Journal of Chromatography, 252 (1982) 325–330
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,299

Note

Separation of styrene oligomers using normal nitrile-bonded phase liquid chromatography with UV and fluorescence detection

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(Received August 2nd, 1982)

Styrene oligomers have been separated using both reversed- and normal-phase chromatography, as well as by size-exclusion chromatography. Snyder and Kirkland[1] presented a chromatogram of the lower styrene oligomers on a C_{18} reversed-phase column using a tetrahydrofuran–water gradient. Later, Kirkland[2], in evaluating a new 7-μm microporous silica derivatized with octadecyl groups, showed a baseline separation of about 11 peaks in 14 min for a polystyrene standard (average molecular weight 600) (PS600) using pure acetonitrile eluent. Holt-Sackett *et al.*[3] analyzed the same PS600 using a 5-μm silica B/5 column and a linear hexane–dichloromethane gradient. In approximately 15 min about 11 peaks appeared on top of a broad envelope. Fukada *et al.*[4] separated styrene oligomers with a degree of polymerization less than 10 (essentially PS600) on a narrow-pore gel permeation column.

We report here the use for this separation of the normal nitrile-bonded phase, which combines the higher resolution of the reversed-phase column with advantages of the silica gel. In addition, both UV absorption and fluorescence detectors were used; the latter has the advantage of much higher sensitivity for styrene oligomers. It could also function as a more selective detector, for example for the analysis of polystyrene in copolymer blends.

EXPERIMENTAL

High-performance liquid chromatography (HPLC)

A Varian 8500 LC was used with a Varian 25 cm × 2 mm I.D. CN-Micropak column for isocratic runs using 20 ml/h of isooctane–dichloromethane (13:1 or 12:1) at ambient temperature. Pure dichloromethane at 10 ml/h was used for some experiments. The Varian UV detector was set at 254 nm. Aliquots (1.0 μl) of the polystyrene standards dissolved in dichloromethane were injected. For gradient elution using 0.77 ml/min gradients of isooctane–dichloromethane, a Micromeritics 7500 LC system was used with the UV detector also set at 254 nm.

Fluorescence

A Perkin-Elmer 650-10S Fluorescence Spectrophotometer was used with a Perkin-Elmer Model 650-0151 10-μl micro flow cell and a Perkin-Elmer Hitachi 057 x–y recorder. The cell also functions as a stopped-flow cell for taking spectra of

0021-9673/82/0000–0000/$02.75

eluting peaks. For continuous monitoring, the wavelengths of excitation were 260 nm or 270 nm and emission was read at 320 nm.

Samples

Polystyrene standards 600 (ArRo Labs., Joliet, U.S.A.) (PS600) and 730 (U.S. National Bureau of Standards) (PS730) were supplied courtesy of Dr. Michael Dong, Perkin-Elmer Corp. Samples were dissolved in dichloromethane at a concentration of approximately 2 μg/ml. Another NBS standard polystyrene of weight-average molecular weight $1.96 \cdot 10^5$ ($PS2 \cdot 10^5$) was supplied courtesy of Dr. Arthur Woodward, City College.

RESULTS AND DISCUSSION

Figs. 1 and 2 are typical chromatograms of the PS600 and PS730, respectively, using the UV detector at 254 nm. In Fig. 1, the eluent was isooctane–dichloromethane (13:1); in Fig. 2 a 12:1 volume ratio was used. One can surmise that solvent programming would improve the separation, which is demonstrated in Fig. 3 for PS730 with a 15-min linear gradient from 0 to 15% (v/v) dichloromethane in isooctane. Resolution is better at the front end, and an additional peak emerges from the noise (degree of polymerization 12), compared to the isocratic chromatogram. Convex gradients produce slight improvements in resolution.

