Density Gradient Sedimentation in Polymer Solutions

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Introduction

Recently, Meselson et al.[1] have originated a new technique of sedimentation analysis, density gradient sedimentation. The reader is referred to their article[1] and to a recent review article[2] for a detailed description of this technique. This technique has been applied to organic polymers by Buchdahl et al.[3] Also, Bresler et al.[4] have reported results on the detection of small structural differences in small density gradients.

The preliminary work reported here was oriented toward the compositional analysis of mixed synthetic polymers and copolymers. Accordingly, some binary solvent systems suitable for the density gradient sedimentation of organophilic polymers have been selected and some preliminary results on rubber and polystyrene are presented here. Rather large density gradients were always used. Schlieren optics was used to record the results of these experiments, because of appreciable absorption in the ultraviolet from all heavy organic solvents considered. A Spinco model E ultracentrifuge was used. When schlieren optics are used, the solvent pair must be nearly matched in refractive index unless wedged windows of large angle are used. If the system is appreciably mismatched, the refractive index gradient from solvent sedimentation is large compared with that from the polymer, and in many cases is even large enough to deviate the gradient trace completely out of the optical system. It is therefore obvious that it is possible to determine activity coefficients in many binary systems of organic liquids by sedimentation equilibrium when an appreciable difference of density and refractive index is present, at speeds low enough so that the excess pressure in a short column of liquid is well under 1 atm.

Systematic Errors in Schlieren Optics

By solution of the equation

$$\frac{d^2r}{dy^2} = \frac{1}{n}\frac{dn}{dr}\left[1 + \left(\frac{dr}{dy}\right)^2\right] \tag{1}$$

for the passage of a ray of light through a medium containing a radial refractive index gradient (y is perpendicular to r) it was established that if

$$\frac{a^2}{\sigma}\left(\frac{dn}{dr}\right)_{\text{max}} < 0.26 \tag{2}$$

where a is the cell thickness, σ the standard deviation of the gradient, and $(dn/dr)_{max}$ the maximum height of gradient, then the true and observed values of σ differ by less than 0.8% and the true and observed values of dn/dr differ by less than 0.1%. All experiments fell within this limit.

Equation (1) can be solved for any arbitrary $C(r)$ where C is the concentration by the substitution $dr/dy = P$. The result is expressed in the form of an integral. Numerical results were obtained from a series approximation to this integral, using the Bendix G-15D computer, and assuming a Gaussian $C(r)$.

Solvent Systems

Data on the binary solvent systems are given in Table I.

TABLE I
Solvent Systems Used for Density Gradient Sedimentation

Heavy component	Light component	n_e, 25°C.	Density, 25°C., g./ml.
Ethylene bromide	Mesitylene	1.5390	2.1648
		1.4972	0.8567
1,2-Dibromo-1,1-difluoroethane	Cyclohexene	1.4457	2.2116
		1.4463	0.8079

Experimental Results

Representative gradient patterns for Hevea smoked sheet, unmilled and milled, and for Shell synthetic cis-polyisoprene are shown in Figure 1.

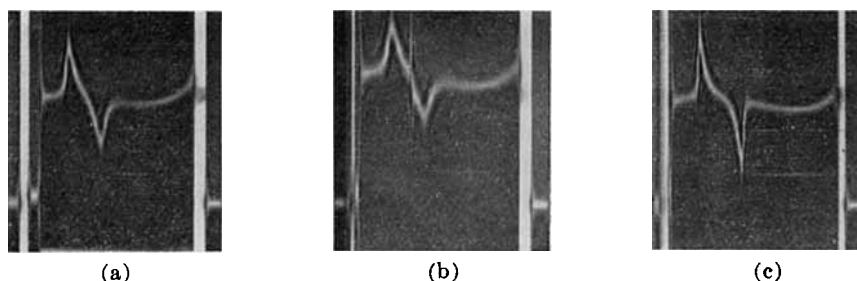(a)   (b)   (c)

Fig. 1. Density gradient sedimentation patterns for rubbers. (a) Hevea smoked sheet 0.1 wt.-% in 25.1% (1,2-dibromo-1,1-difluoroethane-(cyclohexene); (b) same, milled 3 min., 70°C.; (c) shell cis-polyisoprene, 0.145 wt.-% in the same solvent, all at 59,480 rpm; 3 mm. cell.

