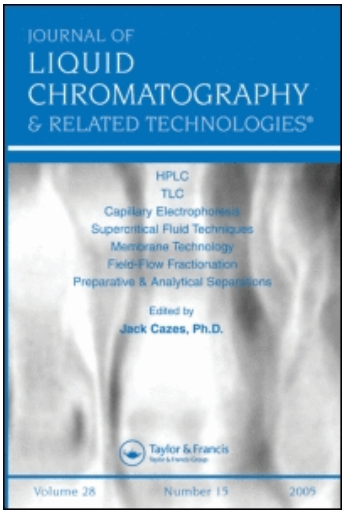


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# THE SEPARATION OF STEREOISOMERS FROM OLIGOMERS OF LOW MOLECULAR WEIGHT POLYSTYRENE ON A CARBON CLAD ZIRCONIA COLUMN USING A REVERSED PHASE MULTIDIMENSIONAL HPLC SYSTEM

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## ABSTRACT

The two dimensional separation of low molecular weight polystyrene oligomers and stereoisomers was achieved using a liquid chromatographic system designed for 'heart-cutting' multidimensional chromatography. The first separation process incorporated a C18 column and a methanol mobile phase to separate the oligomers, whilst the second separation process utilised the change in selectivity associated with a carbon clad zirconia stationary phase and an acetonitrile mobile phase for the separation of the isomers. The two dimensional separation that was achieved using this automated system was comparable to the individual

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separations following manual sample manipulation in each individual separation step. Furthermore, the multidimensional LC system was shown to be highly reproducible in routine continuous operation over an eight hour period.

## INTRODUCTION

Multidimensional techniques have long been employed for use in the separation of complex sample matrices. One-dimensional separations often lack sufficient peak capacities to separate complex mixtures into individual bands. However, using two-dimensional techniques, mixture components are displaced along two axes of separation, providing larger physical spaces in which components of a complex mixture can be ordered.(1) Since multidimensional techniques have the ability to analyse complex samples by conducting two separations in a single system, the need for comprehensive sample preparation is reduced.(2) Multidimensional HPLC techniques may be non-coupled, where columns are not physically joined and require manual sample manipulation between columns. In coupled techniques, the columns are connected in a single instrument using a series of switching valves. Such coupled techniques using an automated system, can provide a more rapid analysis compared with non-coupled techniques with minimal operator input.

There are two main forms of coupled multidimensional HPLC techniques. The first, involves a column switching technique where the effluent from one column flows directly into another column. The second, incorporates a type of trapping column that exists between the two analytical columns. The components are then removed from the trapping column to the second column for analysis. In both forms of coupled multidimensional HPLC, either all or a portion of eluent from one column may be switched to the next column. When only a portion of eluent is switched, the procedure is often referred to as a 'heart-cutting' technique.(2) Often, such techniques use a stationary phase in both columns with similar selectivity and, hence, are in fact not multidimensional. For a system to be truly multidimensional, the essential mechanism of separation in each of the dimensions should differ.(3-5)

The degree to which the two mechanisms of separation differ in a coupled multidimensional HPLC system can be calculated using a procedure described by Slonecker et. al.,(6) where the informational orthogonality of a system can be determined. A qualitative informational similarity technique is described where the informational orthogonality of a two-dimensional chromatographic separation can be projected for complex mixtures from their one-dimensional separations. Thus, a measure of the degree of multidimensionality of the system can be established.

A multidimensional technique is only applicable for samples that are themselves multidimensional. That is, the sample should be able to be described by two or more factors that influence the behaviour of the sample.(7) In many instances, the classification of the sample dimensionality is not possible because there are, in fact, too many constituents within the sample, preventing complete separation and classification. For such samples, the application of multidimensional techniques becomes somewhat more difficult as chaotic peak displacement may result as the dimensions within the sample begin to resolve into additional bands, which overlap bands from the other dimensional separation. To a certain extent, the technique referred to as heart-cutting multidimensional chromatography serves to minimise this chaotic peak displacement, as only a limited fraction of the sample is subjected to the second (or third etc.) dimensional separation process.

An example of a multidimensional sample may be found in low molecular weight polystyrenes. One dimension of the sample is the variation in the molecular weight of each oligomeric unit, while a second dimension of the sample may be described by the stereoisomers that are present within each oligomeric fraction.(8-12) Further dimensionality of the polystyrene may be described depending upon the classification of the types of stereoisomers that may be present within the oligomeric fractions, i.e., tacticity. However, if these additional dimensions within the sample have very little bearing on the properties of the sample, then the sample dimensionality would essentially be equal to that of the dominant factor(s). Such is the case when the molecular weight increases and the behaviour of the polystyrene is dominated by the molecular weight. Hence, low molecular weight polystyrenes may be multidimensional, while high molecular weight polystyrenes are essentially one dimensional.

The multidimensionality behaviour of low molecular weight polystyrenes was recently illustrated by Sweeney et. al.(8,9) In their studies, the dominant sample dimensions were illustrated using various reversed phase systems in which diastereoisomers of the low molecular weight oligomers of polystyrene (average Mw 760 daltons) were separated from the oligomers using a non-coupled HPLC system. The first dimension of the separation process consisted of a reversed phase C18 column and a methanol mobile phase. The oligomers were separated according to molecular weight, with no isomer separation observed. However, by changing the mobile phase to acetonitrile the partial separation of isomers was apparent. However, in both systems, the dominant mechanism of separation was based on the molecular weight. The dominant retention mechanism was changed from one based on the molecular weight dependence to one based on isomer dependence when a carbon clad zirconia column was employed with an acetonitrile mobile phase. Isomers within each oligomeric fraction were then separated according to this dimension. The carbon clad zirconia stationary phase offered a unique reversed phase mechanism that differed

from conventional supports in the reversed phase separation of the isomers of these oligomers.(13,14) Carbon clad zirconia supports are hydrophobic in nature and retain solutes based on electronic ($\pi$-$\pi$) interactions. They are often selective in the separation of isomers, particularly stereoisomers.(13-16) Consequently, by combining these two different reversed phase separations, the sample dimensionality of the low molecular weight polystyrene could be evaluated.

This paper describes the separation of polystyrene oligomers into their stereoisomers using a heart cutting multidimensional HPLC system. While there are many different combinations of columns and solvents that could be employed for this type of separation problem, we chose to employ a system in which the first dimension of the separation contained a C18 column and a methanol mobile phase, with the second dimension of the system employing a carbon clad zirconia column and an acetonitrile mobile phase. This combination was chosen for three reasons: (1) elution of low molecular weight polystyrenes on a C18 column with a methanol mobile phase yielded no expression of the underlying isomer components, thereby, each oligomeric band consisted of a homogeneous sample distribution. Consequently, heart cutting a uniform sample band minimises problems associated with a non representative sample. (2) Previous studies on the carbon clad zirconia column (13) have shown these surfaces to be selective for diastereoisomers, and initial studies in our laboratory support a higher degree of resolving power than conventional reversed phase columns. (3) Because both dimensions of the system are reversed phase, solute transport from one dimension to the next is facilitated by not having to be concerned about solvent miscibility issues that may arise if, for instance, a NP/RP system were employed. Furthermore, by avoiding size exclusion chromatography in the first dimension of the separation, problems associated with solvation of the sample in a strong injection plug after transportation to the second column are avoided.(17)

Numerous coupled multidimensional HPLC system designs that employ column switching techniques have been used in the analysis of samples in environmental, toxicological, pharmaceutical, fuel, and biomedical fields.(2) Ramsteiner(18) has illustrated many of the transfer techniques and switching functions used in coupled column processes from which column switching networks could be designed. The coupled multidimensional HPLC system used in this study is illustrated in Figures 1a-1d, with the operation of this column switching network described later in this paper.

