# EVALUATION OF THE THEORY AND EXPERIMENTAL CONSEQUENCES OF GRADIENT LIQUID CHROMATOGRAPHIC POLYMER SEPARATIONS

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SUMMARY

The separation of synthetic polymers of different molecular weight by non-aqueous, gradient liquid chromatography does not follow traditional chromatographic mechanisms. As a result one can produce separations where late eluting solutes have narrower bandwidths than early eluting solutes, where shorter columns result in higher resolution than identical longer columns, and where the selectivity and resolution are independent of the type of stationary phase used. These results are explained for polystyrene, poly-α-methylstyrene and polyisoprene using recently developed theory. The results of this work are not only pertinent to the practice and theory of chromatography but also to the study of polymers.

INTRODUCTION

The use of various mixtures of thermodynamically good and poor solvents to fractionate synthetic polymers of different molecular weight has been known for some time[1,2]. Not until recently, however, has this phenomenon been used successfully in conjunction with modern liquid chromatographic (LC) systems for the evaluation of molecular weights and molecular weight distributions[3-6]. Recently an extensive theory of homopolymer fractionation via gradient elution chromatography was published[7,8]. The phenomenon described is akin to a highly selective precipitation or dissolution process. Indeed, experimental evidence for such a process is strong for moderate- to high-molecular-weight polymers[3-6]. Previous thin-layer chromatographic work indicated that one must minimize adsorption effects in order to see a molecular-weight dependence on retention[9-12]. Most traditional chromatographic theories assume partitioning or adsorption type behavior. A chromatographic theory largely based on mobile phase control of solute retention is uncommon. Furthermore, any solute which elutes in accordance with this theory would be expected to show unusual behavior (compared to traditional chromatography).

In this work the ramifications of nonaqueous gradient elution are explained and experimentally evaluated using different-molecular-weight polymers. The limitations of the theory and experiments are explored as well. Lastly, a comparison of gradient LC polymer fractionation with traditional chromatography of small solutes is made.

EXPERIMENTAL

*Materials*

The following columns were used in this study: Varian Micropack CN 10 (length 30 cm), Whatman Partisil 10 ODS (length 25 cm), Whatman Partisil 10 $C_8$ (length 25 cm), Whatman custom made Partisil 10 $C_2$ (length 25 cm). Whatman custom made Partisil 10 $C_{18}$ ODS 3 (lengths 10, 5 and 4 cm). All Whatman columns were packed with material from the same silica gel lot under identical conditions. HPLC grade methanol and methylene chloride were obtained from Waters Assoc. The following polymer standards were used. (A) poly-α-methylstyrene from Polymer Laboratories, mol. wt. = 19,500 ($M_w/M_n$ = 1.15); mol. wt. = 87,000 ($M_w/M_n$ = 1.10); mol. wt. = 760,000 ($M_w/M_n$) = 1.10. (B) Polyisoprene from Polymer Laboratories, mol. wt. = 33,300 ($M_w/M_n$ = 1.05); mol. wt. = 113,800 ($M_w/M_n$ = 1.05); mol. wt. = 260,000 ($M_w/M_n$ = 1.07). (C) Polystyrene from Waters Assoc., mol. wt. = 17,500 ($M_w/M_n$ = 1.04); mol. wt. = 35,000 ($M_w/M_n$ = 1.04); mol. wt. = 390,000 ($M_w/M_n$ = 1.04); from Polyscience, mol. wt. = 2000 ($M_w/M_n$ = 1.06); mol. wt. = 3570 ($M_w/M_n$ = 1.06); mol. wt. = 9000 ($M_w/M_n$ = 1.04); mol. wt. = 100,000 ($M_w/M_n$ = 1.06); mol. wt. = 63,000 ($M_w/M_n$ = 1.04); mol. wt. = 233,000 ($M_w/M_n$ = 1.06); mol. wt. = 900,000 ($M_w/M_n$ = 1.10); mol. wt. = 1,000,000 ($M_w/M_n$ = 1.2).

