Short communication

# Molecular differences between deuterated and protonated polystyrenes using reversed-phase high-performance liquid chromatography

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## Abstract

Isotopic substitution is a technique used to highlight particular bonds within a molecule for kinetic, spectroscopic and structure analysis. It is presumed that although some properties such as stretching frequencies will not be the same for substituted analogues, the chemical interactions will not vary appreciably as a function of labelling. Reversed-phase liquid chromatography has been used to demonstrate that there are significant differences between the chromatographic behaviour of a sequence of deuterated and protonated oligomeric polystyrenes. Two-dimensional reversed-phase liquid chromatography was used to show that even the diasteromers of the oligomers ($n = 5$) have retention mechanisms that are dependent on the subtle changes to the molecular conformation and electronic structure, which are a consequence of deuteration.
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## 1. Introduction

Deuterated compounds are generally considered to have very similar chemical and physical properties to their protonated analogues. Deuterated polymers are often used in small angle neutron scattering (SANS) and neutron reflectivity experiments because there is a large difference in the neutron coherent scattering lengths between deuterium and hydrogen, and this allows a range of contrasts to be selected for highlighting segments of the molecule(s) for example in polymer miscibility experiments [1–6]. However, the addition of the neutron to the nucleus of the hydrogen atom to create deuterium changes the nuclear polarizability of the atom. Substitution of hydrogen by deuterium lowers the zero point energy and coupled with the anharmonicity of intermolecular potentials, this gives shorter bond lengths, decreased molar volumes, and smaller bond polarizabilities for deuterated compounds [7]. Bates et al. [7,8] used SANS

to show that 50:50 mixtures of protonated/deuterated polymers do not form ideal solutions, and will phase separate at temperatures below the upper critical solution temperature, and he attributed this to positive segment–segment interaction energies between the polymers in these solutions. Hong et al. [1] found significant surface enrichment of deuterated polystyrene in mixtures of protonated polystyrene and deuterated polystyrene when these were examined by surface enhanced Raman scattering (SERS) [9]. This was attributed to deuterated polystyrene having a lower surface energy than protonated polystyrene. Fenby et al. [6] discussed at length the increase in bond strength, an increase in the enlarged domain and a decrease in London dispersion forces as a result of deuteration, as explained by the Rabinovich model [6]. Wang et al. [10] found deuterated polystyrene had a smaller mean segment size, and a large chemistry-dependent constant than protonated polystyrene. The smaller mean segment size indicated that at the Flory temperature, a deuterated polystyrene chain has a smaller conformation (coils more tightly) than a polystyrene chain of the same length. The larger chemistry-dependent constant indicates that in a good solvent, a

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deuterated polystyrene chain is able to extend more than a polystyrene chain for a given increase of the reduced temperature [10]. Thin layer chromatography [11] can be used for separation of deuterated and protonated polystyrenes. In the present study we illustrate the magnitude of these differences using reversed-phase high-performance liquid chromatography (RP-HPLC). These measurements demonstrate that protonated polystyrene and deuterated polystyrene have different thermodynamic properties, which are a monotonic function of molecular mass.

## 2. Experimental

### 2.1. Chemicals

[$^2H_8$]Styrene (styrene-d$_8$) and styrene were purchased from Sigma–Aldrich. Styrene and styrene-d$_8$ were purified by distillation from calcium hydride under dry nitrogen. n-Butylpolystyrene (weight-average molecular mass, $M_w = 1312$ g mol$^{-1}$) and n-butyl-[$^2H_8$], polystyrene (polystyrene-d$_8$; $M_w = 852$ g mol$^{-1}$) were synthesised by anionic polymerisation using n-butyllithium as the initiator, toluene as the solvent, and methanol for termination. Average molecular masses of both polymers were determined by size-exclusion chromatography and comparison to standard polystyrenes. The chromatography column used for oligomeric separations in this study was a SphereClone C$_{18}$ ODS(2) (150 mm × 4.6 mm, 5 μm Pd), purchased from Phenomenex Australia. Chromatography columns used for the two-dimensional oligomer-diastereomer separations were a C$_{18}$ Valuepak (250 mm × 4.6 mm, 5 μm Pd) purchased from Activon (manufacturer no longer trading), and a Carbon Clad Zirconia (CCZ) column (30 mm × 4.6 mm, 3 μm Pd) packed in the laboratory from stationary phase purchased from ZirChrom Separations (Anoka, MN, USA) using a protocol previously published [12].

