Agilent

Trusted Answers

Creating Sawtooth Gradients with Agilent WinGPC

Introduction

Sawtooth gradients have recently been introduced to polymer separations.[1] In sawtooth gradients, the sample is initially adsorbed or precipitated onto the stationary phase. The sample components are then released based on differences in adsorption strength or solubility. For this purpose, a series of isocratic steps of increasing eluent or solvent strength, separated by further steps of much lower eluent or solvent strength, are applied. The implementation of sawtooth gradients with several steps requires a significant number of entries in a gradient table. These steps often exceed the number of entries foreseen in the gradient tables of software and pump suppliers.

The Agilent WinGPC ChromPilot sequence manager makes it possible to perform sawtooth gradients with the Agilent 1260 Infinity II GPC/SEC System. ChromPilot instrument methods (.spm files) containing different timetables are required for this purpose.

Experimental

Programming a sequence

1. Open the WinGPC ChromPilot sequence manager; if necessary, clear the table.

2. Select **Load instrument settings** and load the .spm file to start.

3. Select **Injection** and enter the parameters for the sample to be injected and separated using the sawtooth gradient.

4. The additional gradients will be started using dummy injections with 0 mL injection volume:

   - Select **Load instrument settings** and load the next .spm file, set the interval to 0, and use append row to add these data to the sequence.

   - Select **Injection**, enter the same vial position and a descriptive sample name (e.g, "start new gradient"), and use **append row** to add this information to the sequence.

   - Modify the injection volume to 0 in the sequence table. Attempts to enter a zero volume in the Row details section will result in an error message—this step must be performed in the table.

5. Continue with step 4 until all files are loaded, and then start the sequence.

Figure 1 shows a sequence where the next gradients are loaded (lines 3, 5, and 7) and immediately started by a zero-volume injection (lines 4, 6, and 8).
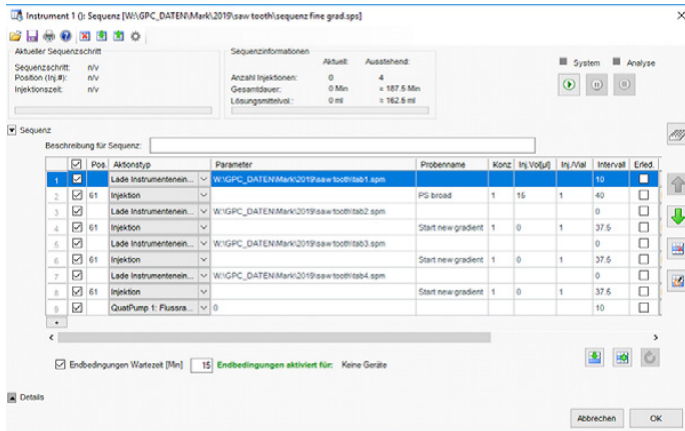

Figure 1. Sequence Manager window

Creation of instrument methods after import of XML files

A convenient way to create the required .spm files is available when:

- Instrument methods are saved after import of XML files containing gradient definitions

- XML files are created using an Excel sheet

Creation of XML files with the Agilent Excel sheet

A Microsoft Excel file "saw tooth.xlsm" for quaternary pumps for gradients using channels A and D can be obtained free of charge from Agilent (Figure 2). Please verify the correct use of this sheet. Agilent takes no responsibility for and does not guarantee long-term support for the use of the Excel sheet, as it is not an official Agilent product.
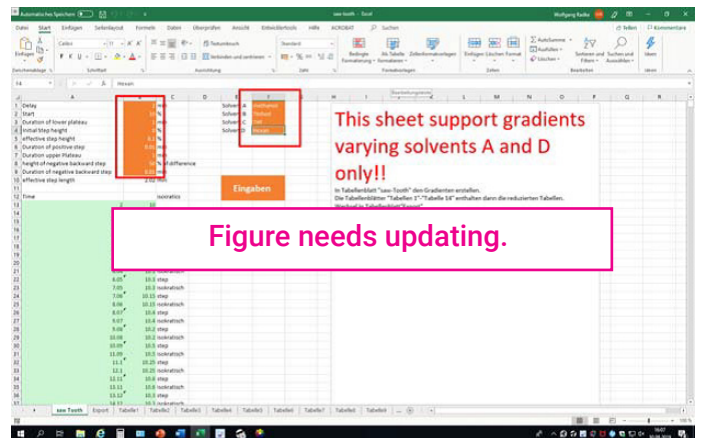