*Methods*

All gradient high-performance liquid chromatographic (HPLC) fractionations of polymers were done with a Varian 5020 Liquid Chromatograph. Polystyrene and poly-α-methylstyrene were detected with a fixed-wavelength 254 nm UV detector while polyisoprene was detected with a Waters Model 450 variable-wavelength detector at a wavelength setting at 240 nm. All polymer standards were dissolved in methylene chloride (0.5 mg/ml of each polymer) and 10 μl injected except in the sample loading experiment where solutions containing one single MW-type of polystyrene at different concentrations were prepared.

RESULTS AND DISCUSSIONS

Polymer fractionation occurs in conventional gradient HPLC by immobilizing a small sample of polymer at the beginning of a column and carrying out a gradient elution (*i.e.*, going from a thermodynamically poor solvent mixture to a thermodynamically good solvent mixture)[3-8]. In the case of polystyrene, poly-α-methylstyrene and polyisoprene, methylene chloride is commonly used as the good solvent and methanol as the poor solvent. A recent statistical mechanical treatment of the equilibrium distribution of isolated flexible polymers between a binary solvent mobile phase and a planar stationary phase can be used to account for many of the unusual chromatographic observations in this system[7].

Unlike traditional chromatographic separations, the capacity factor ($k'$) of a homopolymer (in a system where there is a binary solvent mobile phase but only a single adsorbed solvent) is given by

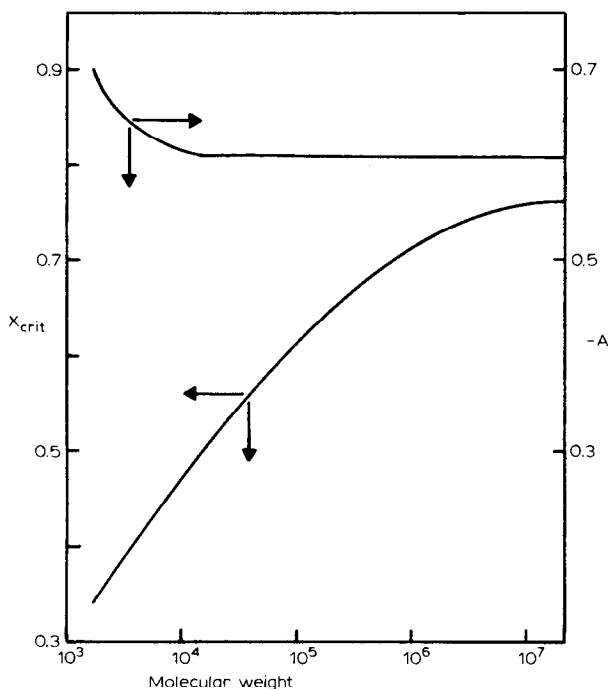$$k' = \exp\left[AM(X - X_c)\right] \tag{1}$$

Fig. 1. A plot showing the molecular weight dependence of the critical solvent composition ($X_c$) and of $A$. Note that $A$ is constant for molecular weights $> 10^4$. The following values were used in plotting this figures: $\chi_{12}^m = 0$, $\chi_{13}^m = -0.1$, $\chi_{23}^m = 0.5$, $\beta(\Delta\varepsilon_{1.2.5}) = -0.115^7$.

where $M$ = the degree of polymerization of a polymer, $X$ = the mobile phase composition (volume fraction) of the good solvent, $X_c$ = the critical mobile phase composition, and $-A$ approaches a positive asymptotic value independent of $M$ and $X$ for high polymers and depends on solvent–solvent and solvent–polymer interchange energies (Fig. 1)[7].

The meaning of capacity factor (*i.e.*, the amount of solute in the stationary phase divided by that in the mobile phase) is well known as is that of $M$ and $X$. However, further explanation of the critical composition ($X_c$) and $A$ are needed to fully understand eqn. 1. The critical composition of a binary solvent system is that composition at which an immobilized polymer will just dissolve or conversely that composition at which a dissolved polymer will just precipitate. Theory predicts[7] using cubic lattice statistics that

$$X_c = X_c(M = \infty) + aM^{-1/2} + bM^{-4/5} + cM^{-1} \ln M \qquad (2)$$

where

$$X_c(M = \infty) = 1 + [\Delta\varepsilon(1, 3; S)/kT + \ln (5/4)] (\chi_{12} + \chi_{13} - \chi_{23})^{-1}$$

and $\chi_{12}$, $\chi_{13}$ and $\chi_{23}$ respectively represent reduced interchange energies between molecules of solvent 1 (methylene chloride), solvent 2 (methanol) and polymer segment 3. $\Delta\varepsilon$ (1, 3; S) is the adhesion free energy for adsorption of a polymer segment

onto the stationary phase surface with the concomitant displacement of a type 1 solvent molecule. The molecular weight independent coefficients *a*, *b* and *c* depend on the interchange energies and coefficients *b* and *c* also depend weakly on solvent composition (see eqn. 37 of ref. 7).

