Mass Distributions of Unfractionated and Fractionated Polymethyl Methacrylates Determined by Ultracentrifugation and Fractional Precipitation

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A new modification of the analysis of sedimentation curves from a velocity ultracentrifugation has been applied on synthetic high molecular weight samples of very different heterogeneity. The high influence of the hydrostatic pressure on organic solvents has been taken into account. The mass distributions obtained in this way have been found to be in good accordance with the results from other methods.

The purpose of this investigation has been to determine the distribution of mass over the different sedimentation constants occurring in a synthetic high polymer by sedimentation analysis of ultracentrifuge data and to test the validity of the method. This has been done in two ways, by comparing the distributions obtained from sedimentation experiments on two different unfractionated polymers with those obtained by precipitation fractionation and by comparing the results of analyses on mixed fractions with those on the separate fractions.

PREVIOUS THEORIES

A knowledge of the distribution of mass over the different molecular species occurring in a sample of a synthetic high molecular weight polymer is in many cases of importance. For instance a determination of the molecular weight frequency function can give a very good picture of the progress of the polymerization reaction itself, and in studying the polymerization kinetics it provides a means to determine the termination mechanism of the growing molecules. The stop mechanism by which the active radicals will turn to dead molecules can involve either a combination of two radicals to one single polymer molecule or a disproportionation between two radicals. In the latter case a hydrogen atom moves from one growing polymer chain to another, giving

rise to two separate macromolecules. These two kinds of deactivation reactions will give different molecular weight distributions. It is also of interest to know the heterogeneity of an unfractionated or fractionated polymer in connection with such physico-chemical determinations as the measurement of intrinsic viscosity, osmotic pressure, light scattering, and diffusion or sedimentation constant of the polymer in solution. Even in the bulk properties of a polymeric material the polydispersity must be considered. For instance it may influence the mechanical properties and the general behaviour of a synthetic plastic.

For this reason, many attempts have been made to determine how the mass is distributed over the different molecular weights or over the different intrinsic viscosities or sedimentation constants occurring in the polymer. The most commonly used methods are fractionation, turbidimetric titration, determination of different average molecular weights with the aid of an equilibrium ultracentrifuge, dB/dx -measurement or analysis of the sedimentation peak in a velocity ultracentrifuge. In the first method the sample is divided into fractions, usually by fractional precipitation or fractional solution. This procedure gives good results but is very time-consuming because of the necessity of having equilibrium between solution and precipitate for every fraction. For this reason it can take two weeks to a month to complete one single fractionation. It is also easy to lose material during the many manipulations necessary. The turbidimetric method has been used on polymethyl methacrylate with success¹, but as a rule it seems to give results inferior to those obtained by fractional precipitation. This is probably due to the difficulty of getting stable and reproducible values of the turbidity itself. The procedure is valuable when one wants a rapid but rough estimate of the polydispersity of a sample. In the third method, an arbitrarily assumed parametric function is fitted to different observed average ultracentrifuge molecular weights obtained by means of an equilibrium centrifuge. This has been employed in many cases, however, the large deviation from ideality of the polymethyl methacrylate solutions will probably make it impractical for this substance. The dB/dx measurement, first developed by Gralén², has been widely used on macromolecular substances with a coiled chain configuration in solution such as occurs with polymethyl methacrylate. In this method, the width (B) of the experimental sedimentation curve is defined as the ratio of area to maximum height of the sedimentation curve:

$$B = \frac{\int_{-\infty}^{+\infty} \frac{dc}{dx} dx}{\left(\frac{dc}{dx}\right)_{\max.}} \quad (1)$$

where x is the distance from center of rotation and c is the polymer concentration at a point x and at a given time (t) after full speed of the rotor. This width is plotted as a function of the position x of the peak of the sedimentation curve. It is found to vary in a linear fashion with the distance from center of rotation. The slope dB/dx of this curve is graphically determined for a series of different

concentrations and extrapolated to infinite dilution. This (dB/dx) , at zero concentration, is then taken as a measure of the heterogeneity of the polymer. This method has been used among others by Gralén and Rånby³ on cellulose derivatives. In all these cases it was possible to neglect the influence of diffusion on the spreading of the sedimentation curve. For polymethyl methacrylate the contribution of diffusion to the width of the curve is appreciable because of the high diffusion constant compared with the low sedimentation constant in fractions of moderate molecular weight. An attempt to correct the experimentally measured width for the influence of diffusion in this case has been made by the author⁴. The (dB/dx) method is rapid but it can never reveal details in the distribution and it fails entirely in the case where the frequency function has more than one maximum as can happen for unfractionated polymers prepared by bulk polymerization at high degrees of conversion⁵⁻⁸.

