Macromol. Symp. **2006**, 237, 18–27

DOI: 10.1002/masy.200650503

Characterization of EO-PO Block Copolymers by Liquid Chromatography Under Critical Conditions

Bernd Trathnigg,[1] *Alexei Gorbunov*[2]

Summary: Block copolymers of ethylene oxide (EO) and propylene oxide (PO) are characterized by liquid chromatography under critical conditions (LCCC) for EO. At the critical adsorption point (CAP) for one structural unit, the non-critical block can elute in size exclusion (SEC) or adsorption (LAC) mode. Depending on the molar mass and architecture of the polymers, different strategies are applied. For samples with a higher molar mass, the SEC separation is the method of choice, while lower molar masses also allow a LAC separation. Examples for both situations are given, which show, that these approaches yield different information. In the SEC mode, homopolymers and diblocks can be separated from the triblocks. In LAC mode, a baseline resolution of individual oligomers can be achieved, in which homopolymers, diblocks and triblocks with the same number of repeat units of the non-critical block have the same elution volume.

Keywords: block copolymer; chromatography; critical conditions

Introduction

Block copolymers of ethylene oxide and propylene oxide are in widespread use in various other applications, which utilize their amphiphilic nature: the EO block is hydrophilic, and the PO block hydrophobic. Especially important are EO-PO-EO triblocks, which are often called poloxamers (the commercial names are Pluronics or Synperonics). These products are produced by ethoxylation of polypropylene glycol (PPG), while PO-EO-PO triblocks are prepared by propoxylation of polyethylene glycol (PEG).

The desired triblocks will only be formed, if alkoxylation proceeds on both ends of the starting material. While the two primary OH groups of PEG may react with the same probability and rate, this is not the case with PPG. A PPG chain typically contains one secondary OH groups, which react more slowly, and sometimes also an allyl end group, which does not react at all. Allyl end groups can be present already in the starting material, but may also be formed during the alkoxylation by hydrogen abstraction from the methyl group of the growing PO chain end, which is thus terminated[1]. This chain transfer reaction results in two side products: homopolymers and diblocks.

Obviously, the properties of these products are strongly influenced by the distributions of molar mass, functionality, chemical composition, architecture, and content of homopolymers and diblocks.

The specification given by the producers are, however, generally far from complete: in most cases the overall molar mass and the overall composition (the average EO content) are given, which does not mean, that they have really been determined: they are typically calculated from (number) average molar mass of the starting material and the monomer consumption in the alkoxylation.

---

[1] Institute of Chemistry, Karl-Franzens-University, Heinrichstrasse 28, A-8010 Graz, Austria, Fax: +43 316 380 9840
E-mail: bernd.trathnigg@uni-graz.at

[2] Institute for Highly Pure Biopreparations, Pudozhskaya 7, 197110 St.Petersburg, Russia

Consequently, there is a strong need for reliable analytical methods, which allow a full characterization of such products: this means an independent determination of the distributions mentioned above.

This can be achieved by different chromatographic techniques, which separate according to different criteria. The mechanism of chromatographic separation is determined by the so-called interaction parameter c, which describes the interaction of a structural unit with the stationary phase [2]. This parameter is negative in Size Exclusion Chromatography (SEC) and positive in Liquid Adsorption Chromatography (LAC).

At the Critical Adsorption Point (CAP) the interaction parameter equals zero, and the correponding block becomes ‘‘chromatographically invisible’’. [3–6] This effect is utilized in Liquid Chromatography under Critical Conditions (LCCC), which allows a separation of polymer homologous series according to their functionality [4,7–9].

In the case of amphiphilic polymers the interaction parameters c_A and c_B of the individual structural units (A and B) may assume different values.

If both c_A and c_B are negative, the molar mass distribution (MMD) can be determined by Size Exclusion Chromatography (SEC), with retention decreasing with increasing molar mass, and only a minor influence of chemical composition. With dual detection, the chemical composition along the peak can be obtained[10–12].

If both c_A and c_B are positive, a separation by LAC (with retention increasing exponentially with the number of repeat units) can be achieved, if one of the blocks is monodisperse. If, however, both blocks are polydisperse and $c_A \neq c_B$, superimposed series of peaks will be observed, which can hardly be resolved.

If one of the parameters is positive and the other one negative, each polymer homologous series will elute in SEC order, but far behind the void volume of the column. This mechanism is called Liquid Exclusion-Adsorption Chromatography (LEAC) [13,14].

It can be utilized in the separation of monofunctional oligomers with a monodisperse adsorbing block or end group. In the analysis of block copolymers, LEAC may only be applied as the second dimension in two-dimensional chromatography.

