Elemental analysis before and after thermal analysis gave the results shown in Table IV.

C. Poly(α,α'-diphenyl-*m*-xylylidene-*o*-phenylenediamine). Analysis of this system was carried out in the same manner as the preceding two. Although the products obtained from this system were the same as those obtained upon thermal degradation of the other two systems, the relative amounts were not the same. The major products were benzene and toluene with lesser amounts of diphenylmethane. Minor products were biphenyl, *m*-benzyltoluene, benzonitrile, and aniline. In addition, *cis*- and *trans*-decahydronaphthalene were also isolated and identified.

Elemental analysis before and after thermal analysis yielded the results given in Table V.

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Fractionation of Polymers by Thin Layer Chromatography. I. Separation

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ABSTRACT: The separation of polymer fractions by thin layer chromatography (tlc) is described. Seven polystyrenes ranging from $\bar{M}_w$ = 10,300 to 1,800,000 were successfully separated on either silica gel or alumina using mixed solvent elution or gradient elution techniques. Six polyethylene oxides ($\bar{M}_w$ = 1500–28,000) were separated by simple mixed solvent elution on silica gel or by gradient elution on alumina. The ability of tlc to separate the polymer fractions appears to be based primarily on the relationship between adsorption and molecular weight.

Perhaps the most important characteristics of a given polymer sample are its molecular weight and polydispersity. Because of this, considerable effort is expended in the analysis and isolation of samples over a wide range of molecular weights and polydispersities.

Gradient elution and precipitation chromatography were the first column methods developed for both analytical and preparative fractionation of polymers. Both these techniques work by partitioning the polymer between a mobile solution phase and a supported stationary macroscopic condensed phase according to the solubility of a given molecular weight.[1,2] The role of adsorption in such fractionations has been discussed[3,4] but no definitive understanding has been reached.

In both analytical and preparative applications the solubility techniques are being supplanted by gel permeation chromatography (gpc). Fractionation in gpc is generally thought to occur *via* increasing exclusion of column volume with increasing hydrodynamic volume of polymer chains. Thus, the largest chains have the least column volume accessible to them, their flow is retarded least and they are eluted first.[5] Recently, an alternative explanation for elution order has been proposed by DiMarzio and Guttman.[6] In general, the conditions of gpc are designed to avoid adsorption.

The predictions of polymer adsorption theories and the observations of experiment indicate a strong influence of polymer molecular weight on both adsorption–desorption rates and the position of the adsorption equilibrium.[7–14] The amount of material adsorbed and the thickness of the adsorbed layer increase with molecular weight.[7,8,10,11] The rate of desorption decreases with molecular weight,[11,12] and strongly adsorbed macromolecules can displace previously deposited material that has less affinity for the substrate.[13,14]

Yeh and Frisch[15] attempted to use adsorption phenomena to fractionate polymers by a column technique. Fractionation was achieved, but flow rates were very low and in several cases the sample was not recovered quantitatively.

Thin layer chromatography (tlc) is a rapid technique

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for the resolution of complex mixtures by adsorption. In general, solutes are deposited as a spot at one point near the edge of a supported layer of adsorbent and eluted by a solvent which is drawn up the adsorbent by capillary action. Solutes migrate distances (their R_f values) which increase with the eluting power of the solvent and decrease as the strength of their own adsorption on the substrate increases. Details of tlc theory and techniques are available in several references.[16–18]

Tlc appears to be an ideal technique with which to study polymer fractionation by adsorption. However, the applications of tlc to polymers so far seem to be limited mainly to the separation of compositionally heterogeneous systems. Polymer blends,[19] stereoisomers,[20] block copolymers,[21] and conventional copolymers[21] have been analyzed by tlc. Inagaki, *et al.*,[22] did not find any effect of molecular weight on R_f values. In recent work by Belenkii and Gankina,[23] however, a strong dependence of R_f value on the molecular weight of homopolymers and copolymers was reported.

Many authors[10,15,22] have pointed out that the solvent–polymer interactions are especially important and must be considered along with solvent–substrate and polymer–substrate interactions in any analysis of experimental results.

The object of this work is to investigate tlc as a new technique for the separation of high polymers according to their molecular weight. The conditions of the tlc experiments will be related to those employed in more conventional adsorption experiments. Finally, methods for determining molecular weight distributions will be discussed.

Experimental Section

Seven fractions of polystyrene (Pressure Chemical Co.) were employed in this study along with a sample of conventional molecular weight distribution (Monsanto). The molecular weight and polydispersity data are given in Table I.

