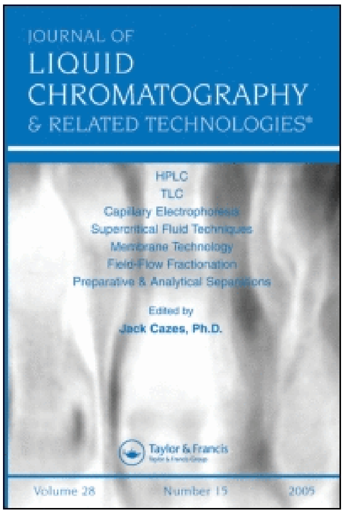

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CARBON CLAD ZIRCONIA AND SEPARATIONS OF THE DIASTEREOISOMERS OF LOW MOLECULAR WEIGHT POLYSTYRENES

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ABSTRACT

The general aim of this work was to illustrate the exceptional resolving power of carbon clad zirconia surfaces for the separation of diastereoisomers of low molecular weight polystyrenes. Separations are reported in which four diastereoisomers of *n*-butyl polystyrene with four configurational repeating units were separated in less than two minutes. Some variability between lot numbers of carbon clad zirconia was observed, which should be noted by chromatographers. Perhaps the most critical aspect that emerged from this study was that stationary phase materials from differing batches should never be mixed, particularly for long term studies.

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INTRODUCTION

The development of new stationary phase surfaces is an important field in separation science. New separations stem from the development of new surfaces, which often provide exceptional resolutions. One such stationary phase that has been developed in recent years is carbon clad zirconia. This stationary phase is one member of the zirconia family of supports that are currently commercially available and gaining widespread use in separation conditions that may otherwise prove to be difficult on conventional silica based supports (1).

Carbon clad zirconia is prepared through the pyrolysis of light hydrocarbons onto the surface of zirconia microspheres (2,3). The resulting product is one that, in many respects, resembles a carbon surface. However, because of the control in pore size distributions and the rigidity of zirconia many of the disadvantages associated with the use of pure carbon stationary phase is avoided. The resultant support also possesses important properties such as, an effective working pH range of 0–14, is thermally stable to 200°C, resistant to solvent shock, and unique selectivity.

Currently there are limited examples of separations on carbon clad zirconia. However, the separations that have been reported indicate that this surface may be extremely useful for the separation of diastereoisomers (1). Carr and coworkers (1) used this surface to separate diastereoisomers of di(phenethyl)amide, resorcinol diether, and *cis-/trans*-stilbene. In addition, they also separated stereoisomeric mixtures of (±)-warfrin and (±)-amino acid esters. Enantiomers of these two pharmaceutically and biologically important compounds were first derivatised using Mosher's reagent, α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, prior to separation. It was found that reversed-phase liquid–solid adsorption chromatography on carbon surfaces often provides excellent resolving power for the range of diastereoisomers evaluated, as compared to RPLC on conventional C18 columns. Weber and Carr used the same support in the separation of a range of structural isomers such as xylenes, nitroxylenes, butylbenzenes, nitrotoluenes, and phenylpropanols (2). Their study highlighted a much greater selectivity for the separation of both polar and nonpolar isomers when compared to conventional reverse-phase supports.

Although the carbon clad supports exhibited lower chromatographic efficiency, these investigators found the stationary phase to be more retentive, having superior resolving power and faster run-times than with conventional ODS columns. Belliardo and coworkers (4) used carbonaceous stationary phases for the separation of *cis-/trans*-stilbenes. The separation was easily achieved on different columns where the available surface area exceeded 1300 m^2/column, even though the particle shape was less than ideal compared to today's modern HPLC packing materials. Although, the column contained a very high surface area, no mention was made to the pore size distribution, and hence, the available surface area for solute retention is unknown. Band broadening in their chromatograms was considerable, which highlights the fact that separation of isomers on these surfaces is easily achievable, and high efficiency columns are often not required to gain these separations.

More recently, Sweeney and coworkers showed that carbon clad zirconia provided exceptional resolving power for diastereoisomers of low molecular weight polystyrenes (5,6). They also demonstrated that the surface could differentiate between polystyrene oligomers containing either *n*-butyl or *sec*-butyl end groups. This led them to develop a process for the analysis of molecular weight distributions and diastereoisomer compositions using a two dimensional column switching process (6). A C18 column was employed for the separation of oligomers by molecular weight and a carbon clad zirconia column was employed for the separation of diastereoisomers from within each oligomeric component. The degree of diastereoisomer separation of the low molecular weight polystyrenes far exceeded the separations that could be achieved using conventional C18 columns.

