ROLE OF POLAR GROUPS IN FRACTIONATING ISOMERIC POLYSTYRENE OLIGOMERS

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SUMMARY

Isomers of polystyrene trimers have been fractionated using column packings of silica derivatized with cyanopropyl, aminopropyl, nitropropyl, and tetranitrofluorene groups. The elution order was independent of the packing and of the solvent used for elution. However, that order was opposite to the one reported earlier for alkyl-derivatized silicas which, when eluted with polar solvents from different locations of the Snyder triangle, all gave the same elution order. Hence, it appears that the polar groups, cyano, amino, and nitro, whether in a solvent or on the surface of an adsorbent provide the same dominant contribution to the polystyrene isomer separation by inducing a dipole in the phenyl groups of polystyrene.

This interaction also can be applied to explain the elution order of polystyrene stereoisomers. For example, the interaction, which is strongest with the nitro group, produces a relatively stronger retention by the isotactic species when the nitro group is attached to the column, but a relatively weaker retention when the nitro group is in the eluting solvent.

INTRODUCTION

Due to its well characterized properties and narrow dispersivity, polystyrene has been used as a model to study the behavior of polymers in chromatography. For polystyrene fractionations, supercritical fluid chromatography (SFC)[1,2], reversed-phase high-performance liquid chromatography (RP-HPLC)[3–7], normal-phase HPLC[8,9] and size-exclusion chromatography (SEC)[10,11] have been examined. In most of these early reports, only oligomer separation was reported. Poorly shaped or split oligomer peaks were attributed to sampling difficulties or to factors other than stereoisomer separation.

The separation of diastereoisomers of various styrene oligomers by recycle gel permeation chromatography was reported by Sato *et al.*[10] using styrene–divinylbenzene gel as the column packing and chloroform as the eluent. The isotactic form eluted before the syndiotactic. Lesec *et al.*[12], using styrene–divinylbenzene copolymer as packing and diisopropyl ether as the eluent found an elution order of the stereoisomers opposite to that in the chloroform. The difference in elution order may have

been related to a different dominant solute-retention mechanism. For example, the chloroform method may have been dominated by the exclusion effect and the diisopropyl ether method, by the adsorption effect. The elution order was studied and established by NMR spectroscopy[13,14].

This work was extended by Lewis et al.[4] using different packings and eluents. The reversed-phase packings, including C_{18}, C_8, C_1 and fluorinated alkyl groups, were capable of polystyrene stereoisomer separation. The elution order on a C_{18} column of isotactic followed by syndiotactic was established by a two-dimensional Fourier transform NMR study[15]. In a later study[16], solvents chosen from different locations of the Snyder triangle were all found to elute the stereoisomers in the same order from a C_{18} column. In addition, mixing acetonitrile with solvents that exhibited different types of primary interaction with the solute did not change the elution order of the stereoisomers.

In the present study, silica packings derivatized with different "polar" groups on the surface were examined. These packings included plain silica as well as silica derivatized with cyano, amino, nitro and tetranitrofluorene (TENF). In addition, a phenyl column, a fluorinated phenyl column, and a polysytrene divinyl benzene column were compared with the C_{18} column. n-Hexane was used as the primary eluent for these polar packings; some polar solvents were added to n-hexane as a modifier to examine their effects on the retention times and elution orders.

EXPERIMENTAL

Chemicals

All solvents were used as received. Chloroform, n-hexane, methanol, acetonitrile, methylene chloride, and toluene were either reagent grade or HPLC grade (J. T. Baker, Phillipsburg, PA, U.S.A.). Nitromethane, pentafluorobenzoyl chloride, propylene carbonate, and proton sponge [1,8-bis(dimethylamino) naphthalene] were purchased from Aldrich (Milwaukee, WI, U.S.A.). Doubly distilled deionized water was prepared from house-distilled water using a home-made purification system consisting of anion and cation exchangers, an activated charcoal bed, and a Corning Mega-Pure 1-l still (Corning Glass Works, Corning, NY, U.S.A.). All mobile phases were filtered through the appropriate Millipore filters (Bedford, MA, U.S.A.). Then, the mobile phases were degassed with helium prior to use. Monodisperse mol. wt. 800 polystyrene samples were obtained from the Pressure Chemical Co. (Pittsburgh, PA, U.S.A.).