If one of the interaction parameters (c_A or c_B) equals zero, the corresponding block becomes ‘‘chromatographically invisible’’, and a separation according to the other block can be achieved (Liquid Chromatography under Critical Conditions: LCCC). Depending of the corresponding interaction parameter, this separation may follow a SEC [15–17] or LAC [18–20] mechanism.

In the case of triblock copolymers, the influence of architecture has to be taken into account [21–26]: while the triblock A-B-A behaves like the diblock A-B at the CAP for A, the B-A-B triblock shows a very different pattern. Even at the CAP for the center block (A), a separation according to its size will be observed, as long as A is rather short. For higher polymers, the difference between A-B-A and B-A-B structures may be rather small. Depending on the architecture, molar mass and chemical composition of block copolymers, there are different strategies for their characterization.

SEC yields the overall molar mass distribution (MMD); with dual detection one may obtain information on the chemical composition along the MMD [12,27,28]. SEC can, however, not discriminate the polymer homologous series in mixtures of diblocks, triblocks, and homopolymers!

At the CAP for the EO unit, a separation according to the PO block(s) may be achieved by a SEC or a LAC mechanism, and vice versa.

The possible situations in LCCC of EO-PO copolymers are summarized in Table 1.

Table 1.
Situations in liquid chromatography of EO-PO block copolymers

Situation	Column	Mobile phase	EO	PO
1	RP	polar	LCCC	LAC
2	RP	non-polar	SEC	LCCC
3	NP	polar	LCCC	SEC
4	NP	non-polar	LAC	LCCC

Critical conditions for the (more polar) EO block have been found on reversed phase columns in aqueous methanol, acetone, or acetonitrile. In such systems, the less polar PO block elutes in LAC mode (situation 1 in Table 1).

In the literature, there are numerous papers on critical conditions for the EO unit: most of them use C18 columns, on which a critical adsorption point (CAP) for EO is observed (typically at 25°C) in methanol-water (80–90%),[20,29] acetonitrile-water (35–45%)[18,19,30,31] and acetone-water (25–30%).,[22,24] On CN columns, critical conditions for EO have been reported in methanol-water (85%)[20] or 44% at 50°C[20]), acetonitrile-water (28%),[31] and dimethoxyethane-water (21.5%).[31]In all of these systems, PO elutes in LAC mode.

This approach is, however, not very favorable, if the molar mass of the PO block is high, as molecules with a long PO block may be strongly retained. Obviously, gradient elution cannot be used to solve this problem, regardless the nature of the gradient: mobile phase composition or temperature[23]: in both cases the critical conditions would be lost.

One may also find critical conditions for the PO unit on a RP column in a different mobile phase composition (with a higher content of organic solvent): in this case EO elutes in SEC mode. The opposite is possible on normal phase columns: at the CAP for the EO unit, PO elutes in SEC mode, while EO elutes in LAC mode at the CAP for PO (provided that such critical points exist).

For the typical molar mass range of poloxamers, situations 1 and 4 are not very favorable, as the retention of the adsorbing block may be too high (or the resolution not sufficient).

As we have shown previously[32], situations 2 and 3 can be successfully applied in the analysis of poloxamers: a separation of the non-critical blocks by SEC allows not only the determination of their MMD, but shows also the presence of homopolymers, if they are sufficiently separated from the block copolymers.

Experimental

These investigations were performed using the density detection system DDS70 (CHROMTECH, Graz, Austria). Data acquisition and processing was performed using the software package CHROMA, which has been developed for the DDS70.

The columns and density cells were placed in a thermostatted box, in which a temperature of 25.0°C was maintained for all measurements on both systems (A and B).

In System A, the mobile phase was delivered by a JASCO 880 PU pump (Japan Spectrosopic Company, Tokyo, Japan) at a flow rate of 0.5 ml/min. Samples were injected manually using a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA, USA) equipped with a 50 μl loop. A Bischoff 8110 refractive index (RI) detector (Bischoff, Leonberg, Germany) was connected to the DDS 70.

Columns were connected to two column selection valves (Rheodyne 7060).

In system B, the mobile phase was delivered by an ISCO 2350 HPLC pump and an ISCO 2360 gradient programmer (from ISCO, Lincoln, Ne, USA) at a flow rate of 0.5 ml/min. Samples were injected using an autosampler Spark SPH 125 Fix (from Spark Holland, Emmen, The Netherlands) equipped with a 20 μl loop.

