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SOLVENT SELECTIVITY IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF ISOMERS OF POLYSTYRENE OLIGOMERS

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SUMMARY

Isotactic, syndiotactic and atactic isomers of oligomers of low-molecular-weight polystyrene were successfully resolved using a highly efficient C_{18} column and an acetonitrile–methylene chloride gradient. Structural assignments for some of the collected trimer peaks were made by gas chromatography–mass spectrometry and ^{13}C nuclear magnetic resonance analysis. After the initial separation, 27 different mobile-phase solvents, several reversed-phase bonded packings, and different column temperatures were tested in an effort to maximize the resolution of the stereoisomers. Peak separation (P_i) for each peak pair and chromatographic response function (CRF) for each chromatogram were calculated and compared. Only a few of the solvents tested produced stereoisomer separation. The sample solubility of polystyrene in the mobile-phase solvent appeared to be the best predictor for an optimum mobile phase. Only "weak" solvents, those solvents that could not easily dissolve polystyrene, gave good isomer separation when used as the mobile phase. Hansen's solubility model appears promising for optimizing the mobile-phase composition for separations of other solutes using reversed-phase high-performance liquid chromatography. A phenyl bonded phase, a C_8 bonded phase, and a synthesized perfluorinated bonded phase were compared to octadecylsilane. Only the C_8 column produced isomer separation. Decreasing the column temperature resulted in better resolution of the stereoisomers at the expense of longer retention times. Based on the high-performance liquid chromatographic data, it has been proposed that the mobile-phase affects both the conformations of the polystyrene stereoisomers and of the long-chain hydrocarbon bonded phases. The resolution of stereoisomers by capillary gas chromatography was found to be significantly less than that obtained by high-performance liquid chromatography with the C_8 and C_{18} columns.

INTRODUCTION

Polystyrene has long been used as a model for predicting the properties and

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behavior of other polymers. It is available in the form of well characterized samples having narrow dispersivity. Ready detectability of its oligomers by ultraviolet (UV) and refractive index (RI) measurements is also an advantage.

Many reports on the separation of polystyrene have been concerned with optimizing the fractionation of oligomers[1-6]. Some of these reports discuss "undesirable" peak splitting or misshapen peaks that have also been observed using supercritical fluid chromatography (SFC)[1], as well as normal-phase[2,3] and reversed-phase (RP) high-performance liquid chromatography (HPLC)[4]. This splitting of oligomer peaks has sometimes been ascribed to sampling difficulties and to effects other than adsorption or the separation of stereoisomers. To date, no reported attempt has been made to increase or optimize the resolution of these split peaks in RP-HPLC. In a recent investigation on the separation of stereoisomers of polystyrene by normal-phase HPLC, the separation was explained in terms of differences of repeat-unit rotational barriers and chain coiling between oligomer isomers, but an optimization scheme was not presented[3].

For several years, there has been increasing interest in the development of a general framework for predicting retention and selectivity in RP-HPLC. The most widely used method for predicting selectivity in liquid chromatography was proposed by Snyder and Kirkland[5]. Their solvent selectivity concept classifies the solvents according to relative proton-donating (X_d), proton-accepting (X_e), and dipole moment (X_n) properties. The contributions of each type of interaction are expressed as fractions of the total rather than on an absolute scale[7]. Not all solvents can be grouped in the triangle, *e.g.*, cyclohexane, carbon tetrachloride. Furthermore, this concept for predicting selectivity does not consider the properties of the solutes that are to be separated.

Several good methods for the prediction of solvent strength for HPLC have been developed which are based on experimental solubility data reported by Rohrschneider[21] and Snyder[5]. The solubility parameter concept has been used extensively in normal-phase HPLC; however, in the special case of reversed-phase systems, a more precise solvent strength parameter, S', for several solvents have been experimentally determined and reported[5].

A similar approach has been used to predict the solubility of polymers[8]. In this case the absolute contributions of the molecular interactions rather than their relative values were employed. The cohesive energy, which is related to the solubility parameter, was divided into three types of interaction forces: hydrogen bonding (δh), dispersion (δd) and polarity (δp) forces. Hansen[9] developed a method for the indirect determination of δh, δd and δp for a number of solvents and polymers. A three-dimensional plot of the polymer and solvents of interest, with each force on a separate axis, was then used to predict solubility. As the distance between the plotted polymer and solvent increases, tendency towards dissolution decreases. This method does have the disadvantage that three-dimensional structures are necessary for a graphical representation of the interaction between polymers and solvents; thus, a two-dimensional method is preferred.

Bagley *et al.*[10] concluded that the effects of dispersion and polarity show close similarity, while the effect of hydrogen bonding is of a considerably different nature. Thus, they introduced the parameter $\delta v = \sqrt{(\delta d^2 + \delta p^2)}$. This led to a diagram in which δv and δh were plotted on two axes. At the present time, this δv–δh diagram

appears to be the most efficient way to represent polymer–solvent interactions. From this plot a predicted circle could be drawn for each solute, *e.g.* a given polymer. Those solvents inside the circle are generally capable of dissolving the polymer while those outside the circle exhibit low or no solubility for the polymer. Since interactions between solute and solvent in the mobile phase are important in governing retention, it seemed worthwhile to examine the ability of this solubility model to predict solvent strength and selectivity in the RP-HPLC separation of isomers of polystyrene oligomers.

Most of the recent work on predicting retention and selectivity in RP-HPLC has focused on the behavior of model compounds containing different functionalities. This large diversity of the substituent groups provides insight into general optimization schemes[5,11]. An interesting alternative is to examine the chromatographic behavior of model compounds that possess very similar structures and properties, such as the separations of stereoisomers of polystyrene oligomers.

The first objective of this work was to examine the effect of different mobile-phase solvents in RP-HPLC on the separation of polystyrene stereoisomers. A second objective was to make structural assignments of some of the stereoisomers which could provide information that would permit an understanding of the basis of the separation. Finally, and most importantly, a theoretical basis for the isomer separations and a scheme for optimum mobile-phase selection for future isomer separations of other polymers and, perhaps, other types of compounds by RP-HPLC was desired.

