

On the Determination of Polydispersity from Ultracentrifugal Sedimentation

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In the physico-chemical characterization of polydisperse, high molecular compounds it is very important to get information about the degree of heterogeneity. If an ultracentrifugal sedimentation* of a polydisperse substance is performed, a separation of the components always occurs because molecules of different sizes have different sedimentation velocities. Sedimentation in the ultracentrifuge therefore is a very convenient way to get the required information. Gralén¹ using this method as a measure of the polydispersity, determined the width (B) of the sedimentation curve (obtained by means of the scale method according to Lamm²) as the quotient between the area (A) and the maximum height (H), *i. e.*

$$B = \frac{A}{H} \quad (1)$$

If the molecular weight of the particles is not too small, the diffusion in case of substances with threadlike molecules can be neglected. Hence it can be assumed, that the variation in width of the curve is due only to the polymolecularity of the substance. As the large molecules always sediment more rapidly than the small ones, the width B will increase with time and have different values at different distances (x) from the centre of rotation. In this way Gralén has studied the derivative dB/dx and as the final measure of the polydispersity introduced dB/dx extrapolated to zero concentration.

Gralén assumes that B varies linearly with x , and points out that this is evident from the experiments. The purpose of this study is to discuss this

* For details about the sedimentation *cf.* T. Svedberg and K. Pedersen, *The Ultracentrifuge* Oxford (1940); subsequently referred to as UC.

relationship more thoroughly, and to show a way in which dB/dx can be calculated in order to get a close relationship between the value, B , and quantities which characterize the frequency-distribution function of the substance. The discussions have hitherto been limited to the case where the sedimentation is not dependent upon the concentration of the solution.

There are mainly three factors which may have an influence on the calculation of dB/dx and for which corrections have to be introduced. These factors arise from the changes occurring in the curve during the course of the sedimentation. Firstly, the width of the sedimentation curve is not only determined from the different sizes of the molecules but also from the increase in the centrifugal field with the distance from the centre of rotation. The width of the curve is larger than what would be expected from the polydispersity. Secondly, the value of dB/dx may change, depending upon whether the calculation is carried out in the originally plotted sedimentation diagram or whether it is made in a diagram where a correction has been made for the dilution due to the sector shape of the centrifuge cell. Thirdly, dB/dx may be affected by the characteristic displacement of the maximum point of the sedimentation curve of polydisperse substances (Kinell³).

In order to study the relation between B and the distance x , we assume that the substance is characterized by the following frequency-distribution function for the sedimentation constants (s)

$$\frac{dc_0}{ds} = \begin{cases} f(s) & \text{for } s_1 \leq s \leq s_2 \\ 0 & \text{for } s < s_1 \text{ and } s > s_2 \end{cases} \quad (2)$$

where c_0 is the analytical concentration. Further we assume that this function has a single maximum for $s = \bar{s}$. We get the following sedimentation curve:

$$\frac{dc}{dx} = \begin{cases} \varphi(x) & \text{for } x_1 \leq x \leq x_2 \\ 0 & \text{for } x_0 \leq x < x_1 \text{ and } x > x_2 \end{cases} \quad (3)$$

or correcting for the sector shape of the cell:

$$\frac{dc_0}{dx} = \begin{cases} \psi(x) & \text{for } x_1 \leq x \leq x_2 \\ 0 & \text{for } x_0 \leq x < x_1 \text{ and } x > x_2 \end{cases} \quad (4)$$

These curves too have only one maximum, for x_m and x'_m respectively. Between dc/dx and dc_0/dx the following relation is valid (cf. Rinde⁴):

$$\frac{dc_0}{dx} = \frac{dc}{dx} \left(\frac{x}{x_0} \right)^2 \quad (5)$$

For the transformation of the dc_0/ds -curve to the dc/dx - and dc_0/dx -curves respectively, the following equations have to be used:

$$x = x_0 e^{\omega^2 s t} \quad (6)$$

$$\frac{dc}{dx} = \frac{dc_0}{ds} \frac{e^{-3\omega^2 s t}}{x_0 \omega^2 t} \quad (7a)$$

$$\frac{dc_0}{dx} = \frac{dc_0}{ds} \frac{e^{-\omega^2 s t}}{x_0 \omega^2 t} \quad (7b)$$

where x_0 is the position of the meniscus in the cell and ω is the angular velocity of the rotor.

