Application Note

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Online LC Monitoring of a Hydrolysis of Organic Acid Esters by Means of a Flow Chemistry Reactor

Using the Agilent InfinityLab Online LC Solution

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Abstract

This application note demonstrates the capability of the Agilent InfinityLab Online LC Solution to be used in combination with flow chemistry reactors. The highly precise direct injection and sampling modes allow the measurement of the influence of different reaction parameters on the overall reaction and its characteristics. Sampling and analysis are completely orchestrated by means of Agilent Online LC Monitoring Software, which enables complete automation of experiment monitoring in a safe and economic way.

Introduction

The use of flow reactors offers the possibility to generate desired products from a chemical reaction in a continuous stream. This is a major difference to batch reactors, which must be loaded and cleaned before and after the reaction. Flow reaction chemistry offers a more economical way to generate small molecule compounds, and therefore, has recently gained significant interest. This application note describes the use of the Agilent InfinityLab Online LC Solution to monitor a flow chemistry reaction. As an example, the hydrolysis of an organic acid was chosen. The Agilent InfinityLab Online LC Solution, together with the Agilent Online LC Monitoring Software, will allow orchestration of the online analysis of the experiment. With the resulting data, the reaction can be optimized to gain a maximum amount of desired product.

Experimental

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 (part number 5067-6680) located at the Agilent 1290 Infinity Valve Drive (G1170A) with Agilent Online LC Monitoring Software
- Thermostat for 1260 Infinity II Online Sample Manager (G7167-60005)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with standard heat exchanger (G7116-60051)
- Agilent 1290 Infinity II Diode Array Detector (G7117B) with Max-Light Cartridge Cell (10 mm, G4212-60008)
- Flow Chemistry Reactor: Corning Advanced Flow Reactor – low flow[1]

Software

- Agilent OpenLab CDS, version 2.6 or later
- Agilent Online LC Monitoring Software, version 1.0

Columns

Agilent ZORBAX Eclipse Plus C18, 4.6 × 50 mm, 1.8 µm (part number 959941-902)

Analytical method

Parameter	Value
Solvents	A) Water + 0.1% formic acid (FA) B) Acetonitrile + 0.1% FA
Analytical Flow Rate	2 mL/min
Isocratic	30% B
Stop Time	1.5 min
Column Temperature	50 °C
Flow Through Injection	Draw speed: 100 µL/min Eject speed: 400 µL/min Wait time after draw: 1.2 s
Sample Volume	1 µL
Needle Wash	3 sec, Water:ACN 1:1, 0.1% FA
Inner Wash and Reconditioning	Solvent S2
Sampling	See direct injection method
Diode Array Detector	243 ±4 nm, Ref.: 390 ±100, data rate 40 Hz

Direct injection from reactor stream

- Draw speed: setting 2 (draw speed: 100 µL/min, wait time: 3.6 seconds).
- For each timepoint, the system was allowed to reach steady state condition (H_2SO_4 and Aspirin feed delivered at the corresponding flow rate for each particular).
- Online HPLC was used to monitor the progress of this process, and once the relevant analytes demonstrated satisfactory consistence in terms of peak area percentage, the values were recorded.

Batch reaction in a single vessel

- Sulfuric acid (H_2SO_4) catalyst used
- Benchtop reaction vessel and agitation provided by a magnetic stirrer
- Hotplate temperature: 75 to 100 °C
- 0.3 g/L Acetylsalicylic acid starting material in H_2O
- Direct injection from reaction feed
- Watson Marlow 120 Series Peristaltic Pump: Connecting the reaction vessel to the interface valve at the Agilent 1260 Infinity II Online Sample Manager with C-Flex tubing used through the pump (C-Flex 6424-13, 0.8 mm id). C-Flex has strong chemical compatibility with many common LC and process solvents. The C-Flex can be connected to PEEK tubing (id: 0.13 to 0.8 mm) to interface with the Online LC system, using inline filters to protect the valve drive. A speed of 25 rpm was used to generate a continuous flow for the reactor interface of the Agilent InfinityLab OnlineLC Soution.

