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STATIONARY PHASE EFFECTS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC FRACTIONATIONS OF STEREOISOMERS OF STYRENE OLIGOMERS

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SUMMARY

The effects of various alkyl, phenyl and fluorinated bonded phases on the selectivity towards stereoisomers of polystyrene oligomers using acetonitrile–water gradients in reversed-phase high-performance liquid chromatography were compared. All alkyl phases tested, from a trimethylsilyl (C_1) to an octadecylsilyl (C_{18}) bonded phase, separated stereoisomers. An increased chain length of the alkyl ligand made only a minor contribution to the resolution. Bonded phases of ethylphenyl and isopropylphenyl, capped with trimethylsilane or non-capped, produced only oligomer separation. Similarly, a synthesized fluorodecyl bonded phase (RPF-10) only gave oligomer separation; however, the corresponding capped fluorinated phase provided some stereoisomer separation. In contrast, commercially available capped and non-capped fluorodecyl phases provided even less isomer selectivity.

INTRODUCTION

There has been considerable progress in understanding the various physicochemical phenomena responsible for retention in liquid chromatography[1]. Much of this attention has focused on solvent and mobile phase phenomena[2], whereas stationary phase effects, which have also been recognized as a contributing factor in selectivity and retention, have been largely ignored. However, the effects of the stationary phase are known to play an important role in the retention of certain compounds, so their contributions should be given more attention[3].

Organic functional groups, such as C_{18} bound to a silica surface, are used as stationary phases to provide adsorption characteristics similar to the liquid extraction behavior exhibited by *n*-octane. Underivatized silanols on the surface modify this

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TABLE I

PROPERTIES OF COLUMNS USED

Column	*Packing*	*Source*	*Particle size (μm)*	*Pore size (μm)*	*TMS capped*	*Packing solvent*	*Plates per column*
A	C_{18} (octadecyl	IBM	5	100	Yes	***	15,300
B	C_{18} (octadecyl)	Macherey, Nagel & Co.	10	100	Yes	CH_3OH	7200
C	C_8 (octyl)	IBM	5	100	Yes	***	14,100
D	C_6 (hexyl)	Phase Separations	5	100	Yes	CH_2Br_2–CH_3OH (1:2)	8400
E	C_1 (trimethyl)	Synthesized*	5	100	**	CH_2Br_2–CH_3OH (1:2)	6700
F	Ethylphenyl	Synthesized*	10	100	No	CH_3OH	5200
G	Ethylphenyl	Synthesized*	10	100	Yes	CH_3OH	5500
H	Isopropylphenyl	Macherey, Nagel & Co.	7.5	100	No	CH_3OH	6000
I	Isopropylphenyl	Macherey, Nagel & Co.	7.5	100	Yes	CH_3OH	6200
J	RPF-10 (fluorodecyl)	Synthesized*	10	100	No	CCl_4	3500
K	RPF-10 (fluorodecyl)	Synthesized*	10	100	Yes	CCl_4	5200
L	RPF-10 (fluorodecyl)	ES Industries	5	60	No	CCl_4	5800
M	RPF-10 (fluorodecyl)	ES Industries	5	60	Yes	CCl_4	5900

* Synthesized in our laboratory using LiChrospher Si 100 particles (E. Merck).

** By definition.

*** Packed by a commercial source.

behavior to some extent. In addition, the pore size of the packing material has a profound influence on retention in liquid chromatography. The overall effect of the stationary phase on retention, however, remains controversial owing to insufficient knowledge of (a) the exact nature of the sorptive surface, (b) the extent and effect of the adsorbed solvent layer on the stationary phase and (c) the orientation of the bonded-phase functional groups.

This investigation extends earlier studies in our laboratory[4] concerning reversed-phase effects on chromatographic fractionations of stereoisomers of styrene oligomers. Several different reversed-phase packings, including alkyl, phenyl and fluorinated bonded phases, have been examined. The effects of decreasing the alkyl chain length and capping the phases on the separation of stereoisomers of polystyrene oligomers have also been investigated.

