

Analysis of Synthetic Polymers using Offline SEC - MALDI TOF MS

Introduction

Size exclusion chromatography, (SEC) is a chromatographic method commonly used to separate large molecules or macromolecular complexes such as proteins and industrial polymers. It operates on the principle of separation of components according to their size or hydrodynamic volume. Particles of differing size will elute through the column's stationary phase at varying rates.

SEC can be used as a measure of both the size and the polydispersity of a synthesized polymer – i.e. the distribution of polymer molecules of varying size. If standards of a known size are analyzed previously, a calibration curve can be created to determine the sizes of polymer molecules of interest. Alternatively, techniques such as light scattering and/or viscometry can be used online with SEC to yield approximate molecular weights without reliance on calibration with standards of known molecular weight. However, these techniques are not particularly accurate and, to complicate matters further, two polymers can have the same molecular weight but differing structures – this added complication can be investigated using mass spectrometry.

Recently, the general applicability of offline-coupled SEC - MALDI TOF MS has been demonstrated by the characterization of a wide variety of synthetic polymers having polydispersities in the range of 1.7-3.0, and extended with end-group analysis from isotopically resolved mass spectra of oligomers.

However, offline SEC - MALDI TOF MS involves fraction collection, evaporation, pipetting, etc. and is laborious and time-consuming. Consequently direct deposition methods in which SEC fractions and MALDI matrix are directly deposited onto the MALDI target are preferred. The following examples show the union of SEC and MALDI via an automated robotic spotting device, the AccuSpot™. The configuration of the whole system is shown in Figure 1.

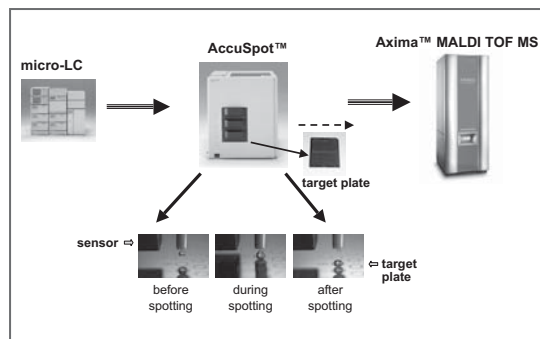


Figure 1. SEC-MALDI TOF MS configuration

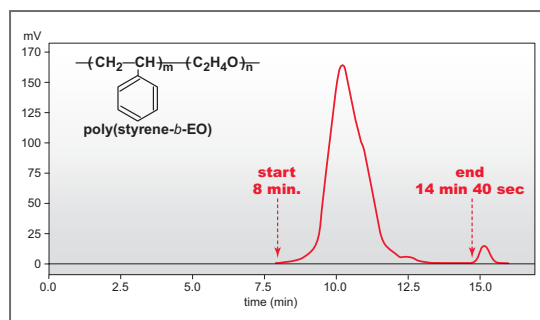


Figure 2. SEC chart of poly(styrene-b-EO)

Methods

SEC conditions

The following typical SEC conditions are utilized to perform the separation component of the analysis. An SEC chromatogram is shown in Figure 2.

Column: Shodex GF310A-1E (1.0 mmID×250 mm)

Flow rate: 10 mL/min

Mobile phase: THF

Detector: UV ($\lambda=220/254\text{nm}$)

Sample amount: 1 μL (40 μg)

A standard AccuSpot™ automated deposition device is modified slightly to accommodate the organic mobile phase used (THF). This modification consists mainly of changing the polyimide coated fused silica delivery capillary to a teflon capillary (0.375 mm OD x 0.05mm ID).

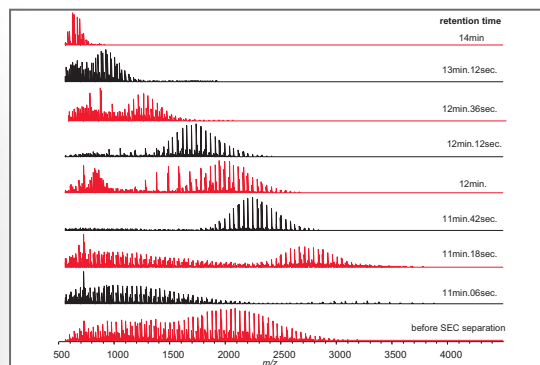


Figure 3. SEC MALDI TOF MS spectra for poly(styrene-b-EO)

Following SEC separation, the column eluent is delivered directly to the AccuSpot™. Here, it is mixed with a MALDI matrix and spotted automatically onto a MALDI target.

