Published in final edited form as:

Anal Bioanal Chem. 2016 January ; 408(3): 677–681. doi:10.1007/s00216-015-9198-9.

Novel behavior of the chromatographic separation of linear and cyclic polymers

J. Rafael Montenegro-Burke^{1,2,3,4}, Jackson M. Bennett^{1,2,3,4}, John A. McLean^{1,2,3,4,*}, and David M. Hercules^{1,*}

¹Department of Chemistry, Vanderbilt University, 7300 Stevenson Center, Nashville, Tennessee 37235, USA

²Center for Innovative Technology, Vanderbilt University, Nashville, Tennessee 37235, USA

³Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, Tennessee 37235, USA

⁴Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University, Nashville, Tennessee 37235, USA

Abstract

In various polymerization processes, the formation of a wide variety of chains, not only in length but also in chemical composition, broadly complicates comprehensive polymer characterization. In this communication, we compare different stationary and mobile phases for the analysis of complex polymer mixtures via size-exclusion chromatography-mass spectrometry (SEC-MS). To the best of our knowledge, we report novel chromatographic effects for the separation of linear and cyclic oligomers for polyesters (PE) and polyurethanes (PUR). A complete separation for the different structures was achieved for both polymer types with a single solvent system (ACN) and without extensive optimization. Additionally, cyclic species were found to show an inverse elution

*corresponding authors: David M. Hercules: david.m.hercules@vanderbilt.edu. John A. McLean: john.a.mclean@vanderbilt.edu. The authors of this paper have no conflicts of interest.

profile compared to their linear counterparts, suggesting distinct physical properties between species.

Keywords

Cyclic and linear polymers; polyurethanes; polyesters; size-exclusion chromatography; electrospray mass spectrometry

Introduction

Polymers exhibit a wide array of structures such as, linear, cyclic, branched chains, copolymers, dendrimers and star shapes [1]. Variations in their molecular mass, chemical composition, end group functionality and topology determine their physical properties and their use as materials for applications ranging from industrial construction products to drug delivery systems in the life sciences. Although significant advances have occurred in polymer synthesis, the focal point of characterization has been mostly on molecular weight distribution (MWD) [2,3]. Typically, the MWD of synthetic polymers is determined by size exclusion chromatography (SEC) and/or matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) [3]. The two techniques are essentially complementary. MALDI-TOF-MS can detect differences in chemical composition and end-group functionality and SEC separations are based on polymer size (hydrodynamic volume). MALDI-TOF suffers from mass dependent response for polymers having relatively wide polydispersities ($M_w/M_n > 1.2$), because of diminished signal contribution by chains with higher degrees of polymerization [4]. Additionally, the effectiveness of GPC depends on the use of proper standards for calibration [5], which oftentimes are not available. Differences in hydrodynamic volumes make a standard calibration for some polymers unfeasible. Even though these limitations were addressed by coupling GPC with MALDI two decades ago [6], complete characterization of complex polymer mixtures still represents a challenging task for polymers with small chemical differences (*e.g.* cyclic and linear chains) [7].

Traditional spectroscopic methods such as infrared (IR) and nuclear magnetic resonance (NMR) have been used to characterize telechelic (functionalized) polymers [8,9]. However, low sensitivity and the inability to deal with functional complexity represent limitations. In contrast, liquid chromatography under critical conditions (LCCC) allows for the separation of complex mixtures solely on the chemical unit or moiety of interest. Entelis pioneered LCCC several decades ago and formulated a theory for operation under critical conditions, where both entropy and enthalpy values (which govern SEC and adsorption separations, respectively) are equal, eliminating interactions with the stationary phase (Gibbs free energy = 0) [10,11]. Under critical conditions, polymers with the same chemical composition will elute at the same time independent of their molecular weight, whereas polymer chains with the same degree of polymerization but with different chemical compositions (*e.g.* end-group chemistry) will be separated, making one part of the analyte “chromatographically invisible” [2]. The downside is that this technique is not as straightforward as other chromatographic techniques. The empirical process of identifying the critical conditions for a specific polymer and maintaining these conditions for chromatography is challenging. For example,

composition changes as little as 0.1% in the mobile phase can alter the LCCC delicate balance and compromise the analysis [10, 11].