The importance of the critical composition (according to eqn. 2) is that it is directly dependent on the polymer molecular weight. This $X_c$–$M$ dependence is a result of the flexibility of the polymer molecule which allows it to change its config-uration and size in response to its environment[4,7,8]. The molecular weight depen-dence of $X_c$ is nicely illustrated by the lower curve in Fig. 1. Also illustrated in Fig. 1 is the molecular weight dependence of *A* which is given by

$$A = \chi_{12} + \chi_{13} - \chi_{23} + dM^{-4/5} + eM^{-1} \tag{3}$$

where *d* and *e* are molecular-weight-independent coefficients that depend on the in-terchange energies and are weakly dependent on solvent composition (see eqn. 44, ref. 7). It is apparent that *A* does not change significantly for polymers of molecular weight greater than 10,000 Daltons (Fig. 1).

The chromatographic behavior of a solute that elutes according to eqn. 1 would be different from that of a small solute goverened by conventional adsorption of partition mechanisms. The use of traditional chromatographic theory to describe the gradient elution of synthetic homopolymers would be deficient[13]. This is because, when dealing with polymers, one must consider segment–segment interactions (in-termolecular interactions) as well as segment–solvent and segment–stationary phase interactions. Conventional theory, of course, does not include segment–segment in-teractions and indeed they are not needed for small solutes and are usually small for oligomers. However, as has been known for years in polymer chemistry, segment–segment interactions can be very important in the solution behavior of macromole-cules[14]. Consequently (as per eqn. 1) molecules of large *M*, have capacity factors that can either be very large or very small. Furthermore, in the ideal case, there would
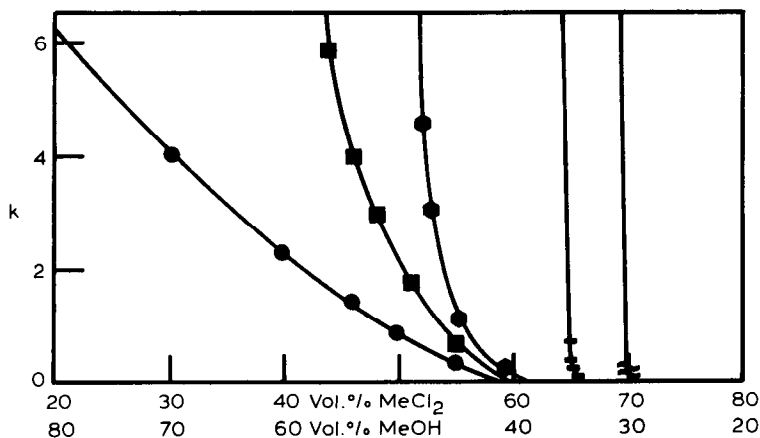


Fig. 2. Plots of the capacity factor (*k'*) of different styrene polymers *versus* mobile phase composition. The *k'* values were evaluated from isocratic HPLC data using a 25-cm, 10-μm, $C_{18}$ reversed-phase column. Polystyrene molecular weights are: (●) $2.0 \cdot 10^3$; (■) $3.6 \cdot 10^3$; (○) $9.0 \cdot 10^3$; (▬) $1.0 \cdot 10^5$ and (≈) $= 9.0 \cdot 10^5$.

be an abrupt change from an exceedingly high to a low $k'$ as a result of a very small change in solvent composition. Thus $k'$ is very large when $0 < X < X_c$, but becomes small when $1 \geqslant X > X_c$[7].

