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# Determination of Critical Conditions of Adsorption for Chromatography of Polymers

Jana Falkenhagen\* and Steffen Weidner

BAM, Federal Institute for Materials Research and Testing, Richard-Willstaetter-Strasse 11, D-12489 Berlin, Germany

Liquid chromatography (LC) at critical conditions of adsorption was used to separate various poly(ethylene oxides), poly(propylene oxides) and their copolymers. For the first time, the determination of the critical conditions by means of Ultra Performance Liquid Chromatography (UPLC) coupled to Electrospray Ionization Time-of-flight Mass Spectrometry (ESI-TOF MS) is reported. In contrast to established, mostly laborious routines to find suitable chromatographic separation conditions, this coupling enables a very fast adjustment of parameters. Similar to LC Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (LC/MALDI MS) coupling, a two-dimensional analysis of homo- and copolymers regarding its functionality type and molecular weight distribution, as well as copolymer composition, can be performed simultaneously. Furthermore, there is no need for using polymer standards for the determination of critical conditions or Size Exclusion Chromatography calibration.

Aside from a molecular weight distribution, polymers almost always exhibit a variety of additional heterogeneities like a functionality distribution, for example, different end groups, a composition distribution (copolymers) and/or a topology distribution, that is, different structures (cyclic, linear, or comblike structures). Size Exclusion Chromatography (SEC) can only be applied for the determination of the polymer molecular weight because its principle is based on a separation of dissolved polymers with different hydrodynamic volumes. For a functionality-based separation, the principle of Liquid Adsorption Chromatography (LAC) has gained significant importance within the last years. In this regard a very special separation mode also referred to as LAC at “critical conditions” (LACCC) is of particular interest. At these conditions polymers with different molecular weights and identical repeat unit structure elute exclusively according to their end group structure.<sup>1–4</sup> A detailed thermodynamic description of this separation state was given by Gorshkov et al.<sup>5</sup> Meanwhile,

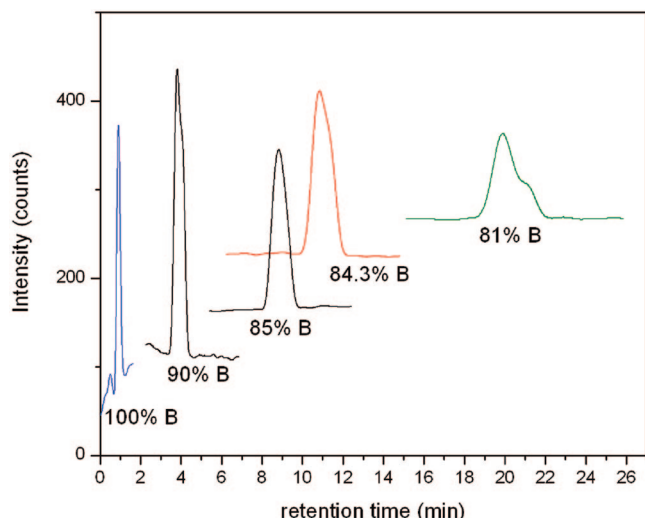
“critical conditions” for many different polymers have been determined.<sup>6–14</sup> The principal approach for any search for “critical conditions” can be described by two basic steps. First, various polymers with identical structure and end groups and different molecular weights have to be dissolved in a thermodynamically “good” solvent. Usually, at least three polymer standards are necessary. At these conditions SEC chromatograms will be recorded, showing an elution beginning with higher masses. In a second step, a thermodynamically “poor” solvent will be added stepwise, until the separation mode changed from SEC toward adsorption. When this mode is reached, a “fine-tuning” by a cautious changing of the solvent mixture composition is necessary to achieve “critical” separation. These conditions are achieved when a co-instantaneous elution of all standards at the same retention time is obtained. However, for many polymers with practical importance standards are not available. Moreover, if the LACCC principle is extended for a separation of copolymers another problem arises. Copolymers show an additional distribution in terms of composition. Aside from the necessity to find two “critical points” (for each repeat unit in the copolymer) the solubility of repeat units might differ drastically. Thus, in an arbitrary chosen chromatographic system one polymer structure may elute in SEC mode, whereas the second structure is separated in LAC mode or, even worse, shows irreversible retention. An alternative very accurate method to determine critical conditions of adsorption and to characterize synthetic polymers was developed by Chang et al. This method was called temperature gradient interaction chromatography.<sup>15</sup>

Comprehensive results about the composition of a complex polymer sample can be achieved by a two-dimensional coupling of LACCC and SEC. Information on the structural heterogeneity and the mean value of the molecular weight and molecular weight

\* To whom correspondence should be addressed. E-mail: jana.falkenhagen@bam.de. Phone: +493081041632.