Figure 2. Microsoft Excel sheet for the creation of XML files

To create XML files in Excel:

1. Open "saw tooth.xlsm" and (if necessary) activate macros.

2. Enter or modify the parameters in the orange cells in the "sawtooth" worksheet. This step will update the complete timetable in this tab. Excel will automatically cut this complete gradient at the lower plateaus into shorter gradients, each of 149 lines (sheets labeled "Tabelle xx").

3. Follow the description as given in the "sawtooth" worksheet to export and save up to 14 different XML files.

To create instrument methods:

1. Open the WinGPC ChromPilot instrument manager (Figure 3), select the **Pump** tab, and import the first XML file.
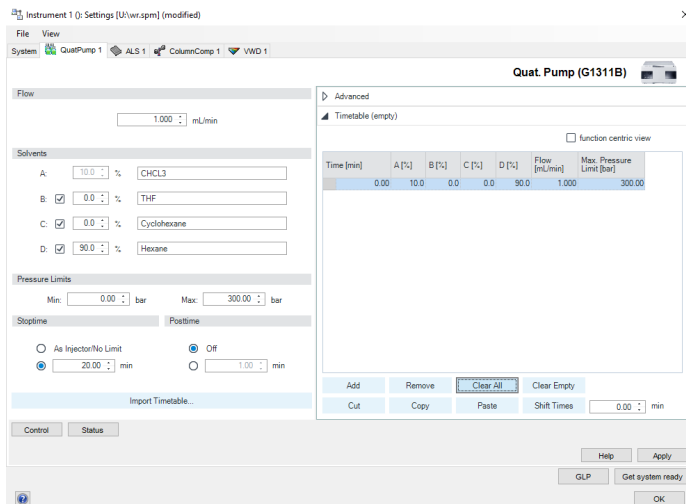

Figure 3. Instrument Manager window

2. Save the instrument settings as an .spm file by clicking **File**, then **Save settings**.

3. Repeat this step until all XML files are saved in an .spm file.

Prerequisites/recommendations

The maximum number of gradient steps per sequence is 46,000.

The number of lines in the WinGPC ChromPilot sequence manager is limited to 255. Each sequence line loading an instrument method can initiate a gradient consisting of up to 184 lines. Thus, a total of approximately 46,000 gradient lines can be executed for all injections in a sequence. The 46,000 lines can be distributed to either more complex (longer) gradients with fewer injections, or vice versa.

Multiple injections from the same vial need separate sequence lines

As the run for a true injection requires triggering of several gradient starts by application of zero volume injections, it is not possible to use the multiple injection per vial option of the WinGPC ChromPilot sequence manager (Inj./Vial needs to be 1). Copy the lines to inject from the same vial.

Load/inject during isocratic portions of the gradient

Downloading a complex gradient to the instrument requires time. For this reason, there may be a delay between the programmed loading and start of the next gradient, and the true gradient start.

Conclusion

The sequence manager function in Agilent WinGPC ChromPilot is an effective tool for performing sawtooth gradients. With capacity for up to approximately 46,000 gradient lines in a sequence, the sequence manager can comfortably host the extensive steps involved in these gradients.

Reference

1. Durner, B.; Ehmann, T.; Matysik, F. M. High-Resolution Polymer High Performance Liquid Chromatography: Application of a Saw Tooth Gradient for the Separation of Various Polymers. *J. Chromatogr A* **2019**, *1587*, 88–100.

www.agilent.com

Agilent

Trusted Answers