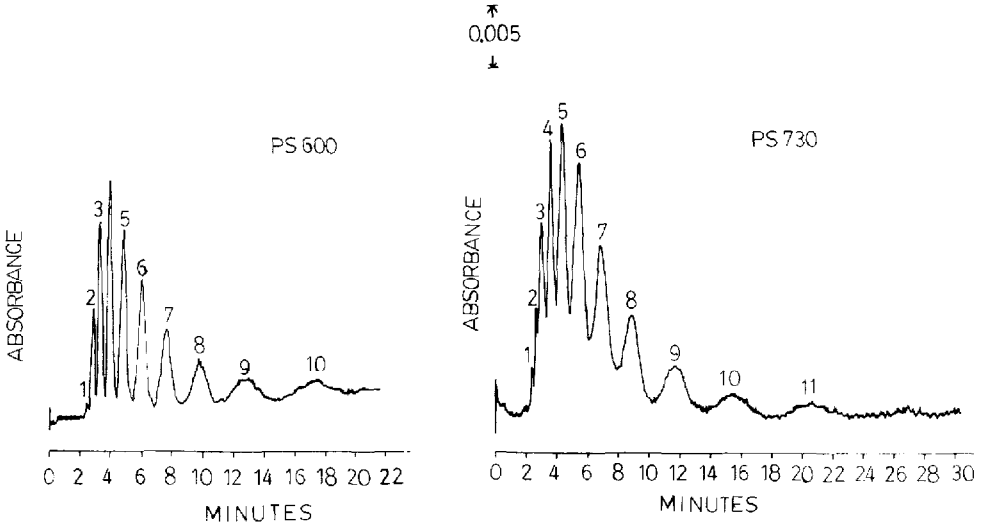

Fig. 1. HPLC separation of components of polystyrene standard PS600. Chromatographic conditions: CN-Micropak column, 25 cm × 2 mm I.D., 20 ml/h isooctane–dichloromethane (13:1), ambient temperature, UV detector at 254 nm.

Fig. 2. HPLC separation of components of PS730. Chromatographic conditions: same as Fig. 1 except the volume ratio of isooctane–dichloromethane is 12:1.

The fluorescence detector, which was not previously applied to polystyrene chromatographic analysis, was used to obtain the chromatograms in Fig. 4. The upper chromatogram in Fig. 4 is for PS600 and the lower for PS730. The signal-to-

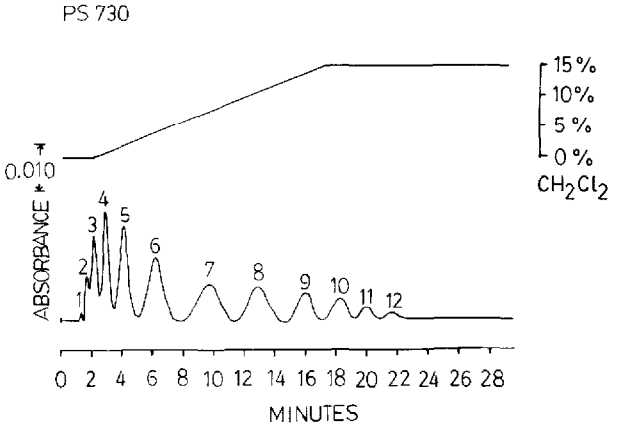

Fig. 3. Gradient elution separation of components of PS730. Chromatographic conditions: same as Fig. 1 except using a linear gradient indicated on the Fig. at 0.77 ml/min.

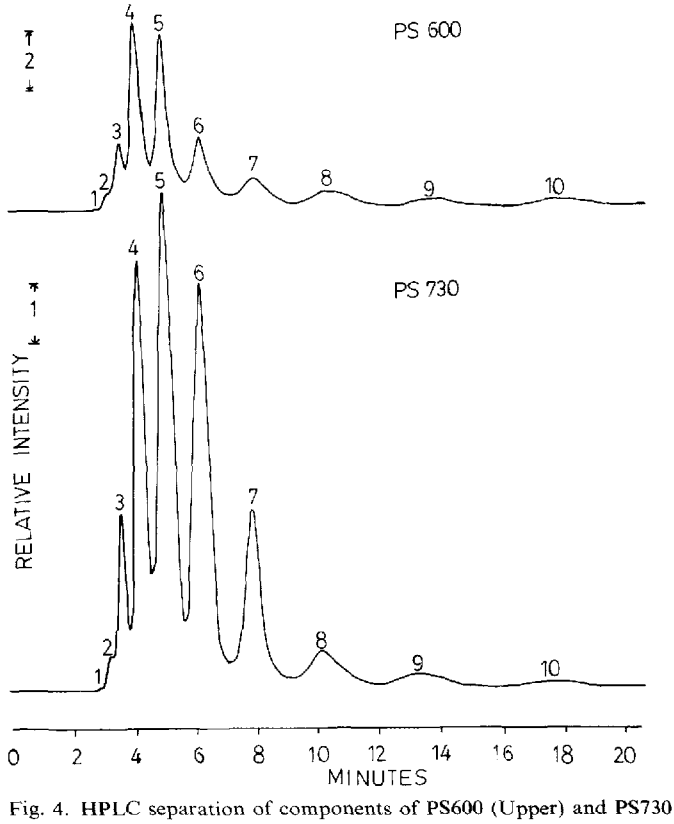

Fig. 4. HPLC separation of components of PS600 (Upper) and PS730 (Lower). Chromatographic conditions: same as Fig. 1 except using a fluorescence detector, wavelength of excitation, 270 nm, and wavelength of emission monitored, 320 nm.

noise ratio is increased by a factor of 10^3 over the UV detector. The eluent in both chromatograms was isooctane–dichloromethane (13:1). The tallest peak in the PS600 chromatogram corresponds to the tetramer of styrene; that in the PS730 is the pentamer.

