Thus if the scattering is reasonably described by eq A.4, we can derive m_0 from q_c using (A.5).

References and Notes

(1) Zsigmondy, R. *Anal. Chem.* **1901**, *40*, 697.
(2) Heller, W.; Pugh, T. *J. Chem. Phys.* **1954**, *22*, 1778.
(3) Mackor, E.; van der Waals, J. *J. Colloid Sci.* **1952**, *7*, 535.
(4) Koelmans, H.; Overbeek, J. *Discuss. Faraday Soc.* **1954**, *18*, 52.
(5) Bontoux, J.; Dauplan, A.; Marignan, R. *J. Chim. Phys. Phys.-Chim. Biol.* **1969**, *66*, 1259.
(6) Glass, J.; Lunberg, R.; Bailey, F. *J. Colloid. Sci.* **1970**, *33*, 491.
(7) Napper, D. H. *J. Colloid Interface Sci.* **1970**, *33*, 324.
(8) Vincent, B. *Adv. Colloid Interface Sci.* **1974**, *4*, 193.
(9) Cain, F.; Ottewill, R.; Smitham, J. *Faraday Discuss. Chem. Soc.* **1978**, *65*, 33.
(10) Klein, J. *Nature (London)* **1980**, *228*, 248.
(11) Meier, D. J. *J. Phys. Chem.* **1967**, *71*, 1861.
(12) Clayfield, E.; Lumb, E. *Macromolecules* **1968**, *1*, 133.
(13) Hesselink, A. *J. Phys. Chem.* **1969**, *73*, 3488. *Ibid.* **1971**, *75*, 2094. *J. Polym. Sci., Polym. Symp.* **1977**, *No. 61*, 439. Hesselink, A.; Vrij, A.; Overbeek, J. *J. Phys. Chem.* **1971**, *75*, 2094.
(14) DiMarzio, E.; Rubin, M. *J. Chem. Phys.* **1971**, *55*, 4318.
(15) Ash, S.; Findenegg, G. *Trans. Faraday Soc.* **1971**, *67*, 2122.
(16) Dolan, A.; Edwards, S. F. *Proc. R. Soc. London, Ser. A* **1974**, *337*, 509. *Ibid.* **1975**, *343*, 427.
(17) Napper, D. H. *J. Colloid Interface Sci.* **1977**, *58*, 390.
(18) Edwards, S. F. *Proc. Phys. Soc. (London)* **1965**, *85*, 613.
(19) Moore, M. *J. Phys. A* **1977**, *10*, 305.
(20) de Gennes, P. G. "Scaling Concepts in Polymer Physics"; Cornell University Press: Ithaca, N.Y., 1979.
(21) de Gennes, P. G. *Macromolecules* **1981**, *14*, 1637.
(22) Widom, G. *Physica* **1979**, *95A*, 1.
(23) Flory, P. "Principles of Polymer Chemistry"; Cornell University Press: Ithaca, N.Y., 1971.
(24) Cahn, J. *J. Chem. Phys.* **1977**, *66*, 3667.
(25) de Gennes, P. G. *Rep. Prog. Phys.* **1969**, *32*, 187.
(26) des Cloizeaux, J. *J. Phys. (Paris)* **1975**, *36*, 281.
(27) Lifshitz, E. M. *Sov. Phys. JETP* **1956**, *2*, 73.
(28) Fisher, M.; de Gennes, P. G. *C. R. Hebd. Seances Acad. Sci., Ser. B* **1978**, *287*, 207.
(29) Daoud, M.; de Gennes, P. G. *J. Phys. (Paris)* **1977**, *38*, 85.
(30) Daoud, M., et al. *Macromolecules* **1975**, *8*, 804.
(31) This observation destroys our confidence in most of the earlier theoretical work which was carried out at the mean field level.
(32) This notation ensures that $\gamma(h \to \infty)$ is the interfacial tension for *one* plate.
(33) One of our referees pointed out the structural analogy between these equations and the classical DLVO theory for overlapping double layers.
(34) Cowell et al. *J. Chem. Soc., Faraday Trans. 1* **1978**, *74*, 337. I am indebted to a referee for mentioning this reference.

Measurement of an Equilibrium Density Gradient of a Solvent Mixture in an Ultracentrifuge

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ABSTRACT: The equilibrium density gradient of a solvent mixture in an ultracentrifuge was analyzed from the thermodynamic viewpoint, including the effects of pressure. Several techniques of the measurement of the gradient were discussed. The technique using a marker polymer and several equilibrium runs, each run sequentially equilibrated at several rotor velocities, was tested experimentally. A sample of polystyrene was used as a marker for the isorefractive mixture of cyclohexane and 1,2-dibromo-1-chloro-1,2,2-trifluoroethane. The rather plausible result for this mixture is believed to be the first detailed density profile measured experimentally in the ultracentrifuge.

