Application Note

Biopharma/Pharma

Dual-Reactor Sampling with Agilent InfinityLab Online LC Solutions

Switching between two reactors by means of an Agilent 1290 Infinity Flexible Cube

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Abstract

This application note demonstrates the capability of the Agilent 1290 Infinity Flexible Cube to be used as a seamlessly integrated sampling device for Agilent InfinityLab Online LC Solutions. This enables full control of sampling with Agilent OpenLab CDS and the Agilent Online LC Monitoring Software. This ease of use allows the user to sample from a reaction vessel according to his or her needs and to connect the reaction vessels to the online LC. As an example, two reactors with a hydrolysis reaction at two different pH conditions were monitored.

Introduction

Efficient and reproducible sampling from the reaction vessel using related sampling devices is an essential building block in the process workflow for the online LC analysis of chemical reactions or biological conversions. The easiest way to sample is with a simple piston pump, a peristaltic pump, a programmable syringe pump[1], or simply an isocratic HPLC pump as a sampling device. Using more complex sampling devices not only enables pumping of the reaction solution to the online LC; samples can also be taken from different reaction vessels, and the reaction solution can be diluted or filtered before infusion into the HPLC instrument. Therefore, these modules often require separate control software and customized connections to the online LC.

The 1290 Infinity Flexible Cube makes it possible to pull reaction solutions from the (reactor) sampling point by use of an internal piston pump. By combining two integrated switching or stream selection valves, the Flexible Cube can be combined with flexible and adjustable configurations for sampling stream diversion, depending on the application. Enabling sampling from multiple sampling points and several reaction vessels, the Flexible Cube is supported by internal pressure monitoring and flushing capability to avoid carryover. The option to install a 4/2-way solvent selection valve (SSV) extends the flushing capability with up to three solvent types, compatible with reaction solvents from various sampling points (Figure 1). In addition, the Flexible Cube is connected to the InfinityLab Online LC Solution via a standard CAN interface and so is fully controlled by OpenLab CDS software and part of the LC acquisition method. With the inclusion in the OpenLab CDS acquisition methods, the Flexible Cube can be used seamlessly with Online LC Monitoring Software.

This application note demonstrates the use of the Flexible Cube integrated into the InfinityLab Online LC Solution setup as a sophisticated sampling device, adjustable to customer's applications. Samples are taken from two reactors running a hydrolysis of acetyl salicylic acid at different pH values.[2] The complete experiment is orchestrated by the Online LC Monitoring Software. The obtained results are visualized in corresponding trending plots.

Experimental

The instrumentation and software used in this study are detailed in Table 1. The method parameters are outlined in Table 2, and reaction conditions in Table 3.

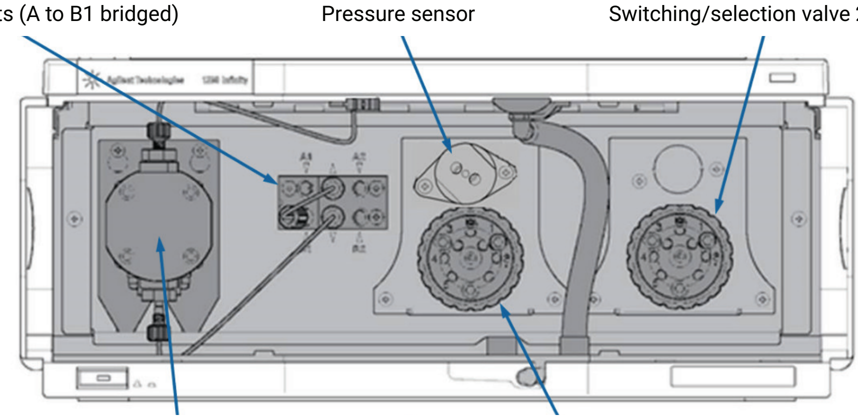

Figure 1. Agilent 1290 Infinity Flexible Cube configuration example.

