APPLICATION NOTE

AN1613: LMW Peptide Characterization by SEC-MALS

Introduction

SEC-MALS is widely recognized as an essential method for absolute characterization of proteins and polymers because it is a first-principles method that does not rely on column calibration or assumptions regarding the conformation or column interactions of the analyte. While often used for larger macromolecules, SEC-MALS is suitable for analysis of the molar mass of peptides despite their low molecular weight (LMW). The non-globular conformation of such peptides, which causes them to elute differently than well-folded proteins, is not an obstacle. In fact, a DAWN® or miniDAWN® multi-angle light scattering (MALS) detector is instrumental in obtaining on-line molar mass determinations for peptides of fewer than a thousand Daltons.

Figure 1 shows the chromatograms obtained from a mixture of proteins and peptides separated on a TSK G2000SWXL size-exclusion chromatography (SEC) column. A miniDAWN MALS detector and Optilab® differential refractive index (dRI) detector are plumbed downstream of the column. The signals are acquired and characterized in terms of molar mass by the ASTRA® chromatography software program. The peptide specific refractive index increment (dn/dc) values were measured using the Optilab by injecting a series of known concentrations.

The molar masses for the four major peaks determined by the MALS measurements are listed in Table 1. They compared favorably with those calculated from the amino acid sequences of the peptides and proteins.

As seen in the last column, the standard deviation of three consecutive runs was 3% or less. The results indicate that this SEC-MALS method provides a rapid, robust and convenient way to characterize peptides—as well as proteins—absolutely.

Peak No.	Protein Name	Sequence *M* (Da)	Measured *M* (Da)	*M* RSD
1	BSA	66,700	64,300	1%
2	Lysozyme	14,400	14,600	1%
3	Bradykinin	1,060	1,090	2%
4	Leucine-Enkephalin	556	592	3%

Table 1. Peptide and protein molar mass values

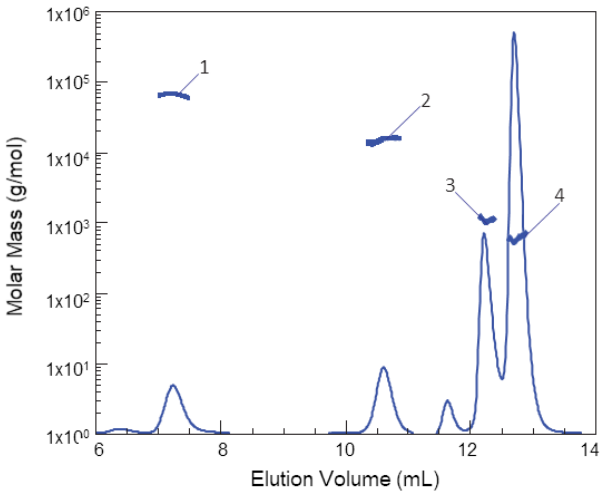

Figure 1. Molar mass determined by SEC-MALS of the four peaks identified, overlaid with the refractive index chromatogram.