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Note

Estimating Sedimentation of Glycogen through Linear Concentration Gradient of Glycerol Solution[#]

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A set of tables is presented for predicting the position of a glycogen particle in linear glycerol density gradient centrifugation. Data about the size and sedimentation coefficient are given.

INTRODUCTION

Several gradient formers, including sucrose, Ficoll and caesium chloride, have been used as media for density gradient centrifugation. For sucrose, the mathematical treatment of fractions has been established.[1] We have used glycerol as a medium for density gradient centrifugation because its properties make it convenient for work with polysacharides.

This paper presents tables by means of which the time required for any glycogen particle to move a given distance at a given rate of rotation can be calculated if a glycerol density gradient is used.

The computation has been made under the assumption that the glycerol concentration varies linearly with the distance from the centre of rotation. The rate of sedimentation varies directly with the density difference between particle and solution, and inversely with solution viscosity.

If we think of a glycogen particle moving from one glycerol concentration to another, we can specify that the linear glycerol distribution passes

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through points (r_1, z_1) and (r_2, z_2); generally, the *top* and the *bottom* of the gradient at the meniscus and bottom of the tube, although any two positions in the gradient can be used. Let z_0 be the (extrapolated) value which z would have at $r = 0$. This consideration follows the procedure of McEwen:[1]

$$S_{20,w} \int_{t_1}^{t_2} \omega^2 dt = \int_{z_1}^{z_2} \frac{G(z,T,\rho_p)}{z - z_0} dz \tag{1}$$

A convenient form for computing z_0 is obtained by linear extrapolation:

$$z_0 = \frac{z_1 r_2 - z_2 r_1}{r_2 - r_1}$$

Generally, z_0 will have a negative value but in cases of shallow gradients and small radii it can be positive.

EXPERIMENTAL

Glycogen was isolated from livers of twelve Wistar rats by a procedure using 45% phenol[2] and then purified by Sevag's procedure using a chloroform-octanol mixture. All particles above 30 s were sedimented at 300,000 × g for two hours in a preparatory rotor with $k = 61$ ($k = (\ln r_{\max} - \ln r_{\min})/\omega^2$) and lighter particles were discarded. After precipitation of glycogen from aqueous solution by ethanol addition, glycogen was dissolved in water and layered over a 10–30 per cent glycerol gradient. Centrifugation lasted 23 minutes at 20,000 rpm in a 3 × 35 ml rotor of a Janetzky MLW ultracentrifuge VAC 602. Fractions of polydispersed glycogen were isolated by pipetting fraction solutions in glycerol into ethanol at –20 °C with some LiCl added. The concentration of glycerol was assessed using Abbe's refractometer.

Eleven fractions were isolated, the heaviest being the sediment at the bottom of the tube. Literature data were used for glycogen partial specific volume ($v_B = 0.63 \times 10^{-3}$ m^3/kg), glycogen density ($\rho = 1630$ kg/m^3) and diffusion coefficients as cited by Geddes.[3]

RESULTS AND DISCUSSION

Table I represents the numerical integration shown in Eq. (1) by Simpson's rule using concentration intervals of 1% for glycogen centrifugation in glycerol density gradient at 5 °C for different rotor geometries (z_0). Numerical value for the right hand side in Eq. (1) is obtained as a difference of the table entries for z_2 and z_1.

By using data from this Table, a glycerol gradient can be used in any swing-out rotor to estimate the sedimentation of glycogen through the gradient.

TABLE I

Values of time integral for glycerol gradient centrifugation at a temperature of 5° and particle density ρ = 1630 kg/m^3

wt. % glycerol	$z_0 = 5.0$	$z_0 = 0.0$	$z_0 = -5.0$	$z_0 = -10.0$	$z_0 = -15.0$	$z_0 = -20.0$	$z_0 = -25.0$	$z_0 = -30.0$	$z_0 = -40.0$	$z_0 = -60.0$	$z_0 = -100$
0.0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2.0	0.0000	0.0000	0.5244	0.2844	0.1953	0.1487	0.1201	0.1007	0.0762	0.0512	0.0309
4.0	0.0000	1.1615	0.9459	0.5431	0.3820	0.2948	0.2401	0.2025	0.1543	0.1045	0.0635
6.0	0.0000	1.8921	1.3079	0.7841	0.5627	0.4393	0.3605	0.3057	0.2345	0.1601	0.0979
8.0	2.1410	2.4495	1.6318	1.0125	0.7391	0.5831	0.4818	0.4106	0.3171	0.2180	0.1342
10.0	3.2034	2.9141	1.9299	1.2320	0.9129	0.7269	0.6044	0.5175	0.4022	0.2784	0.1725
12.0	3.9557	3.3221	2.2101	1.4454	1.0852	0.8714	0.7289	0.6268	0.4901	0.3415	0.2128
14.0	4.5598	3.6929	2.4777	1.6548	1.2572	1.0173	0.8556	0.7388	0.5809	0.4075	0.2554
16.0	5.0786	4.0383	2.7366	1.8619	1.4297	1.1652	0.9850	0.8538	0.6750	0.4765	0.3004
18.0	5.5434	4.3661	2.9898	2.0682	1.6038	1.3157	1.1176	0.9723	0.7727	0.5488	0.3480
20.0	5.9725	4.6820	3.2399	2.2751	1.7803	1.4696	1.2540	1.0948	0.8744	0.6248	0.3985
22.0	6.3773	4.9904	3.4889	2.4840	1.9601	1.6257	1.3947	1.2217	0.9806	0.7047	0.4520
24.0	6.7662	5.2946	3.7388	2.6960	2.1442	1.7902	1.5405	1.3537	1.0916	0.7890	0.5089
26.0	7.1453	5.5979	3.9915	2.9126	2.3337	1.9586	1.6921	1.4915	1.2082	0.8782	0.5695
28.0	7.5196	5.9029	4.2488	3.1351	2.5298	2.1338	1.8504	1.6360	1.3311	0.9728	0.6343
30.0	7.8936	6.2124	4.5128	3.3652	2.7338	2.3170	2.0166	1.7881	1.4612	1.0737	0.7039
32.0	8.2714	6.5292	4.7856	3.6048	2.9473	2.5096	2.1920	1.9491	1.5995	1.1816	0.7789
34.0	8.6569	6.8562	5.0696	3.8558	3.1721	2.7132	2.3781	2.1204	1.7474	1.2977	0.8601
36.0	9.0544	7.1970	5.3678	4.1208	3.4107	2.9301	2.5769	2.3039	1.9064	1.4232	0.9484
38.0	9.4688	7.5554	5.6835	4.4030	3.6657	3.1627	2.7908	2.5019	2.0786	1.5600	1.0452
40.0	8.9055	7.9361	6.0209	4.7060	3.9406	3.4144	3.0228	2.7170	2.2666	1.7099	1.1520
42.0	10.3710	8.3449	6.3853	5.0346	4.2399	3.6891	3.2767	2.9531	2.4735	1.8759	1.2709
44.0	10.8736	8.7890	6.7832	5.3949	4.5692	3.9923	3.5576	3.2148	2.7037	2.0613	1.4045
46.0	11.4236	9.2779	7.2232	5.7950	4.9359	4.3308	3.8719	3.5081	2.9625	2.2709	1.5563
48.0	12.0345	9.8238	7.7166	6.2451	5.3498	4.7138	4.2283	3.8414	3.2575	2.5108	1.7309
50.0	12.7244	10.4433	8.2788	6.7597	5.8241	5.1538	4.6386	4.2257	3.5986	2.7893	1.9347