### 2.2. Instrumentation and separations

All chromatographic experiments were conducted using a Shimadzu LC system (Shimadzu Scientific Instruments, Rydalmere, Australia) incorporating a LC-10ATVP pumping system, SIL-10ADVP auto injector, SPD-10AVP UV detector, SCL-10AVP system controller and Shimadzu Class-VP version 5.03 software on a Pentium II 266 MHz personal computer. Data acquisition was achieved using Lawsons Lab model 203 data acquisition board at 5 Hz. Column switching was achieved using six-port two-position switching valves fitted with microelectric two position valve actuators (Valco Instruments Houston, TX, USA). One additional UV–vis detector was employed to record chromatographic information in the second dimension. A Waters 6000A pump (Waters Associates) was used to control flow in the second dimension. The temperature of the column and all pre-column tubing (including a 2 mL loop prior to the in-

jection valve) were thermostated in a water bath (±0.2 °C). The deuterated and protonated n-butyl polystyrenes were dissolved in dichloromethane and made up to a concentration of approximately 1 mg/mL. All oligomeric separations in a one-dimensional system were conducted using a methanol–7% tetrahydrofuran (THF) (93:7) mobile phase, at a flow-rate of 1.0 mL/min (injection volumes were 5 μL). For two-dimensional separations, where the aim was to resolve oligomers in the first dimension and diasteromers in the second dimension, the mobile phase in the first dimension was 100% methanol (1.0 mL/min) and in the second dimension, the mobile phase was 100% acetonitrile (2.0 mL/min) and maintained at 30 °C. Specific details of the two-dimensional RP-HPLC system have been published previously [12]. The CCZ column was packed using a Haskel air driven fluid pump (Haskel International, Burbank, CA, USA).

## 3. Results and discussion

The generic structure of an atactic polystyrene with five configurational repeating units is shown in Fig. 1.

There are numerous examples of oligomeric separations of polystyrenes using RPHPLC in the literature. Fig. 2 illustrates the separation of deuterated and protonated n-butyl polystyrene, respectively. This separation can be achieved using a standard C$_{18}$ column with a methanol–tetrahydrofuran mobile phase. The polystyrene samples separate into their individual oligomers with their elution time increasing monotonically with molecular weight. Closer inspection of these chromatograms reveals significant retention differences between the deuterated and protonated n-butyl polystyrenes. For the lower order oligomers, the retention time for each oligomer is similar for both the deuterated and protonated polystyrene, whereas for the higher order oligomers, the difference in retention becomes more significant. For example, the $n = 10$ and 14 oligomers have approximately a 9%
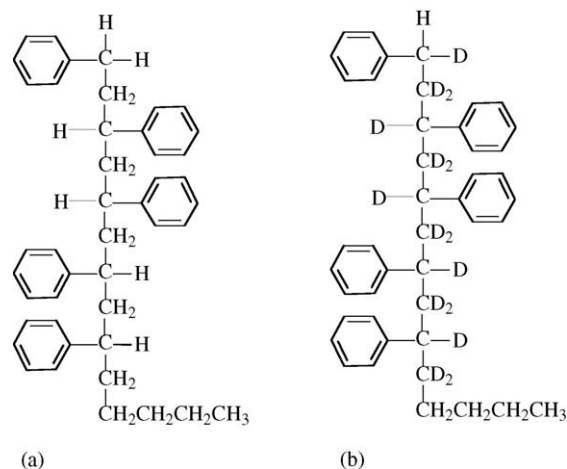


Fig. 1. Molecular structure of the ($n = 5$) butyl oligostyrene (atactic) illustrating the difference between the protonated (a) and deuterated (b) forms.
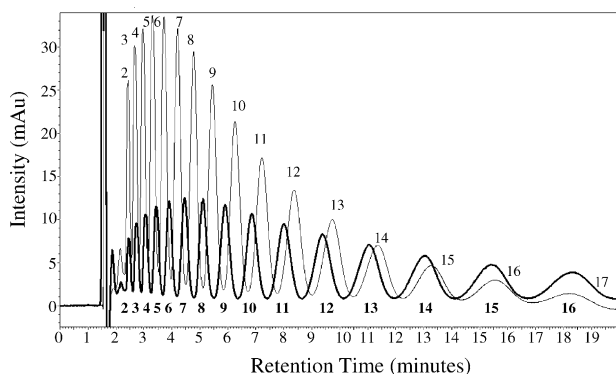
Fig. 2. Chromatogram of deuterated *n*-butyl polystyrene and protonated *n*-butyl polystyrene (bold line) at 55 °C. Mobile phase, methanol–THF (93:7); standard concentration, 1 mg/mL; flow-rate, 1.0 mL/min; Sphereclone $C_{18}$ ODS (2) column; 5 μL injection volume; detection, 262 nm.

and 13% difference in retention times between the deuterated and protonated analogues. The results illustrate that as the oligomer number increases, and therefore, the molecular mass of the polymer, there is an increasing distinction between the deuterated and protonated polystyrene. These retention differences observed chromatographically can further be seen more clearly in the relationship between log $k$ and log molecular mass (Fig. 3). The results are in accordance with the Martin equation.

