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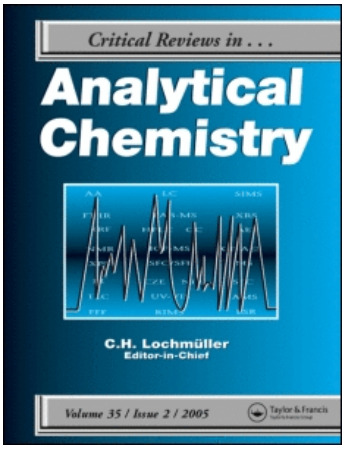

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High-Performance Liquid Chromatography of Polymers: Retention Mechanisms and Recent Advances

C. H. Lochmüller[a]; Chun Jiang[a]; Qicai Liu[a]; Vincent Antonucci[a]; Matti Elomaa[b]
[a] Department of Chemistry, Duke University, Durham, North Carolina [b] Laboratory of Polymer Chemistry, University of Helsinki, SF, Finland

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High-Performance Liquid Chromatography of Polymers: Retention Mechanisms and Recent Advances

C. H. Lochmüller, Chun Jiang, Qicai Liu, and Vincent Antonucci
Department of Chemistry, Duke University, Durham, North Carolina 27708

Matti Elomaa
Laboratory of Polymer Chemistry, University of Helsinki, SF 0014 Finland

SUMMARY: This paper reviews advances in the use of high performance liquid chromatography for the separation of polymers and polymer oligomers. Evidence is presented for ordinary chromatographic behavior up to 2 megadalton molar mass for polystyrenes, polymethylmethacrylates and polyethylene oxides. Examples are given in which static thermal gradients along the column give enhanced separations.

ABSTRACT: The high-resolution separation of polymer oligomers, their breakdown products and isomers is a significant challenge to modern separation science. The historically most successful methods are based on size exclusion strategies and not mobile phase/stationary phase partition in the conventional sense. In the last decade significant progress has been made in understanding the requirements for achieving elution chromatography of high {> 5 kDa} molecular weight organic polymers ranging from quite hydrophobic to quantitatively water soluble in character. This paper discusses retention mechanisms, the dynamics of the partition chromatographic process, evidence for significant delays in polymer equilibration to mobile phase conditions and the effect of the temperature dependence of polymer solubility. Conventional chromatographic behavior is shown to obtain for the separation of polystyrenes, polymethylmethacrylates, polyethylene oxides and polypeptides if proper steps are taken to carry out the experiment. The use of thermal gradients in spatial terms is shown to have significant value for achieving enhanced separations.

KEY WORDS: HPLC, polymers, non-polar, polar, rigid, polystyrene, polymethylmethacrylate, polyethyleneglycol, temperature gradient.

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I. INTRODUCTION

In recent years, there has been a rekindling of interest in methods for the fractionation of macromolecules using high-performance liquid chromatography include reversed-phase (RPLC).[1–16] The driving force of this current interest is to determine whether modern HPLC and especially modern RPLC can bring to the separation, isolation, identification and quantitation of large molecules (molecular weight greater than 1 kDa) advances such as those already seen in the application of these methods to small molecules. The majority of polymer separations are done using some variation on size-exclu-

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sion methods. The gel permeation, gel filtration and even the electromigration methods such as gel "electrophoresis" are based on a size exclusion mechanism. Partition chromatography offers chemical selectivity based on relative solubility and on solute-stationary phase interaction. If it were possible to achieve both isocratic and gradient elution of polymer oligomers and isomers then these separation techniques could provide higher resolution and control than is currently the case in a majority of the applications where size-exclusion *alone* dominates.

If we are to accept the historic models for advances in separation science, the mechanism of separation and the underlying physico-chemical principles of partition separation of polymers must be established. Although there are many reported successful examples of polymer separations[17-20] and several models have been suggested, the retention mechanism of polymers in RPLC remains unclear.

- Glöckner[10,11] suggests a "precipitation-redissolution" model for the gradient elution of polymers. In this model, polymer molecules repeatedly precipitate onto the stationary phase and re-dissolve into the mobile phase until finally eluting at a mobile phase composition at which the polymer is totally soluble. Retention depends solely on the mobile phase with the column playing a passive role providing only a large surface area surface as support for the precipitate.
- Armstrong, Martire, Boehm and Bui[14] proposed a model for critical solvent composition behavior found by some in the isocratic elution of polymers. This model is often called BMAB theory or critical solution theory. This theory was developed from a statistical treatment of the equilibrium distribution of infinitely dilute polymer molecules between a mobile phase and a stationary phase based on the Flory-Huggins model. According to the model, the range of the mobile phase composition within which finite capacity factor (k′) values can be observed under isocratic elution conditions is very narrow for high molecular weight polymers. Plots of log k′ versus the mobile phase composition show slopes that mean that polymer molecules are either infinitely retained or not retained at all. Therefore, isocratic elution should be either impractical or impossible for wide ranges of molecular weight.
- Snyder and co-workers[6-8] assert that no special model is needed for the polymer retention and the traditional models can be used in interpreting the retention behavior of polymers.
- Lochmüller and McGranaghan[15] considered the likely fate of the injected polymer sample in the mobile phase prior to its contact with the column. They found that traditional retention behavior was obtained only when the sample was adequately mixed with the mobile phase using a low dispersion, crocheted mixer before the solute-column contact occurred. They reported that polystyrenes of molecular weight ranging from 20000 to 2,800,000 Daltons could be separated under isocratic elution conditions with binary mobile phases of tetrahydrofuran/H_2O and dichloromethane/acetonitrile. Finite, non-zero k′ values and monotonic relationships of log k′ versus the volume percentage of tetrahydrofuran and dichloromethane were observed.[14] Alhedai, Boehm and Martire[16] have since reported the isocratic elution behavior of polystyrene homopolymers.

The work presented here is a combination of a review of recent advances and new work in our laboratories directed at answering the following questions of chromatographic focus:

- Is isocratic elution of polymers practically possible? (Do polymers which are

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more polar than polystyrene exhibit traditional retention behavior as well?)

- Can isocratic retention can be predicted from gradient elution runs and *vice versa*? (Can the retention of these polymers be predicted based on the Linear-Solvent-Strength model?)
- Do these polymers show traditional chromatographic behavior if uniform solvation conditions can be achieved *without* a pre-column mixer?
- What is the retention behavior of polymers when both solvents in binary mobile phase are relatively "good" solvents?

In addition, it is often worthwhile to apply non-chromatographic methods to obtain further insight into possible retention mechanism details. To this end, some time-dependent light scattering and excimer luminescence experiments are also described. These latter studies are an attempt to confirm the role of the pre-column mixer in the success of isocratic elution separation of polystyrene polymers as first reported in the work of Lochmüller and McGranaghan.[15] The reader will discover that the issue is a complex one at best.

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II. POLYSTYRENES

The discovery[15] that the use of a pre-column, crocheted-mixer permitted normal isocratic elution of polystyrene polymers in the sense that monotonic changes in the composition of a binary mobile phase causes monotonic changes in retention also confirmed that the rate of change of retention volume with composition is increasingly steeper as the molecular weight increases. For the relatively monodisperse polystyrene standards used in those studied, the peak shape is symmetrical and free of peak-doubling anomalies often seen when this type of mixer is not used. Plots of log k′ vs. ϕ (volume percent) tetrahydrofuran {THF} in water and for acetonitrile {ACN} in dichloromethane {MeCl} were shown to be linear for polystyrenes ranging from .6 to 300 kDa. A plot of the "S-value" $\{\Delta \ln k'/\Delta\phi\}$ for the polystyrene experiments is shown as plotted against molecular weight in Figure 1. The anomalous S-value for the 300 kDa sample is apparently the result of size-exclusion because the plot becomes straight including the value for a 300 kDa sample if a column of average pore size 300 Angstrom is used. A test of sample concentration effect was done and there was no evidence for a concentration effect on elution volume over a range of $20 - 2 \times 10^{-3}$ mg/mL {4 orders} in three binary solvents and a factor of 2 difference in elution volume.

If the polymer solutes are behaving conventionally, then the known relation between gradient and isocratic chromatography should obtain. One test of this is to predict isocratic elution volume from gradient methods. A second, and perhaps more demanding, is to determine the predicted S-value from gradient measurements and to compare these to the ones determined by isocratic measurement. Table 1 shows the results for THF/Water and MeCl/ACN {acetonitrile} where the predicted values are gradient derived and the actual data are from individual isocratic runs using several mobile phases.

Remarkably good agreement is achieved given the rather steep nature of the $\Delta \ln k'/\Delta\phi$ values which make slight differences in actual mobile phase composition a major influence on measured retention. At the higher molecular weights, even the purge gas is best pre-saturated with mobile phase to avoid evaporative loss. Table 2 compares the prediction of gradient elution times and gradient composition at elution with that actually observed:

Clearly the addition of a low dispersion mixer which mixes the injected sample plug in the mobile phase before column contact occurs is advantageous in the case of polystyrene chromatography both in normal phase and reversed phase experiments. One interesting anomaly is the observation reported[15]

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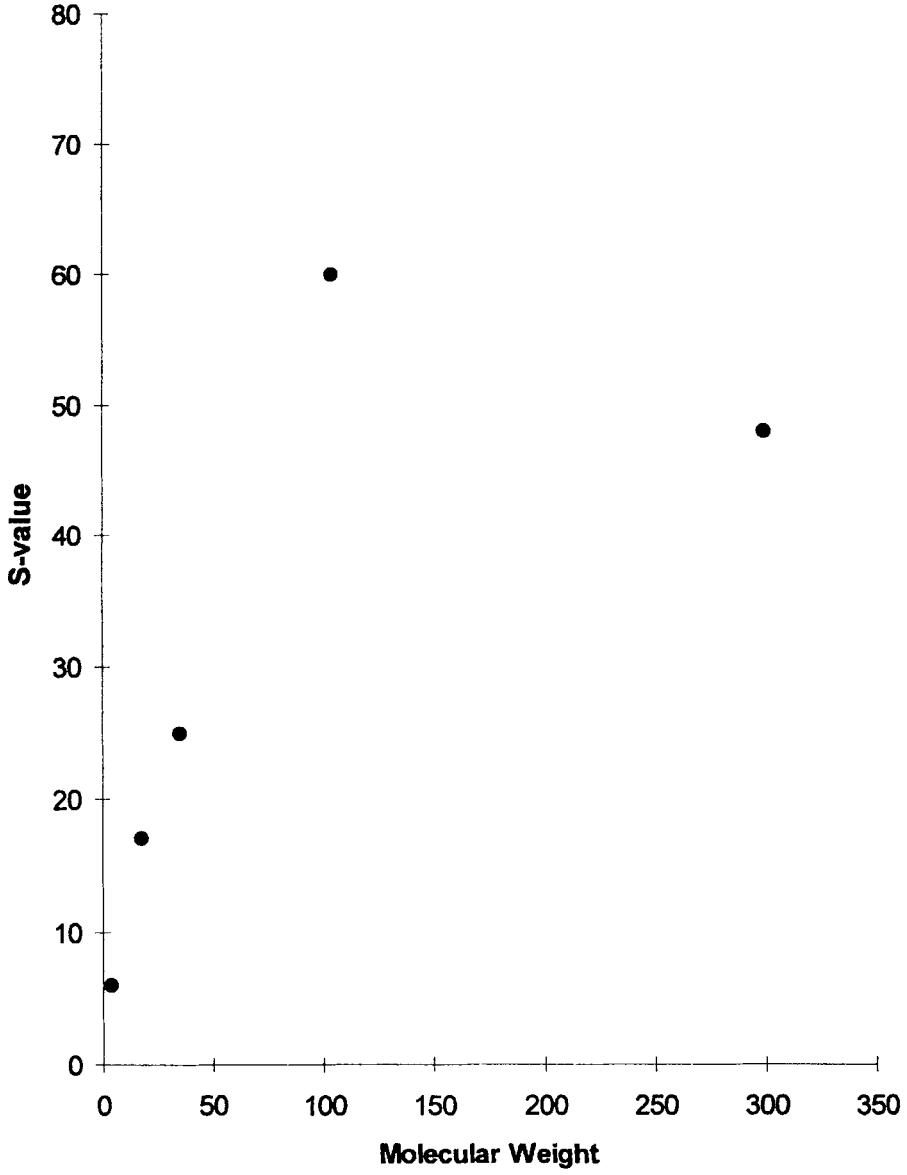

FIGURE 1. Plot of S-value for polystyrene standards on a C-18 column (100 Å pore size) MeCl/ACN mobile phase.

that "normal behavior" was obtained for polystyrenes with mol. wt. > ~75 kDa only if a larger mixing volume was used. The anticipated relation would be that a mixer capable of mixing a nominal 5 microliter sample of 6 kDa polystyrene solution with the mobile phase would suffice for any 5 microliter sample independent of the molecular weight of the polystyrene in the sample.