In an ongoing study, investigating SEC coupled directly to MS for an improved comprehensive characterization of polyesters and polyurethanes, we observed two novel and interesting chromatographic effects. To the best of our knowledge, these effects have not been reported previously. We observed complete separation of cyclic and linear polyesters and polyurethanes with a pure solvent (acetonitrile - ACN) as the mobile phase (unlike LCCC, which requires at least two). Additionally, we observed an inverse elution order for cyclic chains under the same conditions, where shorter chains elute before longer ones, counter to normal SEC behavior. Currently, we are investigating these effects and a complete study for a wider range of polymers is under way to determine if the effect is general. These findings should be of great interest for the polymer community to develop alternative methods for the characterization of complex polymer samples.

Experimental

Materials

All solvents were CHROMOSOLV-grade (Sigma-Aldrich, St. Louis, MO). The polyester (PE 225) was synthesized by melt polymerization using adipic acid (hexanedioic acid) and 1,4-butanediol (Bayer, Leverkusen, Germany) with $M_n=2250$ (end-group analysis). A polyurethane (PUR 262) was synthesized using equimolar amounts of polybutylene adipate ($M_n=1000$) and 4,4'-methylene diphenyl diisocyanate (MDI), (Bayer, New Martinsville, WV). The structures of the oligomers observed are shown in Table 1.

Size Exclusion Chromatography-Mass Spectrometry (SEC-MS)

The SEC-MS experiments were carried out using a Waters (Milford, MA) 515 HPLC pump coupled directly to a Synapt G2-S (Waters, Milford, MA) mass spectrometer via an electrospray ionization (ESI) source. The positive-mode (+) ESI conditions were as follows: capillary, +3.0 kV; sampling cone, 40 V; source temperature, 100 °C; desolvation temperature, 120 °C; desolvation gas flow, 600 L/h; and cone gas flow, 18 L/h, respectively. The instrument was calibrated with sodium-formate in the mass range of 200–2500 amu. Separations were performed at a flow rate of 0.3 mL/min and a temperature of 35 °C. Two types of SEC stationary phases were used: traditional polymer particle substrate (GPC) and ethylene bridged hybrid silica particles (APC) both from Waters (Milford, MA). In both cases two columns with different molecular weight ranges were coupled in series. For GPC experiments, 2 columns in the order of Styragel® HR 3 THF (4.6 x 300mm) and Styragel® HR 0.5 (4.6 x 300mm) were connected. In parallel, the columns ACQUITY APC XT 125 (2.5 μ m, 4.6 x 150mm) and ACQUITY APC XT 45 (1.7 μ m 4.6 x 150mm) were used for APC. Tetrahydrofuran (THF) and ACN were the mobile phases for APC separations, while only THF was used for GPC. In order to obtain a stable electrospray with THF, ACN was infused at 0.3 mL/min via a T shortly before the ESI source. PE 225 and PUR 262 were dissolved and diluted in their respective solvents, 100 ng were injected.

Results and Discussion

The hydrodynamic volume of polymers, and therefore their separation efficiency, strongly depends on the solvent used for the separation. GPC columns typically are certified for a particular MW range for a particular solvent and the use of a different solvent can compromise not only the separation but also the column integrity. Therefore, a stationary phase with a broad solvent compatibility would be of great benefit for polymer characterization. As part of a larger project we evaluated a silica based stationary phase (APC) for the separation of polyesters and polyurethanes and subsequently compared APC to traditional GPC columns. Figure 1A illustrates the elution plots for polyester PE 225 using GPC in THF compared to APC in both THF and ACN. The GPC separation in THF shows a normal profile with both linear (green squares) and cyclic (green circles) chains co-eluting. The elution times of the larger cyclic chains overlap with linear chains of the same size, whereas the shorter cyclic chains tend to elute somewhat after their linear counterparts. On the other hand, the elution profiles for cyclic and linear chains using APC as the stationary phase and THF as the solvent show a small, constant time interval (~45 sec.) between cyclic (red circles) and linear (red squares) chains having the same degree of polymerization.

