employing the equation of Philippoff and Gaskins.[39] The conclusion to be drawn is that the flow curves of Figure 4 may be in slight error if the end corrections are nonnegligible, and in this case the samples 2a and 8a would be slightly more non-Newtonian.

Acknowledgments. The author wishes to express his thanks to Mr. Allan F. Butcher for his painstaking care in obtaining the data discussed in this report. Professor Donald J. Plazek's helpful discussions and suggestion of the origin of the anomalous die swell are gratefully acknowledged.

Analysis of Polydisperse Systems at Sedimentation Equilibrium. II. Single Components in Mixed Solvents[1]

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ABSTRACT: A mathematical function is proposed for the description of preferential interactions of macromolecules with the components of mixed solvents. The function is highly flexible, permitting sufficient scope to encompass many of the forms of interaction previously reported experimentally. Based on this model, an analytical procedure is developed for determining the molecular weight of a polymer and the nature of its interaction with the solvent by means of sedimentation equilibrium studies. Whereas prior applications of the analytical ultracentrifuge to this problem required the assumption of constant preferential interactions, this approach is not so constrained but nevertheless can recognize this effect when it occurs. Although the method has not yet been studied for heterogeneous systems, it is designed to include this possibility as well.

In the first paper of this series,[2] an analytical procedure was presented which had the ability to determine the molecular weights of several independent macromolecular components in simple solvent systems. Such systems are, however, of very limited use in the real world and, accordingly, it is necessary to establish the potential for a good analytical system to be able to operate on realistic solvent systems. The complexity of reality arises in several different ways, of which one, preferential interactions, will be considered in detail in this paper. This phenomenon is a result of the differential binding by a macromolecule of the components of a multicomponent solvent.

The concept of preferential interactions must be distinguished from the more readily appreciated phenomenon of specific binding. The particular type of binding involved is not of direct consequence, nor is the actual magnitude of the binding. What is of concern is the extent to which the composition of the solvent in the immediate environment of the macromolecule is different from the bulk solvent. There, in fact, may be no specific binding as such, but rather a less clear form of intermediate range interaction. Nevertheless, the phenomenon is of great significance.

It has been demonstrated that whenever multicomponent solvents are employed in experiments intended to determine molecular weights by sedimentation techniques, these interactions must be allowed for.[3,4] Several workers[3-7] have also demonstrated the contribution of this phenomenon to light scattering. Thus, it is clear that when determining molecular weights this effect is important. It has also been suggested[4,7] that studies of preferential interactions may be significant toward the understanding of the interplay between solvent composition and protein structure.

There are general treatments of the concept of preferential interactions,[3,4,8] as well as specific experimental considerations of how to estimate their sign and magnitude.[4,7,9-13] For several reasons, generally recognized by their initial proponents, those methods based primarily on the techniques of sedimentation velocity and sedimentation equilibrium are only valid for limited systems in which the samples are homogeneous and the preferential interactions do not depend on the concentration of the cosolute. These techniques really should not be applied when the system under study might not be described within these constraints.[14] In this paper a procedure is presented which, through the use of a generalized computational algorithm, correlates the measured buoyant weights, the solution densities, and the solvent composition to provide estimates for both the molecular weights of the macromolecular components and the preferential interactions between these components and the cosolute as a function of the cosolute concentration. In this initial treatment only solutions of one macromolecular component in two-component solvents will be considered.

Theory

Most of the published considerations of preferential interactions have been based on the general principles of solution thermodynamics. As such, they have been very useful in the development of experimental approaches to the measure-

(1) Presented in part at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 1971.
(2) A. Rosenthal, *Macromolecules*, **4**, 35 (1971).
(3) E. F. Casassa and H. Eisenberg, *Advan. Protein Chem.*, **19**, 287 (1964).
(4) A. Rosenthal, Doctoral Dissertation, University of California at Berkeley, 1969.
(5) J. G. Kirkwood and R. J. Goldberg, *J. Chem. Phys.*, **18**, 54 (1950).
(6) W. H. Stockmayer, *ibid.*, **18**, 58 (1950).
(7) S. N. Timasheff and H. Inoue, *Biochemistry*, **7**, 2501 (1968).
(8) V. N. Schumaker and D. J. Cox, *J. Amer. Chem. Soc.*, **83**, 2445 (1961).
(9) E. Reisler and H. Eisenberg, *Biochemistry*, **8**, 4572 (1969).
(10) M. E. Noelken, *ibid.*, **9**, 4122 (1970).
(11) E. P. Kirby Hade and C. Tanford, *J. Amer. Chem. Soc.*, **89**, 5034 (1967).
(12) J. B. Ifft and J. Vinograd, *J. Phys. Chem.*, **70**, 2814 (1966).
(13) H. K. Schachman, "Ultracentrifugation in Biochemistry," Academic Press, New York, N. Y., 1959.
(14) The method discussed by Reisler and Eisenberg[9] is not quite so constrained.

ment of this quantity. Unfortunately, this approach has no possibility of suggesting the sign, magnitude, or variability of the interactions or even for relating them to secondary parameters, such as solvent composition, which might be used to define a functional relation which could describe them. In the development of this analytical system, the above approach was continued in part, *i.e.*, a generalized function was sought after without any initial assumptions regarding the specific nature of the interactions.