A 25 cm × 4.5 mm I.D. C_{18} HPLC column (IBM Instruments, Yalesville, CT, U.S.A.), having end-capped, spherical, 5-μm particles and 100 Å average pore size, was used as received. Another PRP-1 column obtained from Hamilton (Reno, NV, U.S.A.) was a rigid copolymer of styrene and divinyl benzene in 10-μm particles, and it had an average pore size of 75 Å. Phenyl bonded-phase column packing, having 10-μm particles and 100 Å pore size, was obtained from Macherey-Nagel (Düren, F.R.G.). The column packings having polar functional groups were cyano (Universal Scientific, Atlanta, GA, U.S.A.); amino (E. Merck, Darmstadt, F.R.G.); nitro (Rainin, Woburn, MA, U.S.A.); and TENF (E. S. Industries, Marlton, NJ, U.S.A.). The pure silica packing was obtained from Alltech Assoc. (Norcross, GA, U.S.A.). All of these packings were 10-μm particles and 100 Å pore size.

Apparatus

Two Varian 8500 syringe pumps with a gradient controller (Palo Alto, CA, U.S.A.) were used to deliver the mobile phases. An air-actuated six-port valve, Model ACV-6U HPa (Valco, Houston, TX, U.S.A.) with a 10-μl injection loop and a Beckman (Fullerton, CA, U.S.A.) Model 155 variable-wavelength detector were connected to form the separation system. Chromatograms were recorded using a Model 585 (Linear Instruments, Reno, NV, U.S.A.) dual-pen chart recorder.

Procedures

The pentafluorophenyl-derivatized silica packing was synthesized by a procedure very similiar to that reported by Berendsen and co-workers[17,18] and Unger[19]. Aminopropyl silica gel (4.0 g) was dried in a vacuum oven at 110°C for 24 h. Then, the silica was added to a flame-dried reflux apparatus followed by dry toluene (150 ml) and 0.5 g proton sponge. The whole system was purged with dry nitrogen gas. Pentafluorobenzoyl chloride (7.0 g dissolved in 20 ml dry toluene) was added dropwise, and the silica was refluxed for 12 h at a temperature 10°C below the boiling point of the most volatile component. The silica was then collected and washed successively with toluene, methanol, methanol–water (50:50, v/v), water, methanol, diethyl ether and acetone. Before being packed into a column, the silica was dried in a vacuum oven at 110°C for 24 h.

All chromatographic columns were constructed from precision-bore 316 stainless-steel tubing (25 cm $\times$ 4.5 mm I.D.; Alltech) that had been washed with 6 M nitric acid, followed by distilled water, methanol, and tetrahydrofuran. The tubing was then blown dry with nitrogen. The column was terminated using Swagelock stainless-steel end fittings (Georgia Valve and Fittings, Atlanta, GA, U.S.A.) and 0.5-μm frits from Alltech.

The silica and pentafluorophenyl columns were packed using a modified viscosity down-flow packing method[20,21]. Carbon tetrachloride was placed in the column prior to packing in order to prevent the slurry from setting into the column. Then, a 10% (w/v) slurry of the packing material in cyclohexanol–isopropanol (3:1, v/v) was poured into the slurry packer. A constant-pressure air-driven reciprocating pump (Model 10-600-50, SC Hydraulics, Los Angeles, CA, U.S.A.) was used to force the solvent, methanol, through the column at 11 000 p.s.i. constant pressure. After the pure methanol had been coming out of the column for 30 min, a "slamming" technique was applied several times to consolidate the packed bed[22].

The phenyl, cyano, amino, nitro and TENF columns were packed using a up-flow, balanced density method[23] at 6000 p.s.i. using a Micromeritics Model 705-P slurry packer (Micromeritics, Norcross, GA, U.S.A.) and a Varian Model 8500 pump. The slurry solvents were chloroform–methanol (3:1, v/v) for the phenyl, nitro and cyano columns, chloroform–*n*-hexane (3:1, v/v) for the amino column, and *n*-hexane for the TENF column. All columns were equilibrated by passing the desired composition of mobile phases for 30 column volumes or until a stable baseline had been reached. The mobile phase flow-rate was held at 1.0 ml/min unless otherwise specified.