From equ. (7) it is easy to obtain the following equations, which determine the s -values corresponding to the maximum points of the dc/dx - and dc_0/dx -curves (*cf.* Kinell *l. c.*)

$$\frac{df(s)}{ds} - 3\omega^2 t f(s) = 0 \quad (8a)$$

$$\frac{df(s)}{ds} - \omega^2 t f(s) = 0 \quad (8b)$$

The solution of these equations gives $s_m(t)$ and $s'_m = s'_m(t)$. It is easily seen, that these functions satisfy the relation

$$s_m(t) \leq s'_m(t) \leq \bar{s} \quad (9)$$

where the equality sign has to be used for $t = 0$. The displacement of the maximum point is therefore largest for the original sedimentation curve.

The calculation of dB/dx from the original and corrected sedimentation curves is now possible by means of equ.s (1), (6) and (7). We get the following expressions for B and B_0 as functions of the time:

$$B = \frac{x_2 \int \frac{dc}{dx} dx}{\left(\frac{dc}{dx}\right)_{x=x_m}} = \Phi_1(t) x_0 \omega^2 t \quad (10a)$$

$$B_0 = \frac{\int_{x_1}^{x_2} \frac{dc_0}{dx} dx}{\left(\frac{dc_0}{dx}\right)_{x=x'_m}} = \Phi_2(t) x_0 \omega^2 t \quad (10b)$$

where

$$\Phi_1(t) = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds} e^{-2\omega^2 s t} ds}{\left(\frac{dc_0}{ds}\right)_{s=s_m}} e^{3\omega^2 s_m t} \quad (11a)$$

$$\Phi_2(t) = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds} ds}{\left(\frac{dc_0}{ds}\right)_{s=s'_m}} e^{\omega^2 s'_m t} \quad (11b)$$

By differentiating with respect to x_m and x'_m and observing that s_m and s'_m are functions of the time, we get

$$\frac{dB}{dx_m} = \Phi_1(t) \frac{\left[1 + t \frac{d \ln \Phi_1(t)}{dt}\right] e^{-\omega^2 s_m t}}{s_m \left[1 + \frac{t}{s_m} \frac{ds_m}{dt}\right]} \quad (12a)$$

$$\frac{dB_0}{dx'_m} = \Phi_2(t) \frac{\left[1 + t \frac{d \ln \Phi_2(t)}{dt}\right] e^{-\omega^2 s'_m t}}{s'_m \left[1 + \frac{t}{s'_m} \frac{ds'_m}{dt}\right]} \quad (12b)$$

These expressions are evidently dependent on the time t and hence dB/dx generally cannot be a linear function of x . It also follows, that dB/dx does not give any simple characterization of the frequency-distribution curve. However, if we regard the case $t = 0$, we have

$$\left(\frac{dB}{dx_m}\right)_{t=0} = \frac{\Phi_1(0)}{s} \quad (13a)$$

$$\left(\frac{dB_0}{dx'_m}\right)_{t=0} = \frac{\Phi_2(0)}{s} \quad (13b)$$

Now according to equ.s (11) and (9)

$$\Phi_1(0) = \Phi_2(0) = \frac{\int_{\bar{s}}^{s_2} \frac{dc_0}{ds} ds}{\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}}$$

and hence

$$\left(\frac{dB}{dx_m}\right)_{t=0} = \left(\frac{dB_0}{dx'_m}\right)_{t=0} = \frac{\int_{\bar{s}}^{s_2} \frac{dc_0}{ds} ds}{\bar{s} \left(\frac{dc_0}{ds}\right)_{s=\bar{s}}} \quad (14)$$

If an extrapolation is made to the moment when the sedimentation starts, we obviously get a quantity, which in a very simple manner is related to the frequency-distribution function. This expression is already given by Gralén, but from the treatment here, it is evident that it is valid only at the time $t = 0$, and further, that the same expression is obtained independently, whether or not the correction for the dilution with the sector shape of the cell has been made.