Flow chemistry reaction: Corning Advanced Flow Reactor – low flow.[1] The Corning Advanced Flow Reactor is a configurable, modular microfluidic device with dedicated microfluidic sections, which mix the reactants with subsequent contact microfluidic chips to optimize the reaction. The complete set of microfluidic chips can be thermostatted, and the reactants are fed into the mixing chip by means of syringe pumps (Figure 1).

The heat exchanger was set to 86 °C for all experiments. The concentration of acetylsalicylic acid was 0.016 M. The concentration of sulfuric acid was varied at concentrations of 0.16, 0.375, 0.75, and 1.5 M. The residence time was varied at 1, 5, 10, 30, and 60 minutes (for corresponding reactor feed flow rates, see Table 1).

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).

Additional materials

- Agilent 96 well plates, 0.3 mL, polypropylene (part number 5043-9305)
- Agilent sealing mat, 96 wells, round, preslitted, silicone (part number 5043-9317)
- Agilent Amber wide-opening vial (part number 5182-0716), Agilent conical insert (part number 5181-1270), and Agilent screw cap (part number 5182-0721)

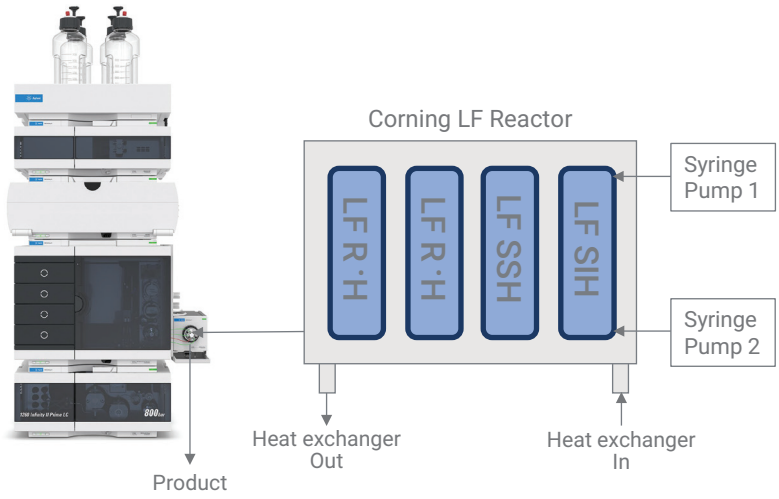


Figure 1. Scheme of the Corning Low Flow Reactor and connection to the Agilent InfinityLab OnlineLC Solution. (LF-SHH: 0.5 mL, one injection zone to contact two reactants. LF-R × H, 0.5 mL mixing chip, "one in one out").

Table 1. Reactor feed flow rate provided by two syringe pumps according to the used residence time of acetylsalicylic acid and HCl in the flow reactor.

Residence Time (min)	Syringe Pump 1 Acetylsalicylic Acid Feed (mL/min)	Syringe Pump 2 H_2SO_4 Feed (mL/min)	Combined Flow (mL/min)
1	1.0	1.0	2.0
5	0.2	0.2	0.4
10	0.102	0.102	0.204
30	0.034	0.034	0.068
60	0.017	0.017	0.034