Sedimentation equilibrium of polymer samples in a density gradient potentially provides a wealth of information about chemical heterogeneity of the sample, its molecular weight, polydispersity, impurities, etc. However, for a proper analysis, the density profile in the cell must be known with a good precision. As far as we know, a satisfactory evaluation of the density gradient (considered as a constant throughout the cell) was achieved only for aqueous solutions of several salts (e.g., CsCl).[1-3] These solutions were used as a density gradient forming media for the study of nucleic acids. The evaluation was based on a detailed knowledge of the activity coefficients of the salts from auxiliary measurements. For a study of most synthetic polymers, mixtures of organic solvents are needed. Some of the solvents are unusual and thermodynamic parameters of the mixtures are unknown. We have shown in a previous paper[4] that even for more common mixtures, the extraction of the necessary data from the literature is rather difficult. A direct evaluation of these thermodynamic parameters requires tedious research with rather sophisticated equilibrium distillation stills and precision calorimetry.

Under these circumstances, it is desirable to develop a method for the measurement of the density gradient using the ultracentrifuge itself. In this paper, we will first discuss the usefulness of the schlieren photographs and ultraviolet scans of the density gradient for its direct measurement and will show that these techniques do not yield information of sufficient quality. In a later part we will analyze the density gradient in detail from the thermodynamic point of view. Using this analysis as a basis, we will then develop a technique that employs a polymer sample as a probe for the evaluation of the density gradient. The technique requires a preparation of several solutions of the same polymer sample in mixtures of the two solvent components with different mass ratios. Each solution is then sequentially brought to an equilibrium at several rotor velocities. The density profile is evaluated from the polymer band positions at all the equilibrium conditions. The method has been applied to a mixture of cyclohexane and 1,2-dibromo-1-chloro-1,2,2-trifluoroethane. This solvent mixture is very convenient for studying samples of polystyrene.

Direct Measurement of a Density Profile

The Spinco Model E ultracentrifuges are equipped with ultraviolet absorption optics with a scanner and with two optical systems based on the refractive index: schlieren and interference. If the two solvents forming a density gradient have rather different UV spectra, then the UV

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scans at a convenient wavelength may lead to the evaluation of the concentration profile and from it to the density profile. In our experience, the use of UV scans yielded the required gradient, but the precision of the measurement was low. We consider it worthwhile to enumerate some experimental problems encountered with the UV method. (1) The species to be measured by the UV scan is one of the solvent components and, as such, it is present at rather high concentration. To prevent a virtual opacity of the solution, the wavelength of the UV light must be selected very far away from the absorption peak. The spectral behavior in such a spectral region is rather sensitive to various types of interactions and to traces of impurities that may be produced in the ultracentrifuge cell during a run lasting up to several days under intermittent irradiation with UV light. (2) The monochromator slit must be kept rather open to obtain a sufficiently strong signal. This leads to an insufficient monochromaticity of the light. In the arrangement of the Spinco scanner, the different parts of the cell are illuminated by light of slightly different wavelengths. That requires rather unwieldy corrections. (3) The compression of the liquid in the cell (which varies with the radial position) changes the optical absorption, again requiring a difficult correction. (4) The noise of the scanner signal and the changing calibration of the recorder did not allow measurement of the absorption with a sufficient accuracy. Because of these problems, we eventually abandoned the UV method.

The interference optics in the ultracentrifuge measures with a great sensitivity the differences of refractive index between the two sectors of the cell. When the differences are too large, a bright band is formed instead of the fringe pattern and no measurement is possible. This happened invariably whenever we tried to design any experiment for the measurement of the density gradient.

The schlieren optics measures the gradient of refractive index in the cell. It is tempting to assume the density gradient to be proportional to the refractive index gradient and to derive the former from the latter. However, when a mixture of two liquids is brought to sedimentation equilibrium, there are two effects that give a rise to a refractive gradient; each of these effects is related to the density gradient in a nonlinear way. (1) At equilibrium, the composition of the mixture is a function of the radial position and so is the refractive index. When the difference between refractive indices of the two components is large, the refractive index is a more or less linear function of density. However, very different refractive indices are undesirable: too steep a gradient deflects the light beam out of the system. Even moderate differences in refractive index are not desirable: when a polymer solution is studied in such a mixture, the refractive *increment* of the polymer is changing with the radial position and the analysis is difficult. Thus, solvent mixtures of components of very close refractive index are most preferable. However, in such mixtures the dependence of refractive index on composition is governed primarily by the change of volume in mixing: the refractive index goes frequently through a minimum. This effect is clearly seen in Figure 1 , which is a schlieren record of a low-speed ultracentrifuge run where cyclohexane was overlaid in a synthetic boundary cell over almost isorefractive 1,2-dibromo-1-chloro-1,2,2-trifluoroethane. (2) Quite a large contribution to the refractive gradient arises due to the pressure gradient. The pressure gradient in the ultracentrifuge is equal to $\rho\omega^2r$, where ρ is the local density, ω is the angular velocity, and r is a radial distance from the rotor axis. The corresponding density gradient is $\alpha\rho\omega^2r$, where α is the local compressibility. The pressure contribution to the refractive gradient is proportional to the pressure contribution to the density. Thus, the shape of the schlieren line represents the density gradient even for isorefractive solvents. (Note: The composition and pressure contribution to the schlieren pattern are distinguished when the rotor speed is quickly reduced: the composition contribution remains essentially unchanged while the pressure contribution decreases with ω^2.) However, it is virtually impossible to evaluate the density gradient from the schlieren photograph: (1) compressibility is an unknown function of composition and pressure; (2) the composition effect is difficult to correct for; (3) precision of schlieren optics is not sufficient for quantitative results.