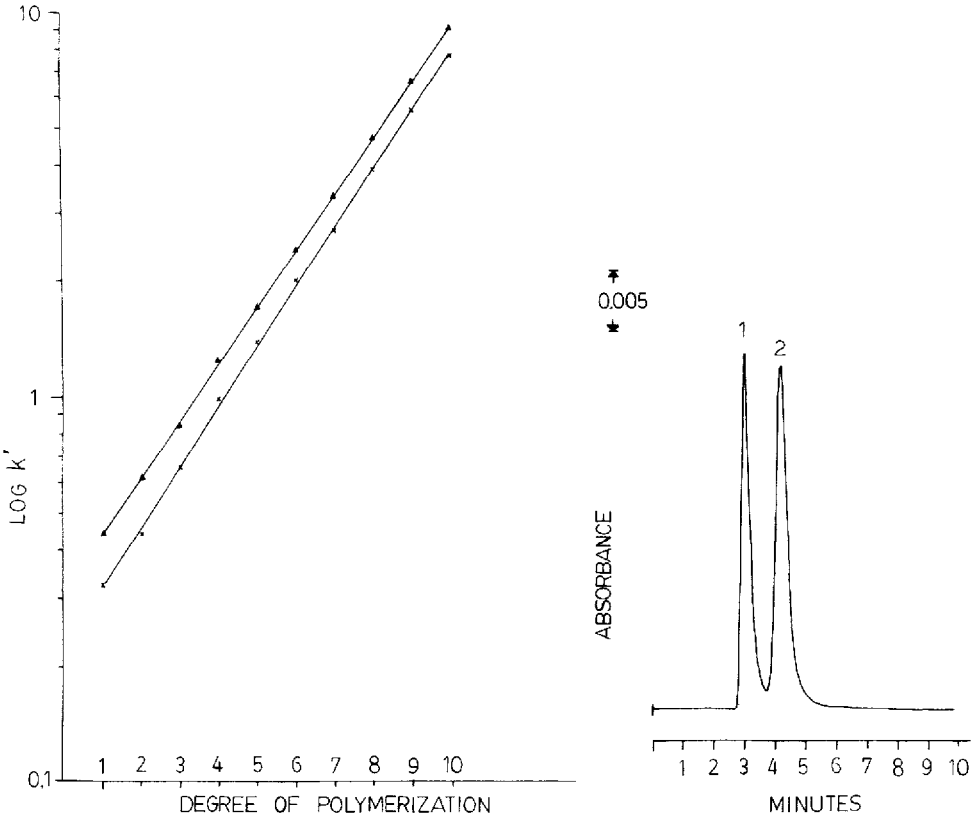

Fig. 5. Log retention as a function of degree of polymerization for PS600 (▲) and PS730 (×). Chromatographic conditions: same as Figs. 1 (▲) and 2 (×).

Fig. 6. CN-Phase functions as a size exclusion column in dichloromethane towards PS2 · 10^5 (1) and PS730 (2). Chromatographic conditions: same as Fig. 1 except eluent is pure dichloromethane at 10 ml/h.

For both standards the plot of log capacity factor (k') (isocratic) *vs.* degree of polymerization is the expected straight line, as shown in Fig. 5. The upper line, for PS600, was obtained with isooctane–dichloromethane (13:1); the lower, for PS730, corresponds to the 12:1 mixture. These lines ultimately converge; the differential solubility between the two solvents decreases with increasing degree of polymerization, as might be expected. From Figs. 3 and 5 it is evident that isocratic elution can handle oligomers with a range of degree of polymerization of about 10; k' varies by a factor of about 50 over this range. Oligomers of higher molecular weight but covering this range could be similarly resolved by increasing the proportion of dichloromethane in isooctane. With gradient elution, however, a far wider range can be separated.

It is interesting to observe that with pure dichloromethane eluent the nitrile-bonded column acts as a size exclusion medium. The solvent is sufficiently polar to

overwhelm any selective solute–stationary phase interactions, and the bonded moiety eliminates active surface hydroxyl sites on the silica. The microporous silica then separates on the basis of molecular size. This is demonstrated in Fig. 6 where a mixture of the PS2 · 10^5 and PS730 standards in dichloromethane was cleanly separated. A higher resolution separation could presumably be achieved with a column longer than the 25 cm one used here.