We further investigated these retention differences by using a two-dimensional heart cutting technique [12], where the $n = 5$ oligomer for both the deuterated and protonated *n*-butyl polystyrene was transported to the second dimension and its respective diastereoisomers were separated. We have previously published detailed information regarding the separation of diasteromers of oligostyrenes and consequently we need not discuss this technique further. Suffice to say, however, that the resolving power the 2D system is increased by virtue of the fact that the first dimension exclusively separates the oligostyrenes according to molecular weight, while the second dimension utilises the shape selectivity of the carbon surface in order to enable the separation of the diasteromers. Fig. 4 illustrates the chromatograms for the second dimension
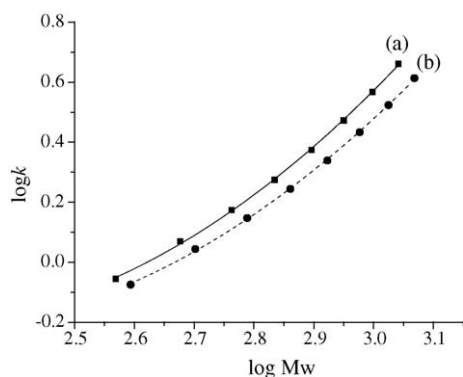


Fig. 3. Plot of log $k$ vs. log molecular mass for (a) protonated and (b) deuterated *n*-butyl polystyrene (at 50 °C).

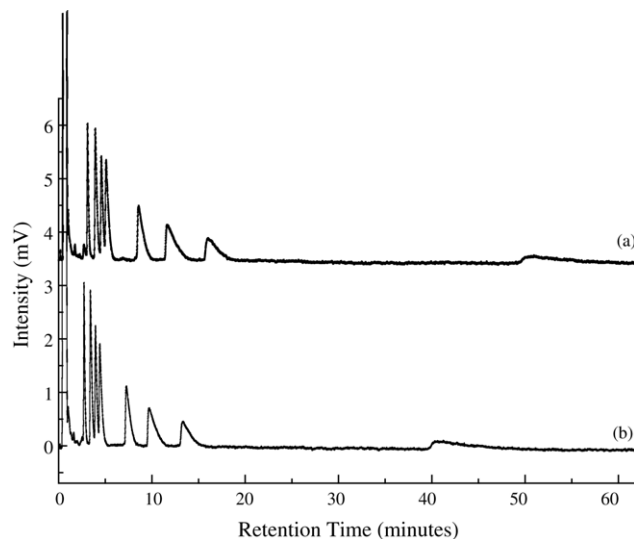

Fig. 4. Diasteromer separation of $n = 5$ oligomer for protonated *n*-butyl polystyrene (a) and deuterated *n*-butyl polystyrene (b) on a CCZ column with 100% acetonitrile mobile phase operated at 2.0 mL/min at 30 °C. UV detection at 262 nm.

separation of the $n = 5$ oligomer for the deuterated and protonated *n*-butyl polystyrene respectively into its diasteromers. The same band profile pattern arises, but with differences in the degree of retention. The difference in retention is minor for the early eluting diasteromers and becomes more marked as retention increases. For example, the last diasteromer for the deuterated *n*-butyl polystyrene (Fig. 4b) elutes approximately 10 min earlier than the same diasteromer for the protonated *n*-butyl polystyrene (Fig. 4a). Similar results were observed for the diasteromer separation of the lower oligomeric members of this series (results not shown).

## 4. Conclusion

The effect of deuteration significantly alters the chromatographic retention process. Not only are there significant differences in retention between different molecular mass oligomers, but these differences extend to the diasteromer level, presumably resulting from subtle differences in molecular conformation and electronic structure of these polymers. The exploitation of the carbon clad zirconia surface to separate diasteromers of the isotopic substituted polystyrenes by π–π type interactions has shown that the molecular shape of the deuterated molecule is a significant factor in the description of the retention process for this system.

## Acknowledgements

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