Some general properties were, however, accepted as essential to a useful function. The first consideration was the ability to reproduce the data form most typically reported as the result of observations in the ultracentrifuge. These studies have most typically suggested that the preferential interactions, expressed as grams of solvent or cosolute per gram of macromolecule, were, in fact, constant.

A secondary requirement was the scope to represent nonconstant interactions of the types that have been reported by Timasheff and coworkers,[7] and by Noelken[10] using refractive index measurements. Similar forms were produced by Reisler and Eisenberg[9] for aldolase. This requirement is basically for preferential binding of cosolute which initially increases with increasing cosolute concentration, but which, at higher concentrations, reverses and may even change sign to indicate binding of solvent.

There are many classes of functions which could lead to these relationships. The simplest and most obvious is a three-term (quadratic) polynomial. This class of function can have the basic properties desired. It did not, however, prove satisfactory. The quality of the available data proved inadequate for a function with three coefficients. The next function considered was a rational fraction. To achieve all of the desired effects this function also required a third coefficient and again reasonable approximations regarding the precision of the available data did not permit accurate analysis of three coefficients. However, the results were better with this function than with a polynomial. A modification of the rational fraction was attempted. Two of the coefficients, the quadratic coefficient in the numerator and the linear coefficient in the denominator, were set equal but opposite in sign. The rational fraction initially employed represented the preferential interaction as

$$\left(\frac{\partial w_3}{\partial w_2}\right)_{T,\mu} = \frac{K_1' w_3 - K_2 w_3^2}{1 + K_2 w_3} \qquad (1)$$

in which w_2 is the concentration of the second component in grams per gram of primary solvent, w_3 is the concentration of component 3, K_1' and K_2 are constants, and μ indicates all diffusible components (1 and 3 in this case). Equation 1 can be rewritten

$$\left(\frac{\partial w_3}{\partial w_2}\right)_{T,\mu} = \frac{K_1 w_3}{1 + K_2 w_3} - \frac{w_3 + K_2 w_3^2}{1 + K_2 w_3} = \frac{K_1 w_3}{1 + K_2 w_3} - w_3 \qquad (2)$$

in which $K_1 = K_1' + 1$. Further, allowing for the behavior indicated in the literature for this parameter, it is reasonable also to constrain K_1 and K_2 to be positive. This is readily achieved if both are determined in the computational algorithm as the result of squaring a secondary coefficient.

Although eq 2 is not being suggested as anything more than a mathematical model, it is imperative to compare the forms of preferential binding which it suggests with those which have been reported from experimental observations. First, one can allow K_1 to equal zero, which will simply hold the interactions to a value of $-w_3$ or one may set K_2 to zero with K_1 varying from 0 to 1 to produce interactions between $-w_3$ and zero but always with a linear dependence on w_3. Secondly, K_1 can be allowed to exceed 1, thereby making the interactions positive but still linear in w_3. Finally, K_1 and K_2 can both be significant, in which case the dependence on w_3 becomes nonlinear.

It has been shown[4,15,16] that $(\partial w_3/\partial w_2)_{T,\mu}$ can be related to $(\partial w_1/\partial w_2)_{T,\mu}$ by

$$\left(\frac{\partial w_1'}{\partial w_2'}\right)_{T,\mu} = -\frac{1}{w_3}\left(\frac{\partial w_3}{\partial w_2}\right)_{T,\mu} \qquad (3)$$

in which w_1' is g_1/g_3 and w_2' is g_2/g_3. This permits consideration of a variation in the concentration of the solvent. Equation 3 is only rigorously true in the limit as w_2 approaches 0.

When one applies eq 3 to those systems where the preferential cosolute binding is negative and varies as a linear function of w_3, one finds that $(\partial w_1'/\partial w_2')_{T,\mu}$ is a positive constant. This is significant since, speaking quite generally, the majority of the ultracentrifuge studies performed by previous techniques have strongly suggested constant preferential binding of solvent.