In order to understand thoroughly the deviations from the linear relationship between B and x , it would be necessary to give an involved discussion of equ. (10). This is however rather laborious and not necessary for the practical calculations as will be shown later. It is only of interest to point out that the following expression is obtained by expansion of equ. (12a) in a power serie of the time t :

$$\left(\frac{dB}{dx_m}\right) / \left(\frac{dB}{dx_m}\right)_{t=0} = 1 + \left(5\bar{s} - 4\bar{s}_w - \frac{6f(\bar{s})}{sf''(\bar{s})}\right) \omega^2 t + \dots$$

where \bar{s}_w is the weight average value of s corresponding to the original frequency-distribution function. It is evident that for small values of $\omega^2 t$, the

curve $B(x)$ can be situated either below or above its tangent at the origin depending upon the sign of the expression within the brackets in the right member. This sign depends upon the nature of the frequency-distribution function. For a high polydispersity \bar{s}_w may have such a large value that $\frac{dB}{dx_m} < (\frac{dB}{dx_m})_{t=0}$, and for low polydispersity the difference between \bar{s} and \bar{s}_w may be so small that $\frac{dB}{dx_m} > (\frac{dB}{dx_m})_{t=0}$. For equ. (12b) it is possible to show that $\frac{dB_0}{dx'_m} > (\frac{dB_0}{dx'_m})_{t=0}$ for all values of t and for every frequency-distribution function. The reason for the difference between the two cases is the dilution with the sector shape of the cell. This brief discussion elucidates the necessity of having complete information about all the phenomena occurring during the sedimentation before judging the results obtained by calculating $\frac{dB}{dx}$.

The relation between B and x for a special frequency-distribution function is shown below.

Let us assume, that the substance is characterized by the logarithmic frequency-distribution function given by Gralén (*l. c.*):

$$\frac{dc_0}{ds} = K_s e^{-\frac{1}{\gamma_s^2} \ln^2 \frac{s}{\bar{s}}} \quad (15)$$

where K_s is the maximum height and γ_s is a distribution coefficient. The range for the s -values is $0 \leq s \leq \infty$. If we apply equ.s (8), (10) and (11) we get

$$B = x_0 \left(\frac{x_m}{x_0}\right)^3 + \frac{3}{2} \gamma_s^2 \ln \frac{x_m}{x_0} e^{-\frac{9}{4} \gamma_s^2 \ln^2 \frac{x_m}{x_0}} \cdot I_1 \quad (16a)$$

where

$$I_1 = \int_0^{\infty} e^{-\frac{1}{\gamma_s^2} \ln^2 \frac{s}{\bar{s}} - \frac{2s}{\bar{s}} \left(\frac{x_m}{x_0}\right)^{\frac{3}{2}} \gamma_s^2 \ln \frac{x_m}{x_0}} d\left(\frac{s}{\bar{s}}\right)$$

and

$$B_0 = x_0 \gamma_s \sqrt{\pi} \left(\frac{x'_m}{x_0}\right)^{\frac{1}{2}} + \frac{1}{2} \gamma_s^2 \ln \frac{x'_m}{x_0} e^{-\frac{1}{4} \gamma_s^2 \ln^2 \frac{x'_m}{x_0}} + \frac{1}{4} \gamma_s^2 \quad (16b)$$

For comparison we have the following expressions, if the displacement of the maximum point is neglected:

$$B = x_0 \left(\frac{x_m}{x_0}\right)^3 \ln \frac{x_m}{x_0} \cdot I_2 \quad (17a)$$

where

$$I_2 = \int_0^{\infty} e^{-\frac{1}{\gamma_s^2} \ln^2 \frac{s}{s} - \frac{2s}{s} \ln \frac{x_m}{x_0}} d\left(\frac{s}{s}\right)$$

and

$$B_0 = x_0 \gamma_s \sqrt{\pi} \left(\frac{x'_m}{x_0}\right) \ln \frac{x'_m}{x_0} e^{\frac{1}{4} \gamma_s^2} \quad (17b)$$

The numerical calculations have been made for $5.0 \leq x \leq 6.0$ cm and for $\gamma_s = 0.1, 0.5$ and 1.0 and the results are given in figs. 1 and 2.

Firstly it is seen that the effect of the displacement of the maximum point cannot be neglected when the polydispersity is high. It is also evident that for high values of γ_s , we get curves which deviate rather much from the straight lines corresponding to the tangents of the curves at the origin. An exception is the curve for $\gamma_s = 1$ in fig. 1. Theoretically, at low values of $\omega^2 t$, the change of the slope shall occur for a γ_s -value determined from the equation

$$5 - 4 e^{0.75 \gamma_s^2} + 3 \gamma_s^2 = 0$$

Solving this we get $\gamma_s = 0.919$. Secondly we see that the curvature of the curves is rather low, and it is easy to make serious mistakes about the nature of the curves. When for instance the points *A*, *B*, *C* and *D* (fig. 2) were experimental points, it is consistent with a rather high degree of accuracy to assume that they represent a linear relationship between *B* and *x*. Taking the slope of such a line we will get too high values of dB/dx .