- (1) Pasch, H.; Trathnigg, B. *HPLC of polymers*; Springer Laboratory: Berlin, 1998.
- (2) Entelis, S. G.; Evreinov, V. V.; Gorshkov, A. V. *Adv. Polym. Sci.* **1986**, *76*, 129–175.
- (3) Gorshkov, A. V.; Much, H.; Becker, H.; Pasch, H.; Evreinov, V. V.; Entelis, S. G. *J. Chromatogr.* **1990**, *523*, 91–102.
- (4) Skvortsov, A. M.; Gorbunov, A. A. *J. Chromatogr.* **1990**, *507*, 487–496.
- (5) Gorshkov, A. V.; Prudskova, T. N.; Guryanova, V. V.; Evreinov, V. V. *Polymer Bull.* **1986**, *15*, 465–468.

- (6) Braun, D.; Esser, E.; Pasch, H. *Int. J. Polym. Anal. Charact.* **1998**, *4*, 501–+.
- (7) Falkenhagen, J.; Much, H.; Stauff, W.; Muller, A. H. E. *Macromolecules* **2000**, *33*, 3687–3693.
- (8) Gancheva, V. B.; Vladimirov, N. G.; Velichkova, R. S. *Macromol. Chem. Phys.* **1996**, *197*, 1757–1770.
- (9) Kruger, R. P.; Much, H.; Schulz, G. *J. Liq. Chromatogr.* **1994**, *17*, 3069–3090.
- (10) Kruger, R. P.; Much, H.; Schulz, G.; Wachsen, O. *Macromol. Symp.* **1996**, *110*, 155–176.
- (11) Mengerink, Y.; Peters, R.; deKoster, C. G.; van der Wal, S.; Claessen, H. A.; Cramers, C. A. *J. Chromatogr. A* **2001**, *914*, 131–145.
- (12) Pasch, H.; Augenstein, M. *Makromolekulare Chemie-Macromol. Chem. Phys.* **1993**, *194*, 2533–2541.
- (13) Pasch, H.; Gallot, Y.; Trathnigg, B. *Polymer* **1993**, *34*, 4986–4989.
- (14) Sauzedde, F.; Hunkeler, D. *Int. J. Polym. Anal. Charact.* **2001**, *6*, 295–314.
- (15) Chang, T. Y.; Lee, H. C.; Lee, W.; Park, S.; Ko, C. H. *Macromol. Chem. Phys.* **1999**, *200*, 2188–2204.



**Figure 1.** Chromatograms of HO-PEO-(CH<sub>2</sub>)<sub>18</sub>-H; 700 g/mol (composite plot) recorded using different A/B ratios; (water (A) - acetonitrile/methanol (B)).

distribution can be simultaneously obtained.<sup>7,16–19</sup> Again, the availability of SEC standards and the required time are limitations. A substitution of SEC by Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) can overcome these problems and offers some advantages.<sup>20,21</sup> MALDI-TOF MS is a fast analytical method for the identification of chromatographically separated fractions. Because of the determination of absolute masses in mass spectrometry, the use of standards is not necessary. The coupling can only be carried out by off-line spraying or spotting by means of different commercially available interfaces. Nevertheless, sample preparation in MALDI-TOF MS is crucial for good spectra quality. The evaporation of solvents during transfer of fractions from chromatography either by spraying or by spotting can result in a formation of inhomogeneous spots. The substitution of MALDI-TOF MS by Electrospray (ESI) MS can avoid such problems. Thus, on line LC/ESI-MS is always preferred in those cases where samples are soluble in electrospray compatible solvents and do not exceed a certain mass region.

Since the first studies of Fenn and co-workers, ESI-TOF MS has become an interesting tool for the characterization of synthetic polymers.<sup>22,23</sup> The first SEC/ESI-TOF MS coupling for the analysis of different polymers was reported by Simonsick and Prokai in the early nineties.<sup>24,25</sup> Later Nielen et al. coupled different LC

modes with ESI-TOF MS.<sup>26,27</sup> Recent investigations on surfactants by LC/ESI-TOF MS were presented by van Leeuwen et al.<sup>28</sup>

Recently, a new type of chromatography was introduced—the so-called Ultra Performance Liquid Chromatography (UPLC).<sup>29</sup> This method provides an ultrafast separation and reduces the sample amounts drastically. By using smaller particles (particle size <2 μm) the time for one analysis can be reduced without any drawbacks in resolution and sensitivity. This technique was introduced for detection of drug metabolites in the pharmaceutical industry.<sup>30</sup> A first applications for synthetic polymers (styrene-acrylonitrile and epoxy resins) was published by Pursch et al.<sup>31</sup> UPLC systems typically operating at flow rates of 0.05–0.6 mL/min. Hence, post-column splitting of flow rates is not necessary for on-line mass spectrometry.