Table 1. Instrumentation and control software.

Product Type	Product Description
Instrument	– Agilent 1290 Infinity II High-Speed Pump (G7120A) – Agilent 1260 Infinity II Online Sample Manager Set (G3167AA): – Agilent 1260 Infinity II Online Sample Manager (G3167A), clustered with external valve (p/n 5067-6680) located at the Agilent 1290 Infinity Valve Drive (G1170A), and Agilent Online LC Monitoring Software – Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) – Agilent 1290 Infinity II Diode Array Detector (G7117B) with Agilent InfinityLab Max-Light Cartridge Cell, 10 mm (G4212-60008) – Agilent 1290 Infinity Flexible Cube (G4227A) equipped with one or two selection valves (G4235A)
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3.0 × 30 mm, 1.9 µm (p/n 691775-302)
Software	– Agilent OpenLab CDS, version 2.6 or later – Agilent Online LC Monitoring Software, version 1.1

Table 2. Method parameters.

Parameter	Value
Analytical Method Conditions: Agilent OpenLab CDS	
Mobile Phase Solvents	A) Water + 0.1% formic acid (FA) B) Acetonitrile (ACN) + 0.1% FA
Mobile Phase Flow Rate	1.0 mL/min
Isocratic Separation Conditions	35% B, stop time: 0.5 min
Column Temperature	45 °C
Feed Injection (Automatic)	80% of analytical flow rate
Flush-Out Solvent	Water (S2)
Flush-Out Volume	Automatic
Injection Volume	1 µL
Needle Wash	3 s, 1:1 water: ACN + 0.1% FA (S1)
Sampling	See sampling methods for sampling to vial
Diode Array Detector	230 ±4 nm, reference: 360 ±50 nm, 20 Hz data range
Sampling to Vial (Dilutions): Agilent Online LC Monitoring Software	
Sampling	Sampling from reactor into deep-well plate sealed with silicone mat
Target Volume	500 µL
Dilution Factor	10
Sample Volume	50 µL
Draw Speed	Setting 1 – Draw speed: 130 µL/min – Wait time: 1.2 s – Dispense speed: 155 µL/min (Ejection of sample into well before dilution)
Dilution Solvent	S2 (Water)
Dilution Eject Speed	10,000 µL/min (after sample ejection for mixing)
Schedule	See Table 3
Sample Delivery Method Conditions: Agilent OpenLab CDS with Agilent 1290 Infinity Flexible Cube	
Pump	Agilent 1290 Infinity Flexible Cube piston pump
Flow Rate	3 mL/min, time based 0.5 minutes each for sampling from reactor and flushing
Reactor Selection (See Figure 2)	– Selection valve position 1 for OL CDS Method used in "PrerunBlank01" Sample (without injection) – Selection valve position 2 for OL CDS Method used in "PrerunBlank 02" Sample (without injection) – Selection valve position 12 for OL CDS Method used in "DilutedToVialSetting 01 and 02" samples (Table 4)

Table 3. Reactor equipment and reaction conditions.

Reaction Conditions	
Reactor	Mettler Toledo EasyMax 102, equipped with two 50 mL reaction vessels, connected with Julabo cooler
Reactant	Acetyl salicylic acid, 50 mg in 50 mL
Solvent	Glycine buffer (45 mL) at pH 9 and pH 10
Stirring	At 25 °C
Reaction Start	Reaction started by adding educt: 50 mg of acetyl salicylic acid in 5 mL of EtOH

Instrument configuration

The 1290 Infinity Flexible Cube was equipped with one or two selection valves. The first stream selection valve was connected to the internal 4/2-way SSV at Port 12 to deliver one of three washing solvents. They should be chosen with consideration of their different dissolution properties to avoid carryover between samplings. Ports 1 and 2 of the first stream selection valve were connected to reaction vessel one and two, accordingly. The central common port of the first stream selection valve was connected to the integrated piston pump to pull the sample from the corresponding reactor to the Agilent 1260 Infinity II Online Sample Manager's external sampling interface (Figure 2). In this setup, the portion of pulled sample stream is disposed of through the external sampling interface, connected downstream, to the separate reactor waste container.