Another series of samples for study were six polyethylene oxides. These samples are all narrow fractions of relatively low molecular weight from Hoechst. Table I also contains the pertinent data on these samples.

The tlc substrates used in this study were precoated alumina or silica gel on 20 × 20 cm aluminum plates (Brinkman Instrument Co). The plates used for experiments on the polystyrene samples contained a fluorescent indicator. Samples (10–20 μg of polymer) were deposited as spots along a line 2 cm from one edge of the adsorbent layer using disposable micropipets.

When the elution was carried out using a constant composition solvent (one or two components) the adsorbent plates were mounted in a sandwich-type cell (Eastman Chromogram apparatus). Gradient elutions were carried out in a cylindrical tank, with the flexible plate curved along the inner wall. The gradient was achieved by the addition of the second solvent from a large variable speed syringe pump (Sage Instrument Co.) *via* Teflon tubing that passed through a stoppered hole in the tank cover. The liquid in the tank was mixed with a large bar magnet stirrer. Vapor phase saturation was achieved by strips of filter paper also placed against the tank wall, one end being below the liquid level.

TABLE I
POLYMER SAMPLES

Sample	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$
	Polystyrenes	
PS-1	10,300	1.06
PS-2	19.800	1.06
PS-3	51,000	10.6
PS-4	160,000	1.06
PS-5	411,000	1.06
PS-6	860,000	
PS-7	1,800,000	1.20
PS-8	277,000[a]	2
	Polyethylene Oxides	
PEO-1	1,500	<1.1
PEO-2	2,000	<1.1
PEO-3	3,000	<1.1
PEO-4	6,000	1.08
PEO-5	10,000	<1.1[b]
PEO-6	28,000	<1.2[b]

[a] $\bar{M}_v$. [b] Based on gpc data.

In carrying out a gradient elution, a scale was penciled on the tlc sheet so that the advance of the front could easily be followed as a function of time. The eluent composition was also monitored during the run so that the composition of the solvent passing the starting point at any time during the experiment was known. After the run was completed and the samples were visualized, the composition of eluent necessary to displace a given molecular weight sample can be calculated. In either gradient or constant composition experiments, elution times are typically 20–60 min.

At the end of the run, the plates were dried and the samples were visualized. For the polystyrenes. short-wave uv illumination indicated the samples as dark spots on the fluorescent background. Simply spraying iodine solutions on the PPO samples revealed dark brown spots on a light background.

Results and Discussion

Constant Eluent Composition. When one component eluents are used, no separation according to molecular weight was observed, with one exception. Acetone partially elutes PS-1 and PS-2 (R_f's of 0.60 and 0.55) leaving all the higher molecular weights behind. This finding can be directly traced to the solubility of these polymers in acetone, where none of the higher molecular weight PS samples dissolve. Because of its polarity acetone is an excellent eluent. On the other hand, CCl_4 dissolves all the PS samples, yet elutes none. In this case the displacing power of CCl_4 is too low to elute the adsorbed macromolecules. Elution of the polystyrenes with a single solvent normally results in either no displacement (CCl_4) or complete displacement (toluene, THF, $CHCl_3$, dioxane) regardless of molecular weight.

Eluting the samples with mixed solvents can provide

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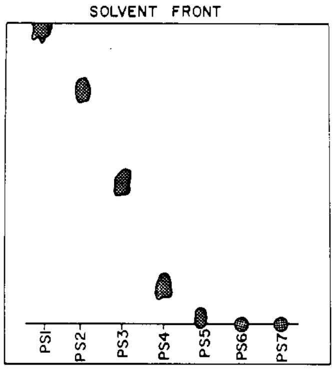

Figure 1. Mixed solvent elution of polystyrenes on silica gel, acetone/chloroform (80/20).

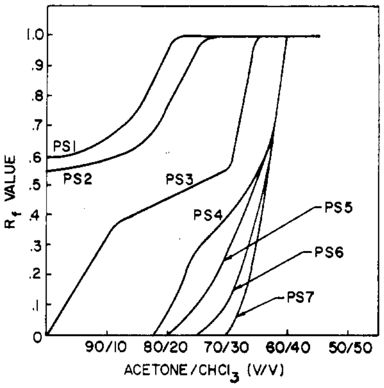

Figure 2. R_f values for the polystyrenes on silica gel in acetone–chloroform mixtures.

