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## Sedimentation Equilibrium of Flexible Chain Molecules

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By L. Mandelkern, L. C. Williams and S. G. Weissberg

## SEDIMENTATION EQUILIBRIUM OF FLEXIBLE CHAIN MOLECULES<sup>1</sup>

BY L. MANDELKERN, L. C. WILLIAMS AND S. G. WEISSBERG

National Bureau of Standards, Washington 25, D. C.

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The thermodynamic theory of sedimentation equilibrium is reviewed with special emphasis on solutions of flexible long chain molecules. The analyses indicate that the weight average molecular weight of this class of solutes, irrespective of the molecular weight heterogeneity, can be obtained in a straightforward manner only in ideal solutions. The molecular weight of a monodisperse polymeric solute in a non-ideal solution can in principle also be obtained from this type of experiment but with a great deal more difficulty. However, if the solute is polydisperse and the solution non-ideal, theory indicates that no reliable molecular weight average can be determined at present from experiments performed in the equilibrium ultracentrifuge. To investigate some of these conclusions it is first demonstrated that ideal solution of flexible chain molecules over a wide range of concentrations and of molecular weights can be achieved in the equilibrium centrifuge. For this initial study polyisobutylene was chosen as the polymer and ethyl *n*-heptanoate as solvent. Determination of the critical consolute temperature as a function of molecular weight indicated that ideal behavior could be attained at 34°, the temperature at which the sedimentation equilibrium experiments were carried out in a Svedberg ultracentrifuge. After the molecular weights were determined from these experiments, measurements were also made in a good solvent, isoöctane, and the methods and difficulties involved in interpreting such data are discussed.

### Introduction

The equilibrium ultracentrifuge was one of the earliest instruments developed to study the thermodynamic behavior of solutions of macromolecules. In principle, from an analysis of the mass distribution of solute along the length of the centrifuge cell, one can obtain information as to the molecular weight of the solute (or the average molecular weight if it is polydisperse) as well as information about deviations from ideal behavior. For thermodynamically ideal solutions a simple and exact analysis exists so that experimental results can be properly interpreted.<sup>2</sup> However, though all solutions must behave ideally as infinite dilution is approached it is usually experimentally impracticable to work at the extremely low concentrations required with solutions of macromolecules.

The introduction of the necessary corrections for non-ideality that are required to properly interpret experiments performed with solutions of flexible chain molecules is extremely difficult. The concentration of a single solute species in equilibrium in a centrifugal field will increase as the distance from the center of rotation increases. As the solute concentration increases, so must the magnitude of the correction for deviations from ideality. If the non-ideality correction is expressed as a power series expansion in concentration, it is *a priori* difficult to tell, except for initially very dilute solutions, how many terms will be adequate. For a heterogeneous solute the situation is further complicated by the fact that both the mass and the distribution of molecular weights will vary along the length of the cell. Recent theoretical and ex-

perimental studies<sup>3,4</sup> on the thermodynamic properties of solutions of chain molecules indicate that the non-ideality corrections will depend very markedly on molecular weight heterogeneity. Thus for this situation the necessary corrections will be further complicated.

A large majority of the experimental results reported<sup>5-7</sup> for long chain molecules have involved good solvents where the non-ideality corrections are severe. To interpret these results the various investigators have of necessity been forced to make certain arbitrary assumptions. It has been assumed that only the second virial coefficient need be considered, and further that the virial coefficient is independent, both of molecular weight and molecular weight distribution. The consequences of these assumptions in determining the molecular weights of long chain flexible molecules are considered in this paper. The deviations from ideality can, of course, be minimized by performing the experiments in poorer solvents. For the more compact macromolecules, such as the globular proteins, the deviations from ideality are not too severe in the accessible concentration range. Such systems will not be considered here.

On the other hand, it is now well known that ideal behavior can be attained for solutions of long chain flexible molecules over a wide range of solute concentrations by an appropriate choice of solvent and temperature. Under these conditions, com-

(3) P. J. Flory and W. R. Krigbaum, *J. Chem. Phys.*, **18**, 1086 (1950).

(4) W. R. Krigbaum and P. J. Flory, *J. Am. Chem. Soc.*, **75**, 1775 (1953).

(5) K. E. Van Holde and J. W. Williams, *J. Polymer Sci.*, **11**, 243 (1953).

(6) M. Wales, J. W. Williams, J. O. Thompson and R. H. Ewart, *THIS JOURNAL*, **52**, 983 (1948).

(7) M. Wales, F. T. Adler and K. E. Van Holde, *ibid.*, **55**, 145 (1951).

(1) Presented in part at the 128th American Chemical Society meeting, Minneapolis, Minn., Sept. 11-16, 1955.

(2) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford, 1940, p. 48.

monly call the " $\Theta$  temperature" or "Flory temperature," the second and all higher virial coefficients vanish for all molecular weights and molecular weight distributions. This situation arises because at the  $\Theta$  point the net interaction between segments of a pair of polymer molecules is zero so that molecules can freely overlap. These conditions are analogous to the Boyle point of a real gas, and therefore polymer solutions obey van't Hoff's law.<sup>8</sup> The rather important theoretical reason for performing sedimentation equilibrium experiments under these conditions has already been emphasized and the contributions that might result from experiments conducted in such a manner have been indicated.<sup>9</sup> The main purpose of this paper is to demonstrate experimentally that ideal solution behavior can be achieved in the equilibrium ultracentrifuge for solutions of long chain molecules over a wide range of molecular weights. Thus the theoretical difficulties in interpreting the experimental data are circumvented. It will be shown that under these conditions the analyses of the experimental data are extremely simple, both for homogeneous and heterogeneous solutes, and that many of the original objectives that led to the development of the instrument by Svedberg might be realizable for solutions of long chain molecules.