A SEDEX 45 ELSD (Sedere, France) was connected to the DDS 70. Nitrogen was used as carrier gas, the pressure at the nebulizer was set to 1.0 bar. Evaporator temperature: 30°C

The following columns were used in both systems:

- Jordi Gel DVB 500 RP: 100% poly(divinylbenzene); 250 × 4.6 mm; particle diameter = 5 μm; nominal pore size = 500 Å, (Jordi, Bellingham, MA, USA)
- Discovery HS PEG, silica-based PEG phase; 250 × 4.6 mm; particle diameter = 5 μm; nominal pore size = 120 Å (Supelco, Bellefonte, PA, USA)
- Nucleosil 100-5 OH 5 μm, silica-based diol phase; 250 × 4.6 mm; particle diameter: 5 μm; nominal pore

size = 100 Å (Macherey-Nagel, Dueren, Germany)

- Novapak C18: silica-based octadecyl phase; 300 × 3.9 mm; particle diameter = 4 μm; nominal pore size = 60 Å (Waters, Milford, MA, USA)

SEC measurements were performed on a modular SEC system comprising of a Gynkotek 300C pump equipped with a VICI injector (sample loop 100 μl), two column selection valves Rheodyne 7060, a density detection system DDS 70 (Chromtech, Graz, Austria) coupled with an ERC 7512 RI detector. Data acquisition and processing was performed using the software CHROMA.

SEC measurements were performed on a set of two columns PLgel (10 μm), $10^3 + 10^4$ Å, 300 × 7.8 mm each (Agilent) at a flow-rate of 1.00 ml/min and a column temperature of 30.0°C. Sample concentrations were 3.0–10.0 g/l.

The solvents (chloroform, acetone, methanol and water, both HPLC grade) were purchased from Roth (Karlsruhe, Germany) and Merck (Darmstadt, Germany).

Mobile phases were mixed by mass and vacuum degassed, their composition was controlled by density measurement using a DMA 60 density meter equipped with a measuring cell DMA 602 M (A. Paar, Graz, Austria).

PEG and PPG samples were purchased from Sigma-Aldrich and FLUKA (Buchs, Switzerland), EO-PO block copolymers from Sigma-Aldrich, BASF and SERVA.

Several polymer samples were also provided by "Blachownia" Institute of Heavy Organic Synthesis, (Kędzierzyn-Koźle, Poland).

Results and Discussion

If triblock copolymers are analyzed by SEC with coupled density and RI detection, one can often observe a shoulder with lower molar mass and a different chemical composition compared to the main fraction.

Figure 1 shows the MMD thus obtained for Pluronic F 108.

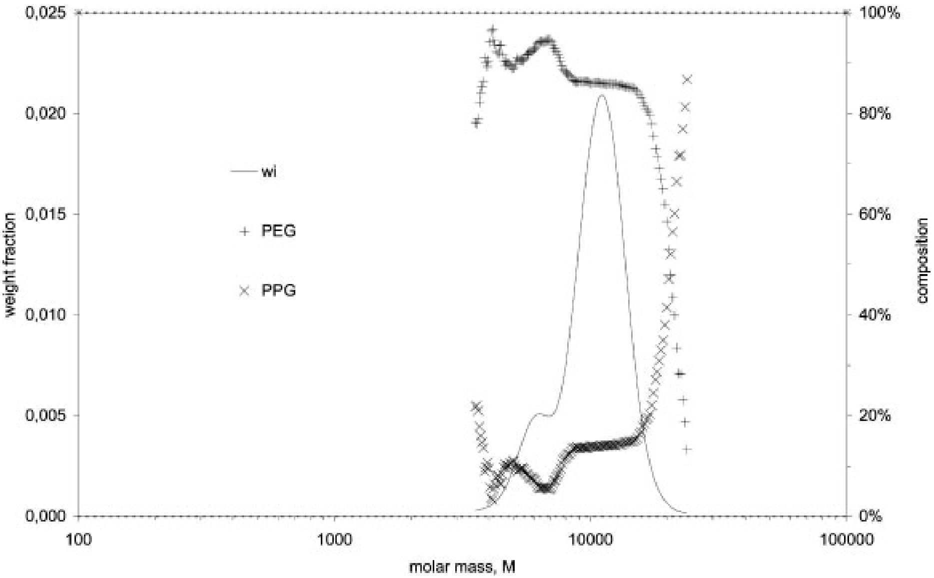


Figure 1.

MMD and chemical composition of Pluronic F 108, as obtained by SEC with coupled density and RI detection (PL gel $10^3 + 10^4$Å, $CHCl_3$).

According to the specification given by the producer (BASF), this sample contains 141 EO and 44 PO units (corresponding to an overall molar mass of 14960 or 2552 for the PO block and 12408 for the sum of the EO blocks).

As can be seen, this shoulder has a considerably lower molar mass and a lower EO content than the main fraction. SEC can, however, not identify this fraction: It may be a diblock, but can as well contain the homopolymers PEG or PPG (as is also the case for the main fraction!).

This information can only be obtained by combination with a different separation mechanism: LCCC. As the individual blocks in this polymer are quite large, one would need a system, in which critical conditions for one block exist, while the other block elutes in SEC regime (corresponding to situations 2 and 3 in Table 1).

