

CHROM. 15,302

EFFECTS OF SALTS ON THE SIZE EXCLUSION BEHAVIOR OF POLY(ETHYLENE GLYCOL)

S. KRAUS* and L. B. ROGERS*

Department of Chemistry, University of Georgia, Athens, GA 30602 (U.S.A.)

(Received August 18th, 1982)**

SUMMARY

Poly(ethylene glycol) (PEG) forms complexes with salts in solutions. Size exclusion chromatography separates macromolecules according to their effective size in solution. In the present work, size exclusion chromatography was used to monitor the changes in the conformation of PEGs of average molecular weights of 8000, 3350, 1000 and 400 influenced by various ions in tetrahydrofuran solutions. Ammonium, barium, potassium and sodium iodides all caused the PEGs to contract and assume smaller effective sizes. The extent of the contraction depended on the concentration of the salt, each salt having a limiting concentration at which all PEGs eluted at the same retention time. Sodium bromide had an effect similar to that of the iodide except at lower concentrations. Lithium bromide had almost no influence on the effective size of the PEGs. The effect of salts on the conformation of PEGs in solution appears to be a combined cation/anion effect.

INTRODUCTION

Poly(ethylene glycol) (PEG) is known to have more than one conformation in the pure state as well as in solutions¹. Lorenz and Rogers² found that PEGs change their properties as gas chromatographic supports when more than five monomer units are in a chain, confirming information from Persinger³. X-ray diffraction studies have also led to the description of two conformers: an open coil, extended conformation, designated "zig-zag", and a more compact helical coiled configuration, "meander". The zig-zag form is predominant in low-molecular-weight PEGs, while the meandering conformation takes over in the PEGs having chains longer than nine units.

Changes in PEG chain conformation are also known to take place in solutions. Salting out of PEGs from aqueous solutions is related to collapse of the polymer chains as indicated by the reduction of the intrinsic viscosity of the aqueous polymer

* Present address: Criminal Identification Division, Israel Police Headquarters, Jerusalem 97999, Israel.

** Publication delayed at the authors request.

solutions⁴. Salts influence not only the molecular conformation but also micelle formation, and the transition between a rod-like micelle and spherical ones⁵.

PEGs react with electrolytes in non-aqueous solutions too. Cook⁶ found that PEGs form complexes with up to three Na⁺ ions per molecule in glycerol solutions of sodium iodide. Favretto *et al.*^{7,8} used the interaction between potassium picrate and PEGs in water to form ion-pairs that were extractable into an organic phase. This was used as a quantitative method for the determination of PEGs in water. Also, association of PEGs with electrolytes in methanolic solutions revealed a "salting in" effect: high-molecular-weight PEGs that were otherwise insoluble in methanol, dissolved in methanolic salt solutions. There was a marked reduction in the viscosity of these solutions with increasing concentration of the salt, indicating a reduction in the hydrodynamic volume⁴.

High-performance liquid chromatographic separations of PEGs have not received extensive treatment. PEGs are known to adsorb irreversibly to silica changing it to a liquid phase⁹. Berek and Bakos¹⁰ reported separations of PEG oligomers from 3 to 9 on a dextran-type column. Melander *et al.*¹¹ investigated the retention behavior of a PEG series on octadecyl-silica (ODS). They concluded that irregularities concerning the dependence of retention factors on temperature, eluent composition, and carbon number of the oligomers could be explained by the existence of an equilibrium between the two PEG conformers. Turner *et al.*¹² used an ODS column to separate PEGs from fatty alcohols. PEGs of molecular weight between 600 and 6000 all eluted in an early peak of invariant retention time, while the alcohols eluted later.

Size exclusion chromatography (SEC) of PEGs is usually carried out on cross-linked polystyrene columns⁹. The factor that determines the retention time of a solute in SEC is the molecular hydrodynamic volume rather than the molecular weight. Grubisic *et al.*¹³ introduced the universal calibration curve based on the concept of hydrodynamic volume. The product $|\eta| \cdot M$ is a direct measure of the hydrodynamic volume (where $|\eta|$ is the intrinsic viscosity of the polymer and M its molecular weight). By plotting $\log |\eta| \cdot M$ vs. retention time, they could construct a calibration curve that fit the data from widely differing polymers.