Using the second stream selection valve installed in the Flexible Cube gives the option of guiding the pulled sample back to the reaction vessel, avoiding loss of reaction solution (Figure 3). Therefore, the dead volume of the connecting tubes has to be taken into account to avoid pollution of the reactor with flushing solvents remaining in the tubing.

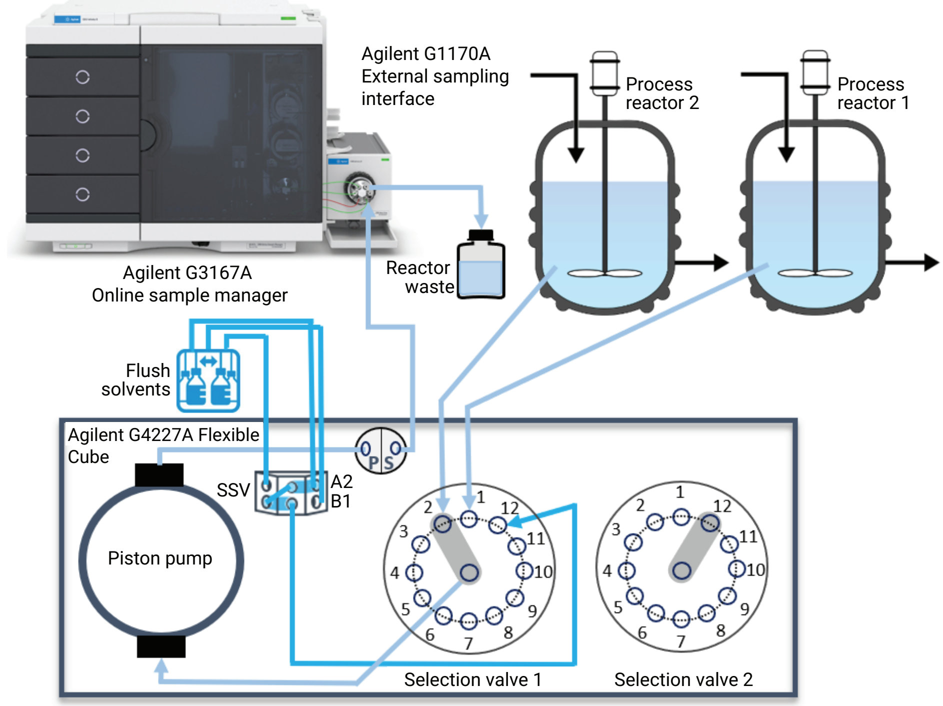

Figure 2. Plumbing diagram with connection of the Agilent 1290 Infinity Flexible Cube as a sampling device for two reaction vessels to the Agilent InfinityLab Online LC System.