There was no difference in the point of zero gradient r_0 in all three cases, when corrected for small variations in meniscus position, under these large density gradients. The density at r_0, when calculated assuming an ideal incompressible binary mixture, was equal to 0.90 in all three cases. (It is

obvious that the assumptions of ideality and incompressibility are not strictly fulfilled, but this was the best that could be done.) The small spike on the milled Hevea pattern is believed to be gel resulting from the milling. Note that the amount of a given component is proportional to the first moment[1] of its gradient about r_0; hence, the amount of gel in Figure 1b is fairly small relative to the rubber. The gel pattern could have been investigated further by running at lower speed, to broaden out the pattern, and at higher total rubber concentration, to increase the absolute amount of gel.

It should be noted in passing that a concentration gradient cannot be recorded by schlieren optics if the suspended particles are too large relative to the wavelength of the light used.[5]

Some representative patterns for polystyrenes are shown in Figure 2. Note that Figure 2a shows a pattern obtained for only 11 ppm total polymer. This was obtained in a 12-mm. cell. In a 30-mm. cell, allowing for
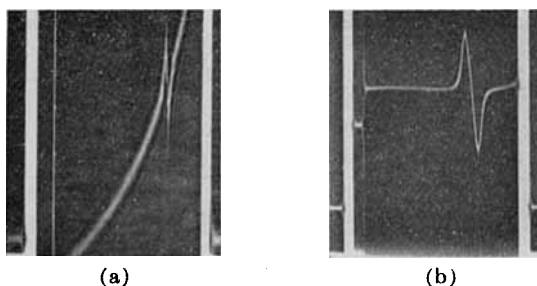

(a)   (b)

Fig. 2. Density gradient sedimentation patterns for polystyrene. (a) 11 ppm emulsion polymer, $M_v = 2.4 \times 10^6$ in 35.35 wt.-% ethylene bromide-mesitylene, 59,780 rpm, 12 mm. cell; (b) 0.098 wt.-% commercial polystyrene $M_w = 2.41 \times 10^5$, in 42.17% 1,2-dibromo-1,1-difluoroethane, 59,780 rpm, 3 mm. cell.

the required decrease in allowable speed of the heavier rotor for the 30-mm. cell, one could still probably detect 5 ppm of polymer in solution at the $M = 10^6$ level.

A series of experiments were run on the commercial polystyrene of Figure 2b, $M_w = 241,000$. These are summarized in Table II.

The quantity 2σ is plotted vs. $\sqrt{\bar{C}}$ in Figure 3. The reason for the concentration dependence is, of course, that the polymer-binary solvent system is nonideal. It can be shown, for an exponential molecular weight distribution, at $C = 0$, that the distance between the maxima of the pattern is proportional to $(M_z)^{-1/2}$, provided that certain conditions[1] (to be discussed later) are fulfilled.

The quantity K_C is defined by:

$$K_0 = \frac{2r_0 G^3 \tan \phi \int_{-\infty}^{+\infty} xy\, dx}{\rho C_{0w}(r_b^2 - r_m^2)} = \frac{\psi_2 La}{F} \qquad (3)$$

where

$$\frac{dn}{dr} = \frac{h \tan \phi}{La}, \quad h = FGy \tag{4}$$

Here, x is the distance from r_0 on an enlargement of the schlieren photograph; y is the height of schlieren pattern on the enlargement; r_0 is the distance from center of rotation of the gradient zero; G = is the magnification factor $r - r_0/X$; F is the cell magnification factor, ca, 2.06; ψ = dn/dc for polymer; L is the apparatus of the constant; ϕ is the phase plate angle; h is the height of pattern on photographic plate; n is the refractive index; a is the cell thickness; ρ is the solvent density, initial; C_{0w} is the initial polymer concentration, wt.-%; and r_b is the radius to cell bottom.

TABLE II

Density Gradient Sedimentation at Various Polymer Concentrations, in 42.17 Wt.-% (1,2-Dibromo-1,1-Difluoroethane)-(Cyclohexene), 59,780 rpm, 3 mm. Cell

Polymer concn., wt.-%	r_0, corrected to r_m[a] = 5.935 cm., cm.	Actual distance between max., 2σ, mm.	K_0
0.01228	6.824	0.56	0.047
0.02457	6.833	0.68	0.036
0.04909	6.835	0.85	0.049
0.09800	6.826	1.09	0.046
0.1942	6.835	1.40	0.047

[a] The data are corrected for small variations in meniscus position, assuming an ideal incompressible solution.