### Experimental Details

**Materials.**—The polyisobutylene fractions used in this study were obtained from two samples of commercially available whole polymer supplied by the Esso Standard Oil Company. Fractions A and F were obtained from the whole polymer designated as B-100 by fractional precipitation with acetone of an initial 0.5% solution in benzene, following the procedure described by Flory.<sup>10</sup> Fractions B, C, D and E were obtained from the whole polymer having the designation LMS. Fraction C was the first fraction obtained by the single precipitation of a 1% benzene solution; fractions B, D and E were obtained by further separation of the second fraction. The above letter designation of the fractions is merely for convenience in the subsequent discussion, and does not reflect the order in which they were obtained. For the present study no special effort was made to obtain sharp fractions. The viscosity average molecular weights of the fractions used are given in Table I and were determined from intrinsic viscosity measurements in benzene at 24° where the relation  $[\eta] = 10.7 \times 10^{-4} M^{1/2}$  has been shown to be valid.<sup>11</sup>

The solvents used, isoöctane and ethyl *n*-heptanoate, were of reagent grade and used as received.

**Precipitation Temperatures.**—The precipitation temperatures of solutions of the polymer fractions in ethyl *n*-heptanoate were obtained following the experimental procedure described by Shultz and Flory.<sup>12</sup> For this system it is important that drying columns be attached to the precipitation tubes in order to obtain reliable results. Precipitation temperatures reproducible to  $\pm 0.1^\circ$  were obtained, and when plotted against concentration for each fraction the curves pass through the expected maximum. The maximum temperature in this plot is the critical consolute temperature.

**Ultracentrifuge.**—A modified Svedberg equilibrium ultracentrifuge, manufactured by LKB Produkter (Sweden), was used in this investigation. The modifications of the standard instrument have been described previously.<sup>13</sup> Four different solutions can be studied at the same time without

the loss of any accuracy. The rotor of this instrument was immersed in a thermostat maintained constant to within  $\pm 0.007^\circ$ . For the systems studied here, equilibrium could be attained in four to fourteen days depending, of course, on the concentration, molecular weight and solvent used. The scale line displacement method was used, reference runs being made at each speed with each of the cells filled with pure solvent. The scale line displacements,  $Z$ , were measured from the reference lines using a Mann comparator having an accuracy of  $\pm 1 \mu$ . A synchropulsed Strobolux light source, appropriately filtered to isolate the 5460 Å line, was used.

The scale line displacement is related to the index of refraction gradient  $dn/dx$  along the length of the cell by the relation

$$Z = Gab \, dn/dx$$

where  $G$  is the scale magnification factor obtained by direct measurement of the scale and scale photograph,  $a$  is the optical path length through the solution of a given cell, and  $b$  is the optical distance from the scale to the center of the cell. The index of refraction gradient is related to the concentration gradient of the solute in the cell by the relation

$$dc/dx = (dn/dx)/(dn/dc)$$

For dilute solutions of polyisobutylene in isoöctane the value of  $dn/dc$  has been reported<sup>5</sup> as  $1.42 \times 10^{-3} \text{ (g./dl.)}^{-1}$ . For polyisobutylene in ethyl *n*-heptanoate  $dn/dc$  was measured at 34°, using a Phoenix Differential Refractometer and is  $1.04 \times 10^{-3} \text{ (g./dl.)}^{-1}$ . To obtain the partial specific volume of this polymer in ethyl *n*-heptanoate at 34°, densities of dilute solutions were measured using a calibrated pycnometer and a semi-microbalance. The partial specific volume was calculated from these data by the method of intercepts as described by Lewis and Randall,<sup>14</sup> and was  $1.106 \text{ cm}^3/\text{g.}$ ; the density of the solvent at this temperature is  $0.8563 \text{ g./cm}^3$ . For polyisobutylene in isoöctane at 25°, the quantity of interest  $(1 - \bar{v}\rho)$  is 0.272.<sup>5</sup>

The scale line displacements were plotted against  $x$ , for about 20 to 30 values of  $x$  for each experiment and a smooth curve drawn through the data from the meniscus to the bottom of the cell. The necessary integrations of the  $Z$  against  $x$  curves to obtain the molecular weights (see Discussion) were performed numerically using Simpson's rule.

### Discussion and Results

**General Theory.**—The analysis of the equilibrium state attained by a solution in the ultracentrifuge represents a special case of heterogeneous equilibrium in a force field. In this case the field can be described by the centrifugal potential  $\phi$  which assumes a definite value at each point  $x$ , the distance outward from the center of rotation. We shall consider a multicomponent solution composed of one solvent component designated by the subscript 1, and  $r - 1$  chemically identical polymeric solute components designated by the indices 2, 3, . . . ,  $r$ . The criterion for equilibrium is that for each of the components the total potential must be uniform throughout the force field. Thus at point  $x$

$$d\mu_i^{(x)} + M_i d\phi^{(x)} = 0 \quad (1)$$

there being  $r$  such equations, one for each component. In equation 1,  $\mu_i^{(x)}$  is the chemical potential of species  $i$  at point  $x$ , and  $M_i$  is the molecular weight of this component. By applying standard thermodynamic methods as described by Pedersen<sup>2</sup> and Goldberg<sup>15</sup> one obtains the relation that

(14) G. N. Lewis and M. Randall, "Thermodynamics," McGraw-Hill Book Co., New York, N. Y., 1923, Chapter IV.

(15) R. J. Goldberg, THIS JOURNAL, 57, 194 (1953).

(8) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 529 and 532.

(9) Ref. 8, p. 307.

(10) P. J. Flory, *J. Am. Chem. Soc.*, 65, 372 (1943).

(11) T. G. Fox and P. J. Flory, *ibid.*, 73, 1909 (1951).

(12) A. R. Shultz and P. J. Flory, *ibid.*, 74, 4760 (1952).

(13) M. Wales, P. G. Sulzer and L. C. Williams, *J. Research Natl. Bur. Standards*, 50, 69 (1953).

$$M_i (1 - \bar{v}_i^{(x)} \rho^{(x)}) \omega^2 x dx = \sum_{k=2}^r (\partial \mu_i / \partial N_k)^{(x)}_{T, p, N_2, \dots, N_1, \dots, N_r} dN_k^{(x)} \quad (2)$$

$N_j \neq N_k$

In equation 2,  $\bar{v}_i^{(x)}$  is the partial specific volume of component  $i$  at point  $x$ ,  $\rho^{(x)}$  the density of the solution at point  $x$ ,  $N_k$  the mole fraction of component  $k$  and the centrifugal potential  $d\phi^{(x)}$  has been replaced by its equivalent— $\omega^2 x dx$  where  $\omega$  is the angular speed of rotation.

