# Development of Comprehensive Liquid Chromatography (LC x LC) Approaches for the Analysis of Complex Copolymer Structures

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## INTRODUCTION

Precise knowledge of the composition and purity of advanced copolymers is of great importance given their influence on the final physical properties since impurities from starting materials, macro initiators or macro monomers are almost always present in the copolymer end product. In the work presented here a comprehensive two-dimensional HPLC separation technique with a slow size exclusion (SEC) separation in the first dimension and a fast reversed phase liquid chromatography (RPLC) separation in the second dimension was therefore developed to make direct impurity analysis of the end-products possible. Three structurally different copolymers were synthesized, based on polyether and a polystyrene backbone. Results are presented in two-dimensional contour plots or three dimensional plots.

## POLYMER STRUCTURES

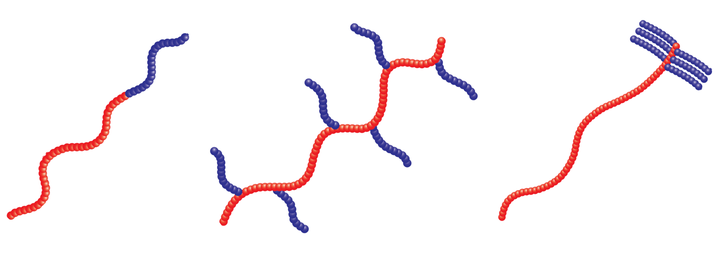

Figure 1: Schematic structure of the three structurally different copolymers. Red: polystyrene, Blue: polyether. Left: block copolymer; middle: graft copolymer; Right: palm-tree copolymer.

## EXPERIMENTAL

| | |
|---|---|
| Instrumentation | Agilent 1100 & 1200 LC system<br>Pump + HP 1050 pump<br>10-port-2 position VALCO valve |
| 1st dimension column | Plgel Mixed-D, 150 mm x 2 mm, 5 µm (SEC) |
| 2nd dimension column | Kinetex 50 mm x 4.6 mm, 2.6 µm, 100 Å $C_{18}$ shell (RPLC) |
| SEC column temperature<br>RPLC column temperature | 40 °C<br>25 °C |
| Sample conc, injection vol. | 40 mg/ml, 10 µl |
| Chemicals used | THF, MeOH, water, DCM, ACN (0.1 vol% formic acid added to DCM and ACN) |
| Detection | UV at 254 nm (PS)<br>ELSD detection (PS + PEO) |

First dimension flow rate was set at 12.5 µl/min. After 1st dimension separation, a make-up flow of 66% water and 34% MeOH (37.5 µl/min) was added to lower the eluotropic strength before the RPLC analysis. Modulation time: 2 min, 2nd dimension gradient: 0.00: 90/10 - 0.18: 60/40 – 1.50: 30/70 – 1.50 ≈ 1.60: 0/100 – 1.60 ≈ 2.00: 90/10 ACN/DCM at a flow rate of 4 ml/min.

Figure 2: 2D-setup used for this study

## RESULTS & DISCUSSION

***First dimension optimization***

To obtain a SEC separation under conditions of a very slow flow rate (necessary because of the limited loop volume on the switching valve), two 2 mm i.d. 150 mm columns were packed in-house with Mixed-D material and used in series. A successful separation of 4 polystyrene standards validated the used packing procedure.

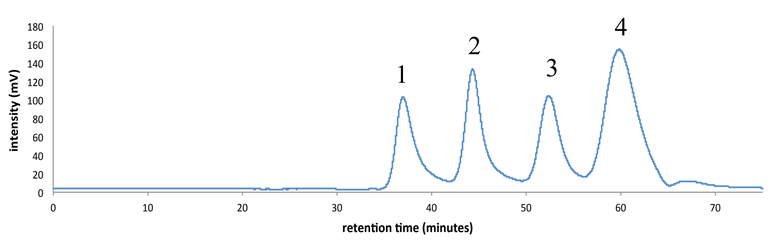


Figure 3: UV signal of the injection of polystyrene standards with molecular weight 299,400 (1); 38,100 (2); 4,920 (3) and 580 (4) g/mol for the calibration of two coupled PLgel Mixed-D columns. THF flow rate 12.5 µl/min.

***Second dimension optimization***

The shorter the second dimension runtime, the more detailed the contour plot will be. A RPLC separation within two minutes was developed. Initially too large and inconsistent $t_0$ peaks containing unretained polymer were observed (Figure 4).

