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The Sedimentation and Diffusion of Polysaccharides from *Penicillium luteum*; Interpretation of the Results Obtained from Polydisperse Material in the Gouy Diffusiometer

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Samples of the dextro- and laevo-rotatory polysaccharides, prepared from *Penicillium luteum* by Freeman & Macpherson (1949), were submitted for the examination of their ultracentrifugal sedimentation and diffusion.

EXPERIMENTAL

The solid materials were dissolved in, and thoroughly dialysed against, buffer of composition: NaCl, 0.2M; KH_2PO_4 , 0.027M; Na_2HPO_4 , 0.027M. The final concentrations were about 1 g./100 ml. and were estimated by refractometry against the buffer.

Fig. 1. Sedimentation diagrams: (a) dextrorotatory polysaccharide 37 min. after reaching full speed; (b) laevorotatory polysaccharide 80 min. after reaching full speed (60,000 rev./min.).

Sedimentation measurements. Sedimentation was observed in a Svedberg oil-turbine ultracentrifuge by the method of Philpot (1938), using the standard conditions of running recommended by Cecil & Ogston (1948).

Both samples proved to be polydisperse. The dextro-rotatory material (Fig. 1a) was composed of two main fractions: a more slowly sedimenting fraction which appeared to be homogeneous and a more rapidly sedimenting fraction which was heterogeneous. The sedimentation constant of the faster material and the relative proportions of the two fractions could not be determined accurately because the boundaries were not clearly resolved. However, separation of the schlieren curve into two more or less symmetrical parts (Fig. 2) gave an approximate estimate of the amounts of the two components. The combined boundary represented only 0.86 of the total refracting material.

The laevorotatory material (Fig. 1b) gave a single boundary, the thickness of which showed that the polysaccharide was polydisperse; it consisted of material sedimenting over a range of rates, symmetrically distributed about a mean value. Integration of this boundary showed that it repre-

Fig. 2. Tracing from sedimentation diagram (full line) of dextrorotatory polysaccharide, 47 min. after reaching full speed (60,000 rev./min.), to show analysis into components (broken lines); gradient of concentration dc/dx against position in cell, x .

sented only 0.64 of the total refracting material; however, this value may be too low, because the lower limit of the boundary appeared to be reaching the bottom of the cell before its upper limit had fully left the meniscus.

The results are given in Table 1.

Diffusion measurements. Diffusion was measured by means of the Gouy diffusiometer (Coulson, Cox, Ogston & Philpot, 1948). Ogston (1949) has shown that this method can be used to determine the diffusion constants of a mixture of two

Table 1. *Data obtained from ultracentrifugal sedimentation*

Material	Component	Fraction of total refracting material in ultracentrifuge diagram	Proportion of component in diagram	$\bar{S}_{20(\text{corr.})} \times 10^{13}$
Dextrorotatory	(1) Monodisperse	0.86	0.43	4.13
	(2) Polydisperse		0.57	5.2 (approx.)
Laevorotatory	Polydisperse	0.64	—	1.79

Table 2. *Data obtained from diffusion*

Material	Component	Proportion of component	$D_{20(\text{corr.})} \times 10^7$	Approx. mol. wt.
Dextrorotatory	(1) Monodisperse	0.27	5.5	50,000
	(2) Polydisperse	0.73	1.9	170,000
Laevorotatory	Polydisperse	—	Av. 8.32	15,000

homogeneous components. Provided that one of the components is present only in small amount, the error introduced by its polydispersity into the estimate of the diffusion constant of a homogeneous main component is small; however, if, as in the case of the dextrorotatory polysaccharide, a main component is polydisperse, considerable errors are introduced into the estimates of the diffusion constants of both components and of their proportions. The values quoted in Table 2 are, therefore, only rough and it is not surprising that the proportion of the components estimated from the diffusion data differs from that obtained from the sedimentation diagram (Table 1).

Any attempt to analyse the diffusion data given by a highly polydisperse material, such as the laevorotatory polysaccharide, in terms of two homogeneous components, is of little value and could do no more than indicate the range of polydispersity. It would be more useful to estimate the mean diffusion constant, for comparison with the mean sedimentation constant. However, the mean diffusion constant which is obtained from the movement of the outermost interference band by the Gouy method is not the weighted mean diffusion constant, but is given by

$$\frac{1}{\sqrt{D}} = S \frac{\alpha_i}{\sqrt{D_i}},$$

where α_i is the fraction of material having diffusion constant D_i . Use of this value, together with the mean sedimentation constant, to calculate the molecular weight would yield a rather curious mean value. A method has therefore been found, and is described below, for obtaining the arithmetic-mean diffusion constant from the Gouy data. The result of this calculation is given in Table 2.