The effect of electrolytes on ionizable polymers was studied by Cha¹⁴. He used as a model a polymer of acrylonitrile into which several vinyl sulfonate groups were copolymerized. When dissolved in dimethylformamide (DMF), this polymer tended to expand and assume an extended conformation due to repulsive forces between the ionized groups. The addition of an electrolyte to the DMF solution neutralized the ionic repulsion by supplying counter-ions so that the polymer assumed a dimension expected for a normal homopolymer of the same molecular weight.

In the present study, the effect of salt complexation of PEGs on their hydrodynamic properties are investigated using SEC. This method is easy to follow and retention is inversely related to the relative molecular dimensions. The mobile phase was chosen to minimize any solute-support interaction other than size exclusion and to be able to dissolve the electrolytes. Knowing the effect of various salts on the chromatographic behavior of PEGs should lead to a better understanding of the properties of PEGs in solutions.

EXPERIMENTAL

Apparatus

Mobile phase was delivered by an Altex 110A pump (Altex, Berkeley, CA, U.S.A.). Samples were introduced into the column via an air-actuated, six-port sampling valve equipped with a 10- μ l sample loop (Valco, Houston, TX, U.S.A.). The effluent was monitored by a refractive index detector (Varian, Palo Alto, CA, U.S.A.). The chromatograms were recorded on an Omniscribe recorder (Houston Instruments, Houston, TX, U.S.A.).

Chemicals and solutions

Polystyrenes (PS) of narrow molecular weight distribution (800, 2200, 4000, 9000, 17,000, 110,000) were obtained from Pressure Chemical (Pittsburgh, PA, U.S.A.). PEG samples of various molecular weights were purchased (Alltech, Deerfield, IL, U.S.A.) as Carbowaxes of gas chromatographic quality. These samples had very broad distributions of molecular weight, nominally 400, 1000, 4000 and 6000. However, their actual average molecular weights were 400, 1000, 3350 and 8000, respectively¹⁵.

Alkali halides (NaI, KI, NH₄I, LiBr, NaBr) and tetrahydrofuran (THF) were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Barium iodide, calcium iodide, and triethylene glycol were purchased from Aldrich (Milwaukee, WI, U.S.A.).

The column packings were based on LiChrosorb Si 60, an irregularly shaped silica gel of 10 μ m mean particle size (E. M. Merck, Darmstadt, G.F.R.). It was deactivated (end-capped) by refluxing overnight with chlorotrimethylsilane in pyridine¹⁶.

A 1% triethylene glycol (TEG)-THF mixture was used as the mobile phase and as the solvent to prepare 10^{-2} M solutions of the salts (except NaBr solution that was $5 \cdot 10^{-4}$ M) and then to dilute them to a working concentration.

Procedures

Column preparation. The columns (220 \times 4.6 mm I.D.) were constructed from precision-bore, 316 stainless-steel tubing (Alltech). Prior to packing, the tubing was washed with 6 M nitric acid followed by distilled water, methanol, chloroform and, finally, THF. The tubing was then dried by a flow of dry nitrogen.

The columns were slurry-packed at 7000 p.s.i. using a "homemade" 37-ml downflow slurry packer connected to a Model 8500 pump (Varian). Silica 60 was slurried in 15 ml of carbon tetrachloride and stabilized by 22 ml of cyclohexanol. End-capped silica was slurried in 15 ml of toluene and stabilized by 22 ml of cyclohexanol.

Column calibration. The columns were calibrated using polystyrene standards dissolved in THF. The concentration of the PS samples was 10 mg/ml and the amount injected was 10 μ l. The flow-rate of the mobile phase during all of the experiments was maintained at 0.23 ml/min (pump set at 0.3 ml/min). The interstitial and the total volumes of the deactivated silica column were determined by the elution volume of the totally excluded PS of molecular weight 110,000 and the totally permeated methanol. The retention time of the totally excluded molecules was 7.7 min and that of the totally permeated was 13.8 min. These retention times corresponded to 1.77 ml for the interstitial volume and 3.17 ml for the total volume of the column. The volumes were

not corrected for extra-column volumes that were small and could be neglected in this study.