Stopped-flow fluorescence spectra were obtained for each peak in the PS600 and PS730 chromatograms. Here, the excitation wavelength was set at 260 nm rather than the 270 nm used in Fig. 4, in order to reduce the influence of scattered light on the fluorescence band at 290 nm. The bands at 290 nm correspond to molecular fluorescence of the oligomers themselves. The bands at 320 nm most evident for the taller chromatographic peaks correspond to the emission of polystyrene excimers, *i.e.* emission from an excited state dimer of a styrene oligomer. Excimer formation in polystyrene of high molecular weight was first reported by Yanari and Bovey[5]; small aromatic molecule excimer emission has been discussed by Birks[6]. This is the first report of excimer emission for lower styrene oligomers. Excimer emission is concentration-dependent; thus the excimer band is most pronounced for the oligomers with degrees of polymerization of 3–7 in Figs. 7 and 8. This also accounts for the differences in the relative peak heights for the earliest and later peaks between the UV

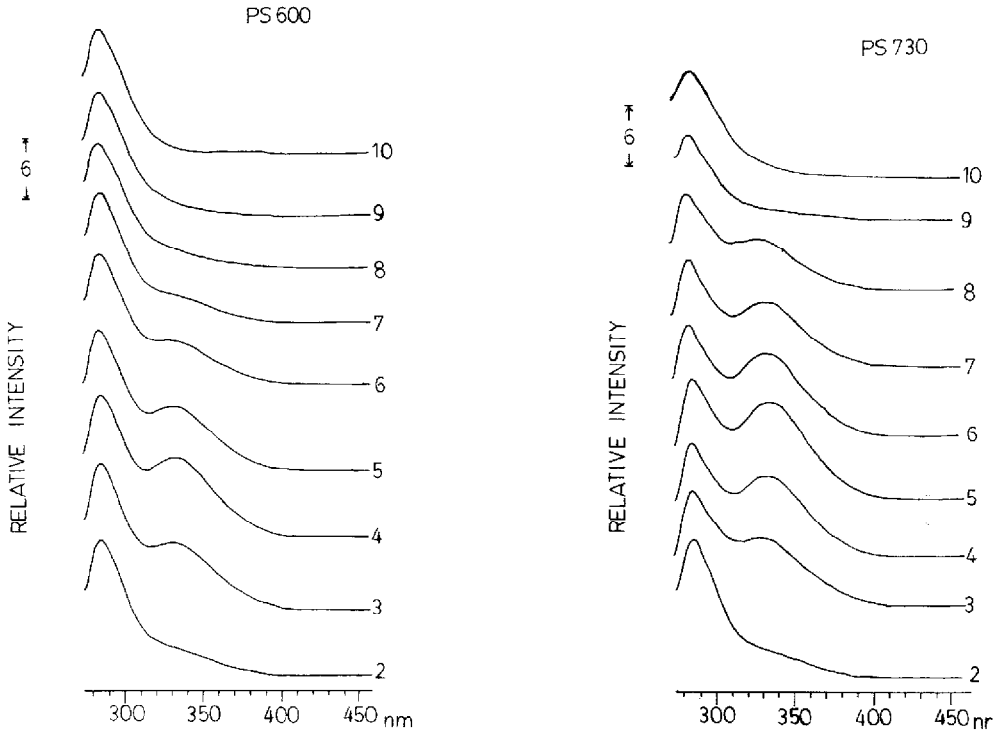

Fig. 7. Stopped-flow fluorescence spectra of each peak in the chromatogram of PS600. Chromatographic conditions: same as Fig. 4. Excitation wavelength, 260 nm.

Fig. 8. Stopped-flow fluorescence spectra of each peak in the chromatogram of PS730. Chromatographic conditions: same as Fig. 4. Excitation wavelength, 260 nm.

and fluorescence detector chromatograms; the latter was set to measure excimer emission to reduce the background from scattered light.

CONCLUSIONS

The potential of normal bonded phase liquid chromatography (LC) has not been fully explored, probably because so much attention has been given to reversed-phase LC, the situation having been discussed by Abbott[7]. The ability of the nitrile phase to separate styrene oligomers has been demonstrated here. In addition we have shown that the nitrile phase acts in polar solvents such as dichloromethane as a size exclusion column. A potentially attractive aspect of the normal phase packings is with the use of LC–mass spectrometry (MS). With reversed-phase LC–MS, evaporation of the water–acetonitrile or water–methanol solvents is a problem; with the more volatile organic solvents used with the nitrile phase, evaporation is easier. Fluorescence detection of polystyrene is far more sensitive than UV detection and should have wider applications in size exclusion chromatography of aromatic polymers.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks the partial support of this work by grants from the National Science Foundation and from the PSC-CUNY Research Award Program (RF13910).

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