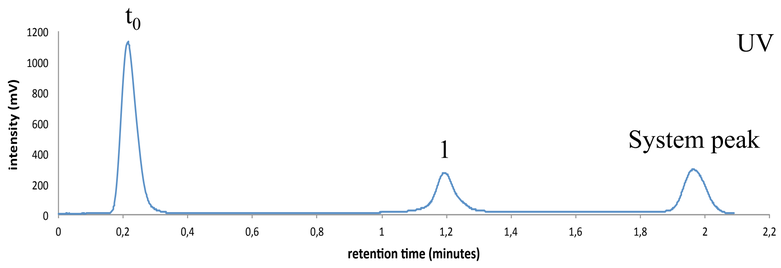


Figure 4: Chromatograms obtained upon analysis of a 200 µl injection of polystyrene (1), 299,400 g/mol in THF at a concentration of 76 µg/ml.

The polymers were therefore focused on the column head by injection in a precipitated state (solvent mixture of 50/25/25 vol% $H_20$/MeOH/THF) (Figure 5). Retained polymer peaks were then obtained.

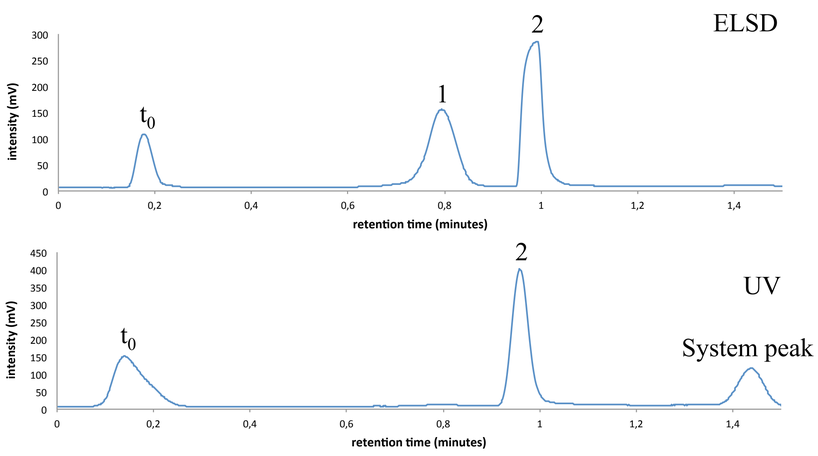


Figure 5: Chromatograms obtained upon analysis of a 50 µg/ml polystyrene (2) and 245 µg/ml polyether (1) with molecular weight 299,400 and 12,000 g/mol respectively, prepared in 50/25/25 volume% water/MeOH/THF.

***Two-dimensional results***

Figure 6,7 and 8 depicts the contour plots from the ELSD and UV signals of the analysis of the block-copolymer, graft copolymer and palm-tree copolymer, respectively.

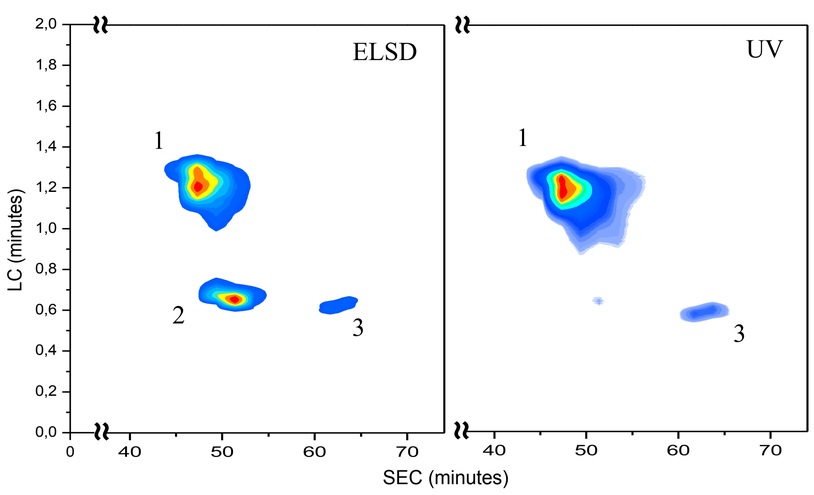


Figure 6: ELSD (left) and UV (right) signal of the comprehensive two-dimensional analysis of a block-copolymer. 1: block-copolymer; 2: polyether; 3: polystyrene.