EXPERIMENTAL

Chemicals

All solvents obtained from various commercial sources were used as received. Fisher Scientific (Rochester, NY, U.S.A.) high-performance liquid chromatography (HPLC) regent grade dimbromomethane and Baker (Phillipsburg, PA, U.S.A.) Photrex grade methylene chloride (DCM), methanol and carbon tetrachloride were used as mobile phases and/or as slurry-packing solvents. Laboratory-distilled water was passed through a deionizing system and a Corning Mega-Pure 1L still (Corning Glass Works, Corning, NY, U.S.A.) and collected in glass bottles. The solvents employed as mobile phases were degassed by purging with helium.

Toluene (Baker, reagent grade) was dissolved in methanol–water (70:30) at a concentration of 5 mg/ml for determining the number of theoretical plates. The oligomers and isomers of some of those oligomers in monodisperse 800 MW polystyrene standard (Pressure Chemical, Pittsburgh, PA, U.S.A.) were fractionated after being dissolved in methylene chloride–acetonitrile (1:19) at a concentration of 20 mg/ml.

Column packings

Octadecyl (C_{18}), octyl (C_8), hexyl (C_6), isopropylphenyl and fluorodecyl (RPF-10) packing materials were obtained from IBM Instruments (Danbury, CT, U.S.A.), Phase Separations (Hauppage, NY, U.S.A.), Macherey, Nagel & Co. (Duren, F.R.G.) and ES Industries (Marlton, NJ, U.S.A.), respectively. C_1 packing was synthesized by reaction of trimethylsilane with LiChrospher Si 100 particles (E. Merck, Darmstadt, F.R.G.). Ethylphenyl packing material was synthesized by reaction of ethyldimethylsilane (Petrarch Systems, Levbittown, PA, U.S.A.) with LiChrospher Si 100. A fluorodecyl (RPF-10) bonded phase was also synthesized in using 1H, 1H, 2H-perfluoro-1-decene (Columbia Organic Chemicals, Columbia, SC, U.S.A.), dimethylmonochlorosilane (Petrarch Systems) and LiChrospher Si 100. The pertinent physical data for the columns are presented in Table I.

Apparatus

Chromatograms were generated using 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 with a 10-μl

injection loop and a Model 852001-901 variable-wavelength UV spectrophotometer. Chromatograms were recorded with a Linear Instruments (Irvine, CA, U.S.A.) Model 385 dual-pen chart recorder.

Procedures

The ethylphenyl bonded phase and one of the fluorodecyl bonded phases were synthesized according to the procedures reported by Berendsen and co-workers[5–7]. Some of the commercial packing materials, as well as those we synthesized, were capped with trimethylchlorosilane (TMS) according to the procedure reported by Berendsen and De Galan[7]. The fines in the packings were removed by repetitive sedimentation in methanol. Carbon and hydrogen determinations were performed by Atlantic Microlab (Atlanta, GA, U.S.A.); fluorine determinations were provided by Galbraith Labs. (Knoxville, TN, U.S.A.).

All columns were constructed from precision-bore 316 stainless-steel tubing (250 × 4.6 mm I.D.) (Alltech, Arlington Heights, IL, U.S.A.) using a previously reported slurry procedure[4]. The columns were packed at a pressure of 70–80 MPa (approximately 10,000–11,500 p.s.i.) using a Model 10-600-50 pneumatic amplifier pump (SC Hydraulic Engineering, Los Angeles, CA, U.S.A.) and a high-pressure slurry packer designed and constructed by machinists at the University of Georgia. Filtered and degassed methanol was used as a purging solvent.

Sample injections of 10 μl were made once the baseline had stabilized. The mobile phase flow-rate was held at 1.0 ml/min for all gradient separations and at 0.8 ml/min for determinations of column efficiency.

Calculations

Column efficiencies were reported as the number of theoretical plates per column, $N = 5.54\ (t_R/W_{1/2})^2$, where t_R is the retention time of a given solute and $W_{1/2}$ is the peak widht at half-height. Toluene was employed as the solute for these measurements.