It is, however, possible to obtain more detailed information about the distribution of mass over the different sedimentation constants contained in a polymer with the aid of a velocity ultracentrifuge. The method of sedimentation analysis was first applied to a high polymer by Signer and Gross⁹. If the polymer solution contains only one species having a constant sedimentation coefficient throughout the entire cell, it is easy to show that the concentration of polymer (c) will be independent of the position in the cell from the boundary between solution and pure solvent down to the bottom. But as a consequence of the sector shape of the centrifuge cell and the increasing strength of the centrifugal field with increasing distance from the center of rotation, the concentration (c) will change with time according to the following square law¹⁰

$$c = c_0 (x_0/x)^2 \quad (2)$$

where c is the actual concentration at the time (t) when the boundary has reached the position (x) in the cell; c_0 is the initial concentration and x_0 the distance from the center of rotation to the position of the meniscus. If the solution consists of more than one component having different but constant sedimentation coefficients, each species will give a separate boundary and a constant concentration in the entire cell from its boundary to the bottom. The square dilution law will still be valid if one uses the x -value of the boundary belonging to the corresponding component.

In the case of a polymer solution with a more or less continuous spectrum of sedimentation constants one can divide the experimental sedimentation curve into a number of strips. The increase of concentration $(dc/dx)dx$ in such an interval $x_1 - dx/2$ to $x_1 + dx/2$, corrected for the dilution effect by multiplication with $(x/x_0)^2$, will then represent the fraction of material having sedimentation constants in the interval $s_1 - ds/2$ to $s_1 + ds/2$, where s_1 is the sedimentation constants for the components having its boundary at the point x_1 at the time of exposure (t).

$$x = x_0 e^{\omega^2 t s} \quad (3)$$

We thus have the expressions

$$dx/ds = x \omega^2 t \quad (4)$$

and

$$g(s) = \frac{dc}{ds} = \left(\frac{dc}{dx} \right) \left(\frac{x}{x_0} \right)^2 x \omega^2 t \quad (5)$$

with the aid of which we can transform the experimental sedimentation curve to a mass frequency function $g(s)$. This procedure is, however, only valid for the case where the sedimentation coefficients are independent of concentration, that is they are real constants, and where the broadening of the boundary peak is entirely due to the distribution of mass over different sedimentation constants and not at all caused by diffusion of the macromolecules. These conditions were fulfilled in the case of the polystyrene fraction investigated by Signer and Gross. If the contribution from diffusion is great enough, the distributions calculated by the method of Signer and Gross from exposures taken at different times after the start of the experiment will not coincide. The frequency curves determined from earlier exposures are broader than those from later ones.

For the case where diffusion cannot be neglected, Baldwin and Williams^{11,12} have developed an extension of the theory by which it is possible to sort out the contribution from sedimentation from that of diffusion. These authors have pointed out that if the concentration is so low that diffusion and sedimentation occur independently of each other the second moment of the experimental sedimentation curve will be equal to the second moment that would have arisen if there had taken place a pure sedimentation process without any diffusion plus the second moment of the curve had there been a pure diffusion without any sedimentation. That is:

$$\sigma_{\text{total}}^2 = \sigma_{\text{sed.}}^2 + \sigma_{\text{diff.}}^2 \quad (6)$$

where σ_{total}^2 = the square of the standard deviation for the experimental curve
 $\sigma_{\text{sed.}}^2$ = the square of the standard deviation for an imaginary curve from a pure sedimentation process
 $\sigma_{\text{diff.}}^2$ = the square of the standard deviation for the curve from a pure diffusion process.

According to Lamm¹³

$$\sigma_{\text{diff.}}^2 = \frac{2Dt}{1 - \omega^2 st} \quad (7)$$

where D = the diffusion constant for a homogeneous sample,
 s = the sedimentation constant,
 ω^2 = the angular velocity of the rotor,
 t = time after the start of the combined sedimentation and diffusion process.