In order to avoid all the difficulties associated with the dB/dx -calculation according to equ. (10), we have to change the method essentially with respect to the displacement of the maximum point. The first quantity we need is an area *A*. For this we choose the area of the corrected sedimentation curve. But since the correction means that this area will be equal to the analytical concentration at every time, it is not necessary to make this correction. We have only to use the analytical concentration (c_0). As Gralén points out, it is obviously correct to assume a constant concentration throughout the experiment. At least this must be the case for a pure substance in a good solvent. If there is any doubt about this, the corrected curve can be traced by means of equ. (5)*. The areas are measured and compared with the analytical

* It is to be noted, that the use of equation $c/c_0 = (x_0/x)^2$ gives a wrong result, because this equation is valid only for a monodisperse substance with a sedimentation, which is not concentration dependent. Using this equation we get

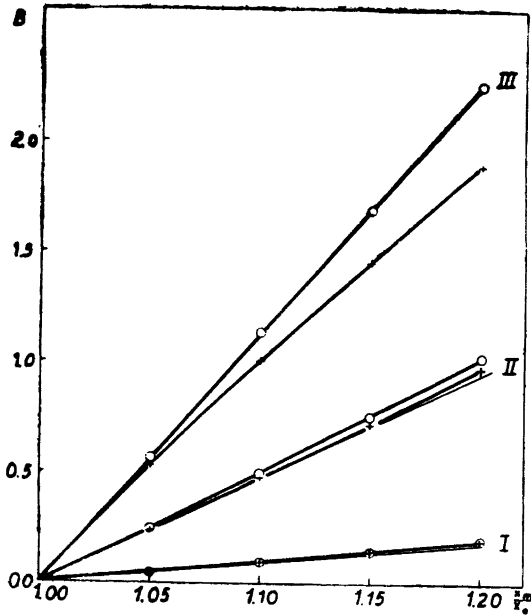


Fig. 1. B as a function of x_m/x_0 for different values of γ_s . I: $\gamma_s = 0.1$; II: $\gamma_s = 0.5$; III: $\gamma_s = 1.0$. \circ values calculated from equ. (16a); $+$ values calculated from equ. (17a). The thin lines are the tangents of the curves at the origin.

concentration. The proper height H to be used must be a quantity corresponding to the maximum height of the frequency-distribution curve. Firstly, it is advantageous from an experimental point of view to take this height from the original sedimentation diagram and secondly, it is necessary to take the height corresponding to a point \bar{x} determined from

$$\bar{x} = x_0 e^{\omega^2 \bar{s} t}. \quad (18)$$

$$\Delta c = \int_{x_1}^{x_2} \frac{dc_0}{dx} dx - \left(\frac{x_m}{x_0}\right)^2 \cdot \int_{x_1}^{x_2} \frac{dc}{dx} dx$$

and if we express this in the frequency-distribution function:

$$\Delta c = \int_{s_1}^{s_2} \frac{dc_0}{ds} \left\{ 1 - e^{-2\omega^2(s - s_m)t} \right\} ds$$

thus Δc is not equal to zero but dependent upon the polydispersity and the position in the cell.