This anomalous effect of sample molecular weight could arise from viscosity differences, from self-aggregation or any of a number of other causes. However, the way a "larger" mixing volume was achieved was by using a *longer* tube in the form of a crocheted mixer. A tube of longer *length* but of the *same internal diameter.* Put in simpler terms, the residence time of the sample in the mixer was increased by the addition of

TABLE 1
Predicted vs. Found Values of the S {Δ ln k'/Δϕ} Value for Polystyrenes

Mol. wt.	Predicted	Found
THF/Water		
4000	11.43	11.51
17500	33.12	35.21
35000	46.14	47.39
MeCl/ACN		
4000	3.46	5.75
17500	16.93	13.03
35000	23.17	17.51
104000	44.02	43.69
300000	91.87	95.33

TABLE 2
Gradient Time Predicted and Found for Polystyrenes on a C-8 (300 Angstrom Pore Size) Column THF Water and Then MeCl/ACN {mol. wt with *}

	t_g		Vol %	
Mol. wt.	Predicted	Found	Predicted	Found
4000	8.14	8.20	79.65	79.95
17500	9.15	9.37	84.75	85.80
35000	9.28	9.57	85.35	86.81
4000*	4.28	4.57	38.68	44.61
17500*	12.45	12.90	45.73	46.85
35000*	14.18	14.49	50.05	50.83
104000*	15.89	16.29	54.33	55.33
300000*	16.83	16.90	56.67	56.85

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more mixing volume simply because the transit time at a given flow rate increases as the path length increases. The question then was whether there was better mixing in a longer tube or was there possibly a *time-dependent* response to the change to a more hostile solvent environment on the part of the polymer solutes which time increased with molecular weight. The time dependence would have to be on a chromatographic time scale of seconds. This led to a study of the mixing behavior of crocheted mixers using small and large solute probes.

III. THE MECHANISM OF PRE-COLUMN MIXING

The geometrical deformation of straight tubing has been used to induce secondary-flow fields in flowing liquid systems for quite some time.[29] Several types of deformations have been used including pinched sections, coils, helices, and many much more elaborate designs.[30–33] The introduction of sharp turns to a length of tubing is one such design. It is desirable because it maximizes the aspect ratio (the ratio of the radius of the

tube to the radius of a single turn) which causes a significant radial flow component to the otherwise parabolic velocity field. Bernoulli's laws predict a pressure gradient from the inner wall (higher pressure and shorter path length for materials to traverse) to the outer wall of each turn (lower pressure and longer path length) which will force material to circulate from the higher velocity regimes to the lower ones continually. The effect seen is the evolution of a significant radial component to the otherwise axially-directed, laminar flow field as described by the Poiseuille equation:

$$\mu = r^2 \Delta P/8\eta L \qquad (1)$$

(where μ is velocity, r is the tube's radius, L is the tube's length, ΔP is the pressure drop, and η is the viscosity of the flowing stream.)

These processes serve to redistribute solute within the zone by repeatedly mixing the leading edge into the zone's interior. The resulting flowing materials experience a significant decrease in dispersion which is displayed as a narrower distribution of solute with time than that produced in straight tubing. The obvious advantages in detection of reducing elution volumes for low concentration chromatographic samples has attracted the chromatographic community for over a decade.[30,31] Katz and Scott have adapted these considerations in designing "zig-zag" or *serpentine,* low-dispersion chromatographic connecting tubing.[31] They demonstrated that this tubing design adds little if any variance-per-unit length to solute distributions as a function of flow rate. Numerous post column reactors have been developed using a related "knitted" or "crocheted" Teflon® tubing design to combine reagent streams and/or carry out post-chromatographic photochemical reactions.[15,33,34] The crocheted style seems most attractive among the designs for reactors since it allows for large aspect ratios (as employed in serpentine tubing) in a dense, mechanically stable configuration that compacts 500 mL into a 7.5 cm × 3 cm fabric.

We endeavor here to identify the physical processes that occur within crocheted mixers or "connectors" and firstly to quantitate sample plug uniformity or "mixing" for small molecule solute systems. A model is developed to describe "quality of mixing" provided by pre-column connectors by way of a common set of criteria or variables. Every effort has been made to minimize the number of variables used in the model in order to ease evaluation but not at the expense of rigor. Conclusions can be drawn from the results about the effectiveness of various connectors as low-dispersion mixers. These flow injection results are then used to extend the investigation to polymer systems in an effort to explain their usefulness as previously reported.[15]

A. Linear Cascade System Model

Several modeling approaches have been used to describe peaks as various combinations of mathematical functions, often as exponentially modified Gaussians.[36–39] Non-symmetrical distributions of solute are accounted for by calculation of peak parameters as variance, skewness, and kurtosis *via* moment analysis. Reviews of principle and use can be found for moment analysis.[40,41] Other physical models for dispersion use moment analysis include a cascade of dead volumes[42] and a stimulus-response technique common to chemical engineering reactor design.[43] The flow-injection profiles discussed here regarding various in-line connectors were modeled with a series of linear, thus interchangeable, elements which describe the connector as a filter.[44] An experimentally generated, narrow and square pulse of equal volume as the actual sample loop was used as the output of the injection valve, *YIN* (Equation 2). A dead time, defined as a dispersionless translation of the input square pulse, accounted for the time delay between sample introduction and detection. A series of N equi-volume, equilibrium mixing stages

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was employed to model the dispersion experienced by sample plugs during transit through the system. The mean residence time of a typical sample molecule in each of the N stages is called τ. An ideal mixer for chromatography would have the lowest number of well mixed stages which provides the desired result — a relatively low N and high τ.

The goal is to model dissimilar elution volumes on the common scale of equilibrium stages to determine not only the dispersion contribution to the system, but also to evaluate the degree of mixing homogeneity achieved per stage. The system transfer function (relation of narrow injection pulse to output peak shape) was defined in Laplace space by[24]:

$$Y_{OUT}(s)/Y_{IN} = e^{(-Ds)(1 + ts)^{-N}} \quad (2)$$

where s is the laplace variable = 2pwj, t is the mean residence time of tracer per volume element in a stage, D is the associated system dead time, and Y_{IN} and Y_{OUT} are the respective input and output for a given s. By using the frequency domain representation, the transfer function is greatly simplified to only three optimized variables. For some experiments, the transfer function was equated with the frequency-domain output expression by defining Y_{IN} as unity. With the simplification, the results were virtually identical to those produced via the convolution of the frequency-domain representation of the input square pulse and the Finite Fourier Transform (FFT) of the flow injection analysis profile.

The output can be deconvoluted from the output produced when the connector is removed to precisely determine the dispersion contribution of the various connectors used. The goal is to compare the *relative* performance of straight and crocheted connectors and therefore the inclusion of the dispersion of system elements such as the injector, detector, and tubing is of less concern.

A second, parallel pathway is offered for a fraction of the sample (a) to travel through the connectors if viscous samples cause non-uniformities in solute equilibration such as stagnant regions along the walls. This can be represented by a simple translation term here added to the above transfer function by the rules of block algebra[45] to give:

$$Y_{OUT}(s)/Y_{IN}(s) = e^{(-DT1\ s)} + e^{(-DT\ s)(1 + \tau s)^{-N}} \quad (3)$$

where DT1 is a second dead time associated with the system. Similarly, a second set of mixing stages could be put in this second path in much the same mathematical form as the first set of N stages, having the effect of broadening the second square pulse into a pseudo-Gaussian shape. Adjustment of the DT1 term allows one to superimpose the second curve on the first to account for such solute distribution phenomena as peak fronting and tailing. The physical interpretations of these approaches are discussed later in this section.

A 500 mL loop was constructed from the Teflon® tubing that contained 13 turns of approximately 15 cm in diameter. The flow field within such tubing should be essentially laminar as in straight tubing. This design was chosen to make transport and usage of the loop more convenient. The crocheted connectors were fabricated from the same tubing in much the same manner as previously mentioned post-column reactors. A #9 needle and single crochet stitch were used to produce the connectors. The resulting vessel is a "fabric" that is about 22 to 23 cm^2 and very durable. Swagelok (Alltech, Deerfield, IL) fittings were placed on the ends of all connectors over a larger diameter piece of Teflon® tubing which acted as a sleeve. The 1 mL crocheted connector is two 500 mL connectors linked together via a stainless steel union. A 1% toluene solution in MeOH (v/v) was used to model the connectors in the small molecule system. A 1.05 ppt 233,000 Dalton polystyrene solution in THF

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was made to model the connector response to large molecule systems. All polystyrene samples were obtained from Polysciences (Warrington, PA) and had Mw/Mn of approximately 1.05 except for the 1.6 *M* Dalton whose polydispersity index approached 1.2. Prodan, a highly fluorescent probe molecule, was used to determine the composition of the sample plugs introduced at a constant flow rate into systems which contained a 500 μL volume of crocheted tubing or the 500 μL loop. The position of emission maximum is a solvent polarity-dependent phenomenon. In protonated or polar solvents, red shifts to higher wavelengths will be seen for prodan and in non-polar environments a blue shift to lower wavelengths is exhibited. Fluorescence measurements were with a 200 μL flow cell and injection volumes were 10 μL. Prodan has an emission maximum at 490 nm in methanol and at 440 nm in methylene chloride, providing a 50 nm wide range for the construction of a calibration curve of emission maximum vs. binary solvent composition. The points on this curve were determined via an emission scan of Prodan in the desired solvent system in a conventional quartz cuvette. A constant excitation wavelength of 336 nm was used. The position of the emission maximum for the flowing systems was determined by a comparison of fluorescence intensities at several emission wavelengths. The percentage conversion data presented are the results of numerous, averaged trials. A second set of experiments attempts to determine the effect of flow rate on the composition of these same sample plugs. The data represented are also the result of several averaged trails for each experimental point given. The goal of both experiments is to determine if the added radial component introduced by secondary flow actually creates greater zone homogeneity as compared to essentially straight tubing of equal volume and inner diameter. Additionally, a dependence of this homogeneity upon flow rate may indicate that pre-column contact time is a significant parameter.

When no connector volume is placed between the injector and the detector system tubing, and a sample in acetonitrile is injected into a methylene chloride flowing stream, the Prodan solute solvent environment in the flow cell is 88.6% $MeCl_2$ as (emiss. Max. = 460 nm). Addition of the 500 μL, 15 cm diameter loop between the injector and the detector only slightly improved conversion to the mobile phase composition, i.e., no significant added mixing for the additional length of tubing. The 500 μL crocheted connector adds nearly three percent or 91% MeCl. This improvement is significant but may be vastly more important to the equilibration of larger molecules. Interestingly, the addition of another 500 μL of crocheted volume increases the apparent volume percent of $MeCl_2$ to 93.1% with less dispersion than the smaller crocheted connector. This is an important observation if polymer/mobile phase contact time is a major factor controlling polystyrene equilibration, thereby necessitating the use of larger mixing volumes. The above measurements were all made at a constant flow rate of 280 μL/min.

For all connectors used, there is a rapid decrease in mixing/% conversion to the $MeCl_2$ mobile phase environment with increasing flow rate until relatively constant values are achieved within the typical flow rate window used experimentally in HPLC. Again, mixing is greatest for crocheted connectors, as compared to the loop and no connector. However, at higher flow rates the added effect of the "doubled" volume for the crocheted tubing become negligible and the elution curves seem to overlap. This seems to say that there essentially is no added mixing or homogenization in the flow rate range of 1.0 to 2.0 ml/min afforded by adding additional crocheted volumes. If the small molecule analogy holds for macromolecules, it implies that the improved chromatographic results for higher MW polystyrenes seen[15] must be related to the added contact time of polymer segments with mobile phase com-

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ponents before column contact. If this is the case, this also would suggest that these polystyrene equilibration events necessary for normal retention behavior must occur on a chromatographic time scale. This could be a time-dependent solvation effect. Alternatively, in higher-molecular-weight polymer chromatography, the added viscosity of the immediate surroundings may make the mixing process less efficient per turn of the connector thus requiring more turns for equilibration. Coupled with this is the requirement that greater volumes of mobile phase must be moved into proximity of the polystyrenes for incorporation within the excluded volume of larger molecules.

The effect of mobile phase composition vs. sample solvent on the size of the polystyrene molecules was studied by pulsed light scattering. On the average, as much as 30 min was allowed for complete equilibration of the polymer in the cuvette of the instrument. If swelling is occurring {negative swelling is shrinking} , this is a longer time that the entire chromatographic experiment and the size should be representative of a maximum size and size-change in that practical sense. With these values as a guide, one can determine if the proposed size exclusion from 100 Å pores is plausible.