On most RP columns, two critical points for PEG are observed in acetone – water mobile phases (Figure 2): at the first one (around 25–30 wt-% acetone) PO elutes in LAC mode with a much too high retention to separate higher oligomers. The second CAP (at about 90 wt-% acetone) is also close to critical conditions for PO and thus not useful in the analysis of poloxamers. The reason for this second critical point is the existence of residual silanol groups, interaction of which with the polar EO group is compensated by exclusion.

On polymer-based RP columns, which do not contain silanol groups, the CAP for PO (at about 90% acetone) can be utilized, as PEG elutes under these conditions in SEC mode, as can be seen in Figure 3.

In a previous paper[32]we have shown, that this system allows a separation according to the length of the EO blocks (situation 2 in Table 1).

In Figure 4 a comparison of poloxamers from different producers, but with similar specification is shown: the peak of the copolymer in Synperonic F 108 is somewhat wider than that in Pluronic F 108, and both polymers do not contain PPG homopolymer (as could be expected: it is not very likely, that none of the end groups in the starting material reacts at all with EO).

The other case is much more important: on a normal phase column, PO would elute in SEC mode aat the CAP for EO (situation

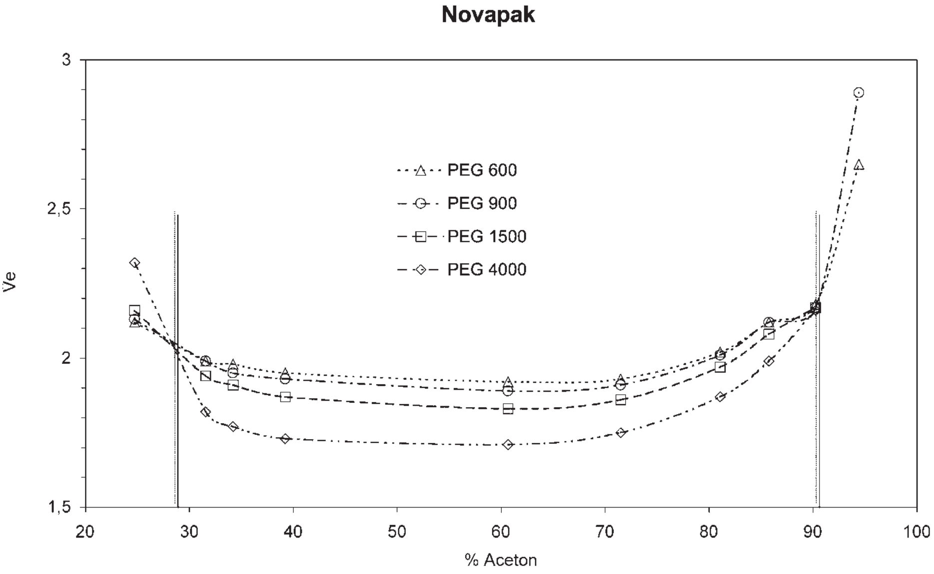


Figure 2.
Elution volumes of PEG with different molar mass on the Novapak column in aceton-water mobile phases of different composition (in wt.%).

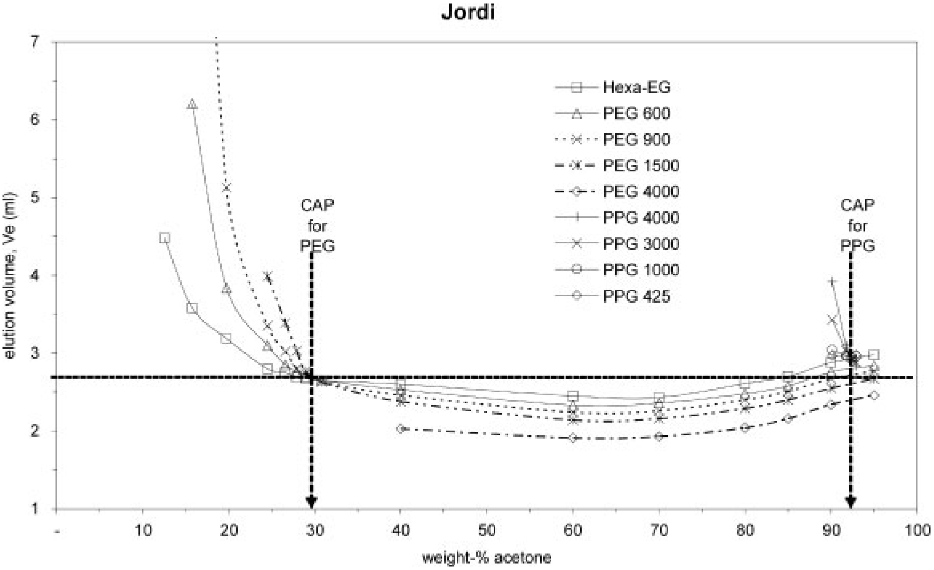


Figure 3.
Elution volumes of PEG and PPG with different molar mass on the Jordi column in aceton-water mobile phases of different composition (in wt.%).