It is also of interest to compare the results for the more complex variation produced by positive values of both K_1 and K_2 with the preferential interactions previously reported by nonultracentrifuge methods. The general forms produced by eq 2 are either uniform negative with constant or decreasing slope or initially positive with decreasing slope going to negative at higher values of w_3 (see Figures 2, 4, and 6 in the Results and Discussion section). Data of this type have been reported by Timasheff and coworkers[7,16] and by Noelken[10] using refractive index measurements. They are also consistent with the results of Reisler and Eisenberg.[9]

It is thus suggested that eq 2 is a reasonably general function which can depict preferential interactions of macromolecules in a broad spectrum of binary solvents. On the other hand, it is very definitely not suggested that later analysis of these coefficients can be seriously used to estimate specific binding constants. There are two distinct values to be gained from this estimate. First, it is required for the correct determination of molecular weights that this parameter be determined or allowed for by procedures detailed by Casassa and Eisenberg.[3] Second, it is reasonable to anticipate that comparison of preferential interactions with measurements of macromolecular conformation may help to unravel the present assortment of explanations of the factors governing the overall structures of biopolymers.

Computational Methods. The analysis of the radial distribution of concentrations presented in the first paper of this series[2] has been modified to allow for the effects of preferential interactions. Equation 1 of the first paper described the distribution of a macromolecular component in a noninteracting solvent rather than in the presence of preferential interactions. It therefore allowed the direct determination of the molecular weight.

It can be shown[3,4] that if the term $(1 - \bar{v}\rho)$ in the earlier paper is not modified when a mixed solvent is employed in order to correct for preferential interactions, an incorrect estimate for the molecular weight is probable. The expression that must be used instead[4] is $[(1 - \bar{v}_2\rho) + (\partial w_3/\partial w_2)_{T,\mu}(1 - \bar{v}_3\rho)]$. Since all of these parameters are assumed to be inde-

(15) S. N. Timasheff and M. J. Kronman, *Arch. Biochem. Biophys.*, **83**, 60 (1959).

(16) M. E. Noelken and S. N. Timasheff, *J. Biol. Chem.*, **242**, 5080 (1967).

pendent of both the protein concentration and the radial locus,[17] the modified equation can be integrated to produce

$$c_r = c_m \exp\left\{AM_2\left[(1 - \bar{v}_2\rho) + \left(\frac{\partial w_3}{\partial w_2}\right)_{T,\mu}(1 - \bar{v}_3\rho)\right](r^2 - r_m^2)\right\} \quad (4)$$

in which c_r is the concentration of the macromolecule at the radial locus r, c_m is its concentration at the meniscus, M_2 is the molecular weight of the polymer, $\bar{v}_2$ is its partial specific volume, and A is defined as

$$A \equiv \omega^2/2RT \quad (5)$$

in which ω is the rotational velocity in radians per second, T is the temperature in degrees Kelvin, and R is the gas constant. Equation 4 differs from eq 7 of the previous paper only in the inclusion of the dependence of the distribution on the preferential interactions.

Since the bracketed expression in eq 4 is an unknown, whereas its counterpart in the earlier treatment was a known, it is necessary to modify the computational algorithm of the first paper slightly. Rather than solving for M_2 directly, the program is altered to determine the buoyant weight, M_{obsd}, as

$$M_{obsd} = M_2\left[(1 - \bar{v}_2\rho) + \left(\frac{\partial w_3}{\partial w_2}\right)_{T,\mu}(1 - \bar{v}_3\rho)\right] \quad (6)$$

Once this parameter is determined for several solution compositions, it remains to resolve the contributions of M_2 and $(\partial w_3/\partial w_2)_{T,\mu}$. It is possible to do this by relating the buoyant weights calculated by the procedures outlined above to the buoyant weight predicted by considerations of preferential interactions. If eq 2 is used to replace $(\partial w_3/\partial w_2)_{T,\mu}$ in the above relation, one obtains

$$M_{obsd} = M_2\left[(1 - \bar{v}_2\rho) + \left(\frac{K_1 w_3}{1 + K_2 w_3} - w_3\right)(1 - \bar{v}_3\rho)\right] \quad (6a)$$

A function of the form of eq 6a has the same difficulty as the ultracentrifuge equation[2] in that it cannot readily be represented by a simple power series. Therefore, the same basic analytical procedure developed for the exponential ultracentrifuge equation[2] has been extended to provide a direct analysis for the molecular weight and for the two preferential binding coefficients of eq 6a.