The molar volume of a coiled chain of polystyrene should be proportional to the molecular weight or number of repeat units that form the chain if molecules can be approximated by spheres. The relationship is reasonably linear over the large MW range studied indicating that in a good solvent the polystyrene coils can be approximated by independent spheres. This is important since most of the evaluations performed by the particle-sizing software assume a spherical-particle rheology. Additionally, the concentrations are varied randomly between 1 to 4 ppt for these solutions, which produces great variability in molarity among the samples. Despite this, no pronounced deviations are produced from a linear relationship, verifying that polystyrene exists as independent spheres under the experimental chromatographic conditions employed in the work cited.[15] The diameters found are large enough for the polymers in excess of 100,000 Daltons to be essentially prohibited from entering the stationary phase pores and thus should show smaller k′ values than predicted. However, the more interesting question is what effect does the introduction of water to the sample solvent have to these equilibrium values? The observed effect is nearly constant over the range of solubility for the polymer in the binary system, suggesting the selective incorporation of the better solvent of a pair into the polymer's internal structure.[35] Once more these equilibrium values would suggest size exclusion claims from 100 Å but not 300 Å pores.

IV. CHROMATOGRAPHY OF POLAR POLYMERS: POLYMETHYLMETHACRYLATE, POLYETHYLENE OXIDE, POLYTRYPTOPHAN

The literature examples for polystyrene, including the work by Lochmüller and McGranaghan,[15] are cases in which both components of the binary mobile phase are at the extremes of good and hostile solvent. Polymer solubility is strongly influenced by small changes in composition. If the right steps are taken and careful measurements made, the behavior is akin to classical chromatographic behavior. It is interesting to examine what the case might be if the polymers become progressive more polar and even ionic in nature. What follows is a synopsis of recent reports of success in the separation of polymethylmethacrylates {PMMA} , of polyethylene glycols/polyethylene oxides {PEG, PEO} and polytryptophans. All polar compounds with varying water solubility and, in the case of PEG, interesting thermal dependence of that property.

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A. PMMA

In reversed-phase liquid chromatography, often a pair of relatively "good" and "poor" solvents are used. In the studies reported here for PMMA for the solvent pairs ACN/water, THF/water, ACN and THF are the good solvents. One needs to be concerned that the solute not become quantitatively insoluble in the mobile phase and the "cloud point" is a measure of the onset of that condition. The cloud-point of 75-kDa PMMA was measured and takes place is 84% ACN and 70% THF in water, respectively. In all of the experiments reported here, the mobile phase compositions were kept well above this "cloud-point" specifically to eliminate the possibility of phase separation.

Although isocratic elution is not used as often in practice for polymer separation as is gradient elution, isocratic elution behavior is of fundamental importance to the understanding of retention mechanism. In Figure 2 are shown the chromatograms of 33.5-kDa PMMA under isocratic elution with different mobile phase compositions. A 4 cm C8 column was used. The 33.5-kDa PMMA appeared partially retained even at 100% ACN (Figure 2a and b). This was confirmed using an identical packing in a longer column. In Figure 2c are shown the chromatograms of 33.5-kDa PMMA with the 15 cm C8 column and, indeed, net retention was observed. With the 4 cm column, the plot of log k′ versus the fraction of ACN (ϕ) (Figure 3) can be described by a linear relationship ($\log k' = A - S\phi$) where A is the intercept and S is the slope. If carefully examined, the line actually is slightly curved and good fits to a quadratic relationship ($\log k' = A - B\phi + C\phi^2$). Both of these relationships have been observed for small molecules, i.e., the overall log k′ versus ϕ plot fits quadratic relationship and within a certain range it fits linear relationship. For 75-kDa PMMA, even with 100% ACN very long retention times were observed. THF is a good solvent for PMMA

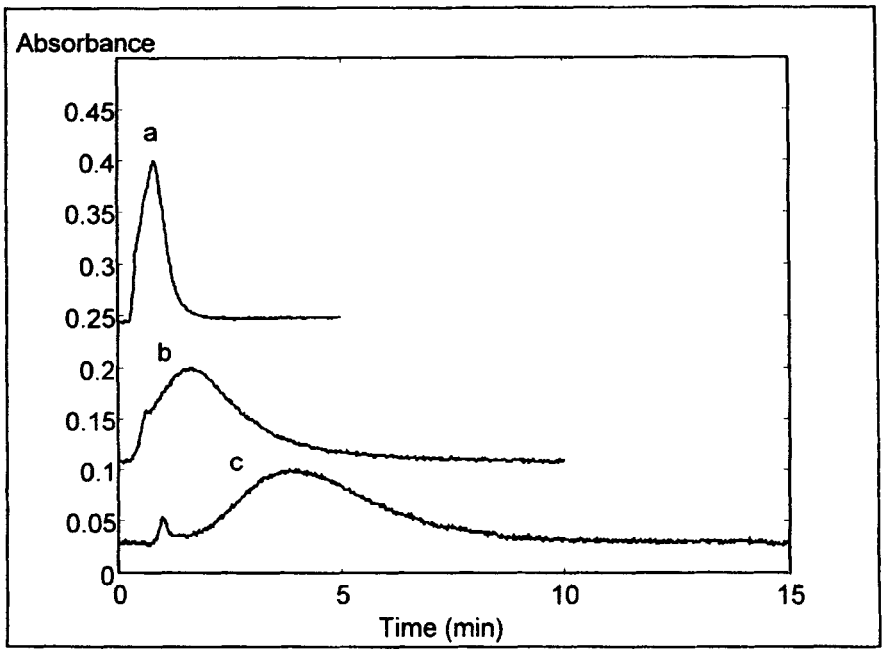

FIGURE 2. Chromatograms of PMMA 33.5-kDa eluted with ACN/H_2O on a 4 cm and a 15 cm Whatman C8 columns. The absorbance is relative in scale. The mobile phase compositions are (a). $\phi_{ACN} = 1$; (b). $\phi_{ACN} = 0.97$; (c). $\phi_{ACN} = 1$.

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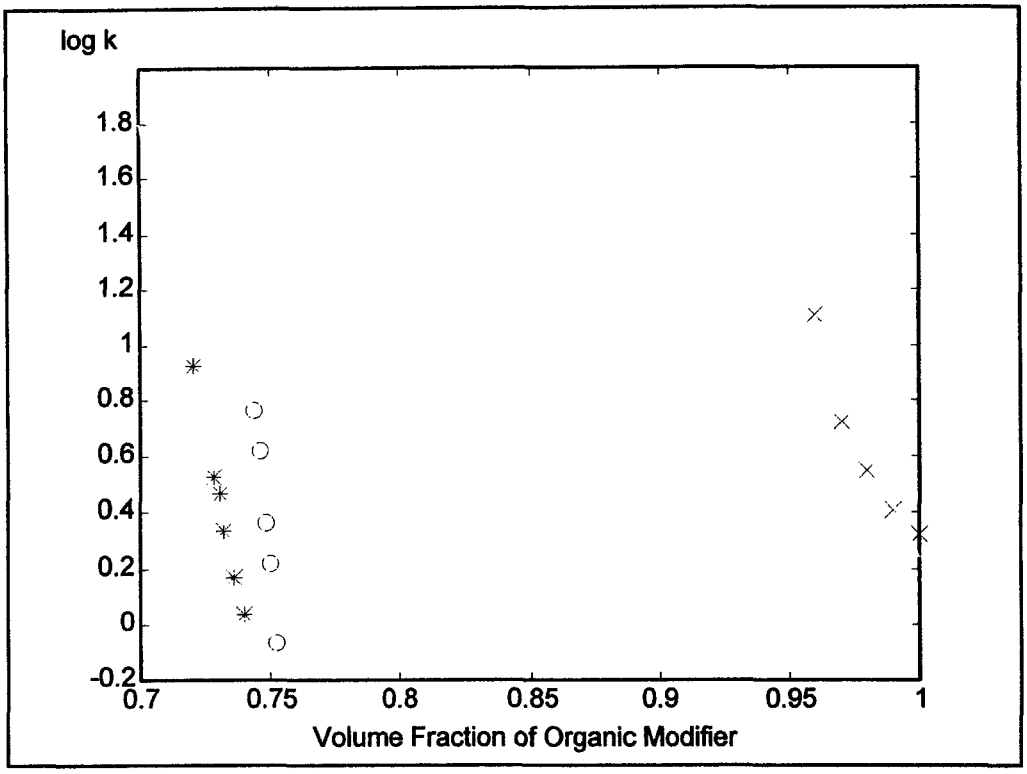

FIGURE 3. Plots of log k′ versus ϕ (linear fit) with a 4 cm C8 column. (X). PMMA 33.5-kDa with ACN/water; (o). PMMA 75-kDa with THF/water; (*). PMMA 33.5-kDa with THF/water.

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and stronger solvent than ACN, the mobile phase of THF/H_2O was used for the rest of the study.

Once again, the prediction of retention from gradient runs was attempted and was successful. The predicted and actual S and t_r values are listed in Table 3. Considering that plots of log k′ versus ϕ are slightly curved over such a wide range of mobile phase compositions, the prediction is quite good. The quality of the results obtained in THF/H_2O mobile phases is quite similar to those obtained with ACN/H_2O.

Many polymers are mixtures of homologous molecules with identical chemical structure but different numbers of repeating units, i.e., oligomers. The resolution of PMMA's was studied at first by separating a mixture of 33.5-kDa and 75-kDa PMMA standards and then by examining the separation of oligomers within one PMMA standard (33.5-kDa). Note that these PMMA samples are standards with very narrow molecular weight distribution. In order to improve observed resolution, a 20 cm C18 column with 2 mm i.d. and 13,000 theoretical plates was used. Figure 4a illustrates the gradient separation of 33.5-kDa and 75-kDa PMMAs. The resolution, Rs, is 0.94. Separation of the oligomers constituting the 33.5-kDa PMMA was examined with elution of a steep gradient and a shallow one (Figure 5). In the steep-gradient (0.5%/min) run, the peak of 33.5-kDa PMMA has a span of 3 minutes. In the shallow-gradient (0.025%/min) run, the PMMA peak has a span of 20 minutes. Even though the polydispersity of this polymers is ~1.05 and thus is a narrow distribution of oligomers, the shallow gradient is a broad peak and fractions could be taken and reinjected to yield narrower bands indicating successful fractionation.

For all PEG samples studied, the plots of log k′ vs. Vol% ACN yielded straight lines.

TABLE 3
Predicted S Values from LSS Model and Actual S Values for PMMAs and PEGs

	$S_{(actual)}$	$S_{(predicted)}$	$tg_{(actual)}$ (min)	$tg_{(predicted)}$ (min)
PMMA 33.5-kDa ACN/water	18.9	17.8	10.7	11.8
PMMA 33.5-kDa THF/water	44.8	45.9	9.7	10.5
PMMA 75-kDa THF/water	103.4	112.4	7.37	7.9
PEG 26-kDa ACN/water	28.6	29.5	12.0	11.63
PEG 46-kDa ACN/water	101.3	108.6	8.9	9.5

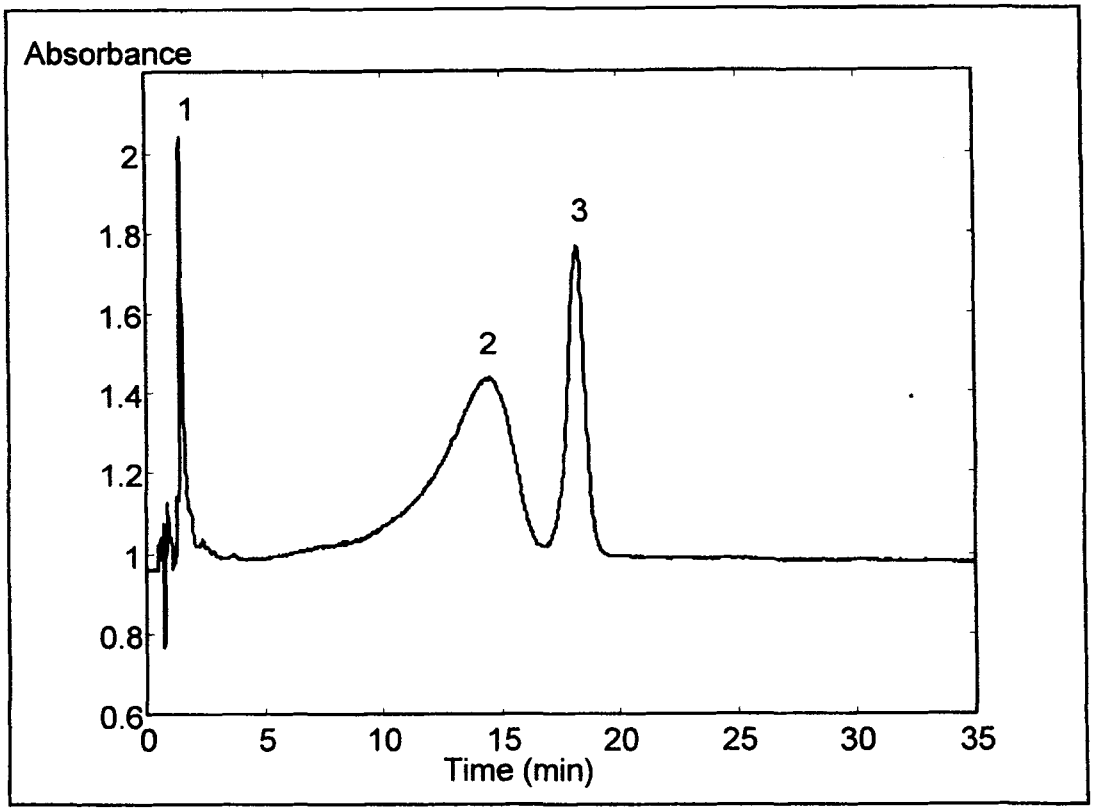

FIGURE 4. Gradient separation of a mixture of PMMA 33.5-kDa (2) and PMMA 75-kDa (3) with mobile phase THF/water from 68/32 to 80/20 in 20 minutes on a Hypersil 20 cm × 2 mm C18 column.