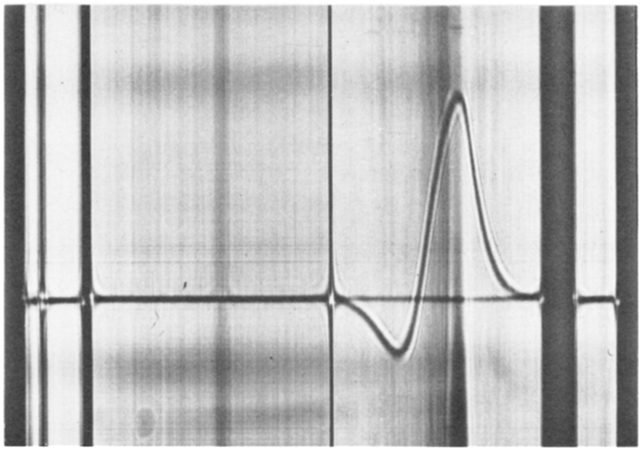

Figure 1. Schlieren photograph of a synthetic boundary run when cyclohexane was laid over 1,2-dibromo-1-chloro-1,2,2-trifluoroethane. Rotor velocity 6000 rpm. Photograph taken 8 min after formation of boundary. Schlieren bar at 75°. The figure demonstrates that the mixture of these solvents exhibits a minimum of the refractive index.

Taking into account all the above difficulties, we decided to measure the density gradient indirectly using a polymer sample as a tracer.

Theory

In this section we will address two problems: (1) the relation between the steepness of the density gradient and the properties of the two solvents and (2) the thermodynamics of the two-component mixtures in an ultracentrifuge field.

To preserve the flexibility in the selection of rotor speed it is convenient to employ solvent pairs that produce rather steep density gradients. We will now study the factors that influence the steepness of the gradient. We will denote the denser solvent by a subscript 2 and the less dense solvent by a subscript 1.

The basic condition of an equilibrium in an ultracentrifuge is that the total potential of any component (i.e., the sum of chemical potential and the potential of the centrifugal field) is the same throughout the cell. This condition is usually expressed as a derivative of the total potential with respect to the radial position r being equal to zero. This condition written for component two in a two-component system reads

$$M_2(1 - \bar{v}_2\rho)\omega^2r = (\partial\mu_2/\partial x)_{P,T}(\mathrm{d}x/\mathrm{d}r) \quad (1)$$

Here, the M_i, μ_i, $\bar{v}_i$, and ρ_i are, respectively, the molecular weight, the chemical potential, the partial specific volume, and the density of the ith component. P is pressure, T is absolute temperature, ρ is the density of the mixture, ω is the angular velocity of the rotor, and r is a distance from the rotor axis. x is any convenient measure of the composition of the mixture: a molar fraction, volume fraction ϕ_i, density, etc.

At this moment we are interested only in guidelines for the selection of the solvent pair. Thus, we may omit the finer points and assume that the two components mix without change of volume and that the mixture is incompressible. For this assumption, it follows

$$\bar{v}_i = 1/\rho_i \tag{2}$$

$$1 - \bar{v}_2\rho = (1 - \bar{v}_2\rho_1)\phi_1 \tag{3}$$

$$\rho = \phi_1\rho_1 + \phi_2\rho_2 \tag{4}$$

Further, it is assumed that the change of the free enthalpy of mixing, ΔG_{mix}, may be expressed by a relation of the Flory–Huggins type

$$\Delta G_{\mathrm{mix}} = RT[n_1 \ln \phi_1 + n_2 \ln \phi_2 + n_1\phi_2 g_{12}] \tag{5}$$

Here, R is the gas constant, n_i's represent the numbers of moles, and g_{12} is a function of the composition of the mixture (also of temperature and pressure), which characterizes the thermodynamic behavior of the mixture. For qualitative considerations, it may be considered as a constant. Aminabhavi and Munk[4] have shown that for a moderately nonideal mixture of benzene and cyclohexane $g_{12} \simeq 0.5$; for more nonideal mixtures of cyclohexane and ethyl acetate $g_{12} \simeq 1.2$.

