. The difference in weight average molecular weights between the two forms does not explain their difference in solubility.

RÉSUMÉ

Au moyen de mesures de sedimentation, de diffusion, et de viscosité on a estimé le poids moléculaire du laminarin soluble émanant de *L. digitata* à 5,300, et celui du laminarin insoluble émanant de *L. cloustoni* à 3,500. Tous deux sont polydispersés. La méthylation douce du laminarin émanant de *L. cloustoni* a diminué le poids moléculaire et a augmenté la polydispersité. La différence des poids moléculaires des deux types dérivés des moyennes des poids n'explique pas la différence de solubilité.

ZUSAMMENFASSUNG

Aus Sedimentation-, Diffusion- und Viskositätsmessungen wurde das Molekulargewicht des lösbaren aus *L. digitata* herstammenden Laminarins mit etwa 5,300, de sunlösbaren aus *L. cloustoni* herstammenden mit etwa 3,500 abgeschätzt. Die beiden Laminarintypen sind polydispers. Beim gelinden Methylieren des Laminarins aus *L. cloustoni* nahm das Molekulargewicht ab, die Polydispersität dagegen zu. Die Verschiedenheit der beiden Typen an Gewichtsdurchschnitts-Molekulargewicht stellt keine Aufklärung der verschiedenen Lösbarkeit dar.

REFERENCES

[1] V. C. BARRY, *Sci. Proc. R. Dublin Soc.*, 22 (1939) 59.
[2] J. J. CONNELL, E. L. HIRST AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1950) 3494.
[3] E. G. V. PERCIVAL AND A. G. ROSS, *J. Chem. Soc.*, (1951) 720.
[4] W. A. P. BLACK, W. J. CORNHILL, E. T. DEWAR AND F. N. WOODWARD, *J. Appl. Chem.*, 1 (1951) 505–517.
[5] J. BÖESEKEN, *Advances in Carbohydrate Chemistry*, IV (1949) 189–210, ed. W. W. PIGMAN AND M. L. WOLFROM, Acad. Press Inc., N.Y.
[6] W. A. P. BLACK, Personal communication (1952) quoting: —
(a) *Ber.*, 40 (1907) 2033.
(b) FLASCHENTRÄGER, *Z. Physiol. Chem.*, 146 (1925) 219.
(c) PREZL-GRANT, *Quantitative Organic Microanalysis*, 4th Ed. (1945).
[7] E. G. PICKELS, W. F. HARRINGTON AND H. K. SCHACHMAN, *Proc. Natl. Acad. Sci. U.S.A.*, 38, No. 11 (1952) 943.
[8] S. CLAESSON, *Nature*, 158 (1946) 843.
[9] T. SVEDBERG AND K. PEDERSEN, *The Ultracentrifuge*, Oxford (1940).
[10] C. O. BECKMANN AND Q. LANDIS, *J. Am. Chem. Soc.*, 61 (1939) 1495.
[11] N. GRALÉN, Inaugural Dissertation, Upsala, (1944) 84–88.
[12] O. L. DAVIS, *Statistical Methods in Research and Production*, Oliver and Boyd (1947).
[13] R. SIMHA, *J. Phys. Chem.*, 44 (1940) 25.
[14] F. PERRIN, *J. Phys. Radium*, 7 (1936) 1.
[15] L. MANDELKERN AND P. J. FLORY, *J. Chem. Phys.*, 20, No. 2 (1952) 212.
[16] J. W. WILLIAMS, R. L. BALDWIN, W. M. SAUNDERS AND R. G. SQUIRE, *J. Am. Chem. Soc.*, 74 (1952) 1542.
[17] H. A. SCHERAGA AND L. MANDELKERN, *J. Am. Chem. Soc.*, 75 (1953) 179.
[18] S. PEAT, W. J. WHELAN AND H. G. LAWLEY, 318th Meeting of the Biochemical Soc., May 16 (1953) 7.
[19] H. GILMAN, (ed.), *Organic Chem.* IV, ed. 4 (1953) 910, John Wiley and Sons, N.Y.

Received December 8th, 1953