Results and discussion

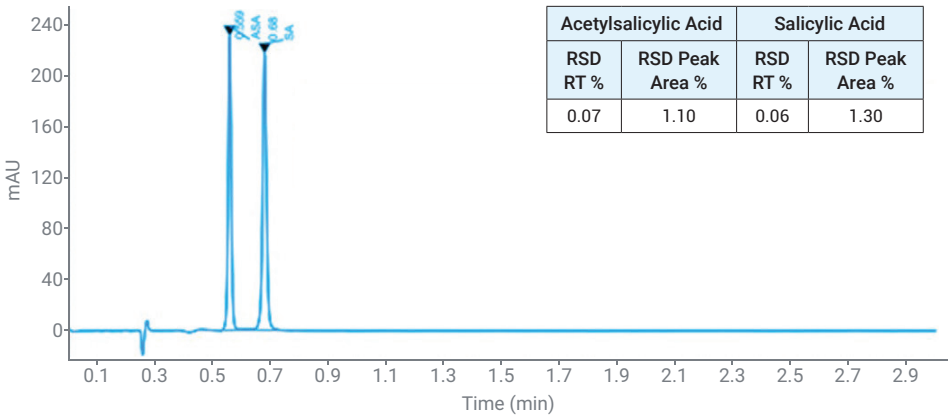
For reliable reaction monitoring, it is necessary to have an instrumental setup that produces peak areas and retention times with high precision. To ensure that the setup is feasible for producing high-quality data, a mixture of 0.2 mg/mL acetylsalicylic acid and salicylic acid was pumped from the reaction vessel to the Agilent InfinityLab OnlineLC Solution, and a sample was drawn every 3 minutes and analyzed immediately. The resulting separation showed a fast separation with high resolution of both peaks. The peak area precision of acetylsalicylic acid and salicylic acid was at 1.1 and 1.3%, and the retention time precision was at 0.07 and 0.06%, respectively (Figure 2). With a peak resolution between acetylsalicylic acid and salicylic acid of 5.8, the chromatography also has potential for sub-30 second separation. These results demonstrate a robust repeatability for both peak area percentage and retention time, as well as robust repeatability for resolution and peak shapes for both analytes. The peristaltic pump used demonstrates strong reliability as a feed mechanism from the reaction vessel.

As a model reaction for the evaluation of the use of the Infinity II Online LC system, in combination with a flow chemistry reactor, the hydrolysis of acetylsalicylic acid (Aspirin) by acidic catalysis was chosen (Figure 3). As described in an earlier application note, this reaction was also used under basic conditions to evaluate the Agilent InfinityLab Online LC Solution in combination with a batch reactor.[3] It was demonstrated that the

sample could be drawn directly from a reactor stream, with subsequent quenching/dilution and analysis. For fast reactions, it was also demonstrated that direct injections are possible.

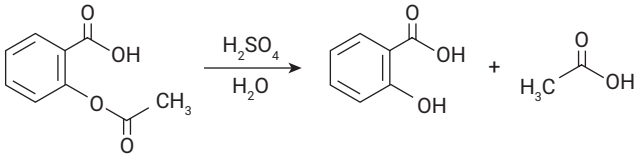
As a first test for a reaction, a batch reactor was used, as described in the Experimental section. Figure 4 shows the fast separation of acetylsalicylic acid and salicylic acid, as the reaction proceeds in the batch reactor. The precision of the retention time is given in the same order as mentioned above.

For the optimization of the reaction under flow reaction conditions, the residence time and the acid concentration were examined more closely. To influence the residence time in the flow reactor, the flow rate at the syringe pumps, which deliver the solution of sulfuric acid and acetylsalicylic acid, were modified accordingly. The temperature and concentration of acetylsalicylic acid were kept constant at 86 °C and 0.016 M, respectively. The flow stream going out from the flow reactor was connected to the Online LC System, and a sample was drawn every 3 minutes. The steady state was reached when the area percent of educt and product were constant. At that point, six samples were drawn to calculate the result.



Acetylsalicylic Acid		Salicylic Acid	
RSD RT %	RSD Peak Area %	RSD RT %	RSD Peak Area %
0.07	1.10	0.06	1.30

Figure 2. Performance test of the instrumental setup, including batch reactor and peristaltic sample pump.



Acetylsalicylic acid Salicylic acid

Figure 3. Acid catalyzed hydrolysis of acetylsalicylic acid (Aspirin) to salicylic acid and acetic acid.