## EXPERIMENTAL

### Chemicals

HPLC grade acetonitrile, methanol, and dichloromethane were obtained from Mallinckrodt, Australia. Milli-Q water was obtained in-house and filtered

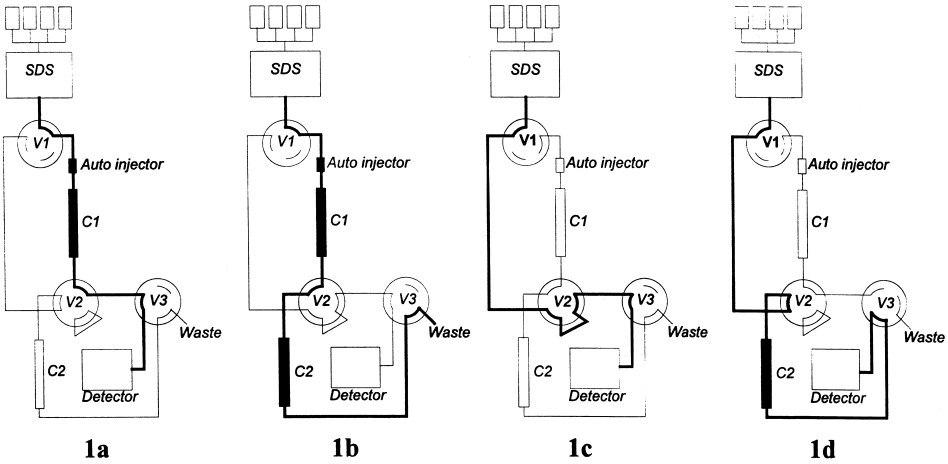


***Figure 1***. Schematic diagram of multidimensional HPLC column switching system. (SDS): Low pressure quaternary solvent delivery system; (*V1-V3*): 6-port 2- position switching valves; (*C1*): Nucleosil C18 10 μm particle diameter (100 × 4.6 mm) column; (*C2*): Carbon clad zirconia (ZirChrom- CARB) 3 μm particle diameter (100 × 4.6 mm) column. a) System configuration for a separation on C1; b. System configuration for the elution of a heart-cut band from C1 onto C2; c) System configuration for the flushing of mobile phase from the solvent delivery system; d) System configuration for the separation of heart-cut band on C2.



through a 0.2 μm filter. All mobile phases were sparged continuously with helium. A polystyrene SEC calibration standard with a molecular weight of 760 daltons, produced via anionic polymerization with *sec*-butyl lithium to produce polymers with *sec*-butyl end groups, was purchased from the Aldrich Chemical Company. The stationary phase materials used for the preparation of columns in this study were either; Nucleosil C18 (silica based), 10 μm particle size with a 10 nm pore diameter (Alltech Associates Australia, Pty. Ltd., Baulkham Hills, NSW, Australia), or carbon clad zirconia (ZirChrom-CARB), 3 μm particle size (ZirChrom Separations, Inc., Anoka, MN, USA). Both stationary phase materials 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-10AT*VP* pumping system, SIL-10AD*VP* auto injector, SPD-10A*VP* UV detector set at 262 nm, SCL-10A*VP* system controller, and Shimadzu Class-

*VP* version 5.03 software on a Pentium II 266 PC. Column switching was achieved using 6-port 2-position switching valves fitted with a micro-electric two position valve actuator (Valco Instruments Co. Inc., Houston, TX, USA). Valve switching was controlled using Shimadzu SCL-10A*VP* system controller and Shimadzu Class-*VP* version 5.03 software. 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 (Lawson Labs Inc, Malvern, PA, USA). Columns were packed using a Haskel air driven fluid pump (Haskel International, Burbank, CA, USA).

## Chromatographic Separations

The polystyrene standard with a molecular weight of 760 daltons was dissolved in 100% dichloromethane. Polystyrene sample injection volumes were 10 µL. Purified oligomer fractions were collected in methanol and, subsequently, injected into the LC system in the same solvent in a volume of 20 µL. All flow rates were 1.0 mL/min.



## Preparation of Chromatography Columns

Two 5 cm carbon clad zirconia columns were prepared using a downward slurry technique in which 6 g of stationary phase was slurried in 35 mL of 90/10 hexane/isopropyl alcohol and stirred for 30 minutes. The slurry was ultrasonicated for a period of 20 minutes, followed by a further 10 minutes of stirring. The slurry was suspended in the packing reservoir over a displacement solvent of dichloromethane contained in the column. The slurry was then compacted with an isopropyl alcohol packing solvent at a pressure of 7000 p.s.i. The column was packed at this pressure until 100 mL of isopropyl alcohol had passed through the bed.

## RESULTS AND DISCUSSION

The chromatogram illustrated in Figure 2 shows the separation of polystyrene oligomers with an average molecular weight of 760 daltons. The separation was achieved on a 10 µm particle size C18 column using a mobile phase of 100% methanol. This separation was reported in previous studies,(8,9) with the first band (labelled as $n = 3$ on Figure 2) containing three configurational repeating units with a terminating *sec*-butyl end group. (Molecular weights of the first two peaks were verified by mass spectral analysis). Thus, subsequent bands rep-

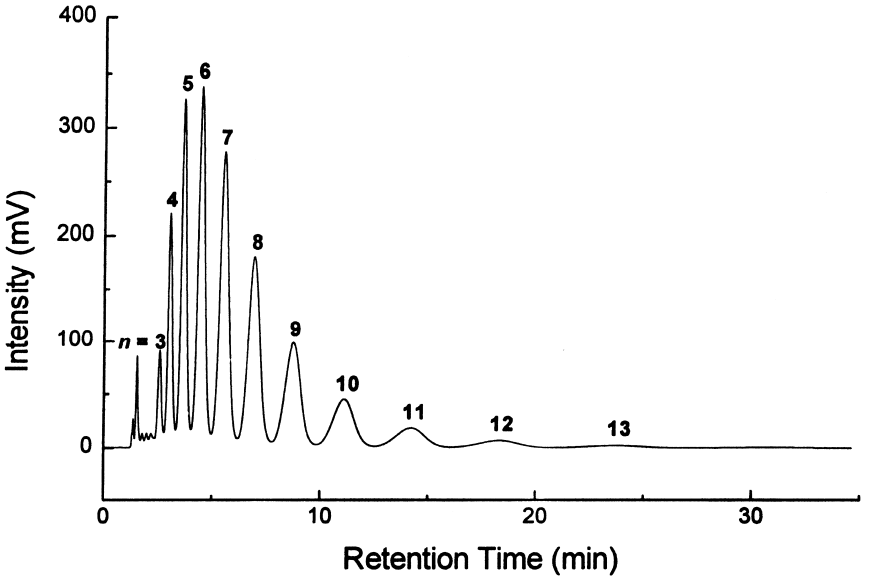


***Figure 2.*** Chromatogram of an oligomer separation of polystyrene with a molecular weight of 760 daltons. Mobile phase 100% methanol, flow rate 1.0 mL/min, injection volume 10 μL. Column C18 Nucleosil 10 μm particle diameter (100 × 4.6 mm).



resent increasing polymer chain lengths of an additional styrene unit, as labeled on Figure 2. It was also previously found, that separation of stereoisomers could be observed for each of the oligomers ($n = 3$ to $n = 7$) on a 5 cm carbon clad zirconia column in a 100% acetonitrile mobile phase. Photodiode array detection of each band eluting from the carbon clad zirconia column, revealed that each band was, in fact, polystyrene and there was no evidence of non polystyrene components underlying any of the peaks.