EXPERIMENTAL

Chemicals

A total of 27 solvents obtained from various sources were used as received unless otherwise noted. Acetic acid, acetone, acetonitrile, carbon tetrachloride, chloroform, cyclohexane, diethyl ether, dimethylformamide, ethyl acetate, ethylene chloride, isopropanol, methanol, methylene chloride, nitromethane, and tetrahydrofuran were either Baker "photrex" or reagent grade (J. T. Baker, Phillipsburg, PA, U.S.A.). Tetrahydrofuran was distilled over 5:1 potassium sodium alloy to remove water and butylated hydroxytoluene (BHT). *n*-Butanol, 1-chlorobutane, 2-cyanoethyl ether, 2-methoxyethanol, nitroethane and propylene carbonate were obtained from Aldrich (Metuchen, NJ, U.S.A.). Technical-grade 2-cyanoethyl ether and nitroethane were distilled to remove impurities. ACS-grade dimethyl sulfoxide and dioxane were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.). Dodecafluoroheptanol and tetrafluoropropanol were obtained from PCR (Gainesville, FL, U.S.A.). Absolute ethanol was purchased from U.S. Industrial Chemicals (New York, NY, U.S.A.). "House" distilled water was passed through a deionizing system and a Corning Mega-Pure 1 L still and then collected in glass bottles. All solvents were thoroughly degassed with helium before use.

Monodisperse 800 molecular weight (MW) polystyrene samples were obtained from Pressure Chemical (Pittsburgh, PA, U.S.A.). Monodisperse 666 MW polystyrene samples were acquired from Arro Labs. (Joliet, IL, U.S.A.). For UV detection, the 800 MW and 666 MW polystyrene were dissolved in methylene chloride–acetonitrile (1:19) at a concentration of 20 mg/ml. For RI detection, the 800 MW polystyrene was dissolved in 1:1 methylene chloride–acetonitrile at a concentration of 200 mg/ml.

For the capillary gas chromatography (GC) separation, the 666 MW polystyrene was dissolved in iso-octane at a concentration of 300 mg/ml. For the GC–mass spectrometric (MS) analysis, the two trimer fractions were dissolved in chloroform at a concentration of 300 mg/ml.

IBM C_{18} and C_8 HPLC columns (IBM Instruments, Yalesville, CT, U.S.A.) (25 cm × 4.5 mm I.D.), having end-capped, spherical, 5-μm particles and 100-Å pore sizes, were used as received. Phenyl bonded-phase column packing having spherical, 7.5-μm particles and 100-Å pore sizes was obtained from Macherey, Nagel & Co. (Düren, G.F.R.). A glass GC column (6 m × 2 mm I.D.) coated with 3% OV-1 on 100–120 mesh Gas Chrom Q (Applied Science, State College, PA, U.S.A.) was used as received for GC–MS analysis. 1H,1H,2H-Perfluoro-1-decene (Columbia Organic Chemicals, Columbia, SC, U.S.A.) dimethyl monochlorosilane (Petrarch Systems, Bristol, PA, U.S.A.) and Licrospher SI 100, 5-μm particles (Batch No. YE605, Charge No. 8554867, E. Merck, Darmstadt, G.F.R.) were used for the preparation of the heptadecafluorodecyl-dimethylsilyl (RPF-10) bonded-phase packing. A fused-silica capillary GC column (15 cm × 0.32 mm I.D.) coated with DB-1 from J. and W. Scientific (Rancho Cordova, CA, U.S.A.) was also used as received.

Apparatus

A DuPont HPLC system consisting of a Model 8800 gradient controller, a Model 870 pump module, an oven compartment for the column, a manually operated Rheodyne Model 7125 injector having a 10-μl injection loop, and a Model 852001-901 UV variable-wavelength spectrophotometer was used to generate solvent gradients. An RI detector from Varian Associates (Palo Alto, CA, U.S.A.) together with an Altex Model 110A solvent metering pump (Altex, Berkeley, CA, U.S.A.) and an air-actuated six-port valve, Model ACV-6UHPa (Valco, Houston, TX, U.S.A.) having a 25-μl injection loop, were used to perform most of the isocratic separations. Chromatograms were recorded using a Linear Instruments Model 385 dual-pen chart recorded (Linear Instruments, Irvine, CA, U.S.A.) for UV detection and an Omniscribe Recorder (Houston Instruments, Houston, TX, U.S.A.) for RI detection.

The two trimer fractions were collected using a Model 270 fraction collector (ISCO, Lincoln, NE, U.S.A.). A Finnigan 4000 quadrupole GC–MS system (Finnigan, Sunnyvale, CA, U.S.A.) and a Nicolet ^{13}C nuclear magnetic resonance (NMR) system (Nicolet Instrument Group, Madison, WI, U.S.A.) were used to characterize the trimer fractions. A Hewlett-Packard 5880 GC with an on-column capillary injector was used to obtain the results for comparison with those obtained by HPLC.

Procedures

The RPF-10 bonded-phase packing was synthesized according to the procedure reported by Berendsen *et al.*[12,13]. The phenyl and heptadecafluorodecyl columns were constructed from precision-bore 316 stainless-steel tubing (25 cm × 4.5 mm I.D.; Alltech, Arlington Heights, IL, U.S.A.) that had been rinsed successively with 6 *M* nitric acid, distilled water, methanol and tetrahydrofuran. The tubing was then blown dry with nitrogen. The column was terminated using Swagelock® stainless-steel fittings (Crawford Fitting, Solon, OH, U.S.A.) and 0.5-μm frits from Alltech. The column was then packed 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 equipped with a solvent programmer. The packing solvent for the phenyl and heptadecafluorodecyl bonded phases were chloroform–cyclohexane (2:1) and 100% carbon tetrachloride, respectively. After the packing pressure had been reached, the slurry was forced into the column until it was filled.

Before each successive run, all columns were prepared for sample injection by passing 20 column volumes of tetrahydrofuran or methylene chloride through before returning to the desired mobile-phase composition for the next injection. Fewer column volumes, *ca.* 10, of tetrahydrofuran or methylene chloride were later used and found to have no effect on the chromatograms.

Sample injection was performed in the following manner. Once the baseline had stabilized, the sample loop was filled and the sample injected. The mobile phase flow-rate was held at 1.0 ml/min for all separations and confirmed volumetrically by collecting the column effluent for an appropriate length of time. In solvent gradient studies, the gradient was initiated at the time of injection. Two gradients were run on the C_{18} and C_8 columns. The first gradient went from 100% acetonitrile to acetonitrile–methylene chloride (50:50) in 30 min. The second gradient went from acetonitrile–water (80:20) to 100% acetonitrile in 30 min, was held at 100% acetonitrile for 10 min, and was then changed to acetonitrile–methylene chloride (50:50) in 30 min. The gradient performed on the phenyl column started at acetonitrile–water (70:30), was ramped to acetonitrile–water (90:10) in 30 min, and was held at this composition until the end of the run. The mobile-phase composition was held at acetonitrile–water (60:40) for the RPF-10 column.