In order to discuss the behavior of polymer solutions in the equilibrium ultracentrifuge it will be convenient to express the results obtained above in terms of the osmotic pressure at point  $x$ . This can be accomplished by multiplying both sides of equation 5 by  $n_i^{(x)}$  the number of moles of component  $i$  at point  $x$ , and summing over all polymer species. Then

$$\sum_{i=2}^r n_i^{(x)} M_i (1 - \bar{v}_i^{(x)} \rho^{(x)}) \omega^2 x dx = \sum_{i=2}^r \sum_{k=2}^r n_i^{(x)} (\partial \mu_i / \partial N_k)^{(x)}_{T, p, N_2, \dots, N_1, \dots, N_r} dN_k^{(x)} \quad (3)$$

$N_j \neq N_k$

the assumption being made that the partial specific volume is independent of molecular weight. By use of the relation

$$\mu_1^{(x)} - \mu_1^{(x,0)} = -\pi^{(x)} \bar{V}_1^{(x)} \quad (4)$$

and the Gibbs-Duhem relation in the form

$$\sum_{i=1}^r n_i^{(x)} (\partial \mu_i / \partial N_k)^{(x)}_{T, p} = 0 \quad (5)$$

there is obtained

$$\sum_{i=2}^r (n_i^{(x)} M_i / n_1^{(x)} \bar{V}_1^{(x)}) (1 - \bar{v}_i^{(x)} \rho^{(x)}) \omega^2 x \left( \frac{dc}{dx} \right)^{-1} = (\partial \pi / \partial c)^{(x)} \quad (6)$$

where  $\mu_1^{(x,0)}$  is the chemical potential of pure solvent at point  $x$ ,  $\pi^{(x)}$  is the osmotic pressure at point  $x$ , and  $c_x$  is the concentration at point  $x$  expressed as grams of solute per unit volume of solution. The expression

$$\sum_{i=2}^r n_i^{(x)} M_i / n_1^{(x)} \bar{V}_1^{(x)}$$

is equal to the number of grams of solute per unit volume of solvent at  $x$ . In dilute solution this quantity can be set equal to  $c_x$ . The error introduced is insignificant so that equation 6 can be written as

$$(1 - \bar{v}^{(x)} \rho^{(x)}) \omega^2 x c_x / (dc/dx)^x = (\partial \pi / \partial c)^x \quad (7)$$

Equation 7 is a general relation describing the equilibrium of a dilute solution in the ultracentrifuge. It is applicable to ideal as well as non-ideal solutions irrespective of the polydispersity of the solute. However, in interpreting the behavior of such solutions it is convenient to consider separately the problems involved in treating ideal and non-ideal solutions.

**Ideal Solutions.**—Over the concentration range in which a solution is ideal, van't Hoff's law is obeyed so that

$$\pi^x = (RT/M_n^{(x)}) c_x \quad (8)$$

where  $M_n^{(x)}$  is the number average molecular weight of the solute at  $x$ . For the special case of a single component solute equation 7 becomes

$$dc_x/dx = [(1 - \bar{v}^{(x)} \rho^{(x)}) \omega^2 x / RT] c_x M \quad (9)$$

which can be written in the alternative form as

$$H_u^{(x)} c_x / (dc/dx)^{(x)} = RT/M \quad (9-1)$$

As Goldberg<sup>15</sup> has pointed out a close analogy exists between equations 9 and 9-1 and the expression relating the excess turbidity of a polymer solution at zero angle and infinite dilution to the polymer molecular weight. The significant difference between the expressions is that in the light scattering case the equation is applicable to the solution as a whole; while for sedimentation equilibrium it is only applicable at point  $x$ .

For a multicomponent solute

$$(dc_x/dx)^x = \sum_{i=2}^r dc_{ix}/dx = [(1 - \bar{v}^{(x)} \rho^{(x)}) \omega^2 x / RT] \sum_{i=2}^r c_{ix} M_i \quad (10)$$

Since the weight average molecular weight at  $x$ ,  $M_{wx}$  is defined as

$$\sum_{i=2}^r c_{ix} M_i / c_x$$

we obtain the result that

$$M_{wx} = [RT / (1 - \bar{v}^{(x)} \rho^{(x)}) \omega^2 x] (dc_x/dx) c_x \quad (11)$$

Equation 11 gives the weight average molecular weight at point  $x$ , while the quantity of interest is the weight average molecular weight of the polymer. This quantity can be obtained by integrating equation 11 over the length of the centrifuge cell, taking cognizance of the fact that a sector shaped cell is used.<sup>16</sup> Thus for the weight average molecular weight of the polymer

$$M_w = \int_{x_a}^{x_b} M_{wx} c_x dx / \int_{x_a}^{x_b} c_x dx \quad (12)$$

where the limits of integration represent the extremities of the cell,  $x_a$  being the meniscus position and  $x_b$  the bottom of the cell. If the necessary integrations are performed using equation 11 the result is

$$M_w = [2RT / (1 - \bar{v} \rho) \omega^2] [1/c_0 (x_b^2 - x_a^2)] \int_{x_a}^{x_b} (dc/dx) dx \quad (13)$$

where  $M_w$  is the weight average molecular weight of the solute and  $c_0$  is the initial solution concentration in the centrifuge cell. In performing the integration we have assumed that  $\bar{v}$  and  $\rho$  are independent of  $x$  and hence of polymer concentration and of pressure; these assumptions would appear to be adequate in dilute solutions.