In the current study, we further illustrate separation studies of diastereoisomers of low molecular weight polystyrenes on carbon clad zirconia stationary phase supports. In this work, we illustrate high-speed separations and point out aspects of the separation problem that are important for chromatographers wishing to undertake long term studies employing these surfaces.

EXPERIMENTAL

Chemicals

HPLC grade acetonitrile, methanol, 95% *n*-hexanes, dichloromethane, and *iso*propanol were purchased from Mallinckrodt Australia. A polystyrene standard with an average molecular weight of 580 daltons and a *n*-butyl end group was purchased from the Aldrich Chemical Company. Individual oligomers from $n = 2$ to $n = 5$ were fractionated in-house using methods previously described (5,6). Carbon clad zirconia (ZirChrom-CARB – 3 μm particle diameter) stationary phase materials used for the preparation of columns in this study were purchased from ZirChrom Separations, Inc. (Anoka, MN, USA). All stationary phases were used as supplied from the manufacturer.

Equipment

All chromatographic experiments were performed on a Shimadzu LC system (Shimadzu Scientific Instruments, Rydalmere, NSW, Australia) incorporating a LC-10ATvp pumping system, SIL-10ADvp auto injector, SPD-10Avp UV detector set at 262 nm, SCL-10Avp system controller, and Shimadzu Class-VP version 5.03 software on a Pentium II 266 PC. Data acquisition was achieved using a Lawson Labs model 203 serially interfaced 20-bit data acquisition system, with a custom ± 1 volt gain range operated at 5 Hz and 10 Hz, as appropriate (Lawson Labs Inc, Malvern, PA, USA). Columns were packed using a Haskel air driven fluid pump (Haskel International, Burbank, CA, USA).

Chromatographic Separations

Purified polystyrene oligomer fractions were collected in methanol and, subsequently, injected into the LC system in an acetonitrile mobile phase. All flow rates were 1.0 mL/min unless stated otherwise. Column temperature was regulated by submersion of the column in a heated water bath at temperatures noted appropriately in the text. All injection volumes were 20 μL.

Preparation of Chromatography Columns

A range of carbon clad zirconia columns (50 × 4.6 mm and 30 × 4.6 mm) were prepared for this study. These columns were prepared from two different lot numbers of carbon clad zirconia purchased from the same supplier. Each lot of stationary phase support was kept separate throughout the study. All columns were prepared using a downward slurry packing technique, in which 6 g of stationary phase was slurried in 35 mL of a 90% hexane and 10% *iso*propanol solvent mixture (5,6). The slurry was stirred for 30 minutes, followed by 20 minutes of ultrasonication and a further 10 minutes of stirring. A dichloromethane displacement solvent was employed in the column blank and the column was packed at 7000 p.s.i. using an *iso*propanol packing solvent. Packing continued until 100 mL of *iso*propanol passed through the bed.

RESULTS AND DISCUSSION

In previous studies, we have shown the exceptional resolving power of carbon clad zirconia for the separation of diastereoisomers of low molecular weight polystyrenes (5,6). An interesting aspect of these separations is that elution is predominantly dependent upon the isomeric form, rather than the molecular weight. Whereas in conventional reversed phase separations of diastereoisomers of low molecular weight polystyrenes that employ C18 columns, the separation of the diastereoisomers occurs in distinct molecular weight groupings corresponding to oligomeric fractions. On the carbon clad zirconia columns, injection of the neat polystyrene sample yields chaotic band displacement – necessitating the fractionation of oligomeric components and then the individual analysis of each oligomeric fraction (5,6). An example of the chaotic band displacement is shown in Figure 1.

Diastereoisomer separations of individual oligomer fractions on a 5 cm carbon clad zirconia column (lot # 5-146) at 30°C are illustrated in Figure 2. These chromatograms show the highly specific nature that this type of surface provides for the separation of the diastereoisomers. Although, for the oligomer $n = 5$, complete baseline resolution of the eight possible diastereoisomers was not achieved. Nevertheless, the resolution was still substantially greater than separations performed on conventional C18 columns, (5) even when the C18 separations where optimised in gradient conditions.