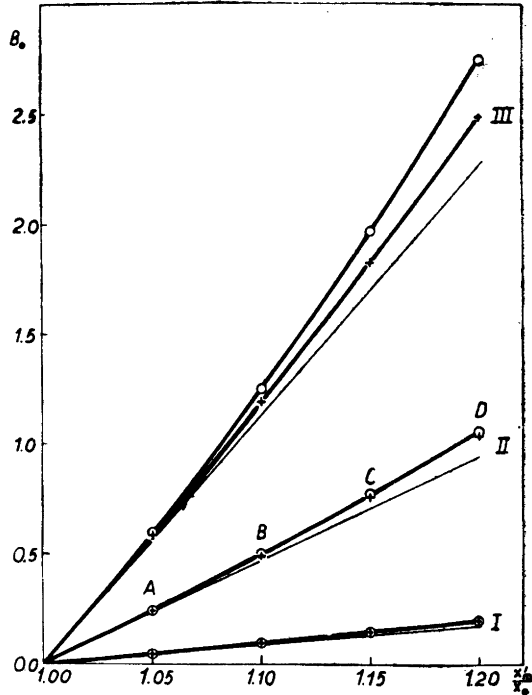


Fig. 2. B_0 as a function of x'_m/x_0 for different values of γ_s . I : $\gamma_s = 0.1$; II : $\gamma_s = 0.5$; III : $\gamma_s = 1.0$. \circ values calculated from equ. (16b); + values calculated from equ. (17b). The thin lines are the tangents of the curves at the origin.

The value \bar{s} can be found if we plott the s -values obtained at different times t in the ordinary way against t and then extrapolate to $t = 0$. Then \bar{x} can be calculated from equ. (18) for different times, and the heights corresponding to these points can be measured in the sedimentation diagram. Thus we get for H :

$$H = \left(\frac{\bar{x}}{x_0}\right)^2 \left(\frac{dc}{dx}\right)_{x=\bar{x}} = \left(\frac{dc_0}{ds}\right)_{s=\bar{s}} \cdot \frac{e^{-\omega^2 \bar{s} t}}{x_0 \omega^2 t} \tag{19}$$

and thus for the width B :

$$B = \frac{c_0}{\left(\frac{\bar{x}}{x_0}\right)^2 \left(\frac{dc}{dx}\right)_{x=\bar{x}}} = \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds} ds}{\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}} x_0 \omega^2 t e^{-\omega^2 \bar{s} t} \tag{20}$$

If we further apply equ. (18), we get as the final expression:

$$\bar{B} = \frac{c_0}{\left(\frac{x}{x_0}\right)^2 \left(\frac{dc}{dx}\right)_{x=\frac{x}{x_0} \ln \frac{x}{x_0}}} = \frac{s_1}{s} \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds} ds}{\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}} \bar{x}, \quad (21)$$

and taking the derivative with respect to \bar{x} :

$$\frac{d\bar{B}}{d\bar{x}} = \frac{s_1}{s} \frac{\int_{s_1}^{s_2} \frac{dc_0}{ds} ds}{\left(\frac{dc_0}{ds}\right)_{s=\bar{s}}}. \quad (22)$$

This expression is independent of \bar{x} , and is in a very simple way related to quantities characterizing the frequency-distribution function. If the displacement of the maximum point can be neglected, this method of calculation mainly corresponds to Gralén's, except that the height is corrected by multiplying with the factor $\ln x/x_0$.

One of the main problems in most of the calculations based on the sedimentation diagrams is to obtain good base lines. The peak in the diagram is often rather good but the horizontal parts of the curve, from which the base line have to be drawn, are in many cases irregular and hence the drawing of this line may be a little arbitrary. Gralén has tried to avoid this difficulty by drawing a base line and adjust its position in such a way that the area of the curve corresponds to the analytical concentration. Of course even this involves some arbitrariness, because the lowest part of the curve has to be constructed as extensions of the original curve. Furthermore, the shape of the curve is not in agreement with that one which would be obtained if the correction were made according to equ. (5). This may have some influence on the height of the curve. The method suggested here does not involve any arbitrariness, but does depend upon the ability to draw a good base line.

It is difficult to say anything about the application of equ. (21) for a substance with concentration dependent sedimentation. The formulas for the correction of the dilution due to the sector shape of the cell etc. involve in this case functions which characterize the concentration dependence. For

concentration dependences, not greatly pronounced, however, equ. (21) may be expected to give useful results.*

In order to show the application of the new method, a calculation of $d\bar{B}/d\bar{x}$ has been carried out on a fraction of commercial polymeric methyl methacrylate. The fractionation was made in the ordinary way by precipitation with cyclohexane from a benzene solution. One of the middle fractions was run in the ultracentrifuge in an acetone solution. The sedimentation was studied by means of the scale method of Lamm (*l. c.*); the following concentrations were used: 0.396, 0.298, 0.198, 0.104 and 0.047 g/100 ml. Lower concentrations could not be used, because they did not give any observable peak.