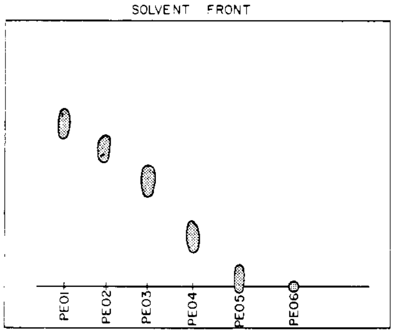

Figure 3. Mixed solvent elution of PEO fractions: substrate, silica gel; solvent, ethylene glycol–methanol (80/20).

good separation over a limited molecular weight range. Figure 1 shows a separation achieved in an 80/20 (v/v) acetone–CHCl$_3$ mixture. Figure 2 shows how the R_f values of the individual PS samples changes with eluent composition. These results lead directly to the use of gradient elution to cover the wide molecular weight range of the PS series.

The PEO samples were strongly bound to either adsorbent and strong eluents were required to get any displacement. Figure 3 shows a separation achieved in a 80/20 (v/v) ethylene glycol–methanol mixed solvent elution.

Gradient Elution. The experiment begins with acetone or CCl$_4$ as the eluent. After an elution of 1 or 2 cm from the starting point, a second (stronger) eluent is added from the syringe pump for the remainder of the run. Figure 4 shows the tracing of a typical gradient elution tlc plate. The elution curves derived from the height–composition data are shown in Figure 5 for some representative runs. The values for solvent composition necessary to elute a given molecular weight obtained in the gradient experiment agree with those found in constant composition tests. In general, the reproducibility is very good if plate activity, temperature and sample size are carefully controlled. Some downward tailing of the sample spots in the mixed solvent separations was noted, but the spots in the gradient elution are sharply defined because of the increasing eluting power of the solvent behind the mobile spot. The differences between the elution profiles shown in Figure 5 demonstrate how differences in the polymer–solvent–substrate interactions can affect the outcome of an experiment. These differences also indicate the potential power of this technique for carrying out enhanced separations at either end of the molecular weight range. A gradient elution result for PEO on alumina is pictured in Figure 6.

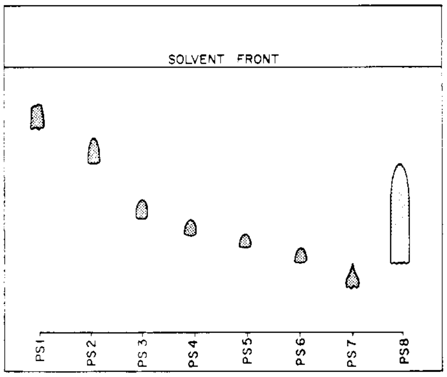

Figure 4. Gradient elution results: substrate, aluminum oxide; solvents, acetone → acetone/THF (70/30).

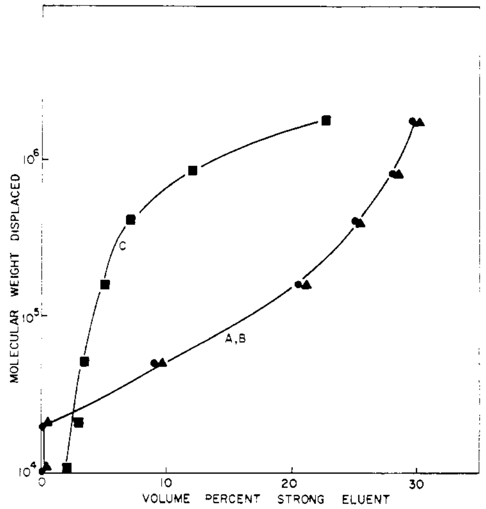

Figure 5. Molecular weight eluted *vs.* solvent composition from gradient elution experiments: ●, acetone → acetone/chloroform (silica gel); ▲, acetone → acetone/toluene (aluminum oxide); ■, CCl$_4$ → CCl$_4$/THF (aluminum oxide).

With the separating ability of tlc established, attention must be directed at the phenomena occurring during elution. Based on the weight of adsorbent per unit area of tlc plate, an estimate of adsorbent surface area based on particle size, the density of the adsorbent layer and the sample weight, we calculate that, as normally spotted, the sample coverage amounts to 2–3 $\times$ 10^{-4} mg of polymer/cm^2 of adsorbent surface. If the mobile solvent were to elute the sample com-

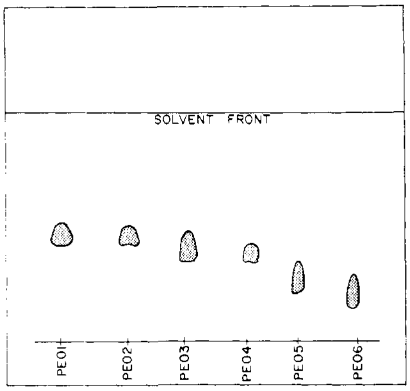

Figure 6. Gradient elution of PEO samples: substrate, aluminum oxide; solvent, methanol → methanol/DMF (80/20).