TABLE II

Molecular weights and radii of particles of glycogen fractions isolated at a temperature of 5 °C, $\rho = 1630$ kg/m^3 and $z_0 = -0.405$.

Fraction	Distance from the axis (cm)	Middle distance of fraction (cm)	wt. % glycerol	Integral	s_B ($\times 10^{-13}$s)	D_B ($\times 10^4$m^2/s)	M_B (B) ($\times 10^6$)	M_B(S) ($\times 10^6$)	r (B) (nm)	r (S) (nm)
0	5.2		10.0	5.6042						
1	5.9	5.5	11.7	5.9386	477	5.54	52	54	23	24
2	6.5	6.2	12.0	5.9976	561	5.14	66	68	25	26
3	7.2	6.9	12.7	6.1235	740	4.26	101	108	29	30
4	7.8	7.5	13.3	6.2313	894	3.64	134	154	32	34
5	8.5	8.2	14.5	6.4413	1194	3.18	206	234	37	39
6	9.2	8.8	17.5	6.9336	1896	2.59	413	457	47	48
7	9.8	9.5	21.5	7.5499	2775	2.38	731	723	57	57
8	10.5	10.2	25.0	8.0739	3522	2.19	1045	1006	64	63
9	11.1	10.8	26.8	8.3430	3907	2.30	1221	1061	67	64
10	11.8	11.5	28.3	8.5691	4229	2.45	1375	1078	70	64
11		12.1	28.6	8.6149	4295	2.48	1407	1081	70	65
	12.5									

Integral values for $z_0 = -0.405$ (our rotor 3 × 35 ml) in Eq. (1) determine s_B. Average molecular weights of the fractions can be calculated by using the Svedberg formula:[4]

$$M_B(S) = \frac{RT\, s_B}{D_B(1-\nu_B\rho)}$$

and Brammer's formula:[5]

$$M_B(B) = 5\sqrt{10} \cdot 10^{22} \cdot s_B{}^{3/2}$$

Effective radii of spherical glycogen particles were calculated by Stokes' formula:[6]

$$r = \left(\frac{3M_B\nu_B}{4\pi N}\right)^{1/3}$$

for both $M_B(B)$ and $M_B(S)$.

In Table II, the results of experiments and calculations for glycogen preparations centrifuged through a linear glycerol gradient are presented.

The tables can be used to predict the position of material of a given sedimentation coefficient and density, or, alternatively, the sedimentation coefficient can be calculated from the measured position of the fraction.

REFERENCES

1. C. R. McEwen, *Anal. Biochem.* **20** (1967) 114–149.
2. R. Geddes and G. C. Stratton, *Biochem. J.* **163** (1977) 193–200.
3. R. Geddes, J. D. Harvey. and P. R. Wills, *Biochem. J.* **163** (1977) 201–209.
4. R. Geddes, Glycogen: A Structural Viewpoint, in: G. O. Aspinall, *The Polysacharides*, Academic Press, 1983, vol. 3, pp. 283–336.
5. G. L.Brammer, M. A. Rougvie, and D. French, *Carbohydr. Res.* **24** (1972) 343–354.
6. E. A. Dawes, *Quantitative Problems in Biochemistry*, 5th Edition, Churchill Livingstone, Edinburgh & London, 1972, p. 32.

SAŽETAK

Određivanje sedimentacije glikogena u linearnom gradijentu koncentracije otopine glicerola

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Dane su tablice za proračun vremena potrebnog da pri centrifugiranju čestica glikogena proputuje kroz linearni gradijent koncentracije glicerola od jedne zone do druge. Za svaku česticu daju se podaci o njezinoj veličini i sedimentacijskom koeficijentu.