Baldwin and Williams calculated the square of the standard deviation of a pure sedimentation curve and found that

$$\sigma_{\text{sed.}}^2 = p^2 \omega^4 x_m^2 t^2 \quad (8)$$

where p^2 = the square of the standard deviation of the distribution of sedimentation constants,
 x_m the distance from the center of rotation to the first moment of the gradient curve.

From eqns. (6), (7), and (8) it follows that the fraction of the total standard deviation of the experimental sedimentation curve which is due to the contribution from diffusion will be less important with increasing time (t) and zero when t goes to infinity. In eqn. (7) we have to use average values for the diffusion and sedimentation constants for the inhomogeneous polymer. Baldwin and Williams calculate an apparent distribution $g(s)^*$ from each sedimentation curve obtained from the different exposures taken during the same run by means of the equation

$$g(s)^* = \frac{(dc/dx)}{A_{t=0}} \left(\frac{x}{x_0} \right)^2 x \omega^2 t \quad (9)$$

where $A_{t=0}$ is the area of the sedimentation curve extrapolated to zero time. In order to get the real distribution $g(s)$ undisturbed by the influence of diffusion they then determine $g(s)^*$ for a series of fixed s -values and plot these $g(s)^*$ values against $1/t$. These values extrapolated to $1/t = 0$ then give the real distribution of sedimentation coefficients. Gosting¹⁴ has examined the question of extrapolation in detail and has found that if t is fairly large $g(s)^*$ is a closely linear function of $1/xt$.

Up to this point we have only discussed the problem for the case where the sedimentation is independent of the polymer concentration or the hydrostatic pressure in the cell. The first of these two questions has been treated independently by both Baldwin¹⁵ and Williams^{16,17}. If the sedimentation is concentration-dependent, the concentration of each species will no longer be constant throughout the cell as was the case when the sedimentation coefficient was independent of the concentration. Instead, the concentration of the different species will now change with the position in the cell in such a way that a slower component will appear to have a higher and a faster one a lower concentration if calculated in the usual manner. That is, one obtains a so-called Johnston-Ogston effect¹⁸. Secondly, one does not obtain the distribution of sedimentation constants but rather a distribution of sedimentation coefficients for different concentrations. The concentration is highest for the fastest component and decreases with decreasing sedimentation rate of the species down to zero concentration for the slowest one. Both these effects can be eliminated if we extrapolate the observed distribution function to zero concentration. According to the method of Williams, which is purely graphical, the distributions of sedimentation coefficients derived from a series of concentrations are extrapolated to infinite dilution. This is done by determining the $g(s)$ values for given s values and by plotting these $g(s)$ values as a function of concentration c . The procedure given by Baldwin is, however, analytic. By this method it is possible to calculate the final distribution of sedimentation constants from one single sedimentation run. But this theory requires a knowledge of how the different sedimentation coefficients depend on concentration. This dependence is, however, not identical with the usually determined s - c function as evaluated from the midpoints of the sedimentation peaks. In the

Fig. 1. The centrifuge cell.

Baldwin method one must determine the value of s for a particular species and this value is not identical with that which would be obtained if one could isolate a narrow range of species around the one in question and determine its sedimentation coefficient. Every narrow range of components is influenced by the presence of a much broader distribution of sedimentation coefficients and this is as a rule not symmetrically arranged around the range in question. It is possible to determine the desired function experimentally but because of the large amount of work involved the method has not been used in the present investigation. Both Baldwin and Williams have applied their theories to aqueous solutions of dextran and gelatin¹⁵⁻¹⁷. Both have obtained good results.

PRESENT IMPROVEMENTS

When organic solvents are used, as is necessary for polymethyl methacrylate, the great influence of the high hydrostatic pressure in the cell on the density and viscosity of the solvent must be taken into account. This pressure varies from about 1 atm. at the meniscus to 200—400 atm. at the bottom of the cell depending on the density of the solvent used and on the speed of the rotor. The sedimentation coefficient has to be reduced to the value of 1 atm. by means of the formula¹⁹

$$s_1 = s_p \frac{\eta_p(1-V_1\delta_1)}{\eta_1(1-V_p\delta_p)} = s_p \cdot f(x) \quad (10)$$

where s and V are the sedimentation coefficient and partial specific volume of the solute, η and δ the viscosity and density of the solvent, and the subscripts 1 and p refer, respectively, to the values at one and p atmospheres. As a rule there is no available data on how the partial specific volume varies with the pressure but it is generally assumed that this variation is small enough that in calculating the correction factor $f(x)$ one may put V_p equal to V_1 .