In studies of the fractionation of the isomers of polystyrene trimer, a preliminary isolation of oligomers 2–5 was first performed. A 2-g sample of monodisperse mol.wt. 800 polystyrene standard was dissolved in a minimum amount of *n*-hexane.

Then, this sample was applied to a low-pressure glass column which contained 200–250 ml of silica gel (J. T. Baker). The silica column was eluted successively with 10% toluene in hexane (the effluent contained mostly the impurities and the mono-mer), 20% (n-mers 2–5), 40% (n-mers 6–8), and 60% (to clean the column). The solvent was removed by fractional distillation followed by a vacuum oven. The poly-styrene n-mers were redissolved in pure acetonitrile or n-hexane depending upon the mobile phase used for the elution-order studies.

Collection of the two fractions of trimer stereoisomers was carried out using a Vydac semi-prep C_{18} column (25 cm × 10 mm I.D., 10-μm particle size), (Sepa-rations Group, Hesperia, CA, U.S.A.) using acetonitrile–chloroform (97:3, v/v) as the eluent. The molecular structures of collected fractions have been analyzed and assigned by Ray et al.[15] using a two-dimensional NMR method. These fractionated isomers were used separately to spike different samples of the 20% toluene (in hexane) fraction (n-mer 2–5), so as to determine the isomer elution order.

To compare different separations of polystyrene stereoisomers, a peak sepa-ration factor was calculated for each column. The separation factor, $p = f/g$, where f is the depth of the valley below a straight line connecting two adjacent peak maxima and g is the length of that line after it has been extended to the baseline[24,25].

RESULTS AND DISCUSSION

In comparing three different solvents on a C_{18} column, Fig. 1 shows that nitromethane produced almost a baseline separation of the trimer stereoisomers, acetonitrile was the next best, and methanol was the worst, a poor separation of stereoisomers being observed only for the higher oligomers. The trend of isomer
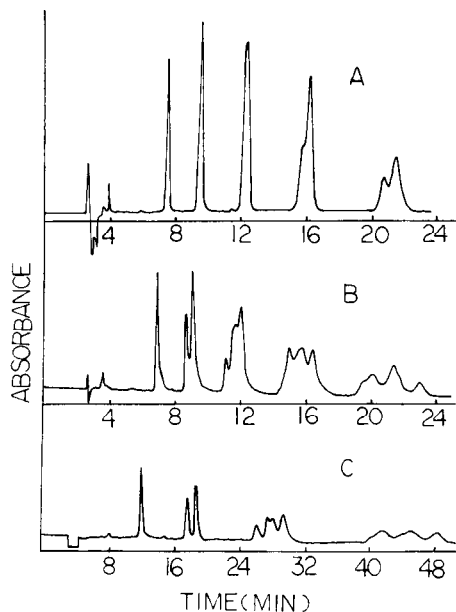

Fig. 1. Chromatograms for a C_{18} column using different mobile phases. (A) Methanol, (B) acetonitrile and (C) nitromethane.

separation is roughly parallel to the polarity of the mobile phase: nitromethane which gave the best separation is the strongest with a value of 6.0 (ref. 26); acetonitrile with a value of 5.8 gave the second best separation; while methanol at 5.1 gave the worst separation of stereoisomers.