From the comparison of the slopes in Figure 6, it was found that the change of the mobile phase composition affected higher molecular weight PEG's to a greater extent. Figure 7 is a plot of slope (S) against logarithm of the molecular weight for some PEG

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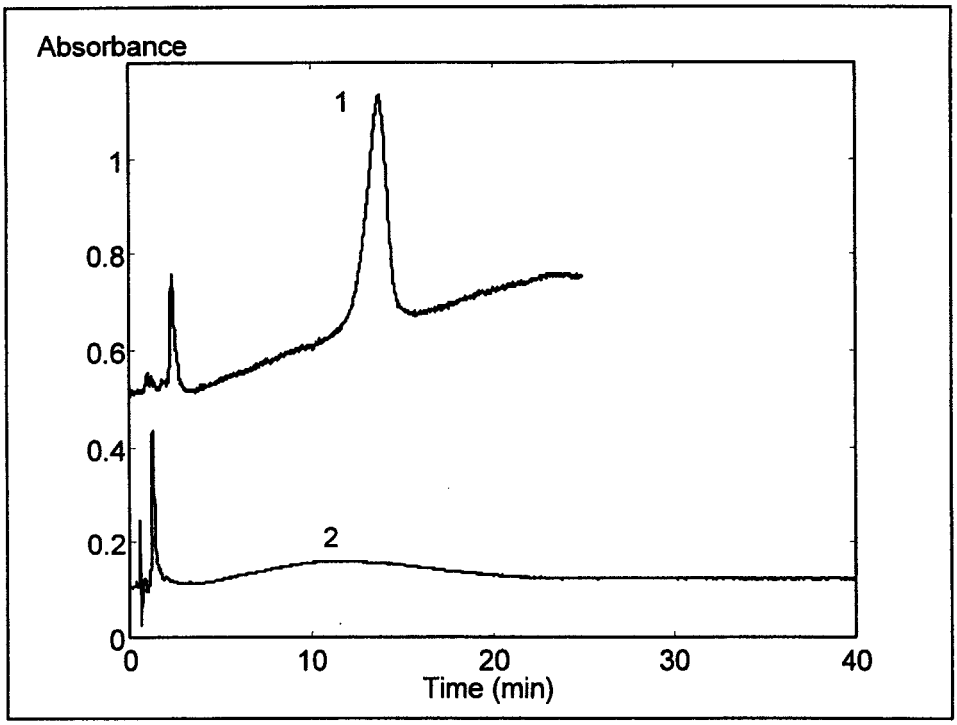

FIGURE 5. Gradient elution of PMMA 33.5-kDa eluted with THF/water mobile phase. (1) from 68/32 to 78/22 in 20 minutes; (2) from 75/25 to 75.5/24.5 in 20 minutes.

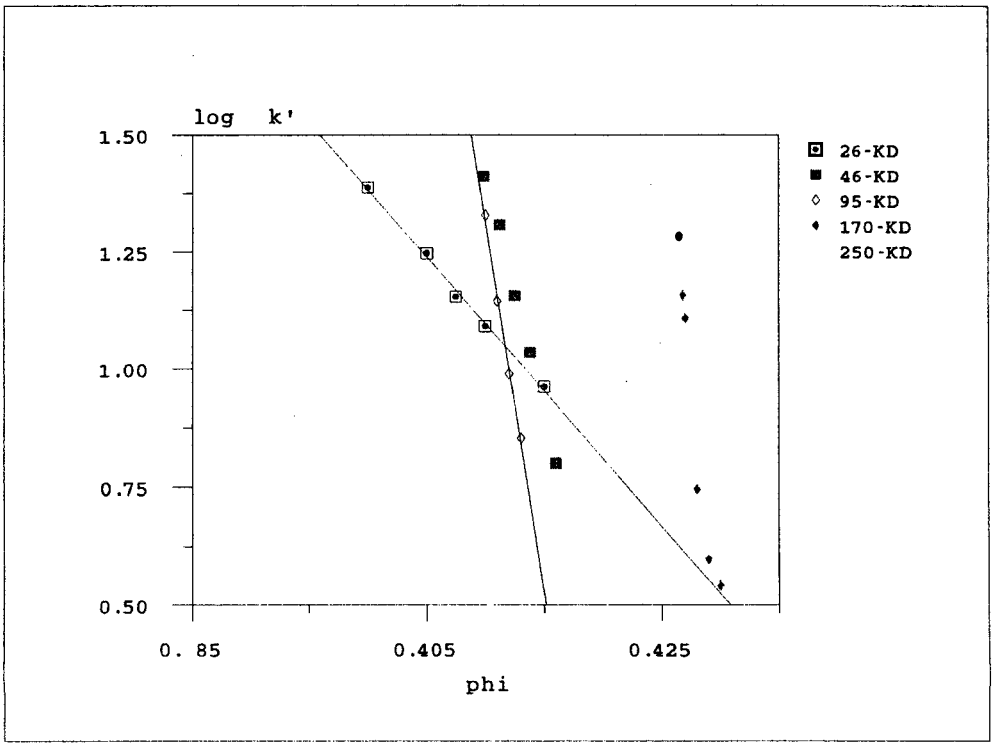

FIGURE 6. Plot of log k′ vs. volume fraction of THF for PEG samples.

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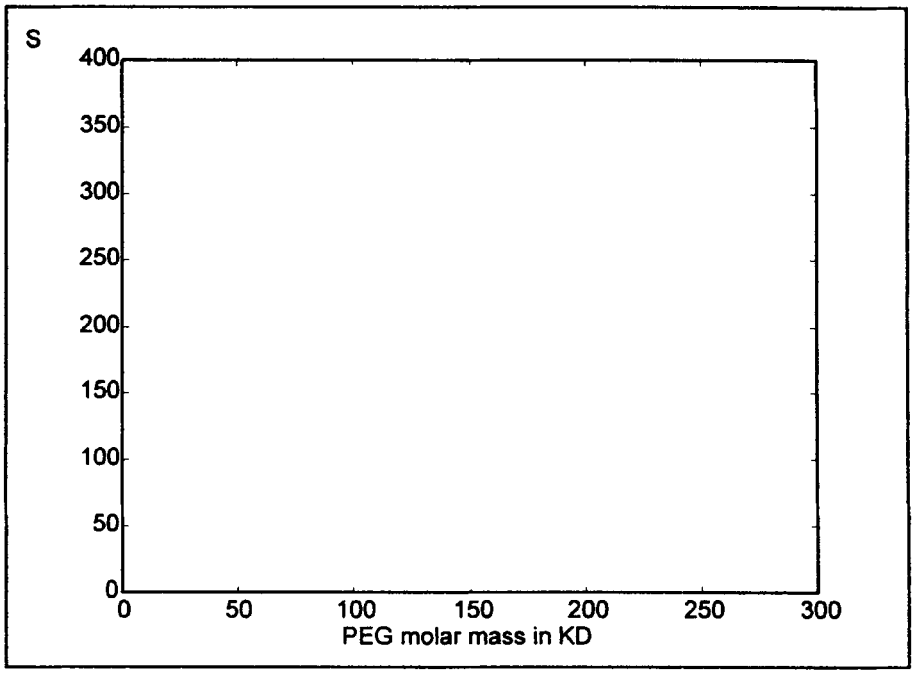

FIGURE 7. Plot of S values from isocratic retention data versus the molar mass of PEG.

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samples. The S value increases monotonously with the increase of molecular weight until the molecular weight reaches 170-kDa and then suddenly goes down. This mirrors the observations with polystyrenes and ca. 100 Å pores size column materials.

The sample concentration effect with respect to retention mechanism in reversed-phase chromatography of polymers was discussed in other reports.[46–49] The concern is that as the concentration of polymers increases, inter-segment interactions become more important. The polymer molecules in solution indeed can form an entangled network at high concentrations. The crossover concentration (c*)[50] at which PEG forms a network can be calculated by C* = M/V, where $V = (4/3)\pi rg^3$ where M is the molecular weight and V is the volume of the polymer coil calculated from the radius of gyration r_g. The radius of gyration r_g for PEG in water can be estimated as $rg = 0.1323\ M^{0.62}$. For PEG molecules of 4 megadaltons, c* = 0.22%, that is, 2.2 mg/mL. In the studies discussed here, the concentrations of polymer samples are at weight fractions of ~0.2%. Since the molecular weight of the PEG samples that were used in chromatographic experiments is well below 4 megadaltons, it can be assumed that PEG exists as individual coils. The retention of 46-kDa of 4 different concentrations of 2 mg/mL, 4 mg/mL, 8 mg/mL and 12 mg/mL was examined. For 46-kDa PEG, the crossover concentration c* in pure water is 0.7% (weight percentage). In Figure 8 is shown the plot of k′ against the logarithm of concentration of PEG 46-kDa. The plot shows that within a range of sample concentration below c* up to 4 mg/mL, the retention is not affected by the concentration; however, when the concentration is as high as 8 mg/mL, there is significant decrease in retention time. This decrease could well be due to chain entanglement and the formation of a network. Such a network could behave as a pseudo-polymer of much larger apparent molecular weight.

Gradient elution was also carried out to examine the prediction of isocratic retention behavior based on the LSS model described above. Two samples (26-kDa and 46-kDa

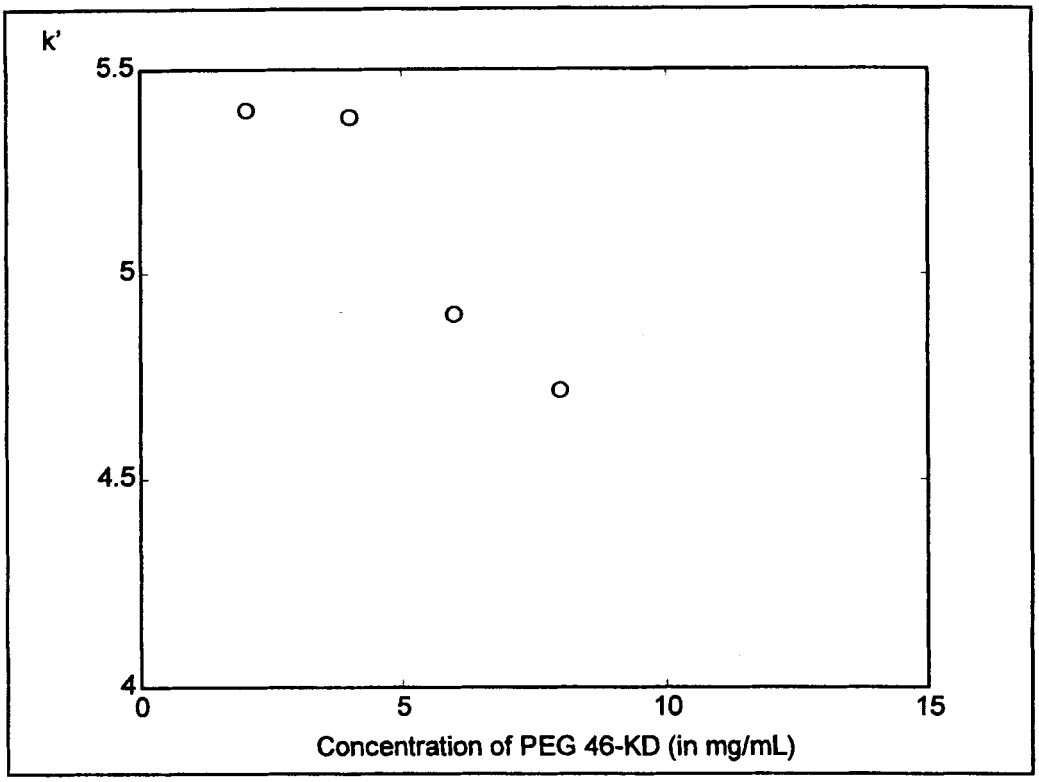

FIGURE 8. Plot of capacity factor k' versus concentrations of PEG 46 kDa.