In combination with ESI-TOF MS, which enable fast data acquisition rates paired with excellent mass resolution, UPLC should be an excellent method for polymer separation in different modes of chromatography. Because of its polydispersity, every polymer (even standards with narrow polydispersities of <1.2) can be regarded as a mixture of different length polymer chains. Depending on the separation mode (SEC, LAC, or LACCC) their elution order within a chromatographic peak must be different. Vice versa, the inherent mass distribution of a single peak reveals the mode of separation. This assumption, representing a basically new approach, was investigated using poly(ethylene oxide)s (PEO)s and poly(propylene oxides) (PPO)s.

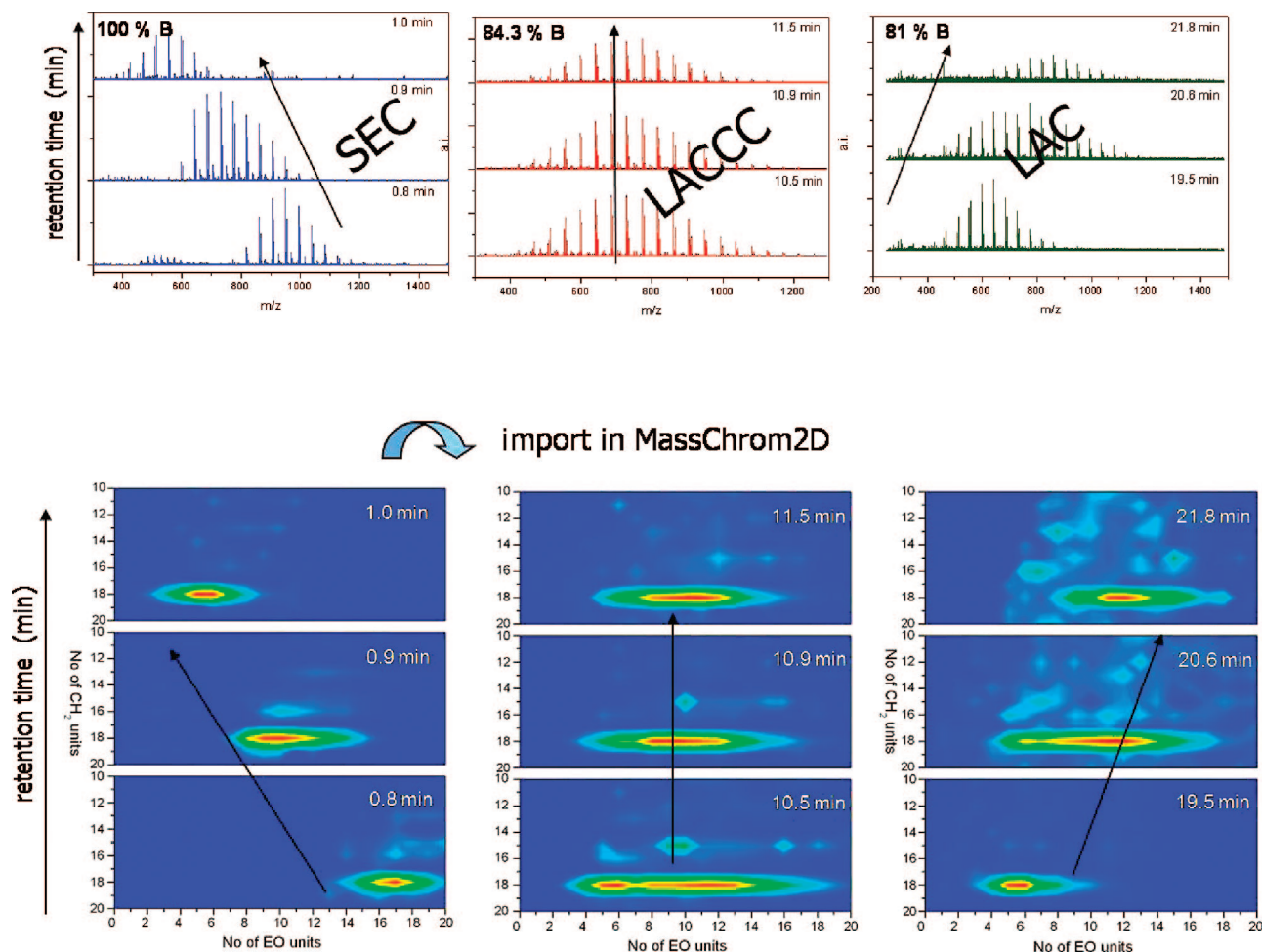
In addition, mixtures of various ethoxylated fatty acid alcohols were analyzed. Because of their application as nonionic surfactants, antifoaming and wetting agents, dispersants, thickeners, and emulsifiers, these compounds, although intensively investigated, are still of great interest.