RESULTS

Alkyl bonded phases

These columns provided excellent stereoisomer separations, as shown by the chromatograms in Fig. 1. The number of theoretical plates and the carbon and hydrogen content (Tables I and II) increased as the chain length of the hydrocarbon ligand increased. As expected, the efficiency of the 10-μm C_{18} column was significantly less than that of the 5-μm C_{18} column. By comparing the stereoisomer separations of these phases, it can be seen that as the chain length decreased, the stereoisomer resolution decreased slightly. Thus, additional carbons in the chain on the stationary phase made only a very small contribution to stereoisomer selectivity.

Phenyl bonded phases

These phases, with and without capping, did not provide any diastereomer selectivity. Only oligomer separation, as shown in Fig. 2, was observed. The number of theoretical plates was approximately the same for all phenyl phases (slightly higher for the 7.5-μm material) and was similar to the efficiency obtained with 10-μm C_{18}.

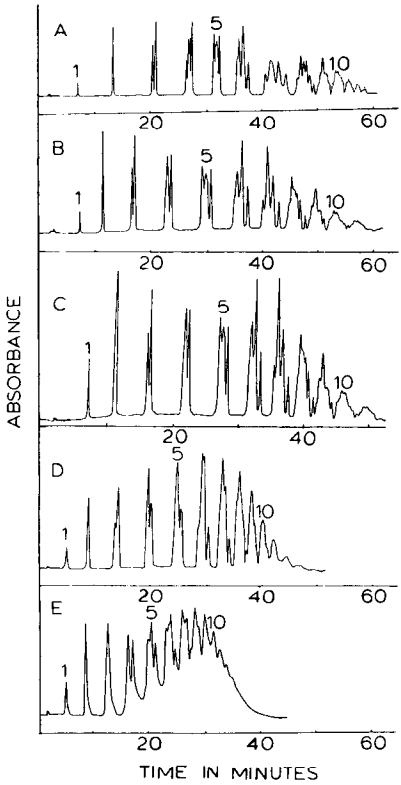

Fig. 1. Separations of 800 MW polystyrene on alkyl phases. Gradient: acetonitrile–water (80:20) to acetonitrile in 30 min, held at acetonitrile for 10 min, then changed to acetonitrile–dichloromethane (50:50) in 30 min. (A) C_{18} (5 μm); (B) C_{18} (10 μm); (C) C_8; (D)n C_6; (E) C_1.

However, significantly shorter retention times were observed compared with the 10-μm C_{18} column.

Fluorinated bonded phases

Elemental analyses (Table III) indicated that synthesized, non-capped RPF-10 had approximately one third the coverage (8.38% F) of the commercial non-capped RPF-10 (21.11% F). The number of theoretical plates per column (Table I) was

TABLE II

ELEMENTAL ANALYSES OF THE ALKYL BONDED PHASES

Column	*Carbon (%)*	*Hydrogen (%)*
A	16.92	3.01
B	17.38	3.33
C	9.93	2.11
D	8.35	1.82
E	3.49	0.99