Previous studies have shown that improvements in resolution by reduction in the solvent strength would be limited,(8,9) so as a means of increasing the resolution of the isomer separations on the carbon clad zirconia column, the effect of temperature was examined for oligomers $n = 3$ to $n = 5$. Temperatures of –15, 0, 30, 60, and 80°C were evaluated. The chromatograms shown in Figure 3, for three of these temperatures, illustrate the influence of temperature on the isomeric separation of the oligomer $n = 4$ on a 5 cm carbon clad zirconia column. In general, the separation improved as the temperature decreased, as shown in Figure 3. However, the temperature dependant retention behaviour is more complex than this figure suggests, as for some higher order isomers, there was an improvement in resolution at low temperatures, whilst for other isomers the reso-

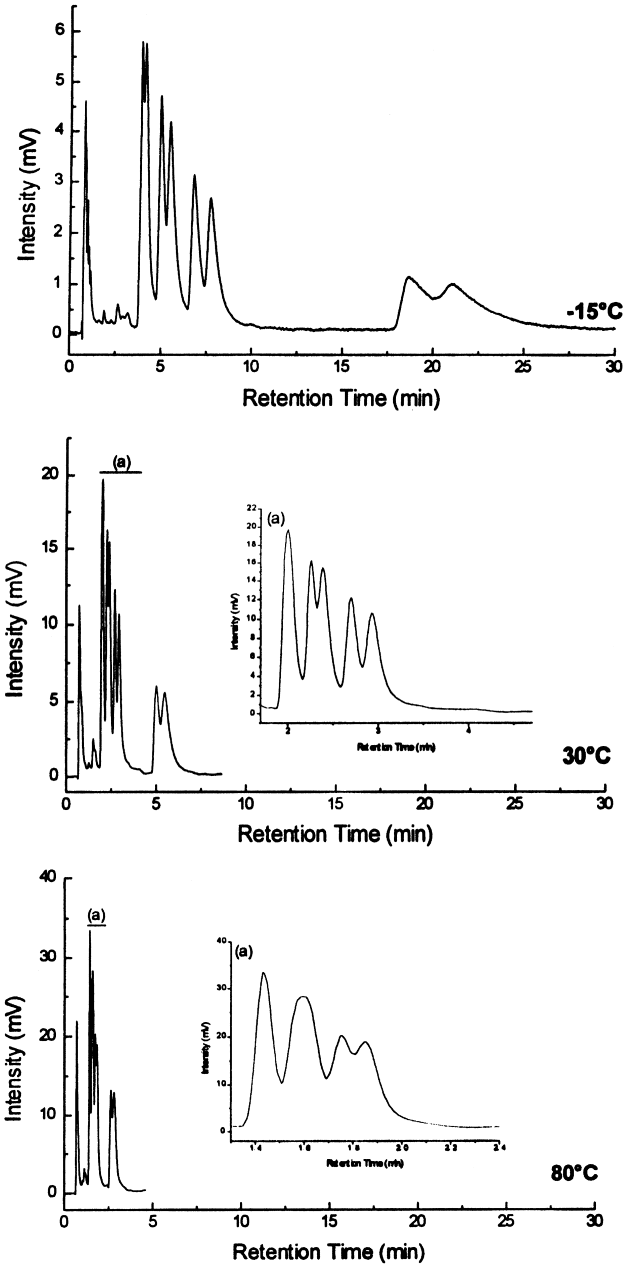


***Figure 3***. Chromatogram of an isomer separation of oligomer $n = 4$ (from Figure 2) on the carbon clad zirconia column (3 μm particle diameter, 50 × 4.6 mm) at temperatures of -15°C, 30°C, and 80°C. Mobile phase 100% acetonitrile, flow rate 1.0 mL/min, injection volume 20 μL.

lution is decreased at these lower temperatures due to severe peak distortion. This phenomenon is currently under investigation. The best compromise for the isomer separation was found to occur in the temperature range from ambient to 30°C, with the higher temperatures (60°C and 80°C) resulting in a further reduction in resolution of isomers. As a result of this complex temperature dependent elution behaviour, temperature programming was not employed as a means of gaining further resolution between isomers.

Consequently, in order to increase the resolution of the isomers on the carbon clad zirconia column, the column length was increased to 10 cm by coupling two five centimeter columns, which effectively doubled the number of theoretical plates. The series of chromatograms in Figure 4 compare the separations of the isomers ($n = 4$) on the 5 cm and 10 cm columns, showing the improvement in resolution gained by the increase in column length. Isomer separations on the carbon clad zirconia column were also attempted for the oligomers $n = 6$ and $n = 7$, however, some of the components were very strongly retained and their elution required the addition of dichloromethane to the mobile phase. We have not yet fully explored the optimisation of the isomer separation on the carbon clad zirconia column, and studies are currently under way to investigate the retention behaviour in detail. Nevertheless, for the oligomer $n = 6$, 27 out of a possible 32 diastereoisomers were observed to elute, and this indicates the excellent potential this surface may offer for isomer separations of low molecular weight polymers once the behaviour is more thoroughly understood.

One problem that is often encountered in the coupling of multidimensional separations, is that of solvent incompatibility between the separations in each dimension. Both separation dimensions in this system are reversed phase and, consequently, incompatibility between solvents has been minimized, despite great differences in the separation mechanisms on the two stationary phases. Consequently, coupling of both separations in a heart cut mode appeared to be feasible. Heart-cutting was chosen, primarily, due to the expected chaotic peak displacement that would result if the entire eluent from the C18 column was directly channeled through the carbon clad zirconia column.(8)

The multidimensional HPLC system that was used in this study is illustrated in Figures 1a-1d, which incorporated three PC controlled switching valves (*V1-V3*) and a low-pressure quaternary solvent delivery system. In Figure 1a, a mobile phase of 100% methanol was used to elute the polystyrene sample on *C1* (10 μm C18 Nucleosil) in order to separate the oligomers on the basis of increasing molecular weight. When the oligomer that is to be further fractionated elutes from *C1*, the system would switch into the position illustrated in Figure 1b, where the oligomer of interest was heart-cut from *C1* and directed onto *C2* (3 μm carbon clad zirconia). The system could then be operated in one of two ways, where either it could switch back to finish elution on *C1* (Figure 1a) before beginning the separation on *C2* (switching method 2); or alternatively, the separation of the