The statistical factors are obviously not the same for this problem as they were for the general sedimentation problem. It does not seem reasonable to treat all of the data points evenly, since there are two distinct factors suggesting differing degrees of reliability. When the average value of M_{obsd} is determined, there is, obviously, a standard deviation to the average. The deviations are not equal, although generally they do not differ greatly from one value to the next. Also, the potential sources of error do not decrease significantly as M_{obsd} decreases, but rather remain approximately constant. In consequence, they are proportionately greater for small M_{obsd} than for large. A weighting factor which accounts for both of these aspects, the value of M_{obsd} divided by the standard deviation in M_{obsd}, was chosen as reasonable for these data.

Advantage has also been taken of the facility provided by this analytical approach, and discussed in detail in the previous paper,[2] to apply constraints to the coefficients evaluated. Since it seems reasonable to assume that the constants and the molecular weight be positive, all of the unknown coefficients of eq 6a were constrained to be positive or zero by the simple expedient of defining them to be the squares of secondary parameters which serve as the actual unknown coefficients in the solution of eq 6a.

Data Generation

The basic data generation scheme was exactly as described in the first paper[2] except for the addition of preferential interactions. A subroutine was added to the data generator which computed the preferential interactions for each concentration of solute according to eq 2. This same routine also used the defined values of the partial specific volumes of the components to compute the density of the solution according to the relation

$$\rho = (1 + \sum g_i)/(1 + \sum \bar{v}_i g_i) \quad (7)$$

These values of the preferential interaction, partial specific volumes, and the density were then used with eq 4 to compute the mass distribution throughout the ultracentrifuge cell. A pseudorandom error was introduced, as indicated in the previous paper.[2]

In the first paper it was demonstrated that results were acceptable over a broad range of experimental conditions, and no attempt was made to define these conditions. In the course of the present work, a purely empirical general test parameter was developed for one-component systems. The parameter basically considers the amount of useful information available in terms of the difference in concentration across the cell and the length of the cell containing usable data. The test can basically be described as accepting any experiments for which the observations fit the relation

$$(c_{max}/c_{min})(r_{max}^2 - r_{min}^2) \gtrsim 200 \quad (8)$$

in which c_{max} is the highest readable concentration; c_{min} is the lowest, but not less than the experimental uncertainty in a reading; r_{max} is the greatest radius at which data are accepted; and r_{min} is the radius of the first significant point after the meniscus. No work has been done as yet to properly test this function for more complex systems.

For convenience, data have been generated in three basic groups. The first considers dense cosolutes corresponding to salts. The second includes medium-density solutes with partial specific volumes in the range of urea and guanidinium chloride. The third set is for low-density substances of the alcohol or detergent class. Within each set the apparent binding parameters were varied to allow for constant preferential binding of primary solvent, constant exclusion of primary solvent, and generally variable solvent binding as was suggested earlier. The first two binding categories are considered in terms of linear concentration dependence of the solute interactions. These variations were accomplished by selecting the appropriate coefficients for eq 2.

Results and Discussion

Since each of the solvent conditions employed for each model was used in several hypothetical experiments at a variety of angular velocities, it was necessary to have some means of selecting the final value of M_{obsd} to be used for sub-

(17) The implicit assumption is being made at this point that the preferential interactions will not depend on the concentration of the macromolecule. Clark[18] and Kirby Hade and Tanford[19] have presented some evidence suggesting that this assumption is valid at least for proteins.

(18) J. B. Clark, Doctoral Dissertation, University of California at Berkeley, 1964.

(19) E. P. Kirby Hade and C. Tanford, *J. Amer. Chem. Soc.*, **89**, 5034 (1967).

TABLE I
DETERMINATION OF MOLECULAR WEIGHTS AND INTERACTION COEFFICIENTS

System	Input k_1	Input k_2	Return k_1	Return k_2	Return M_2
			$\bar{v}_3$[a] = 0.2		
1	2.0	0.0	2.03	0.018	100,010
2	2.0	2.0	2.04	2.08	99,828
3	4.0	8.0	4.03	8.05	100,100
4	10.0	18.0	9.60	17.2	101,380
			$\bar{v}_3$[b] = 0.7		
5	1.0	0.0	0.999	0.000	100,360
6	0.5	0.0	0.500	0.000	100,310
7	0.1	18.0	0.58	8.11	100,170
8	2.0	2.0	2.31	2.42	97,849
9	4.0	8.0	3.51	6.88	101,600
10[c]	4.0	8.0	3.72	7.26	100,600
11	10.0	18.0	10.7	19.2	99,612
12[c]	10.0	18.0	10.9	19.6	99,289
			$\bar{v}_3$ = 0.9		
13	2.0	0.0	1.99	0.000	100,290
14	1.0	0.0	1.002	0.005	100,110
15	0.5	0.0	0.478	0.001	100,280
16[d]	0.1	18.0	0.001	36.7	100,370
17[d]	0.01	18.0	0.000	26.5	100,680
18	2.0	2.0	2.17	2.10	99,592
19	4.0	8.0	3.55	7.27	100,810
20	10.0	18.0	10.4	18.0	99,585

[a] The data in the next four rows are for a simulated concentration range of $0.025–0.5g_3/g_1$. [b] The data in the remainder of the table are for a simulated concentration range of $0.05–1.0g_3/g_1$ unless otherwise indicated. [c] The data in this row are for a concentration range of $0.025–0.5g_3/g_1$. [d] The data in this row did not converge directly, but rather diverged completely in the subsequent iteration.

