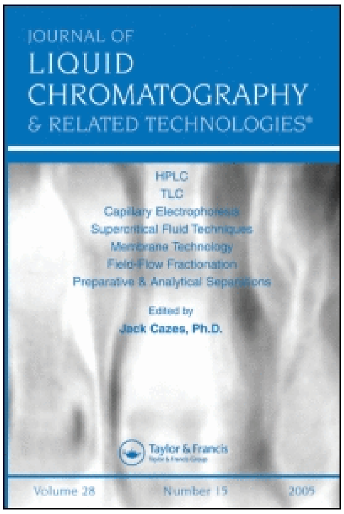

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Ultra-High Resolution Separation of Diastereomers on Carbon Adsorption Stationary Phases

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Abstract: Reversed-phase adsorption liquid chromatography was used to successfully resolve diastereomers in a complex mixture of low molecular weight polystyrene oligomers. The separation was achieved by incorporating a two-dimensional liquid chromatography system where the polystyrene oligomers were transported from the first dimensional column, a C_{18}, to an adsorbent phase in the second dimension, a carbon clad zirconia (CCZ) column. Using this system up to 46 diastereomers, each consisting of eight configurational repeat units, were able to be resolved in ultra high separations.

Keywords: Diastereomer, Liquid solid chromatography, Carbon-clad zirconia, Low molecular weight polystyrene oligomer

INTRODUCTION

Generally, silica based reversed-phase stationary phases, such as the C_{18}, give retention that, at least for homologues, is dependent upon the molecular weight according to Martin's Rule:[1]

$$\text{In}\, k = \text{Bn} + \text{In}\, \text{A} \tag{1}$$

in which A and B are empirical coefficients and k increases exponentially with the number of repeat units, n within a homologous series of isomers. This dependence upon molecular weight often inhibits high resolution separations

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of diastereomers, since mixtures of diastereomers have identical molecular weights. To some degree, the stereochemistry of isomers may be controlled or expressed by the use of different mobile phases. The C_{18} phase gives good separation of low molecular weight polystyrene oligomers using a methanol mobile phase,[2,3] and by changing the mobile phase to acetonitrile the isomeric separation also exhibited some diastereomer resolution.[2,3] This was also evidenced by other works employing acetonitrile as the mobile phase for the separation of oligostyrenes using a C_{18} column.[4–6] However, the extent of the selectivity for diastereomers is often limited due to the retention being dominated by the molecular weight. Hence, the underlying resolution of the isomeric components must occur within a distinct region defined by their molecular weight and, as a consequence, the separation capacity is limited.

A more useful chromatographic method for the separation of diastereomers is adsorption chromatography, referred to as liquid solid chromatography (LSC). This mode of separation is usually very sensitive to molecular shape since solutes interact with adsorption sites, and their interactions are largely shape dependent. LSC can be either normal- or reversed-phase. In normal-phase LSC, silica stationary phases are used in HPLC, and alumina phases are prevalent in TLC applications. In reversed-phase LSC, stationary phase choice is limited to carbon phases, which explains the small number of examples reported for reversed-phase LSC separations. This is due to two reasons: (a) Carbon stationary phases are generally not robust and (b) They have poor reproducibility. Regardless of these problems, carbon stationary phases offer the potential for unique retention and present an alternative mechanism of separation to conventional bonded phase reversed-phase stationary phases. The extensive delocalised network on these carbon adsorption surfaces allows for the establishment of electronic (π-π) bonding[7] and offers stereoselectivity of diastereomers that are capable of undergoing these π-π type interactions.

The porous graphitized carbon (PGC) stationary phase was developed as an alternative to the bonded phase supports[8,9] and offers an increased selectivity for solutes with closely related structures including stereoisomers.[10] PGC consists of porous carbon particles comprised of flat sheets of hexagonally arranged carbon atoms, and is stable across a large pH and temperature range.[9] The preparation of this surface is achieved by multiple steps that involve the chemical and thermal treatment of a polymerised resin impregnated within the pores of a silica gel template that is then subjected to carbonisation, the dissolution of the silica template, and finally graphitisation. The spherical shape and particle size of the original silica template are, thus, retained by the carbon.[11] PGC has proven useful for the separation of isomers, where the spatial arrangement of the diastereomers rather than molecular weight of the isomer can determine selectivity. Examples of diastereomer separation on PGC include the quantitation of diastereomers in plasma,[12] the separation of *cis*- and *trans*-stilbenes,[13] *cis*-*trans* isomers of potential anti-asthma agents,[14] *cis-trans* isomers of proline-containing dipeptides,[15] and diastereomeric gluronides of almakalant.[16] However, poor mechanical stability, low surface area, the heterogeneous nature of the surface, and the non-uniform pore structure (which have effects on the loading capacity) have been listed as possible shortcomings of PGC.[17,18]