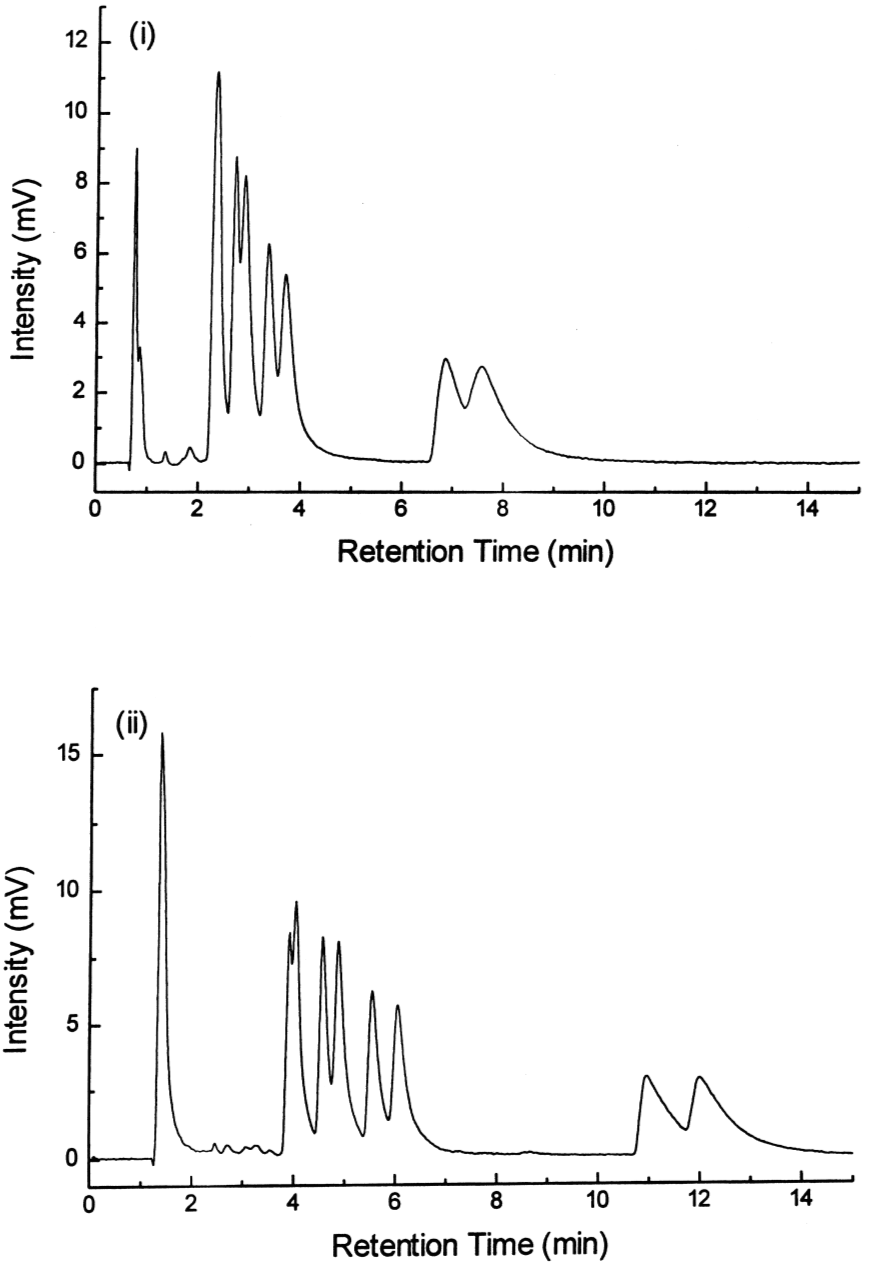


***Figure 4***. Chromatogram of isomer separations of oligomer $n = 4$ (from Figure 2) on the carbon clad zirconia column (3 μm particle diameter). (i) 50 × 4.6 mm and (ii) 100 × 4.6 mm. Conditions as in Figure 3.

heart-cut oligomer could be completed on *C2* (Figures 1c and 1d) before completing the separation on *C1* (switching method 1).

In Figure 1c, acetonitrile mobile phase is flushed through the system to remove methanol mobile phase remaining in the solvent delivery system. In the quaternary solvent delivery system used in this study, all mobile phases elute through a single channel. Therefore, when changing from the methanol mobile phase used in *C1* to the acetonitrile mobile phase used in *C2*, acetonitrile must firstly displace residual methanol from the solvent delivery system before elution onto *C2* (hence the flush to waste in Figure 1c). Figure 1d illustrates the elution of the heart-cut section on *C2* using a mobile phase of 100% acetonitrile, except for $n = 6$ oligomer where 85% acetonitrile:15% dichloromethane mobile phase was used.

The system was operated using timed event tables that were computer controlled using appropriate software. Examples of these event tables are shown in Tables 1 and 2, illustrating the event tables for the coupled multidimensional separation of the $n = 4$ oligomer for the two switching methods used in this study. Clearly, the complexity of these timed events demand that coupled multidimensional techniques require precise valve switching, which could only be achieved using a PC controlled system.

Figure 5 illustrates an overview of the heart-cutting procedure that was employed in this study, using the $n = 4$ polymer as an example. In the first instance, the polystyrene sample is separated into individual oligomers on the basis of increasing polymer chain length, on a C18 column in methanol mobile phase, as shown in Figure 5, chromatogram (i). Using the multidimensional system, it was possible to heart-cut or isolate a specific oligomer of interest, in order to conduct another separation on that oligomer using a different separation mechanism (dimension), which in this study was a carbon clad zirconia column. For the $n = 4$ oligomer, in the first C18 dimension, a single band was observed to elute, which is highlighted in the hashed box. The band (or portion of the band) could then be isolated from the first dimension separation, as indicated by the hashed region, and directed onto the second dimensional separation. In this second dimension, the band is then separated on the basis of spatial arrangement, in that the oligomer is then further divided into isomers that make up that oligomer as shown in Figure 5, chromatograms (ii) and (iii). Chromatogram (iii) is an expansion of the separation that occurred in the second dimension on the carbon clad zirconia column. A reduction in peak intensity occurred because the sample that eluted as a single band in chromatogram (i) is now further divided into seven bands. Upon completion of the separation in the second dimension, one is then able to revert back to the initial dimension if required. Thus, two separations that employ differing separation mechanisms can be conducted using a single system, without manual intervention, and reported as a single output.

***Table 1.*** Timed Event Table for the Coupled Multidimensional HPLC Separation of the $n = 4$ Oligomer Using Switching Method 1

| Time (Minutes) | Event | Value |
|---|---|---|
| 0.01 | B.Conc | 100.0 |
| 0.01 | C.Conc | 0.0 |
| 2.75 | Event | 2.0 |
| 3.15 | Event | 12.0 |
| 3.16 | B.Conc | 100.0 |
| 3.16 | C.Conc | 0.0 |
| 3.17 | C.Conc | 100.0 |
| 3.18 | T.Flow | 1.0 |
| 3.30 | T.Flow | 4.2 |
| 4.30 | T.Flow | 4.2 |
| 4.31 | T.Flow | 0.2 |
| 4.49 | T.Flow | 0.2 |
| 4.50 | Event | 13.0 |
| 5.00 | T.Flow | 1.0 |
| 22.50 | Event | 12.0 |
| 22.51 | C.Conc | 100.0 |
| 22.51 | B.Conc | 0.0 |
| 22.52 | B.Conc | 100.0 |
| 22.52 | C.Conc | 0.0 |
| 22.53 | T.Flow | 1.0 |
| 22.60 | T.Flow | 4.2 |
| 23.60 | T.Flow | 4.2 |
| 23.70 | T.Flow | 0.2 |
| 23.99 | T.Flow | 0.2 |
| 24.00 | Event | 0.0 |
| 25.00 | T.Flow | 1.0 |
| 50.00 | T.Flow | 1.0 |

B.Conc: Methanol Concentration (%); C.Conc: Acetonitrile Concentration (%); T.Flow: Flow Rate (mL/min); Event: Valve Switch (1:*V1*, 2:*V2*, 3:*V3*, from Figure 1a-1d).