THEORETICAL

Method of obtaining an arithmetic-mean diffusion constant by the Gouy method

Where the diffusion boundary is made up of a range of superimposed Gaussian boundaries, if α_i is the fraction of the total refractive increment due to each component i having diffusion constant D_i and if v is the total refractive increment (expressed as numbers of wavelengths of phase difference), then the phase difference r of light passing through the

boundary at distances $+x$ and $-x$ from its centre and the angular deflexion θ_r of such light at time t are given by

$$r = \frac{2v}{\sqrt{\pi}} S \{ \alpha_i f(z_i) \},$$

where

$$f(z_i) = \int_0^{z_i} e^{-z^2} dz - z_i e^{-z_i^2} \quad \text{and} \quad z_i = x/\sqrt{(4D_i t)};$$

and

$$\theta_r = \lambda S \left\{ \frac{\alpha_i}{\sqrt{(4\pi D_i t)}} e^{-z_i^2} \right\}.$$

It follows that

$$\left(\frac{\partial r}{\partial \theta_r} \right)_t = -2x/\lambda.$$

Now

$$\begin{aligned} \bar{D}t &= S(\alpha_i D_i) t = \frac{1}{2} x^2 \\ &= \int_0^\infty \left(\frac{\lambda}{2} \frac{\partial r}{\partial \theta_r} \right)^2 \theta_r dx \bigg/ \int_0^\infty \theta_r dx \\ &= \frac{\lambda^2}{24v} \int_0^\infty \theta_r \cdot d \left\{ \frac{\partial r}{\partial \theta_r} \right\}^2. \end{aligned} \quad (1)$$

This quantity can be approximately computed from the Gouy interference pattern by the following procedure. The values of r for the interference minima, from without inwards are $\frac{1}{2}, 1 + \frac{1}{2}, \dots$; the corresponding displacements of the minima from the optic axis, X_r , are measured at a given time t . In addition, the maximal displacement X_{max} , is calculated from X_0/β_0 (Coulson *et al.* 1948; Ogston, 1949), which corresponds to $r=0$. r , X_r , δ_r , δX_r , $\frac{\delta r}{\delta X_r}$, $\left(\frac{\delta r}{\delta X_r} \right)^2$ and $\delta \left(\frac{\delta r}{\delta X_r} \right)^2$ are then tabulated. Since $\theta = X/F$, where F is the focal distance, equation (1) is approximated by

$$\bar{D}t = \frac{F^2 \lambda^2}{24v} S \left\{ X_r \delta \left(\frac{\delta r}{\delta X_r} \right)^2 \right\}.$$

This sum is computed over the whole range of interference bands, including the optic axis where $X=0$ and $r=v$, but omitting the interference minimum next to the optic axis, since its proper value of r is uncertain (Kegeles & Gosting, 1947). By thus computing $\bar{D}t$ at two or more values of t , the value of $\bar{D}$ is obtained.

This method was tested on a record obtained with a nearly homogeneous sample of lactoglobulin. The mean diffusion

constant (in buffer at 20°) obtained from $\frac{1}{\sqrt{D}} = S \frac{\alpha}{\sqrt{D}}$ was 7.15×10^{-7} , while the application of the above method gave $\bar{D} = 6.94$ and 7.02×10^{-7} , from two intervals of time. The mean diffusion constant of a mixed solution of lactoglobulin and sucrose (Ogston, 1949) was found to be 10.82×10^{-7} , the expected value being 10.85×10^{-7} .

DISCUSSION

It is clear from the sedimentation diagrams that both polysaccharides are polydisperse, the dextrorotatory consisting of homogeneous and heterogeneous fractions and the laevorotatory being entirely heterogeneous. In addition, in neither case does all the refracting material appear in the sedimentation boundaries, which indicates that a proportion of the material may sediment too quickly or too slowly or may be too highly polydisperse to contribute to the boundary diagrams. Thus, while the mean sedimentation constants of the material can be regarded as established, in view of the uncertainties and errors discussed above neither the proportions of the components nor their diffusion constants should be regarded as more than rough estimates, and the same is true of the estimates of molecular weights,

given in Table 2. These were derived from the sedimentation and diffusion constants assuming a value of 0.62 for the partial specific volumes.

The value of the mean diffusion constant of the laevorotatory polysaccharide, calculated by the method described, would give a reliable estimate of its mean molecular weight if it were certain that the average applied to the same range of material as does the estimate of the sedimentation constant. The fact that only 0.64 of the refracting material appears in the sedimentation boundary shows that this may not be so.

SUMMARY

1. Measurements of the sedimentation and diffusion of two samples of polysaccharide from *Penicillium luteum* are described.

2. A new method is given for analysing the diffusion data obtained with the Gouy diffusimeter, so as to obtain a value of the arithmetic-mean diffusion constant which is comparable with the mean sedimentation constant.

3. Approximate values for the amounts and constants of the components of the polysaccharides have been deduced.

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Metabolic Products of *Trichothecium roseum* Link

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The work described in this paper was carried out independently at Stevenston and the London School of Hygiene and Tropical Medicine. When the separate investigations were communicated to the Biochemical Society (Freeman & Morrison, 1948; Michael, 1948) the results from the two laboratories were found to be so similar that it was decided to present a joint account of the work. This paper deals with the isolation of three crystalline metabolic products of *Trichothecium roseum* which had not hitherto been described. Two of these products which are present mainly in the mycelium, and only in smaller amounts in the culture fluid, were named

rosein I and rosein II, while the name rosein III is proposed for the third compound which has been found only in the culture fluid. The three new products are additional to trichothecin, the antifungal substance which was described by Freeman & Morrison (1949a).

It has been known for many years that fruits attacked by the pink rot caused by *T. roseum* Link contain a bitter principle. The latter was isolated in the form of a crude syrup and its solubility described by Iwanoff (1904). Antagonism between *T. roseum* and certain plant pathogenic fungi was reported by Whetzel (1909), Boning (1933), Koch (1934) and