In order to obtain a relation between the concentration of a component (i) and the position coordinate x we may consider two cylindrical surfaces I and II through the centrifugal cell (see Fig. 1). At any given time these two surfaces will move with the same velocities as the (i) components having the same position coordinates. At the time $t = t_1$ the surfaces I and II will be at the points $x^I_{t_1}$ and $x^{II}_{t_1}$ and at the time $t = t_2$ at $x^I_{t_2}$ and $x^{II}_{t_2}$, respectively. By integration of the equation defining the sedimentation coefficient:

$$\omega^2 dt = \frac{dx}{x} \frac{f(x)}{s} \quad (11)$$

the following expressions are obtained

$$\omega^2 (t_2 - t_1) = \int_{x^I_{t_1}}^{x^I_{t_2}} \frac{f(x)}{x} \frac{dx}{s(c^I)} = \int_{x^{II}_{t_1}}^{x^{II}_{t_2}} \frac{f(x)}{x} \frac{dx}{s(c^{II})} \quad (12)$$

This equation can be rearranged to

$$\begin{aligned} \int_{x^I_{t_1}}^{x^{II}_{t_1}} \frac{f(x)}{x} \frac{dx}{s(c^I)} + \int_{x^{II}_{t_1}}^{x^I_{t_2}} \frac{f(x)}{x} \frac{dx}{s(c^I)} &= \int_{x^{II}_{t_1}}^{x^I_{t_2}} \frac{f(x)}{x} \frac{dx}{s(c^{II})} + \\ + \int_{x^I_{t_1}}^{x^{II}_{t_1}} \frac{f(x)}{x} \frac{dx}{s(c^{II})} \end{aligned} \quad (13)$$

In these equations, $f(x)$ is the correction factor introduced in order to take into account the influence of the hydrostatic pressure. The surface II will always move in a more concentrated solution than the surface I so it may be valid to assume that

$$c^I(x) \leq c^{II}(x) \quad (14)$$

where $c^{II}(x)$ is the total polymer concentration at the point x at the time when the surface II passes this point and $c^I(x)$ has a corresponding meaning for the surface I. As a first approximation it can further be assumed that the sedimentation coefficient s of the component (i) decreases with increasing total concentration. We thus obtain the following inequality with the aid of (14)

$$\frac{1}{s(c(x))} \leq \frac{1}{s(c^{II}(x))} \quad (15)$$

This expression and equation (13) give the following inequality

$$\int_{x^I_{t_1}}^{x^{II}_{t_1}} \frac{f(x)}{x} \frac{dx}{s(c^I)} \geq \int_{x^I_{t_1}}^{x^{II}_{t_1}} \frac{f(x)}{x} \frac{dx}{s(c^{II})} \quad (16)$$

And if the interval x^I to x^{II} is sufficiently small

$$\left\{ \frac{f(x)}{x} \frac{dx}{s(x)} \right\}_{x_{t_1}} \geq \left\{ \frac{f(x)}{x} \frac{dx}{s(x)} \right\}_{x_{t_1}} \quad (17)$$

Denoting the concentration of component (i) between the two surfaces by c , the volume element between these two surfaces by v and remembering the sector shape of the cell it is possible to write

Fig. 2. The relative concentration as a function of $(x-x_0)$ calculated from equation (19) for different positions of the meniscus.

$$\frac{c_{t_1}}{c_{t_2}} = \frac{v_{t_1}}{v_{t_2}} = \frac{x_{t_1}(dx)_{t_1}}{x_{t_2}(dx)_{t_2}} \leq \frac{(x_{t_1})^2 f(x_{t_1}) s(x_{t_1})}{(x_{t_2})^2 f(x_{t_2}) s(x_{t_2})} \quad (18)^*$$

As a special case valid without any approximation at all

$$\frac{c_{t_1}}{c_{t_2}} = \frac{(x_{t_1})^2 f(x_{t_1})}{(x_{t_2})^2 f(x_{t_2})} \quad (19)$$

if there is no concentration dependence of the sedimentation coefficient. With the aid of this equation the concentration in the cell has been calculated for different positions and at different times for the case of polymethyl methacrylate in ethyl acetate. As can be seen from Fig. 2 the concentration increases with increasing values of x even for sedimentation independent of concentration but dependent on the pressure in the cell **. This increase in concentra-