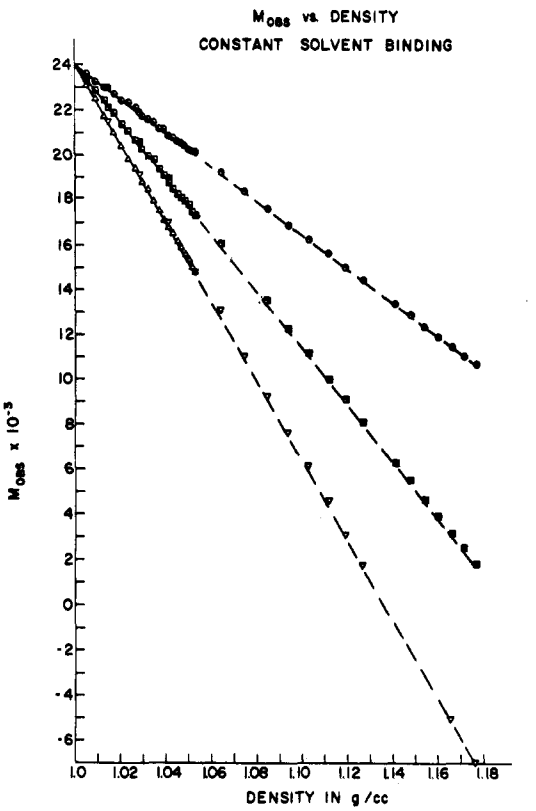

Figure 1. Buoyant mass *vs.* density for systems generated with constant preferential binding of primary solvent. Systems with $\bar{v}_3$ = 0.9 cm³/g include no preferential binding (●), preferential solvent binding of 0.5 (◥) and 1.0 (△). Systems with $\bar{v}_3$ = 0.7 cm³/g include no preferential binding (◑), preferential solvent binding of 0.5 (◥) and 1.0 (▽). The broken lines represent the theoretical curves.

sequent analysis. A weighted average of all of the values computed from experiments acceptable in terms of the test function of inequality 8 was developed. The weighting factor employed was the standard deviation in the representation of c *vs.* r^2. These averages were then utilized for the second stage of the analysis, where the molecular weight and interaction coefficients were determined. As was discussed above, it is not reasonable to assume that all of the values determined in the first stage are equally reliable. While there is no *a priori* basis for assigning a weighting factor, the percentage uncertainty in the determination of M_{obsd} not only seems to be an acceptable weighting factor, but, in fact, proved to be very well chosen. Data sets which failed to converge on any answers, let alone the proper one, without weighting, converged rapidly to the correct results when weighted by this parameter.

The results for all of the systems generated and analyzed are presented in Table I. In each case the molecular weight of the macromolecule was set at 100,000. The largest deviation, 2.151%, is seen in row 8, where a molecular weight of 97,489 was returned. While there are no distinct patterns to the results, some of the particular systems and their results are worth separate comment.

Rows 1, 5–7, and 13–17 are for systems involving constant interactions with the primary solvent, produced by a linear dependence of cosolute interaction on the concentration of the added cosolute. The 1st and 13th relate to the preferential exclusion of solvent, the 5th and 14th to zero interaction, and the others provide preferential interaction of 0.5 and 1.0 g of solvent per gram of polymer. It can be clearly seen that these situations are readily recognized with a good precision. It should be noted that the apparently poor return of the specific coefficients for preferential solvent binding of 1.0 g per gram of polymer is not significant. The net form of these coefficients is set to cause the first term on the right-hand side of eq 2 to approximate zero. While the actual solution is highly unstable and does not return a unique set of coefficients, it is very clear from the solution that this term is close to zero and that the value for M_2 is returned properly. The other constant-interaction terms are determined with a fairly high degree of precision.