3 in Table 1). This can be achieved on a diol column in about 80 wt-% acetone, as we have shown previously[32].

Under these conditions, one can see, that there is some PEG in these samples, but also a shoulder, which should contain the diblock.

For samples with rather short PO blocks, one might prefer the situation 1 (LAC for PO at the CAP for EO).

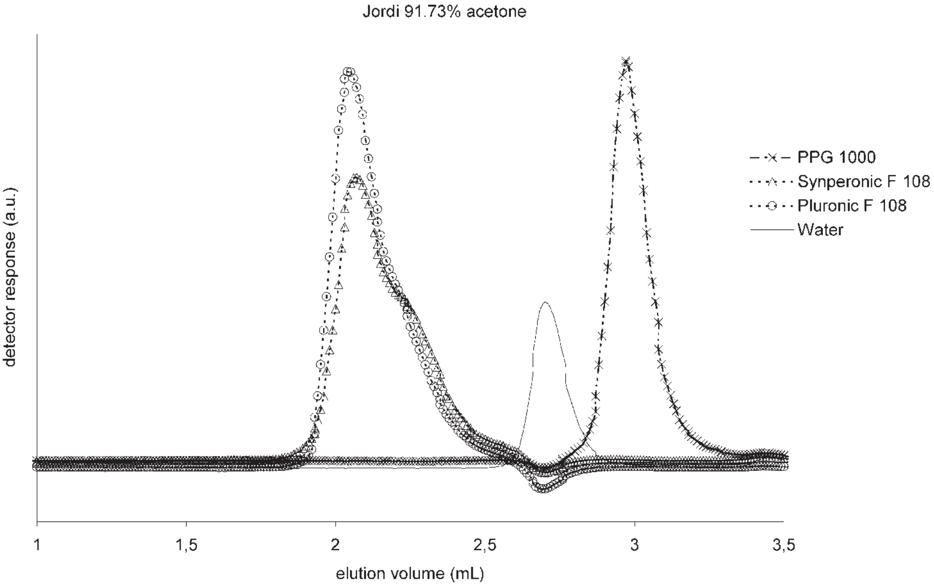


Figure 4.
Chromatograms of PPG 1000, water, and two poloxamers, as obtained on the Jordi column in 91.73 wt-% acetone – water. Detection: RI.

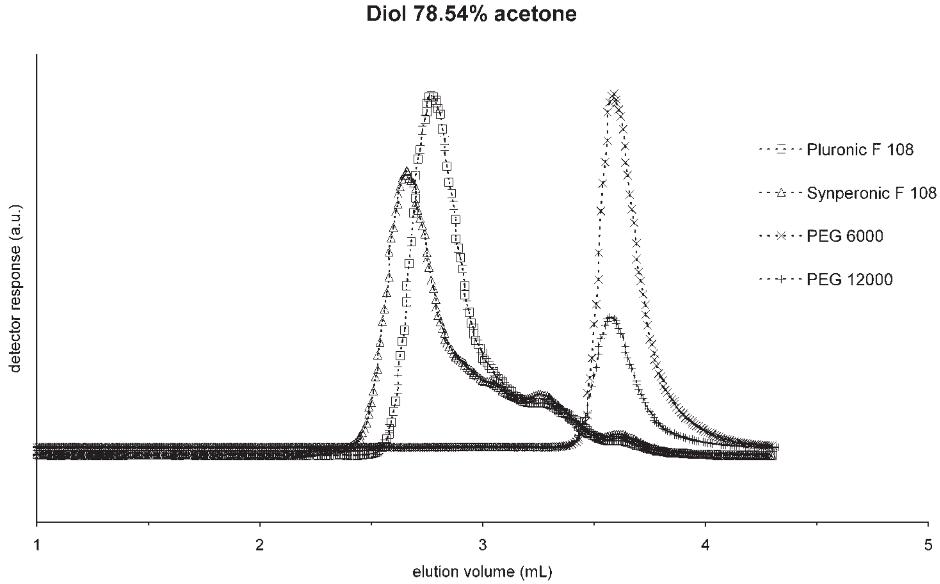
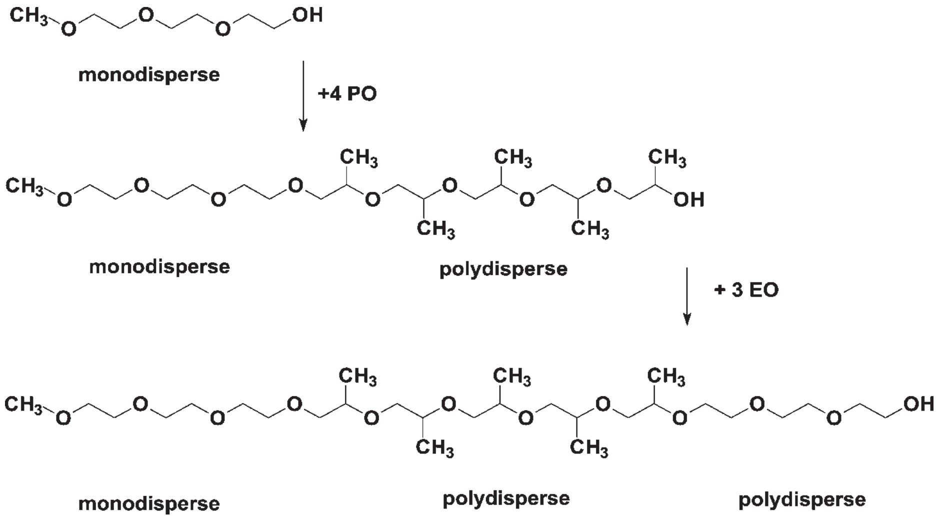