Increasing the column temperature resulted in a systematic decrease in the degree of retention, as shown by the separations for the $n = 4$ and $n = 5$ oligomers in Figure 3. The resolution between adjacent bands decreased slightly over the

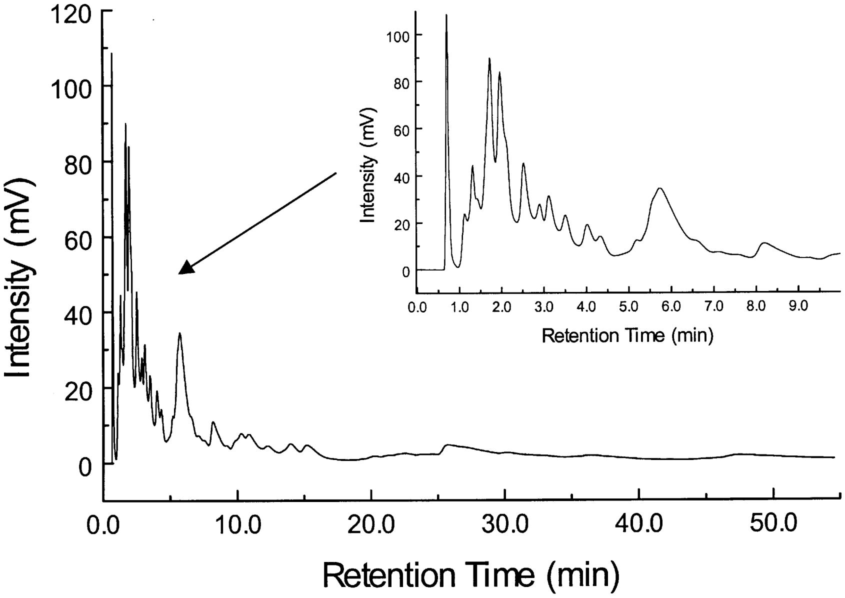

Figure 1. Chromatogram showing the elution profile of a polystyrene standard with a molecular weight of 580 Daltons on a carbon clad zirconia column. Elution conditions: Stationary phase lot number 5-146 carbon clad zirconia in a 50 × 4.6 mm column, mobile phase 100% acetonitrile, flow rate 1.0 mL/min, column temperature 30°C, injection volume 20 μL.

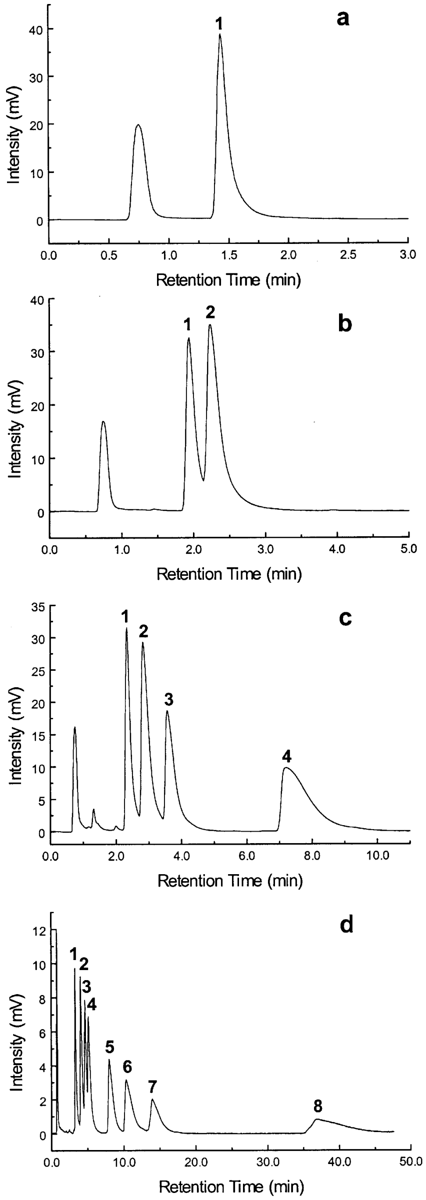

Figure 2. Diastereoisomer separations of individual oligomer fractions. Chromatographic conditions as described in Figure 1. Injection volume 20 μL. 2a, diastereoisomers of oligomer $n = 2$. 2b, diastereoisomers of oligomer $n = 3$. 2c, diastereoisomers of oligomer $n = 4$. 2d, diastereoisomers of oligomer $n = 5$.