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PEGs) are used. The predicted S and retention time t_r values agree well with the actual ones (Table 1). Apparently, the same, conventional chromatographic behavior obtains in the case of these two polymer polymers as in the case of polystyrene.

V. RIGID POLYMERS: POLYTRYPTOPHANS

Characterization of rigid polymers with size-exclusion chromatography is difficult because the calibration curve that is derived from flexible polymers is no longer applicable.[51–54] If a polymeric sample contains both rigid and flexible polymers, it is even more difficult to assign the peaks in the size-exclusion chromatogram. RPLC is a potentially good method for characterizing rigid polymers because of its partition/adsorption retention mechanism and its independence of calibration. It is also highly desirable to be able to control the retention with current available separation optimization knowledge developed from small molecules, such as the "third solvent" strategy.[55] Here, we review work reported on the separation of poly(l-tryptophan)s and poly(d,l-tryptophan)s. These are good model rigid polymers because:

- The former is rigid, rod-like and helical and the latter a flexible and globular polymer.
- A very small amount (less than 0.5 nanogram) of poly)tryptophan)s can be detected with fluorescence detection and this insures that solution of poly(tryptophan) is close to infinitely dilute in a chromatographic sense.

It was found that poly(l-tryptophan)s show longer retention times than poly(d,l-

tryptophan) of the corresponding molecular weight. For all poly(tryptophan) samples, linear plots of the logarithm of the capacity factor (k′) versus volume fraction (f) of the strong solvent were obtained. Typical chromatograms obtained under isocratic elution conditions using THF/Water are shown in Figure 9. It is interesting to observe that at the same mobile phase composition, poly(l-tryptophan) 11.5 kDa has longer retention time than its counterpart 14.5 kDa poly(d,l-tryptophan)s within the range of mobile phase compositions used in the experiments. The difference in retention time between 5.4 kDa poly(l-tryptophan) and 5.7 kDa poly(d,l-tryptophan)s is small. The predicted order of solute retention is rigid-rod solutes > plate solutes > flexible chain solutes.[56] There have been many reports and debates on whether the retention mechanism for small molecules is an adsorption process ("Solvophobic" model), or a partition process, or both.[57] For the high molecular weight polymer case, if retention only involves adsorption mechanism in which solute-stationary phase interaction only takes place at the surface of the stationary phase, the retention of poly(l-tryptophan)s should be shorter than that of poly(d,l-tryptophan)s because the average surface area of the polymer solute in contact with the stationary phase of poly(l-tryptophan) is smaller than that of poly(d,l-tryptophan). The bulky globular poly(d,l-tryptophan)s may not be able to enter the bonded phase of alkyl chains, whereas a part of the rod-like poly(l-tryptophan)s may actually intercalate in the bonded phase mass. If the molecular weight is low, there may not be such a dramatic difference. The results here support that proposal that, even for the RPLC of polymers, the stationary phase plays a very important role. In Figure 10 are shown plots of log k′ versus the volume fraction of THF in binary THF-water mobile phases. Again the conventional behavior of small solutes appears to obtain as in the case of the polystyrenes, the PMMA's and the PEG's.

Mobile phase plays an important role in RPLC.[55–57] Many optimization techniques

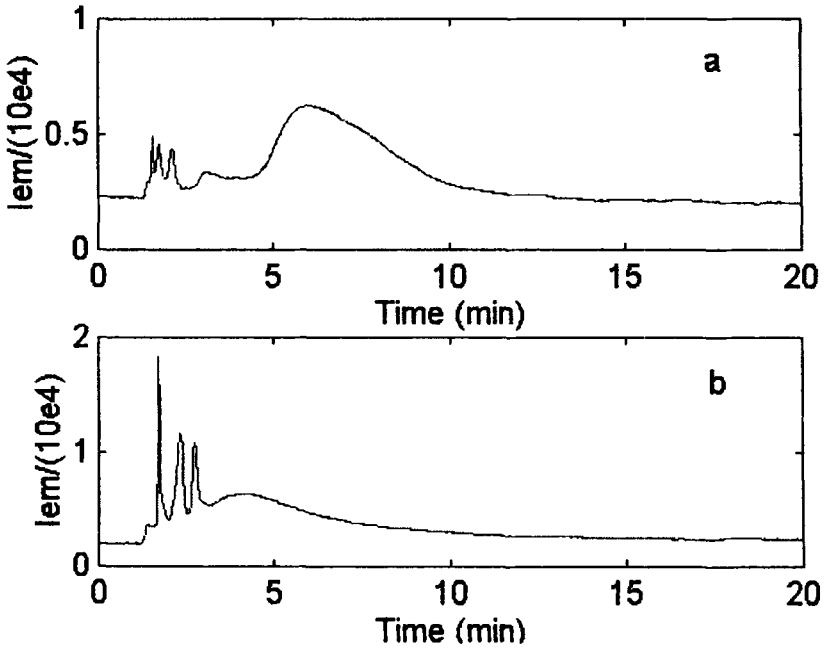

FIGURE 9. Chromatograms of PTRP. Mobile phase: THF/H_2O (77/23). (a). P-dl-TRP 14.5 kDa; (b). P-l-TRP 11.5 kDa.

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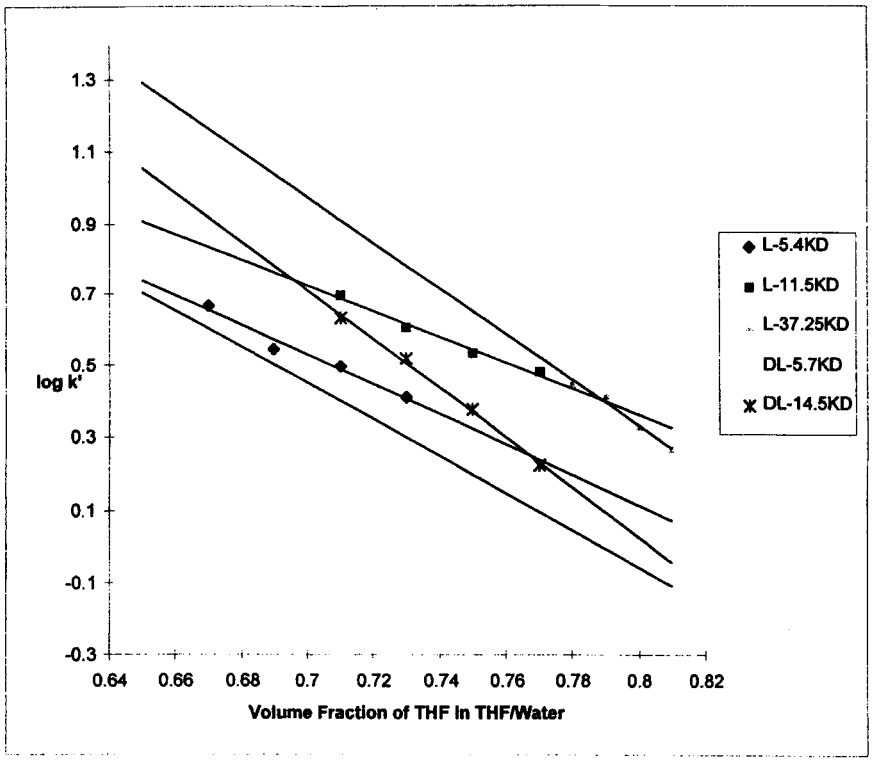

FIGURE 10. Plots of log k' versus volume fraction of THF poly(l-tryptophan)s and poly (dl-tryptophan)s.

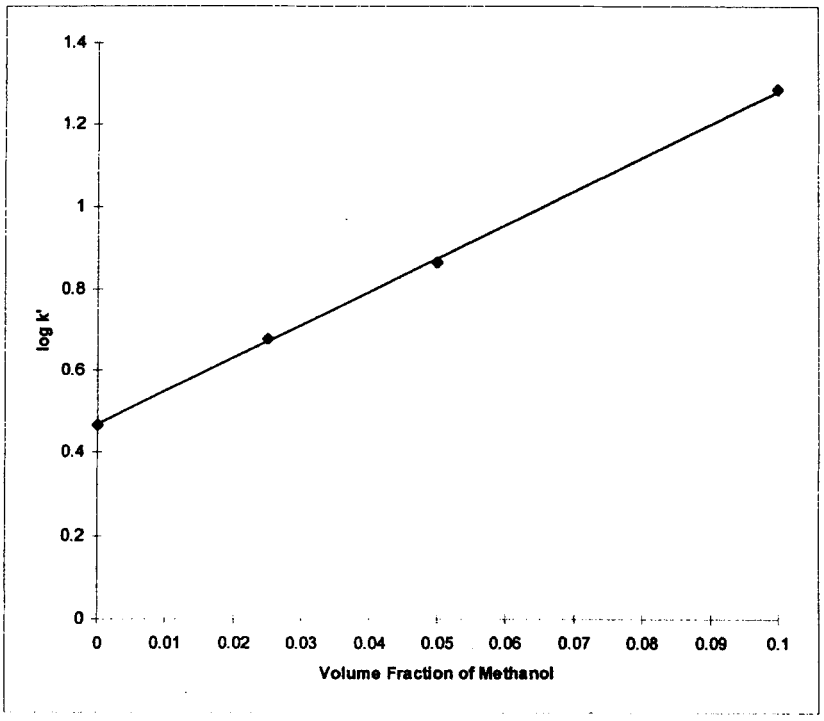

FIGURE 11. Plot of log k' versus volume fraction of methanol in THF-water-methanol ternary mobile phase.

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TABLE 4
Predicted S Values and Actual S Values*

	37.25 kDa (l)	11.5 kDa (l)	5.4 kDa (1)	14.5 kDa (d,l)
Predicted S	6.68	4.2	4.5	7.5
Actual S	6.42	3.6	5.1	6.94

center around using combinations of different mobile phase. Using ternary solvent mixture as mobile phase to precisely control the elution strength and polarity of mobile phase has been studied for a long time.[55] There appear to be no reports involving the effect of ternary mobile phase on the polymer retention prior to the work reported here. The change in behavior was studied as methanol is added into THF-water mobile phase as a third solvent. Methanol is a poor solvent for poly(tryptophan)s, but it is a better solvent than water.

While the volume fraction of THF is kept constant, the fraction of water is decreased with the increase of the fraction of methanol. Since methanol is a better solvent than water, one would expect that replacing water with methanol should decrease retention of poly(l-tryptophan) 11.5 kDa. However, the opposite was observed. A plot of log k′ versus the volume fraction of methanol shows a linear relationship (Figure 10). A similar unusual retention behavior was observed for small molecules by Lochmüller and co-workers.[57] They found that water actually was freed from the methanol-water associates when a small amount of acetonitrile or THF was added into the methanol-water mobile phase and the increased content of "free" water led to the increase of the retention time within a certain range.

VI. TEMPERATURE GRADIENTS AND POLYMER SEPARATION BY HPLC

The discussion and review to this point has dealt with gradient and isocratic mobile phase conditions and evidence that larger molecules can behave in the manner predicted by conventional chromatographic models and theory. In this section is presented the outcome of an attempt to use temperature variation to study the thermodynamics of the separation and the mechanism. We also present the original and some very recent examples of using isocratic elution with a column whose inlet and outlet are deliberately at different temperatures creating a gradient in temperature from end-to-end. For this goal, isocratic retention data of poly(ethylene glycol)s (PEGs) and poly (ethylene oxide)s (PEOs) of 9 different molecular weights were obtained at different temperatures. These data were used to construct Van't Hoff plots and to obtain both apparent changes in enthalpy ($\Delta H°$) and changes in entropy ($\Delta S°$).

Factor analysis can be useful technique to analyze multivariate data without the knowledge of an explicit equation to describe the data, i.e., a "soft model" approach.[70–72] Lochmüller and co-workers have demonstrated already that a factor analytical model can be applied to precise retention prediction in RPLC.[72] In the studies of polymer elution as a function of temperature, factor analysis is used to correlate the retention data with the molecular properties of polymers, e.g., average molar mass. If this can be done {if the data are Factor analyzable} than polymer properties might be obtained or predicted from retention data. For the measurements reported here, the retention data set (ln k′) of 7 different PEG samples (row designee) under different temperatures is used in Principal Component Analysis and target-transformation factor analysis (TTFA).[72]

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In chromatographic process, the *quantitative* contributions to the retention from molecular interactions, such as London forces and hydrogen bonding, are still unknown after years of study. However, since the chromatographic process is an equilibrium distribution of molecules between a stationary phase and a mobile phase, the changes of state functions, such as apparent standard enthalpy ($\Delta H°$) and standard free energy ($\Delta G°$), can be calculated from the equilibrium constants (K) via the following relationships (Equations 1, 2, 3);

$$\ln k' = -\frac{\Delta H°}{RT} + \frac{\Delta S°}{R} - \ln\left(\frac{V_s}{V_M}\right)$$

One fundamental question that remains to be answered in chromatography is whether the phase ratio V_S/V_M is constant over a range of temperatures. The volume of the mobile phase V_M, the same as the void volume of the column for a specific solute has been the object of many studies. For the purposes here, the phase ratio is assumed constant over a temperature range of 25°C. This situation results in a linear plot of ln k′ versus (1/T) for a constant mobile phase, where 1000/T is used for scaling purposes. This linear relationship has been widely observed in the study of small molecules.[58–69] From the plot of ln k′ versus (1/T), the slope is the measure of $-\Delta H°/R$, and the intercept is $\Delta S°/R$ plus the term of $(-\ln(V_S/V_M))$. Since $-\ln(V_S/V_M)$ is assumed to be a constant, the intercept is used as $\Delta S*/R$ hereafter. Because the phase ratio is usually between 0.3 and 0.9[73], $\Delta S*$ is a good approximation of $\Delta S°$.