According to Svedberg⁵ the sedimentation constant at the temperature T and the distance x from the centre of rotation (*cf.* also Kinell, *l. c.****) $s_T(x)$ is given by the expression:

$$s_T(x) = \frac{M[1 - V_T \rho_T(x)]}{f_T(x)} \quad (23)$$

where M is the molecular weight, V_T the partial specific volume of the solute, $\rho_T(x)$ the density of the solvent, and $f_T(x)$ the molar frictional coefficient of the solute at the distance x from the centre of rotation. The dependence on x for these last two quantities has been introduced, because they are dependent on the hydrostatic pressure in the cell. We write $f_T(x) = \eta_T(x) \cdot F(M, V, h)$, where $\eta_T(x)$ is the viscosity of the solvent and F a function which is determined by the size, shape, and solvation of the molecule. In a first approximation, we can assume this function to be independent of small changes in temperature and pressure. We now reduce $s_T(x)$ to a standard condition $T = 20^\circ \text{C}$ and the pressure 1 atm, then we get (*cf.* UC p. 35 and Mosimann and Signer⁶) the following expression:

$$s_T(x) = s_{1,20} \frac{\eta_{20}(x) [1 - V_T \rho_T(x)] \cdot \eta_{20}(x_0) [1 - V_{20} \rho_{20}(x)]}{\eta_T(x) [1 - V_{20} \rho_{20}(x)] \cdot \eta_{20}(x) [1 - V_{20} \rho_{20}(x_0)]}$$

* Gralén's treatment of the sedimentation curves before the $d\bar{B}/d\bar{x}$ -calculation makes it probable that his calculations theoretically should correspond to a case between our cases represented by eqs. (10a) and (10b). A linear relationship is not then excluded with regard to the discussions on p. 340. Furthermore the concentration dependence may have an influence upon the deviations from the linear relationship but a final decision cannot be made before the mathematical calculations for the concentration dependent case have been carried out.

** The dependence of s on the changes of concentration in the cell has been disregarded in the following treatment.

$$= \frac{s_{1,20}}{[1 + \delta(T)] [1 + \varepsilon(x)]} \quad (24)$$

The correction for the temperature is made under the assumption that the pressure is constant. In the equation above $1 + \delta(T)$ may be regarded as independent of the time and the distance x . This is true only as an approximation, but if the centrifuge run has been carried out carefully the variations in the temperature are rather small and serious disturbances can occur only at very low concentrations (*cf.* Jullander⁷ p. 54). The function $1 + \varepsilon(x)$ is at low speeds of rotation a linear function of x and can be written in the form $1 + R(x - x_0)/x_0$. According to Svedberg (*l. c.*) we have

$$s_T(x) = \frac{1}{\omega^2 x} \frac{dx}{dt} \quad (25)$$

and hence we get from equ. (24)

$$\left[1 + \frac{R}{x_0} \cdot (x - x_0)\right] \frac{dx}{x} = \frac{s_{1,20}\omega^2 dt}{1 + \delta(T)} \quad (26)$$

Integrating this and assuming that ω is independent of time we get

$$(1 - R) \ln \frac{x}{x_0} + \frac{R}{x_0} \cdot (x - x_0) = \frac{s_{1,20}\omega^2 t}{1 + \delta(T)} \quad (27)$$

Here we can develop $R \ln x/x_0$ in a power series of $\Delta x/x_0$, where $\Delta x = x - x_0$. In our case, where R has a rather low value, it is sufficient to have only two terms and then we get

$$[1 + \delta(T)] \left[\ln \frac{x}{x_0} + \frac{R}{2} \left(\frac{\Delta x}{x_0}\right)^2 \right] = s_{1,20}\omega^2 t \quad (28)$$

This formula is valid with an accuracy of a few tenth of a percent. If we plott the left member of this equation against $\omega^2 t$, we obtain straight lines if s is not affected by the decrease of concentration with the sector shape of the cell and by the polydispersity.