Hence, the percentage of free polystyrene in an unknown mixture of overall concentration is equal to $100K_c/K_0$. (The value of K_0 is established from experiments on pure polymer; K_c is based on total solids in an unknown solution.)

It is suspected that the second experiment in Table II may not have reached equilibrium, possibly because of some unknown disturbance in an overnight run. The calculated value of K_0, using ψ_2 obtained from the refractive index of the solvent system by the method[6] of Outer et al. was 0.0485.

Some more experiments were done in the ethylene bromide-mesitylene system on an emulsion polystyrene, $M_v = 2.4 \times 10^6$ and on a lithium butyl-catalyzed polystyrene, $M_v = 2.4 \times 10^5$. It is found from what data are available so far that r_0 for polystyrene is independent of concentration, mode of preparation of the polymer, and molecular weight. It should be noted, however, that all these experiments involve large density gradients; it is possible that small differences might have been detected in small

gradients. It was also possible to make a rough estimate of the quantity[2] Γ' which is a measure of the excess amount of one of the solvents preferred by the polymer; for 1,2-dibromo-1,1-difluoroethane at an initial concentration of 42.17 wt.-% in cyclohexane, $\Gamma' \simeq +0.13$ (in this experiment the concentration of heavy component at r_0 is estimated to be about 49 wt.-%).

For an incompressible system,

$$\Gamma' = \frac{(1 - \overline{V}_2\rho_0)}{(1 - \overline{V}_3\rho_0)} \text{ where } \rho_0 \text{ is } \rho \text{ at } r = r_0 \qquad (5)$$
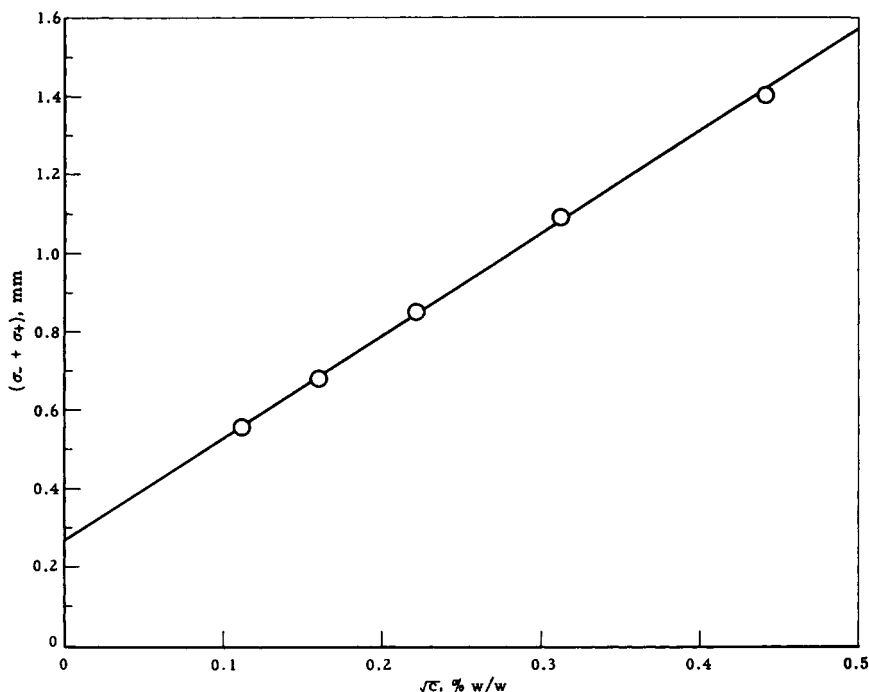

Fig. 3. Plot of band width vs. (concentration)$^{1/2}$, commercial polystyrene in 42.17% dibromodifluoroethane, 59,780 rpm. Data from Table I.

The partial specific volume $\overline{V}_2$ refers to polymer (taken as 0.91) and $\overline{V}_3$ in this case was taken to be $1/\rho$ for the heavy solvent. The density ρ_0 was 1.168 (calculated assuming an ideal incompressible solvent system, see Appendix). It is far easier in practice to obtain Γ' from light-scattering measurements.[7] Our data would indicate that Γ' is independent of polymer molecular weight, since r_0 is also independent of the molecular weight. This conclusion is not unexpected. Solvation of long polymer molecules would not be expected to depend on chain length on a weight basis.