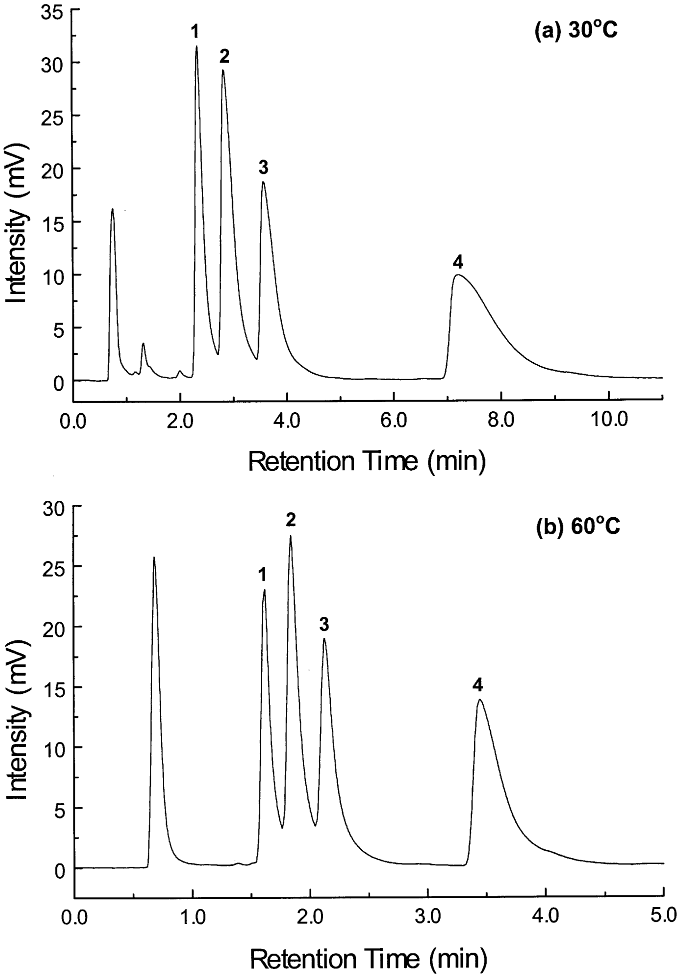

Figure 3. Diastereoisomer separations of individual oligomer fractions at various temperatures for the oligomers (a) $n = 4$ and (b) $n = 5$. Chromatographic conditions as described in Figure 1.

(continued)

thirty degree temperature range studied. The capacity factor for the band labelled as 4 in Figure 3(a) (30°C) was 8.54, and at 60°C (Figure 3(b)) the capacity factor for the same band had decreased to 4.02. Also, there was a marginal increase in resolution between the two least resolved bands (labelled as bands 1 and 2). The

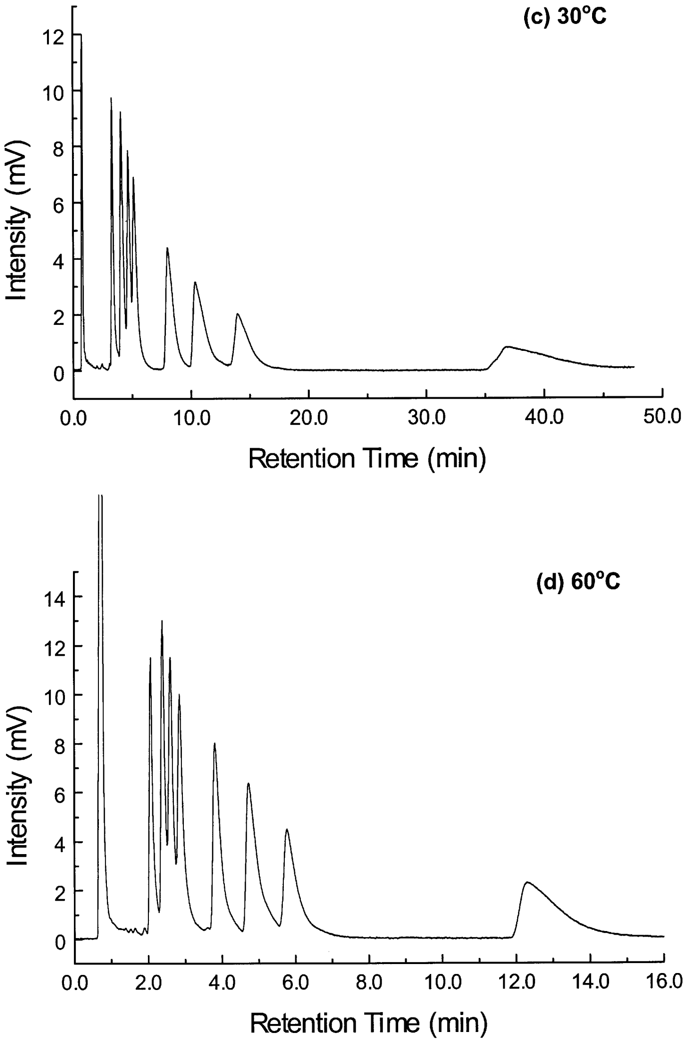

Figure 3. Continued.

resolution for these 2 bands increased from 0.790 at 30°C to 0.880 at 60°C (see Table 1 for further information). Essentially, the same effect was observed for all oligomer fractions that were tested, for example, Figures 3(c) and 3(d) ($n = 5$). Information on capacity factors, selectivities, and resolution for these separations in Figures 2 and 3 are given in Table 1.