The veracity and limitations of eqn. 1 can be determined experimentally by evaluating $k'$ values for different molecular weight polymers at different solvent compositions. Results are shown in Fig. 2. It is apparent that for the higher-molecular-weight styrene polymers the behavior approaches that of a true critical concentration (i.e., ca. 70% methylene chloride) for polystyrene of mol. wt. $9 \cdot 10^5$ and ca. 65% for polystyrene of mol. wt. $1 \cdot 10^5$. The change in $k'$ for these solutes is abrupt and large. Next consider the curve for polystyrene of mol. wt. 9000. There is a more gradual change in $k'$ in the solvent composition range of ca. 53 to 60% methylene chloride. Outside this range, the polymer is either totally retained or totally unretained as per eqn. 1. The solvent composition range where a gradual change in $k'$ occurs is referred to as the transition region[4]. As the molecular weight of a polymer becomes smaller the transition region increases (Fig. 2). It is believed that separations in the transition region occur by traditional partition or adsorption type mechanisms. Thus, as the molecular weight of a polymer approaches that of a small solute, the mechanism by which the separation occurs does likewise. Analogous results were obtained for poly-$\alpha$-methylstyrene and polyisoprene. By carefully controlling the solvent composition (and type) one can separate oligomers and some small polymers entirely in the transition region[13,15,16]. This is impossible for high polymers, however.

It is apparent from the data in Fig. 1 that there is no linear or regular relationship of polymer $k'$ values versus solvent composition except in the transition region. Once one exceeds this range, the $k'$ values rapidly go to infinity (on the poor solvent side) or to zero (on the good solvent side). Larmann et al. (Fig. 4 in ref. 13) also made plots of $k'$ versus solvent composition. Their experiments were reproduced over a wide range of solvent compositions and the same discontinuities were found (as indicated above) for all but the smaller oligomers. One avoids seeing these discontinuities by limiting the plots to data in the transition region.

For high polymers the plot of $k'$ versus solvent composition is nearly vertical (Fig. 2) as predicted by eqn. 1. There is no significant transition region and the point at which the line intersects the x-axis is very near the critical concentration of that polymer. Since each polymer of different molecular weight has a unique critical concentration (Figs. 1 and 2) fractionation must occur during gradient elution (i.e., when going from a thermodynamically poor to a thermodynamically good mobile phase).

The effect of isocratic elution of a low-molecular-weight polymer standard in its transition region is shown in Fig. 3. Small increases of methanol in the mobile phase result in increased retention volumes and band broadening. It is believed that the band broadening is due to two factors. First, mass transfer is significant in the transition region where more traditional equilibrium processes occur (it is not as significant outside this region). Since the mass transfer of polymers is relatively inefficient, band broadening is significant. Secondly, the polymer standards used are never monodisperse but consist of a narrow range of similar molecular-weight polymers. These homologs tend to partially separate in the transition region adding to band broadening (Fig. 3).

It is apparent that the gradient separation of high polymers is accurately described by eqn. 1. In particular it follows from eqn. 1 that a given difference in solvent
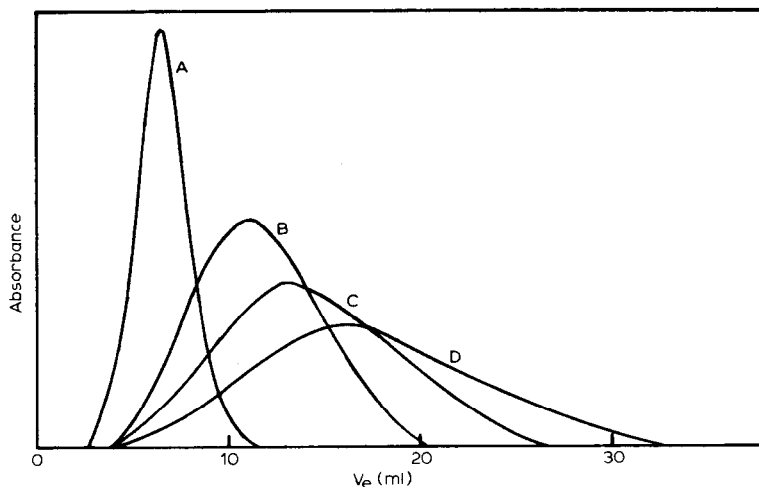
Fig. 3. Four superimposed LC chromatograms of a polystyrene standard (mol. wt. = 3570, $M_w/M_n$ = 1.06) which illustrate the effect of isocratic elution with different solvent compositions (in the transition region) on peak shape. Note that as one elutes with a "poorer" solvent both the retention volume and band width increase. The increase in band width is due to poor mass transfer in the transition region and the fact that the polymer standards are polydisperse. The mobile phase (methylene chloride–methanol) compositions are: (A) 50:50; (B) 47:53; (C) 46:54; (D) 44:56.