Preparation of samples. Polymer samples (PEG and PS) were dissolved in the mobile phase to prevent spurious peaks and to maintain baseline stability¹⁷. The concentrations of the PEG samples were 30–40 mg/ml, a value high enough to produce adequate detector response without overloading the column. The concentration of the solute in the mobile phase at the column outlet could be estimated from the peak width. For example, for an average peak width at half height of 1.4 min, the volume was 0.32 ml. The concentration of PEG of average molecular weight 8000 in the mobile phase was thus *ca.* $1.6 \cdot 10^{-4} M$ (based on a 10- μ l injection of a 40 mg/ml sample).

RESULTS

Solvent study

Cook⁶ found that PEG formed complexes with sodium ions in glycerin solutions that could be detected by mass spectrometry (MS). Unfortunately, that solvent is very viscous and has a refractive index beyond the range of the available detector. In contrast, although ethylene glycol has a refractive index in the working range of the detector, its viscosity is too high to produce a stable baseline and well-shaped peaks. Other solvents such as acetone, ethanol, water, and THF failed to elute PEGs from the Si 60 column, being irreversibly adsorbed as noted also by other workers⁹. Our study was limited to those solvents that, like Cook's glycerol, could dissolve sufficiently large amounts of the salts.

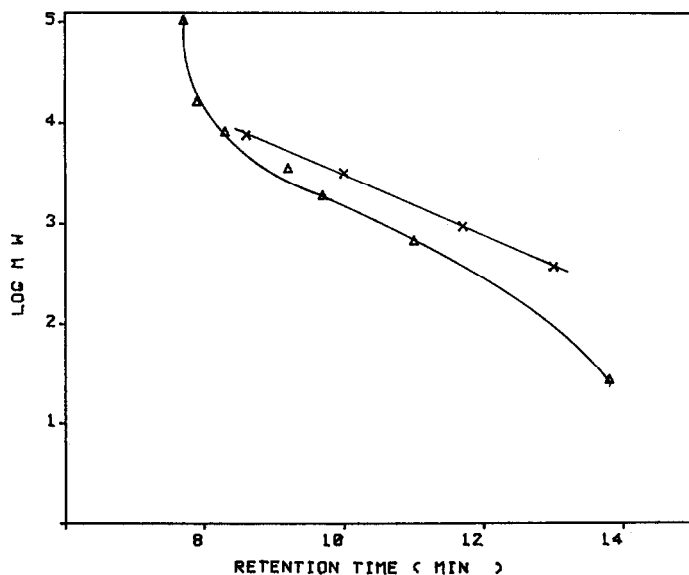


Fig. 1. Size exclusion calibration curve for PEGs (X) and PS (Δ). Column 22 \times 0.46 cm I.D. TMCS end-capped Si 60. Mobile phase, 1% triethylene glycol in THF. Flow-rate, 0.23 ml/min. Detector, refractive index. MW = Molecular weight.

It was evident that a silica column could not be used for size exclusion of PEGs with the types of solvent we were using. End-capping the active silanols on the silica surface reduced the adsorption of the PEGs significantly, yet it did not eliminate the adsorption totally. Adsorption of the PEGs was indicated by the fact that the PEG of molecular weight 400 eluted after benzene when THF was used as mobile phase. The other solvents mentioned above also failed to elute the PEGs in the size exclusion range.

The adsorption of PEGs onto the end-capped silica was prevented by modifying the THF by the presence of a competing agent. A solution of 1% TEG in THF as a mobile phase allowed the PEGs to elute within the size exclusion range. Samples of PEGs from 8 mg/ml to 80 mg/ml eluted at the same retention time. As a result, 30–40 mg/ml was used throughout this work without evidence of overloading effects.

Calibration curves

Fig. 1 presents log molecular weight vs. retention time of PS and PEGs. The molecular weight assigned to each PEG was the average molecular weight of each range. The fact that the two curves did not coincide was not surprising. Although both polymers are linear molecules with random coil conformation, their molecular-size dependence on the molecular weight could be different. Dubin *et al.*¹⁸ found that they needed to use the hydrodynamic volume in order to fit PS, PEG and other linear polymers, having different regular calibration curves, to a common curve.

