Characterization of Biopolymers and Synthetic Polymers using Asymmetric Flow Field-Flow Fractionation (AF4) and Thermal Field-Flow Fractionation (TF3) coupled to Multi-Angle Light Scattering (MALS)

Bassem Sabagh, Gerhard Heinzmann, Evelin Moldenhauer, Florian Meier, Thorsten Klein*

Introduction

Field-Flow Fractionation (FFF) has become a reliable tool for the separation and characterization of (bio)polymers, proteins and nanoparticles. Four different FFF techniques are commonly used today: Asymmetrical Flow FFF [1], Centrifugal FFF [2], Thermal FFF [3] and Gravitational FFF [4]. By coupling FFF to Multi-Angle Light Scattering (MALS), Dynamic Light Scattering (DLS), UV and Refractive Index Detection (RI) the molecular weight distribution as well as the molecular structure (degree of branching, copolymer composition, aggregation) of synthetic polymers and biopolymers can be determined.

Separation Principle in FFF

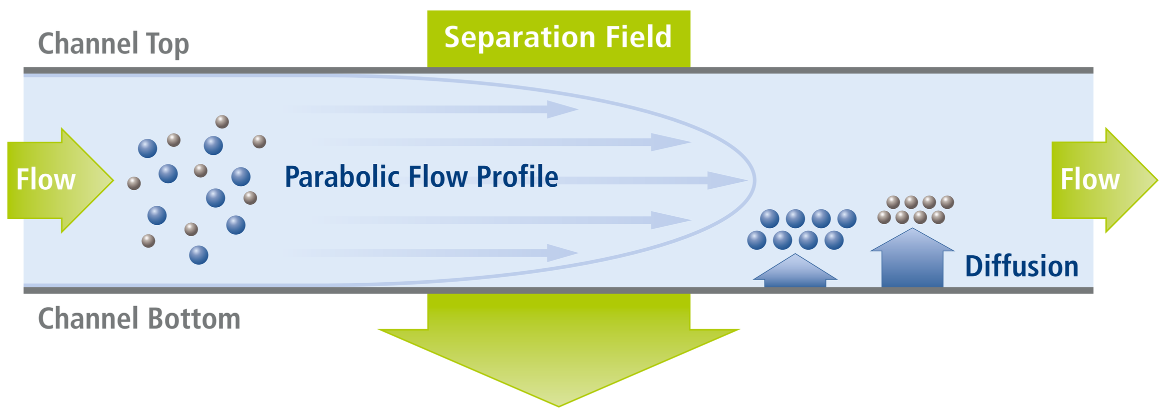

Figure 1: Basic scheme of the separation principle in FFF.

Multi-Angle Light Scattering (MALS)

Determination of Molar Mass and Radii of Gyration of synthetic- and bio-polymers, proteins, antibodies, viruses, engineered and natural nanoparticles after FFF-fractionation.

Specifications of the PN3621 MALS detector:
21 Light Scattering Angles: 7° - 164°
Molar Mass Range: approx. 1 kDa - 1 GDa
Radius of Gyration Range: approx. 8 nm - 500 nm

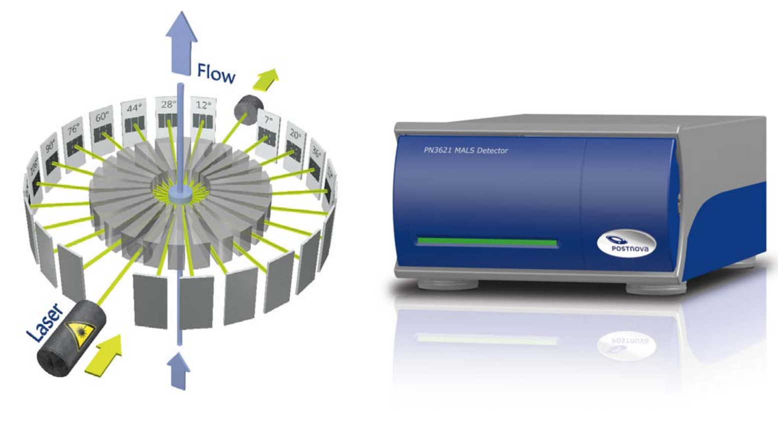

Figure 2: Basic principle of MALS and Postnova PN3621 State-of-the-Art 21-Angles MALS detector.