composition ($X$) has a much stronger influence in the high-molecular-weight region than in the low-molecular-weight region. Hence a linear composition gradient promotes high peak compression in the high molecular weight part of the chromatogram. There are other important chromatographic consequences for solutes that separate by this type of mechanism. For instance, the nature of the bonded stationary phase would make little difference to the separation. This is because the precipitation or dissolution process is mainly controlled by the mobile phase. Consequently the chemistry of the stationary phase should play a subordinate role provided the physical properties (*i.e.*, particle size, pore size, packing geometry *etc.*) are similar. Furthermore, separation efficiency, selectivity and resolution are controlled by the type of gradient one uses. The validity of these statements can be shown theoretically (*vide supra*) and experimentally (*vide infra*).

   Table I shows the effect of column length on the retention volume and peak width of different-molecular-weight polymers. Note that for any given column length, the elution volume increases with increasing solute molecular weight. Conversely, the peak width decreases for the later eluting, higher-molecular-weight polymers. Both the compression of the later eluting peaks and the additional band broadening of the smaller polymers (due to inefficient mass transfer in their transition region) is apparent from this data.

   The retention volumes of the high polymers (Table I) are largely independent of column length while the $V_e$ values of the lower molecular weight polymers decrease with decreasing column length. This, once again, is a reflection of the fact that the high polymers will only move when their critical mobile phase concentration is exceeded. The lower molecular weight polymers, of course, move continuously in their transition region. The peak widths of the high polymers (Table I) decrease with

TABLE I

THE EFFECT OF COLUMN LENGTH ON THE SEPARATION EFFICIENCY OF DIFFERENT-MOLECULAR-WEIGHT STYRENE POLYMERS

The stationary phase was 10-$\mu$m Whatman ODS-3 (see Experimental). Polymers were eluted with identical linear gradients (*i.e.*, from methylene–methanol (45:55) to 100% methylene chloride in 27.5 min). Net elution volumes ($V_e$) are given in ml and peak widths at half peak heights ($W_{1/2}$) are given in cm.

| Column length (cm) | Molecular weight | | | | | | | | | | | |
| | 3570 | | 9000 | | 35,000 | | 100,000 | | 390,000 | | 900,000 | |
| | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25.0 | 8.0 | 2.42 | 10.2 | 1.20 | 13.6 | 1.10 | 16.8 | 0.75 | 18.1 | 0.50 | 18.7 | 0.50 |
| 10.0 | 5.8 | 3.15 | 9.5 | 2.10 | 13.9 | 1.00 | 15.5 | 1.07 | 18.1 | 0.35 | 18.3 | 0.29 |
| 5.0 | —* | —* | 8.4 | 2.60 | 13.3 | 1.68 | 16.9 | 1.08 | 18.5 | 0.22 | 18.7 | 0.19 |
| 4.0 | —* | —* | 7.63 | 2.87 | 13.1 | 1.97 | 17.1 | 0.91 | 18.5 | 0.20 | 18.7 | 0.18 |

* Solute eluted at the void volume of the column.

decreasing column length. This, coupled with the fact that the net elution volume ($V_e$) is nearly constant, yields the surprising result that one can obtain significantly better resolution for these solutes using shorter columns. Conversely, smaller polymers elute from shorter columns with wider peak widths. This band broadening is a result of the increased fractionating ability of the mobile phase and the poor mass transfer of the polymer in the transition region (Fig. 3). In longer columns the small partially retained polymer has a longer residence time and is not completely eluted until the mobile phase composition is that of a good solvent. As previously demonstrated, elution with a good mobile phase results in very sharp peaks.