Effects of salts on retention behavior

The addition of electrolytes to mobile phases is recommended in separations of

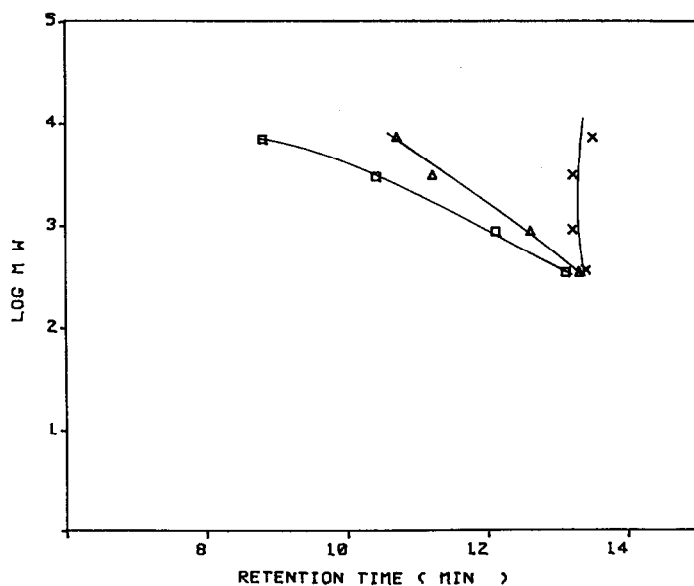


Fig. 2. Size exclusion curves of PEGs in the presence of ammonium iodide at three concentrations, $1.4 \cdot 10^{-3} M$ (\square), $3.5 \cdot 10^{-3} M$ (\triangle), $7 \cdot 10^{-3} M$ (\times). Conditions as in Fig. 1 except for the addition of salt to the mobile phase.

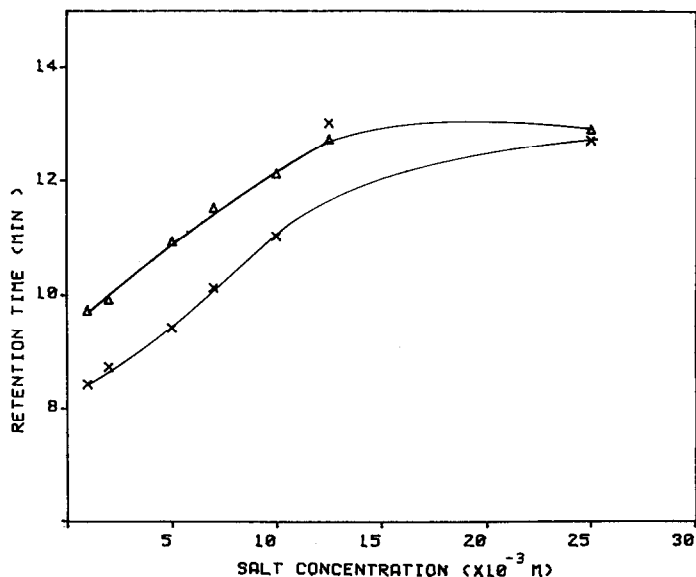


Fig. 3. The effect of various concentrations of sodium iodide dissolved in the mobile phase on the retention time of PEGs, molecular weight 8000 (x) and molecular weight 3350 (Δ). Conditions as in Fig. 1 except for the addition of salt to the mobile phase.

polymers having ionizable groups^{14,18}. The electrolyte suppresses the abnormal expansion of the ionic polymer by supplying counter-ions to the charged sites of the polymer backbone and restoring the expected dimensions of the neutral molecule¹⁷.

The addition of sodium iodide to the mobile phase had a very significant effect on the hydrodynamic dimensions of the non-ionic PEGs. Fig. 2 demonstrates the effect of various concentrations of salt on the retention times of the PEGs. Notice that retention times were prolonged, which means a smaller effective size of the solutes. A surprising result was that even the largest PEG (molecular weight 8000) eluted at the same retention time as the smallest PEG tested (molecular weight 400). All of the PEGs eluted within the size exclusion range, so the phenomenon cannot be one of selective adsorption of the complexes formed.

Fig. 3 illustrates the relationship between the retention times of PEGs 8000 and 3250 and the concentration of NaI between $1.0 \cdot 10^{-3} M$ and $2.5 \cdot 10^{-2} M$. The retention time gradually shifted to longer times until it reached the limiting elution time of the smaller molecules. Ammonium iodide had the same effect on retention of PEGs as sodium iodide except that it was somewhat more effective. This salt attained its maximum effect at $7 \cdot 10^{-3} M$. Potassium iodide was effective at even lower concentrations reaching its maximum effect at $1.0 \cdot 10^{-3} M$.