Another carbon stationary phase that has proved useful as a reversed-phase adsorption surface is carbon-clad zirconia (CCZ). CCZ is a carbon coated zirconia particle prepared by chemical vapour deposition (CVD) of hydrocarbons over porous zirconia microspheres at elevated temperatures.[17–19] This method offers a mechanically and chemically stable support.[20] CCZ has been used for the separation of *cis-* and *trans*-stilbenes and was shown to offer superior resolution when compared to conventional bonded phase reversed-phase surfaces. As each diastereomer had a unique shape, a different surface of electron density was available to the CCZ phase.[21] CCZ has also demonstrated high resolution of diastereomers of low molecular weight polystyrenes.[4–6,22–28] Similar to the PGC surfaces, the CCZ stationary phase also suffers from poor reproducibility in the manufacturing process,[23] which could explain the limited applications reported in the literature.

The purpose of the present communication is to illustrate the high resolving power of the CCZ stationary phase for the separation of some polystyrene diastereomers (to nine configurational repeating units). These reported separations may encourage the use of adsorption reversed-phase HPLC, which in turn may promote development in a technique that offers potentially superb separation performance for structurally related compounds.

EXPERIMENTAL

Chemicals

HPLC grade methanol, acetonitrile, chloroform, and dichloromethane were obtained from Lomb Scientific, Australia. *tert*-Butyl polystyrene (molecular mass ~580 Da) was synthesised using anionic polymerisation of styrene initiated with *tert*-butyl lithium. The Pursuit XRs C18 column (250 mm × 4.6 mm) used for the separation of the low molecular weight oligostyrenes was kindly donated by Varian. A carbon clad zirconia (CCZ) column (100 mm × 10.0 mm)[4] was used to separate the diastereomers of oligomer fractions. The bulk CCZ packing material used to prepare this column was purchased from ZirChrom Separations, Inc., (Anoka, MN, USA). The stationary phase was used as supplied from the manufacturer.

Equipment

In order to demonstrate the superb diastereomer selectivity of the carbon based stationary phase, the complex polystyrene sample matrix required simplification. This was readily achieved using a two-dimensional HPLC system. While two-dimensional HPLC is not the focus of this work, it has been extensively employed for the separation of the isomers of low molecular weight polystyrenes and, hence, is the perfect conduit for the transportation of the diastereomers onto the carbon stationary phase. Manual processes of sample fractionation could have been employed to illustrate the outcome of this separation, but the two-dimensional separation approach is clearly more efficient. Subsequently, the diastereomer separations were undertaken using a 2D chromatographic system that incorporated two 600 controllers, 717plus autosampler, two 2487 dual wavelength UV detectors, and Millenium^{32} Version 4.00 software running on a Compaq EVO D500 Pentium 4 1.6 GHz personal computer with 256 Mb RAM (Waters Associates). Column switching was achieved using two 6-port, 2-position switching valves fitted with micro-electric two position valve actuators (Valco Instruments, Houston, TX, USA), controlled via the onboard Millenium^{32} software.

Chromatographic Separation

tert-Butyl polystyrene was dissolved in methanol:chloroform (80:20). Separations were performed in the first dimension (C_{18}) under linear gradient conditions (100% MeOH to 80% MeOH:20% DCM over 4 minutes). The second dimension separations (CCZ) were also performed under linear conditions (80% ACN:20% DCM to 40% ACN:60% DCM over 80 minutes for the $n = 7$ oligomeric fraction, 80% ACN:20% DCM to 40% ACN:60% DCM over 40 minutes for the $n = 8$ oligomeric fraction, and a linear gradient of 100% ACN:0% DCM to 0% ACN:100% DCM over 20 minutes for $n = 9$ oligomeric fraction). Mobile phases were sparged continually with helium. Flow rates were 1.0 mL/min in the first dimension and 3.0 mL/min in the second dimension, injection volumes were 30 μL with the exception of $n = 9$, which was 200 μL. The cutting volumes from the first dimension to the second were 1350 μL, 1600 μL, and 2050 μL for $n = 7$, $n = 8$, and $n = 9$ respectively, which corresponded to approximately 98% for $n = 7$ and $n = 8$, and 94% for $n = 9$ of the oligomeric fractions. UV detection was set at 262 nm. All experiments were conducted under ambient temperature conditions.