We will now employ eq 1 using volume fraction ϕ_2 for the quantity x. The derivative $(\partial\mu_2/\partial\phi_2)_{P,T}$ is calculated from eq 5 as

$$(\partial\mu_2/\partial\phi_2)_{P,T} = RT\phi_1[1/\phi_2 + (V_2/V_1)(1/\phi_1 - 2g_{12})] \tag{6}$$

where V_i's are the molar volumes. Substitution of eq 2, 3, and 6 into eq 1 yields

$$\frac{M_2(\rho_2 - \rho_1)\omega^2 r \, \mathrm{d}r}{RT\rho_2} = \left[\frac{1}{\phi_2} + \frac{V_2}{V_1}\left(\frac{1}{\phi_1} - 2g_{12}\right)\right] \mathrm{d}\phi_2 \tag{7}$$

Taking into account the relation between the density ρ and the volume fractions, we may conveniently write eq 7 as

$$\frac{\mathrm{d}\rho}{\mathrm{d}(r^2\omega^2/2)} = \frac{(\rho_2 - \rho_1)^2}{RT\left[\dfrac{1}{V_1\phi_1} + \dfrac{1}{V_2\phi_2} - \dfrac{2g_{12}}{V_1}\right]} \tag{8}$$

The density gradient increases with the square of the rotor velocity and with the square of the density difference between the solvent components. It also increases with increasing molar volumes of both solvents. The density gradient increases hyperbolically with increasing values of g_{12}. For the special case $V_1 = V_2$ and $\phi_1 = \phi_2 = {}^1/_2$, the density gradient doubles its value when g_{12} increases from 0 to 1 (other parameters being unchanged); it reaches infinity (phase separation) at $g_{12} = 2$.

When a solvent miture is selected for an analysis of some polymer sample, the composition of the mixture must be selected in such a way as to match the density of the mixture to the apparent density of the polymer, ρ^*, at some convenient point in the cell (often in the middle). Therefore, it is interesting to calculate the density gradient at a point where $\rho = \rho^*$. The result reads

$$\left(\frac{\mathrm{d}\rho}{\mathrm{d}(r^2\omega^2/2)}\right)_{\rho=\rho^*} = \frac{\rho_2 - \rho_1}{RT\left[\dfrac{1}{V_1(\rho_2 - \rho^*)} + \dfrac{1}{V_2(\rho^* - \rho_1)} - \dfrac{2g_{12}}{V_1(\rho_2 - \rho_1)}\right]} \tag{9}$$

Thus, not only $\rho_2 - \rho_1$ should be large but the differences $\rho_i - \rho^*$ should have high absolute values, too. For typical organic polymers with relatively low values of ρ^*, the density of the less dense solvent should not be to high, i.e., not too close to ρ^*.

Detailed Density Profile. Let us consider a very long sedimentation column in a strong centrifugal field. The solvent near the top of the column would be almost pure component 1 and the solvent near the bottom would be almost pure component 2. Obviously, the density profile (i.e., the dependence ρ vs. r^2) would be S-shaped. In the previous section, we discussed the steepness of the middle part of this profile. When a short column is employed, only a part of the whole pattern will appear. It is obvious that this partial pattern may be convex, concave, or almost straight, depending on the part of the whole pattern that is applicable to the short-column experiment. The detailed form of the profile depends on the factors discussed in the previous section plus on those that were neglected: the pressure and composition dependence of the function g_{12}, compressibility and its dependence on composition, nonlinearity of the dependence of density on volume fraction, and dependence of partial specific volumes on composition and pressure. Under these circumstances, it is virtually impossible to develop a useful thermodynamic expression for the calculation of the detailed density profile. Instead of that, we will analyze the profile from a phenomenological viewpoint and describe it as a series. Before proceeding further, we will define a new variable u as

$$u \equiv r^2\omega^2/2 \tag{10}$$

It is obvious from eq 8 and 9 that u is the appropriate variable for the ultracentrifuge experiments.

A mixture of two liquids at a given temperature is fully described by two independent variables: pressure P and composition (expressed in any units; we will use a symbol x for it). The density, ρ, as well as other properties of the mixture (partial specific volumes, chemical potentials) are unique functions of P and x. Let us consider some density profile at sedimentation equilibrium. The profile will depend on the initial composition of the mixture, on the radius of the top and bottom of the column, and on the rotor speed. At some particular location in the cell (r_0, u_0) the pressure and composition will have some particular values P_0 and x_0. According to the laws of hydrostatics, the following relation is applicable in a centrifugal field:

$$\mathrm{d}P = \rho \, \mathrm{d}u \tag{11}$$

Integration of this relation combined with the boundary condition (expressed as values u_0, P_0, x_0) yields pressure as a unique function of $u - u_0$. This is a result of ρ being a unique function of P and x.

The dependence of x on the position in the cell is described by a rearranged eq 1.

$$(\partial\mu_2/\partial x)_{P,T} \, \mathrm{d}x = M_2(1 - \bar{v}_2\rho) \, \mathrm{d}u \tag{12}$$

Noting again that μ_2, $\bar{v}_2$, and ρ are unique functions of P and x and using the boundary condition (u_0, P_0, x_0), we find that the composition x is also a unique function of $u - u_0$. Eliminating the variable $u - u_0$ from the two functional expressions $\mathrm{P}(u - u_0)$ and $x(u - u_0)$, we obtain a unique relation between P and x for this particular experiment. In the plane P,x this relation describes a curve going through point (P_0, x_0); the points on the curve correspond to various locations in the cell and are parameterized by the values of $u - u_0$.