Figures 6a-6d illustrate the separations achieved for the oligomers $n = 3$ to $n = 6$ using the coupled multidimensional HPLC system, where separation of the heart-cut oligomer was conducted on *C2* immediately after the cutting process. Once the separation was completed on *C2*, elution of the remaining polystyrene oligomers was concluded on *C1*. For each of the four oligomers separated using this column switching technique, the resolution of the isomers was comparable to injection of individual oligomers onto the carbon clad zirconia, which is evident

***Table 2.*** Timed Event Table for the Coupled Multidimensional HPLC Separation of the $n = 4$ Oligomer Using Switching Method 2

| Time (Minutes) | Event | Value |
|---|---|---|
| 0.01 | B.Conc | 100.0 |
| 0.01 | C.Conc | 0.0 |
| 2.75 | Event | 2.0 |
| 3.15 | Event | 0.0 |
| 40.01 | Event | 12.0 |
| 40.02 | B.Conc | 100.0 |
| 40.02 | C.Conc | 0.0 |
| 40.03 | C.Conc | 100.0 |
| 40.04 | T.Flow | 1.0 |
| 40.20 | T.Flow | 4.2 |
| 41.20 | T.Flow | 4.2 |
| 41.21 | T.Flow | 0.2 |
| 41.39 | T.Flow | 0.2 |
| 41.40 | Event | 13.0 |
| 42.40 | T.Flow | 1.0 |
| 60.01 | Event | 12.0 |
| 60.02 | C.Conc | 100.0 |
| 60.02 | B.Conc | 0.0 |
| 60.03 | C.Conc | 0.0 |
| 60.03 | B.Conc | 100.0 |
| 60.04 | T.Flow | 1.0 |
| 60.30 | T.Flow | 4.2 |
| 61.30 | T.Flow | 4.2 |
| 61.31 | T.Flow | 0.2 |
| 61.49 | T.Flow | 0.2 |
| 61.50 | Event | 0.0 |
| 62.50 | T.Flow | 1.0 |

B.Conc: Methanol Concentration (%); C.Conc: Acetonitrile Concentration (%); T.Flow: Flow Rate (mL/min); Event: Valve Switch (1:*V1*, 2:*V2*, 3:*V3*, from Figure 1a-1d).

by comparing the separation for the $n = 4$ oligomer in Figure 4 (ii) and Figure 6b. However, a slight loss in resolution was observed, probably as a result of large transfer volumes from *C1* to *C2* during the switching process (ranging from 300-600 μL, depending on the oligomer). Despite these large volumes, the resolution of the isomer separations on *C2* is still maintained to a large degree.

The separation of the remaining oligomers on *C1*, following the switch and separation on *C2*, is comparable to that in Figure 2, despite the oligomers spend-

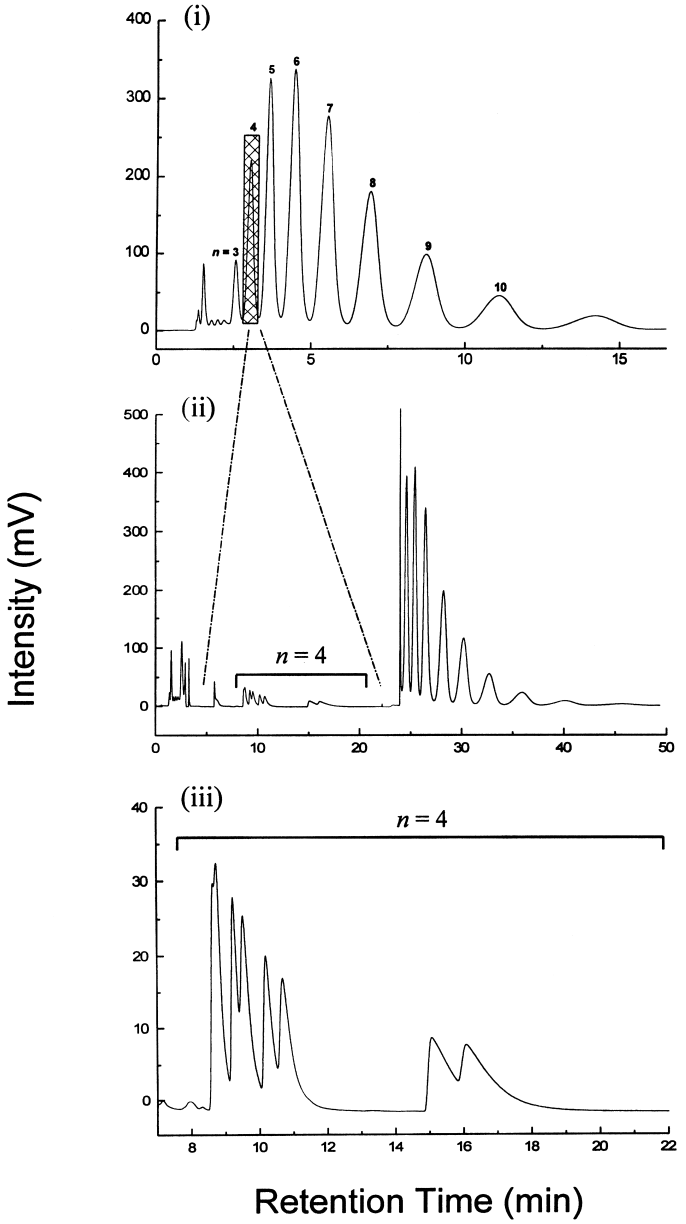


***Figure 5***. Overview of the heart-cutting technique employed using the coupled multidimensional HPLC system (Figures 1a-1d). (i) Polystyrene (Mw = 760 daltons) oligomer separation on C18 in methanol mobile phase. (ii) Chromatogram of the isomer separation of n = 4 oligomer (from Figure 2) from polystyrene (760 daltons) using the coupled multidimensional HPLC system (Figures 1a-1d). (iii) Expansion of isomeric separation of n = 4 oligomer on carbon clad zirconia from Figure 4 (ii).