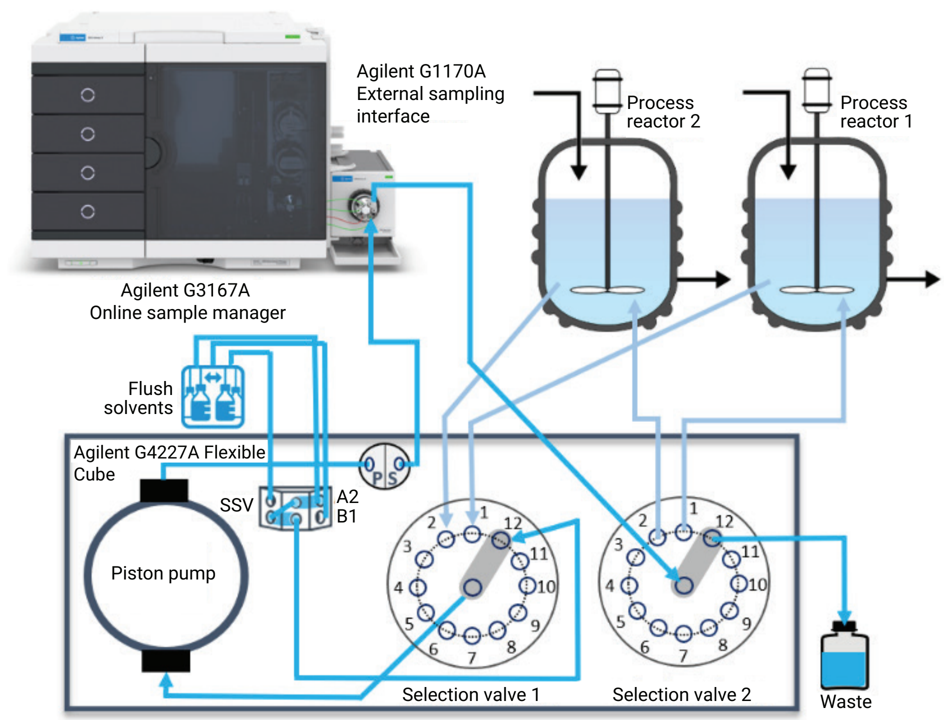

Figure 3. Plumbing diagram with connection of the Agilent 1290 Infinity Flexible Cube as a sampling device for two reaction vessels to the Agilent InfinityLab Online LC Solution, including a second stream selection valve for sample return to the reactor.

Chemicals

- Acetyl salicylic acid
- Salicylic acid
- Formic acid
- Glycine
- NaCl
- NaOH
- EtOH

Glycine buffer solutions

- Stock solutions
  - **A) Glycine:** 0.1 mol/L + NaCl 0.1 mol/L (7.507 g of glycine, 5.844 g of NaCl)
  - **B) NaOH:** 0.1 mol/L (3.999 g NaOH)
- **Glycine buffer pH 9:** 884 mL of solution A + 116 mL of solution B
- **Glycine buffer pH 10:** 625 mL of solution A + 375 mL of solution B

Other materials

- Agilent 96-deepwell plates, 1 mL, polypropylene (part number 5043-9305)
- Agilent sealing mat, 96 wells, round, preslitted, silicone (part number 5043-9317)
- Connection of reactor to selection valve: 1.6 mm od PTFE tubing (part number 5041-2191), ferrule (part number 5022-2154), and PTFE nuts (part number 50222158)
- Connection of Flexible Cube pressure valve to sampling interface: SST capillary 0.17 mm id, 900 mm length (part number 5500-1217)
- Connection of sampling interface to waste or second selection valve: 1.6 mm od PTFE tubing, fittings, and ferrule (part number 5065-4454)

Solvents and chemicals

All solvents were purchased from Merck, Germany. Chemicals were purchased from VWR, Germany. Fresh, ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Results and discussion

In this application note, the hydrolysis of acetyl salicylic acid to salicylic acid under basic conditions at two different pH values was used as an example, as recently published in another application note.[2] The hydrolysis was performed at a pH value of 10.0 and 9.0 at the same temperature in two independent reaction vessels. The reaction vessels were filled with 45 mL of glycine buffer pH 10.0 (reactor 1) and pH 9.0 (reactor 2). In reactor 1, the reaction was started by adding 50 mg of acetyl salicylic acid dissolved in 5 mL of ethanol, simultaneously with the first sampling triggered by the Online LC Monitoring Software. Taking the sample from reactor 1 connected to Port 1 of the first stream selection valve in the Flexible Cube, this valve was switched to position 1 for transporting the reaction solution to the external sampling interface of the Online LC Sample Manager.

