Molecular weight determination of Gelatin

Application Note Food Analysis

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Reproducible and easy-to-use GPC/SEC analysis for all types of Gelatins can be done using a SECcurity GPC system and PROTEEMA columns. Exact sample preparation, following a strict protocol, and the choice of the proper solvent is a crucial step for successful analysis. Determination of the molar mass distribution and the molar mass averages can be done using PSS WinGPC Software. A very comprehensive characterization for multimodal samples, like the Gelatins, can be done using the WinGPC multi area evaluation.

Introduction
Gelatin is a polypeptide produced from hydrolysis of collagens in skin and bones. It is obtained from acidic or from basic hydrolysis and contains poly amino acids. Gelatin is used in the food and beverages industries, in the pharmaceutical industry, as a stabilizer for tablets, and also for many other applications.

The isoelectric point of Gelatin produced by basic hydrolysis is between 4.7 and 5.2; the Gelatin produced by acidic hydrolysis has an isoelectrical point of 7.5 to 9.3. Gelatin produced by basic hydrolysis can be easily measured using standard conditions (see PSS Column Application notes 10271 and 10272).
Gelatin with an isoelectric point between 7 and 9 must be measured using low pH values or at pH-values > 9 (not recommended) in order to achieve sufficient solubility.
The following experimental conditions can be applied to Gelatins independent of their isoelectric point and the hydrolysis process. However, exact sample preparation following a strict protocol is a crucial step for the GPC/SEC analysis of Gelatins

System Requirements

	Conditions
Pump	PSS SECcurity GPC1260 isocratic pump • flow rate [mL/min]: 0.5-1.0 • mobile phase: Phosphate Buffer pH 5.5 (acidic gelatin) or 6.6 (basic gelatin) + 0.2 M NaCl + 0.5% SDS
Injection system	PSS SECcurity GPC1260 Autosampler
Columns	• PSS PROTEEMA precolumn (8*50 mm) • PSS PROTEEMA, 5 µm 1 000 Å, 300 Å, 100 Å (8x300 mm each)
Calibration	PSS Pullulan kit, 10 standards: 342 - 710 000 Da (alternative: PSS Poly(styrene sulfonate) sodium salt kit, 10 standards: 100 - 1 000 000 Da)
Loading	• 2 mg/mL, 100 µL injection volume
Detectors	• PSS SECcurity DAD, 214 nm +/- 4 nm
Software	PSS WinGPC UniChrom • optional for FDA 21CFR11 compliance: Compliance Pack

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Procedure, Results & Discussion

In this application three different Gelatins with different Bloom values have been investigated. The Bloom value refers to the firmness of gelatin and is determined using a Bloom Gelometer. This instrument measures the rigidity of a gelatin film. The higher the number, the more viscous the product. Gelatin used in food applications has Bloom values between 125 and 250.

For all three samples the relative molar mass distribution and the molar mass averages have been determined based on conventional calibration with narrow Pullulan molar mass standards.

Figure 1 shows an overlay of the samples while table 1 compares the numerical results.

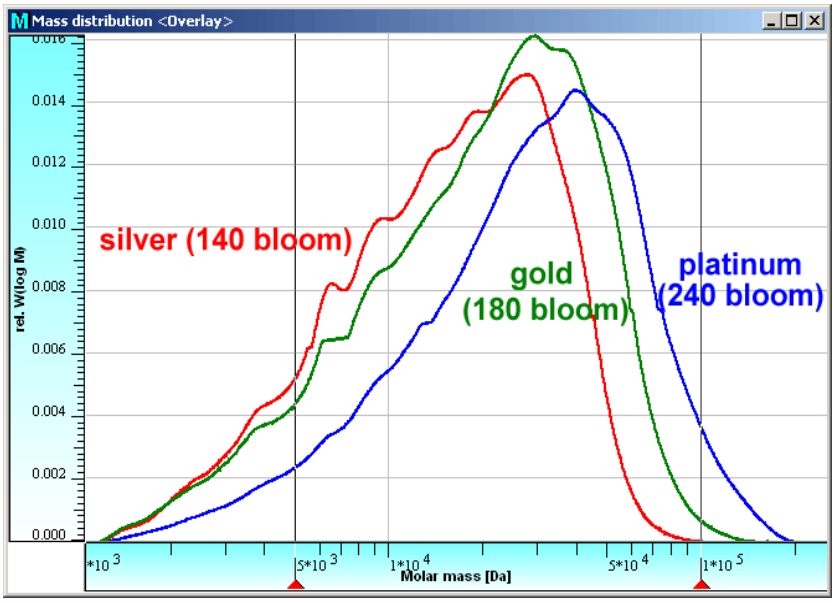

Fig. 1: Molar mass distributions for different gelatins from Carl Roth Chemicals, Karlsruhe, Germany

Table 1: Numerical results and molar mass averages for the three different gelatins.