## EXPERIMENTAL SECTION

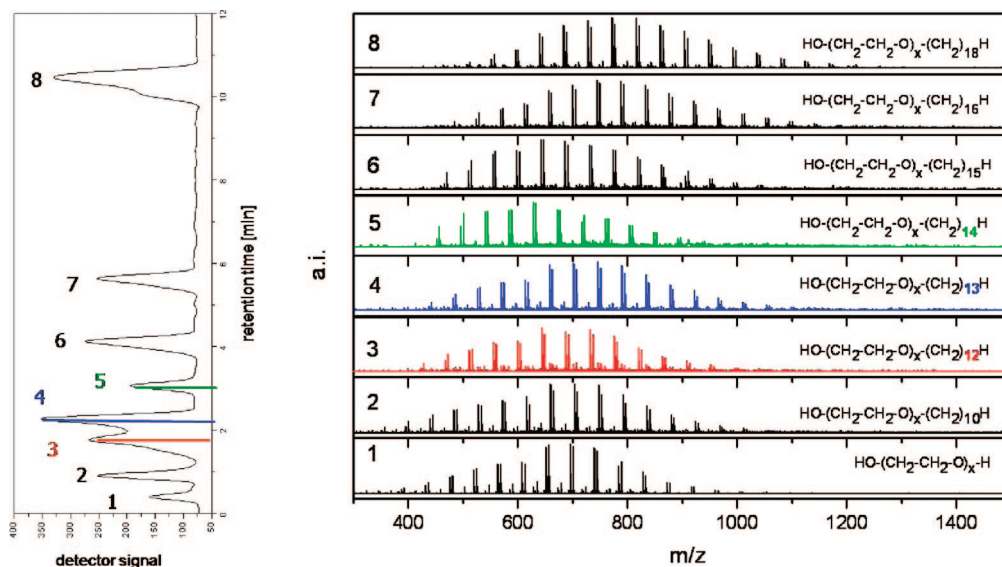
**Chromatography.** A UPLC system (Waters) working at a pressure of about 750 bar was used for separation. The system was equipped with one BEH (Bridged Ethane Hybrid particle) C18 column with a length of 50 mm and a diameter of 2.1 mm (Waters GmbH). The particle size was 1.7 μm. Column and autosampler were kept at a constant temperature of 30 °C. Different binary and ternary solvent mixtures were used as mobile phase. The determination of critical conditions of adsorption of PEO and the characterization of different PEO's was performed using mixtures of water with 0.1% of formic acid (component A) and acetonitrile/methanol 30:70 (v/v) (component B). The flow rate was 0.35 mL/min. For the determination of critical conditions of PPO and PPO/PEO copolymers, mixtures of water with 0.2% of formic acid (A) and tetrahydrofuran (B) were used. Various flow rates from 0.2 to 0.5 mL/min were tested. Two microliters

- (16) Adler, M.; Rittig, F.; Becker, S.; Pasch, H. *Macromol. Chem. Phys.* **2005**, *206*, 2269–2277.
- (17) Kilz, P.; Kruger, R. P.; Much, H.; Schulz, G. *Chromatogr. Charact. Polym.* **1995**, *247*, 223–241.
- (18) Pasch, H.; Adler, M.; Rittig, F.; Becker, S. *Macromol. Rapid Commun.* **2005**, *26*, 438–444.
- (19) Adrian, J.; Esser, E.; Hellmann, G.; Pasch, H. *Polymer* **2000**, *41*, 2439–2449.
- (20) Weidner, S.; Falkenhagen, J.; Krueger, R. P.; Just, U. *Anal. Chem.* **2007**, *79*, 4814–4819.
- (21) Weidner, S. M.; Falkenhagen, J.; Maltsev, S.; Sauerland, V.; Rinken, M. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2750–2758.
- (22) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Mass Spectrom. Rev.* **1990**, *9*, 37–70.
- (23) Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. *Anal. Chem.* **1985**, *57*, 675–679.
- (24) Prokai, L.; Simonsick, W. J. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 853–856.

- (25) Simonsick, W. J.; Prokai, L. *Chromatogr. Charact. Polym.* **1995**, *247*, 41–56.
- (26) Nielen, M. W. F. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1652–1660.
- (27) Nielen, M. W. F.; Buijtenhuijs, F. A. *Anal. Chem.* **1999**, *71*, 1809–1814.
- (28) van Leeuwen, S. M.; Tan, B. H.; Grijpma, D. W.; Fejen, J.; Karst, U. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2629–2637.
- (29) Swartz, M. E. *J. Liq. Chromatogr. Relat. Technol.* **2005**, *28*, 1253–1263.
- (30) Plumb, R.; Castro-Perez, J.; Granger, J.; Beattie, I.; Joncour, K.; Wright, A. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2331–2337.
- (31) Pursch, M.; Schweizer-Theobaldt, A.; Cortes, H.; Gratzfeld-Huesgen, A.; Schulenberg-Schell, H.; Hoffmann, B. W. *LCGC Europe* **2008**, *21*, 152.