Table II shows the effect of the stationary phase type on the gradient frac-

TABLE II

THE EFFECT OF DIFFERENT BONDED STATIONARY PHASES ON THE NET RETENTION VOLUME ($V_e$) AND PEAK WIDTHS AT HALF PEAK HEIGHTS ($W_{1/2}$) FOR DIFFERENT MOLECULAR WEIGHT STYRENE POLYMERS

Experimental conditions (*i.e.*, gradient) were identical to that indicated in Table I. All columns were 25 cm in length except for the propylnitrile column which was 30 cm in length (see Experimental). $V_e$ in ml and $W_{1/2}$ in cm.

| Type of 10 $\mu$m bonded stationary phase | Molecular weight | | | | | | | | | | | |
| | 3570 | | 9000 | | 35,000 | | 100,000 | | 390,000 | | 900,000 | |
| | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_{18}$ | 8.0 | 2.42 | 10.2 | 1.20 | 13.7 | 1.10 | 16.8 | 0.75 | 18.1 | 0.45 | 18.7 | 0.40 |
| $C_8$ | 4.4 | 2.82 | 8.1 | 2.41 | 12.8 | 1.70 | 16.3 | 0.80 | 17.9 | 0.46 | 18.3 | 0.30 |
| $C_2$ | —* | —* | —* | —* | 12.9 | 1.75 | 15.9 | 0.98 | 18.3 | 0.48 | 18.6 | 0.43 |
| Propyl nitrile | —* | —* | —* | —* | 13.2 | 2.06 | 17.5 | 0.58 | 19.5 | 0.21 | 19.6 | 0.20 |

* Solute eluted at the void volume of of the column.

TABLE III

THE EFFECT OF GRADIENT VARIATION ON THE NET RETENTION VOLUME ($V_e'$) AND PEAK WIDTHS AT HALF PEAK HEIGHT ($W_{1/2}$)

All elutions were done on a 25-cm $C_{18}$ column beginning with methylene chloride–methanol (45:55) and enriched in methylene chloride at the rate indicated. Note that the experimental conditions for the 2% gradient are identical to those for the 25-cm column in Table I. $V_e'$ in ml and $W_{1/2}$ in cm.

| Gradient change (vol.%/min) | Molecular weight | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3570 | | 9000 | | 35,000 | | 100,000 | | 390,000 | | 900,000 | |
| | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ | $V_e$ | $W_{1/2}$ |
| 1% | 8.4 | 4.00 | 14.0 | 3.86 | 21.4 | 1.31 | 28.3 | 1.70 | 32.4 | 0.51 | 33.1 | 0.52 |
| 2% | 8.0 | 2.42 | 10.2 | 1.20 | 13.7 | 1.10 | 16.8 | 0.75 | 18.1 | 0.45 | 18.7 | 0.60 |
| 3% | 6.8 | 1.60 | 8.7 | 1.03 | 10.5 | 0.42 | 12.4 | 0.56 | 13.1 | 0.35 | 13.6 | 0.36 |
| 4% | 6.2 | 1.31 | 7.6 | 0.70 | 8.8 | 0.31 | 9.7 | 0.42 | 10.5 | 0.28 | 10.9 | 0.34 |

tionation of polystyrene. Four different bonded stationary phases were used including $C_{18}$, $C_8$, $C_2$ and propylnitrile. As predicted, the high polymers showed no significant difference in retention volume, peak width or peak shape when different stationary phases were used. On the other hand, the lower-molecular-weight styrene polymers (which have transition regions) begin to show traditional retention behavior (*i.e.,* increased retention volumes with the more hydrophobic stationary phases). Analogous results were obtained for poly-α-methylstyrene and polyisoprene.

The effect of gradient variation on macromolecular separations is shown in Table III. Once a good–poor solvent pair has been selected, the main control one has on separation efficiency and resolution is through changes in the gradient. As would be expected, the retention volumes and peak widths decrease with increasing gradient steepness. The decrease in peak width is much greater for the low-molecular-weight polymers however. Furthermore, the peak to peak separation (Table III) is greatest for the slowest changing gradient. A more complete evaluation of the resolution and efficiency of this technique is given elsewhere[5,6].