	Gelatin silver standard	Gelatin gold standard	Gelatin platinum standard
M_n [Da]	10 300	12 100	16 900
M_w [Da]	19 200	25 300	36 000
D (PDI) [-]	1.87	2.10	2.14
M_p [Da]	27 800	29 200	39 200
Area [ml*V]	0.01269	0.01401	0.01219
% < 5 000 Da	11.17	9.12	4.98
% > 100 000 Da	0.01	0.31	3.34

These data show that the higher the Bloom value, the higher the molar mass averages and the molar mass fractions above 100 000 Da (based on Pullulan calibration).

Figure 1 also shows that Gelatins have a broad molar mass distribution and that multiple peaks can be present in the sample. Therefore, simultaneously to the characterization of the complete sample, a very detailed analysis of the Gelatin silver sample has been done using the WinGPC multi area data evaluation option.

Here up to 8 regions can be defined; the region definition can be done based on
- elution volumes
- elution times
- molar masses.

The user can select if the values are fixed for all samples or if WinGPC should identify the peaks by searching for minima.
In addition the user can select which GPC/SEC results should be determined, either for every single regions or even for a combination of regions. This approach can be automated and allows a comprehensive analysis as well as a powerful easy-to-use quality control for multimodal Gelatin samples.

In the example below the silver gelatin has been divided into 7 different regions, A to G, by setting elution volume borders. WinGPC was configured to search for the minima. The regions are visualized in figure 2.

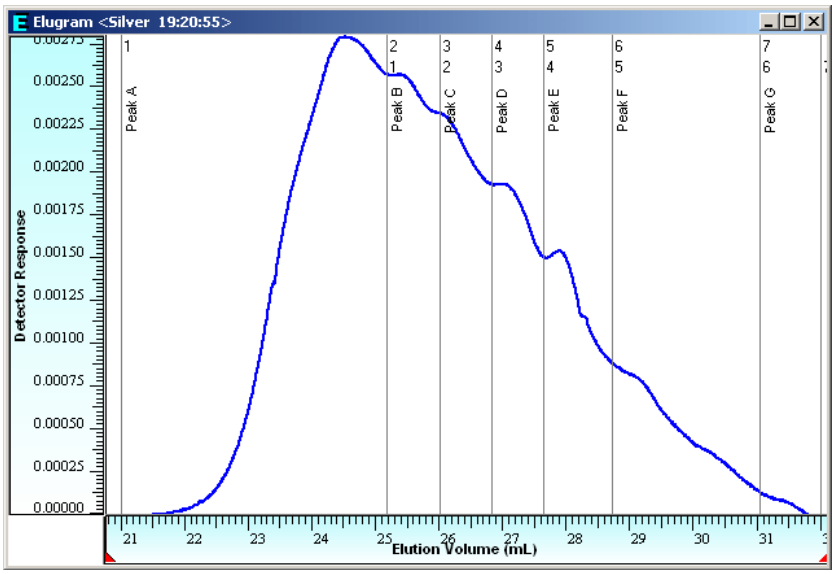

Fig. 2: Detailed characterization and reliable quality control applying the WinGPC multi area detector evaluation for sample Gelatin silver. 7 regions have been defined so that GPC/SEC results for 7 peaks are available in a single analysis (together with the overall sample results shown in table and figure 1).

Figure 3 shows the selection criteria for the 7 regions and the required numerical results.

With this very detailed analysis a comprehensive characterization is available. The multi area analysis can be automated, so that no additional user input is required. Therefore a method for quality control can be established quite easily.

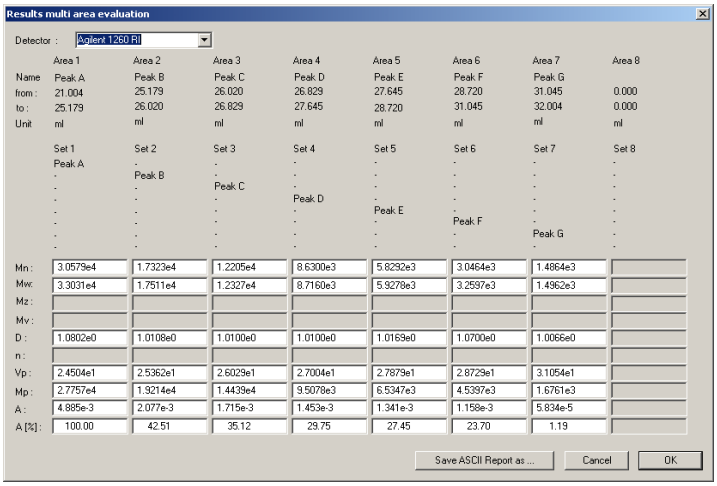

Fig. 3: Overview regions and results for the 7 regions. Only the results selected by the users are displayed (not selected: grayed out; areas are referenced to Peak A).

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