Figure 5.
Chromatograms of poloxamers and PEGs, as obtained on the Diol column in 78.54 wt-% acetone - water.

This can be achieved on RP columns at the first critical point in acetone – water with about 30 wt-% acetone. In such a mobile phase, retention is rather high, which allows a separation just of the lowest PO oligomers in a reasonable time.

The main question is here, whether the prediction of the theory[24,33] is correct: at the CAP for A and LAC conditions for B, the elution behaviour of ABA triblocks and AB diblocks should be the same. Moreover, one could ask, whether symmetry in triblocks has to be taken into account.

For this purpose, various block copolymers with one monodisperse EO block were synthesized at ICSO by stepwise alkoxylation of triethylene glycol monomethyl ether according to Scheme 1.



Scheme 1.
Reaction scheme of sequential alkoxylation of a monodisperse EO oligomer.

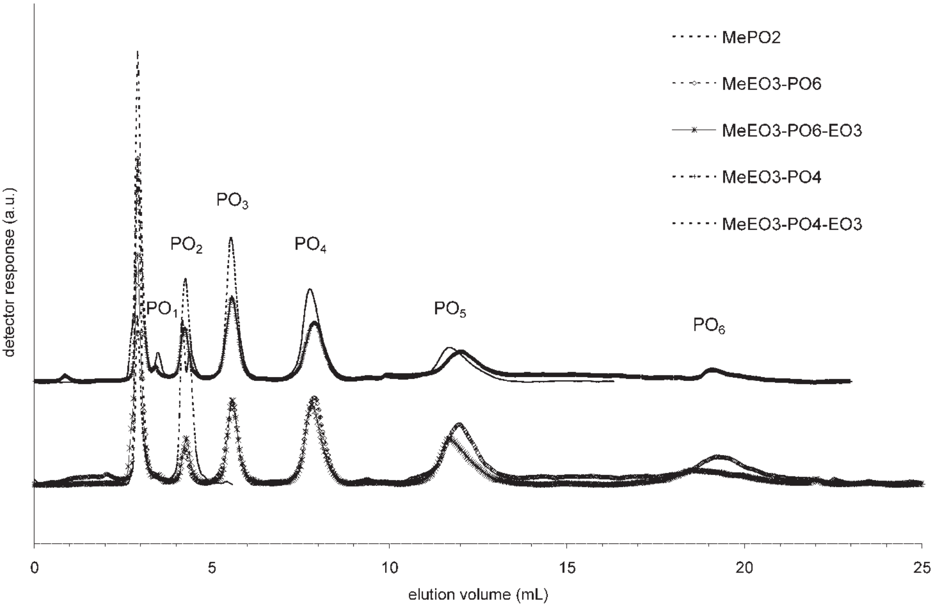


Figure 6.
Chromatograms of different diblock and triblock oligomers and dipropylene glycol monomethyl ether, as obtained on the Jordi column in 30 wt-% acetone – water. Detection: RI.

The synthesis will be described in full detail in another communication.

Obviously, the individual oligomers with the same number of PO units elute at the same position, regardless their EO content and architecture. The double peak of dipropylene glycol monomethyl ether is due to the existence of isomers.

If this technique shall be applied to EO-PO_EO triblocks with longer PO blocks, a different mobile phase must be used: as can be seen in Figure 7, a CAP for EO exits on the Novapak column in 80 wt-% methanol.

In such a mobile phase, PPG with an average molar mass of up to 1000 can be separated by LAC almost to the baseline.

