



Aqueous SEC analysis of pectins used in pharmaceutical products

Application Note

Author

Stephen Luke
Agilent Technologies, Inc.

Introduction

Pectin is a natural product used for coating capsules. It is produced from plant raw materials such as apple, citrus and beet. The extracts are processed to derive pectins with specific properties. Although pectin chemical composition is key to its application, rheological behavior is critical to performance, and determination of the molecular weight distribution can help to predict rheological behavior. Size exclusion chromatography and Agilent PL aquagel-OH MIXED-H 8 μm columns are ideal for resolving pectins. With their wide molecular weight resolving range (up to 10 million g/mol relative to PEO/PEG) and high efficiency (>35,000 plates/meter), PL aquagel-OH MIXED-H 8 μm are the columns of choice for this application.



Agilent Technologies

Pectin samples were prepared at 2 mg/mL, left to fully dissolve overnight and filtered through a 0.45 µm membrane. The column set was calibrated with narrow pullulan standards and, therefore, all molecular weight values quoted are relative to these. The calibration curve is shown in Figure 1.

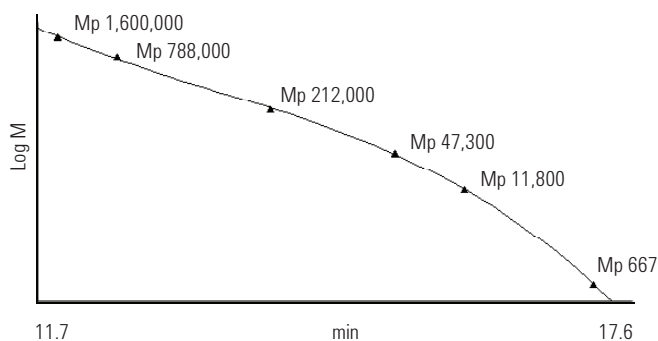


Figure 1. Pullulan standard calibration curve for the Agilent PL aquagel-OH MIXED-H 8 µm column

Raw-data chromatograms for the pectin samples are illustrated in Figure 2.

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 7.5 x 300 mm
(Part No. PL1149-6800)
Eluent: 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ at pH 7
Flow Rate: 1.0 mL/min
Temp: 50 °C
Detector: RI

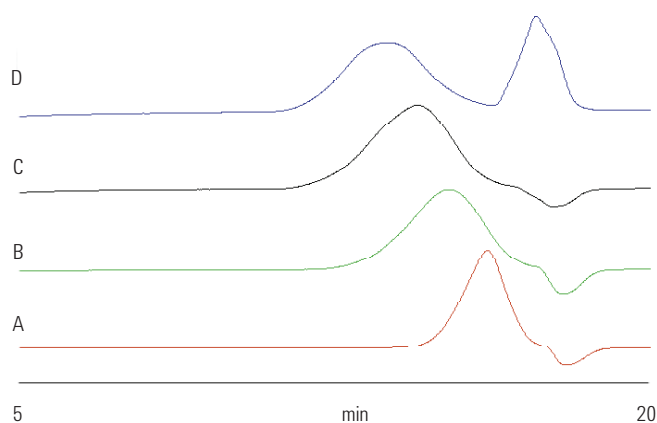


Figure 2. Chromatograms of four pectin samples on Agilent PL aquagel-OH MIXED columns

Overlaid molecular weight distribution plots are shown in Figure 3.

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 7.5 x 300 mm
(Part No. PL1149-6800)
Eluent: 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ at pH 7
Flow Rate: 1.0 mL/min
Temp: 50 °C
Detector: RI

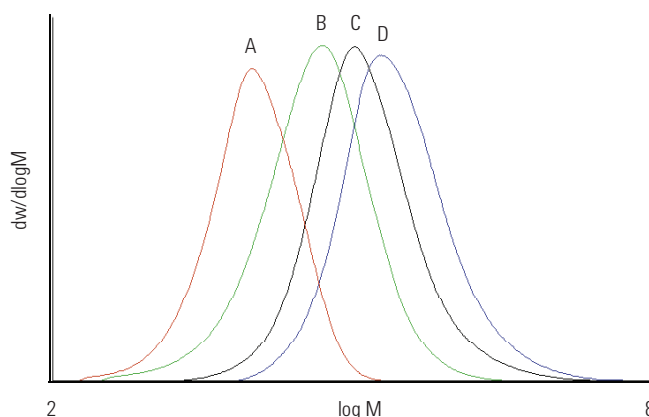


Figure 3. Molecular weight distributions of four pectins

Unlike the other samples, sample D exhibits a strong, positive peak around total permeation. This sample is a slow-setting grade and contains buffer salts added to modify its properties. Molecular weight averages for the samples are given in Table 1.

Table 1. Molecular weight averages and polydispersity for four pectin samples

Sample	Molecular weight average		Polydispersity (Mw/Mn)
	Mn	Mw	
A	6,520	17,560	2.7
B	21,720	88,480	4.1
C	67,980	243,120	3.6
D	128,360	459,990	3.6

The samples vary in molecular weight and in polydispersity (Mw/Mn).

The wide molecular weight operating range of PL aquagel-OH MIXED-H 8 µm 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 pectins reduces interaction between the sample and the columns, ensuring that good chromatography is obtained.

www.agilent.com/gpc-sec

This information is subject to change without notice.
© Agilent Technologies, Inc. 2014
Published in UK, February 18, 2014
5991-4086EN



Agilent Technologies