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

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 we developed 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. Two structural differences were observed: based on polymer and a polyester backbone. Results are presented in two-dimensional contour plots or three-dimensional plots.

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 mm i.d. 150 mm columns were packed in-house with Mixed-D material and used in series. A successful separation of four polystyrene standards validated the used packing procedure.

Second dimension optimization

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

The polymers were therefore focused on the column head by injection in a precipitated state (solvent mixture of 50/25/25 v/v% H2O/MeOH/THF) (Figure 5). Retained polymer peaks were then obtained.

Experimental

Instrumentation

Agilent 1100 & 1200 LC system
Pump + HP 1050 pump
10-port-2 position VALCO valve

1st dimension column
Picolin Mixed-D, 150 mm x 2 mm, 5 μm (SEC)

2nd dimension column
Ninardex 50 mm x 4.6 mm, 2.6 μm, 100 Å C18 shell (RPLC)

SEC column temperature
40 °C

RPLC column temperature
25 °C

Sample conc, injection vol.
40 mg/mL, 10 μL

Chemicals used
THF, MeOH, water, DCM, ACN

Detection
UV at 254 nm (PS)
ELSD detection (PS + PED)

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.

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.

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.