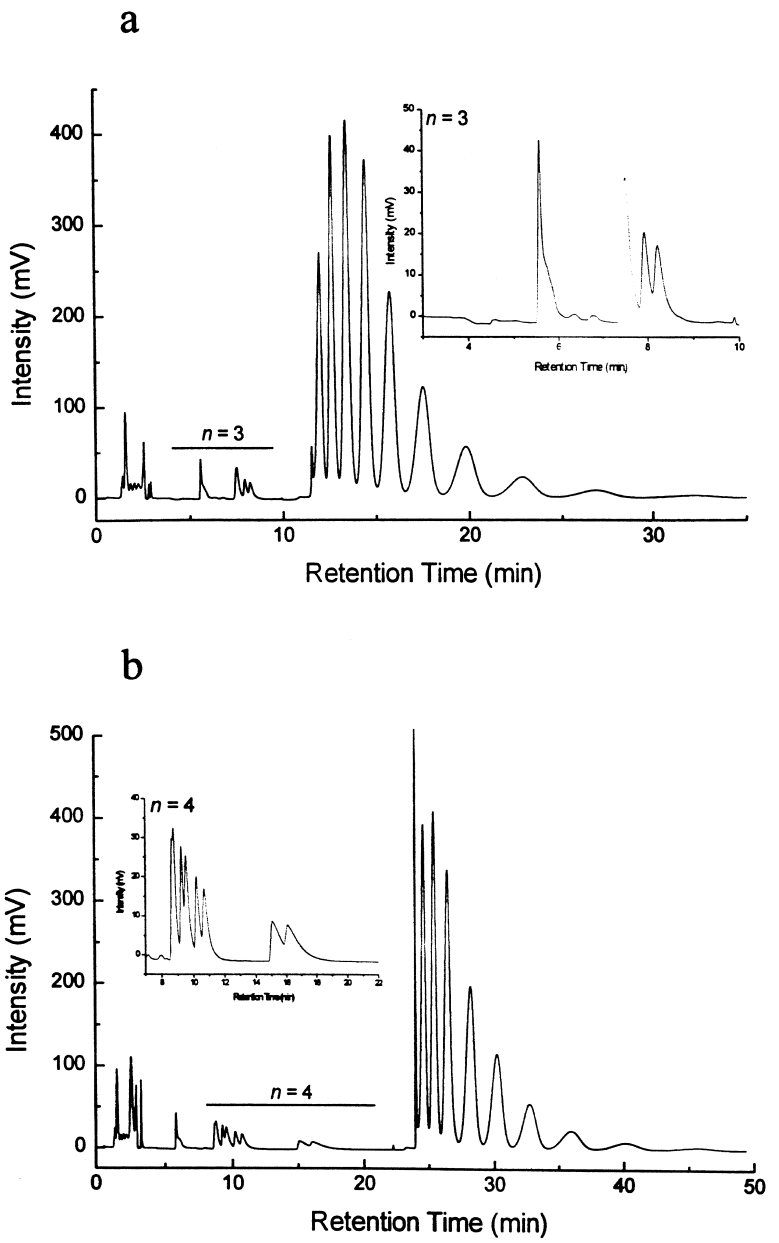


***Figure 6***. Chromatogram of isomer separations of oligomers $n$ = 3 and $n$ = 6 (from Figure 2) from polystyrene (760 daltons) using the coupled multidimensional HPLC system (Figures 1a-1d). Switching method 1. a) Oligomer n = 3. b) Oligomer n = 4. c) Oligomer n = 5. d) Oligomer n = 6.

*(continued)*

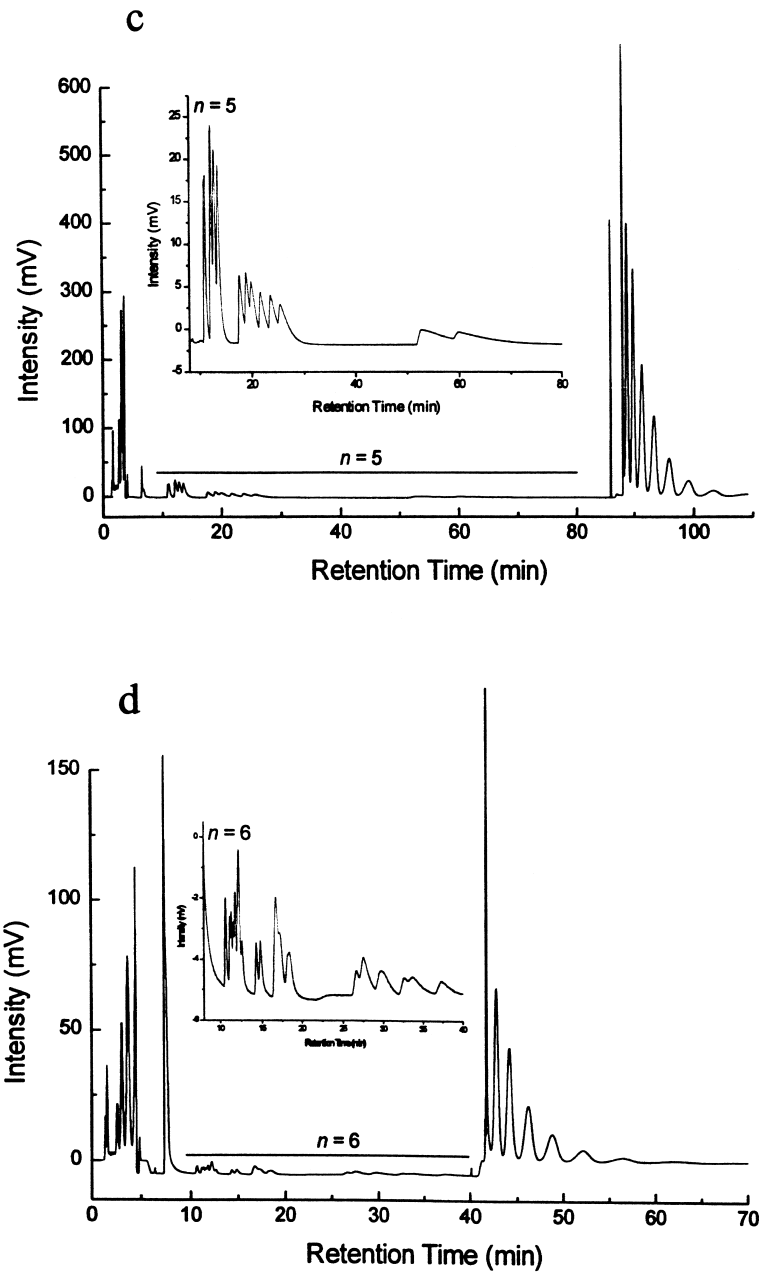


*Figure 6*. Continued.

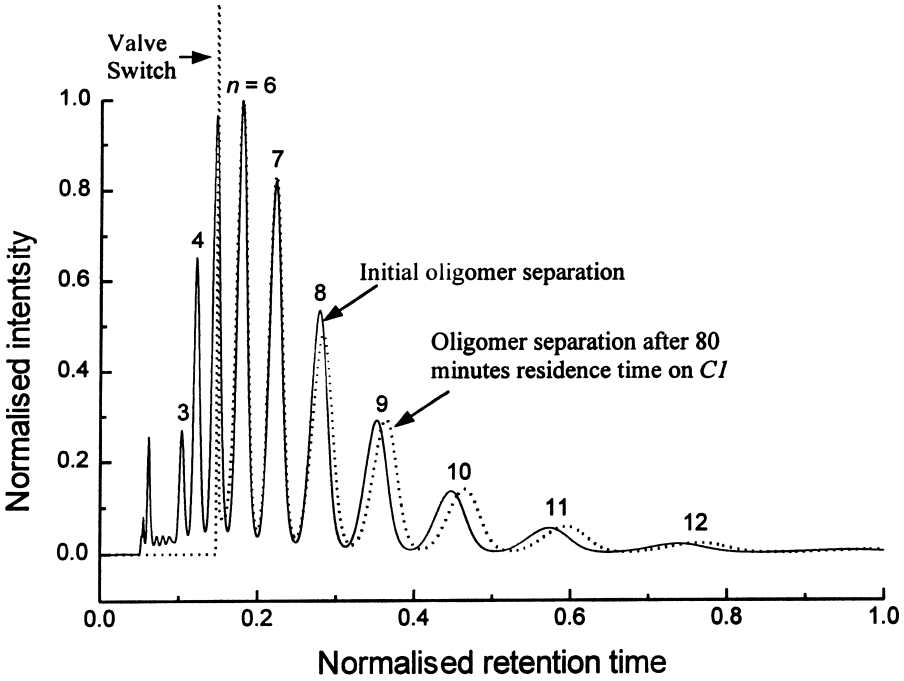


***Figure 7.*** Illustration of the band broadening due to the diffusion of the polystyrene oligomers during the eighty minute residence time on C1 under conditions of zero flow. (a) Chromatogram of the polystyrene oligomers under continual flow (solid curve). (b) Chromatogram of the polystyrene oligomers after a period of 80 minutes without flow (dotted curve).

ing a long period of time on *C1* (as long as 80 minutes as in Figure 6c) without migration. This shows that diffusion on *C1* during this period was minimal. This is illustrated in Figure 7, which compares the band widths of the oligomers in a separation on *C1* without a heart-cut fraction, to one in which a heart-cut has been made and the remaining polymer allowed to sit on *C1* for a period of 80 minutes. Note, that in this figure, the time axis has been normalised to the retention time of the most concentrated oligomer.