Polar packings

The polar packings included plain silica, cyanopropyl, aminopropyl, nitropropyl and TENF. Fig. 2 shows the extent of stereoisomer separation on each column when *n*-hexane was used as the mobile phase under isocratic conditions. The peak resolutions of the stereoisomeric trimers by these columns are listed in Table I. The nitro column had the best peak separation factor (and the greatest retention) followed
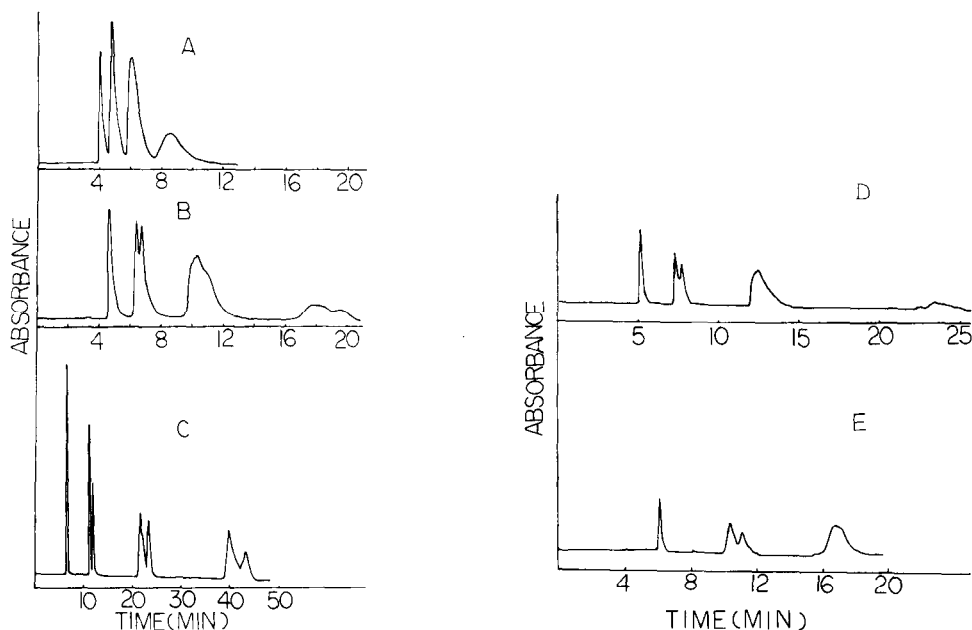

Fig. 2. Chromatograms for different columns and the same mobile phase (*n*-hexane). (A), silica; (B), cyanopropyl; (C), nitropropyl; (D), aminopropyl; (E), TENF.

TABLE I

SEPARATION OF POLYSTYRENE STEREOISOMERS ON –CN, –NH$_2$, TENF AND –NO$_2$ COLUMNS USING *n*-HEXANE AS THE MOBILE PHASE

Mobile phase: *n*-hexane; flow-rate: 1 ml/min; peak separation: $p = f/g$.

Column	Retention time (min)		p
	Syndiotactic	Isotactic	
Cyano	6.2˙	6.6	0.29
Amino	7.2	7.5	0.43
Nitro	11.0	12.0	0.93
TENF	10.6	11.3	0.69

by TENF, amino, and cyano. Plain silica separated only the oligomers. Note the similarity to Fig. 1 where the resolution of the stereoisomer peaks got worse as the polar group attached to methyl on the mobile phase changed from nitro to cyano to hydroxyl. These sets of results indicate that C_{18} on the packing material or n-hexane as the solvent was not the dominant factor in the stereoisomer separation. Instead, the polar functional groups (–OH, –CN and –NO$_2$), either on the packings or in the mobile phases, were the dominant factor for the isomer separation.

Elution order

The elution order of polystyrene stereoisomers from a C_{18} column using ace-tonitrile as the mobile phase has been established by Ray *et al.*[15]; the isotactic isomer of trimer eluted before the syndiotactic isomer. In our previous study[16], that same elution order was observed on the C_{18} column even though the mobile phase was changed from acetonitrile to methanol or nitromethane. However, we shall show that the inverted elution order of trimer isomers was found on cyanopropyl, aminopropyl, nitropropyl and TENF columns using n-hexane as the mobile phase.

The chromatogram for stereoisomer separation of the trimers on the TENF column is shown in Fig. 3. It is obvious that the second peak of trimer isomers was enhanced in 3B when the polystyrene sample was spiked with the isotactic isomer. Spiking with the syndiotactic isomer enhanced the first peak in 3C instead of the second. Hence, the isotactic isomer was retained longer than the syndiotactic isomer. Obviously, the elution order on the TENF column was the reverse of that on the C_{18} column. Table II shows that similar results were obtained for cyano, amino and nitro
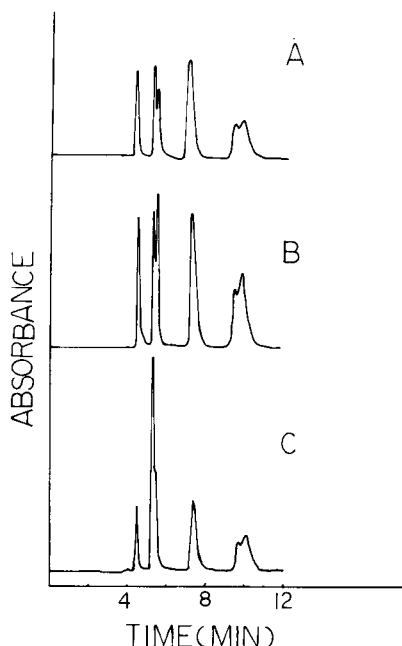