Table 1. Capacity Factors, Selectivities, and Resolution Information for *n*-Butyl Polystyrene Isomers from Oligomers

	Stationary Phase (Lot #5-146)						Stationary Phase (Lot #25-109)					
	30°C			60°C			30°C			60°C		
Isomers	k'	$\alpha_{i,j}$	$Rs_{i,j}$	k'	$\alpha_{i,j}$	$Rs_{i,j}$	k'	$\alpha_{i,j}$	$Rs_{i,j}$	k'	$\alpha_{i,j}$	$Rs_{i,j}$
$n=2$												
1	0.915			0.54			2.28			1.32		
$n=3$												
1	1.61			0.90			4.97			2.66		
2	2.02	1.25	0.64	1.07	1.19	0.49	6.48	1.30	1.11	3.22	1.21	0.83
$n=4$												
1	2.09			1.36			7.71			4.12		
2	2.72	1.30	0.79	1.69	1.24	0.88	11.1	1.44	1.41	5.45	1.32	1.25
3	3.70	1.36	1.03	2.10	1.24	0.87	15.5	1.40	1.42	7.12	1.30	1.26
4	8.54	2.30	2.95	4.02	1.91	2.86	42.2	2.67	6.00	15.9	2.23	3.54
$n=5$												
1	3.32			1.84						7.39		
2	4.32	1.30	1.35	2.29	1.23	1.19				9.85	1.33	1.65
3	5.12	1.18	0.98	2.60	1.13	0.70				11.3	1.15	0.85
4	5.75	1.12	0.65	2.93	1.12	0.66				13.4	1.18	0.99
5	9.52	1.65	2.21	4.27	1.45	2.13				31.9	1.63	2.60
6	12.6	1.32	1.19	5.53	1.29	1.49				31.4	1.42	1.77
7	17.4	1.37	1.54	6.99	1.26	1.47				40.5	1.29	1.32
8	47.5	2.73	4.00	16.0	2.29	4.24				114	2.81	6.21

Performed using column temperatures of 30°C and 60°C on 50 mm columns prepared using lot numbers 5-146 and 25-109 carbon clad zirconia stationary phase materials (from $n=2$ to $n=5$).

As a stationary phase material, carbon clad zirconia is relatively new, and there are few papers in the literature that describe separations using these surfaces. As such, little is known regarding the performance of the material in a wide range of conditions. Furthermore, the relatively low demand of this type of stationary phase has not allowed for the extensive development of the surface to take place in the same manner that has resulted for the production of the more widely used reversed phase surfaces, such as the C18. It was not until comparatively recent years that manufacturers have been able to produce highly reproducible C18 stationary phases, and this was illustrated in a series of detailed studies by Kele and Guiochon (7–10). Consequently, a similar development process is undoubtedly required before carbon clad zirconias become highly reproducible from batch to batch preparations. With this in mind, we performed several separations on a new batch of stationary phase material (lot # 25-109). Two columns from this batch of stationary phase were prepared and the retention behaviour was compared to the separations that employed the lot number 5-146. We were not surprised to observe variations in the retention behaviour of the surface, albeit the deviation was substantially greater than expected.

Separations of the same oligomer fractions are shown in Figure 4. The retention time increased substantially on these columns, to the extent that complete elution of all diastereoisomers for $n = 5$ were not observed at 30°C. Despite this, the resolution of the diastereoisomers that were eluted under the same solvent conditions as employed for the first batch of stationary phase material, was improved. To compare the stationary phase supports, the column temperature was raised to 60°C and the resulting chromatograms are shown in Figure 5. Clearly, these stationary phase surfaces provided widely differing degrees of retention. Retention information (k', selectivity and resolution) is given in Table 1 for comparison between the two batches of the stationary phase material. An example of the difference in the surface retention properties between these batches is highlighted by the widely differing retention behaviour of the last eluting diastereoisomer of the $n = 5$ oligomer. On the stationary phase material lot number 5-146 the capacity factor was 16.0, whilst the same diastereoisomer on the stationary phase lot number 25-109 was 114. Typically, the extent of the variation in the capacity factors of the diastereoisomers between each batch of stationary phase was more than 2-fold, but for some of the more strongly retained diastereoisomers, the difference increased to as much as 10-fold. In comparison, variability between capacity factors for the two columns prepared from the same batch of stationary phase (lot # 25-109) was typically less than 12%, based on $n = 4$ at 60°C.