Figures 8a-8d show the separation of oligomers $n = 3$ to $n = 6$, using the system in a manner where the oligomer is heart-cut from *C1* to *C2*, with elution of the cut section completed on *C2* following the complete separation of the polystyrene oligomers on *C1*. Once again, only slight losses in resolution of the isomer separations was observed using the column switching method, with the two

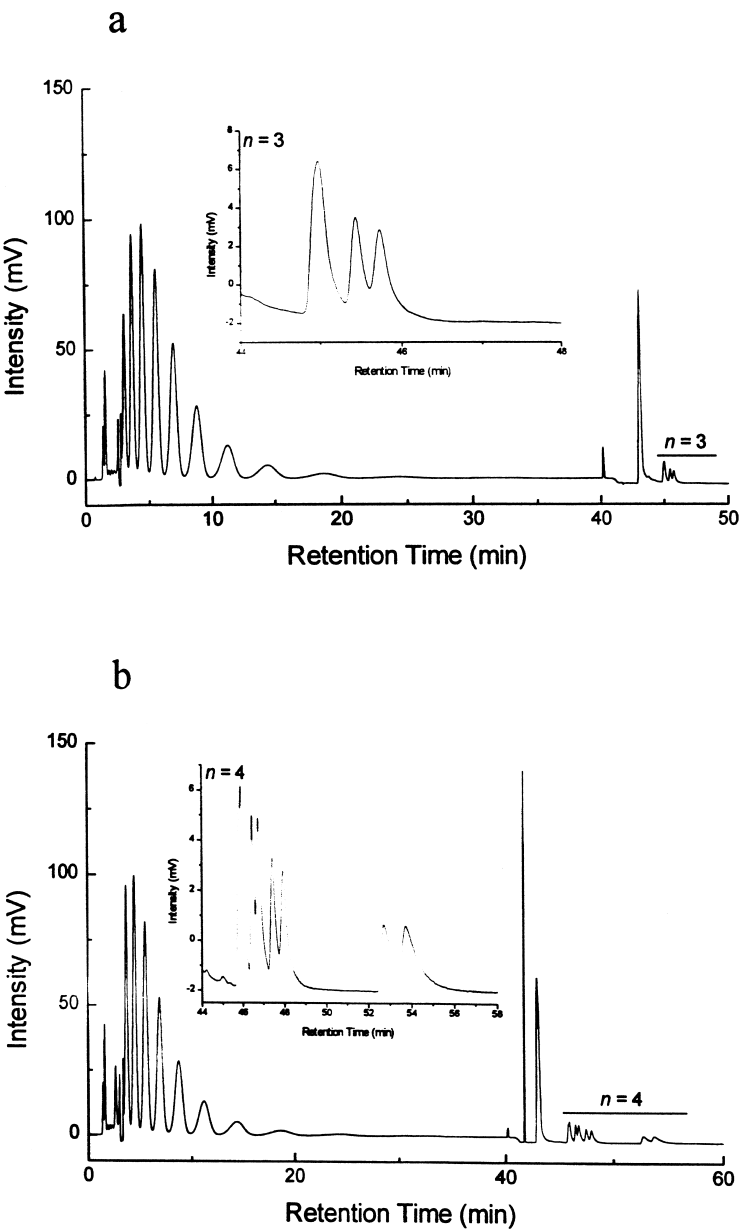


***Figure 8***. Chromatogram of isomer separations of oligomers $n = 3$ to $n = 6$ (from Figure 2) from polystyrene (760 daltons) using the coupled multidimensional HPLC system (Figures 1a-1d). Switching method 2. a) Oligomer n = 3. b) Oligomer n = 4. c) Oligomer n = 5. d) Oligomer n = 6.

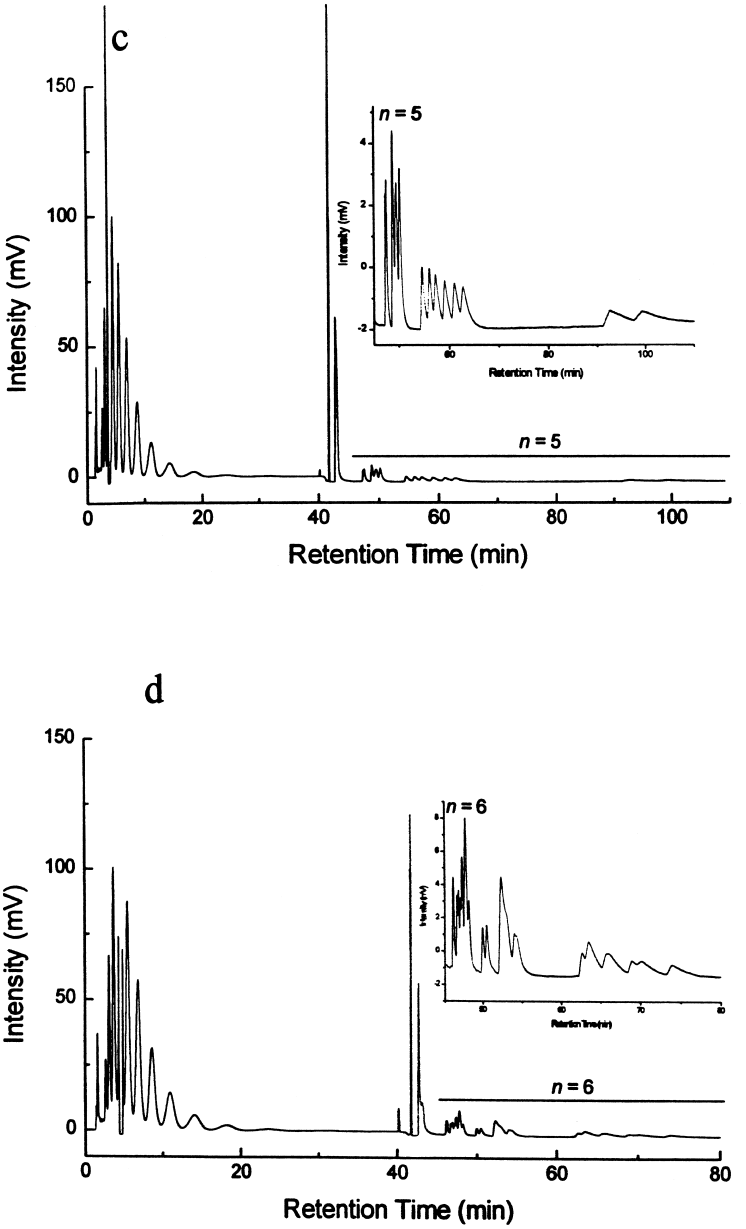


*Figure 8*. Continued.