Let us now consider another equilibrium experiment with different top and bottom positions, different original composition of the mixture, and different speed. However,

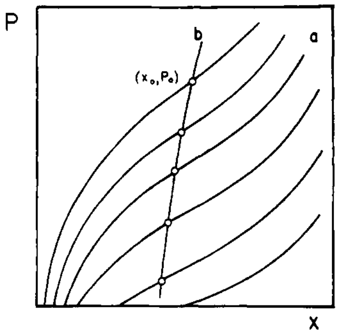

Figure 2. Sketch in the composition (x)-pressure (P) plane of the equilibrium properties of a solvent mixture in the centrifugal field. The family of curves a represents different initial and boundary conditions. Curve b represents the dependence of density (and composition) of the mixture, in which the marker polymer is buoyant, on the pressure. The intersections of curves a with curve b correspond to reference pressure P_0 at different conditions of the run.

let us select these parameters in such a way that somewhere in the cell the conditions correspond to the same values (P_0, x_0) as in the previous experiment. Obviously, in the P,x plane the same curve as before will describe the density profile. However, if the experimental conditions were selected so that at no location in the cell the conditions (P_0, x_0) exist, another curve is defined. Thus for a description of all possible experimental conditions, a family of curves is needed. Such a family is sketched in Figure 2 (curves a).

For an analysis of a particular experiment, it is necessary to find which particular curve from the family is applicable. The density is then expressed as that function of the parameter $u - u_0$ that is applicable to this curve. The reference values P_0 and x_0 may be selected arbitrarily. However, it is convenient to select them in such a way as to provide an easy identification of the (P_0, x_0) point. The position of a marker polymer in the density profile yields such an identification.

When a polymer sample is brought to sedimentation equilibrium together with the solvent mixture, it bands around a position in the cell where the quantity $1 - \bar{v}_3{}^*\rho$ is equal to zero. Here, the subscript 3 identifies the polymer and the asterisk refers to a quantity measured at constant chemical potential of both solvent components. The quantity $\bar{v}_3{}^*$ is strongly influenced by the preferential adsorption of solvent components onto the polymer and—through it—on the details of the thermodynamics of the three-component system. Specifically, $\bar{v}_3{}^*$ depends on pressure not only due to the compressibility of the polymer but also due to the dependence of the preferential adsorption on pressure. However, at each pressure there exists some composition of the solvent mixture that has the same density as the solvated polymer at this pressure. The dependence of this composition on pressure is sketched in Figure 2 as curve b. We will use the intersections of curve b with the curves a as a definition of the reference values (P_0, x_0). It should be stressed that not only the position of the curve b but also its shape depend on details of the interaction of that particular polymer sample with the solvent mixture. However, different samples of the same homopolymer should give essentially identical curves.

As the next step in the analysis we will develop the functional expression of density as a function of u into a Taylor series around the point u_0. Of course, the series will be different for different members of the family of curves a; i.e., the coefficients must be expressed as a function of pressure P_0. Truncating the series at a quadratic term of $u - u_0$ and writing the coefficients as linear functions of pressure, we get the expression for density as

$$\rho = a_0 + b_0P_0 + (a_1 + b_1P_0)(u - u_0) + (a_2 + b_2P_0)(u - u_0)^2 \qquad (13)$$

For finding the values of the coefficients a_0, a_1, a_2, b_0, b_1, and b_2, we employ the law of mass conservation in the ultracentrifuge cell: the mass of the solvent mixture in the absence of the ultracentrifugal field (the density is $\rho_{\rm or}$) must be equal to the mass at equilibrium.

The mass W of the mixture in the cell at equilibrium is obtained by integration as

$$W = \int_{\rm t}^{\rm b} \rho \, {\rm d}V \qquad (14)$$

where dV is an element of volume and t and b refer to the top and bottom of the sedimentation column, respectively. Law of mass conservation requires

$$W = \rho_{\rm or}V_{\rm or} \qquad (15)$$

where $\rho_{\rm or}$ is the known density of the original mixture and $V_{\rm or}$ is the volume of the mixture introduced into the cell. Due to the compression of the liquid, this volume is larger than the volume at equilibrium. To simplify the calculations, we will assume that the compressibility α of the mixture is independent of the composition and pressure. Then it is possible to introduce d$V_{\rm or}$ as a volume element that, after the hydrostatic compression, would yield dV

$${\rm d}V_{\rm or} = {\rm d}V(1 + \alpha P) \qquad (16)$$

Here, the pressure P is a function of the position in the cell. Integration of eq 16 combined with eq 14 and 15 yields

$$\rho_{\rm or}\int_{\rm t}^{\rm b} (1 + \alpha P) \, {\rm d}V = \int_{\rm t}^{\rm b} \rho \, {\rm d}V \qquad (17)$$