A significant difference is quite evident when ACN is used as the mobile phase, significant separation of linear (blue squares) and cyclic (blue circles) oligomers is noted. There is a 2–3 minutes longer elution time for linear chains and 3–4 minutes for cyclic chains as compared to THF for both stationary phases. More importantly, linear and cyclic chains were completely separated regardless of their close MW and chemical composition. It should be noted that the elution profiles with ACN are steeper as compared to THF, which might suggest a smaller number of theoretical plates in the former. Also, the separation seems to widen as the degree of polymerization increases. The reproducibility of these separations was determined by calculating the difference of elution times of equal polymerizations degree for linear and cyclic chains. This was analyzed for 3 separate runs in all conditions and it was determined that even though small elution time changes, commonly observed in chromatography experiments, were detected, the times between the elution of same length linear and cyclic chains showed low variability under all conditions (1–6 seconds in 0.3 to 2.1 minute separation of linear and cyclic oligomers).

Given that different polymers will have different hydrodynamic volumes, and that these will be affected by the solvent (polarity), we selected a simple polyurethane (PUR 262) for study, to see if increased chain rigidity caused by the addition of MDI to the chains would have an effect for solvents with different polarities (THF and ACN relative polarities are 0.207 and 0.460 respectively) [10]. Changes in flexibility can have an effect on the folding capability of the polymers, altering their hydrodynamic volumes. Figure 1B illustrates comparisons between the separations of PE 225 and PUR 262 on an APC column in both THF and ACN. The difference between both polymer elution profiles with THF is minimal; the plots for PE 225 (red) and PUR 262 (black) overlap completely. Of greater interest is the behavior observed for both the polyester and polyurethane in ACN, where the linear and cyclic chains are separated and the PE 225 (blue) and PUR 262 (orange) data points completely overlap, showing the same separation of linear and cyclic oligomers. These

results suggest that an increase in mobile phase polarity reduces the hydrodynamic volumes of these polymers, with the effect being greater on cyclic chains even in more rigid structures. Changes in elution times by altering the mobile phase composition are not unusual. Moreover, it is one of the main factors to be considered in any chromatographic experiment. However, the increase in separation between linear and cyclic chains under APC conditions with ACN as mobile phase provides an effective means to separate these architectures.

These separations were performed rapidly (less than 15 minutes) and minimal optimization was needed due to the use of a single solvent system, which increases the reproducibility and ease of analysis. In critical chromatography (LCCC), the selection of the solvents is determined by their interactions with the polymer and stationary phase. Typically, a good polymer solvent with a column showing good SEC behavior is selected. Subsequently, a non-solvent for the polymer is added to the mobile phase to induce enthalpic interactions until a delicate solvent balance is reached [11,12]. Previously, critical conditions for a single solvent system have been reported for polyisoprene (PI) [13]. In that study PI chains eluted at the same time regardless of their MW with 1,4-Dioxane at exactly 47.7°C and small temperature changes (0.1°C) altered the balance. However, these conditions were not appropriate for the separation of block-copolymers (polystyrene-polyisoprene). This demonstrated that polymers with different chemical compositions need a two solvent system in order to successfully separate complex polymer samples using LCCC for the separation.