Calcium and barium ions have ionic radii similar to those of sodium and potassium, respectively. Therefore, it was of interest to investigate their effects. Unfortunately, calcium iodide was not sufficiently soluble in THF to be tested. However, barium iodide was found to have the same effect and the same effective range of concentration as sodium iodide (10^{-3} to $10^{-2} M$).

Bromides were not expected to have any additional effect on the PEGs if only

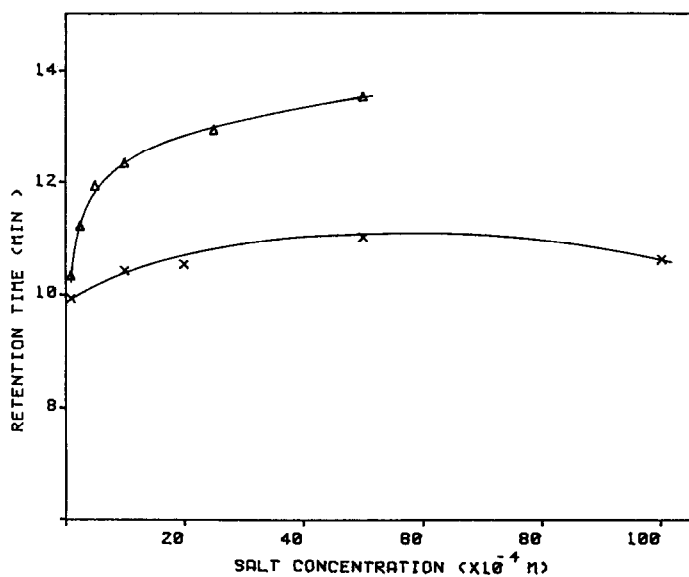


Fig. 4. The effect of various concentrations of sodium bromide (Δ) and lithium bromide (\times) on the retention times of PEG of molecular weight 3350.

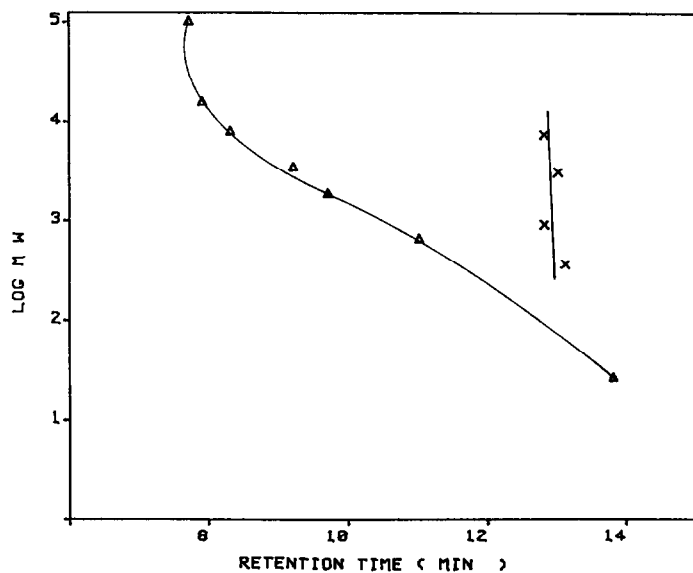


Fig. 5. Size exclusion calibration curves for PEGs (\times) and PS (Δ). Conditions as in Fig. 1 except for the addition of potassium iodide to the mobile phase ($10^{-3} M KI$).

the cation was responsible for the phenomenon of molecular contraction. However, sodium bromide was found to be more effective than sodium iodide in contracting the PEGs. It influenced the separation behavior when its concentration was as low as $10^{-5} M$, and it reached its maximum effect at $5 \cdot 10^{-4} M$. Hence, there was clearly a bromide effect, or better, a sodium bromide (combined) effect. In attempting to test the bromide effect, potassium bromide was found to have too low a solubility in THF to be tested. Lithium bromide had no significant effect on the PEGs up to the concentration of $10^{-2} M$. Fig. 4 demonstrates the difference between the effects of NaBr and LiBr on the PEG of molecular weight 3350.

Selectivity of the salt effect. Fig. 5 demonstrates the selectivity of the influence of salt on the PEGs by comparing PS and PEGs under the same conditions. Both sets of calibration runs were performed in $10^{-3} M$ KI in the mobile phase. The PS calibration curve was not disturbed at all while all of the PEGs eluted at a similar retention time.