Figure 1 shows the M_{obsd} *vs.* density curve used to simulate the data presented in rows 5–7 and 14–16 of Table I. Although the basic function and the computational approach treat directly with the cosolute interaction, it is possible to determine the interactions in terms of the primary solvent by means of eq 3. The hypothetical correct and the calculated interactions for the systems presented in Figure 1 are illustrated in Figure 2. The close comparison between the input and the output in both figures for this type of system shows that, even though the extreme preferential solvent binding leads to an unstable solution, the algorithm developed can treat systems of this nature. Since there already exists a large volume of ultracentrifuge data suggesting constant solvent interactions,[4,20,21] it is very important that this proce-

(20) D. J. Cox and V. N. Schumaker, *J. Amer. Chem. Soc.*, **83**, 2433 (1961).
(21) S. J. Edelstein and H. K. Schachman, *J. Biol. Chem.*, **242**, 306 (1967).

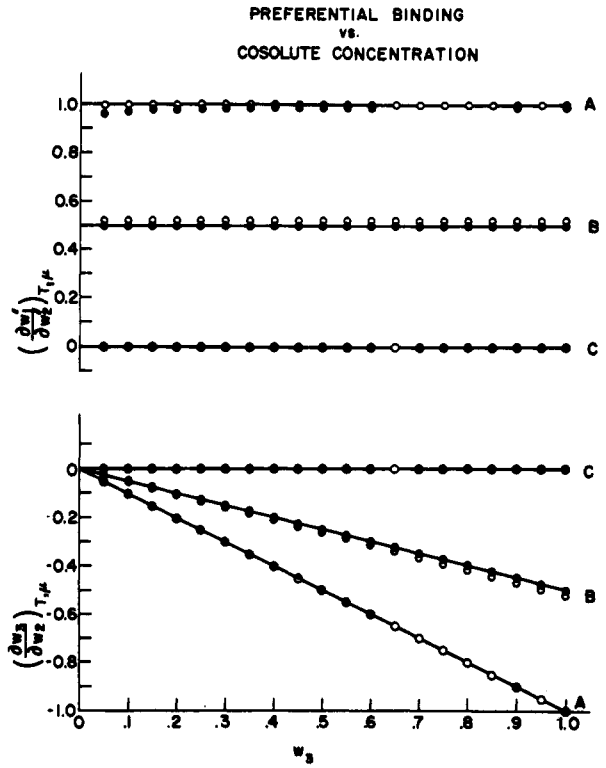

Figure 2. Preferential binding *vs.* cosolute concentration for systems generated with constant preferential binding of primary solvent. Preferential binding is presented in terms of primary solvent (upper scale) and cosolute (lower scale) for systems with constant interactions of 1.0 (A) and 0.5 g of solvent per gram of macromolecule (B) as well as no interactions (C). Values are shown for systems with $\bar{v}_3 = 0.7$ cm³/g (○) and 0.9 cm³/g (●). The solid lines represent the hypothetical binding pattern used to generate the data.

dure be able to handle data for this form, especially since it was not constrained to do so.

The systems presented in Figures 3 and 4 serve to illustrate the value of being able to analyze for constant interactions without having to constrain the system to that limited model. Curve D represents a linear dependence of cosolute binding on w_3 or constant preferential solvation. Accordingly, M_{obsd} *vs.* ρ should be a straight line. On the other hand, for curves A, B, and C of Figure 3, it is possible to select relatively long regions of the M_{obsd} *vs.* ρ function that appear linear. These curves are, however, not based on linear cosolute interactions and, therefore, do not have constant solvent binding. In curve C, for example, if only the solvent values with w_3 equal to or greater than 0.1 are considered, it would be quite easy to believe that M_{odsd} varied linearly with ρ. This illustrates the importance of having a fitting function that does not impose this bias, or at the very least, of testing for constancy of solution by some criterion more stringent than linearity.

If one were to assume curve C to be linear and use the conventional procedure for estimating the interactions from the extrapolated density value producing M_{obsd} equal to 0, it would be possible to compute the value of M_2 for each sedimentation experiment separately. By doing this, it would be made obvious that in order for $(\partial w_1'/\partial w_2')_{T,\mu}$ to be a constant, M_2 could not be. This test, suggested by the author,[4] should be applied any time the conventional constrained analysis is employed. If, on the other hand, one were to accept the extrapolated value of M_{obsd} at $\rho = 1.0$ g/cm³ as producing a correct value for $M_2(1 - \bar{v}_2\rho)$ exclusive of preferential binding effects,[21] the calculated value of M_2 would be erroneously high by more than 25%. Similar, though less dramatic, conclusions would issue from curves A and B as well, since these are also for nonlinear interactions but with a lesser concentration dependence.