TABLE IV

THE EFFECT OF INCREASING THE AMOUNT OF SAMPLE INJECTED, ON THE NET RETENTION VOLUME ($V_e'$) OF DIFFERENT MOLECULAR WEIGHT STYRENE POLYMERS

Experimental conditions (*i.e.,* gradient) were identical to that indicated in Table I.

| Polymer concentration (g/ml) | $V_e$ (ml) | | | | |
|---|---|---|---|---|---|
| | Molecular weight | | | | |
| | 9000 | 63,000 | 100,000 | 233,000 | 900,000 |
| 10 | 8.3 | 14.5 | 15.0 | 16.0 | 16.7 |
| 1 | 8.3 | 13.1 | 14.1 | 15.8 | 16.6 |
| 0.1 | 8.2 | 12.3 | 13.6 | 15.7 | 16.1 |
| 0.01 | —* | 11.6 | 13.3 | 15.6 | 16.0 |

* Not detectable.

One of the simplification made in the theoretical treatment for precipitation chromatography is that the polymer solute is present at infinite dilution. In reality one must include polymer–polymer interactions especially for the higher-molecular-weight polymeric solutes[7,8]. The influence of polymer–polymer interactions can be detected via a sample loading experiment (see Table IV). In the absence of polymer–polymer interactions, one would obtain identical retention volumes upon injection of different concentrations of the same polymer. It is apparent from the results in Table IV that there is a small but significant decrease in the $V_e$ values of all polymers when less material is injected. This, again, is different from results reported in other work[13,15]. Development of a theoretical treatment which includes polymer–polymer interactions has recently been completed and further supports the conclusions of this work[8].

It is important to remember that both the theoretical and experimental data presented here describe the equilibrium distribution of flexible, homopolymer molecules between a binary solvent mobile phase and a stationary phase[3–8]. These results arise from the fact that the polymer can change its configuration in response to its environment (i.e., the mobile phase composition). Not only can the environment be altered by changing the mobile phase composition (i.e., to a better or poorer solvent) but one can theoretically and experimentally obtain analogous results by altering the temperature of an isocratic solvent system[2,7,14]. This would result in a much greater temperature dependance in the $k'$ of polymers than would be observed for small solutes. One should be cautioned not directly to apply the conclusions of this work to biopolymers which are generally not flexible homopolymers. Proteins, for example, are not only made up of a variety of subunits (i.e., amino acids) in a variety of proportions but also have secondary and tertiary structure which can be stabilized by disulfide bonds, hydrogen bonds and hydrophobic effects. Separation of these macromolecules can occur by a variety of useful techniques and mechanisms[17] which are not necessarily related to those described in this work.

## ACKNOWLEDGEMENT

## REFERENCES

1 C. A. Baker and R. J. P. Williams, *J. Chem. Soc.*, (1956) 2352.
2 M. J. R. Cantow, R. S. Porter and J. F. Johnson, *J. Polymer Sci.*, 1 (1963) 187.
3 D. W. Armstrong and K. H. Bui, *Anal. Chem.*, 54 (1982) 706.
4 D. W. Armstrong, K. H. Bui and R. E. Boehm, *J. Liq. Chromatogr.*, 6 (1983) 1.
5 K. H. Bui and D. W. Armstrong, *J. Liq. Chromatogr.*, 7 (1984) 29.
6 K. H. Bui and D. W. Armstrong, *J. Liq. Chromatogr.*, 7 (1984) 45.
7 R. E. Boehm, D. E. Martire, D. W. Armstrong and K. H. Bui, *Macromolecules*, 16 (1983) 466.
8 R. E. Boehm, D. E. Martire, D. W. Armstrong and K. H. Bui, *Macromolecules*, (1984) in press.
9 E. P. Otocka and M. Y. Hellman, *Macromolecules*, 3 (1970) 362.
10 E. P. Otocka, *Macromolecules*, 3 (1970) 691.
11 H. Inagaki, F. Kamiyama and T. Yagi, *Macromolecules*, 4 (1971) 133.
12 B. G. Belenkii and E. S. Gankina, *J. Chromatogr.*, 141 (1977) 13.

13  J. P. Larmann, J. J. DeStefano, A. P. Goldberg, R. W. Stout, L. R. Snyder and M. A. Stadaluis, *J. Chromatogr.*, 255 (1983) 163.
14  P. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, NY, 1971.
15  L. R. Snyder, personal communication, March 13th, 1983.
16  F. Eisenbeiss, E. Dumont and H. Henke, *Angew. Makromol. Chem.*, 71 (1978) 67.
17  J. Porath, J. Carlsson, I. Olsson and G. Belfrage, *Nature (London)*, 258 (1975) 598.