Equation 13, valid only for ideal solutions, expresses the weight average molecular weight in terms of directly measurable quantities. The term  $(dc_x/dx)$  is directly related to the scale line displacements and the necessary integrations can be performed either numerically or graphically. The  $M_w$  calculated from equation 13 should be the

(16) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford, 1940, p. 7.

same for different initial polymer concentrations, alleviating the necessity of making any arbitrary and hazardous extrapolations of the data to infinite dilution. Thus, if ideal solution behavior can be achieved in the ultracentrifuge,  $M_w$  can be very simply determined without reference to whether the solute is monodisperse or polydisperse, or to the nature of the polydispersity. The determination of  $M_w$  in the ultracentrifuge independent of total concentration and over a wide composition range can be taken as a sufficient criterion that ideal solution behavior has been achieved.

The attainment of ideal behavior for a given polymer involves the appropriate selection of solvent and temperature. This choice is not unlimited since the operation and design of the centrifuge itself impose certain restrictions. The temperature selected must, of course, lie within the operating range of the instrument. The solvent-polymer system must be such that the term  $(1 - \bar{v}\rho)$  is not so small as to make the rate of sedimentation too slow. Also the inherent lack of precision in measuring a small  $(1 - \bar{v}\rho)$  would be reflected in inaccurate molecular weights. Finally the indices of refraction of the polymer and solvent must differ appreciably so that the scale line displacement method can be used to measure the concentration gradient along the length of the cell. Though these conditions appear formidable they are not too difficult to fulfill. We have selected for our initial study the system polyisobutylene as polymer and ethyl *n*-heptanoate as solvent. We shall describe how the "θ temperature" or ideal solution conditions can be determined independent of the sedimentation equilibrium experiments and discuss the results of such experiments when performed under these conditions.

According to thermodynamic theory of polymer solutions the critical miscibility temperature,  $T_c$ , of a binary polymer-solvent system depends on molecular weight according to the following relation<sup>12</sup>

$$1/T_c = (1/\Theta) [1 + (1/\psi_1)(1/X^{1/2} + 1/2X)] \quad (14)$$

when  $X$  is the ratio of the molar volume of the polymer to that of the solvent; *i.e.*, the number of segments per polymer chain.  $\psi_1$  is an entropy parameter, and  $\Theta$  is the critical miscibility temperature for polymer species of infinite molecular weight, being defined as the maximum temperature for the coexistence of two liquid phases. At the temperature  $T = \Theta$ , it has been shown theoretically<sup>8</sup> and verified experimentally<sup>17,18</sup> that polymer solutions of any molecular weight behave ideally. That is, van't Hoff's law is followed at all concentrations, for all molecular weights and molecular weight distributions. Thus the conditions for ideal solution behavior can be obtained from studies of the dependence of  $T_c$  on  $M$ . From such studies the  $\Theta$  temperature can be deduced.<sup>11,12</sup> In Table I the values of  $T_c$  for fractions of polyisobutylene in ethyl *n*-heptanoate are given and the data are plotted in Fig. 1 according to the suggestion of equation 14. A linear plot results and  $\Theta$  is

found to be  $33 \pm 1^\circ$  which is in very good agreement with the previous estimate of Fox and Flory.<sup>11</sup>

TABLE I  
CRITICAL MISCIBILITY TEMPERATURE OF POLYISOBUTYLENE  
FRACTIONS IN ETHYL *n*-HEPTANOATE

Fraction	$M_v$	$M_w$	$T_c, ^\circ\text{C.}$
D	78,500	85,000	5.0
B	268,000	278,000	16.2
E	215,000	...	15.6
A	560,000	588,000	22.6
F	2,830,000	...	28.3
C	...	131,000	...

Exploratory experiments utilizing the equilibrium ultracentrifuge were performed with this system in the temperature range of 32–34° and as a result of these measurements the  $\Theta$  temperature was taken as 34°. The imposition of  $\Theta$  conditions does not prevent the attainment of sedimentation equilibrium in a reasonable length of time; five to twelve days were usually required for the system studied here. Sedimentation equilibrium experiments were then made under these conditions with four of the polymer fractions over a wide initial concentration range for each. Weight average molecular weights were calculated from the data by means of equation 13 and the results are summarized in Table II. These calculations are, of course, performed without any regard to the polydispersity of the sample. For each of the fractions, the values of  $M_w$  that are obtained are independent of the initial concentration of polymer. The absolute value of the molecular weight of each fraction must of course reflect the uncertainty in the factor  $(1 - \bar{v}\rho)$ . Since for the system under consideration this quantity is equal to 0.053, a 1% error in the determination of  $\bar{v}$  can result in a 10% error in the molecular weight. However, any corrections from this cause would be uniformly applied to all molecular weights.

TABLE II  
 $M_w$  CALCULATED FROM EQUATION 13 FOR FRACTIONS OF  
PIB IN ETHYL *n*-HEPTANOATE AT 34.0°

Fraction A		Fraction C	
co, g./dl.	$M_w$	co, g./dl.	$M_w$
0.122	604,000	0.5011	133,000
.122	594,000	.8150	130,000
.229	583,000	.9696	133,000
.308	578,000	1.1695	128,000
.381	582,000		
av.	588,000 ± 4,000	av.	131,000 ± 1,000
Fraction B		Fraction D	
0.269	272,000	0.605	84,400
.302	279,000	0.879	83,400
.425	278,000	1.236	87,000
.555	276,000	1.5116	83,000
.636	282,000	1.8178	86,000
av.	278,000 ± 2,000	2.0600	85,700
		2.3882	85,000
		av.	85,000 ± 500

The deviation of molecular weights of each of the fractions at different concentration is small and

(17) W. R. Krigbaum and P. J. Flory, *J. Am. Chem. Soc.*, **75**, 5254 (1953).