The transport of the reactor solution was done by the Flexible Cube piston pump as per parameters defined in the first "PrerunBlank 01" sample run execution (Table 4, start time: 00 hours 00 minutes). Then, as part of parameters for the following "DilutedToVialSetting 01" sample run execution (Table 4, start time: 00 hours 01 minute), the reactor 1 solution was diluted and analyzed. The stream selection valve was then switched to position 12. This position was connected to the Flexible Cube 4/2-way SSV with appropriate solvent for flushing the sampling path to avoid carryover between the reaction solutions taken from two different reactors. At 5 minutes, the first sampling from reactor 2 was triggered as a part of "PrerunBlank 02" sample run execution (Table 4, start time: 00 hours 05 minutes), simultaneously with the start of the reaction, as described for reactor 1. Taking the sample from reactor 2 connected to Port 2, the first stream selection valve was switched to position 2 and the described sampling and flushing steps were repeated. This set of steps for one sampling cycle of both reactors was repeated 21 times, every 30 minutes, taking approximately 10 hours for a complete experiment.

Table 4. Schedule used in Agilent Online LC Monitoring Software experiment setup for sampling from two reactors.

Type	Setting	Start time	Interval	Count	Start last action
Blank Sample	PrerunBlank 01	00 h 00 m	00 h 30 m	21	10 h 00 m
Diluted to vial	DilutedToVialSetting 01	00 h 01 m	00 h 30 m	21	10 h 01 m
Blank Sample	PrerunBlank 02	00 h 05 m	00 h 30 m	21	10 h 05 m
Diluted to vial	DilutedToVialSetting 02	00 h 06 m	00 h 30 m	21	10 h 06 m

The results for both reaction vessels were displayed as curves in an Online LC Monitoring Software trending plot, reflecting the real-time reaction components (Figure 4). For this purpose, two OpenLab CDS Data Analysis methods were applied, one for the data obtained from reactor 1 and another for the data obtained from reactor 2. Since the monitored components in both reactors are identical, the component names were provided with different suffixes derived from reactor identifiers 1 or 2 (Table 5, Figure 5). The results were displayed in "Area %" units for the composition of acetyl salicylic acid and salicylic acid in collected samples over the duration of the monitoring experiment run. The hydrolysis of acetyl salicylic acid at pH 10.0 in reactor 1 (Figure 4, blue curve) to salicylic acid (Figure 4, green curve) occurs rapidly, with 50% conversion at approximately 70 minutes. At the same time, the reaction at pH 9 of acetyl salicylic acid in reactor 2 (Figure 4, purple curve) to salicylic acid (Figure 4, orange curve) does not even reach 50% conversion after 10 hours.

Since the samples were taken in an alternating manner, the odd sample numbers belong to reactor 1 and the even sample numbers belong to reactor 2. Table 5 shows the composition in sample 5 (reactor 1 at 60 minutes) and sample 42 (reactor 2 at approximately 10 hours). They show similar conversion rates expressed in "Area %", proving the slower reaction at pH 9.0 in reactor 2.

A fast, isocratic chromatographic separation of acetyl salicylic acid and salicylic acid gave a retention time of 0.250 minutes for acetyl salicylic acid and 0.310 minutes for salicylic acid (Figure 5).