Compared to the chromatograms obtained with two EO-PO-EO triblocks with a similar PO block, but different EO blocks shows a remarkable pattern: The peaks of the individual oligomers with the same number of PO units elute at the same position, only for the polymer with long EO blocks they are slightly shifted towards lower elution volumes. This may be due to the fact, that the Novapak column has a rather low pore diameter. In the sample with 10% EO the peaks of the lower oligomers are considerably higher, which might indicate, that the coversion of the lower PO oligomers with EO could be higher than that of the higher ones. It must be mentioned, that the sensitivity of the ELSD for EO is somewhat higher than that for PO (at least in such a mobile phase).

Conclusions

In the characterization of EO-PO block copolymers different strategies have to be chosen. Size exclusion chromatography with coupled density and RI detection provides information on the overall molar mass distribution as well as the chemical composition along the MMD. An unambiguous identification of low molecular fractions as homopolymers of diblocks can only be achieved by a combination of SEC with a different chromatographic separation under critical conditions for one of the blocks, while the other one elutes in SEC or LAC mode. For samples with higher molar mass, the first option is the method of

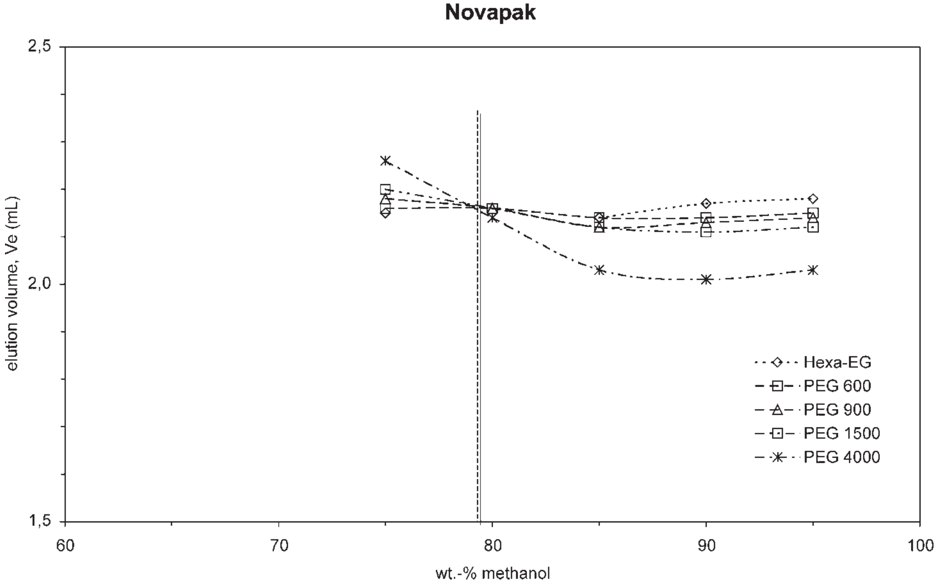


Figure 7.
Elution volumes of PEG with different molar mass on the Novapak column in methanol-water mobile phases of different composition (in wt.%).

choice. This situation (CAP for one block and SEC for the other one) can be observed on different columns. Lower di- and triblocks can also be analyzed at the CAP for EO under LAC conditions for PO. In methanol-water a baseline separation of oligomers in PPG up to an average molar mass of 1000 can be achieved. At the CAP for A, the same elution behaviour is observed for ABA triblocks, AB diblocks

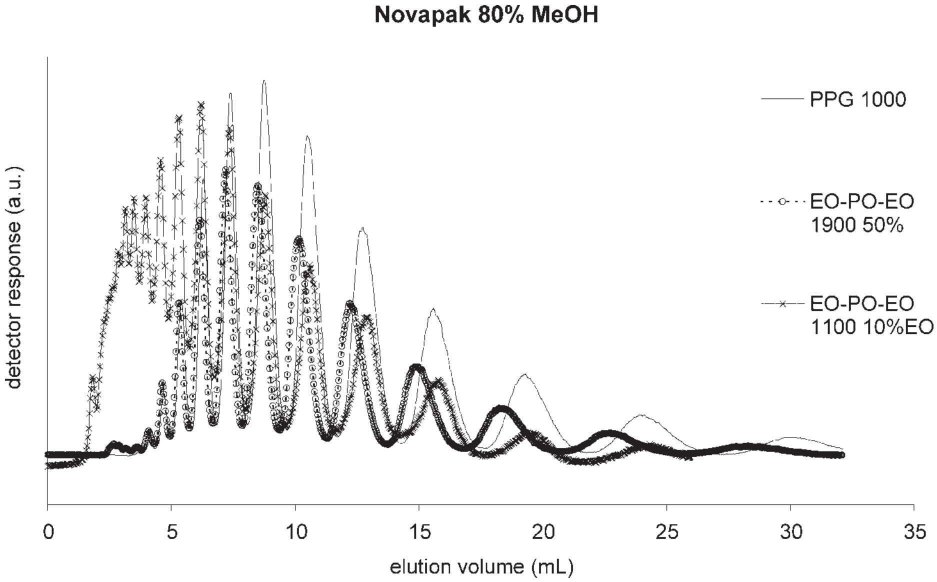


Figure 8.
Chromatograms of PPG 1000 and different triblock copolymers, as obtained on the Novapak column in 80 wt-% acetone – water. Detection: ELSD.

and B homopolymers, as is predicted by the theory.