Mixed-solvent studies using isocratic and gradient profiles were performed on the C_{18} column in an effort to duplicate the Snyder selectivity properties of the gradient that started with acetonitrile and changed to acetonitrile–methylene chloride (50:50) in 30 min. The solvent combinations and proton-donating (X_d), proton-accepting (X_e) and dipole moment (X_n) properties are presented in Table I.

The rest of the separations were performed isocratically. After elution of the polystyrene pentamer, higher oligomers were quickly removed by elution using only strong solvent. For isocratic separations, water-miscible solvents were appropriately diluted in order to maintain the capacity factor, k', of the two trimer peaks between 9 and 10. Since k' is a measure of the solvent strength of the mobile phase, approximately equal k' values will provide relatively constant mobile phase solvent strengths, which is necessary for selectivity comparisons. The extent of dilution was determined in the following way. The percentage of water necessary in the mobile phase was first approximated by using the reported values[5] for the solvent strength parameter, S, then further adjusted, based on the polymer retention data obtained, until the k' values were within the desired range.

Fraction collection of the two trimer peaks was carried out using the C_{18} column and a gradient from acetonitrile–water (80:20) to 100% acetonitrile in 20 min. After an appropriate number of injections (usually at least 50), the solvent was removed from the two collected fractions by a flash evaporator (Buchler, Fort Lee, NJ, U.S.A.) and the fractions were dried *in vacuo* over PCl_5. These fractions were then further purified by repeating the process until a single peak for each of the two trimer fractions was obtained (>99% purity).

Approximately 1 μl of each of these two trimer peaks (*ca.* 300 μg/ml) was injected into a GC–MS system using the following chromatographic conditions. The

TABLE I

ISOCRATIC AND GRADIENT SOLVENT COMBINATIONS AND THEIR SNYDER CONSTANTS

	X_e	X_d	X_n
UV detection —30-min gradient			
Chloroform	0.25	0.41	0.33
Tetrahydrofuran	0.38	0.20	0.42
Ethylene chloride	0.30	0.21	0.49
Initial chloroform–tetrahydrofuran–ethylene chloride (33:33:34)	0.31	0.27	0.41
Final chloroform–tetrahydrofuran–ethylene chloride (10:10:80)	0.30	0.23	0.48
RI detection —in five isocratic steps			
Nitroethane	0.28	0.29	0.43
Tetrahydrofuran	0.38	0.20	0.42
Ethylene chloride	0.30	0.21	0.49
Initial nitroethane–tetrahydrofuran (50:50)	0.33	0.25	0.43
Final nitroethane–tetrahydrofuran–ethylene chloride (25:25:50)	0.31	0.23	0.46
Original system			
Acetonitrile	0.31	0.27	0.42
Methylene chloride	0.29	0.18	0.53
Initial acetonitrile	0.31	0.27	0.42
Final acetonitrile–methylene chloride (50:50)	0.30	0.23	0.48

injector and detector temperatures were maintained at 100°C and 300°C, respectively. The oven temperature was initially held at 100°C for 4 min, then changed to 400°C at 160°C/min. Electron impact (EI) (70 eV) spectra were obtained on each peak. ^{13}C Nuclear magnetic resonance (NMR) spectra were obtained from *ca.* 3 mg of the first trimer peak and 9 mg of the second trimer peak. Using 10^5 scans on each sample, spectral regions of 14–45 ppm and 120–140 ppm were recorded.

The 666 MW polystyrene was separated by capillary GC. A 1-μl aliquot of a 300 μg/ml solution was injected. The column head pressure was maintained at 20 p.s.i.g. (helium), providing a linear flow-rate of 120 cm/sec. The injector and detector temperatures were 50°C and 300°C, respectively. The oven temperature was initially set at 50°C for 4 min, then changed at 8°C/min to a final temperature of 350°C.

Calculations

The capacity factor, k', was calculated according to the following equation:

$$k' = \frac{t_R - t_0}{t_0}$$

where t_R is the retention time of a particular component (in this case the trimer of polystyrene) and t_0 is the value for an unretained peak. Values of t_0 were obtained by injecting a small volume of solvent having a slightly weaker composition than the actual mobile phase. The RI detector was then used to monitor the first peak resulting from this injection (avoiding the total exclusion peak) to determine the t_0 value.

In order to compare the separation quality of the isocratic separations ob-

tained with each solvent, the peak separation of the isomers of each oligomer from trimer through pentamer was calculated for those solvents that gave isomer separations. Peak separation is defined as follows:

$$P_i = \frac{f}{g}$$

where P_i is the peak separation of the ith pair of peaks in a system with k total pairs of interest, f is the depth of the valley below a straight line connecting two adjacent peak maxima and g is the height of the straight line above the baseline of the valley[14,15]. The peak separation numbers were then used to calculate the chromatographic response function (CRF), which allows one to judge conveniently the overall separation quality or chromatographic performance of each set of conditions. In the CRF, overall resolution of all peaks is important:

$$\text{CRF} = \sum_{i=1}^{k} \ln P_i$$

The total number of peak pairs of interest in the isocratic separations was seven; *i.e.*, one for the trimer and three each for the tetramer and pentamer.

RESULTS AND DISCUSSION

Structural assignments

The first gradient, performed with acetonitrile–methylene chloride on a highly efficient octadecylsilane column, produced multiple peaks for each oligomer of the 800 MW polystyrene, as shown in Fig. 1A. The excellent resolution observed for the multiple peaks of polystyrene is the best reported thus far for RP-HPLC. Addition of water to the mobile phase greatly improved the separation but at the expense of longer retention times, as seen by comparison of Fig. 1A and B and by examination of the peak separation factors and CRF values in Table II. Since the second gradient gave almost baseline resolution for the two trimer peaks, these conditions were used for fraction collection.

Structural elucidations of the two presumed trimer peaks were performed by GC–MS and ^{13}C NMR. The GC–EI-MS of the two collected fractions (Fig. 2) produced the same fragmentation pattern and molecular ion (370.3 *m/e*). Therefore, both peaks collected were trimers having the same general structure $ArCH_2CH_2CH(Ar)CH_2CH_2(Ar)(CH_2)_4CH_3$, where $Ar = C_6H_5$ (Fig. 3). The *n*-butyl end-group of each oligomer arose from the polystyrene polymerization process in which *n*-butyllithium was added as an initiator[16,17]. Structures I and II and structures II and IV are enantiomeric pairs which are distinguishable by NMR.