* This formula is valid for a polydisperse or a monodisperse solute in a solvent, where the influence of the hydrostatic pressure can not be neglected. Working with aqueous solutions Trautman²⁰ and Schumaker have previously treated the case of no hydrostatic pressure effect. They found that in a region where $(dc/dx) = 0$, the dilution will follow the square law

$$c/c_0 = (x/x_0)^2$$

This problem will also be discussed by Moring-Claesson²³ and Kinell^{24,25}. Investigating the Ogston effect in a system of a few discrete components Moring-Claesson obtains the same formula as Trautman but in a different way; Kinell will consider in more detail the assumptions made by Trautman *et al.*

** It can be shown that the expression

$$(\partial c / \partial x)_t = 0 \quad (1)$$

is only possible if the correction function $f(x)$ fulfils the conditions that

$$\frac{d \left(x^2 \frac{1}{f} \right)}{x \, dx} = \text{constant} \quad (2)$$

Cont. on p. 368.

tion is more serious than the corresponding Johnston-Ogston effect caused by the concentration dependence of sedimentation because it can not be eliminated by extrapolation to infinite dilution in the usual way. It will, however, be so small that it will not be further considered here. For the dilution at the boundary one obtains

$$c_0 = c \left(\frac{x}{x_0} \right)^2 \cdot \frac{1}{f(x)} \quad (20)$$

since the pressure correction factor is equal to 1 at the meniscus. Not only will the dilution law change, but it follows from the integrated form of equation (11) that the derivative $\frac{dx}{ds}$ must now be written

$$\frac{dx}{ds} = \frac{1}{f(x)} x \omega^2 t \quad (21)$$

Instead of eqns. (5) and (3) the following expressions are valid for a concentration independent but pressure sensitive sedimentation

$$g(s) = \left(\frac{dc}{dx} \right) \left(\frac{x}{x_0} \right)^2 \frac{1}{f(x)^2} x \omega^2 t \quad (22)$$

$$s = \frac{1}{\omega^2 t} \int_{x_0}^x \frac{dx}{x} f(x) \quad (23)$$

When it is impossible to keep the temperature of the centrifuge solution and the angular velocity of the rotor perfectly constant the s -values must be corrected to the same temperature and the same speed of the rotor. The temperature is therefore read at short intervals of time and the sedimentation coefficient calculated for this interval reduced to a temperature of 20° C by means of the formula

$$s_{20} = s_t \frac{\eta_t}{\eta_{20}} \frac{(1 - V_{20} \rho_{20})}{(1 - V_t \rho_t)} \quad (24)$$

Cont. from p. 367.

This follows from the equation of continuity

$$\left(\frac{\partial c}{\partial t} \right)_x = - \frac{\omega^2 \partial \left(x^2 \frac{1}{f} c s \right)}{x \partial x_t} = - \omega^2 c s \frac{d \left(x^2 \frac{1}{f} \right)}{x dx} - \frac{\omega^2 x}{f} \frac{\partial (c s)}{\partial x_t} \quad (3)$$

which in the case of condition (1) reduces to

$$\left(\frac{\partial c}{\partial t} \right)_x = - \omega^2 c s \frac{d \left(\frac{1}{f} x^2 \right)}{x dx} \quad (4)$$

Equations (1) and (4) can only be simultaneously valid if

$$\frac{d \left(x^2 \frac{1}{f} \right)}{x dx} = \text{constant}$$

where all symbols have their usual significance. The subscripts 20 and t refer to the temperature 20 and t °C. Small errors in the determination of temperature or of rotor speed will cause a shift in the whole $g(s)$ curve. This in turn may produce serious errors in the extrapolation of $g(s)$ to infinite time and infinite dilution. For this reason formula (22) has not been used in this investigation. Another procedure to calculate the distribution has therefore been developed. The experimental sedimentation curve dc/dx against x is read at constant x intervals and the dc/dx values corrected for dilution effects with the aid of eqn. (20). This corrected frequency curve is then integrated and starting from the origin the x_p values were determined for successive 10 % increments of the integral distribution curve. The index p refers to an increase of p % in the integral mass distribution. By means of equation (23) the ratio s_p/s_{50} is then calculated from the different x_p . In order to eliminate the contribution from diffusion the ratios s_p/s_{50} from different exposures are plotted against $1/(s_{50} \cdot t)$ and extrapolated to infinite time. The extrapolated values of the ratio s_p/s_{50} have to be converted to s_p -values. This can be done with the aid of eqn.

