

SEC Analysis of Gelatin with Online Light Scattering Detection

Application Note

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Introduction

Food-grade gelatins are biologically derived materials used in the food industry as thickening agents. SEC analysis of gelatins yields critical molecular weight information upon which the physical properties of the polymer (such as the setting properties) depend. Linear Agilent PL aquagel-OH MIXED-H 8 μm columns were used. These columns resolve up to $10,000,000 \text{ g}\cdot\text{mol}^{-1}$ (polyethylene glycol/oxide equivalent), offering high performance columns with excellent resolution over a very wide range of molecular weights, simplifying column selection and providing a versatile analytical system.



Conditions

The eluent was prepared as a buffer with its pH adjusted by the addition of 0.1 M NaOH. The samples were accurately prepared as 1.0 mg/mL solutions in the eluent. The light scattering detector was first calibrated using a pullulan polysaccharide standard. The standard was Mp 186,000 and prepared at 1.0 mg/mL. From the known concentration, Mp and dn/dc of the calibrant, the detector constants and inter-detector volume for the system were calculated.

Sample: Gelatin
Columns: 2 x PL aquagel-OH MIXED-H 8 μ m, 300 x 7.5 mm (part number PL1149-6800)
Eluent: Water + 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ at pH 7
Flow Rate: 1.0 mL/min
Detection: RI and Agilent PL-LS 15/90 Light Scattering Detector

Results and Discussion

From the RI chromatogram, dn/dc was calculated for the gelatin sample as the sample had been prepared at known concentration. This value of dn/dc was then used to calculate a bulk Mw value from the 90° and the 15° light scattering data.

The RI and light scattering data was also used to perform an SEC slice-by-slice molecular weight calculation for the gelatin sample using both LS signals. The bulk Mw values were 174,000 (90°), 189,850 (15°) and 184,800 (SEC).

Figure 1 shows the RI and the 90° and 15° light scattering data for the gelatin sample. Light scattering detection is more sensitive to higher molecular weight species, hence the 90° and 15° light scattering chromatograms placed more emphasis on high molecular weight material than the RI chromatogram. The RI chromatogram also contained a negative peak due to compositional differences between the sample, solvent and eluent, which was not observed by light scattering.

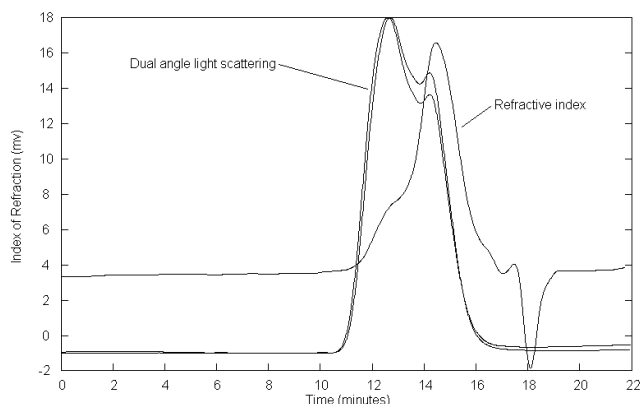


Figure 1. RI and 90° and 15° light scattering data for the gelatin sample

Conclusion

The wide molecular weight operating range of PL aquagel-OH MIXED-H 8 μ m columns makes them particularly suited to the analysis of water soluble polymers with intermediate to high molecular weight. The use of a simple buffer solution as the eluent for the analysis of gelatins reduces interaction between the sample and the columns ensuring that good chromatography is obtained.

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