For a sector-shaped cell, the volume element dV may be expressed as

$${\rm d}V = h \, {\rm d}(r^2) \qquad (18)$$

where h is an inconsequential geometric factor. Combination of eq 10, 17, and 18 yields

$$\rho_{\rm or}\int_{u_{\rm t}}^{u_{\rm b}} (1 + \alpha P) \, {\rm d}u = \int_{u_{\rm t}}^{u_{\rm b}} \rho \, {\rm d}u \qquad (19)$$

It is convenient to introduce new variable y as

$$y \equiv u - u_{\rm t} \qquad (20)$$

and transform eq 19 into

$$\rho_{\rm or}\int_0^{y_{\rm b}} (1 + \alpha P) \, {\rm d}y = \int_0^{y_{\rm b}} \rho \, {\rm d}y \qquad (21)$$

Proceeding now to the evaluation of the integrals in eq 21, we calculate the local pressure P by integrating eq 11 (and using eq 20) as

$$P = P_{\rm t} + \int_0^y \rho \, {\rm d}y \qquad (22)$$

Assuming that the pressure $P_{\rm t}$ at the top of the column is equal to zero (assumption of $P_{\rm t}$ being atmospheric pressure would lead to equivalent results; P_0 would become excess pressure), substituting the truncated series eq 13 into eq 22, and realizing that $u - u_0 = y - y_0$, we find the integral in eq 22 as

$$P = y[a_0 - a_1y_0 + a_2y_0^2 + a_1y/2 - a_2yy_0 + a_2y^2/3 + P_0(b_0 - b_1y_0 + b_2y_0^2 + b_1y/2 - b_2yy_0 + b_2y^2/3)] \qquad (23)$$

Further integration yields

$$\int_0^{y_b} P\,dy = (y_b^2/2) \times [a_0 - a_1 y_0 + a_2 y_0^2 + a_1 y_b/3 - 2a_2 y_0 y_b/3 + a_2 y_b^2/6 + P_0(b_0 - b_1 y_0 + b_2 y_0^2 + b_1 y_b/3 - 2b_2 y_0 y_b/3 + b_2 y_b^2/6)] \quad (24)$$

Similarly, the other integral in eq 21 reads

$$\int_0^{y_b} \rho\,dy = y_b[a_0 - a_1 y_0 + a_2 y_0^2 + a_1 y_b/2 - a_2 y_0 y_b + a_2 y_b^2/3 + P_0(b_0 - b_1 y_0 + b_2 y_0^2 + b_1 y_b/2 - b_2 y_0 y_b + b_2 y_b^2/3)] \quad (25)$$

The pressure at the polymer peak, P_0, is obtained from eq 23 written for the location $y = y_0$, where $P = P_0$. The result reads

$$P_0 = y_0 \frac{a_0 - a_1 y_0/2 + a_2 y_0^2/3}{1 - b_0 y_0 + b_1 y_0^2/2 - b_2 y_0^3/3} \quad (26)$$

Substituting eq 24–26 into eq 21, we obtain a rather unwieldy relation (not given) between the known original density, ρ_{or}, and the experimentally accessible values y_0 and y_b. The relation contains as parameters the constants α, a_0, a_1, a_2, b_0, b_1, and b_2; it may be written as a known expression of the form

$$f(\rho_{or}, y_0, y_b, \alpha, a_0, a_1, a_2, b_0, b_1, b_2) = 0 \quad (27)$$

If the values ρ_{or}, y_0, and y_b are measured for a sufficient number of sedimentation equilibria, the parameters may be obtained by a suitable least-squares analysis.

Once the parameters are known, the density profile may be calculated from eq 13 for any sedimentation equilibrium experiment which used the studied mixture of solvents. However, eq 13 represents a family of dependences, and it is necessary to decide which value of P_0 is applicable to each particular experiment. If the polymer, which was used for the analysis of the density profile, is present at the equilibrium experiment and the position of its peak y_0 is accessible, then P_0 is easily calculated from eq 26. In this case, knowledge of ρ_{or} is not even necessary; it may be obtained from eq 27. In practice, the agreement between the experimental value of ρ_{or} and the value calculated from eq 27 serves as a check of the internal consistency of the measurement.

If the marker polymer is absent from the mixture and/or its y_0 is not experimentally accessible, it is calculated from the experimental ρ_{or} using eq 27; P_0 is then obtained from eq 26, and the density profile is again given by eq 13.

Experimental Part and Results

The mixture selected for the study was composed of cyclohexane (ρ_{20} = 0.7788 g/mL; n^{20}_D = 1.4258; Aldrich Chemical Co. (99+%)) and 1,2-dibromo-1-chloro-1,2,2-trifluoroethane (ρ_{20} = 2.2467 g/mL; n^{20}_D = 1.4278; PCR Research Chemicals). This almost isorefractive mixture is very convenient for studying polystyrene samples. Polystyrene sample lot 13a from Pressure Chemical Co. ($\bar{M}_w$ = 670 000, manufacturer's value) was used as a marker polymer.