[1] H. Altinok, G. E. Yu, S. K. Nixon, P. A. Gorry, D. Attwood, C. Booth, Langmuir 13 (1997) 5837–5848.

[2] A. A. Gorbunov, L. Y. Solovyova, A. M. Skvortsov, Polymer 39 (1998) 697–702.

[3] A. A. Gorbunov, A. M. Skvortsov, Vysokomolekulyarnye Soedineniya Seriya A 30 (1988) 895–899.

[4] A. M. Skvortsov, A. A. Gorbunov, J. Chromatogr. 507 (1990) 487–496.

[5] B. G. Belenkii, E. S. Gankina, V. N. Zgonnik, Malchova, II, E. U. Melenevskaya, J. Chromatogr. 609 (1992) 355–362.

[6] H. Pasch, Macromolecular Symposia 110 (1996) 107–120.

[7] A. V. Gorshkov, H. Much, H. Becker, H. Pasch, V. V. Evreinov, S. G. Entelis, J. Chromatogr. 523 (1990) 91–102.

[8] H. Pasch, I. Zammert, J Liq Chromatogr 17 (1994) 3091–3108.

[9] A. M. Skvortsov, A. A. Gorbunov, D. Berek, B. Trathnigg, Polymer 39 (1998) 423–429.

[10] B. Trathnigg, Journal Of Liquid Chromatography 13 (1990) 1731–1743.

[11] B. Trathnigg, J. Chromatogr. 552 (1991) 507–516.

[12] B. Trathnigg, S. Feichtenhofer, M. Kollroser, J. Chromatogr. A 786 (1997) 75–84.

[13] B. Trathnigg, A. Gorbunov, J. Chromatogr. A 910 (2001) 207–216.

[14] B. Trathnigg, J. Chromatogr. A 915 (2001) 155–166.

[15] H. Pasch, M. Augenstein, B. Trathnigg, Macromol Chem Physics 195 (1994) 743–750.

[16] H. Pasch, Y. Gallot, B. Trathnigg, Polymer 34 (1993) 4986–4989.

[17] H. Pasch, M. Augenstein, Makromol Chem Macro Chem Phys 194 (1993) 2533–2541.

[18] A. V. Gorshkov, H. Much, H. Becker, H. Pasch, V. V. Evreinov, S. G. Entelis, J. Chromatogr. A 523 (1990) 91–102.

[19] H. Pasch, C. Brinkmann, H. Much, U. Just, J. Chromatogr. A 623 (1992) 315–322.

[20] W. Batsberg, S. Ndoni, C. Trandum, S. Hvidt, Macromolecules 37 (2004) 2965–2971.

[21] C. M. Guttman, E. A. Di Marzio, J. F. Douglas, Macromolecules 29 (1996) 5723–5733.

[22] A. Gorbunov, B. Trathnigg, J. Chromatogr. A 955 (2002) 9–17.

[23] I. Park, S. Park, D. Y. Cho, T. Y. Chang, E. Kim, K. Y. Lee, Y. J. Kim, Macromolecules 36 (2003) 8539–8543.

[24] C. Rappel, B. Trathnigg, A. Gorbunov, J. Chromatogr. A 984 (2003) 29–43.

[25] B. Trathnigg, C. Rappel, R. Hodl, S. Fraydl, Tenside Surfactants Detergents 40 (2003) 148–154.

[26] A. A. Gorbunov, A. V. Vakhrushev, J. Chromatogr. A 1064 (2005) 169–181.

[27] B. Trathnigg, X. Yan, J. Appl. Polym.. Sci.: Appl.-Polym. Symp. 52 (1993) 193–203.

[28] B. Trathnigg, in T. Provder (Editor), Chromatography of Polymers: Hyphenated and Multidimensional Techniques, American Chemical Society, 1999, p. 1.

[29] B. Trathnigg, B. Maier, D. Thamer, J Liq Chromatogr 17 (1994) 4285–4302.

[30] H. Pasch, B. Trathnigg, HPLC of Polymers, Springer, Berlin, 1997.

[31] K. Baran, S. Laugier, H. Cramail, Journal of Chromatography B 753 (2001) 139–149.

[32] B. Trathnigg, Polymer 46 (2005) 9211–9223.

[33] A. Gorbunov, B. Trathnigg, J. Chromatogr. A 955 (2002) 9–17.