Fig. 3. Separation order on the TENF column. (A), Polystyrene; (B), polystyrene spiked with isotactic trimer; (C), polystyrene spiked with syndiotactic trimer. Mobile phase: n-hexane–chloroform (90:10, v/v).

columns. Furthermore, the same elution order for two plain silica columns connected in a series was reported by Mourey et al.[27].

Polar diluents

Both acetonitrile and chloroform are good polystyrene solvents according to the solubility diagram proposed by Hansen[28] and modified by Bagley et al.[29]. When n-hexane was saturated with acetonitrile and then mixed with pure n-hexane in different ratios for use as the eluent with the TENF column, the chromatograms shown in Fig. 4 were obtained. Note that as one went across the table from cyano to nitro, the p values increased and so did the percentage of acetonitrile-saturated hexane at which a p value could be measured.
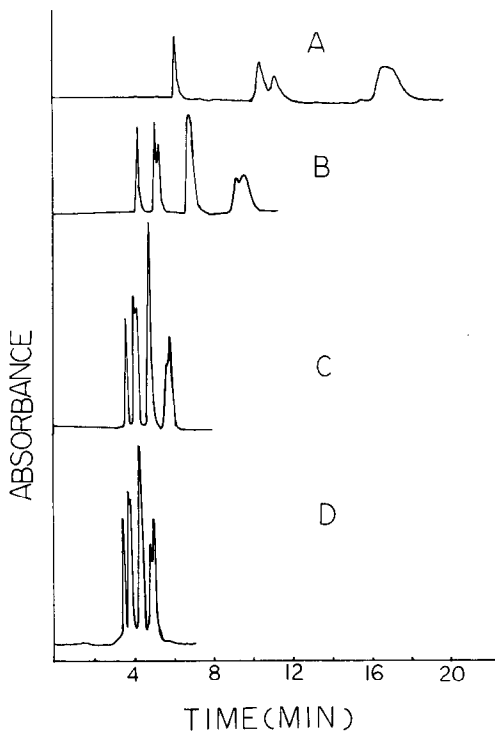

Fig. 4. Effect of mobile phase composition on the separation of polystyrene trimer stereoisomers on the TENF column. Mobile phase, n-hexane–n-hexane saturated with acetonitrile (v/v), (A) 100:0, (B) 90:10, (C) 50:50 and (D) 0:100.

The effects of acetonitrile on the fractionations observed for cyano, amino, TENF and nitro columns are compared in Table II. Clearly, only a very small amount of polar acetonitrile reduced the retentions and peak resolution of the stereoisomers.

The effect of chloroform on the fractionations by cyano and nitro columns are listed in Table III. Again, only a small amount of the polar species, chloroform, decreased the retention times and peak resolution. Note also that a given amount of chloroform had a greater effect on the nitro data than on the cyano.

TABLE II

EFFECT OF A SMALL AMOUNT OF ACETONITRILE IN THE MOBILE PHASE ON THE FRACTIONATION OF ISOMERIC TRIMERS

Syn. = Syndiotactic; Iso. = isotactic.