The equilibrium experiments were performed at 20 °C in a Spinco Model E ultracentrifuge equipped with an electronic speed control, a titanium ANH rotor, a Kel-F double-sector centerpiece, sapphire windows, and schlieren and interference optics. One sector of the cell was loaded with a polystyrene solution ($c \sim 6 \times 10^{-4}$ g/mL) and the other with the corresponding mixture of solvents. Both sectors were filled completely to obtain the longest possible column length. Several experiments were performed, each starting with a solvent mixture of different composition.

In each experiment, the rotor was first brought up to the maximum speed (60 000 rpm). The polymer peak usually formed after about 12 h. However, after that the peak slowly drifted from its original position. The schlieren and interference photographs were taken at intervals up to 48 h and the peak positions were evaluated. The peak positions were then extrapolated to inifinite time, assuming an exponential approach toward the equilibrium position. The position at 48 h usually differed less that 0.005 cm from the extrapolated position. After about 48 h the rotor speed was reduced and the procedure was repeated. The spreading of the peak (especially at higher rotor speeds) was relatively fast and was essentially completed after a few hours. However, the drift of the peak toward its new equilibrium position was slow; another 48 h was required for a reasonable approach to equilibrium. The extrapolation was performed for each rotor speed. The rotor speed was sequentially reduced several times until the peak either spread so much that the measurement of its position was ambiguous or until it drifted all the way to the top or bottom of

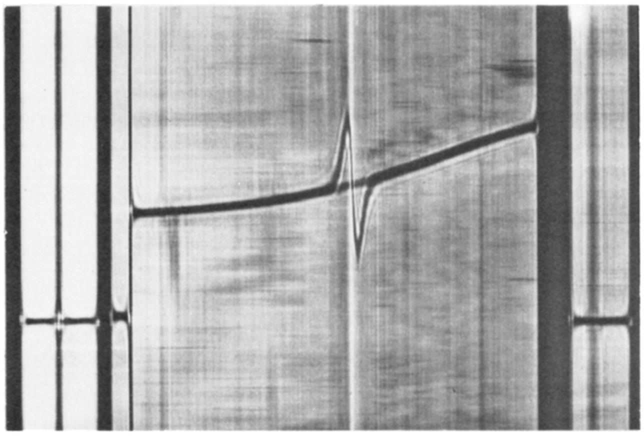

Figure 3. Schlieren photograph of a sedimentation equilibrium. Marker polymer (polystyrene) in a mixture of cyclohexane and 1,2-dibromo-1-chloro-1,2,2-trifluoroethane; 60 000 rpm; original density ρ_{or} = 1.222 g/mL, schlieren bar angle 75°.

Table I
Experimental Values of the Positions of the Top and Bottom of the Column and of the Polymer Peak for Several Equilibrium Experiments: Polystyrene in Mixtures of Cyclohexane and 1,2-Dibromo-1-chloro-1,2,2-trifluoroethane at 20 °C

			radial position, cm		
ϕ_2[a]	ρ_{or}, g/mL	rpm	top	peak	bottom
0.145	0.991	60 000	5.840	7.029	7.182
		56 000	5.830	7.073	7.181
0.182	1.043	60 000	5.837	6.913	7.185
		52 000	5.821	6.964	7.184
		44 000	5.809	7.078	7.183
0.244	1.138	60 000	5.845	6.723	7.184
		52 000	5.835	6.730	7.183
		44 000	5.827	6.761	7.185
		36 000	5.820	6.847	7.185
		30 000	5.816	6.941	7.186
0.302	1.222	60 000	5.853	6.576	7.182
		52 000	5.844	6.545	7.181
		44 000	5.835	6.510	7.185
		36 000	5.828	6.474	7.186
		30 000	5.823	6.439	7.187
		26 000	5.820	6.411	7.186
0.342	1.276	60 000	5.861	6.494	7.184
		52 000	5.850	6.425	7.183
		44 000	5.847	6.373	7.186
		36 000	5.838	6.261	7.187
		30 000	5.838	6.123	7.189
		26 000	5.830	6.027	7.190
0.406	1.369	60 000	5.848	6.359	7.185
		52 000	5.845	6.264	7.184
		44 000	5.843	6.151	7.186

[a] Volume fraction of 1,2-dibromo-1-chloro-1,2,2-trifluoroethane calculated from the weights of the components.

Table II
Parameters of Eq 13 Calculated from Experimental Data for Several Values of Compressibility

$10^{11}\alpha$, cm^2/dyn	a_0, g/mL	$10^9 a_1$	$10^{18} a_2$	$10^{11} b_0$	$10^{19} b_1$	$10^{27} b_2$
6.0	1.2009	2.725	4.527	5.29	−5.99	−1.34
8.0	1.1996	2.751	4.546	7.91	−4.64	−0.86
10.0	1.1984	2.777	4.561	10.55	−3.27	−0.37
12.0	1.1971	2.803	4.572	13.20	−1.89	+0.12
14.0	1.1958	2.831	4.579	15.89	−0.52	+0.62

the cell. In all cases the peak position was evaluated both from interference and schlieren pictures. The match was within the reading error of the schlieren photographs. A typical schlieren photograph is displayed in Figure 3.

