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Comprehensive two-dimensional liquid chromatography for the separation of protonated and deuterated polystyrene☆

Pritish Sinha[a], Gareth W. Harding[a,1], Khumo Maiko[a], Wolf Hiller[b], Harald Pasch[a,*]

[a] Department of Chemistry and Polymer Science, University of Stellenbosch, Private Bag X1, 7602 Matieland, South Africa
[b] Faculty of Chemistry, Technical University Dortmund, Otto-Hahn-Strasse 6, 44221 Dortmund, Germany

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ABSTRACT

Liquid chromatography at critical conditions (LCCC) has been shown to be a powerful method for the separation of complex polymers regarding chemical composition, functionality, or molecular topology. LCCC has never been used, however, to separate polymers according to the degree of deuteration. This is a very challenging task since polymers shall be separated that are identical regarding molar mass, endgroups and chemical composition. In the present work, critical conditions were established in such a way that one component of a complex mixture elutes at critical conditions, whereas the other component shows size exclusion chromatography (SEC) behaviour. Blends of protonated (h) and deuterated (d) polystyrene (PS) were separated by LCCC at critical conditions of both h-PS and d-PS. Depending on the molar masses of the blend components, baseline separation could be achieved. In order to improve the separation further, comprehensive two-dimensional liquid chromatography was carried out on a number of model blends. In the first dimension LCCC was used, which separated the blends according to isotopic effects whereas in the second dimension the separation took place with respect to hydrodynamic volume. In order to further improve the separation of a number of blends a separation protocol was used where one component shows SEC conditions whereas the other component shows liquid adsorption chromatography (LAC) conditions. This separation protocol was achieved by varying the column temperature.

1. Introduction

The physical properties of deuterated polymers have been widely studied for many decades [1,2]. With respect to their protonated analogues they are generally considered to possess very similar physical and chemical properties. These interesting polymers are used in a number of applications. For example, deuterated polymer foams are used for thermo nuclear fusion targets [3], deuterated compounds are used in laser and optical technology [4–9], synthetic deuterated fibres may be incorporated in currency notes [10], and a large number of deuterated drugs have been synthesised in order to study their movement and metabolism in bodies of humans and other animals [11–13]. These materials are extensively characterised by small-angle neutron scattering (SANS) [14–20], neutron reflectivity (NR) [21] and surface enhanced Raman scattering (SERS) [22,23].

There are very few chromatographic separation studies performed on deuterium-labelled polymers. In the case of small molecules [24] the chromatographic differentiation of deuterated molecules from their hydrogenous analogues was realised a long time ago. In polymer science thin layer chromatography (TLC) was used for separating isotopically different polymers. This technique was used for the separation of deuterated and protonated polystyrenes [25]. The hydrophobic interactions of deuterated aromatic compounds were studied using reversed phase liquid chromatography (RPLC) [26]. The chromatographic separation of racemates on the basis of isotopic chirality has been reported [27,28]. A number of authors have reported on the RPLC separation of several isotopic compounds [29–31]. An extensive study on the isotopic effects on the separation of deuterium and tritium labelled compounds from unlabelled ones using RPLC separation was carried out [32]. There is a significant isotope effect in the RPLC retention of polybutadiene as reported by Perny et al. [33]. Kayillo et al. [34,35] reported on the deuterium isotope effect in the RPLC retention of oligostyrenes. They found that the deuterated polymers are retained less than their hydrogenous analogues, similar to small molecules. Kim et al. [36] used RPLC and NPLC (normal phase liquid chromatography) and examined the retention of deuterated PS relative to protonated PS. Ahn et al. [37] reported on the characterisation of comb-shaped polystyrene, where deuterated

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* Corresponding author. Tel.: +27 21 8083173; fax: +27 21 808 4967.
E-mail address: hpasch@sun.ac.za (H. Pasch).

[1] Current address: Business Support, Planning & Technology Sasol Polymers, PO Box 321, 1947 Sasolburg, South Africa.

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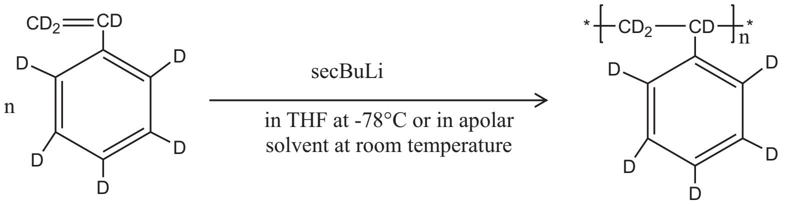
PS branches were attached to the protonated backbone. They used two-dimensional liquid chromatography for the characterisation of comb shaped polymers where one LC method is more sensitive to the deuterium content whereas the other LC method is more sensitive to the molar mass. Kayillo et al. investigated the chromatographic behaviour of deuterated and protonated oligostyrenes [34] using adsorption chromatography. They found that the differences in retention times were not sufficient to separate h-PS and d-PS of similar molar masses. To the best of our knowledge, LCCC has never been used for the separation of deuterated and protonated polymers.