Figure 12 shows the chromatograms of PEG 95-kDa at different temperatures. In this case, an increase in temperature results in an increase in retention. Such a retention behavior correlates with the inverse solubility-temperature relationship of PEG molecules, that is, the solubility of PEGs in water solution decreases as temperature increases.[74] In addition, the temperature coefficient of unperturbed dimension of PEGs in athermal aqueous solvent was reported[47] as $d(\ln\langle r^2\rangle_o)/dT \sim 2.3 \times 10^{-4}\ C^{-1}$. Therefore, an increase in temperature will result in an increase in the size of PEG molecules. The enlarged random coils of PEG under higher temperature may experience more total interactions with the stationary phase due to the increased surface area.

Figures 13A–13E show the Van't Hoff plots of ln k′ versus (1000/T) of PEG samples at 5 different mobile phase compositions. The slope and intercept values were converted to $\Delta H°$ and $\Delta S*$ and are listed in Table 5.

As seen in Table 1, $\Delta H°$ and $\Delta S*$ increases rapidly as the molar mass of PEG increases. Note that the sign of $\Delta H°$ is positive, indicating the retention process for PEGs is endothermic. The values of $\Delta S*$ are also much larger than those of small molecules,[73] indicating that the retention of polymers onto the stationary phase results in large changes in the conformations and configurations of polymers and surface structures of the bonded phase. Both $\Delta H°$ and $\Delta S*$ values in Table 5 reach apparent maxima which occur at 42.1% ACN. Why this is the case is not yet known.

The plots of $\Delta H°$ values of PEG samples versus their molar masses show linear relationships (Figure 3). Similarly the plot of the $\Delta S*$ values versus PEG molar mass is also a linear relationship (Figure 4). From Figures 13 and 14,

$$\Delta H° = n\,\Delta H°_m + \Delta H°_{end}$$

$$\Delta S° = n\,\Delta S°_m + \Delta S°_{end}$$

where $\Delta H°_m$ and $\Delta S°_m$ are the standard enthalpy change and standard entropy change, respectively, for a repeating unit, $-CH_2CH_2O-$. Constant $\Delta H°_{end}$ and constant $\Delta S°_{end}$ can be regarded as $\Delta H°$ and $\Delta S°$ contributed by end groups. The total free energy of the retention process can be expressed by:

$$\Delta G° = \Delta H° - T\,\Delta S° = n\,\Delta G°_m + \Delta G°_{end}$$

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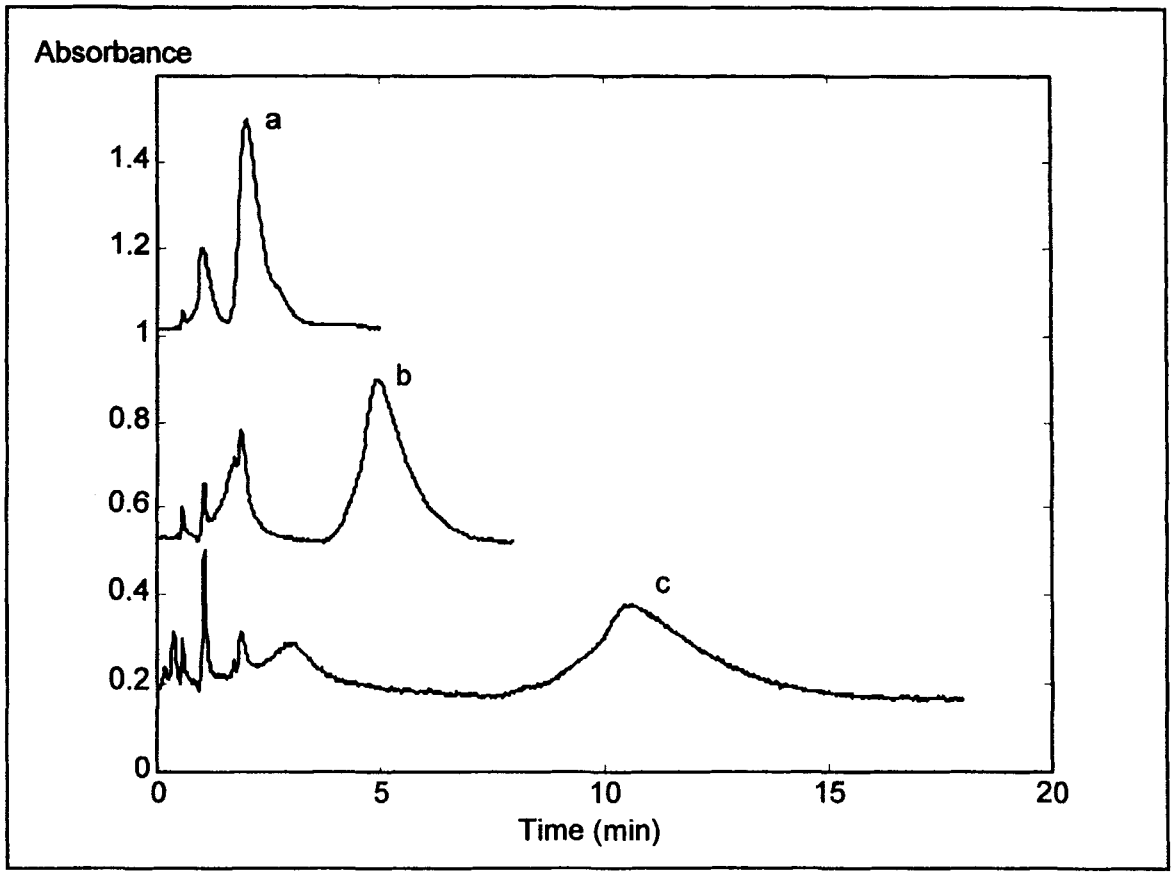

FIGURE 12. Chromatograms of PEG 95-kDa eluted with mobile phase ACN/water 50/50: (a) t = 40.98°C; (b) t = 42.24°C; (c) t = 43.10°C. Note the early eluting small peaks are system peaks and the peaks for the sample of lower molar mass.

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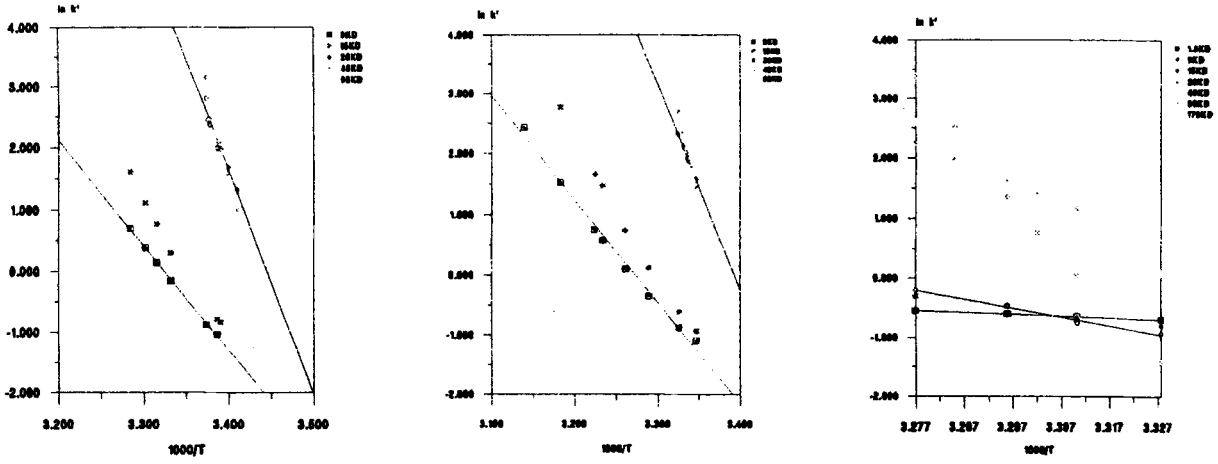
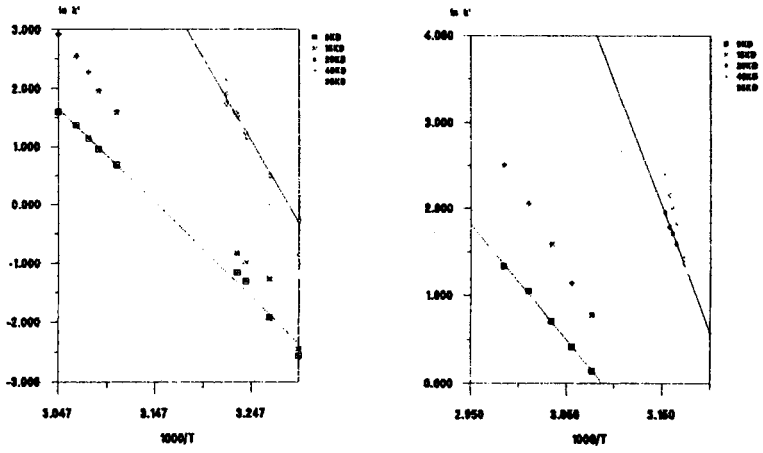
where $\Delta G°_m$ and $\Delta G°_{end}$ are the changes of the standard free energy for a repeating unit and end groups, respectively. Thus, there is a linear relationship between $\Delta G°$ and the molar mass, which correlates well with the conformational studies on polymers in the adsorbed state. The conformation of a globular polymer at the adsorbed state is either a cylindrical disc (train-type) or a globular one.[75] Each of the conformations has a contact area with the stationary phase proportional to the molar mass of the polymer. Therefore, total adsorption energy can be expected to be also proportional to the molar mass.

If this is indeed the case, these linear relationships might use analytical curves for determination of PEG molar masses. The $\Delta H°$ value for the same type of the sample but with unknown molar mass can be calculated from its retention time at two different temperatures. The molar mass of the unknown sample can be found from an existing plot of $\Delta H°$ versus molar mass constructed from standards. This strategy was tested with PEG standards. It was found that the calculated molar masses closely track the actual reported values. These results further support Martin's assumption[76] that the total free energy for transfer of a molecule from the stationary phase to the mobile phase, $\Delta G°$, can be fragmented according to the compound structure. That is, $\Delta G°$ is a sum of the free energies of "transferring" each fragment of the compound from the stationary phase to the mobile phase.

$$\Delta G° = a\Delta G°_A + b\Delta G°_B + c\Delta G°_C + d\Delta G°_D + \cdots$$



(A) (B) (C)



(D) (E)

FIGURE 13. Van't Hoff Plots of different ratios of ACN-water: A, 40.0:60.0; B, 42.1:57.9; C, 44.2:55.8; D, 47.1:52.9; and E, 50.0:50.0.

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TABLE 5
ΔH° and ΔS^* Values for 5 PEG Samples

ΔH (kJ/(mole K))					
PEG	ACN%	ACN%	ACN%	ACN%	ACN%
(kDa)	50	47.1	44.2	42.1	40
9	13.2	16.5	17.8	17.7	17.8
15	19.0	22.2	22.9	23.7	22.6
26	28.6	33.7	32.8	36.9	32.5
46	48.8	51.8	51.7	57.8	51.8
95	91.8	95.6	98.7	102.4	96.6
ΔS^* (J/(mole K))					
PEG	ACN%	ACN%	ACN%	ACN%	ACN%
(kDa)	50	47.1	44.2	42.1	40
9	40.7	51.9	57.4	58.0	59.3
15	59.3	70.7	74.4	78.1	75.3
26	92.8	110.4	109.5	124.9	112.3
46	156.3	168.9	171.8	194.8	177.6
95	292.7	310.7	326.8	343.9	329.6

Note: *ΔS^* listed is the pseudo-ΔS from the intercept on Van't Hoff Plots, and it should contain the constant term of the phase ratio.