The ^{13}C NMR spectra obtained on the two trimer peaks are presented in Fig. 4A and B, and they show clear differences, suggesting two different enantiomeric pairs. Based on work reported by Breitmaier and Bauer[18] concerning the ^{13}C NMR chemical shifts of aromatic carbons in atactic and isotactic polystyrene, the differences in the region *ca.* 128.2 ppm in the two spectra permit one to identify the first trimer peak as atactic (in this case syndiotactic) isomers (structures III and IV). The second

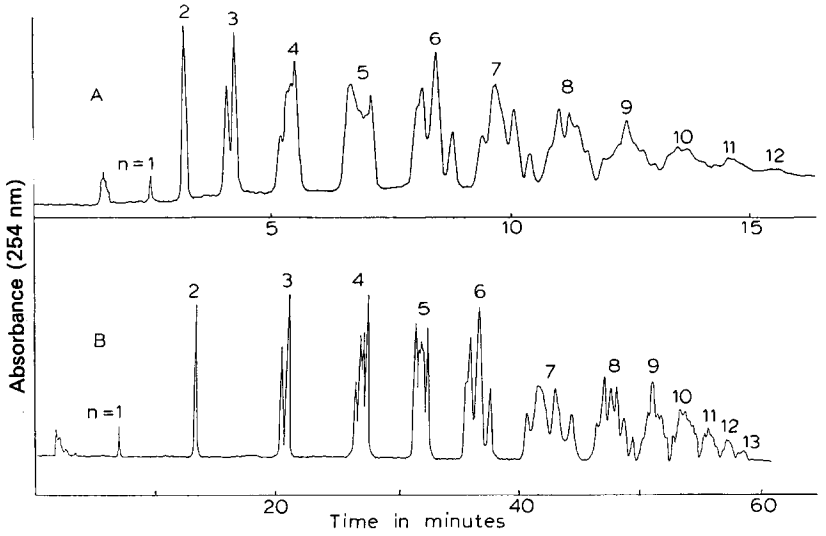


Fig. 1. Chromatograms of 800 MW polystyrene separation on a C_{18} column with gradient (A), acetonitrile to acetonitrile–methylene chloride (50:50) in 30 min, and (B), acetonitrile–water (80:20, v/v) to acetonitrile in 30 min, held at acetonitrile for 10 min, then changed to acetonitrile–methylene chloride (50:50) in 30 min.

trimer peak is isotactic (structures I and II). On the basis of steric factors, the isotactic isomer should interact to a greater extent with the C_{18} stationary phase than the syndiotactic or atactic isomers and thus be retained longer, an expectation consistent with the ^{13}C NMR data obtained.

Solvent studies

A few solvents that did not give isomer separation were selected and mixed together so that the Snyder selectivity properties (proton donating, proton accepting, and dipole moment) were approximately equivalent to those of the initial acetonitrile–methylene chloride gradient (see Table I). The severe limitations on solvent selection were primarily miscibility problems with solvents from different selectivity groups. These mixed-solvent attempts failed to produce any isomer separation in isocratic runs.

TABLE II

CALCULATED PEAK SEPARATIONS (P_i) AND CHROMATOGRAPHIC RESPONSE FUNCTIONS (CRF) FOR GRADIENTS PERFORMED ON A C_{18} COLUMN

Gradient	*Peak pair*							
	Trimer, 1–2	*Tetramer*			*Pentamer*			*CRF*
		3–4	*4–5*	*6–7*	*7–8*	*8–9*	*9–10*	
A	0.88	0.61	0.07	0.16	0.14	0.01	0.21	−13.3
B	0.91	0.67	0.22	0.61	0.27	0.07	0.54	−6.7

* See gradient profiles in Fig. 1.

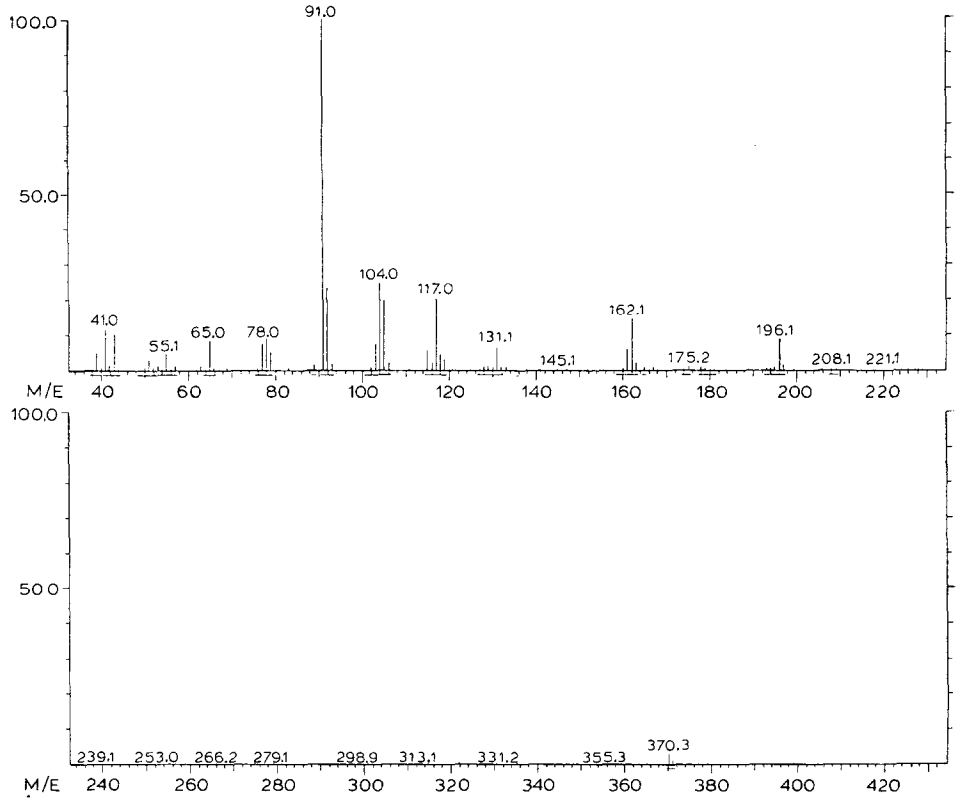
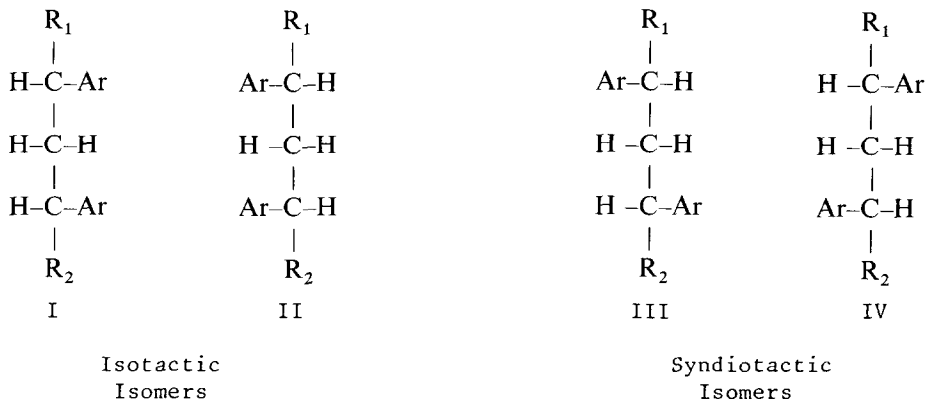


Fig. 2. Electron impact mass spectra of the two trimer peaks using gradient (B) of Fig. 1.