Mobile phase: n-hexane–n-hexane saturated with acetonitrile	Cyano column			Amino column			TENF column			Nitro column		
	Retention time (min)		p	Retention time (min)		p	Retention time (min)		p	Retention time (min)		p
	Syn.	Iso.		Syn.	Iso.		Syn.	Iso.		Syn.	Iso.	
0:100	3.2*	3.2*	—	4.0*	4.0*	—	3.9	4.0	0.04	3.8	3.9	0.16
20:80	3.7**	3.8**	—	—	—	—	4.0	4.1	0.05	4.0	4.1	0.19
50:50	3.7**	3.8**	—	4.2*	4.2*	—	4.2	4.3	0.06	4.3	4.4	0.25
80:20	4.7	4.8	0.05	4.7	4.8	0.11	4.6	4.7	0.15	5.0	5.2	0.34
90:10	5.4	5.7	0.25	5.0	5.2	0.18	5.2	5.4	0.30	—	—	—
97:3	—	—	—	5.7	5.9	0.33	6.5	6.9	0.67	—	—	—
100:0	6.2	6.6	0.29	7.2	7.5	0.43	10.6	11.3	0.69	11.0	12.0	0.93

* Oligomer separation only.
** Shoulder on peak.

TABLE III

EFFECT OF CHLOROFORM ON ISOMER FRACTIONATION BY CYANO AND NITRO COL-
UMNS USING *n*-HEXANE

Mobile phase: n-hexane–chloroform	Cyano column			Nitro column		
	Retention time (min)		p	Retention time (min)		p
	Syn.	Iso.		Syn.	Iso.	
95:5	6.2	6.6	0.29	3.5*	3.5*	—
98:2	5.4	5.6	0.05	—	—	—
99:1	5.7	5.9	0.13	4.2*	4.2*	—
100:0	6.2	6.6	0.29	11.0	12.0	0.93

* Oligomer separation only.

Packings having phenyl groups

    PRP-1. A column was packed with rigid particles of polystyrene–divinylben-
zene copolymer. Chromatograms for the column when using *n*-hexane and acetoni-
trile- or water-saturated *n*-hexane as an eluent are shown in Fig. 5. *n*-Hexane satur-
ated with acetonitrile gave a better (but still poor) fractionation of the oligomers
than *n*-hexane or *n*-hexane saturated with water. Using acetonitrile alone as an eluent
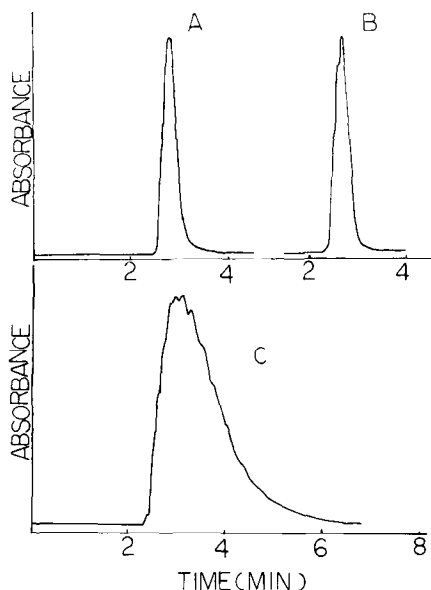for the PRP-1 column, the fractionation of oligomers in a sample containing only

Fig. 5. Effects on the polystyrene fractionation of using a PRP-1 column with eluents of *n*-hexane alone
and after saturation with either water or acetonitrile. (A), *n*-Hexane; (B), *n*-hexane saturated with water;
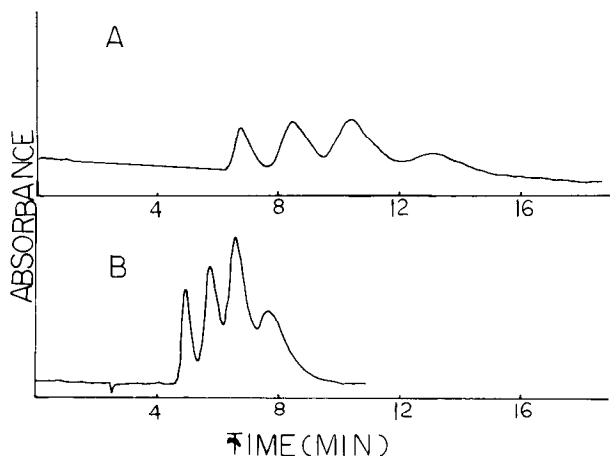(C), *n*-hexane saturated with acetonitrile.