AccuSpot™ conditions

Spot interval: 6 seconds

Loading: 1 mL/well

Total loading (mixture of matrix and cationizing reagent): 0.2 mL/well

The resultant samples were then analyzed by MALDI TOF MS to generate a profile of each well.

MALDI TOF MS conditions

Matrix: Dithranol-20 mg/ml

Cationizing reagent: Na-TFA-10 mg/mL

Instrument: AXIMA Performance™

Co-polymer used in this study :-

poly(styrene-*b*-ethyleneoxide): poly(styrene-*b*-EO)

Results

The MALDI TOF MS spectra displayed in Figure 3 represent the SEC separated fractions for poly(styrene-*b*-EO) with time intervals indicated, in addition to the complex unseparated sample. The detection of homopolymer units is shown in Figure 4.

Individual precursor ions may be easily selected from the MS spectra as candidates for MS/MS. The spectra in Figures 5 and 6 show the valuable structural information that may be gained from MS/MS analysis.

Conclusions

By combining micro-LC (SEC) with the AccuSpot™ and Axima Performance™, an offline SEC - MALDI TOF MS analytical system has been developed.

Using the SEC - MALDI TOF MS analytical system,

- it is possible to collect SEC fractions without detriment to the separation capacity
- frequently used SEC organic solvents (for example, tetrahydrofuran(THF)) can be utilized in combination with a spotting device and MALDI analysis
- MS analyses are achieved with adequate peak sensitivity from a practical viewpoint
- semi-automatic analysis may be performed
- individual precursor ions may be selected for MS/MS analysis providing valuable structural information

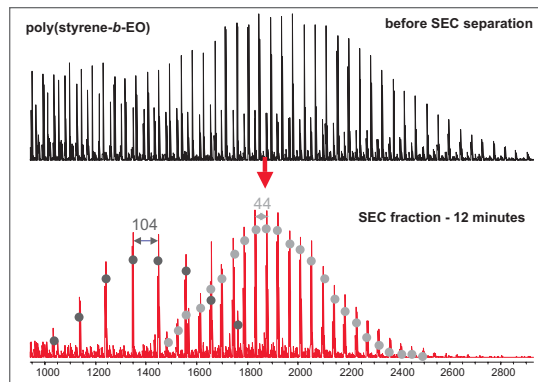


Figure 4. Detection of homopolymer units for poly(styrene-*b*-EO)

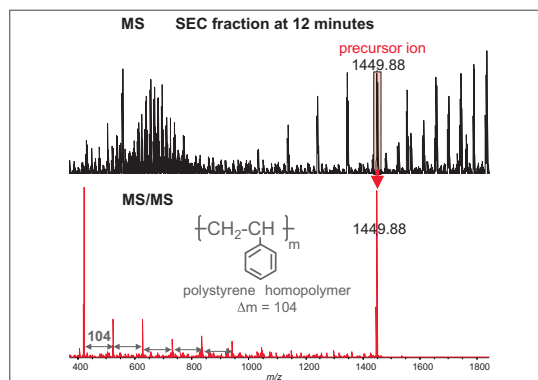


Figure 5. MS/MS of m/z 1449 showing Δm of 104 indicating a polystyrene structure

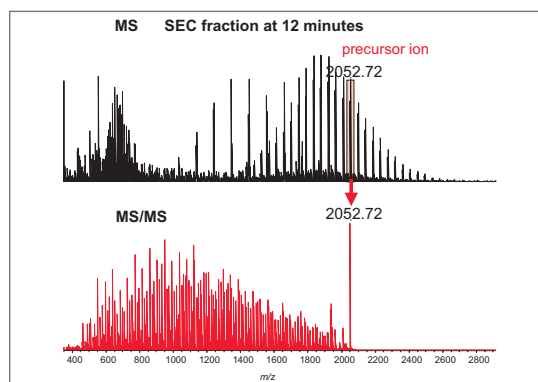


Figure 6. MS/MS of m/z 2052