Ar = C_6H_5, R_1 = $ArCH_2CH_2$, R_2 = $(CH_2)_4CH_3$

Fig. 3. Four possible structural configurations for the trimer of polystyrene.

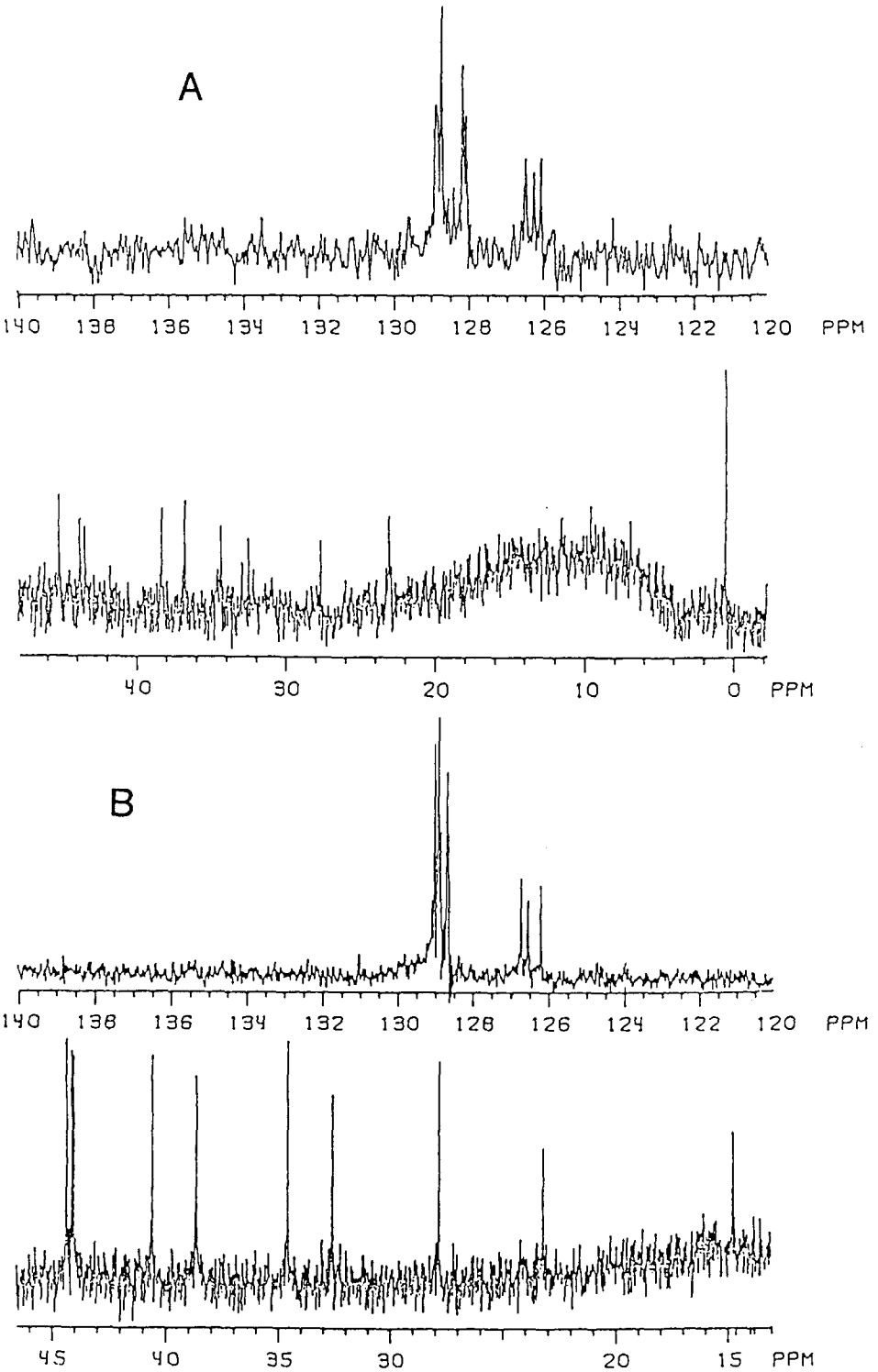


Fig. 4. ^{13}C NMR spectra of the two trimer peaks using gradient (B) of Fig. 1.

TABLE III

CALCULATED PEAK SEPARATIONS (P_i) AND CHROMATOGRAPHIC RESPONSE FUNCTIONS (CRF) FOR SOLVENTS PRODUCING ISOMER SEPARATION ON A C_{18} COLUMN

Solvent composition	*Peak separations (P_i)*							
	Trimer, peak pair	*Tetramer*			*Pentamer*			*CRF*
	1–2	*3–4*	*4–5*	*6–7*	*7–8*	*8–9*	*9–10*	
Propylene carbonate (100)	1.00	0.81	0.16	0.96	0.49	0.28	1.00	−4.05
Nitromethane (100)	0.95	0.82	0.34	0.79	0.72	0.15	0.83	−4.00
Dimethyl sulfoxide–water (90:10)	0.92	0.71	0.34	0.38	0.33	0.19	0.54	−6.63
Acetonitrile–water (80:20)	0.80	0.56	0.34	0.31	0.40	0.15	0.34	−8.94
2-Cyanoethyl ether–water (80:20)	0.70	0.54	0.14	0.25	0.35	0.03	0.42	−9.74
Ethanol–water (87:13)*	–	(	0.26	)	(	0.09	)	$-\infty$
Methanol–water (90:10)*	–	(	0.14	)	(	0.32	)	$-\infty$

* For the methanol and ethanol mobile phases, peak separations were calculated on the basis of a single peak for trimer and only two peaks for tetramer and pentamer.