Each of these carbon clad zirconias were prepared from different base zirconias (11). The small differences in the surface area, pore volume, and pore diameter of the zirconia support materials would not account for the variation in the retention behaviour, especially since the material lot # 5-146 has the higher surface area and pore volume; but isomers experience less retention on this surface (see Table 2). Variations in the retention properties of carbon clad zirconias has also been noted in the early days of the stationary phase preparation, and was attributed to the source of the carbon used in the preparation of the stationary phase (12). A plot of the scaled capacity factor on surface 5-146 against the scaled capacity factor on surface 25-109 showed direct correlation between both surfaces, with almost all retention data being aligned along the diagonal, as shown in Figure 6. Consequently, we can conclude that the retention process on both stationary phase supports was essentially the same, differing only in the extent of retention.

With such a large variability between the two batches of stationary phase our initial thoughts were towards this type of surface being unreliable and,

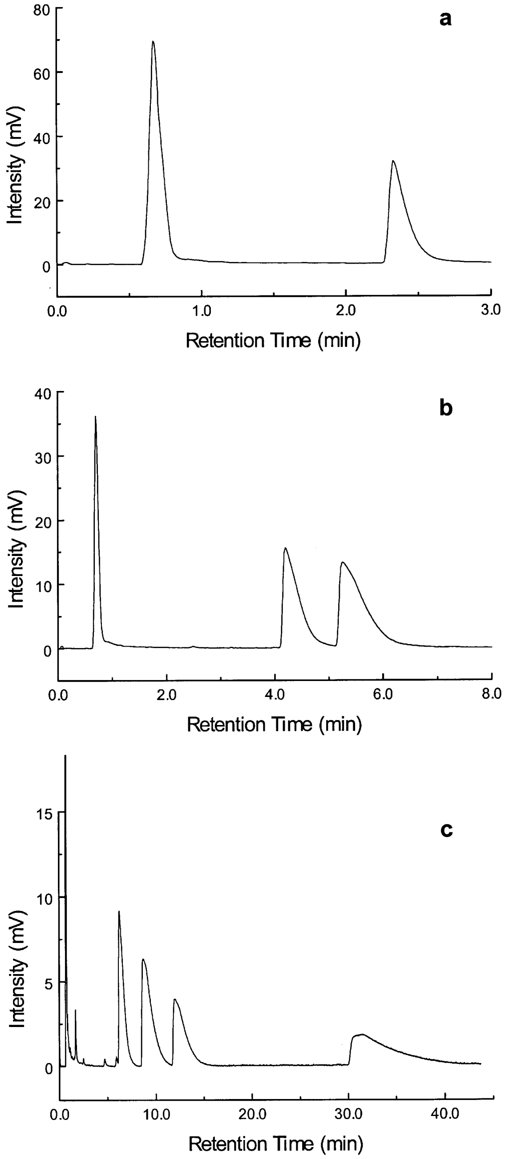

Figure 4. Diastereoisomer separations of individual oligomer fractions on the carbon clad zirconia column prepared from lot # 25-109. Mobile phase 100% acetonitrile, flow rate 1.0 mL/min, column temperature 30°C, injection volume 20 μL. 4a, diastereoisomers of oligomer $n = 2$. 4b, diastereoisomers of oligomer $n = 3$. 4c, diastereoisomers of oligomer $n = 4$.

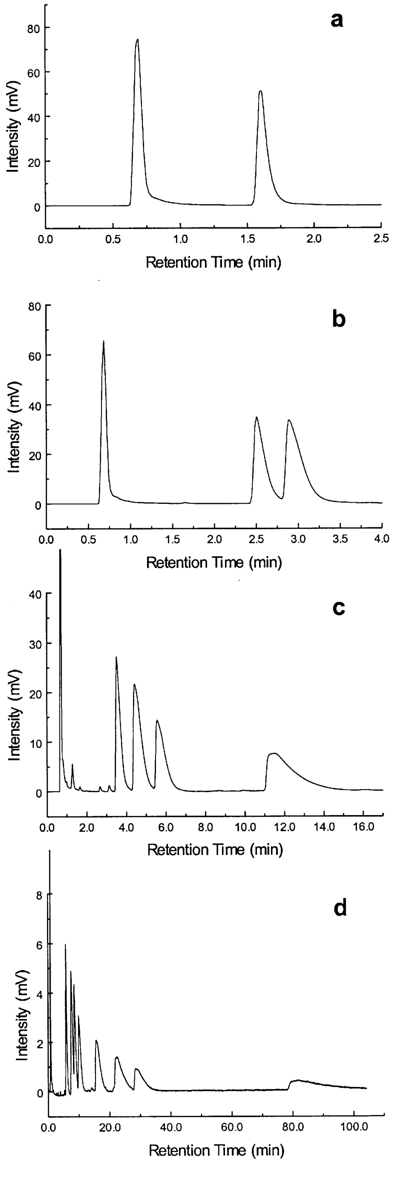

Figure 5. Diastereoisomer separations of individual oligomer fractions on the carbon clad zirconia column prepared from lot # 25-109 operated at 60°C. Chromatographic conditions as described in Figure 4. 5a, diastereoisomers of oligomer $n=2$. 5b, diastereoisomers of oligomer $n=3$. 5c, diastereoisomers of oligomer $n=4$. 5d, diastereoisomers of oligomer $n=5$.