The coupling of LCCC's unique sorting capability with MALDI offers great potential for the characterization of complex polymer samples. The first separation allows for fractionation based on chemical composition followed by measurement of the MW distribution. For example, for the characterization of PE 225 and PU 262, linear and cyclic chains would first be separated in LCCC, and fractions would be collected for MW distribution measurements with MALDI. Interestingly, in the present work we observe not only the separation of linear and cyclic chains but also an SEC behavior for linear chains, where chain length is inversely proportional to elution time. To the contrary, an inverse SEC behavior for cyclic chains was observed. Figure 2A displays a magnified region of the PE 225 (blue) and PUR 262 (orange) elution profiles of cyclic chains shown in figure 1B. With APC and ACN as the stationary-mobile phase system, cyclic chains with lower degrees of polymerization elute before chains with higher degrees of polymerization. In the case of the polyester (blue), a linear elution profile ($R^2 = 0.98$) is observed for the first 3 species ($n=2$ to $n=4$). Nonetheless, this profile changes for longer chains ($n=5$ to $n=8$), where elution times are observed in a narrower time range, reminiscent of LCCC separations. This is not the case for polyurethane chains (orange), which show a more nearly linear elution profile ($R^2 = 0.88$) for all observed species.

The two novel effects reported in this work can be visualized in the extracted ion chromatograms (EICs) shown in Figure 2B. The first eluting peaks correspond to linear polyester species, with longer chains eluting before shorter chains ($n=6$ to $n=1$). The later peaks correspond to the cyclic species with the opposite elution order ($n=2$ to $n=6$). The

separation between both species is evident and given the well resolved characteristics of the separation, the possibilities of co-elution are minimal.

The reason(s) for these interesting differences under APC and ACN conditions are not entirely clear. It is understood from LCCC theory that entropy governs SEC separations while enthalpy governs adsorption separations. In SEC, as described above, longer chains have shorter elution times than shorter chains. On the other hand, in adsorption separations longer chains have longer elution times, due to the higher interactions between long chains and the stationary phase. One possible explanation for the effects we observe is that, at the specific conditions for ACN on APC columns, entropy governs the separation of linear chains while enthalpy governs the separation of cyclic chains. This would suggest that ACN is what would be considered a good solvent in LCCC theory only for linear chains but a non-solvent for cyclic chains under the same conditions. These differences between linear and cyclic chains are supported by the fact that, smaller hydrodynamic volumes, lower viscosities, higher thermostabilities, higher self-diffusion coefficients, enhanced fluorescence as well as both lower and higher glass transition temperatures have been reported for cyclic chains as compared to their linear counterparts [14–16].

Conclusions

In this preliminary communication, we have reported novel behavior observed for complex polymer samples using APC columns with ACN as a solvent, resulting in the separation of linear and cyclic oligomers. The methodology developed does not involve extensive optimization, utilizes an electrospray friendly solvent and has the potential for significantly improving polymer characterization. We are currently pursuing a better understanding of this effect to see how general it might be. We will study different types of polymers, solvent effects and the influence of other experimental variables.

Acknowledgments

The authors thank Dr. Hartmut Nefzger and Dr. Stefan Wershofen of Bayer MaterialScience AG, Leverkusen, Germany for providing the polymer samples. Research reported in this publication was supported by the National Center For Advancing Translational Sciences of the National Institutes of Health under Award Numbers 5UH3TR000491-04 and 3UH3TR000491-04S1. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors also acknowledge support for this work provided by the Vanderbilt University Center for Innovative Technologies, the Vanderbilt Institute for Chemical Biology, the Vanderbilt Institute for Integrative Biosystems Research and Education and Vanderbilt University College of Arts and Science.

References

1. Montaudo G, Samperi F, Montaudo MS. Characterization of synthetic polymers by MALDI-MS. *Prog Polym Sci.* 2006; 31:277–357.
2. Crotty S, Weber C, Baumgaertel A, Fritz N, Altunta E, Kempe K, Schubert US. Semi-automated multi-dimensional characterization of synthetic copolymers. *Eur Polym J.* 2014; 60:153–162.
3. Pretorius NO, Willemse CM, de Villiers A, Pasch H. Combined size exclusion chromatography, supercritical fluid chromatography and electrospray ionization mass spectrometry for the analysis of complex aliphatic polyesters. *J Chromatogr A.* 2014; 1330:74–81. [PubMed: 24472839]
4. McEwen CN, Jackson C, Larson BS. Instrumental effects in the analysis of polymers of wide polydispersity by MALDI mass spectrometry. *Int J Mass Spectrom Ion Processes.* 1997; 160:387–394.