In the present study, blends of deuterated and protonated polystyrene are analysed by liquid chromatography at critical conditions. Critical conditions will be established for both protonated as well as deuterated polystyrene. For the comprehensive analysis of the blend components two-dimensional liquid chromatography will be carried out. In the first dimension liquid chromatography at critical conditions will be employed which separates the blend components according to isotopic effects whereas in the second dimension size exclusion chromatography is employed where separation is accomplished with respect to hydrodynamic volume. The aim of the study is to investigate the capability of LCCC and 2D-LC to separate h-PS and d-PS of similar molar masses and chemical compositions. Further to improve the separation of the blends of h-PS and d-PS a separation protocol will be used, where one component will show SEC behaviour whereas the other component will show LAC behaviour and finally two-dimensional liquid chromatography will be carried out at the above mentioned conditions.

2. Experimental part

2.1. Synthesis of deuterated PS

Deuterated PS was synthesised via living anionic polymerisation of deuterostyrene (styrene d-8) using secondary-butyl lithium as the initiator and THF as the solvent at −78 °C or by using an apolar solvent like cyclohexane at room temperature. The synthesis process was terminated by using degassed methanol.



2.2. Samples

Protonated PS and deuterated PS samples were produced by Polymer Laboratories/Agilent (Church Stretton, England) and Polymer Standards Service GmbH (Mainz, Germany). All samples had the same endgroups, i.e. a sec-butyl and a methoxy group. The HPLC grade solvents tetrahydrofuran (THF), acetonitrile (ACN) and cyclohexane were purchased from Sigma Aldrich. The samples had the following molar masses (M_p): h-PS 2590, 5120, 10,210, 19,640, 38,640 and 72,450 g/mol; d-PS 2090, 5750, 15,900, 28,400, 49,000, 112,000, 211,000 g/mol. The molar masses of the blends of h-PS and d-PS used at critical conditions of both h-PS and d-PS are summarised in Table 1.

2.3. HPLC

The experiments were carried out using two different instruments: (1) an Alliance 2690 Separation module HPLC system (Waters Corporation, Milford, MA, USA) consisting of a solvent mixer, vacuum degasser and an auto sampler with a 100 μL sample loop. The system was connected to an evaporative light scattering ELS 1000 detector (Polymer Laboratories/Varian). For the ELSD nitrogen was used as the carrier gas. The ELSD settings used were: nebulisation temperature, 40 °C; evaporative temperature, 90 °C; and the gas flow rate, 1.0 standard litre per min (SLM). Recording and processing of data was carried out by using WinGPC Unity (version 7) software (Polymer Standards Service GmbH, Mainz, Germany), (2) an Agilent 1200 series system (Agilent Technologies, Böblingen, Germany) consisting of a quaternary pump, vacuum degasser, auto sampler, thermostatted column compartment, variable wavelength UV detector, instant pilot and an Agilent 1260 infinity evaporative light scattering detector. The ELSD setting used was: nebulisation temperature, 90 °C. Recording and processing of data was carried out by using WinGPC Unity (version 7) software (Polymer Standards Service GmbH, Mainz, Germany). The experiments described in Figs. 1–6 were conducted with instrument 1 while the experiments described in Figs. 7–10 were conducted with instrument 2.

For establishing the critical conditions of h-PS, a Nucleosil C_{18} 300 Å (Macherey-Nagel, Düren, Germany) column with a size of 250 mm × 4.6 mm i.d. and 5 μm average particle size was used. THF and ACN were used as the mobile phase at a flow rate of 0.5 mL/min. The mobile phase was pre-mixed by volume. The column oven temperature was maintained at 31.5 °C. 0.5 mg/mL of the samples were dissolved in the mobile phase composition and 10 μL was injected.

For establishing the critical conditions of d-PS, a Nucleosil Si 300 Å (Macherey-Nagel, Düren, Germany) column with a size of 250 mm × 4.6 mm i.d. and 5 μm average particle size was used. Normal HPLC grade THF and cyclohexane were used as the mobile phase at a flow rate of 0.5 mL/min. The mobile phase was premixed by volume. The column oven temperature was maintained at 30 °C. 0.5 mg/mL of the samples were dissolved in the mobile phase composition and 10 μL was injected.

Blends of h-PS and d-PS were prepared and measured at both the critical conditions. 2 mg/mL of each component was dissolved in the mobile phase composition and 50 μL was injected.

For establishing the separation protocol where one blend component shows SEC behaviour and the other blend component shows LAC behaviour a Nucleosil C_{18} 300 Å (Macherey-Nagel, Düren, Germany) column with a size of 250 mm × 4.6 mm i.d. and 5 μm average particle size was used. Normal HPLC grade THF and ACN were used as the mobile phase at a flow rate of 0.5 mL/min. The mobile phase composition of THF–ACN 47:53 (v/v) was used. The column oven temperatures used were 41 °C, 45 °C and 54 °C, respectively. 1 mg/mL of the samples were dissolved in the mobile phase composition and 10 μL was injected. Blends of h-PS and d-PS were prepared and 2 mg/mL of each component was dissolved in the mobile phase composition and 50 μL was injected.

2.4. Two-dimensional liquid chromatography

For the two-dimensional liquid chromatography experiments, sample fractions from the first dimension were transferred to the second-dimension column via an electronically controlled