

The Agilent WinGPC 3D Spectra Add-On for UV-Vis and Fluorescence Online Spectra

Introduction

Changes in UV-Vis or fluorescence spectra as a function of molecular weight can provide useful information for the analysis of copolymers and polymer mixtures. The Agilent WinGPC 3D spectra Add-On enables detection and recording of online spectra for each chromatographic slice of a GPC/SEC measurement. Spectra are displayed in a 2D or 3D plot of wavelength against elution volume and signal intensities. This technical overview uses an Agilent 1260 Infinity DAD as an example for the setup and evaluation of a 3D measurement.

Experimental

System setup

To enable measurements and evaluations, WinGPC 3D Spectra Add-On must be activated in the Login window (Figure 1), similarly to other Add-Ons such as the Copolymer or Light Scattering and Viscosity Add-Ons. In the WinGPC login window, select PSS-UDC 810 as the interface in the PSS UDC Devices window.

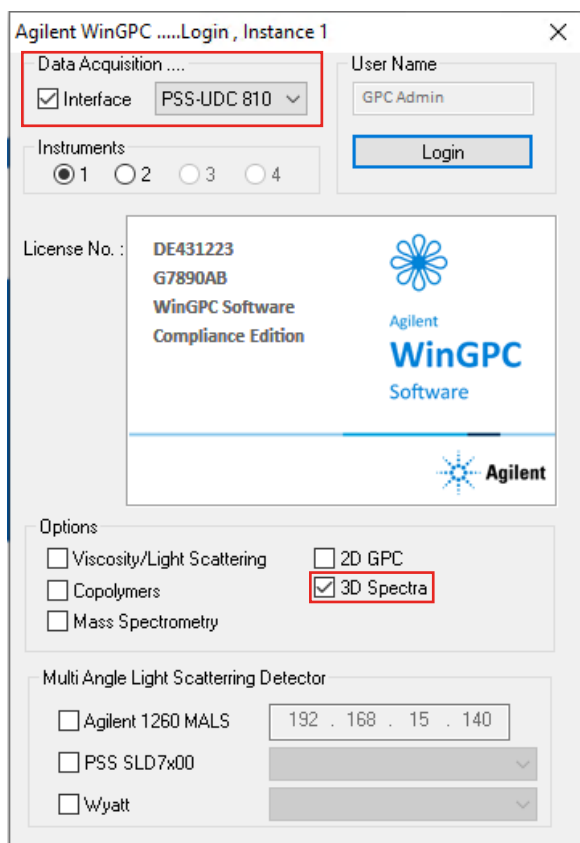


Figure 1. Agilent WinGPC Login window.

Click **ChromPilot** in the Method window, click the **DAD 1** tab, and then choose **Advanced** (Figure 2). Once the All option is chosen from the Store drop-down menu in the Spectrum panel, 3D measurements are activated. The wavelength range and steps can then be specified between the single points of the spectra. The configuration dialog boxes can be slightly different for other detectors. For example, with fluorescence detectors, emission and excitation can be distinguished from one another.

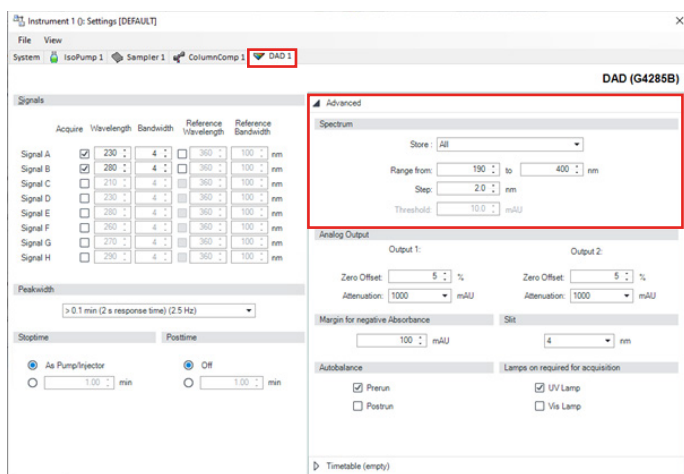


Figure 2. Agilent WinGPC ChromPilot Instrument Manager.

Once all 3D spectra parameters are set correctly, they will be displayed in the ChromPilot simplified user interface (in the DAD panel in the System tab; see Figure 3). This indicates that 3D measurement is activated. If 3D measurement is not activated, "Off" will be displayed. Measurements can be started by creating a sequence in the Sequence Manager and clicking Start sequence. The spectra are acquired automatically and simultaneously with the GPC/SEC measurement.