For the substance investigated here, the speed of the rotor was 30000 r. p. m., the correction for the changes in temperature was less than 2 %, and the correction for the pressure increased to about 5 % for a sedimented distance of about 10 mm. The R -value was calculated to 0.232 for all concen-

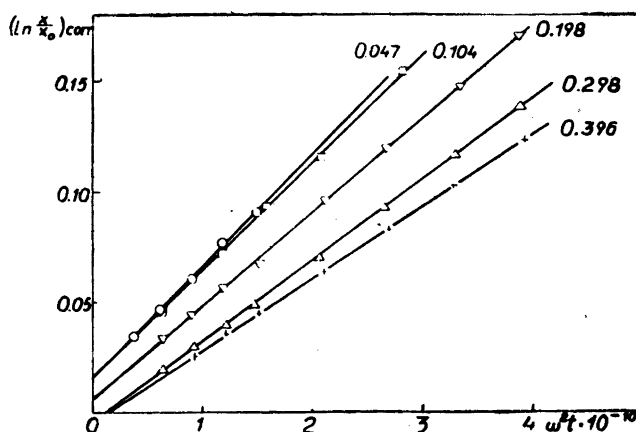


Fig. 3. Diagram according to equ. (28) for different concentrations.

trations used. In fig. 3 the left member of equ. (28) is plotted against $\omega^2 t$. The lines (calculated from the experimental values) do not intersect the $\omega^2 t$ -axis in the origin. The reason for this is the difficulty to determine the exact starting time, because the substance always starts its sedimentation before the rotor has reached its full speed. Further the meniscus can have an effect on the sedimentation during the first moments. The following intersection points have been calculated: $\omega^2 t = + 147.3 \cdot 10^7, + 134.3 \cdot 10^7, - 143.0 \cdot 10^7, - 310.9 \cdot 10^7$ and $- 317.6 \cdot 10^7$. Correcting the $\omega^2 t$ -values used in fig. 3 for these values we can use equ. (28) to calculate the s -values corresponding to each point in the cell. The values obtained follow from table 1 where x is in cm and s in S -units. It is evident that we do not have any changes in the s -values

Table 1. Values of $s_{1,20}$ for different concentrations.

$c_0 = 0.396$		$c_0 = 0.298$		$c_0 = 0.198$		$c_0 = 0.104$		$c_0 = 0.047$	
x	$s_{1,20}$	x	$s_{1,20}$	x	$s_{1,20}$	x	$s_{1,20}$	x	$s_{1,20}$
5.840		5.838		5.837		5.839		5.874	
5.991	32.6	5.954	39.4	6.031	43.3	6.109	48.6	6.074	50.4
6.045	32.9	6.018	38.0	6.095	42.8	6.207	50.5	6.146	50.1
6.101	32.7	6.076	36.5	6.170	42.8	6.274	48.4	6.228	48.5
6.213	32.2	6.133	36.3	6.251	41.0	6.404	49.1	6.332	51.4
6.333	32.3	6.262	36.1	6.407	42.3	6.548	48.4	6.414	49.7
6.462	32.7	6.400	36.5	6.556	42.6	6.778	49.3		50.0
6.595	32.6	6.549	36.8	6.735	42.5		49.0		
	32.5	6.688	37.3	6.881	42.4				
			36.9		42.4				

which can be due to decrease in the concentration or to the polydispersity of the substance. In any case these changes are less than the experimental accuracy.

Plotting s against $c.s$, we get for s at zero concentration, $s_0 = 59.0 S$, and for the characterization of the concentration dependence, $k = 2.0$ (cf. Gralén, *l. c.* p. 12). Thus the dependence of concentration is rather low.

Having characterized our substance and shown that there is no observable displacement of the maximum point of the sedimentation curve, we can proceed to the calculations according to equ. (21). Since this equation is valid only in a case where we do not have any changes in the hydrostatic pressure, we have to introduce the above mentioned corrections. At the same time, we can reduce the \bar{B} -values to the temperature $20^\circ C$ and the pressure 1 atm. For equ. (28) we easily get the necessary corrections. The final result is:

$$\bar{B} = \frac{c_0}{\left\{ \left(\frac{\bar{x}}{x_0} \right) \left[1 + \frac{R}{2} \left(\frac{\Delta \bar{x}}{x_0} \right)^2 \right] \right\}^{2[1 + \delta(T)]} \left(\frac{dc}{dx} \right) \cdot \frac{\ln \frac{\bar{x}}{x_0} + \frac{R}{2} \left(\frac{\Delta \bar{x}}{x_0} \right)^2}{1 + R \cdot \frac{\Delta \bar{x}}{x_0}}} \quad (29)$$

or in an abbreviated form

$$\bar{B} = \frac{c_0}{\left\{ \left(\frac{\bar{x}}{x_0} \right)^2 \right\}_{\text{corr.}} \left(\frac{dc}{dx} \right) \left\{ \ln \frac{\bar{x}}{x_0} \right\}_{\text{corr.}}} \quad (30)$$