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Where a, b, c, d, ..., are the coefficients and ΔG°_A, ΔG°_B, ΔG°_C, ΔG°_D..., are the free energies of the fragments. It follows that ln k′, can be expressed as:

$$\ln k' = \sum_i \ln k'_i + \text{constant}$$

where $\ln k'_i$ is the logarithm of the capacity factor for fragment *i* of the molecule. Therefore, for a polymer Martin's assumption is expressed as

$$\ln k' = f(n) \ln k_i + \text{constant}$$

where $\ln k_i$ is the logarithm of the capacity factor for the repeating unit *i* of the polymer, f(n) is a function related to the number of repeating units (n), i.e., the molar mass of the polymer.

The retention data structure (ln k′) of 7 different PEG samples (row designee) under different temperatures forms a data structure that can be treated by Principal Component Factor Analysis. As indicated in Table 6 by the columns of *Eigenvalues, Variance Explained* and *Probability Test,* there are two principal factors. It is important for the reader to keep in mind these are abstract not physical factors.

The data set was then reduced by using two factors. After the solute submatrix was regenerated, Target Transform Factor Analysis (TTFA) was performed with the *reported molar masses* of the PEG standards as a test target vector for the row vector matrix because the molar mass is one of the primary molecular properties. There is a good correlation between the test vector (column 1, Table 7) and the predicted vector (column 2, Table 7) and the vector of molar mass was found to be the real vector. The molar mass of one PEG sample was deliberately left out as a *"free-floating"* point and, as shown in Table 7, the molar mass of this unknown can be predicted. Because the number of data points is small, the target-testing provides a good estimation and a reasonable check of the PEG molar masses.

These thermodynamic studies of PEG retention can be applied to optimize the sepa-

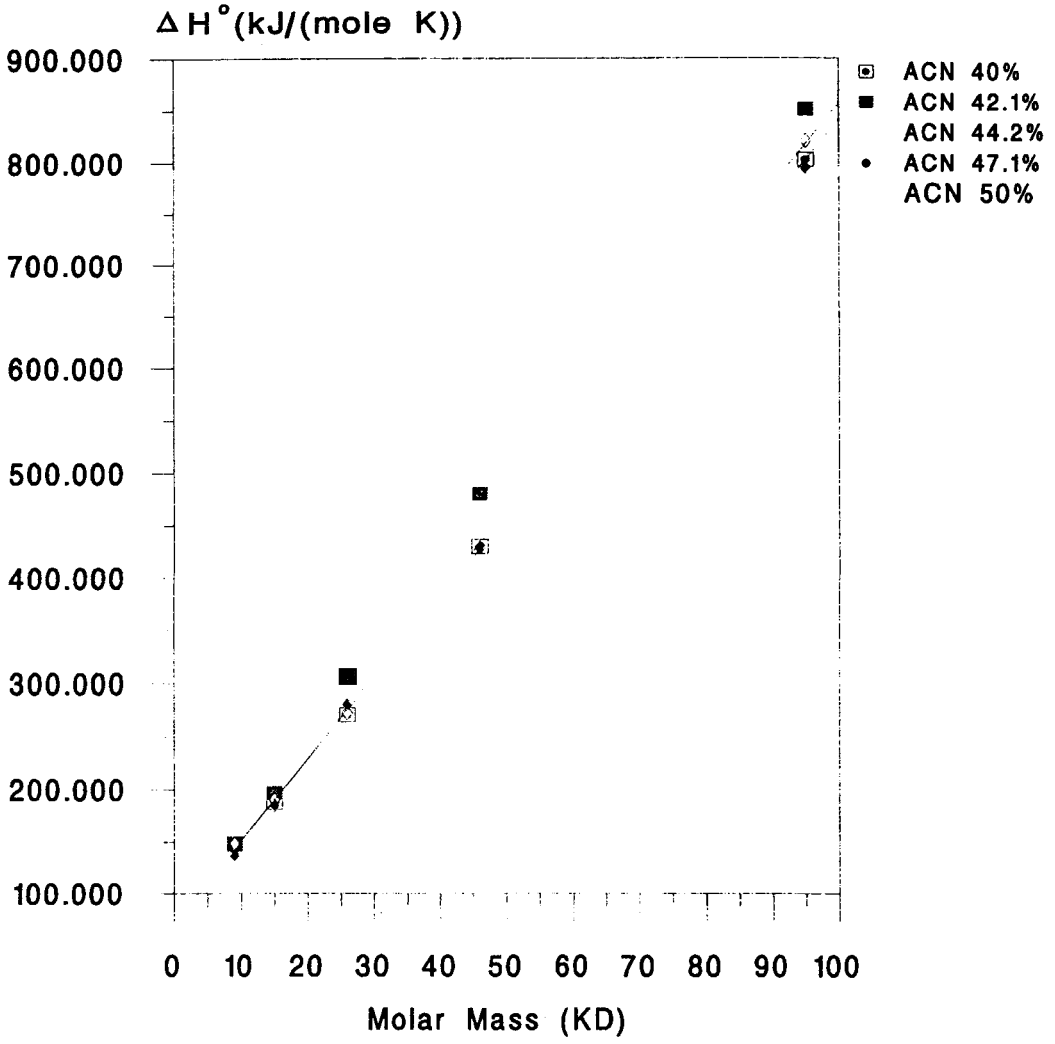

FIGURE 14. Plots of ΔH° (kJ/(mole K)) versus molar mass of PEGs (kDa). The mobile phase compositions (percentage of ACN) are: 40%, 42.1%, 44.2%, 47.1%, and 50%.

TABLE 6
PCFA of Retention Data of 7 PEG Samples

No. of factors	Eigenvalue	Variance explained	Probability test[49]
1	1.89e + 03	93.00	0.001
2	1.42e + 02	7.00	0.0
3	7.60e − 27	0.00	0.08
4	1.66e − 27	0.00	0.141
5	3.69e − 28	0.00	0.293
6	1.30e − 28	0.00	0.387
7	2.75e − 29	0.00	1.000

ration of the polymers. Figure 9 shows the chromatogram of the separation of a mixture of 7 PEG polymers using a solvent gradient.

To improve the separation without a solvent gradient, two types of temperature gradients were employed: gradient T raises or lowers

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TABLE 7
TTFA of PEG Solute Retention Data Space with Molar Mass as Target Factors

Test vector	Predicted vector	Free floating calculation
1.5	3.47	
9	12.86	11.47
15	18.06	16.08
26	34.03	31.13
46	51.46	45.76
95	94.68	
170	166.17	

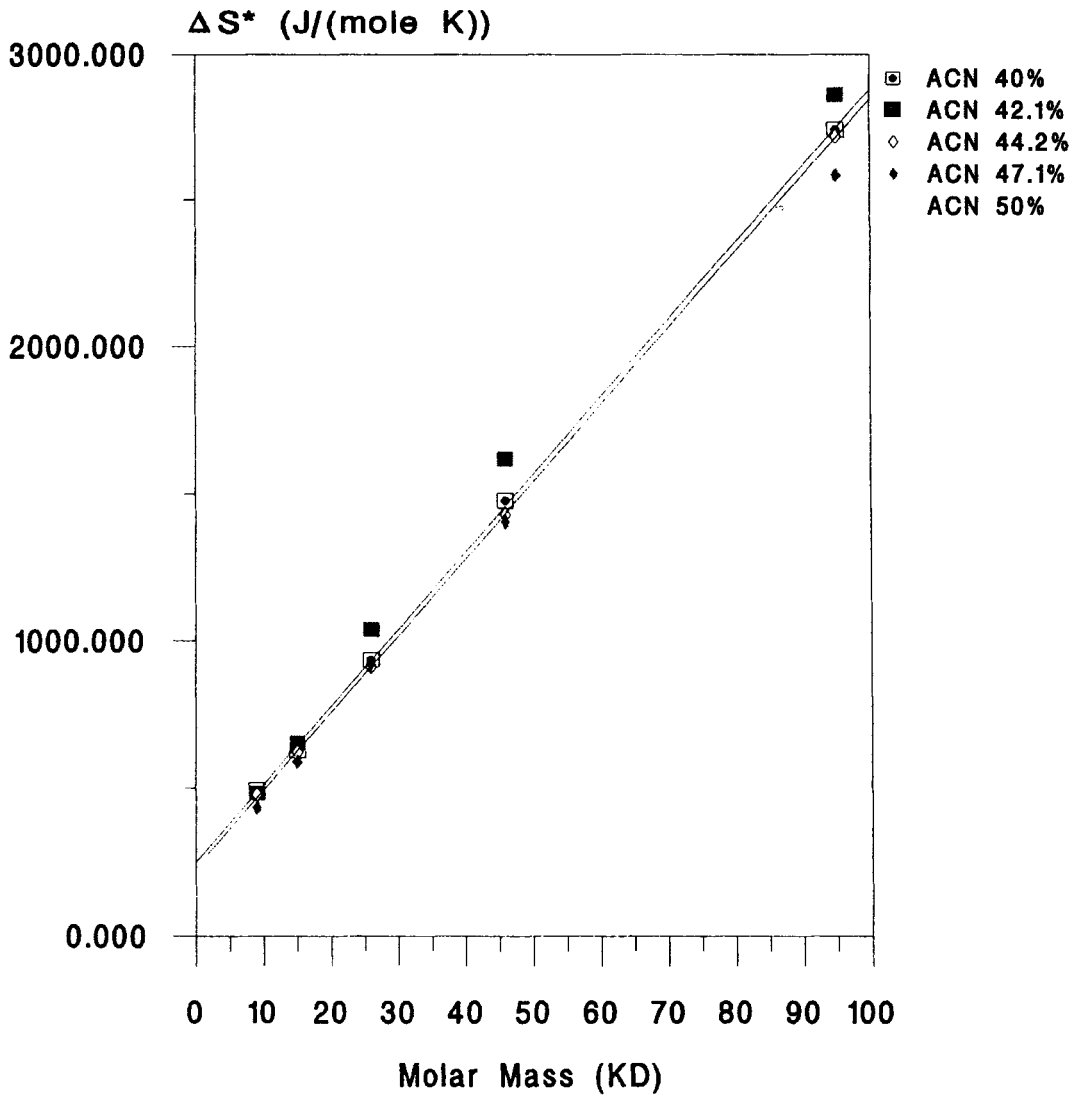

FIGURE 15. Plots of ΔS* (J/(mole K)) versus molar mass of PEGs (kDa). The mobile phase compositions (percentage of ACN) are: 40%, 42.1%, 44.2%, 47.1%, and 50%.

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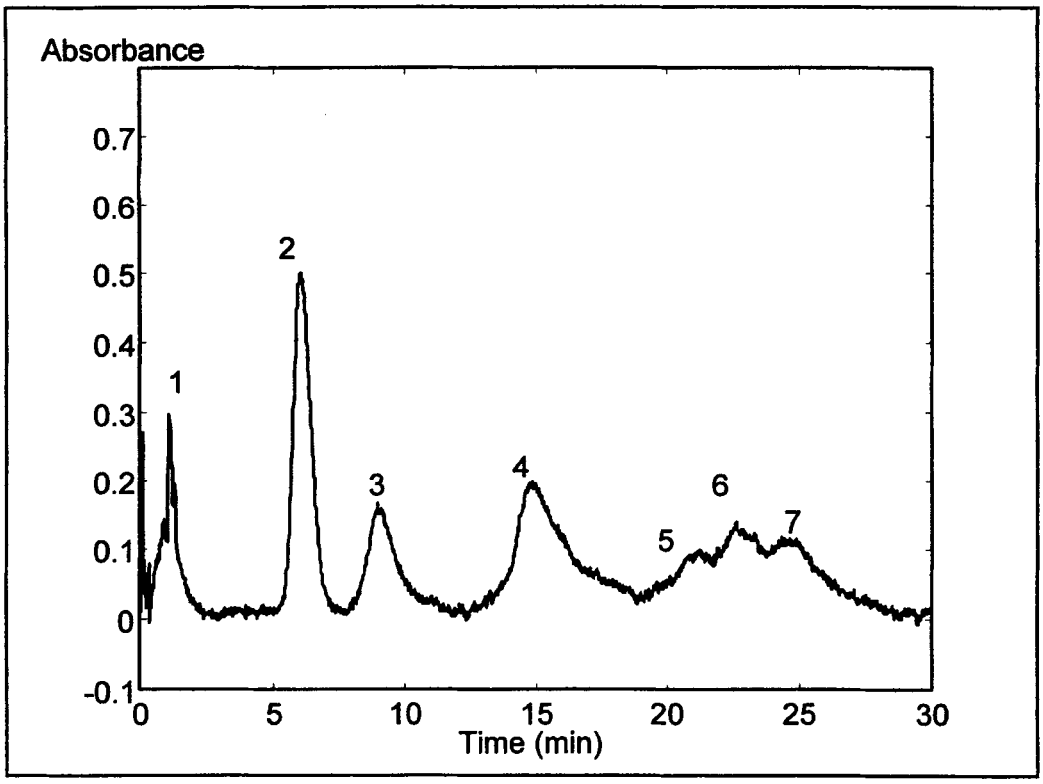

FIGURE 16. Gradient elution of a mixture of 7 PEG standards with ACN/water from 41/59 to 43/57 in 45 minutes. From 1 to 7 are PEG (in kDa) 26, 46, 95, 170, 250, 510, 913, among which the sample sizes of 2 and 4 were spiked.