(23) using the experimentally determined values of the integral $\int_{x_0}^{x_{50}} \frac{f(x)}{x} dx$ which are found to be a linear function of time.

This curve crosses the t axis at a small negative value indicating that the sedimentation starts a little before the rotor has reached the operating speed. The s_{50} -values calculated in this way are found to lie close to the $s_{\max.}$ -value determined in the usual manner from the maximum point of the sedimentation diagram. So in the case of homogeneous fractions it may be preferable to use values of s_{50} calculated from the known relation

$$\frac{1}{s} = \frac{1}{s_{\max.}} (1 + kc) \quad (25)$$

where $s_{\max.}$ is the sedimentation constant of the maximum point extrapolated to infinite dilution and k is a constant expressing the concentration dependence of the sedimentation coefficient. Both $1/s_{\max.}$ and k have of course been determined by means of the method of least squares from a long series of experimentally found $1/s, c$ pairs. In order to obtain the real distribution, $1/s_p$ has to be plotted against c and extrapolated to infinite dilution. This procedure is facilitated by the fact that $1/s_p$ is a closely linear function of c .

EXPERIMENTAL AND RESULTS

Materials. The method of determining the distribution of mass over the different sedimentation constants in a synthetic high polymer has been tested on polymethyl methacrylate in ethyl acetate as solvent. In order to obtain polymers of highly different heterogeneity covering a broad interval of molecular sizes, samples of unfractionated and fractionated polymers as well as mixed fractions have been investigated. The polymethyl methacrylates have been prepared by bulk polymerization with benzoyl peroxide as initiator. Both the concentration of initiator and the degree of conversion have been varied. The reaction conditions will be given in detail later²². The polymers were fractionated by precipitation and the sedimentation constants were determined for every

fraction in ethyl acetate. Details of fractionation and sedimentation technique are given in Ref.²¹. The ethyl acetate used was "Baker Analyzed Reagent" and was not further purified.

Influence of the correction factor. The choice of correction factor has only a small influence on the distribution s_p/s_{50} calculated from a sedimentation diagram. This can be seen from Table 1, where the ratio

$$\frac{s_p/s_{50} \text{ corrected with the factor } (x/x_0)^2}{s_p/s_{50} \text{ not corrected for dilution}}$$

has been given for different percents (p) and at five times during the same run. The sample used was an unfractionated polymer /B(714)/ and therefore had an unusually broad mass distribution. The concentration was 0.157 g per 100 ml solution. The dilution-correction will only affect the value of s_{50} . The value of s_{50} calculated by the method of least squares after applying the correction factors, $(x/x_0)^2$ and $(x/x_0)^2 \cdot 1/f(x)$ and no correction, was 13.95 and 13.84 and 13.72, respectively.

Table 1. The ratio $\frac{s_p/s_{50} \text{ corrected with the factor } (x/x_0)^2}{s_p/s_{50} \text{ not corrected for dilution}}$ for different percents (p) and at five times during the same run in the ultracentrifuge.

Time after full speed	10	20	30	Percent (p) 40	60	70	80	90
625 sec.	1.007	1.006	1.006	1.000	1.005	1.004	1.000	1.000
935	1.001	1.005	1.001	1.000	1.000	1.000	1.000	0.999
1 285	1.006	1.004	1.000	1.000	1.000	1.002	1.003	1.003
2 400	1.001	1.002	0.999	0.999	0.998	0.997	0.998	0.998
3 025	1.003	1.002	1.000	0.999	0.999	0.998	0.998	0.997
Mean value	1.004	1.004	1.001	1.000	1.000	1.000	1.000	0.999