(18) W. R. Krigbaum, *ibid.*, **76**, 3758 (1954).

well within the experimental error. These results, obtained under  $\Theta$  condition, are the theoretically expected ones and satisfy the condition for ideal solution behavior. For ideal solution the determination of the weight average molecular weight is, from a theoretical point of view, extremely simple. Extrapolation to infinite dilution is unnecessary since the molecular weight can be determined from an experiment involving only a single concentration. The thermodynamic analysis which allows the molecular weight to be calculated is almost exact; the only approximations are those pertaining to the solution concentration which introduce negligible error. Experiments performed in this manner yield absolute weight average molecular weights. Thus when sedimentation equilibrium experiments are performed under these conditions important and useful unambiguous information can be obtained. Though attainment of ideal behavior in the equilibrium centrifuge has only been demonstrated for a specific polymer-solvent system at an appropriate temperature there is no reason to expect that similar studies cannot be carried out with other polymer-solvent systems. If more heterogeneous polymeric solutes are studied under these conditions, then higher molecular weight averages could be obtained by methods of analysis previously described.<sup>15,19</sup> In practice, the determination of the higher molecular weight averages, such as  $M_z$  and  $M_{z+1}$ , require accurate values of the scale line displacement at the extremities of the cell. The optical system used with the centrifuge does not allow the direct determination of  $Z$  at  $x_a$  and  $x_b$ ; these quantities are usually determined by extrapolation.<sup>20,21</sup> For very polydisperse solutes this fact makes the reliable determination of the molecular weight averages greater than  $M_w$  difficult even under ideal conditions, but does not prevent the determination of  $M_w$ .

**Non-ideal Solutions.**—The results that have been obtained under " $\Theta$  conditions" can be immediately used to investigate the applicability of this experimental technique to the study of non-ideal solutions. In discussing the sedimentation equilibrium of non-ideal polymer solutions it is advisable to divide the discussion into two portions: One part deals with the problem of a monodisperse solute while the other portion considers the case where the solute is heterogeneous with respect to molecular weight.

For these purposes it is convenient to expand the osmotic pressure, at point  $x$  in the centrifugal field, in virial form. Thus

$$(\pi/c_x)^x = RT [A_1^{(x)} + A_2^{(x)}c_x + A_3^{(x)}c_x^2 + \dots] \quad (15)$$

If we consider first a monodisperse solute, then  $A_1$  can be set equal to  $1/M$  and the superscript  $x$  can be omitted from the higher virial coefficients since the molecular weight will be uniform throughout the entire centrifuge cell. Equation 15 can also be written in an alternative form<sup>3</sup>

(19) M. Wales, *THIS JOURNAL*, **52**, 235 (1948).

(20) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford, 1940, p. 319.

(21) J. B. Nichols and F. D. Bailey, Chapter XIII in "Physical Methods of Organic Chemistry," Vol. I, Part I, Edited by A. Weissberger, Interscience Publishers, New York, N. Y., 1949.

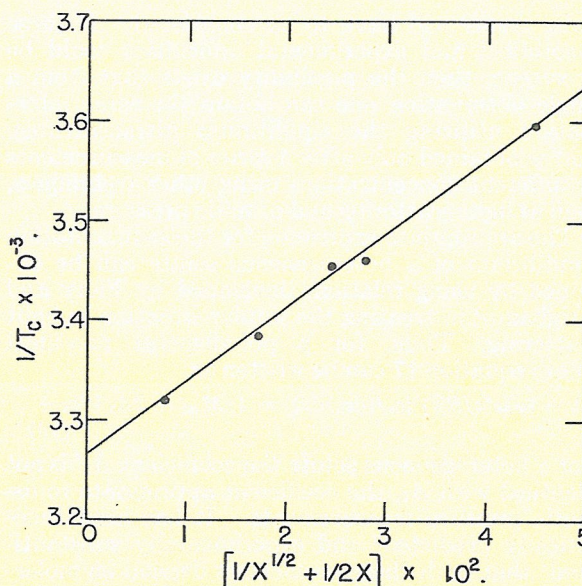


Fig. 1.—Plot of the reciprocal of the critical temperature against the molecular size function of equation 18 for polyisobutylene fractions in ethyl *n*-heptanoate.

$$(\pi/c_x)^x = (RT/M) [1 + \Gamma_2 c_x + \Gamma_3 c_x^2 + \dots] \quad (16)$$

where  $\Gamma_2 = MA_2$  and  $\Gamma_3 = MA_3$ . Relating the third virial coefficient to the second by the expression<sup>22</sup>  $\Gamma_3 = g\Gamma_2^2$  we obtain the following relation valid at any point  $x$  for a single solute component

$$((1 - \bar{v}\rho)\omega^2 x / RT) c_x / (dc_x/dx) = (1/M) [1 + 2\Gamma_2 c_x + 3g\Gamma_2^2 c_x^2 + \dots] \quad (17)$$

In good solvents  $g$  will have the value of about one third so that equation 17 becomes

$$((1 - \bar{v}\rho)\omega^2 x / RT) c_x / (dc_x/dx) \cong (1/M) [1 + \Gamma_2 c_x]^2 \quad (18)$$

Thus if  $dc_x/dx$  and  $c_x$  are known as a function of  $x$ , a point by point analysis can be made of a single experiment and the molecular weight and virial coefficients obtained.<sup>23</sup> By appropriately integrating the experimentally determined  $(dc_x/dx)$  against  $x$  curve, the values of  $c_x$  can be obtained. However, extremely accurate values of  $dc_x/dx$  are required for this purpose and the conditions of the experiments must be such that low values of  $dc_x/dx$  are avoided, otherwise the quantity of interest  $c_x/(dc_x/dx)$  will be unreliable. A further difficulty presents itself in that one does not know *a priori* how many terms in the virial expansion need be considered. In light scattering or osmotic pressure measurements, solutions sufficiently dilute can be studied so that the consideration of only the first three virial coefficients is usually adequate. However, in the equilibrium ultracentrifuge, for comparable initial concentrations, the mass of solute distributes itself along the length of the cell so that the concentration near the bottom of the cell may be two to three times the initial concentration. Thus at each point in the cell a different number of

(22) W. H. Stockmayer and E. F. Casassa, *J. Chem. Phys.*, **20**, 1560 (1952).