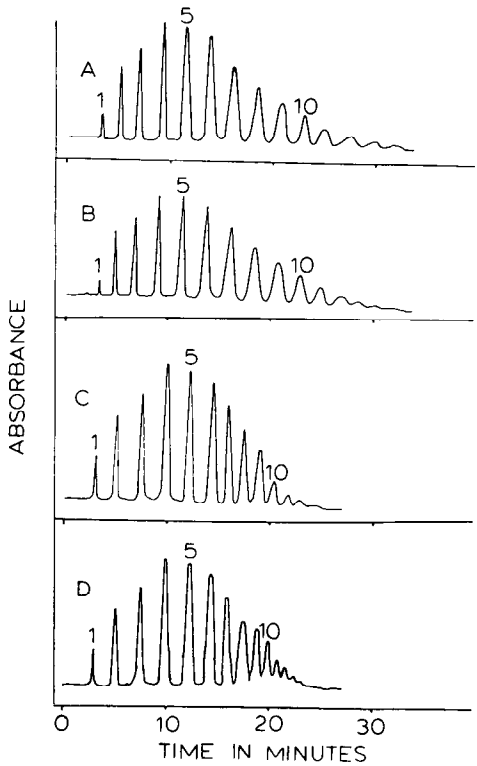

Fig. 2. Separations of 800 MW polystyrene on phenyl phases. Gradient: acetonitrile–water (80:20) to acetonitrile in 30 min. (A) Ethylphenyl; (B) capped ethylphenyl; (C) isopropylphenyl; (D) capped isopropylphenyl.

similar for the synthesized, capped RPF-10 column and the two commercial RPF-10 columns. A lower plate number was obtained with the synthesized uncapped column. The chromatograms in Fig. 3 show that only oligomer separation was achieved with non-capped, synthesized RPF-10 while the commercial RPF-10, with and without capping, provided some additional stereoisomer selectivity. No significant difference in resolution was observed when the commercial RPF-10 was end-capped. The best isomer separation was obtained when the synthesized RPF-10 packing was later capped. This suggested that the hydrogenated alkyl groups were more effective than the fluorinated groups in producing stereoisomer separations. It also indicated that silanol groups on the surface did not enhance isomer fractionation.

TABLE III

ELEMENTAL ANALYSES OF THE FLUORINATED BONDED PHASES

Column	*Carbon (%)*	*Hydrogen (%)*	*Fluorine (%)*
J	3.74	1.00	8.38
K	6.03	1.40	7.69
L	9.45	0.07	21.21
M	10.12	0.18	19.96

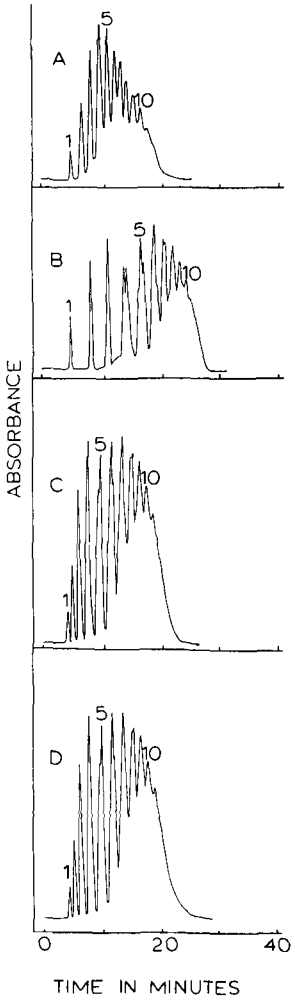

Fig. 3. Separations of 800 MW polystyrene on fluorinated alkyl phases. Gradient: acetonitrile–water (70:30) to acetonitrile in 30 min. (A) Synthesized RPF-10; (B) capped, synthesized RPF-10; (C) commercial RPF-10; (D) capped, commercial RPF-10.

DISCUSSION

All the alkyl bonded phases tested provided stereoisomer separation. Chromatographic resolution factors, which provide an indication of stereoisomer resolution, did not differ significantly as the alkyl chain length was varied[8,9]. It has been postulated that the enhanced selectivity of long-chain hydrocarbon phases, compared with other reversed-phases, was due to their ability to adapt to a "matted" or associated form in certain mobile phases[10]. However, it is unlikely that the small C_1 (TMS) phase can achieve an associated form because of its smaller molar volume. It is possible, however, that the matted form of the long-chain hydrocarbon ligands has surface characteristics similar to those of the TMS phase. We earlier reported

that only certain types of solvents, *e.g.*, acetonitrile, propylene carbonate and nitromethane, provided isomer separation with C_{18} and C_8 phases[4]. Since that time, we have also found that the TMS phase only gave isomer resolution with those same solvents, which suggests that the diastereomers undergo conformational changes in certain mobile phases and these changes enhance the separation. This would be consistent with the generally accepted theory that retention is governed primarily by the "solvophobic effect", a mobile phase phenomenon[11]. In addition, the presence of an adsorbed layer of the mobile phase on the hydrocarbonaceous surface may shield the surface silanols and siloxane groups. Thus, specific solvation effects in that adsorbed layer may be controlling the selectivity to a large extent, particularly with the TMS phase.