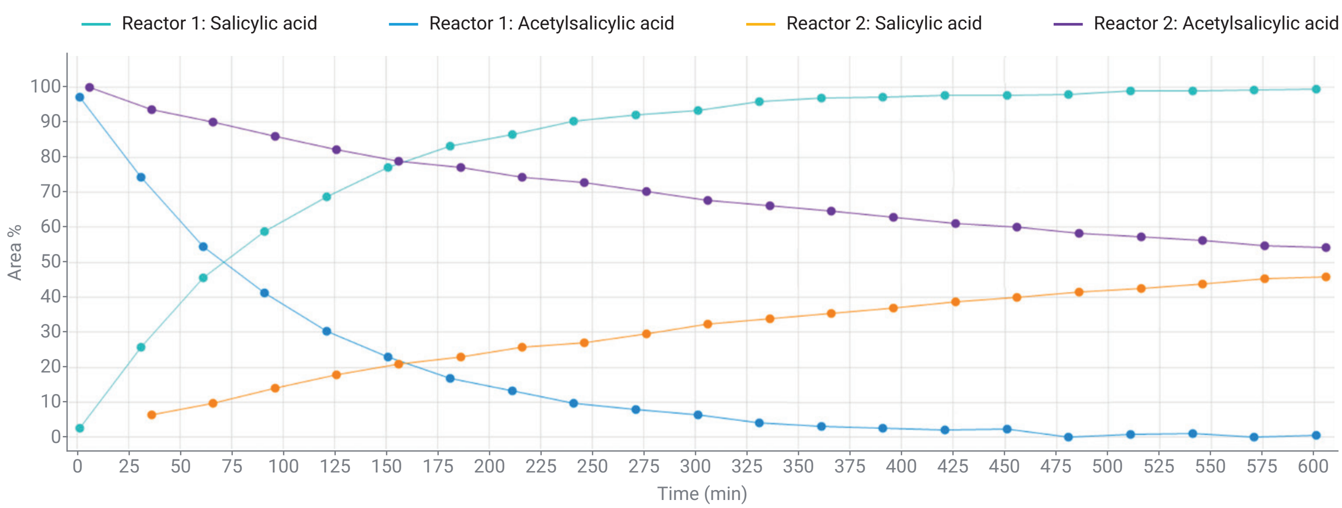

Figure 4. Trending plot for monitoring results of two reaction vessels with hydrolysis of acetyl salicylic acid: at pH 10.0 in reactor 1 (blue and green curve), and at pH 9.0 in reactor 2 (orange and purple curve).

Table 5. Composition in reactors 1 and 2 in samples 5 and 42, respectively.

Results

Sample	Compound <ALL>	RT (min)	Area%
Sample-5	Acetylsalicylic acid 1	0.259	54.450
	Salicylic acid 1	0.310	45.550
Sample-42	Acetylsalicylic acid 2	0.259	54.250
	Salicylic acid 2	0.309	45.750

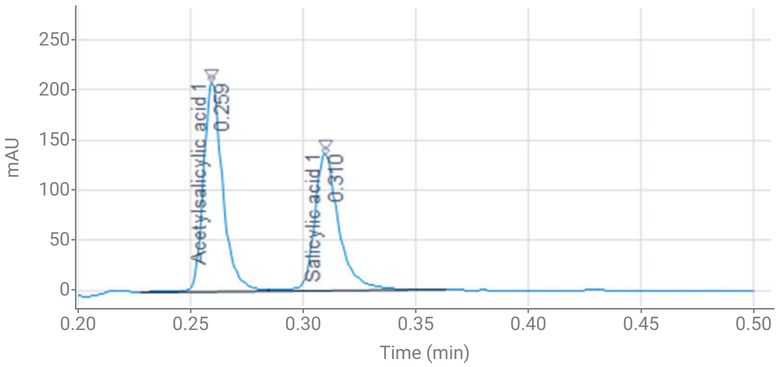

Figure 5. Chromatogram for the isocratic separation of acetyl salicylic acid at 0.259 minutes and salicylic acid at 0.310 minutes. The chromatogram shows the composition in reactor 1 at a reaction time of 60 minutes (sample 5).

Conclusion

This application note demonstrates the use of the Agilent 1290 Infinity Flexible Cube with the Agilent InfinityLab Online LC Solution for sampling from two reaction vessels. The entire analytical method, including the seamlessly integrated Flexible Cube, is controlled by the Agilent OpenLab CDS acquisition software. Agilent Online LC Monitoring Software is able to control the complete experiment, and the data obtained from both reaction vessels can be displayed in real time with complete evaluation after the experiment in a time-saving manner. This enables the user to easily handle sampling, analytical runs, and data analysis within one system.

References

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2. Naegele, E.; Kutscher, D. Online Reaction Monitoring by the Agilent InfinityLab Online LC Solutions. *Agilent Technologies application note*, publication number 5994-3528EN, **2021**.

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