A number of mobile-phase solvents were evaluated for their ability to separate oligomer stereoisomers. Solvents were selected from each of Snyder's solvent selectivity groups. These separations were performed isocratically using an RI detector. In all, 27 solvents were investigated. Fig. 5 shows the solvents tested in their Snyder selectivity triangle and Table III lists the separation factors and the CRF values for those solvents that produced isomer fractionation. Partial or complete isomer separation was observed for seven solvents. From the CRF values, propylene carbonate and nitromethane appeared to give the best overall isomer separation, followed by dimethyl sulfoxide, acetonitrile, and 2-cyanoethyl ether, respectively. Methanol and ethanol each gave a slight hint of isomer fractionation in the form of a slight shoulder. Examples of chromatograms from solvents that produced complete (propylene carbonate), partial (methanol), and no (tetrahydrofuran) stereoisomer separation are shown in Fig. 6. The shapes of the resolved peaks suggested the same order of the isomers in different solvents.

It is evident from Fig. 5 that the Snyder solvent selectivity scheme, although good for a first approximation, does not accurately predict selectivity for this separation. Solvents from *different* groups gave isomer separations, yet solvents within the same group showed widely different selectivity for the isomers of the oligomers. Thus, one can not determine accurately which of the Snyder selectivity properties (proton accepting, proton donating or dipole moment) is important in the separation.

Sample solubility in a *pure* solvent appeared to be extremely important in achieving isomer separation, and it was a much better predictor for selecting those mobile phases that eventually provided isomer separations. This factor also appeared to be important in the separation of oligomers[2]. Using too strong a pure solvent as the mobile phase, *i.e.*, one that too readily dissolved the polystyrene, *even after adjusting solvent strength with water*, resulted in no isomer resolution. The strengths of solvents that gave good isomer separations were determined to be substantially lower than those reported in the literature[5] (see Table IV). Addition of water to the mobile

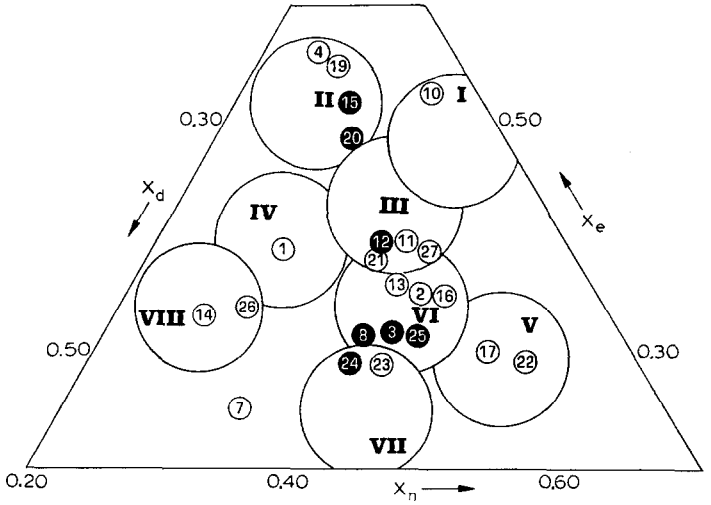


Fig. 5. Snyder selectivity triangle for the solvents tested. Solid numbers correspond to those solvents producing isomer separations. Clear numbers represent those solvents producing no isomer separations. The numbers correspond to: 1 = acetic acid; 2 = acetone; 3 = acetonitrile; 4 = *n*-butanol; 5 = carbon tetrachloride; 6 = 1-chlorobutane; 7 = chloroform; 8 = 2-cyanoethyl ether; 9 = cyclohexane; 10 = diethyl ether; 11 = dimethylformamide; 12 = dimethyl sulfoxide; 13 = dioxane; 14 = dodecafluoroheptanol; 15 = ethanol; 16 = ethyl acetate; 17 = ethylene chloride; 18 = hexane; 19 = isopropanol; 20 = methanol; 21 = 2-methoxyethanol; 22 = methylene chloride; 23 = nitroethane; 24 = nitromethane; 25 = propylene carbonate; 26 = tetrafluoropropanol; 27 = tetrahydrofuran. *Note:* Not all solvents tested could be plotted on this diagram: carbon tetrachloride, 1-chlorobutane, cyclohexane, hexane.

phase, however, did not appear to change the selectivity of the organic solvent, weak or strong, but only increased the retention time. For example in tetrahydrofuran, a 10% increase in the water component resulted in an increase in retention time by a factor of *ca.* 2.

A plot in Fig. 7 of Hansen's hydrogen bonding forces *vs.* combined dispersion

TABLE IV

PERCENTAGE OF WATER EXPECTED AND OBSERVED WITH VARIOUS SOLVENTS FOR EQUIVALENT SOLVENT STRENGTH

Solvent	*Water expected (%)**	*Water necessary (%)***	*Reported S' value used*
Acetonitrile	–	20	3.1
Acetone	22	63	3.4
Dioxane	23	65	3.5
Ethanol	23	13	3.6
Isopropanol	27	23	4.2
Methanol	19	10	3.0
Tetrahydrofuran	28	68	4.4

* Percentage of water expected for an equivalent solvent strength of an acetonitrile–water (80:20) mixture using reported *S'* values[5].

** Water necessary in the mobile phase in order to make the capacity factor of the trimer of polystyrene equivalent in each chromatogram.

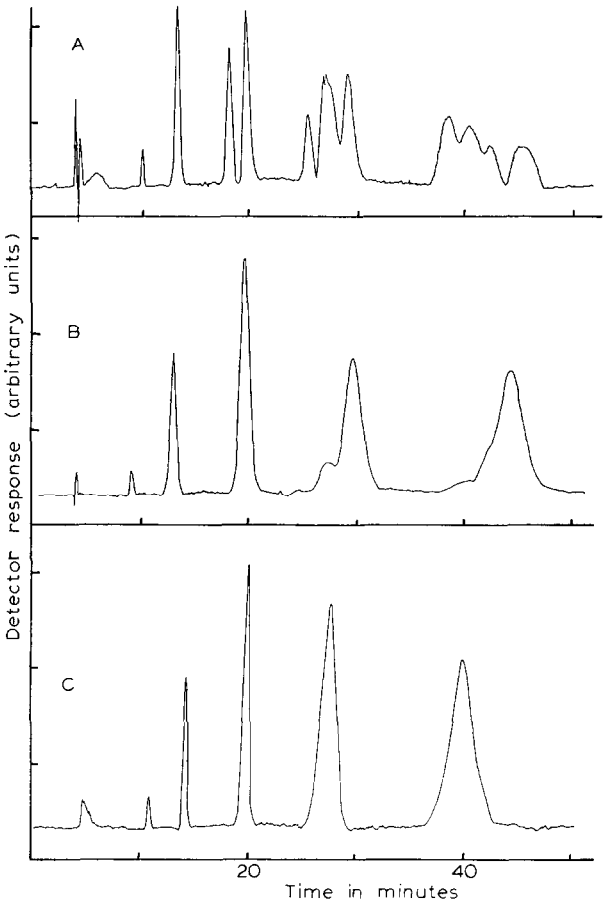


Fig. 6. Chromatograms of 800 MW polystyrene under isocratic conditions showing typical separations by solvents producing (A) good isomer resolution (propylene carbonate), partial isomer resolution (methanol–water, 90:10) and no isomer resolution (tetrahydrofuran–water, 60:40).