Finally, an attempt was made to map out the density-radius profile in 44.83% (1,2-dibromo-1,1-difluoroethane)-(cyclohexene) by the use of "markers:" small droplets or crystals of substances of known density

which would come to rest at a level where their density was equal to that of the medium, at equilibrium. This has recently been suggested by Szybalski.[8] In the case of D_2O and aqueous markers, marker solution was pre-equilibrated with the solvent in all cases. It was necessary to coat the cell windows with Dri-Film to prevent adherence of very small drops of liquid. Manipulations involving D_2O were all done in a dry box and the solvent system was dried carefully beforehand, over anhydrous Na_2SO_4 and fresh Drierite. Data are presented in Table III and Figure 4. The aqueous markers were assumed to have the compressibility of water. The density of aniline hydrochloride was determined by a liquid displacement method.
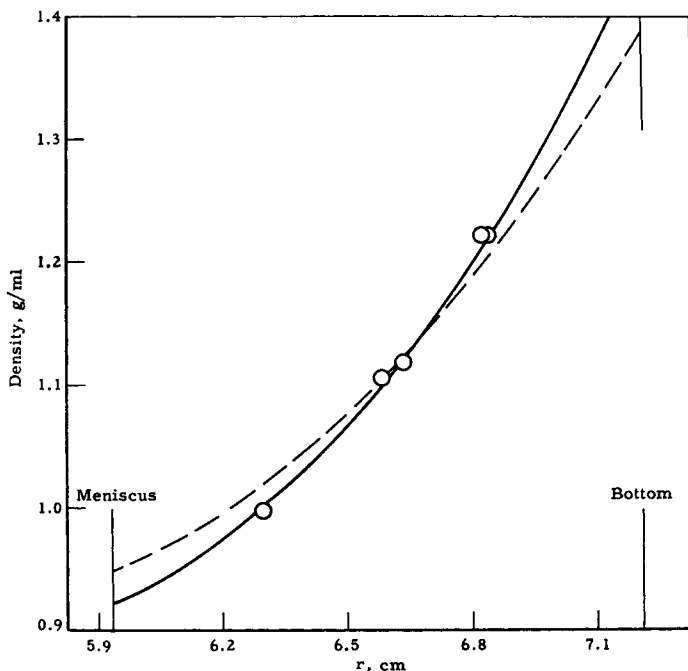

Fig. 4. Density vs. distance from center of rotation, 44.83% dibromo-difluoroethane, 59,780 rpm. (——) Experimental, (– – –) calculated, ideal incompressible solution.

The "incompressible-ideal" assumption is not very far off in this case. It appears that this solvent system exhibits a small positive deviation from ideality, as would be expected.

It is estimated that the column of solvent, at equilibrium, at 59,780 rpm contains approximately 19 wt.-% of the heavy solvent at the meniscus and 67% at the bottom at an overall concentration of 42.17 wt.-%. This represents quite an extensive redistribution of components in contrast to the $CsCl$-H_2O system used for proteins.[1] In the 44.83% bromofluoroethane system (the increased concentration of heavy component was intended to shift the polystyrene peak toward the cell center) r_0 ($r_m = 5.935$) was

TABLE III

Density Distribution in 44.83% (1,2-Dibromo-1,1-difluoroethane)-(Cyclohexene), 59,780 rpm

Marker	Measured marker density 1 atm., g./ml.	Rest point, r_0, cm.	Corr. density at r_0[a]	Meniscus position, r_m	Corrected rest point at $r_m = 5.935$	Max. estd.[b] pressure at r_0, atm.
Distilled water	0.997	6.274	1.000	5.877	6.296	96
D$_2$O	1.106	6.578	1.114	5.933	6.579	175
CaCl$_2$ solution	1.1176	6.616	1.126	5.876	6.634	203
Aniline hydrochloride[c]	1.227	6.804	—	5.883	6.824	—
(Crystals)	1.227	6.844	—	5.950	6.838	—
	1.227	6.860	—	6.026	6.826	—

[a] Used maximum pressure and compressibility of water.
[b] Calculated from $\rho\omega^2(r_0^2 - r_m^2)$.
[c] No correction applied because of generally very low compressibility of ionic crystals.