RESULTS AND DISCUSSION

Under the chromatographic conditions described for the first dimension it was possible to separate the *tert*-butyl oligomers ($n = 2$ to $n = 10$) with baseline resolution. Such separations have been extensively reported in the literature and require no further discussion. Of interest in this work were oligomers 7, 8, and 9. These oligomers were heart cut from the first dimension to the second dimension where their diastereomers were separated on the CCZ column. The number of diastereomers for each oligomer can be calculated according to Equation (2), where n is equal to the number of configurational repeat units.

$$\text{No. of diastereomers} = 2^{n-2} \tag{2}$$

Therefore, for an oligomer containing seven configurational repeating units, the number of diastereomers is 32, for eight configurational repeating units, the number of diastereomers is 64, and for an oligomer containing nine configurational repeating units there are 128 diastereomers.

The chromatogram illustrated in Figure 1 shows the separation of 28 out of a possible 32 diastereomers of an $n = 7$ oligostyrene. In previous work by Mourey et al.,[29] normal-phase LSC was employed for the separation of diastereomers of low molecular weight oligostyrenes. They used two 4.6 mm × 250 mm serially coupled silica columns running a linear gradient, increasing at 0.1%/mL from 89% to 11% n-hexane/dichloromethane at a flow rate of 1.0 mL/min. A total of 11 diastereomers were resolved for

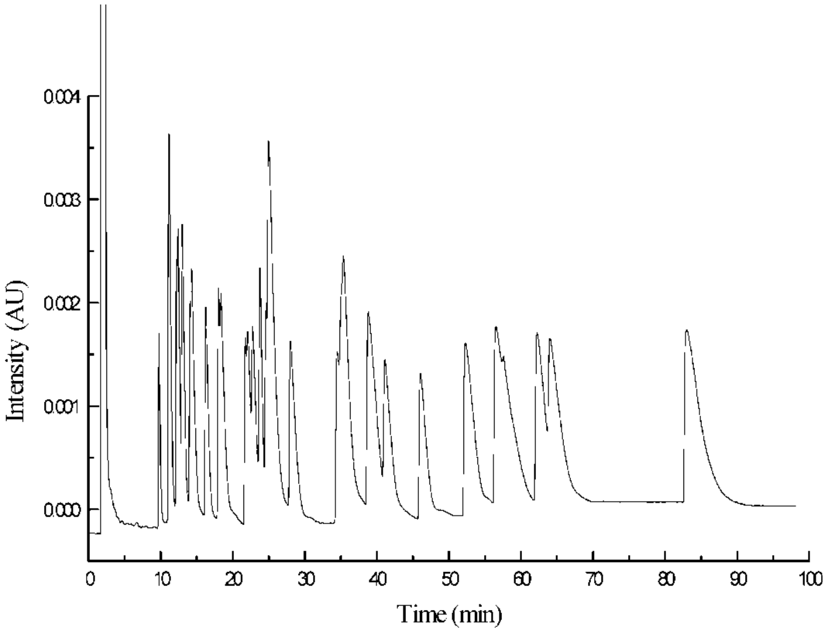

Figure 1. Diastereomer separation of the $n = 7$ oligostyrene on CCZ column. Mobile phase: linear gradient 80:20 ACN:DCM to 40:60 ACN:DCM over 80 minutes, flow rate 3.0 mL/min, injection volume 30 μL.

the $n = 7$ isomer. The efficiency of the reversed-phase LSC separation on CCZ relative to that of the normal-phase LSC separation on silica is quite remarkable given the bed length in the reversed phase mode was 10 cm, compared to 50 cm in the normal-phase mode.

Figure 2 shows the separation of the diastereomers of the eighth oligomer on the CCZ column. In accordance with Equation (2), 64 diastereomers are possible, and using the CCZ stationary phase a total of 42 diastereomers were resolved. This is a remarkable separation given the column length was 10 cm. Comparable separations are not possible using conventional C_{18} surfaces. Development of ultra performance liquid chromatography (UPLC) may give the higher resolution required to give similar high resolution diastereomer separations, but even then it is doubtful.

Even with the excellent resolving power that was evident in the separations shown in Figures 1 and 2, the peak capacity of this column has been exceeded for the separation of the diastereomers of the ninth oligomer fraction, as shown by the separation in Figure 3. The 128 diastereomer mixture eluted essentially as a continuum and, hence, a larger capacity column would be required for resolution of the components.

