Science Together

KNAUER

Molecular weight distribution of a broad polystyrene standard

Juliane Böttcher, Lisa Loxterkamp, Jan Wendrich, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin; www.knauer.net

SUMMARY

Gel permeation chromatography (GPC) and size exclusion chromatography (SEC) describe the separation of molecules based only on their molecular size. Using an AZURA® GPC it is possible to run GPC/SEC applications with aqueous as well as organic solvents, when changing the system modules with the suitable compatibility.

INTRODUCTION

GPC and SEC are based on the same separation principle, size exclusion. GPC can characterize polymers with determining the molecular weight distribution and the refractive index. In former times the term SEC was used for aqueous applications with stationary phases, e.g. dextran. GPC on the other side was applied with organic solvents and for instance polystyrene stationary phases. Nowadays both definitions are used side by side for the same separation. The peculiarity of GPC and SEC is that analysis is performed over one column volume, which results in a negative partition coefficient (k) for the analytes. The complete separation is therefore taking place over the selective permeation range which depends on the pore and exclusion volume[1]. The analyte needs to be dissolved in the eluent during separation to determine the exact molecular weight distribution. Sorption or chemical interactions with the stationary phase or wetted parts are objectionable. GPC is a relative molecular weight determination, where the best alternative is using the direct standard calibration in which case the standard is the same polymer type as the sample[2]. This application gives an example of the mentioned appliance. A calibration with narrow polystyrene standards on a polystyrene stationary phase was performed. With this calibration a broad polystyrene standard was analyzed. The obtained results are compared to the given values of the certificate of the broad standard to illustrate that KNAUER AZURA devices are suitable for GPC/SEC implementation.

Molecular weight distribution of a broad polystyrene standard

RESULTS

The elugram in **Fig. 1** shows an overlay of the RID trace of the broad standard and the determined calibration curve. Here it can be seen that the elution volume or the peak maximum of the broad standard lies within the calibration range. By using the ClarityChrom GPC/SEC extension it is easy to obtain the desired information about the molecular weight distribution of the analyzed samples. **Fig. 2** exemplarily shows the distribution of the broad standard. The certificate of the broad standard provides the following values: Mw=248000 Da and Mn=143000 Da. The polydispersity is given with a value of PD=1.73 [Mw/Mn]. In comparison the calculated result with ClarityChrom are Mw=247163 Da, Mn=126765 Da and PD=1.95 [Mw/Mn]. The deviation of the calculated values compared to the certificate might be a result of the used column with mixed porosities resulting in a broader weight distribution. This column normally is used for screening purposes and did not provide the best resolution. In addition, the broad standard signal is not completely within the calibration range, which makes the deviation of Mn and PD plausible. Another effect could be the approximated K and α coefficients which are not exactly according the measurement conditions due to the different temperature. For exact coefficient determination a viscosity detector should be used.

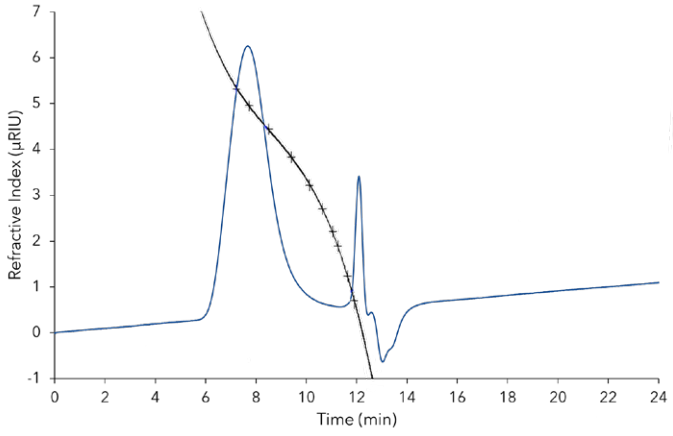

Fig. 1 Polystyrene broad standard elugram (blue) with the calibration curve (black) in the background .

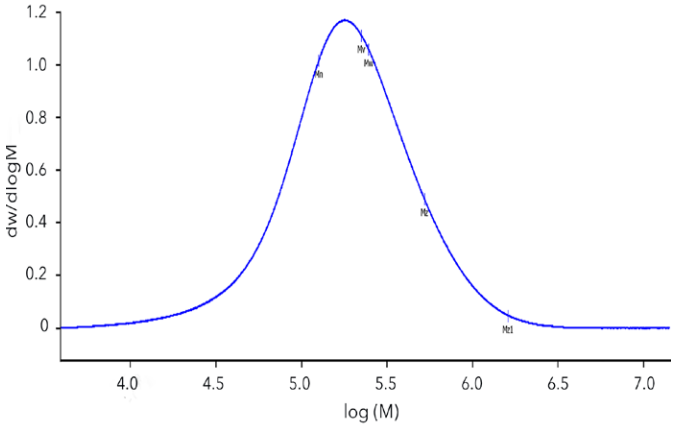

Fig. 2 Molecular weight distribution of the polystyrene broad standard.