**Figure 2.** ESI-TOF mass spectra of HO-PEO-(CH<sub>2</sub>)<sub>18</sub>-H; 700 g/mol recorded at different retention times using various solvent compositions, corresponding 2D composition plots.



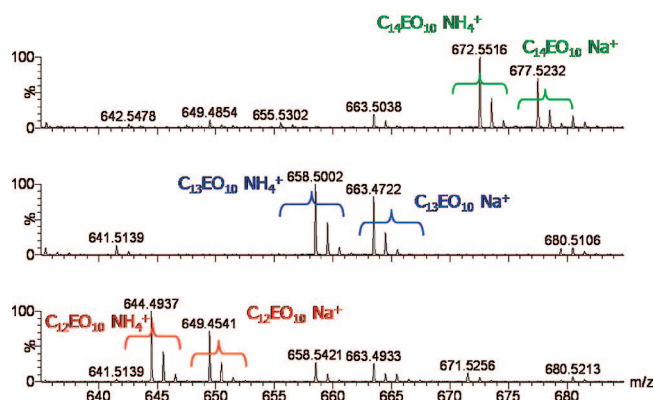
**Figure 3.** Separation of 8 different ethoxylated fatty alcohols at critical conditions of adsorption of PEO (left) and corresponding ESI-TOF mass spectra of peaks separated by LACCC (right).

of a sample (concentration 0.2 mg/mL) were injected. UPLC/MS grade solvents were used for chromatography (Biosolve Ltd., Valkenswaard, The Netherlands).

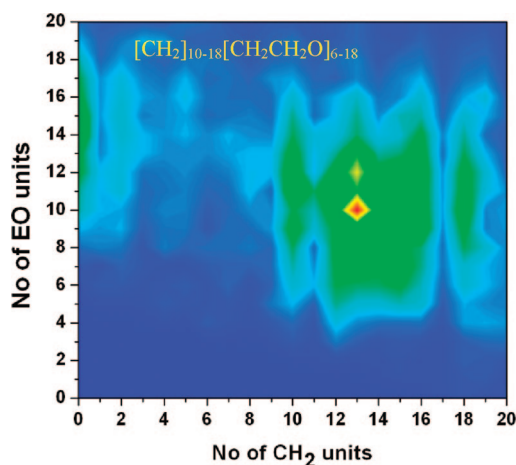
**ESI-TOF Mass Spectrometry.** A Q-TOF Ultima ESI-TOF mass spectrometer (Micromass) running at 3.4 kV, at a source

temperature of 120 °C and a desolvation temperature of 180 °C, was applied. The mass spectrometer was operating in the positive ion mode. The calibration of the system was performed using phosphoric acid and a PEO ( $M_p = 970$  g/mol, PD = 1.03) polymer standard (PSS GmbH, Mainz, Germany).

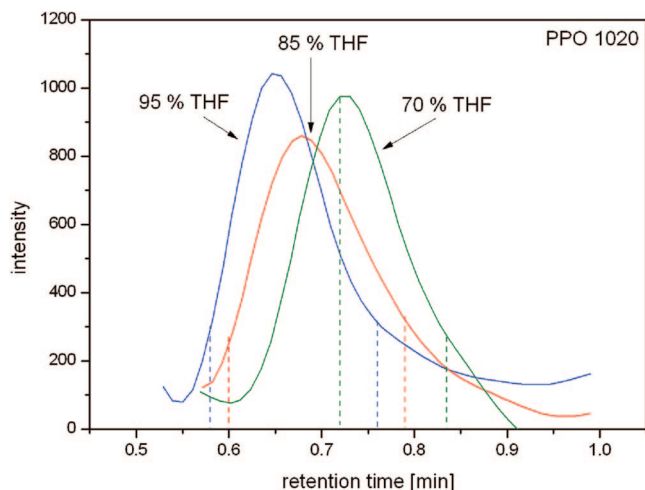




**Figure 4.** Extracted region of spectra 3, 4, and 5 (see Figure 3, right) with assignment of peaks.



**Figure 5.** 2D composition plot of an ethoxylated fatty alcohol mixture.



**Figure 6.** Chromatograms of PPO 1020 g/mol (composite plot) recorded using different A/B ratios; (water (A) - THF (B)), flow rate 0.2 mL/min; TIC from ESI-MS.