(23) Alternatively equation (17) can be integrated over the length of the centrifuge cell and the molecular weight and virial coefficients determined from the resulting equations.

virial terms will have to be considered. If these theoretical and experimental difficulties could be overcome, then the possibility exists that from a single observation one can obtain the same information utilizing the equilibrium ultracentrifuge that is obtained only after a series of measurements at different concentrations using other techniques, such as light scattering and osmotic pressure.

The appropriate expression for the sedimentation equilibrium of a heterogeneous solute can be obtained by using relations developed by Flory and Krigbaum<sup>3</sup> in treating the similar situation in light scattering. Thus, for a polydisperse non-ideal solute equation 17 can be written as

$$[(1 - \bar{v}_p)\omega^2 x/RT] [c_x/(dc_x/dx)] = 1/M_{wx} + 2A_2'^{(x)}c_x + \dots \quad (19)$$

For a heterogeneous solute the coefficient  $A_2'$  is not identical with  $A_2$ , the coefficient appropriate to osmotic pressure measurements. It has been theoretically predicted<sup>3</sup> and experimentally substantiated<sup>4</sup> that both these coefficients depend on molecular weight and molecular weight heterogeneity, though each in a different manner. Equation 19 is only valid at point  $x$ , and to determine the weight average molecular weight of the sample the integration indicated by equation 12 must be performed. However, since the distribution of molecular weights must vary with  $x$ , the term  $A_2'^{(x)}$  must in general also vary with  $x$ . Thus to perform the necessary integration, the dependence of  $A_2'^{(x)}$  on molecular weight and molecular weight distribution must be specified. However, the present theoretical and experimental understanding of the thermodynamic behavior of polymer solutions, does not allow the appropriate expression to be formulated in any tractable form. Hence, the necessary integration to obtain the weight average molecular weight of the polymer under these experimental conditions cannot be accomplished. From these considerations we conclude that unambiguous information cannot be obtained from a study of the sedimentation equilibrium of a non-ideal solution of polydisperse long chain molecules.

It has been suggested that the condition that  $A_2'$  must vary with molecular weight and molecular weight distribution can be relaxed. Thus procedures have been developed for treating the sedimentation equilibrium of non-ideal polydisperse polymer solutions by assuming  $A_2'$  is independent of  $x$  in these cases. Under these conditions equation 19 can then be written as<sup>19</sup>

$$1/M_{wx}^\circ = 1/M_{wx} + 2A_2'c_x \quad (20)$$

where  $M_{wx}^\circ$  represents a "molecular weight" at  $x$ , calculated as if the solution behaved ideally at this point, *i.e.*, the molecular weight calculated according to equation 13. Following the treatment of Williams and Van Holde<sup>5</sup> equation 20 is written as

$$M_{wx} = M_{wx}^\circ / (1 - 2A_2'c_x M_{wx}^\circ) \quad (21)$$

If the denominator is expanded in a power series

$$M_{wx} = M_{wx}^\circ [1 + 2A_2' M_{wx}^\circ c_x + 4A_2'^2 M_{wx}^\circ c_x^2 + \dots] \quad (22)$$

By multiplying each term in equation 22 by  $c_x dx$  and integrating over the extremities of the centrifuge cell there is obtained

$$1/M_w^\circ \cong 1/M_w + \frac{2A_2}{c_0/2(x_b^2 - x_a^2)M_w^\circ} \left[ \int_{x_a}^{x_b} M_w^\circ c_x^2 dx \right] \times \left\{ \frac{1 + 2A_2 \int_{x_a}^{x_b} M_w^\circ c_x^3 dx}{\int_{x_a}^{x_b} M_w^\circ c_x^2 dx} \right. \\ \left. \frac{1 + 2A_2 \int_{x_a}^{x_b} M_w^\circ c_x^2 dx}{\int_{x_a}^{x_b} M_w^\circ c_x dx} \right\} \quad (23)$$

By assuming that the term in the brackets is approximately unity Van Holde and Williams<sup>5</sup> obtained

$$1/M_w^\circ = 1/M_w + \frac{2A_2}{c_0/2(x_b^2 - x_a^2)M_w^\circ} \int_{x_a}^{x_b} M_w^\circ c_x^2 dx \quad (24)$$

or

$$1/M_w^\circ = 1/M_w + 2A_2 c'_0 \quad (25)$$

or where  $c'_0$  is a new concentration variable defined as

$$c'_0 = [2/(c_0(x_b^2 - x_a^2)M_w^\circ)] \int_{x_a}^{x_b} M_w^\circ c_x^2 dx$$

For the concentration and molecular weights studied here  $c'_0$  exceeds  $c_0$  by about 15 to 30%. In this procedure only the second virial coefficient has been considered and its dependence on molecular weight and molecular weight distribution has been neglected. Furthermore, the series expansion of equation 21, which leads to equation 22, tacitly assumes a low value of the virial coefficient, since effectively only the second term is retained. On the other hand equation 25 offers the advantage that the weight average molecular weight of the solute is very simply expressed in terms of quantities readily obtainable from the experimental data. It is thus important to assess the validity or limitations of this analysis. To study this point, sedimentation equilibrium experiments were undertaken using a good solvent, isoöctane, with the same polymer fractions previously studied under "Θ conditions."

The data obtained from these studies are shown in Fig. 2 where according to equation 25 the reciprocal of  $M_w^\circ$  is plotted against  $c'_0$ . When the data are plotted in this manner straight lines result and if the analysis is correct the intercept at  $c'_0 = 0$  should represent the reciprocal of the weight average molecular weight. However, the horizontal dashed lines in this figure represent the results obtained for the same polymers under ideal solution conditions (the data of Table II), where the actual molecular weight is, of course, independent of concentration. The molecular weights obtained in the good solvent and the "Θ solvent" differ by about 25% in the higher molecular weight range. As the true molecular weight decreases this discrepancy becomes less and for a molecular weight of 85,000 the two results are virtually identical. Thus for low molecular weights, where the deviations from ideality will be less severe, the above analysis seems to be adequate. We therefore conclude that the suggested method of treating non-ideal solutions, equation 25, would be inaccurate except when ap-

plied to solutions of low molecular weight solutes. Other methods<sup>15,19</sup> suggested to treat this problem are similar in principle to the one just described and offer no substantial improvement in results.