pletely, the polymer concentration would be about 1 mg/ml. When the polymer is displaced these values will decrease because of diffusion enlargement of the spot size. With constant initial spot area, we increased sample weights to determine the point at which the adsorbent became obviously overloaded. While the behavior differed somewhat with sample molecular weight, substrate and solvent, we find that for the polystyrenes on alumina, coverages of $6\text{–}8 \times 10^{-4}$ mg/cm^2 of adsorbent surface result in obvious flooding of the substrate. These values are in good agreement with the equilibrium adsorption of $\sim 7 \times 10^{-4}$ mg/cm^2 found by Stromberg[11] for a number of substrates.

With the knowledge that our tlc conditions are normally well below substrate saturation, we may now consider the adsorption and desorption occurring during elution of a given sample in an eluent of constant composition. The adsorption rate at the "head" of the sample and desorption rate at the "tail" of the sample must be rapid relative to the rate of motion of the solvent in order for the distribution of polymer along the substrate (or within the mobile solvent) to be an accurate reflection of the molecular weight and polydispersity.

Under conditions where R_f 0, the polymer does not enter the mobile phase, either because of insolubility or insufficient displacing power of the solvent. Where R_f 1.0, it is obvious that the polymer entered the mobile phase rapidly (<2 min) and stayed there, without significant adsorption during elution. For $0.0 < R_f < 1.0$ we must determine whether the equilibrium criteria mentioned previously are met. This is especially critical in view of other studies[12,13] showing that desorption rates in constant composition solvents are low and decrease with increasing molecular weight.

To examine the extent of nonequilibrium behavior resulting from low desorption rates, samples of each molecular weight polystyrene were eluted to an R_f value 0.3–0.6 with an appropriate two-component solvent. After outlining the sample as visualized by the fluorescence method, the region below the spot was reeluted using toluene. This should displace any small amount of material present on the plate as a "tail" and deposit it as a spot at the limit of toluene solvent flow (R_f 1.0 in toluene). This second elution was terminated within 0.5 cm of the original spot, the plate was dried and again examined. Only for PS-6 and PS-7 was there enough material in the second spot to be detected visually.

Thus, nonequilibrium adsorption can occur during the tlc of polymers, primarily under three conditions: constant composition mobile phase, high molecular weight polymer, and rapid mobile phase rate of travel. There is another type of nonequilibrium condition which occurs during elution but its relevance is somewhat obscure at the present time. It has been shown[11] that there are long term changes in the conformation of the polymer in the adsorbed layer. We feel that the time scale of the experiment is probably too short to allow significant rearrangement within the adsorbed layer between the successive adsorption–desorption cycles taking place in a sample with $0 < R_f < 1.0$.

The conventional separation of small molecule compounds depends primarily upon the relative strengths of solute–substrate interaction. Normally these differences in strength are derived from differences in solute chemical structure. In this work, all the solutes have the same structure (tacticity variation was ruled out by nmr studies) and the difference in solute–substrate interaction strength appears to be derived from the number of adsorption sites per molecule, which is a function of molecular weight.

The potential value of tlc to the study of polymers goes beyond what has been found here. The ability of any technique to separate molecular weights must be complemented by a means of determining the amount of material at any given molecular weight. The obvious breadth of PS 8 shown in Figure 4 is of little value unless an exact analysis of the polymer density as a function of distance along the elution path can be made. There are a number of techniques available for obtaining quantitative information from tlc plates.[16–18] One of the newest is scanning spectrodensitometry, where a monochromatic light beam (uv–vis) is either transmitted through the adsorbent layer or reflected from it to a detector. When the wavelength is set on a strong adsorption peak for the sample, the amount of light reaching the detector is a function of the amount of sample in the beam. The results of such analysis will be the subject of future communications.

Conclusions

Polymers may be separated in tlc by either constant composition mixed solvent or gradient elution. As normally carried out, the concentration of polymer in the mobile phase is comparable to conventional adsorption experiments, and surface coverages are below equilibrium plateau values. Under conditions of high molecular weight and rapid elution, some nonequilibrium conditions may be encountered. As methods for the determination of concentration along the elution path are developed, tlc fractionation can become a valuable analytical tool in molecular weight and polydispersity determinations.

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