**Materials.** Ethoxylated fatty acid alcohols ( $\text{HO}-(\text{CH}_2-\text{CH}_2-\text{O})_x-(\text{CH}_2)_y-\text{H}$ , with  $y = 10-18$ ,  $M_w = 700-900$  g/mol) and PPO/PEO copolymers were obtained from BASF AG (Ludwigshafen, Germany). The polydispersities of all samples were between 1.03 and 1.06. A PPO homopolymer ( $M_p = 1020$

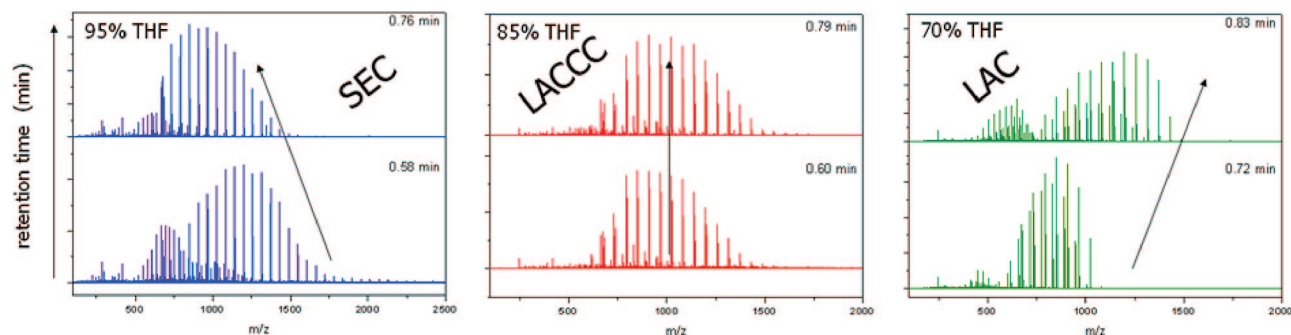
g/mol, PD = 1.04) used for determination of critical conditions was purchased from PSS GmbH (Mainz, Germany).

## RESULTS AND DISCUSSION

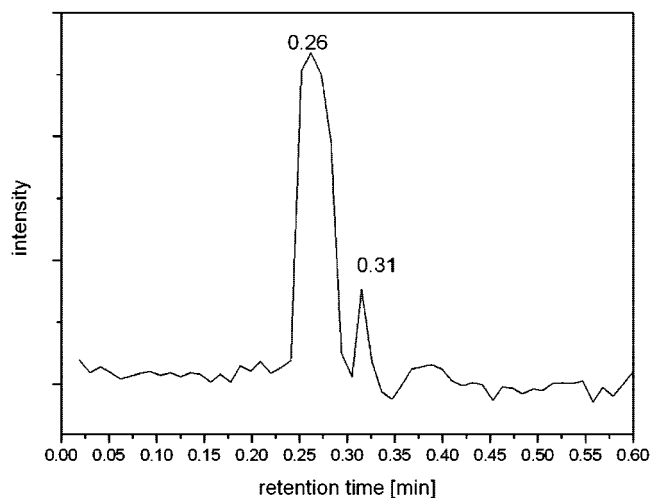
**Determination of Critical Conditions of Adsorption of Ethylene Oxide Repeating Units.** For the determination of critical conditions of adsorption of PEO, an ethoxylated fatty acid C 18 alcohol ( $M_w = 700$  g/mol) was used. In Figure 1 various peaks are shown taken from 5 chromatograms recorded at different solvent compositions. With increasing amount of solvent A (water), the elution shifts toward longer retention times. Simultaneously, the peaks became broader. This indicates increasing interactions between polymer molecules and the stationary phase, which is typical for a LAC mode. It is quite obvious that the sharp peaks observed at low water amount cannot be investigated by an off-line LC-MALDI-TOF MS coupling. The already poor chromatographic resolution would have been swept off by a remixing during spraying onto the MALDI target. However, using on-line ESI-TOF MS even this peak (at 100% B) can be investigated. The resulting ESI-TOF spectra taken at 0.8, 0.9, and 1.0 min are presented in Figure 2. Well resolved mass spectra with peak-to-peak distances of 44.05 Da (typical for PEO repeat units) were obtained. An additional peak series differs by only 5 Da. These series can be explained by a formation of sodium ( $\text{Na}^+$ ) and ammonium ( $\text{NH}_4^+$ ) adduct ions. In the spectra, recorded at 0.9 and 0.8 min (upper left), a third distribution with a maximum of about 400 g/mol and a peak-to-peak distance of 22 Da, representing double charged ions, can be seen. The maximum of the distribution of single charged ions shifts from higher molecular weight (850 g/mol) at a retention time of 0.8 min toward lower masses (550 g/mol) at 1.0 min. This clearly indicates a SEC mode of separation.