Table 2. Surface Area, Pore Volumes, and Pore Diameters of the Zirconia Support Material Used in the Preparation of the Carbon Clad Zirconias

Base Material Lot #	Surface Area (m^2/g)	Pore Volume (mL/g)	Pore Diameter (nm)
5-146	30.1	0.22	29.2
25-109	22.8	0.16	28.7

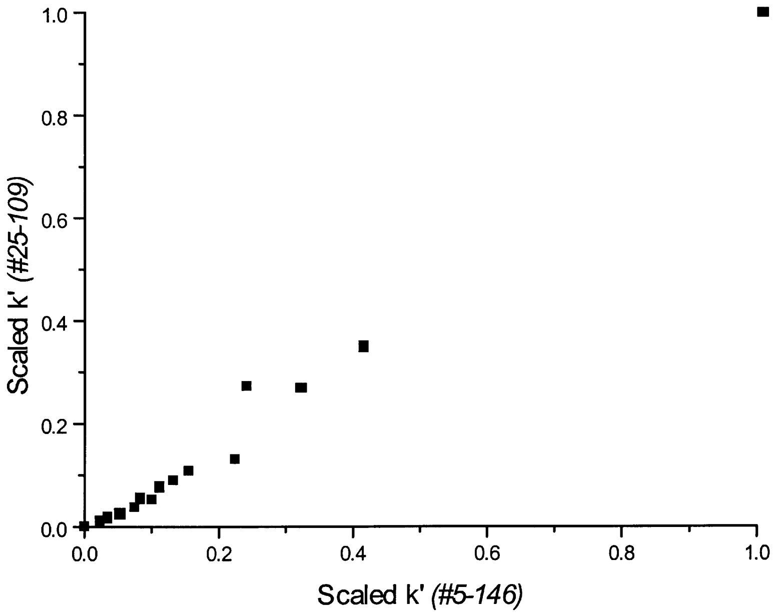

Figure 6. Plot of the scaled capacity factors of each diastereoisomers eluting from both batches of carbon clad zirconia stationary phase.

consequently, less important for long term studies on the separation of diastereoisomers. However, the increased degree of retention and the marginally improved resolution gained on the second batch of stationary phase proved to be an advantage in the separation. The greater resolution meant that shorter columns could be prepared, and because of the lower pressure drop, higher flow rates could be used. As a result, a 30 × 4.6 mm column was prepared in the same manner as described in the experimental. The separation of the diastereoisomers from oligomer $n = 4$ that resulted on this column are shown in Figure 7. The series of chromatograms represent the separations that were achieved at flow rates from 1.0 mL/min up to 5.0 mL/min at 60°C.