Asymmetrical Flow-FFF (AF4)

Asymmetrical Flow FFF is based on the separation in a narrow ribbon-like channel or a hollow fiber module. Perpendicular to the laminar eluent flow in the channel or hollow fiber a cross flow is applied. The sample molecules or particles are separated by their hydrodynamic size.

Figure 3: Postnova AF2000.

Applications of AF4-MALS

Polysaccharides

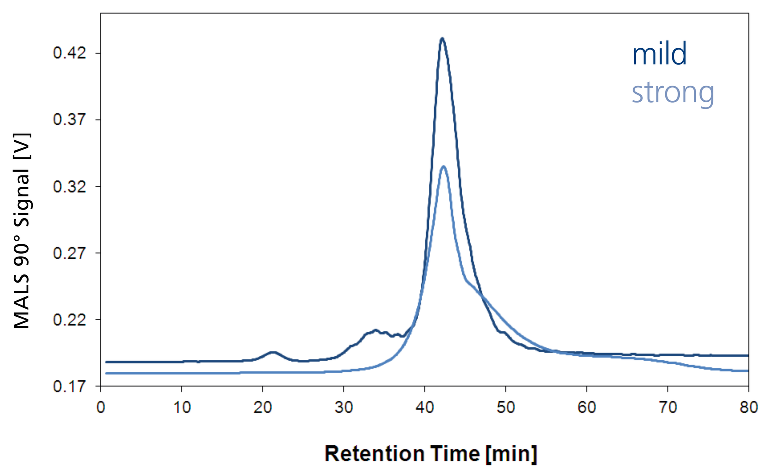

*Figure 4: **Polysaccharides**: Overlay - Light Scattering (LS) fractogram after mild and strong high-pressure treatment (size-sensitive detection).*

Synthetic and Natural Rubber

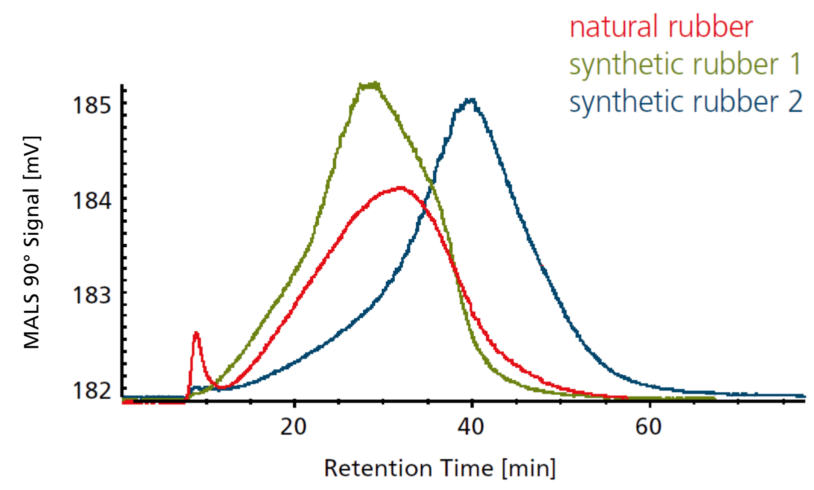

*Figure 5: **Synthetic and Natural Rubber**: Comparison of 90° MALS signal of different rubber samples.*