For the next experiments, the content of water in the solvent was increased stepwise to 19%. Again ESI-TOF mass spectra at three different elution times were recorded. Compared to the previous results completely reverse elution behavior can be seen. The maximum of the molecular weight distribution increases with increasing retention times (upper right, from 19.5 to 21.8 min). This represents a behavior which is typical for LAC. According to theory, a “critical mode of adsorption” should exist somewhere between these solvent compositions. Using a mixture of 15.7/84.3 (A/B, v/v) “critical” separation conditions were realized. The ESI-TOF mass spectra recorded at 10.5, 10.9, and 11.5 min (Figure 2, upper middle) did not show any change of the maximum of the mass peak distribution. Finally, all spectra were imported in an in-house software tool (MassChrom2D). This software transforms the mass information of the spectra into composition information. As a result the number of PEO units is plotted versus the number of  $\text{CH}_2$ -groups in a two-dimensional graph (Figure 2, bottom figures). These images clearly underline the above-mentioned different separation mechanisms. The results impressively demonstrate the ability of the chosen coupling method. Without using any polymer standard, a “critical” point of adsorption could be determined by only a few fast chromatographic runs.

These “critical” conditions were applied for a separation of a mixture of 8 fatty acid ethoxylated alcohols. As presented in Figure



**Figure 7.** ESI-TOF mass spectra of PPO 1020 g/mol, at two different positions of the chromatographic peak shown in Figure 6.

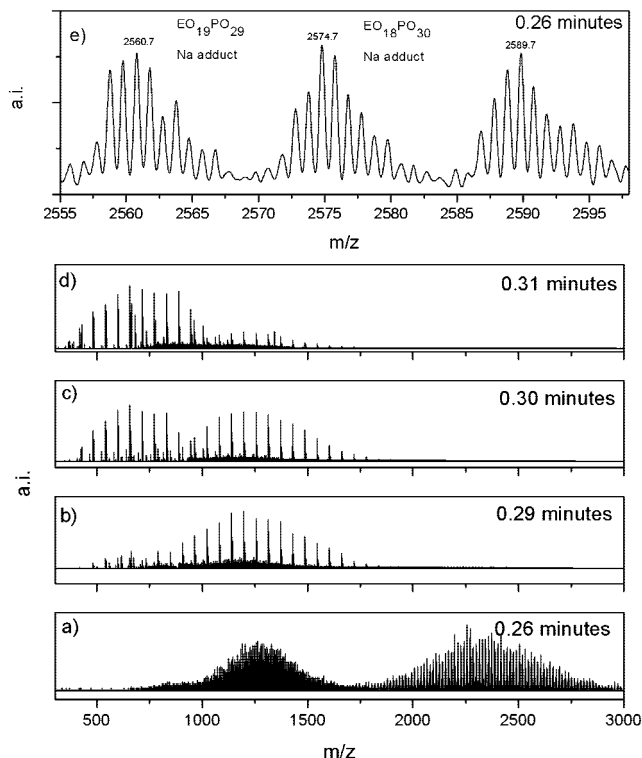


**Figure 8.** Elution of a PPO/PEO copolymer at critical conditions of PPO; mobile phase: THF/water, 85:15 (v/v); flow rate 0.5 mL/min; TIC from ESI-MS.

3 (left) the mixture could be separated in 10 min, which is just a fourth of the time previously needed.<sup>32</sup> The corresponding ESI-TOF mass spectra of the peak maxima are shown in Figure 3 (right). Even at lower chromatographic resolution, for example, between fraction 3 and 4, a clear assignment of polymer structures was possible. As seen in Figure 4 the distribution of  $\text{Na}^+$  and  $\text{NH}_4^+$ -adduct ions in the 635–685 Da mass region could be attributed to  $\text{C}_{14}\text{EO}_{10}$ ,  $\text{C}_{13}\text{EO}_{10}$ , and  $\text{C}_{12}\text{EO}_{10}$  species.

A 2D composition plot of the mixture is presented in Figure 5. It clearly shows the presence of  $[\text{CH}_2]_x[\text{PEO}]_y$  species with 10 to 18  $\text{CH}_2$  units and 5 to 18 PEO units. Additionally, a PEO homopolymer  $(\text{PEO})_{9-18}$  can be seen at the left image border.