6.782 cm., corresponding to a calculated ρ_0 of 1.175 (see Appendix) and a ρ_0 estimated from Figure 4 of 1.196. This latter value is characteristic of a pressure of the order of 200 atm.; one must then know $\overline{V}$ for polystyrene at 200 atm. If it is assumed that all densities increase by 2% in going from 1 to 200 atm., a good guess for Γ' gives $+0.14$ in this case, corresponding to positive net adsorption of heavy component. The estimated concentration of heavy component at r_0, estimated from ρ_0, was 51 wt.-%. Both pure components are solvents for polystyrene; cyclohexene is a "poor" solvent but better than cyclohexane. This estimate of Γ' is probably better than that at 42.17% overall heavy component (49% at r_0) because an experimental ρ_0 was used, the calculated ρ_0 being somewhat low.

Note also that the density gradient is quite variable in contrast to the previous work[1] on aqueous solutions. Hence, we no longer have the simple theory[1] applying in this case. There are opportunities for getting unsymmetrical gradient patterns even with polymers of uniform composition. We can distinguish between at least two different kinds of asymmetry: (1) the two gradient loops have equal areas, but unequal first moments; (2) unequal areas under the two gradient loops. Even in solvents of matched refractive index, one can easily see that rapid variation of the density gradient with distance would yield the first kind of asymmetry, even with a polymer of uniform composition. Furthermore, Γ' is certainly a function of solvent composition; the effective density of the polymer may change nonlinearly with position.[9] The virial coefficients of the polymer are also functions of solvent composition. Thus, one can conceive of at least three ways of obtaining the first kind of asymmetry.

The second kind of asymmetry, unequal areas, can easily arise when the solvent refractive indices are not perfectly matched; this leads essentially to a value of dn/dc for the polymer which varies with position in the cell. There is also some reason to believe that mixtures of two liquids of equal refractive index but different cohesive energy density may, in fact, have refractive indices which vary with composition and are always somewhat less than that of the pure components. This effect is related to the well-known increase in volume on mixing in regular solutions.[10] Furthermore, it can be shown that, if the solvent gradient on each side of the loops is merely extrapolated through the pattern, a small imbalance in area with respect to the resulting baseline will always result without affecting the first moments. This derivation will not be reproduced here. There is also a possible effect of pressure on ψ_2. In the bromofluoroethane system the area imbalances were usually small and it was questionable whether or not they were caused by baseline uncertainties.

It can be seen at this point from the preceding discussion that any linear relation between $(M_z)^{-1/2}$ and the distance between maxima may be subject to some uncertainty, over and above the extrapolation of this distance to infinite dilution. There may also be some systematic variation of K_0 with pattern width, but our experiments have not revealed any.

The solutions used were centrifuged 18 hr. or longer. In some explora-

tory experiments, little if any change in pattern was noticed between 12 and 24 hr. It is believed that the extensive redistribution of the solvents is important in the approach[1] to equilibrium, since when the length of the column of solution was reduced from 1.3–0.8 cm., little change in pattern occurred after 6 hr. of sedimentation. It should be noted that for assessing the amount of a polymer component, complete equilibrium need not be attained, provided that $C = 0$ on both sides of the gradient pattern. If material of extremely low molecular weight is present in appreciable amounts, this will not accumulate in the gradient and may lead to error.

Appendix

Relations Used to Correct r_0 for Small Variations in Meniscus Position, r_m

For the ethylene bromide-mesitylene system a linear regression of r_0 vs. r_m was used; an "expected" r_0 could be calculated for a standard r_m.

For the bromofluoroethane–cyclohexene system, it was possible to correct r_0 theoretically and to calculate the density at r_0 assuming ideality and incompressibility, since the molar volumes of these two solvents are nearly equal. (For unequal molar volumes, this calculation is troublesome even by machine methods.) For an ideal incompressible solution,

$$\rho = \frac{\rho_1 \rho_2}{\rho_1 + X_1(\rho_2 - \rho_1)}$$

where ρ refers to solution and ρ_1 and ρ_2 to components and X is a weight fraction, and

$$X_1(r) = \frac{\rho_1(e^z - 1)}{\rho_1(e^z - 1) + \rho_2 \exp \{AM_1(r_b{}^2 - r^2)\} - \rho_2 \exp \{Z - AM_1(r^2 - r_m{}^2)\}}$$

where

$$A = \frac{\omega^2(\rho_1 - \rho_2)}{2RT\rho_1}$$

and

$$z = \frac{AM_1\rho_2 X_{10}(r_b{}^2 - r_m{}^2)}{X_{10}(\rho_2 - \rho_1) + \rho_1}$$

where ω is angular velocity, r_b and r_m are radii at cell bottom and meniscus, M is solvent molecule weight, and X_{10} is the overall initial weight fraction of solvent (1).