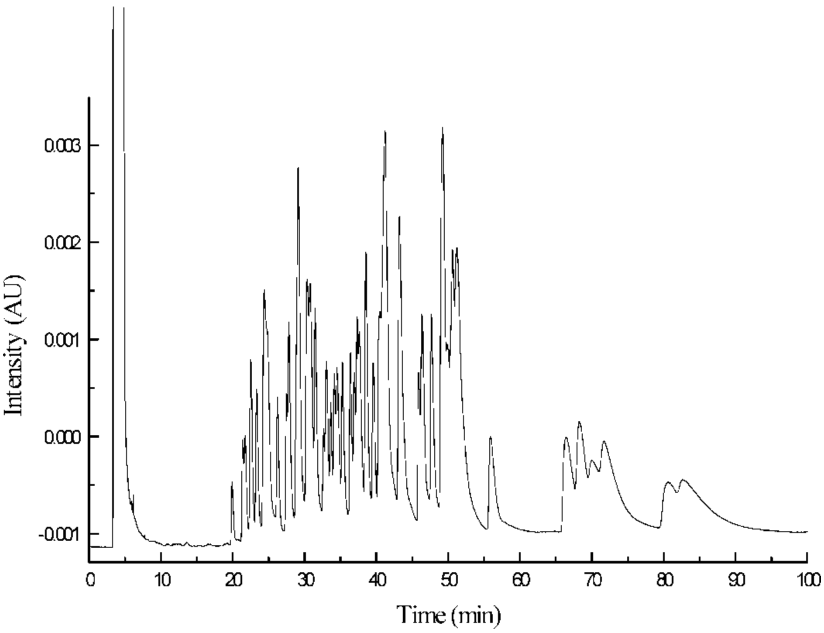

Figure 2. Diastereomer separation of the $n = 8$ oligostyrene on CCZ column. Mobile phase: linear gradient 80:20 ACN:DCM to 40:60 ACN:DCM over 40 minutes, flow rate 3.0 mL/min, injection volume 30 μL.

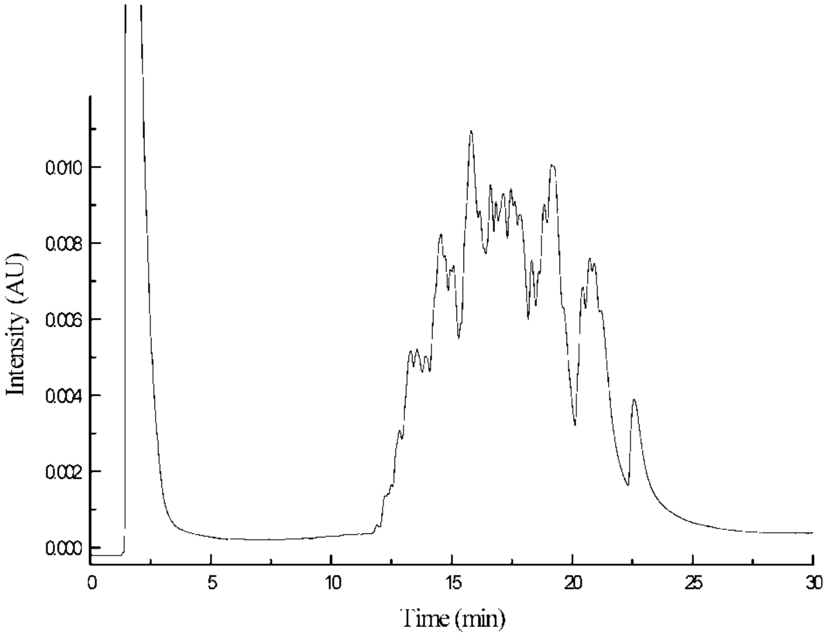

Figure 3. Diastereomer separation of the $n = 9$ oligostyrene on CCZ column. Mobile phase: linear gradient 100% ACN to 100% DCM over 20 minutes, flow rate 3.0 mL/min, injection volume 200 μL.

CONCLUSION

The separations illustrated demonstrate the superb diastereomer selectivity that was gained using a carbon adsorption stationary phase. Analysts who seek to resolve similar complex mixtures should consider reinvestigating this now often overlooked separation mode, by employing carbon supports that are designed for the rigours of HPLC. Granted, the irreproducible nature of the stationary phase can be problematic, however, the gains that can be obtained far outweigh those limitations. Variability can, to some extent, be controlled by purchasing carbon columns and stationary phases from the same batch.

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