Natural Rubber

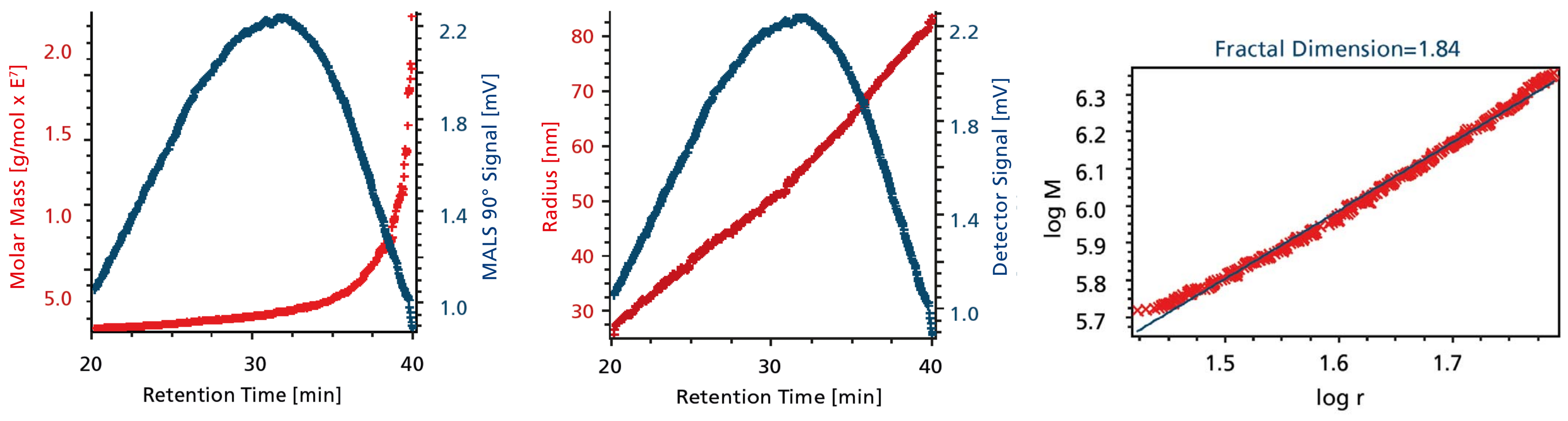

*Figure 6: **Natural Rubber**: Determination of molar mass, radius of gyration and fractal dimension.*

Thermal FFF (TF3)

Thermal FFF uses a temperature gradient for separation of sample molecules. The temperature gradient is generated between a upper hot plate and a lower cold plate in a narrow separation channel. The sample molecules or particles are separated by their hydrodynamic size and their chemical composition.

Figure 7: Postnova TF2000.

Applications of TF3-MALS

Natural Rubber

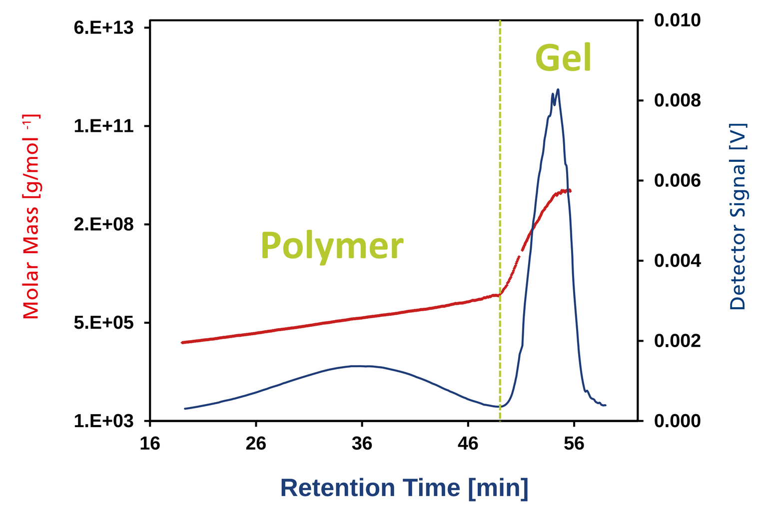

*Figure 8: **Natural Rubber:** Overlay of molar masses and radii of gyration with 90° MALS signal.*

Polystyrene

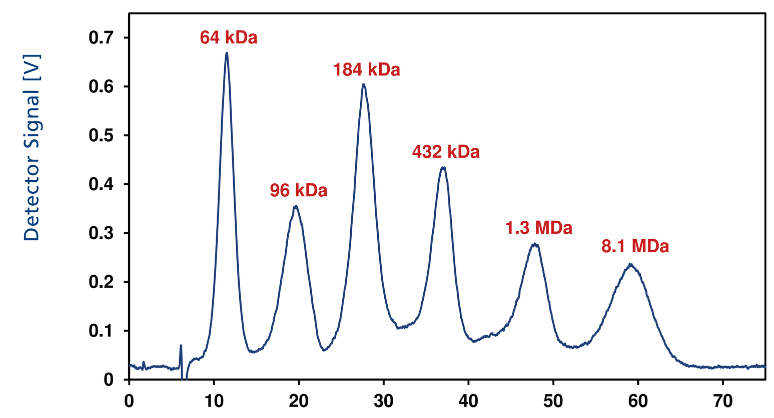

*Figure 9: **Polystyrene:** Fractionation of a mixture of Polystyrene of six different molar masses (90° MALS signal).*