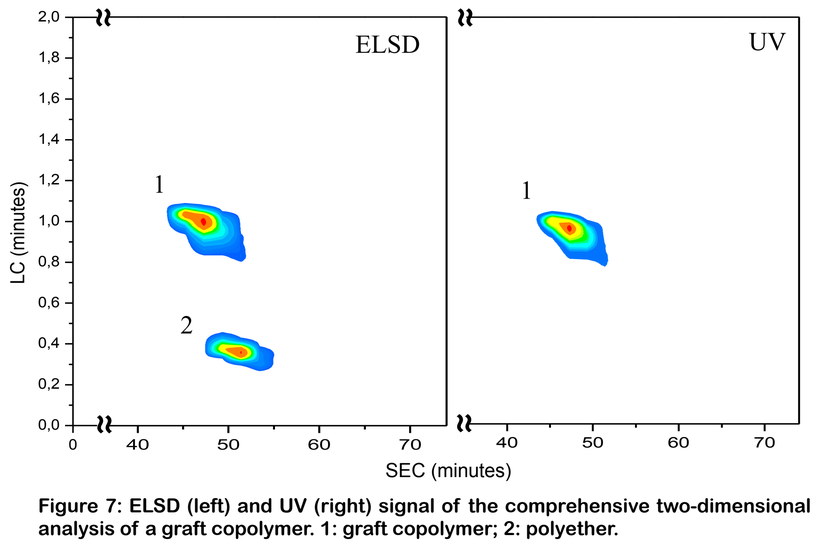


Figure 7: ELSD (left) and UV (right) signal of the comprehensive two-dimensional analysis of a graft copolymer. 1: graft copolymer; 2: polyether.

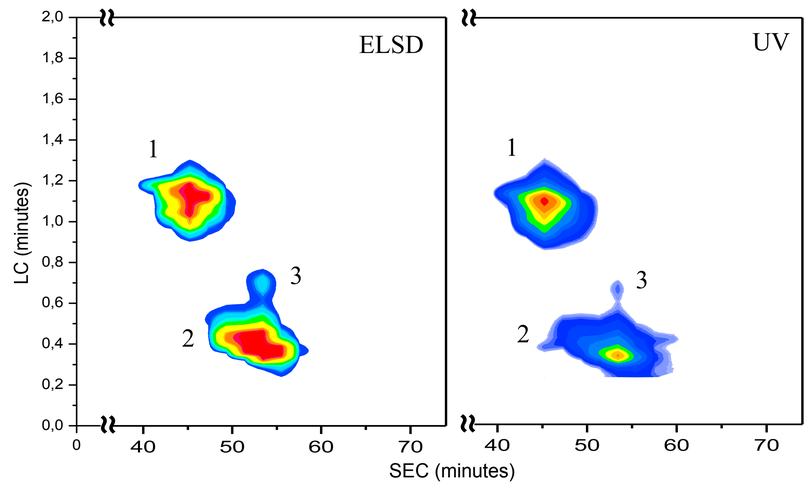


Figure 8: ELSD (left) and UV (right) signal of the comprehensive two-dimensional analysis of a palm-tree copolymer. 1: graft copolymer; 2: styrene-functionalized polyether); 3: polystyrene.

- Good baseline separated peaks
- Additional info with two detectors

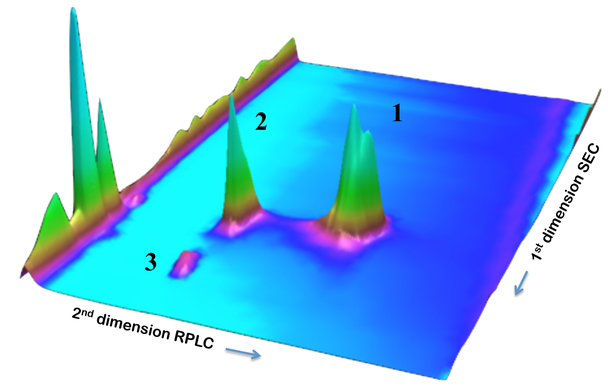


Figure 9: 3D-plot of the ELSD contour plot obtained in Figure 7 (top).

The relative standard deviations of the peak volumes of consecutive runs varied from 4%, 8% and 12% for peaks 1,2 and 3 respectively. This shows a good reproducibility (see also Figure 10). In further work a detailed quantification study will be executed.

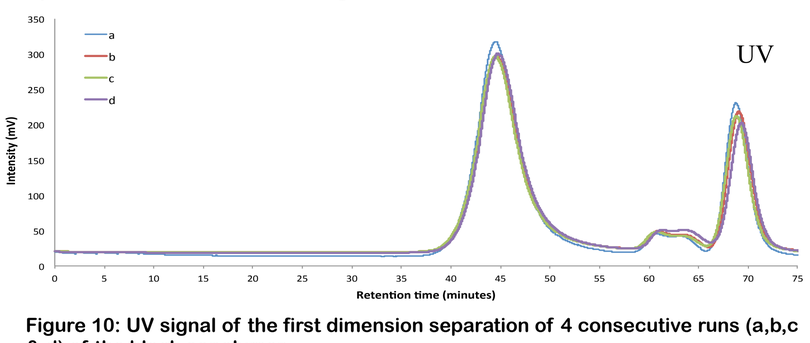


Figure 10: UV signal of the first dimension separation of 4 consecutive runs (a,b,c & d) of the block copolymer.

## CONCLUSION

- Modular method development is a successful approach.
- successful in-house manufacturing of SEC-material in narrow (2 mm i.d.) columns.
- analysis time only 70 min.
- good separation and reproducibility for further quantitative calculations.
- Further development and research for creating an as generic possible method.
- Satisfactory figures of merit.