The solvent densities were measured at 20 °C with a DMA 02C digitial precision density meter manufactured by A. Paar, K. G., Austria.

In Table I are collected the values of the densities of the original solvent mixtures, the approximate volume fractions of the heavy component ϕ_2, the rotor velocities, and the equilibrium radial positions of the top of the column, polymer peak, and bottom of the column. The variation of the position of the cell bottom reflects the precision of the evaluation of the schlieren photographs and, possibly, also the cell deformation. The changes of the position of the top of the liquid column with changing rotor velocity result from the compression of the liquid and from the deformation of the centerpiece.

When the data from Table I were subjected to least-squares analysis, it was found that the sum of the squares of the deviations of the original and back-calculated density was rather insensitive to the compressibility α. Consequently, the value of α cannot be obtained from this analysis. We have evaluated the remaining parameters a_0, a_1, a_2, b_0, b_1, and b_2 from the least-squares analysis based on eq 27 for several values of α. (Note that the compressibility of pure cyclohexane is about 11 × 10^{-11} cm^2/dyn). The results are compiled in Table II; in our calculations u is expressed in units cm^2 s^{-2} and pressure in dyn/cm^2. It is obvious that the parameters a_1 and a_2, which make the major contribution to the density gradient, are rather insensitive to the assumed value of the compressibility. The parameters b_1 and b_2, which make about 10% contribution to the density gradient, are more sensitive. For all assumed values of α, the mean deviation of back-calculated ρ_{0r} from the experimental value was about 0.003 g/mL. An example of the calculated density profile is shown in Figure 4. The profile is markedly nonlinear.

Discussion

The mean difference between the measured original density of the solvent mixture and its value calculated back from our eq 13 (using the data from Table II) and from the experimental peak positions is about 0.003 g/mL. This value should be compared with the difference of densities between the top and bottom of the cell, which at the highest experimental speeds was about 0.9 g/mL. Thus, the agreement seems quite satisfactory. The remaining deviation is caused not only by experimental errors in measurements of peak positions and solvent densities but also by the truncation of the series in eq 13. The possible error caused by unknown compressibility is not more than a few percent of the value of the density gradient; it may be further reduced by measuring the compressibility by some independent method.

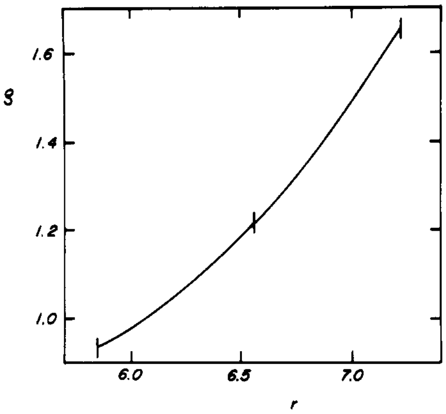

Figure 4. Dependence of the density on the radial position in the cell calculated according to eq 13 ($\alpha = 10 \times 10^{-11}$ cm^2/dyn; parameters from Table II) for an equilibrium experiment at 52 000 rpm using a solvent mixture with an original density of 1.222 g/mL. The short vertical lines correspond to the top of the column, to the peak, and to the bottom of the column, respectively.

Assuming the compressibility $\alpha = 10 \times 10^{-11}$ cm^2/dyn and neglecting the volume changes in mixing, we have calculated ϕ_2 at the isopycnic point of polystyrene (zero excess pressure) at $\phi_2^* = 0.285$. From eq 8 we have then found the thermodynamic parameter $g_{12} = 0.534$. Thus, the mixture of cyclohexane with 1,2-dibromo-1-chloro-1,2,2-trifluoroethane is only moderately nonideal. The observation that the time needed for equilibration of the solvent mixture was much longer than the time for equilibration of the polymer band was somehow unexpected. It seems that the long time for solvent equilibration follows from the fact that the solvents have to equilibrate (diffuse) across the whole column length while the polymer is diffusing only across a much narrower band. The long equilibration times led to rather extensive time requirements for this calibration experiment. However, the calibration is independent of the instrument and it has to be done only once for each solvent system.

Conclusions

The proposed method of the indirect measurement of the equilibrium density profile in the ultracentrifuge cell yields plausible and internally consistent results. To our knowledge, it is the first time the complete density profile has been measured experimentally.

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References and Notes

(1) Ifft, J. B.; Voet, D. H.; Vinograd, J. *J. Phys. Chem.* **1961**, *65*, 1138.
(2) Hearst, J. E.; Ifft, J. B.; Vinograd, J. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 1015.
(3) Schmid, C. W.; Hearst, J. *Biopolymers* **1971**, *10*, 1901.
(4) Aminabhavi, T. M.; Munk, P. *Macromolecules* **1979**, *12*, 607.