Synthetic Rubber

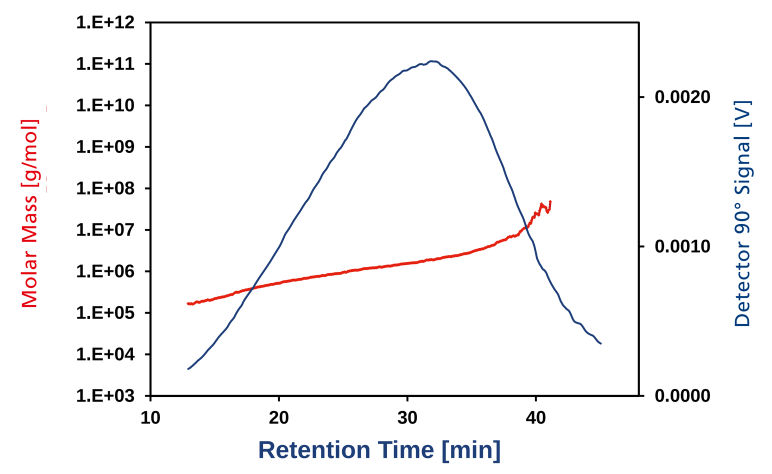

*Figure 10: **Synthetic Rubber:** Overlay of 90° MALS signal and molar mass.*

Polystyrene and PMMA

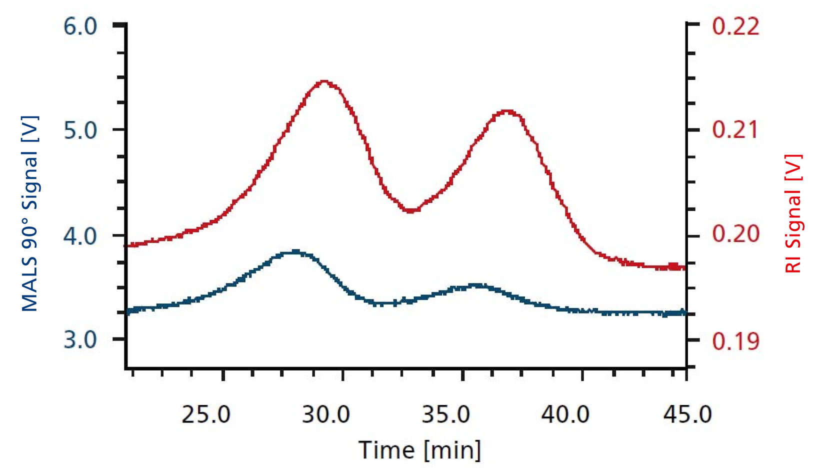

*Figure 11: **Mixture of 226 kDa Polystyrene and 242 kDa PMMA**: TF3 Fractogram showing RI and 90° MALS signal.*

Conclusions

In this presentation, the application of AF4 for the characterization of biopolymers in aqueous solvents and TF3 for the characterization of synthetic polymers in organic solvents was demonstrated. Several application examples showed the possibilities of the FFF techniques and the results that can be achieved when FFF separation methods are coupled with Multi-Angle Light Scattering (MALS) detection.

References

[1] Gogos A., Kaegi R., Zenobi R., Bucheli T.D., *Environ. Sci. Nano*, **2014**, 6(1), 584-594.
[2] Cascio C., Gilliland D., Rossi F., Calzolai L., Contado C., *Anal. Chem.*, **2014**, 86(24), 12143-12151.
[3] Greyling G., Pasch H., *Anal. Chem.*, **2015**, 87(5), 3011-3018.
[4] Kowalkowski T., Szparaga A., Pastuszak M., *Environ. Earth Sci.*, **2015**, 73, 1179-1788.

Postnova Analytics GmbH
86899 Landsberg, GERMANY
T: +49 8191 985 688 0

Postnova Analytics UK Ltd.
Worcestershire, WR14 3SZ, UK
Tel: +44 1684 585167

Postnova Analytics Inc.
84102 Salt Lake City, USA
T: +1 801 521 2004

Postnova North Europe
01630 Vantaa, FINLAND
T: +358 9 8545 510

info@postnova.com
www.postnova.com