The difficulties involved in treating the sedimentation equilibrium in good solvents can be realized if results with the same solute in good solvents and under ideal solution conditions are treated as though they behave as ideal two component system.<sup>5</sup> Lansing and Kraemer,<sup>24</sup> starting with equation 12, have derived an expression that is applicable to an ideal two component system. Their results are

$$\ln(Z/x) = \ln(Z/x)_a + (M(1 - \nu\rho)\omega^2/2RT)(x^2 - x_a^2) \quad (26)$$

Thus for an ideal two component solution a plot of  $\ln(Z/x)$  against  $x^2$  should be linear and the slope is directly related to the molecular weight. It has already been shown that weight average molecular weights can be determined unambiguously from equation 13 without having to assume that the solute consists of only one species. Thus a plot according to equation 26, of the data obtained under  $\Theta$  conditions will yield information as to the polydispersity of the solute since the slope at any point  $x$  is representative of the molecular weight, calculated as though the solute were monodisperse. For the experiments involving polyisobutylene in ethyl *n*-heptanoate at 34° straight lines should result if the fractions are monodisperse; deviations from linearity will be due solely to polydispersity. Once information regarding the polydispersity of the solute is obtained, treatment of experiments performed in isoöctane according to equation 26 should yield qualitative information as to the effect of deviations from ideality on the apparent molecular weight. It must be realized that non-ideal behavior will give an apparent molecular weight at any point which will be less than the true molecular weight of the solute.

Representative data obtained in both ethyl *n*-heptanoate and isoöctane for two different concentrations of each fraction are plotted in Fig. 3 according to equation 26. For illustrative purposes, apparent "ideal molecular weights" were calculated from the slopes at two points, the meniscus and at the bottom of the cell. These results are summarized in Table III. For fraction A straight lines result in these plots for the experiments performed under  $\Theta$  conditions (Fig. 3a). Since the slopes of these lines yield a molecular weight of 570,000, which is in excellent agreement with that previously deduced from equation 13, we can conclude that this sample is monodisperse as far as observations in the equilibrium ultracentrifuge are concerned. The results for the same solute in isoöctane are given in Fig. 3a, and curves with gradually decreasing slopes are obtained, indicating that the "apparent" ideal molecular weight is continuously decreasing. The initial apparent molecular weight is about 460,000 and gradually decreases to about 200,000. These observations can be explained by the fact that the concentration increases as the distance from the center of rotation

(24) W. D. Lansing and E. O. Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935).

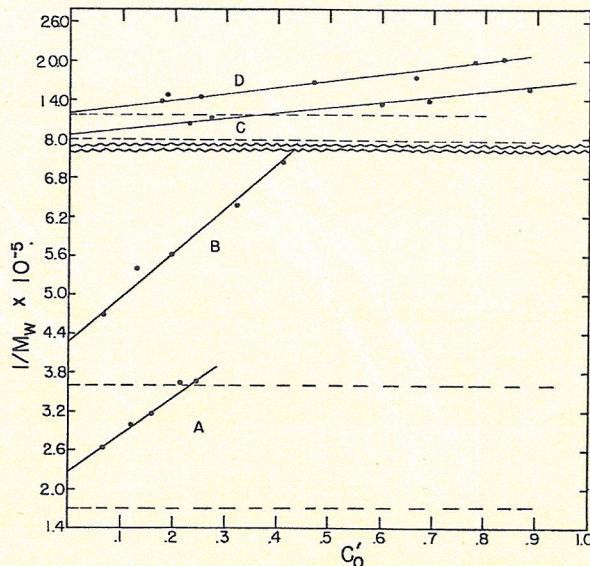


Fig. 2.—Plot of the reciprocal of the weight average molecular weight, calculated as though the solution were ideal, against the concentration variable  $c_0'$ . Solid lines for polyisobutylene fractions in isoöctane at 25°; dashed lines for same fractions in ethyl *n*-heptanoate at 34°.

increases. Since the non-ideality contribution to the total potential at any point is greater, the

TABLE III

APPARENT IDEAL MOLECULAR WEIGHTS AS DEDUCED FROM FIGURE 3 ACCORDING TO EQUATION 26<sup>a</sup>

Fraction		In ethyl <i>n</i> -heptanoate 34°	Isoöctane, 25°
A	(a) Meniscus	570,000	460,000
	Base		193,000
	(b) Meniscus	578,000	470,000
	Base		206,000
B	(a) Meniscus	296,000	272,000
	Base		163,000
	(b) Meniscus	306,000	268,000
	Base		159,000
D	(a) Meniscus	63,000	60,500
	Base	173,000	
	(b) Meniscus	60,000	59,000
	Base	173,000	

<sup>a</sup> (a) Results at lower concentration. (b) Results at higher concentration.

greater the concentration, it will increase with increasing  $x$ . Therefore the apparent ideal molecular weight must be continually decreasing. The non-ideality contribution is appreciable in this case and these results illustrate the difficulties in trying to determine the true molecular weight from experiments in good solvents by calculating apparent ideal molecular weights at finite concentrations even when polydispersity complications are absent. Since fraction A behaves as a monodisperse solute, then the true molecular weight and higher virial coefficients should be obtainable from the experiments in isoöctane by use of equation 17 or 18. Unfortunately in these experiments, the scale line displacements at the low values of  $x$  were relatively small. Although this presents no difficulty in evaluating integrals of the type