forces and polarity forces[9] was a much better model for predicting solvent strength and selectivity for this particular separation. All of the solvents tested that lay inside the solubility circle gave no diastereomer separation while all of the tested solvents that produced at least some hint of isomer separation lay outside the circle. Furthermore, solvents in a particular area of the graph or those with high dispersion and polarity contributions as well as medium hydrogen-bonding contributions gave excellent diastereomer separations. Dimethylformamide was the exception only because this model was not able to predict solubility or solvent strength in all cases. In other words, polystyrene was observed to be very soluble in dimethylformamide and, therefore, it was not expected to give diastereomer separation. This is still consistent with the idea that the diastereomer separation is controlled by the forces governing the solubility of polystyrene in the pure solvent, particularly dispersion and polarity forces.

Stationary-phase effects were not thoroughly tested, but results on the C_8,

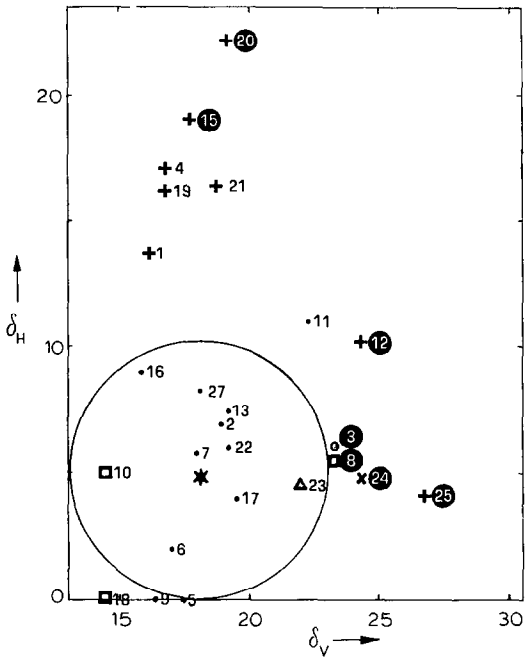


Fig. 7. Solubility of polystyrene in solvents tested using a δv ($\delta p^2 + \delta d^2$ Hansen's dispersion and polarity forces) *vs.* δh (Hansen's hydrogen bonding forces) diagram. The symbol, ⋆, corresponds to polystyrene. The other symbols correspond to whether polystyrene is soluble (·), almost soluble (△), strongly swollen (□), slightly swollen (×), or has no effect (+) in the various solvents tested. See the caption of Fig. 5 to identify the solvents.

phenyl, and RPF-10 columns were used for comparison with those on the C_{18} column. The C_8 column gave very similar diastereomer separations (Fig. 8A). However, the chromatograms obtained from the gradient performed on the phenyl and RPF-10 columns (Fig. 8B and C) showed no diastereoisomer separation and much shorter retention times compared with the C_{18} column. The shorter retention times would be expected for the phenyl column because the surface area was smaller and also because, as a result of steric effects, the oligomers might not interact as well with the isopropylphenyl groups bonded on the silica surface.

The data do suggest that the C_{18} and C_8 stationary phases have significantly better interactions than the other stationary phases with the polystyrene, particularly in certain solvents. Reasons for this are not clear; however, some indirect evidence[19,20] indicates that C_{18}, C_8 and phenyl bonded phases may have a "matted" or associated form in certain mobile phases and a "brush" or "bristle" form in others. Perhaps the C_{18} is in a matted form with the solvents that gave isomer separation, thereby contributing to the increasing selectivity. The phenyl column may have also been in a matted form with these solvents, but it probably could not interact as well with the polystyrene due to steric effects or the smaller molecular volume of the isopropylphenyl-bonded phase. The fluorinated stationary phase may have been ineffective because it was not in a matted or coiled configuration with the solvents tested or because it did not interact as strongly with the polystyrene. Additional investiga-

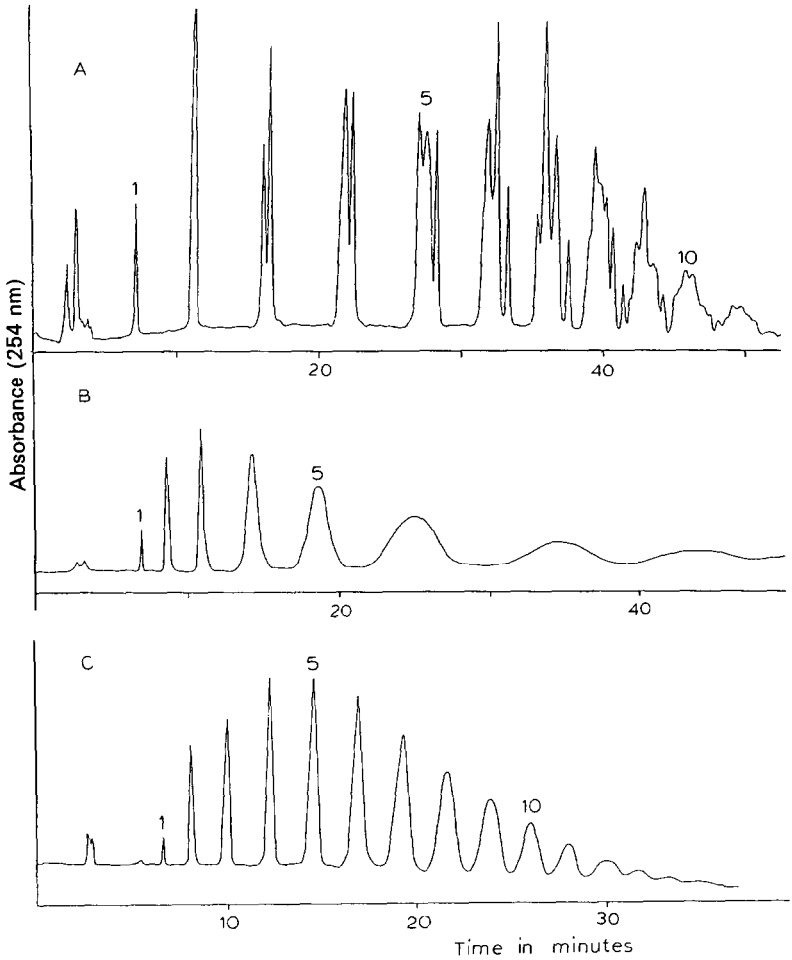


Fig. 8. Chromatogram of the 800 MW polystyrene separation on (A) a C_8 column using gradient (B) in Fig. 1, (B) a fluorinated bonded phase (RPF-10) column holding the mobile-phase composition at acetonitrile–water (60:40), and (C) a phenyl bonded-phase column using a gradient starting at acetonitrile–water (70:30), ramped to acetonitrile–water (90:10) in 30 min and held at this composition until the end of the run.