One other difficulty of the conventional linear function interpretation is the general difficulty encountered in long extrapolations. Since the value of $1/\rho$ for which M_{obsd} goes to zero is required, it is necessary either to work at high cosolute concentrations or to make a dangerously long extrapolation. The results displayed in Figures 1, 2, 5, and 6 as well as the low-density values in Table I show clearly that the comparable problem does not plague the present method. For the system presented in row 3 of Table I, the high-density points were progressively eliminated to test the effect of not having them. When only the first seven points remained, with a density maximum of 1.1354 g/cm³, M_2 was still evaluated as 98,989 and K_1 and K_2 were 4.23 and 8.61, respectively. No further reduction was attempted, since it was felt that fitting three coefficients with six points was as meaningless as drawing a straight line between two points, and even if the system gave good results, they should not be considered as particularly significant. For all of the data sets with a $\bar{v}_3$ of 0.9 cm³/g, the maximum density created was 1.053 g/cm³. The systems presented in rows 18 and 20 of Table I were also treated with as few as 13 points each and were found to give essentially the same results presented in Table I.

Although all of the studies presented above deal with

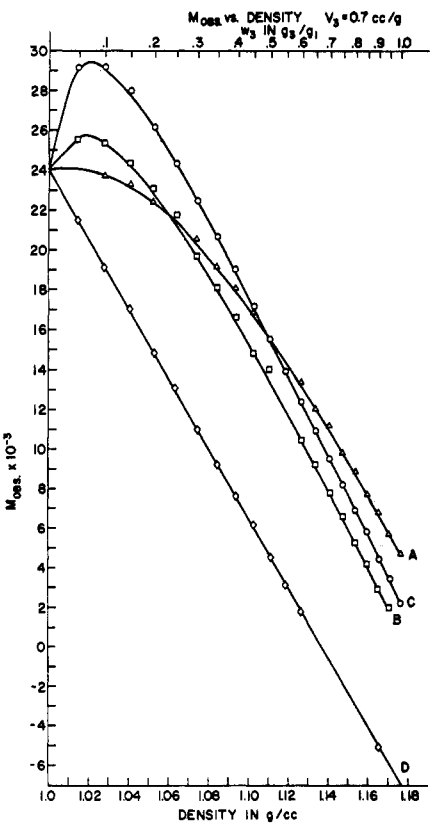

Figure 3. Buoyant mass *vs.* density for systems generated with $\bar{v}_3 = 0.7$ cm³/g and selected binding parameters: (A, △) data for $K_1 = K_2 = 2.0$, (B, □) $K_1 = 4.0$ and $K_2 = 8.0$, (C, ○) K_1 and $K_2 = 10.0$ and 18.0, respectively. Constant preferential solvent binding of 1.0 g per gram of macromolecule is presented in curve D (◇). The solid lines represent the hypothetical values from which the data were generated.

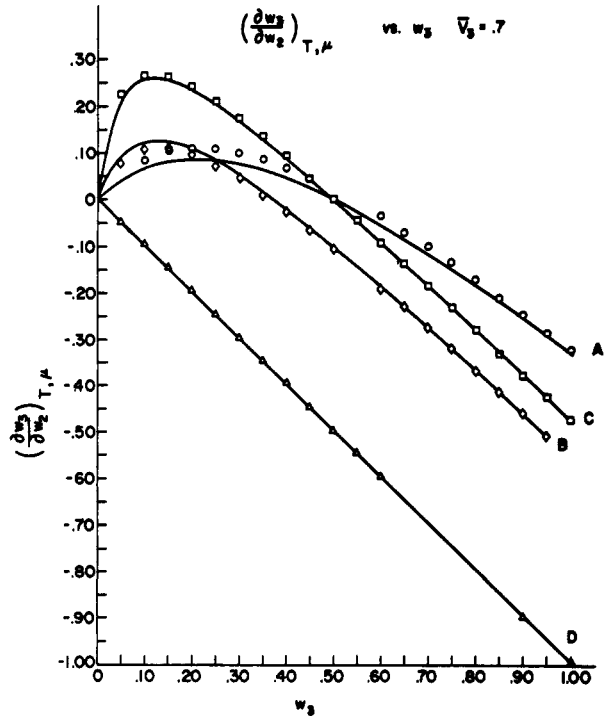

Figure 4. Preferential binding of cosolute *vs.* cosolute concentration for systems generated with a $\bar{v}_3$ equal to 0.7 cm³/g and selected interaction parameters: (A, ○) $K_1 = K_2 = 2.0$, (B, ◇) $K_1 = 4.0$ and $K_2 = 8.0$, (C, □) for K_1 and $K_2 = 10.0$ and 18.0, respectively. Constant preferential solvent binding of 1.0 g per gram of macromolecule is presented in curve D (△). The solid lines represent the hypothetical values used to generate the data.