The preceding yields the relation used for reducing positions of gradient zero to a standard meniscus position:

$$r_0{}^2 - r'{}_0^2 = \frac{1}{AM_1} \ln \left\{ \left[\frac{1 - e^{\frac{-z\rho_1(1 - X_{10})}{\rho_2 X_{10}}}}{1 - e^{\frac{-z'\rho_1(1 - X_{10})}{\rho_2 X_{10}}}} \right] \left[\frac{1 - e^{z'}}{1 - e^z} \right] \right\}$$

where the prime refers to the r_m corresponding to r_0'. The approximation $\ln x = x - 1$ is usually valid here.

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Synopsis

Density gradient sedimentation of polystyrenes, Hevea rubber and synthetic cis-1,4-polyisoprene was studied in the systems ethylene bromide-mesitylene and (1,2-dibromo-1,1-difluoroethane)-cyclohexene. The latter system has very closely matched refractive indices at atmospheric pressure. This work was oriented toward the analysis of mixed polymer and copolymers. Large density gradients were used with schlieren optics. No difference between the points of accumulation of polystyrene from different types of polymerization nor between Hevea and cis-polyisoprene could be found under the conditions employed. Narrow gradient patterns could be produced for polymers of $M_v = 200{,}000$. These broadened with increasing polymer concentration. At $M = 10^6$ it is believed to be possible to estimate semiquantitatively concentrations of polystyrene of 5 ppm. A method of estimating the amount of a component present in terms of its gradient pattern is given. The density distribution in the bromofluoroethane system was studied by the use of inert marker substances of known density.

Résumé

On a étudié les gradients de densité par sédimentation de polystyrènes, de caoutchouc d'Hévéa et de cis-1,4-polyisoprène dans les systèmes bromure d'éthylène-mésitylène et (1,2-dibromo-1,1-difluoroéthane), cyclohexène. Dans le dernier système les indices de réfraction sous pression atmosphérique sont extrémement rapprochés. Ce travail était orienté vers gradients de densité etalés à l'aide d'une optique de schlieren. Dans les conditions employées, on n'a pu trouver de différences entre les points d'accumulation de polystyrènes polymérisés de différentes façons ni entre l'Hévéa et le cis-polyisoprène. On peut obtenir des diagrammes de gradient reserrés pour des polymères de $M_v = 200.000$. Ceux-ci s'étalent avec l'augmentation de la concentration en polymère. Lorsque $M = 10^6$ on suppose qu'il est possible d'estimer semi-quantitativement les concentratixns de polystyr'ne de 5 ppm. On donne une méthode d'estimation de la quantité de substance présente sur la base de ses diagrammes de gradient. La distribution de den-

sité dans le système bromofluoroéthane a été étudié grâce à des substances indicatrices inertes de densité connue.

Zusammenfassung

Dichtegradientsedimentation von Polystyrolen, Heveakautschuk und synthetischem *cis*-1,4-Polyisopren wurde in den Systemen Äthylenbromid-mesitylen und 1,2-Dibrom-1,1-difluoräthan-Cyclohexan untersucht. Das letztgeannte System besitzt bei Atmosphärendruck sehr ähnliche Brechungsindices. Die vorliegende Arbeit behandelt die Analyse von Misch- und Copolymeren. Es wurden grosse Dichtzgradienten mit Schlierrenoptik verwendet. Unter den angewendeten bedingungen konnte kein Unterschied in den Akkumulationspunkten von Polystyrol verschiedenen Polymerisationstyps und auch nicht zwischen Hevea und *cis*-Polyisopren gefunden werden. Enge Gradientendiagramme konnten für Polymere mit $M_v = 200.000$ erzeugt. wreden. Sie verbreiterten sich mit steigender Polymerikonzentratixn. Es wird angenommen, dass bei $M = 10^6$ ein ehalbquantitative Bestimmung von Polystyrolkonzentrationen von 5 ppm möglich ist. Es wird eine Methode zur Ermittlung des Anteils einer Komponente aus ihrem Gradientendiagramm angegeben. Die Dichtzverteilung im Bromfluoräthansystem wurde mit inerten Markierungssubstanzen bekannter Dichte untersucht.