Since there is no change in the s -values, \bar{x} is equal to the x previously used. $dc/d\bar{x}$ can be calculated from the heights (Z) measured in the sedimentation diagrams by means of the formula (cf. UC p. 259)

$$\frac{dc}{d\bar{x}} = \frac{Z}{Gaba} \quad (31)$$

where a is the refractive index increment, G the photographic enlargement, a the thickness of the cell, and b the scale distance. For the case of simplicity, $dn/d\bar{x}$ can be calculated instead of $dc/d\bar{x}$, and at the same time, c_0 in the numerator of equ. (30) can be expressed in refractive index units by multiplication with a . The value of a for the substance used here, determined in acetone, was $134 \cdot 10^{-5}$.

Table 2. Values of $d\bar{B}/d\bar{x}$ calculated according to equ. (21).

$c_0 = 0.396$			$c_0 = 0.298$			$c_0 = 0.198$		
\bar{x}	B	$d\bar{B}/d\bar{x}$	\bar{x}	B	$d\bar{B}/d\bar{x}$	\bar{x}	B	$d\bar{B}/d\bar{x}$
5.840			5.838			5.837		
5.991	1.46	0.24	5.954	2.40	0.40	6.031	2.83	0.47
6.045	1.38	0.23	6.018	2.06	0.34	6.095	2.47	0.41
6.101	1.28	0.21	6.076	1.72	0.28	6.170	2.57	0.42
6.213	1.43	0.23	6.133	1.95	0.32	6.251	2.69	0.43
6.333	1.31	0.21	6.262	1.99	0.32	6.407	2.69	0.42
6.462	1.47	0.23	6.400	2.06	0.32	6.556	2.42	0.37
6.595	1.37	0.21	6.549	2.00	0.31	6.735	2.47	0.37
			6.688	1.93	0.29	6.881	2.96	0.43
$c_0 = 0.104$			$c_0 = 0.047$					
\bar{x}	B	$d\bar{B}/d\bar{x}$	\bar{x}	B	$d\bar{B}/d\bar{x}$			
5.839			5.874					
6.109	3.37	0.55	6.074	2.95	0.49			
6.207	3.00	0.48	6.146	3.07	0.50			
6.274	3.41	0.54	6.228	3.01	0.48			
6.404	3.53	0.55	6.332	3.88	0.61			
6.548	3.25	0.50	6.414	4.37	0.68			
6.778	3.50	0.52						

The performance of all the numerical calculations is omitted here. The final values follow from table 2. Theoretically the \bar{B} -values should increase with increasing distance from the centre of rotation. This is not quite obvious from the values given in the table, but the $d\bar{B}/d\bar{x}$ -values show a rather good consistency for each concentration. Notwithstanding the difficulties in measuring the heights of the sedimentation curves, the result is quite satisfactory. The following mean values (with mean deviations from the mean) are obtained

c_0	$d\bar{B}/d\bar{x}$
0.396	0.22 \pm 0.01
0.298	0.32 \pm 0.03
0.198	0.41 \pm 0.02
0.104	0.52 \pm 0.03
0.047	0.55 \pm 0.07
0	0.61

The last value is extrapolated to zero concentration.

This example shows that the new formula derived for a non concentration dependent sedimentation can be used even in the case of concentration dependence. One advantage of this method over Gralén's is, that the values of dB/dx obtained for different distances from the centre of rotation theoretically shall be independent of this distance. The example given presents experimental evidence that this is the case even for substances with concentration dependent sedimentation. It is consequently possible to get evidence that the quantities measured in the sedimentation diagrams are correct.

A further use of the method will show if it is possible to apply the simple formula (21) even to substances having greater concentration dependence.

SUMMARY

The dB/dx -method introduced by Gralén to characterize polydispersity of high molecular compounds has been studied as regards the influence of 1) increase in centrifugal field with the distance from the centre of rotation, 2) dilution with the sector shape of the cell, and 3) displacements in the maximum point of the sedimentation curve. The result is that generally the width B is not a linear function of x , however, it is shown that a linear relationship can be obtained, if corrections are introduced for the above mentioned influences. The new method has been applied with a good result to a polymeric methyl methacrylate.

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