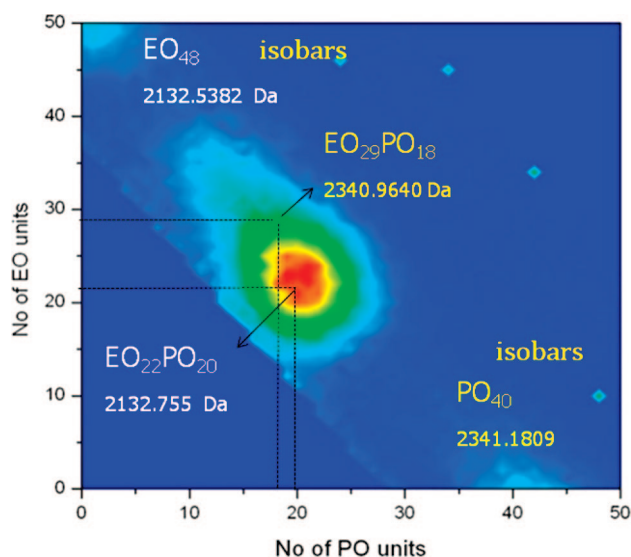
**Characterization of PPO/PEO Copolymers.** Because of the amphiphilic properties of PPO/PEO copolymers in general and strong hydrophobic properties of the PPO units, acetonitrile/MeOH/water systems are inapplicable for the “critical” chromatography. In previous studies a reversed separation phase system running with a THF/water eluent has been shown to be useful for adjusting critical conditions of adsorption of PPO.<sup>21</sup> Therefore, various solvent mixtures containing 5 to 30% water with 0.2% of formic acid were chosen for this investigation. In Figure 6 PPO elution curves at three different solvent compositions are shown. The obtained ESI-TOF mass spectra recorded



**Figure 9.** ESI-TOF mass spectra at different elution times of the copolymer sample (a–d) shown in Figure 8; enlarged mass region showing a typical copolymer peak distribution (e).

at two positions of the elugrams (dotted lines in Figure 6) are shown in Figure 7. With increasing water concentration in the eluent the mode of elution changed from SEC (5% water, left) to LAC (30% water, right). At a solvent composition of 85:15 THF/water (v/v), which represents the “critical” mode of separation of PPO (middle), the PPO/PEO copolymer was injected. At a flow rate of 0.5 mL/min only after 0.35 min a complete elution could be observed (Figure 8). The ESI-TOF mass spectra recorded at different retention times obviously reveal different peak series (Figure 9). A typical copolymer spectrum with single and double charged polymer ions is shown in Figure 9a. Their average molecular weight is about 2200 g/mol. The spectrum reveals characteristic peak series with distances of  $m/z = 58$  (PO units) and  $m/z = 44$  (EO units) overlapping each other. The resulting peak-to-peak distance, therefore, is  $m/z = 12$ , which is typical for those copolymers. A small mass region is exemplarily shown in Figure 9e. Assuming the formation of sodium adduct ions, a possible assignment of peaks to copolymer structures is given, for

(32) Falkenhagen, J.; Friedrich, J. F.; Schulz, G.; Kruger, R. P.; Much, H.; Weidner, S. *Int. J. Polym. Anal. Charact.* **2000**, *5*, 549–562.



**Figure 10.** 2D composition plot of the PPO/PEO copolymer.

example, the mass  $m/z = 2574.7$  can be attributed to an  $\text{EO}_{18}\text{PO}_{30}$  copolymer. A much easier way to get the composition of the copolymer offers the use of the previously mentioned MassChrom2D software tool. The ESI-TOF data of the 1700–3000 Da region of Figure 9a were used to create a 2D composition plot shown in Figure 10.

However, two additional distributions can be observed in Figure 9 after separation by UPLC. At 0.29 min (Figure 9b) a peak series with distances of  $m/z = 58$  and a residual mass of 18 Da is visible. These peaks can be clearly attributed to hydroxyl terminated PPO homopolymer structures. At 0.3 min (Figure 9c) a second distribution appeared. Their peak-to-peak distance is  $m/z = 58$  Da, too. However, no residual mass could be calculated. Thus, this distribution can be attributed either to a cyclic PPO homopolymer or to a linear PPO homopolymer with allyl end groups, which cannot be distinguished by mass spectrometry.

Finally, at a retention time of 0.31 min (Figure 9d) only the unknown distribution could be observed. For identification a reference substance PPO with allyl end groups was injected, which eluted exactly at the same retention time as the unknown fraction. This was a first hint for the presence of allyl end groups. Thus, this fraction was investigated by  $^1\text{H}$  NMR. In addition to characteristic signals of the PPO chain, at 4 ppm and between 5 and 6 ppm multiple signals were obtained which can be attributed to protons of an allyl group. These results clearly confirm the formation of allyl end groups.

This example clearly demonstrates the potential of the UPLC/ESI-TOF coupling. Even with very low chromatographic resolution, the separation and identification of complex polymer mixtures seems to be possible in an extremely short time span.

## CONCLUSIONS

On-line UPLC/ESI-TOF MS can be applied for characterization of synthetic polymers of a certain mass range with high mass accuracy. This coupling technique can be used for extremely fast determination of critical conditions of adsorption of polymers. Furthermore, no specific standards for calibration are necessary. The applicability of this new approach was successfully proved for the characterization of a narrowly distributed low molar mass copolymer. Even with poor separation conditions in UPLC, the structure, composition, and molecular weight of a PEO/PPO copolymer was obtained in less than 1 min.

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