Fig. 6. Effect on the fractionation of oligomers by a PRP-1 column of adding 5% chloroform to aceto-nitrile. (A), 100% acetonitrile; (B) acetonitrile–chloroform (95:5, v/v).

n-mers 2–5 was much improved, as shown in Fig. 6. However, when chloroform was added as a modifier, the retention times and resolutions were decreased. No stereo-isomer separation was obtained.

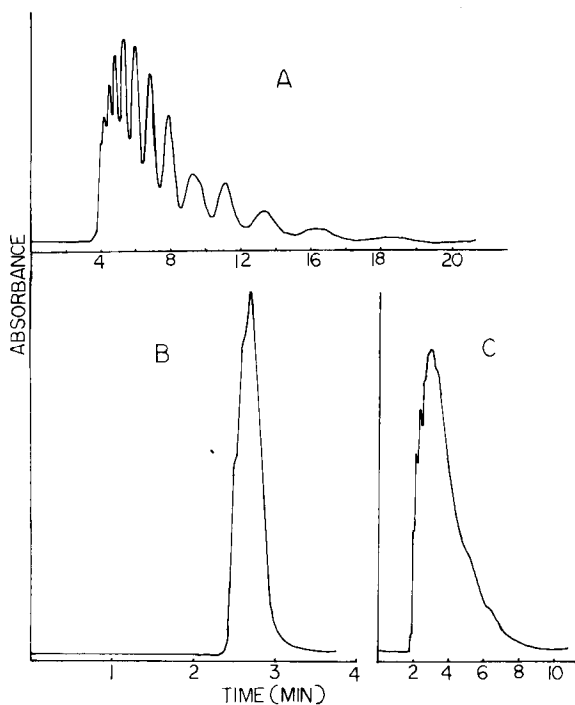*Other phenyl packings.* According to Fig. 7, a conventional phenyl column was

Fig. 7. Fractionation of polystyrene using different phenyl-containing packings and *n*-hexane as the mobile phase. (A), Phenyl column; (B), polystyrene column; (C), pentafluorophenyl column.

far superior to a pentafluorophenyl column and to a PRP-1 column for fractionation of oligomers when using *n*-hexane as the mobile phase. However, there was no hint of isomer separation. Although all of these packings contained phenyl groups, they differed substantially in their behaviors toward polystyrene.

CONCLUSIONS

The results of the present study and those of an earlier one are consistent. In our first study[16], it was shown that the location of a solvent in different parts of the Snyder triangle did not influence the order of elution of the isomers of the trimer from an alkyl-derivatized silica column. The isotactic species were retained less than the syndiotactic, presumably because of a stronger dipole–induced dipole interaction between, for example, the NO_2 group in the solvent and two adjacent phenyl groups in the trimer.

In contrast, the present study reports the opposite elution order when the polar functional groups were on the derivatized silica and the eluent was non-polar. Again, the stereoisomer elution order was independent of the nature of the polar species. Hence, the polar species, whether in the mobile phase or on the surface of the packing, determined the order of the stereoisomers. Presumably, these polar groups (*e.g.*, hydroxyl, cyano, amino, and nitro groups) induced a dipole in the phenyl groups in the polystyrene. For the polystyrene trimers, the isotactic isomer had more interaction because the phenyl groups were on the same side of the polystyrene backbone. As a result, the isotactic isomer was retained longer when the polar function was on the packing but had a greater preference to stay in the polar mobile phase when in contact with an alkyl-derivatized column. Hence, the elution order on the C_{18} column was opposite to that for the polar packings.

The nitro groups, whether in a solvent (*e.g.* nitromethane) or on the surface of an adsorbent (*e.g.* nitropropyl silica), gave better resolution than the cyano groups (*e.g.* acetonitrile as the mobile phase or cyanopropyl silica as the packing). In turn, the CN group provided better resolution than OH. (This assumes that the plate numbers for each of the columns were nearly the same.)

Introduction of a trace of a polar solvent (*e.g.*, water, chloroform or acetonitrile) into the eluent, *n*-hexane, decreased the resolution of the stereoisomer peaks, presumably due to the competition for the polar sites on the column. At the same time, the retention times became shorter.

ACKNOWLEDGEMENTS

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