The capped phenyl phases provided only oligomer separation and gave very similar chromatograms to the corresponding non-capped phenyl phases. Apparently, the presence of residual silanols on the silica surface is not a major factor in the separation mechanism, unless the phenyl ligands prevent efficient solvation of the remaining silanols. Inefficient solvation of the silanols, the slightly more polar character of phenyl phases and the lower surface area may all contribute to the significantly decreased retention times of the styrene oligomers compared with those obtained with the alkyl bonded phases. As a C_6 and even a C_1 phase produced stereoisomer selectivity, the chain length or molecular volume of the phenyl phases must not be an important factor affecting isomer resolution. However, the bulkiness or steric hindrance of the phenyl groups, even in a "matted" form, may not allow the stereoisomers to assume the same conformations as with the alkyl ligands. Also, the slightly more polar character of the phenyl phases may not allow the diastereomers to coil up as the less polar alkyl phases.

The capped, synthesized RPF-10 provided isomer separation whereas the non-capped version did not. This result can apparently be attributed to a high loading of the TMS, which by itself provided good selectivity. This high loading resulted because of the poor fluorinated coverage obtained on the synthesized RPF-10. No significant difference was obtained between the highly covered commercial RPF-10 columns, whether capped or non-capped. The less polar nature of the fluorinated phases, compared with the corresponding alkyl phases, was expected to provide improved selectivity for diastereomers of styrene oligomers. The reasons for the drastically decreased stereoisomer selectivity with fluorinated phases are unclear. One possibility may be that the highly non-polar fluorinated phase is not sufficiently solvated by the mobile phase. This could be tested by breakthrough studies of the type reported by Berendsen *et al.*[12]. Alternatively, the fluorinated phase may behave as a true "brush", being sterically fixed in any mobile phase because of the larger C–F covalent radii. In that event, it would not provide as many contact sites for adsorption, particularly for large molecules such as the styrene oligomers. This has been suggested by Haas *et al.*[13], who also stated that the fluorinated chain, although larger in diameter than a normal hydrocarbon chain, is not able to adapt to the solute molecule by "clustering". Thus, as the contact area decreases owing to the rigidity of the fluorinated chain, the capacity factor (k') becomes smaller for the same mobile phase composition.

This study also addressed two other questions. First, because the C_{18} column in Fig. 1A had 15,300 plates, there was a distinct possibility that the poorer resolu-

tions obtained using the phenyl and fluorodecyl phases were due to their lower plate counts, of less than 6000 each. For that reason, a C_{18} column (Fig. 1B) having 7200 plates and a C_1 column having 6700 plates (Fig. 1E) were examined and found to produce much better stereoisomer resolutions than the phenyl and fluordecyl phases. Second, there is the question of the importance of the differences in the plate counts for columns having the same type of stationary phase. Comparison of Fig. 1A and B shows that the stereoisomers were resolved fairly well by both C_{18} columns, one having 15,300 and the other 7200 plates. In fact, some, but not all, of the stereoisomers appeared to have been separated better by the column having the lower plate count! As a result, we believe that the evidence suggests that stereoisomer resolution was more dependent on the *type* of stationary phase than the plate count.

In conclusion, it is apparent that minor changes in the characteristics of the stationary phase result in large selectivity differences between the stereoisomers of styrene oligomers. Further investigations into the structure of the solute on the solvated stationary phase, perhaps using solid-state NMR spectroscopy[14], will be necessary in order to obtain a better understanding of the complexity of the chromatographic process and to arrive at more definitive conclusions about the possible interactions.

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