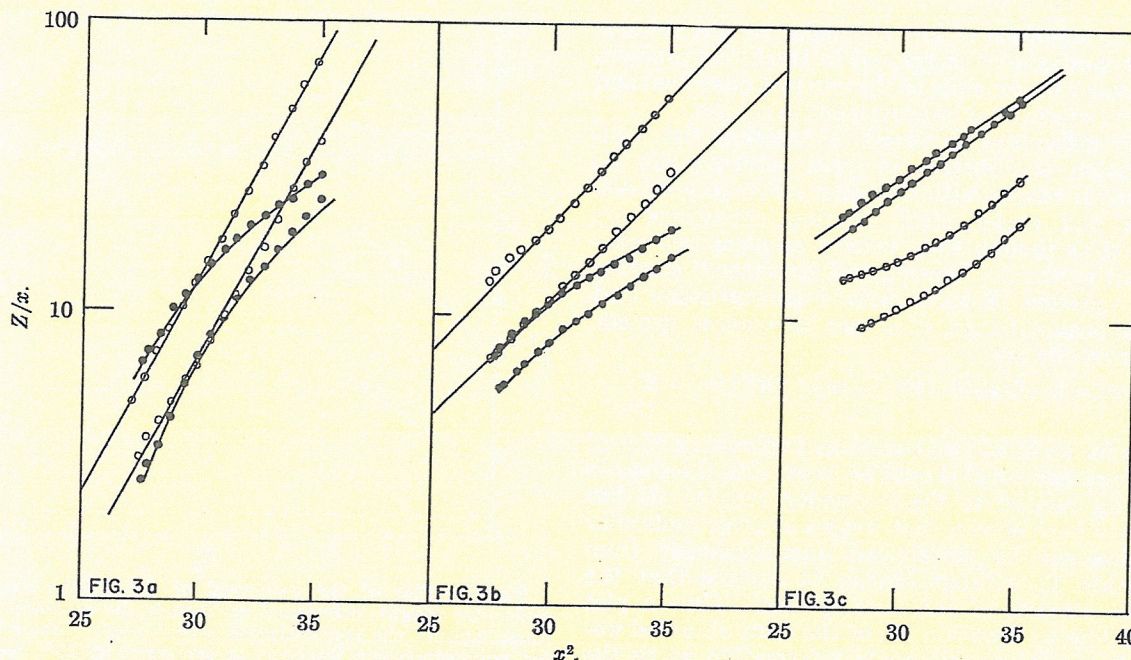


Fig. 3.—Plot of  $\log(Z/x)$  against  $x^2$ . *a* for fraction A, in ethyl *n*-heptanoate at 34° O; upper curve  $c_0 = 0.229$  g./dl.; lower curve,  $c_0 = 0.122$  g./dl. in isoöctane at 25° ●; upper curve  $c_0 = 0.214$  g./dl.; lower curve  $c_0 = 0.118$  g./dl. *b* for fraction B, in ethyl *n*-heptanoate at 34° O; upper curve  $c_0 = 0.555$  g./dl.; lower curve  $c_0 = 0.302$  g./dl. In isoöctane at 25° ●; upper curve  $c_0 = 0.285$  g./dl.; lower curve  $c_0 = 0.235$  g./dl. *c* for fraction D, in ethyl *n*-heptanoate at 34° O; upper curve  $c_0 = 0.879$  g./dl.; lower curve  $c_0 = 0.605$  g./dl. In isoöctane at 25° ●; upper curve  $c_0 = 0.778$  g./dl.; lower curve  $c_0 = 0.614$  g./dl.

$$\int_{x_a}^{x_b} (dc/dx)_x dx$$

for use with equation 23, it is extremely difficult to obtain accurate values of the ratio  $c_x/(dc_x/dx)$  as a function of  $x$ , which is required for the correct evaluation of  $M$  and  $\Gamma_2$  according to equation 18.

Similar results are obtained when the data for fraction B,  $M_w$  278,000, are treated in this manner (Fig. 3b). Although under “ $\Theta$  conditions” the data can be fitted by a straight line, the molecular weight calculated from the slope of this line is slightly higher than the true molecular weight; the curve is probably slightly concave upward, indicative of a small amount of polydispersity. In the good solvent the data can be interpreted as for fraction A.

The results for fraction D, plotted in Fig. (3c), are quite different. For experiments in ethyl *n*-heptanoate at 34° straight lines do not result which can only mean that the solute is polydisperse. The “apparent ideal molecular weights” vary from 60,000 to 173,000, the higher molecular species occurring at the higher values of  $x$ , *i.e.*, in the direction of sedimentation. The weight average molecular weight of this sample is 85,000 which is consistent with the above observations. In the good solvent, surprisingly enough, straight lines do result and give an apparent molecular weight of 60,000. For this polydisperse solute at the higher  $x$  values, with its greater solute concentration and greater concentration of higher molecular weight species, the deviations from ideality are sufficient to give the same apparent ideal molecular weight that is obtained at lower values of  $x$  where for the lower molecular weight species and concentrations

involved the deviation from non-ideality should not be severe. However, even though straight lines are obtained when the data in a good solvent are treated in the manner described, the apparent molecular weight obtained is still appreciably lower than that obtained by the correct method.

### Conclusions

From the preceding discussion and consideration of experimental results it is clear that ideal solution behavior of flexible chain molecules can be attained in the equilibrium ultracentrifuge. An unambiguous and simple analysis can then be made of the experimental data. Thus the weight average molecular weight of a polymeric solute can be obtained without regard to its heterogeneity. The heterogeneity of a polydisperse polymeric solute is then describable by the relations previously developed.<sup>6</sup>

The difficulties of treating such experimental data obtained in non-ideal solution have been discussed and the inadequacies of the present empirical method of treatment have been indicated. On the other hand for a monodisperse polymeric solute, the possibility exists that the equilibrium centrifuge could be an important tool in studying the thermodynamic behavior of polymer solutions.<sup>25</sup> Verification of this latter point must await further experimental investigation.

NOTE ADDED IN PROOF.—Recent theoretical considerations of the thermodynamics of dilute polymer solutions by Orifino and Flory<sup>26</sup> indicate the possibility that the temperature at which the second virial coefficient vanishes might

(25) M. Wales, *J. Applied Phys.*, **22**, 735 (1951).

(26) T. A. Orifino and P. J. Flory, presented before 130th Meeting of the American Chemical Society, Atlantic City, N. J., September, 1956.

depend slightly upon the polymer molecular weight. Such an effect would not, however, significantly alter any of the major conclusions of this paper, which are concerned with much greater deviations from ideality.

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