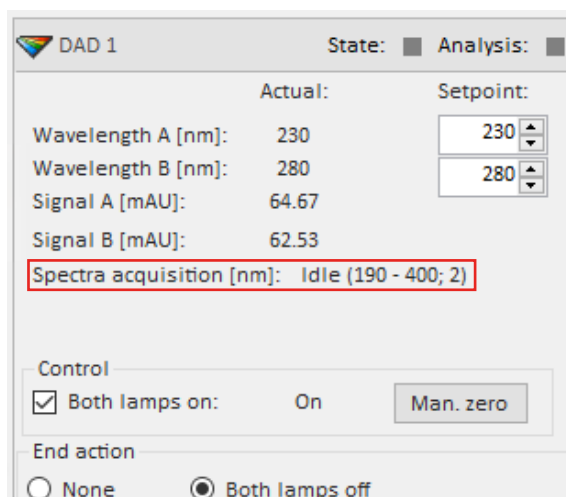


Figure 3. DAD 1 panel in the System tab of the Agilent WinGPC ChromPilot Instrument Manager.

Spectral analysis

When the measurement is finished, the recorded 3D spectra can be opened. Select the desired injection to activate the elugram window and select **File > ...to 3D Spectra**. A window displaying the 3D spectra will appear (Figure 4).

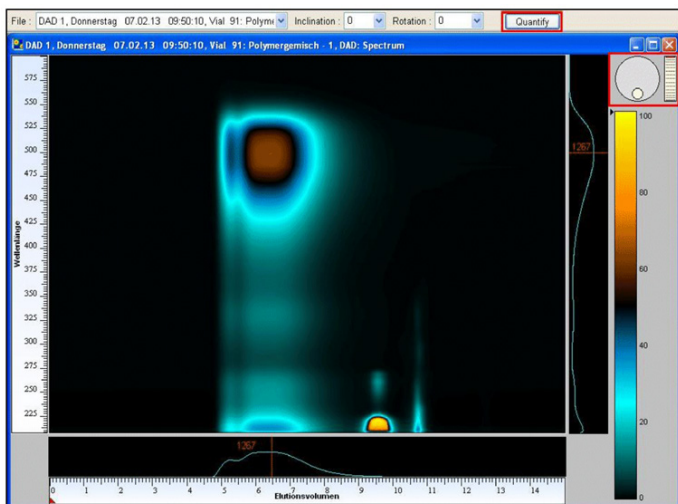


Figure 4. Example 3D spectrum.

In the Preset view, the elution volume is plotted on the X-axis, and the wavelength of the UV/VIS or fluorescence spectra on the Y-axis. In this 2D picture, different colors represent varying signal intensities. Click **Quantify** to toggle between axis scales.

While the mouse pointer moves over the 2D landscape to any coordinate, the corresponding spectrum (projection) will be displayed on the right, and the related elugram will be displayed below the 2D screen. Like other scalable WinGPC windows, both axes can be shifted and stretched; press and hold the left mouse button to zoom into a region.

There are more sophisticated display options in the upper right corner of the 3D screen. With the round "horizontal wheel", the spectrum can be rotated, and with the rectangular "vertical wheel", the angle of view can be adjusted, allowing the spectra to be flipped into the third dimension. Both angles can also be entered manually in Rotation and Inclination.

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Quantitative evaluation can be activated by clicking Options, Grid, then Design Mode in the menu. The region of interest can be outlined by clicking with the left mouse button and defining the region point by point (Figure 5). Click **ESC** once the region is completely enclosed. WinGPC will then assign a number to this region. Following the above process, other evaluation regions can be added. The results can be found by clicking through the file path from Analysis to Results.

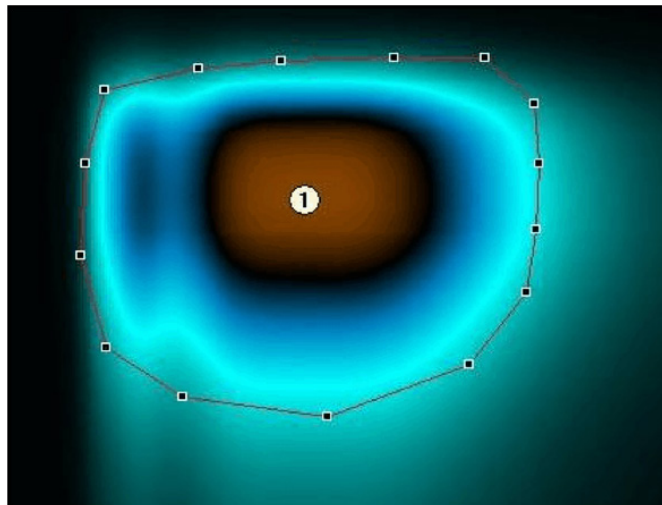


Figure 5. An outlined region within a 3D spectrum.

3D spectra are stored in a file ending .SPX in the same folder as the WinGPC project files, which comprise other file types: .ATX, .FSX, .INX, .LDX, .MDX, and .SAX. SPX files are crucial for the evaluation of 3D spectra, and so must be included if WinGPC projects are copied or moved.

Conclusion

The Agilent WinGPC 3D spectra Add-On enables automatic acquisition of 3D spectra simultaneously with GPC/SEC analysis. The module makes it possible to determine polymer fractions of copolymers or mixtures against wavelength and molecular weight, or to quantify extinction coefficients.