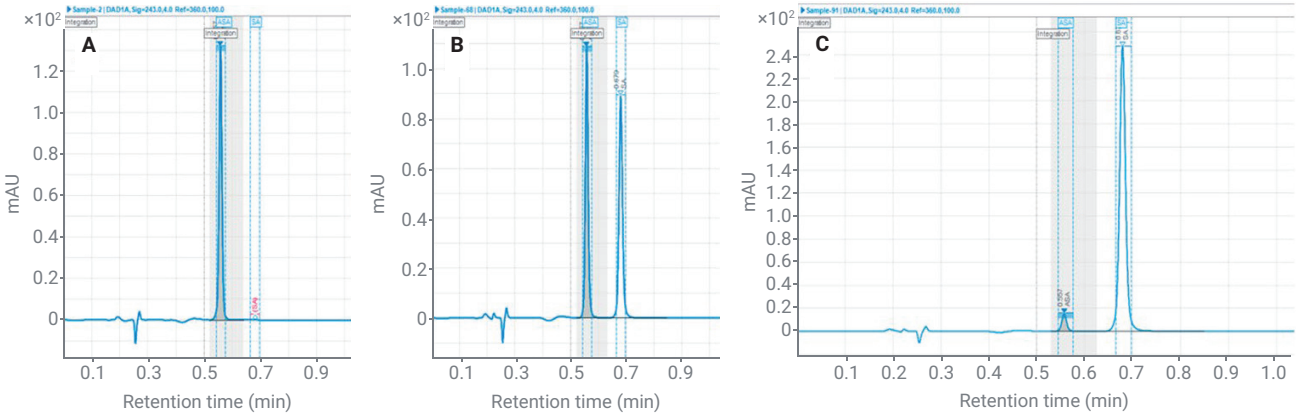


Figure 4. Progress of the acid-catalyzed hydrolysis of acetylsalicylic acid in a batch reactor. (A) Start of the reaction with acetylsalicylic acid. (B) Approximately half of the acetylsalicylic acid is already hydrolyzed to acetylsalicylic acid. (C) Nearly complete reaction.

The first parameter evaluated was the residence time of the educts in the flow reactor. The flow rates at the syringe pumps were set to achieve residence times of 1, 5, 10, 30, and 60 minutes (Table 1). The achieved peak area percentage precisions were calculated from six measurements after reaching the steady state (Table 2). For instance, at a residence time of 30 minutes, the peak area of acetylsalicylic acid was 70.10 area percentage, and the value for salicylic acid was 29.90 area percentage. The respective RSD values were 0.01% and 0.02%, respectively.

The resulting decline of the educt area percentage and the generated product area percentage are shown in Figure 5. For instance, at a residence time of 60 minutes, the peak area of the educt acetylsalicylic acid declined by 54.81%, the area percentage of the formed product, salicylic acid, was at 52.80%, and 2.01% of impurity was generated in the mixture leaving the reactor (Table 2).

Table 2. Performance of the Agilent InfinityLab OnlineLC Solution for measured peak area percentage.

Residence Time (min)	Area % ASA Avg	Area % ASA StDev	Area % ASA RSD	Area % SA Avg	Area % SA StDev	Area % SA RSD	Area % Imp. Avg	Area % Imp. StDev	Area % Imp. RSD
1	95.02	0.27	<0.01	4.98	0.27	0.05	N/A	N/A	N/A
5	89.84	0.19	<0.01	9.82	0.20	0.02	0.34	0.01	0.02
10	84.30	0.27	<0.01	15.70	0.27	0.02	N/A	N/A	N/A
30	70.10	0.52	0.01	29.90	0.52	0.02	N/A	N/A	N/A
60	45.19	0.23	0.01	52.80	0.18	<0.01	2.01	0.06	0.03

ASA: acetylsalicylic acid, SA: salicylic acid, Imp.: impurity, Avg: average, StDev: standard deviation, RSD: relative standard deviation