DISCUSSION

We believe that we have obtained evidence for the formation of complexes between PEGs and salts. Although the influence of such complexation on the molecular conformation was expected, the extent of a reaction strong enough to produce a uniform retention volume for all PEGs was not. Potassium iodide was found to be more effective than sodium iodide. Hence, it might be of interest to test KI in Cook's system of mass spectrometry using glycerol solution.

The differences among the various alkali halides were not expected, based on information available concerning the influence of salts on PEG viscosity in aqueous solutions⁴. The main influence in aqueous solutions is that of the anion: trivalent > bivalent > monovalent. The very high activity of sodium bromide vs. sodium iodide clearly demonstrates that the anion itself or a combination of the anion and the cation can affect the complexation. In Cook's work, the species detected were the positively charged sodium-PEG complexes. It is possible that negative-ion MS could reveal the presence of negatively charged species carrying the anions.

We conclude that the interaction between PEG and salt is a combined cation-anion effect. The selectivity of the salt effect on the PEGs (Fig. 5) clearly shows that there was no major change in the mobile phase or in the stationary phase with respect to other solutes (PS).

There are no details of intrinsic viscosity available for solvent mixtures and solutes used in this study. However, a four-fold decrease in the viscosity of a methanolic solution of PEG 500 has been observed when increasing amounts of KI were added⁴. It would be of interest to confirm the conformational changes in the salt solution by viscosity measurements. We expect that the product of the intrinsic viscosity and the molecular weight would be constant in the solutions of salts in THF at the limiting concentrations.

It would also be of interest to compare the present results with those from other techniques, such as light scattering. In our laboratory, preliminary investigations of the salt-PEG complexes by means of Raman spectroscopy was not successful due to solvent background. Nuclear magnetic resonance of ^{23}Na might prove to be more informative¹⁹.

ACKNOWLEDGEMENTS

We thank Kelsey Cook of the University of Illinois at Urbana-Champaign for very helpful discussions and for providing portions of his PEG samples.

This work was supported by the U.S. Department of Energy Division of Basic Energy Sciences through Contract Number DE-AS09-76R00854.

REFERENCES

- 1 M. Rösch, in M. J. Schick (Editor), *Non-Ionic Surfactants*, Marcel Dekker, New York, 1967, Ch. 22.
- 2 L. Z. Lorenz and L. B. Rogers, *Anal. Chem.*, 43 (1971) 1593.
- 3 H. E. Persinger, Technical Center, Union Carbide Corp., South Charleston, WV, personal communication, May 1969.
- 4 F. E. Bailey, Jr. and J. V. Koleske, in M. J. Schick (Editor), *Non-Ionic Surfactants*, Marcel Dekker, New York, 1967, Ch. 23.
- 5 K. Kalayanasundaram and J. K. Thomas, *J. Phys. Chem.*, 80 (1967) 1492.
- 6 S. T. F. Lai, K. W. Chan and K. D. Cook, *Macromolecules*, 13 (1980) 953.
- 7 L. Favretto, B. Stancher and F. Tunis, *Analyst (London)*, 103 (1978) 955.
- 8 L. Favretto, B. Stancher and F. Tunis, *Twelfth Annual Symposium on the Analytical Chemistry of Pollutants, Amsterdam, April 14-16, 1982*, abstracts, p. 127.
- 9 R. M. Cassidy and C. M. Niro, *J. Chromatogr.*, 126 (1976) 787.
- 10 D. Berek and D. Bakoš, *J. Chromatogr.*, 91 (1974) 237.
- 11 W. R. Melander, A. Nahum and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 129.
- 12 L. P. Turner, D. McCullough and A. Jackewitz, *J. Amer. Oil Chem. Soc.*, 53 (1976) 691.
- 13 Z. Grubisic, P. Rempp and H. Beniot, *J. Polym. Sci., &art B*, 5 (1967) 753.
- 14 C. Y. Cha, *J. Polym. Sci., Part B*, 7 (1969) 343.
- 15 M. Windholz (Editor), *The Merck Index*, Merck and Co., Rahway, NJ 9th ed., 1976, p. 983.
- 16 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 2nd ed., 1979, p. 397.
- 18 P. L. Dubin, S. Koontz and K. L. Wright III, *J. Polym. Sci.*, 15 (1977) 2047.
- 19 A. F. Findeis, National Science Foundation, Washington D.C., personal communication, April 2, 1982.