SAMPLE PREPARATIONS

A calibration in a range from 580 Da to 364 kDa using the EasiCal PS-2 calibration kit was prepared. Standards were prepared directly in the vial and dissolved in approximately 1.5 ml THF. 30 mg of the broad standard polystyrene were weighed in a volumentric flask and dissolved in 10 ml THF overnight. The used K and α Houwink coefficient were provided by the ClarityChrom software with K=14.1 [dl/g*10^5] and α=0.7 at 25 °C. The elugrams of the measured calibration standards are shown in **Fig. 3** and **Fig. 4**.

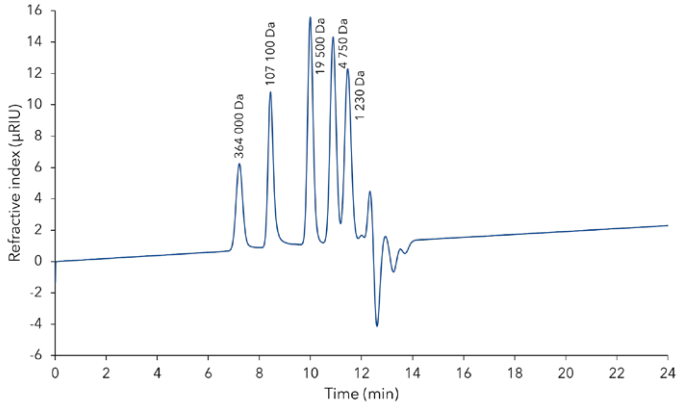

Fig. 3 Polystyrene Standard PS-2 A.

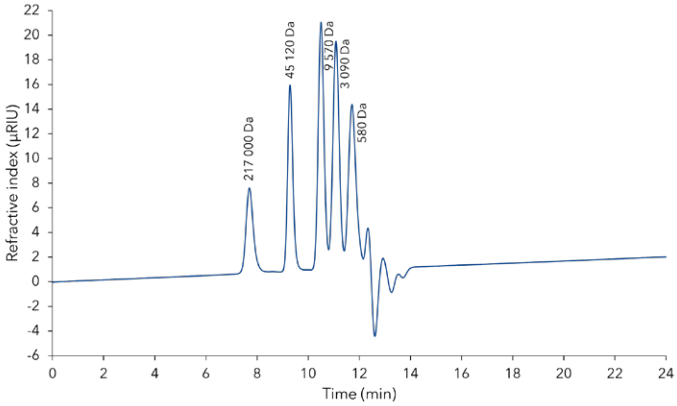

Fig. 4 Polystyrene Standard PS-2 B.

CONCLUSION

As pumps are available for normal phase operation, aqueous/organic and aqueous mode KNAUER devices are perfectly suitable for this application.

MATERIALS AND METHODS

Tab. 1 Method parameters

Column temperature	23 °C
Injection volume	20 µl
Injection mode	Partial loop
Detection	RI
Data rate, Time constant	20 Hz, 0.05 s
Temperatur of RID	35 °C

Tab. 2 Pump parameters

Eluent	Tetrahydrofuran stabilized with BHT
Compressibility	0.97
Flow rate	1 ml/min
Pressure	~ 35 bar
Pump program	isocratic

Tab. 3 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L HPG	APH35ED
Degasser	2 channel GPC degasser	A5335
Autosampler	AZURA AS 6.1L	AAA00AA
Detector	AZURA RID 2.1L	ADD31
Thermostat	AZURA CT 2.1	ATC00
Column	AppliChrom® ABOA StyDiViBe-P-10E5A-BPT, 5 µm, GPC column 300 x 8 mm	30GW470ABJ
Software	ClarityChrom 8.2.2	A1670
Software	ClarityChrom GPC Extension	A1678

Tab. 4 Standards and samples

Name	Molecular weight data
Polystyrene Standard EasiCal PS-2, Agilent	
Standard A	364 000, 107 100, 19 500, 4 750, 1 230 Da
Standard B	217 000, 45 120, 9 570, 3 090, 580 Da
Polystyrene PSS-psb250k Lot: psbr240k	Mw: 248 000 Da Mn: 143 000 Da PDI: 1.73

REFERENCES

[1] Wu, C. Handbook of size exclusion chromatography. Marcel Dekker (1995).

[2] Teraoka, I. Polymer Solutions: An introduction to physical properties. John Wiley & Sons, Inc. (2002).

RELATED KNAUER APPLICATIONS

VFD0153 – GPC cleanup of olive oil samples

VFD0166 – LC-FLD of 4 PAHs in olive oil samples using AZURA® GPC Cleanup System

VFD0172 – A D E K - Easy separation of fat-soluble vitamins using GPC/SEC