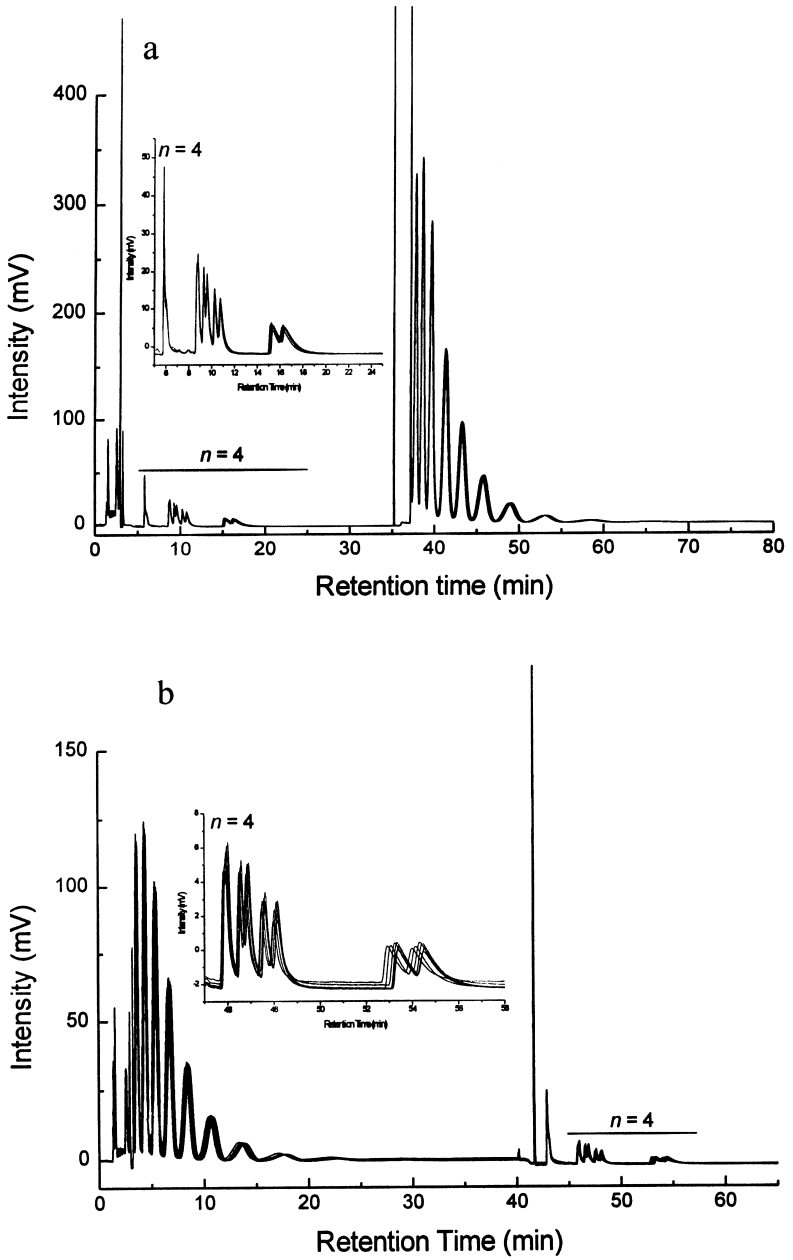


***Figure 9***. Coupled multidimensional HPLC system (Figures 1a-1d) reproducibility test, using oligomer $n = 4$. a) Switching method 1. b) Switching method 2.

methods of column switching yielding comparable results in terms of the separations on *C1* and *C2*.

Given that the method of heart-cutting used in this system was based on timed events using retention time information, it was necessary to test the reproducibility of the system in its ability to consistently heart-cut bands from *C1* from one run to another. An initial test run of the first separation is required to establish the timing of the events in Tables 1 and 2. For a coupled HPLC system to function effectively, it must be able to reproduce the separation required in an automated fashion from one run to the next. This is because the advantages of using a coupled multidimensional HPLC system, apart from the obvious advantages of providing two dimensional separations, is that once established it should operate automatically with minimal operator supervision. Also, using coupled systems, the need for manual sample fractionation from the first dimension, followed by concentration and preparation of collected fractions, is eliminated. This could be advantageous where relatively non-volatile solvents are required in the first dimension that could make sample concentration difficult.

Figures 9a and 9b illustrate the reproducibility of the system over an eight-hour period, a typical working day. Firstly, an injection of the polystyrene standard was injected onto *C1* in order to establish the retention times of each of the oligomers. Oligomer $n = 4$ was then used as a test sample on which to examine



***Table 3.*** Comparison of RSD for Coupled Multidimensional HPLC System Reproducibility Test over a Period of Six Injections for Switching Methods 1 and 2 Using Oligomer $n = 4$

| Switching Method 1 | | | Switching Method 2 | | |
|---|---|---|---|---|---|
| Average Rt | RSD Rt (%) | RSD Area (%) | Average Rt | RSD Rt (%) | RSD Area (%) |
| 8.772# | 0.133 | 3.305 | 3.628* | 1.625 | 3.719 |
| 9.274# | 0.172 | 3.632 | 4.404* | 1.743 | 3.487 |
| 9.569# | 0.167 | 3.775 | 5.407* | 1.741 | 3.570 |
| 10.243# | 0.258 | 3.276 | 6.718* | 1.731 | 3.810 |
| 10.745# | 0.304 | 3.751 | 8.437* | 1.688 | 5.083 |
| 15.253# | 0.561 | 3.402 | 45.932# | 0.091 | 12.649 |
| 16.278# | 0.592 | 3.798 | 46.505# | 0.106 | 11.562 |
| 37.781* | 0.033 | 2.062 | 46.819# | 0.113 | 15.385 |
| 38.588* | 0.045 | 2.685 | 47.531# | 0.149 | 1.299 |
| 39.623* | 0.057 | 2.670 | 48.061# | 0.170 | 15.698 |
| 41.297* | 0.155 | 2.572 | 53.183# | 0.363 | 11.476 |
| 43.225* | 0.197 | 2.313 | 54.278# | 0.403 | 20.660 |

# Isomer bands on carbon clad zirconia/100% acetonitrile; * Oligomer bands on C18/100% methanol.

the system reliability. Figure 9a shows the separation of $n = 4$ using the column switching method, where elution on *C2* was conducted before completion of the polystyrene oligomer separation on *C1*. Six replicate injections, each having an 80 minute run time, were completed with an overlay of each run shown in Figure 9a. From this figure, it can be seen that little change in peak shape or retention time was observed for each of the replicates in both the separations on *C1* and *C2*. Table 3 contains information on the reproducibility of retention times and peak areas for bands in Figure 9a. The same six replicate injections for the separation of $n = 4$ oligomer was also conducted for the switching technique, where elution on *C2* was completed following elution of oligomers on *C1*. An overlay of each run is illustrated in Figure 9b. The reproducibility of peak areas and retention times of some bands are also given in Table 3.

The results in Table 3 show that the RSD of the retention time for both methods of column switching was less than 2% (less than 0.6% for switching method 1). The RSD of peak area of polystyrene isomers on carbon clad zirconia was less than 4% using switching method 1, but using switching method 2, the peak area RSD was up to 20%, indicating that for quantitative analysis switching method 1 would be most appropriate.



## CONCLUSION

A coupled multidimensional HPLC system was employed for the separation of polystyrene oligomers that utilised a heart-cutting column switching technique. Using this system, the separation of polystyrene isomers from oligomers consisting of 3-6 configurational repeating units was performed in an automated mode using a series of switching valves. The system was found to be reproducible over a period of eight hours continuous operation, using two different column-switching techniques. The resolution of the isomer separations on the carbon clad zirconia column following the automated heart-cut from the oligomer separation on the C18 column, was comparable to the resolution that was obtained when purified oligomer fractions were manually injected onto the carbon clad zirconia column. As a result, this paper demonstrated the advantages of column switching techniques for the analysis of complex multidimensional mixtures.

Furthermore, this paper illustrates, that the application of automated multidimensional HPLC in the analysis of polymers will allow for the rapid evaluation of sample composition. That is, the technique could be readily adapted to evaluate the tacticity of polymers and may be suitable for studies involving rates of polymerisation.

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