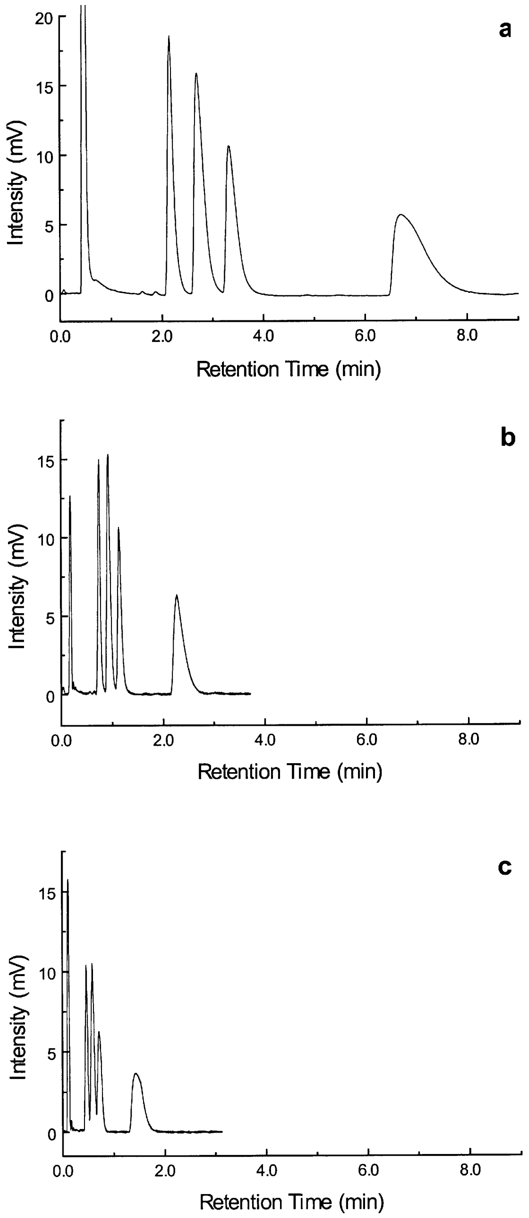

Figure 7. The separation of the diastereoisomers from oligomers $n = 4$ at flow rates (a) 1.0 mL/min, (b) 3.0 mL/min, and (c) 5.0 mL/min. Column temperature: 60°C. Other chromatographic conditions as in Figure 4.

Table 3. Capacity Factors, Selectivities, and Resolution Information for *n*-Butyl Polystyrene Isomers from Oligomers

	30 mm Lot #25-109			50 mm Lot #25-109			50 mm Lot #5-146		
Isomers	k'	$\alpha_{i,j}$	$Rs_{i,j}$	k'	$\alpha_{i,j}$	$Rs_{i,j}$	k'	$\alpha_{i,j}$	$Rs_{i,j}$
$n=2$									
1				1.32			0.54		
$n=3$									
1				2.66			0.90		
2				3.22	1.21	0.83	1.07	1.19	0.49
$n=4$									
1	3.59			4.12			1.36		
2	4.75	1.32	1.22	5.45	1.32	1.25	1.69	1.24	0.88
3	6.09	1.28	1.27	7.12	1.30	1.26	2.10	1.24	0.87
4	13.4	2.19	3.99	15.9	2.23	3.54	4.02	1.91	2.86
$n=5$									
1	6.29			7.39			1.84		
2	8.35	1.32	1.79	9.85	1.33	1.65	2.29	1.23	1.19
3	9.55	1.14	0.96	11.3	1.15	0.85	2.60	1.13	0.70
4	11.1	1.16	1.08	13.4	1.18	0.99	2.93	1.12	0.66
5	17.6	1.56	2.94	31.9	1.63	2.60	4.27	1.45	2.13
6	24.6	1.39	1.92	31.4	1.42	1.77	5.53	1.29	1.49
7	31.2	1.26	1.57	40.5	1.29	1.32	6.99	1.26	1.47
8	83.0	2.65	6.56	114	2.81	6.21	16.0	2.29	4.24

Flow rate 1.0 mL/min with the column temperature set at 60°C on columns prepared using two different batches of carbon clad zirconia stationary phase material (from $n=2$ to $n=5$).

Despite the increase in flow rate the resolution was essentially maintained throughout each flow rate increment. As a result, the separation of the diastereoisomers was complete within 1.8 minutes. However, resolution was observed to decrease more significantly at the higher flow rates when the same process was applied to the $n=5$ oligomer (result not shown). A comparison in the capacity factors, selectivities, and resolutions between three columns prepared from the two lots of stationary phase is given in Table 3. From these results, we see that there is very little difference in the separation power of the 30 mm column compared to the 50 mm column containing lot number 5-146 stationary phase material.

CONCLUSION

Finally, we have shown that carbon clad zirconia surfaces can provide exceptional resolving power of diastereoisomers. However, the chromatographer should be aware that the surface is subject to some variability. The variability noted in this manuscript covered two batches of stationary phase material purchased one and a half years apart. We have been informed that the manufacturing process has been changed, and if we had tested stationary phase materials from more recent batches, the batch to batch reproducibility would have been better (11). However, since we are not in the business of evaluating stationary phase surfaces from commercial suppliers, we did not wish to further exam this aspect. Rather, our aim was to make the user of speciality surfaces aware of the fact that, as the manufacturing process develops, so too may the stationary phase surface. Consequently, when using these types of stationary phase materials, the analyst should expect variability and adjust their separation conditions to suit the surface properties of the stationary phase support.

Perhaps the most important aspect that we gained from this exercise was that under no circumstances should stationary phase materials be blended from different lot numbers. If long term studies are planned, sufficient stationary phase material should be purchased if altering conditions of operation during extended studies may pose problems. We should also caution that, at this point in time, we have only used this surface to study the behaviour of low molecular weight polystyrenes and consequently, the degree of variability for different types of compounds is unknown. Clearly, this will be the case, as our results show vast differences in the degree of retention for individual diastereoisomers.

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