Extrapolation of s_p/s_{50} to infinite time. With the method used here to get the mass distribution it is not possible to give an unequivocal theoretical justification for the procedure of extrapolating the ratio s_p/s_{50} to infinite time. In two special cases, however, $(s_p - s_{50})^2/s_{50}^2$ will be a linear function of $1/t$, namely if the substance is monodisperse or if the mass is distributed over the different s values in accordance with a Gaussian frequency curve and the diffusion constant is the same for all molecules. In both these cases it can be shown that the experimental sedimentation curves will be Gaussian if diffusion and sedimentation occur independently of each other in the cell. It was experimentally found that for polymethylmethacrylate s_p/s_{50} plotted against $1/(s_{50}t)$ gives a straight line. In order to investigate if different extrapolation methods could give different results, all s_p/s_{50} values for each concentration used (fraction B(712) XIV) were treated in two different ways, in one case s_p/s_{50} and in the other $(s_p/s_{50})^2$ was plotted against $1/(s_{50}t)$. This material has a high diffusion constant but a low sedimentation constant and hence the dependence of s_p/s_{50} on time after the start of the experiment is unusually large. The final distribution extrapolated to zero concentration did not show any systematical trend with the two methods. The mean difference between two corresponding s -values was less than 0.5 % and can be

Fig. 3. Extrapolation of s_p/s_{50} to infinite time for two concentrations of B(712) XIV.

A Concentration equal to 0.792 g/100 ml
B Concentration equal to 0.199 g/100 ml

Fig. 4. Extrapolation of $(s_p/s_{50})^2$ to infinite time for the same fraction and at the same concentrations as in figure 3.

Fig. 5. Extrapolation of s_p/s_{50} to infinite time for the unfractionated polymer B (714) at the concentrations 0.358 (A) and 0.157 (B) g/100 ml.

considered to lie within the experimental accuracy of the procedure. As the difference can be expected to be still less for a more polydisperse substance or fraction with a lower diffusion constant, the method of extrapolating s_p/s_{50} as a linear function of $1/(s_{50}t)$ was chosen in order to save time. The two methods of extrapolation are illustrated in Figs. 3 and 4 for the two concentrations 0.792 and 0.199 g polymethyl metacrylate per 100 ml ethyl acetate solution. The fact that the dependence of s_p/s_{50} on time is much smaller for a more polydisperse sample is shown in Fig. 5 for the two concentrations 0.358 and 0.157 g/ml. The sample used was an unfractionated polymer B(714).

Fig. 6. $1/s_p$ as a function of concentration. In order to spread out the data the lines have been displaced from each other by 0.025 units. Sample B(714).

Comparison of the distributions of mass obtained by means of the velocity ultracentrifuge with those obtained by means of precipitation fractionation. The contribution of diffusion to the total width of the sedimentation peak is relatively small for a polymer with a reasonably heterogeneous composition of different molecular sizes. This is of course quite natural as the spreading from diffusion is determined only by the average diffusion constant and not at all by the width of the distribution of this constant. However, the spreading due to sedimentation is proportional to the mean sedimentation constant multiplied by the standard deviation of the function expressing the mass frequency over this constant. This is the reason why s_p/s_{50} is almost independent of t for all percents (p) except for the last fraction with the highest molecular weight. The function s_p/s_{50} plotted against $1/(s_{50}t)$ was shown in Fig. 5. If the average molecular size is not extremely high it is further possible to use more concentrated solutions in order to minimize the errors from the inaccuracy in the drawing of the base line of the gradient curve, dc/dx against x . The uncertainty in the determination of this line is greatest for polydisperse material and for low concentrations, where the sedimentation peak is very broad and flat. The mass distribution of two unfractionated polymers with an average sedimentation constant of 16.80 and 11.38 have been determined by analysis of the sedimentation diagrams. The first polymer is designed by B(714) and the second by B(712). The $s_{50}(c)$ values used were obtained from an eqn. similar to eqn. 25, the constants being derived by the method of least squares using the experimental s_{50} values for 8 and 5 concs., resp. in the 2 above cases. The coefficients $1/s_p$ were in every case found to lie on a straight line as shown in Fig. 6 for the polymer B(714). The final mass distribution over the sedimentation constant is shown in Fig. 7. In these figures the integral distributions obtained from precipitation fractionation are also given. These have been constructed under the assumption that half the mass of a fraction has a sedimentation constant lower than the average value determined for the fraction. The result is that the curves obtained from the two methods lie close to one another. A part of the small parallel displacement of the curves can possibly be explained by the fact that the sedimentation measurements on the different fractions were performed some years before the determinations on the unfractionated polymer. During this time the thermo-elements giving the rotor temperature have been exchanged and therefore the zero point of the temperature scale re-determined. The method of determining the integral distribution of mass by means of analysis of the sedimentation peaks is considered to be as accurate as the laborious and time-consuming technique of precipitation fractionation. Another advantage with the method of sedimentation analysis is that it can be applied directly on the initial reaction product diluted with the monomer. In this way it will be unnecessary to separate the polymer from the unreacted monomer.