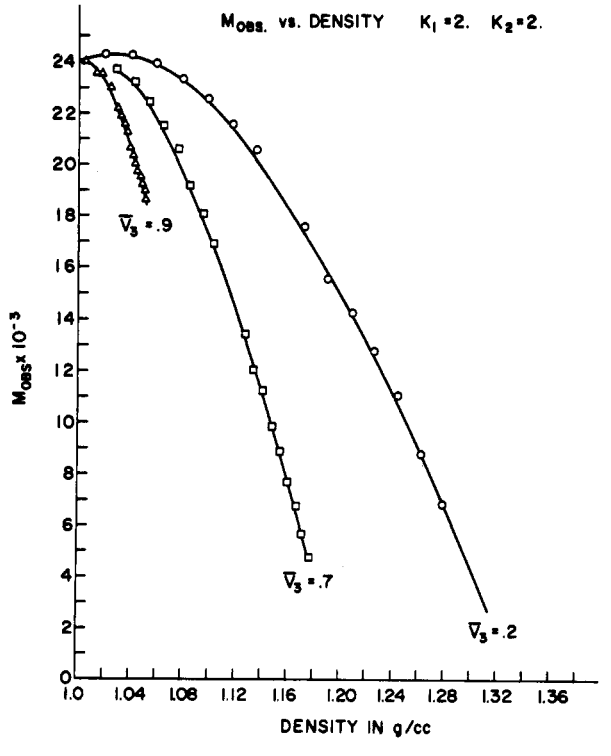

Figure 5. Buoyant mass *vs.* density for systems with K_1 and $K_2 = 2.0$ and $\bar{v}_3 = 0.2$ (○), 0.7 (□), and 0.9 cm³/g (△). The solid lines represent the hypothetical curves from which the data were generated.

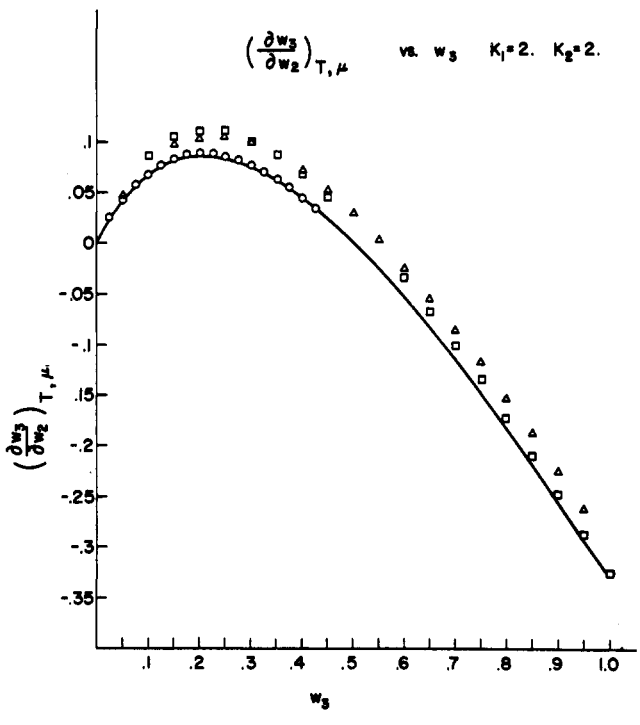

Figure 6. Preferential binding of cosolute *vs.* cosolute concentration for systems with $K_1 = K_2 = 2.0$ and with $\bar{v}_3 = 0.2$ (○), 0.7 (□), and 0.9 cm³/g (△). The solid line represents the hypothetical preferential interaction pattern for these coefficients.

homogeneous systems, the basic procedure is intended to deal with the more complicated case of polydispersity. At the present time only one four-component system, two macromolecules and a two-component solvent, has been generated for analysis. This system is based on a monomer of molecular weight 100,000 with 0.5 g of solvent preferentially bound per gram of monomer and a dimer, molecular weight 200,000, with no preferential interactions. The first return on this system showed molecular weights of 92,565 and 197,560 for the two components. While the first component is found to have a variable interaction, the total range is only from 0.37 to 0.47 g of solvent per gram of macromolecule. The analysis shows a preferential cosolute binding for the second component of less than 0.02 g per gram of macromolecule at the maximum. These preliminary results are indeed exciting and will be expanded at length in subsequent work.

Summary

It is evident that the analytical procedure presented above has the facility for simultaneously determining the molecular weight of a macromolecule and the nature of its interaction with the components of a binary solvent. Preliminary studies suggest that the evaluation of systems with two polymer species should also be amenable to analysis by this method. These results and the requisite correction factors for the study of nonideality are being pursued and will be presented subsequently. Fortran V listings (Univac 1108 Fortran code) are available from the author.

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