tions would be necessary to arrive at a better understanding of the results, but it is proposed that the solvents affect both the conformations of the polystyrene stereoisomers, resulting in differences in molecular volumes for each stereoisomer, and of the C_{18} chains on the stationary phase. Furthermore, the bulky phenyl groups on the syndiotactic and atactic stereoisomers may affect the conformation of the long-chain hydrocarbon stationary phase. A proposed mechanism consistent with the results is that the hydrocarbon chain on the stationary phase lines up in a coiled fashion with the polystyrene chain when using "weak" solvents, *e.g.*, acetonitrile, nitromethane, etc., and as many bulky phenyl rings on the polystyrene as possible are pointing away from the C_8 or C_{18} as shown in Fig. 9. Fraction collection and structural identification of the stereoisomers of the tetramer and pentamer could be used

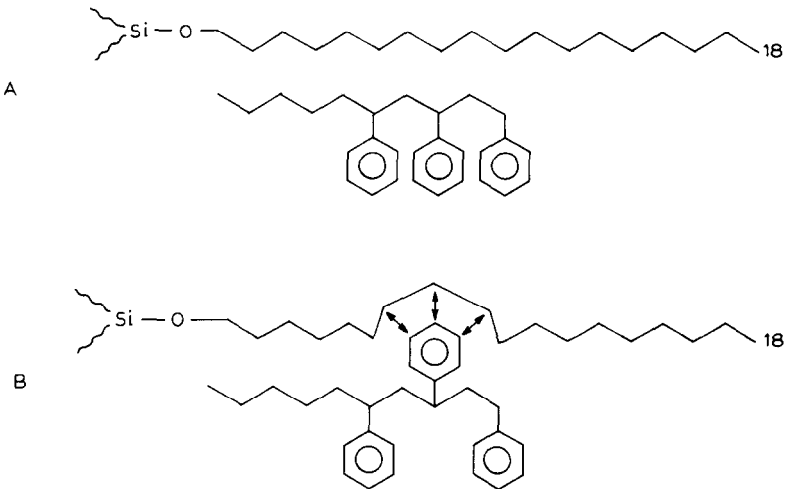


Fig. 9. Proposed mechanism for separations using the C_{18} column: (A) represents the isotactic trimer interaction and (B) represents the syndiotactic trimer interaction with the C_{18} chain and of the two pairs of the trimer diastereomers of polystyrene.

to test this idea; however, obtaining the necessary amounts of the *pure* species for ^{13}C NMR analysis would be rather difficult owing to the poor resolution between certain stereoisomers and the small amounts of material collected from each injection.

Temperature effects

Temperature effects on the resolutions of the isomers were tested using the C_{18} column and 100% acetonitrile (isocratic conditions). Separations carried out at *ca.* 4°C (by cooling with ice), 25°C, and 40°C indicated that increasing the temperature resulted in decreasing the retention times at the expense of decreasing the isomer resolution. However, when the k values were made nearly equal by addition of water to the mobile phase, the resolution was approximately the same at all three temperatures. Water added to the 4°C, 25°C and 40°C experiments was 0, 10 and 15%, respectively.

Gas chromatographic separations

The capillary GC work presented in Fig. 10 was performed for comparison with the RP-HPLC separations. Under the reported conditions, elution of oligomers through $n = 9$ was possible. There are clearly two trimer and two tetramer peaks. In general, resolution for GC was considerably less than in LC where at least four tetramer peaks were noted. Although difficult to discern, the higher oligomers also showed multiple peaks in the GC chromatogram, but they were not nearly as well resolved as in the HPLC separations.

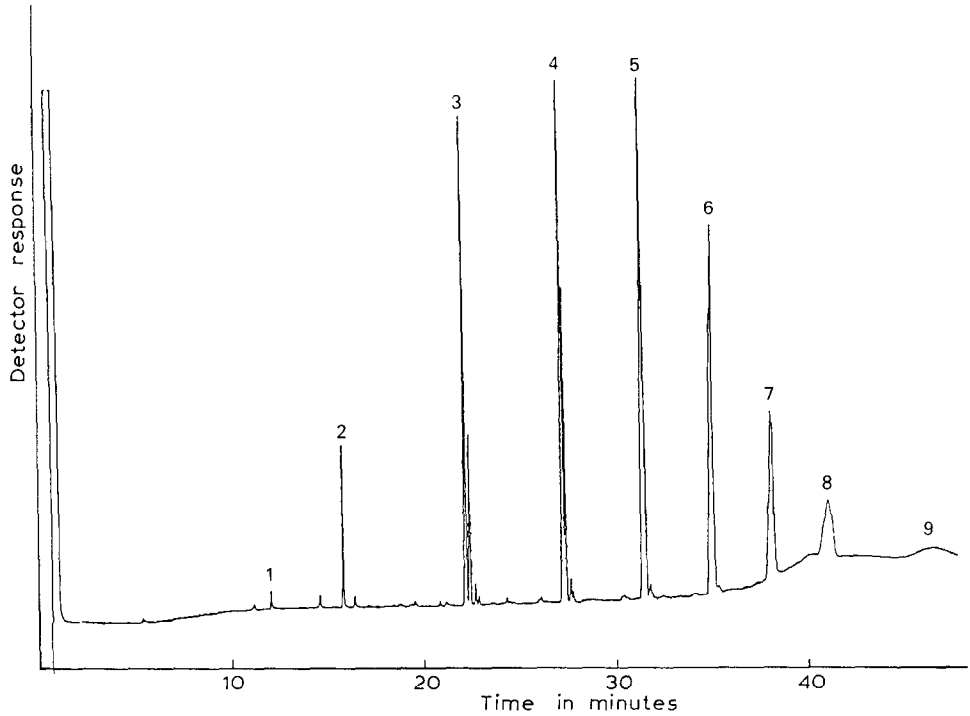


Fig. 10. Capillary GC chromatogram of 666 MW polystyrene separation using a DB-1 column. Column head pressure of $1.4 \cdot 10^5$ Pa (20 p.s.i.) provided a linear flow-rate of 120 cm/sec. Oven temperature was set at 50°C for 4 min, then ramped at 8°C/min to a final temperature of 350°C.

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