Comparison of the distribution of mass obtained for mixtures of two fractions by means of the velocity ultracentrifuge with those obtained on the separate fractions by means of the same method. In order to get further proof of the usefulness of sedimentation analysis on high polymers, the investigation has been extended to higher molecular sizes and to more homogeneous material composed of one single fraction or mixtures of two fractions in equal parts. The more

Fig. 7. Integral distributions obtained from sedimentation analyses (fuldrawn lines) and from fractional precipitation (dotted lines). A for B(714) and B for B(712).

essential properties of the fractions used in this study are summarized in Table 2. The values of $s_{\max}$ are the sedimentation constant as determined from the maximum point of the sedimentation curve. The experimentally determined relation $1/s = 1/s_{\max} (1 + k_s c)$ has been used to calculate s_{50} . This has been done because it was found that the s_{50} values for this rather homogeneous sample are very nearly equal to $s_{\max}$ and because there was a greater number of data available on which to apply the method of least squares for the determi-

Table 2. Summary of the properties of the polymethyl methacrylate fractions that have been used for the sedimentation analyses.

Fraction		$s_{\max}$	Weight of the fraction in percent of the total weight of the polymer product	Weight of material in the initial polymer product hav- ing a higher sedimentation constant than that of the fraction used
B(712)	XIV	6.29	7.68	89.25
B(709)	X	16.38	9.22	69.69
B(709)	XIII	10.50	6.75	90.09
B(709)	X + XIII	13.09 (s_{50})	—	—
B(713)	IV	30.36	4.40	3.40
B(713)	VI	27.05	5.45	10.00
B(713)	IV + VI	28.43	—	—

Fig. 8. Integral distribution of B(712) XIV.

nation of the constants $1/s_{\max}$ and k_s . The different $s_{\max}$ are given in the second column of Table 2. In the next column the amount of each fraction is given in weight percent of the total polymer product, and the last column shows the total amount of material in the initial polymer product having higher sedimentation constant than that of the fractions used. As can be seen from the table, in polymer B(709) the mass of the two fractions between X and XIII was about 11 % of the total polymer. This was enough to give the mixture of the two fractions X and XIII a double peaked sedimentation curve. It was therefore impossible to determine any $s_{\max}$. In this case the observed values of the sedimentation coefficient s_{50} for the five concentrations employed for the sedimentation analysis were used instead to calculate the sedimentation coef-

Fig. 9. Integral distributions of B(709) X, B(709) XIII and a mixture of equal parts of these two fractions. The dotted line is the distribution of the mixture calculated from the data of the separate fractions.

Fig. 10. Integral distributions.

- I. of B(713) IV
- II. of B(713) VI
- III. of the two fractions mixed in equal parts.

ficient at infinite dilution and the constant k_s that expresses the concentration dependence of this coefficient. Five or six concentrations have been used in the sedimentation analysis of each fraction or mixture of fractions. The lowest concentration in each series was about 0.1 g per 100 ml. The upper concentration limit was 1.0, 0.6 and 0.4 g per 100 ml for polymers designated by B(712) B(709) and B(713), respectively. In these concentration intervals it has been possible to extrapolate $1/s_p$ linearly with concentration. The calculated integral distributions of mass are shown in Figs. 8, 9 and 10. The dotted line in Fig. 9 is the mass distribution of the mixture of equal parts of the two fractions B(709) X and B(709) XIII, that has been calculated from the experimentally determined mass distributions of the two fractions separately. As can be seen from the figure this line is in reasonably good accordance with the mass distribution that has been obtained from sedimentation analysis applied directly on the mixture. A part of the small discrepancy may have been caused by errors in the determinations of the different $1/s_{50}$ values at infinite dilution. The result of the sedimentation analysis on the mixture of equal parts of the two fractions B(713) IV and B(713) VI is also very good (Fig. 10). Unfortunately the two fractions lie close together. It is therefore a little difficult to judge the extent to which the good agreement is due only to the reliability of the method. It may be that part of the agreement is due to the fact that the method gives approximately the same errors in all these three cases. It can, however, be concluded from these experiments that the analysis of the sedimentation curves will give integral mass distributions that are at least in broad outline correct even for relatively homogeneous materials.

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