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the temperature across the whole column linearly with respect to time and gradient L generates a thermal gradient along the column. To examine the effects from both temperatures, the mixture of three PEG polymers of molar mass 26-kDa, 46-kDa, 95-kDa was used. Figures 6A, 6B, and 6C show the chromatograms of the mixture of three PEG samples. Both elution modes were isocratic, and one had gradient T and the other had gradient L. Under isothermal isocratic conditions, PEG samples were hardly separated, whereas imposing a thermal gradient T or L can facilitate separation. It should be noted that these results are preliminary. Studies on the precise control of the thermal gradients of both T and L to obtain high resolutions are in progress.

Some recent results are shown in Figures 18 to 20. Figure 18 is a chromatogram achieved by spatial temperature gradient use column during the separation run. Figure 19 is a chromatogram is a solvent gradient run at 38°C. Figure 20 is a temperature programmed run where the start temperature is close to the inlet temperature of that in Figure 18. The results are equivalent except that the spatial gradient does not require any re-equilibration time delay between samples.

CONCLUSIONS

There is clearly a great deal more to be done both from a fundamental and practical view before a full evaluation of the potential

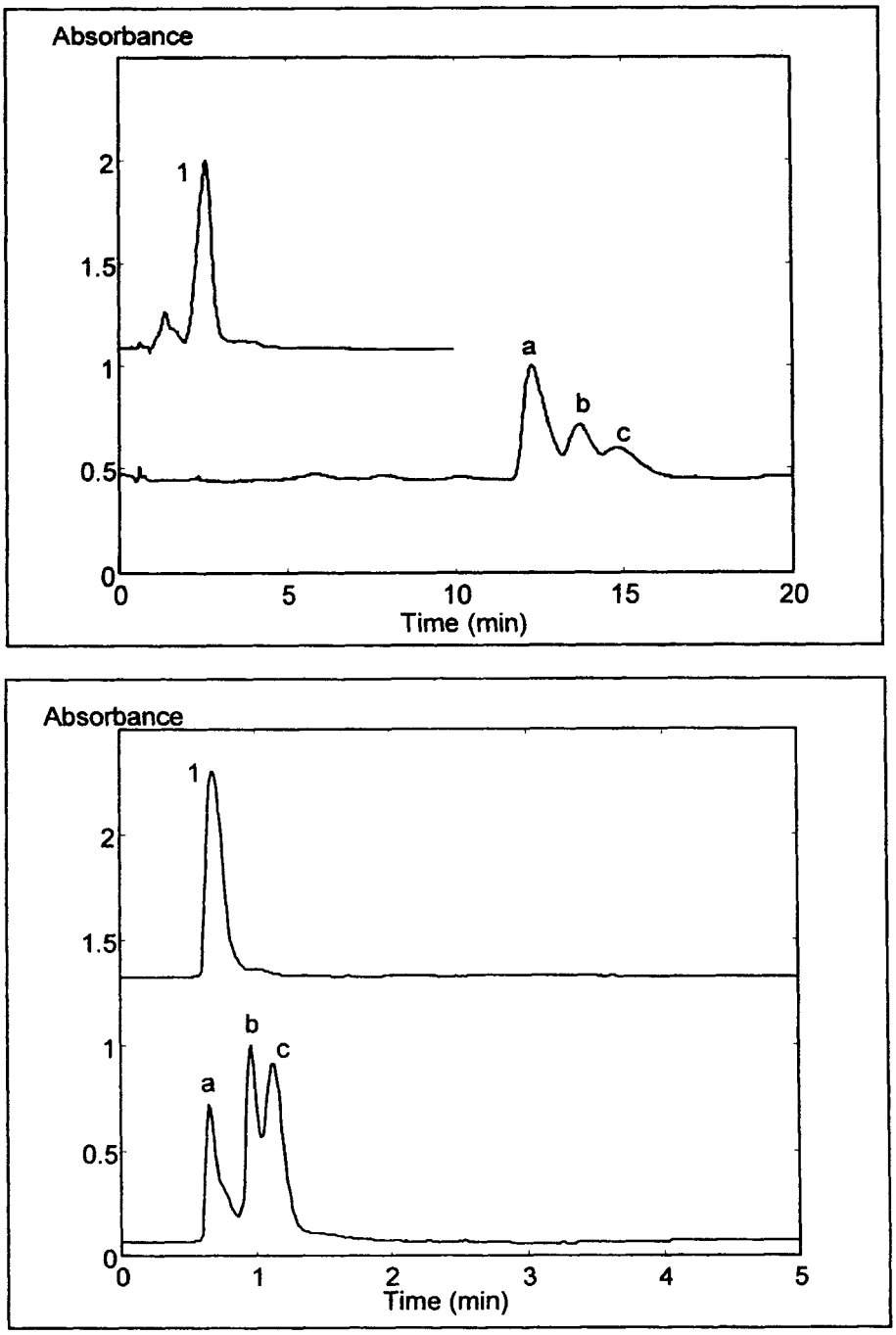

FIGURE 17. Chromatograms of a mixture (*l*) of PEG 26-kDa (*a*), 46-kDa (*b*) and 95-kDa (*c*) **(A):** ACN/H_2O 42/58; Top, t = 23°C; Bottom, the thermal gradient was −0.05°C/min started from 28°C. **(B):** Top, t = 23°C; Bottom, t_{inlet} = 40°C, t_{outlet} = 23°C, the gradient was 0.7°C/cm along the 10 cm column.

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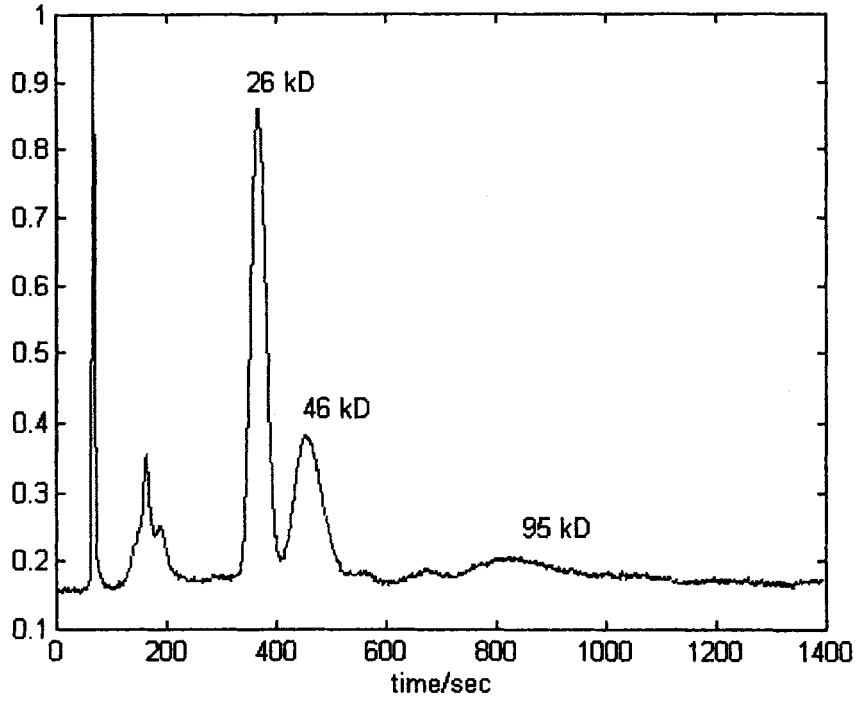

FIGURE 18. Spatial temperature gradient separation column YMC-pack ODS-AP, 5 micro spherical particles, 300 Å pores, 150 by 4.6 mm. Mobile phase: 50/50 ACN/water premixed sample: 26 kDa, 46 kDa, and 95 kDa PEO's 2 mg/mL each in 50/50 ACN/water. Temperature: column inlet = 45.1 C, outlet = 41.0 C.

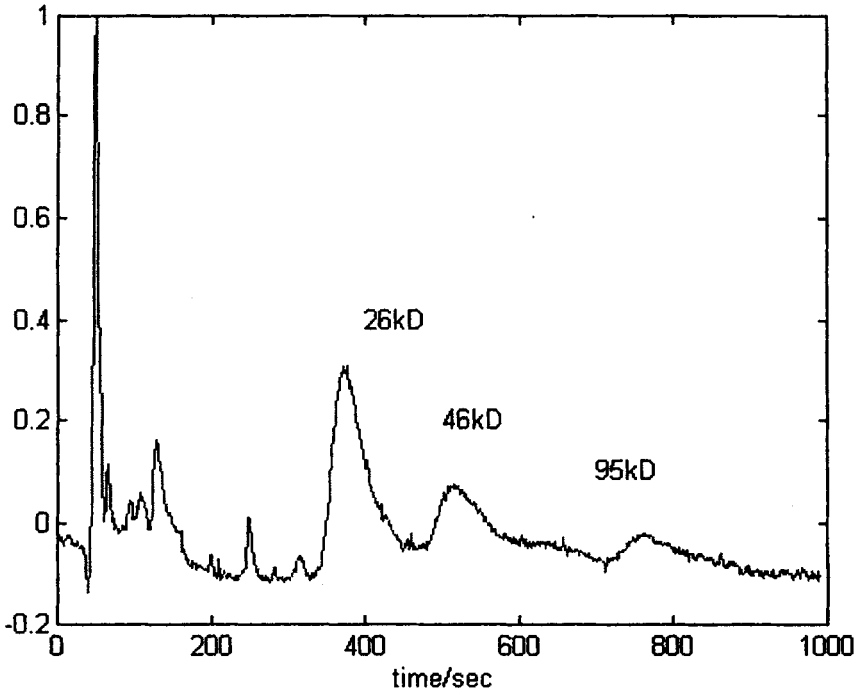

FIGURE 19. Solvent gradient separation. Conditions - Column: HP C18, 120 Å pore, 5 micron, 100 by 4.6 mm. Temp: 38.0°C. Mobile Phase: A - 52/48 ACN/Water, B - 48/52 ACN/Water. Solvent gradient from B to A.

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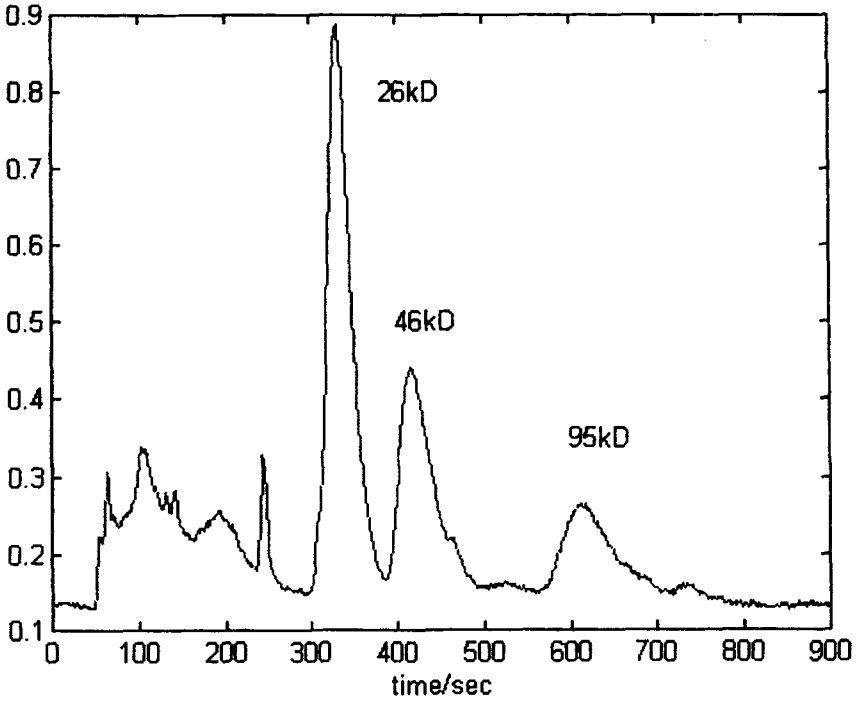

FIGURE 20. Temperature programmed separation. Temperature program. 48/52 ACN/Water. HP column. Flow rate = 1 mL/min. Temp = 39.81 - 0.1687*time.

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of conventional HPLC in the separation of polymers and polymer oligomers and isomers can be made. The positive information at this date is that conventional chromatographic models or "theory" seem to obtain for non-polar, polar, rigid and non-rigid polymers based on the model compounds studied. It will require more, well-planned and careful studies of representative polymers including block and random co-polymers to see how general the observations made so far indeed are. Work proceeds toward that goal.

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