5. Hanton SD, Liu XM. GPC Separation of Polymer Samples for MALDI Analysis. *Anal Chem.* 2000; 72:4550–4554. [PubMed: 11028609]
6. Montaudo G, Montaudo MS, Puglisi C, Samperi F. Molecular weight distribution of poly(dimethylsiloxane) by combining matrix-assisted laser desorption/ionization time-of-flight mass spectrometry with gel-permeation chromatography fractionation. *Rapid Commun Mass Spectrom.* 1995; 9:1158–1163.
7. Chen H, He M. Quantitation of synthetic polymers using an internal standard by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Am Soc Mass Spectrom.* 2005; 16:100–106. [PubMed: 15653369]
8. Iván B, Kennedy JP, Chang VSC. New telechelic polymers and sequential copolymers by polyfunctional *Initiator-transfer* agents (Inifers). VII. Synthesis and characterization of α,ω -di(hydroxy)polyisobutylene. *J Polym Sci Polym Chem Ed.* 198010.1002/pol.1980.170181104
9. Hiltunen K, Härkönen M, Seppälä JV, Väänänen T. Synthesis and Characterization of Lactic Acid Based Telechelic Prepolymers. *Macromolecules.* 1996; 29:8677–8682.
10. Reichardt, C.; Welton, T. *Solvents and Solvent effects in Organic Chemsitry.* Wiley-VCH; Weinheim: 2003.
11. Entelis SG, Evreinov VV, Gorshkov AV. Functionality and molecular weight distribution of Telechelic polymers. *Adv Polym Sci.* 1986; 76:129–175.
12. Olesik SV. Liquid chromatography at the critical condition. *Anal Bioanal Chem.* 2004; 378:43–45. [PubMed: 14614584]
13. Lee W, Park S, Chang T. Liquid Chromatography at the Critical Condition for Polyisoprene Using a Single Solvent. *Anal Chem.* 2001; 73:3884–3889. [PubMed: 11534711]
14. Bielawski CW, Benitez D, Grubbs RH. Synthesis of Cyclic Polybutadiene via Ring-Opening Metathesis Polymerization: The Importance of Removing Trace Linear Contaminants. *J Am Chem Soc.* 2003; 125:8424–8425. [PubMed: 12848534]
15. Laurent BA, Grayson SM. Synthetic approaches for the preparation of cyclic polymers. *Chem Soc Rev.* 2009; 38:2202–2213. [PubMed: 19623344]
16. Kricheldorf HR. Cyclic Polymers: Synthetic Strategies and Physical Properties. *J Polym Sci Part A: Polym Chem.* 2010; 48:251–284.

Figure 1.

Elution plots of the molecular weights of cyclic (circles) and linear (squares) chains versus their elution times in SEC. A) Elution profiles for PE 225 and B) elution profiles for PE 225 and PUR 262 are color coded by polymer and separation conditions. PE 225 (green- GPC in THF, red- APC in THF and blue- APC in ACN), PUR 262 (black- APC in THF and orange- APC in ACN).

Figure 2.

A) Expanded SEC elution plot of the molecular weights of cyclic chains of PE 225 (blue) and PUR 262 (orange) versus time. B) Extracted ion chromatograms (EIC) of linear and cyclic PE-225 B chains. Chains are color coded by their degree of polymerization (*e.g.* $n=2$ is red... $n=6$ is yellow).

Table 1

Structural assignments for PE 225 and PUR 262 oligomers.

Species	Structure	Mass Na adduct
Linear PE 225		513.3 (n=1)
		713.4 (n=2)
		913.5 (n=3)
Cyclic PE 225		423.2 (n=2)
		623.3 (n=3)
		823.4 (n=4)
Linear PUR 262		453.2 (n=0)
		653.3 (n=1)
		853.4 (n=2)
Cyclic PUR 262		563.2 (n=1)
		763.3 (n=2)
		963.4 (n=3)