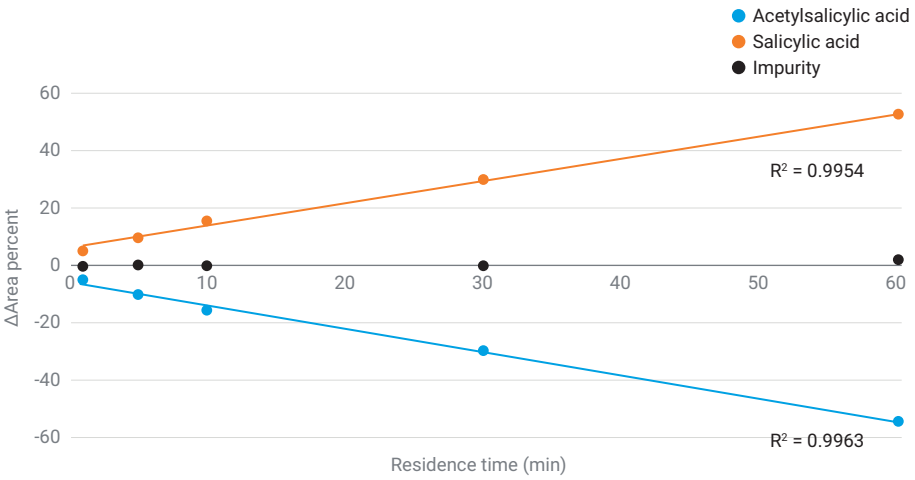


Figure 5. Composition of the reaction components leaving the reactor after the chosen residence time. An occurring impurity is also shown.

The conditions to achieve 1 hour of residence time of the reaction mixture in the flow reactor were kept constant, and the concentration of sulfuric acid was varied to examine the influence on the progress of the reaction. As shown in Figure 6, the reaction of hydrolysis of the educt is nearly complete when using 1.5 M sulfuric acid, but with the drawback that the concentration of the occurring impurity increases. Under these conditions, a decrease of 95.55% of acetylsalicylic acid, and a conversion to 78.88% salicylic acid with a byproduct formation of 15.87% was achieved (Table 3).

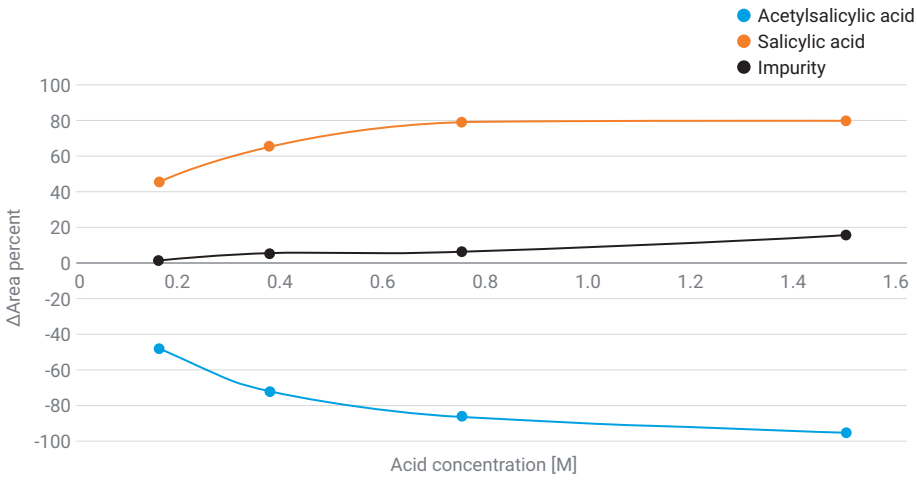


Figure 6. Composition of the reaction components leaving the reactor, depending on the chosen concentration of sulfuric acid. An occurring byproduct is also shown.

Table 3. Resulting composition of the educt acetylsalicylic acid and the product salicylic acid, depending on the concentration of sulfuric acid in the flow reactor. An occurring Impurity is also shown (AP = Area percent).

Acid Conc. (M)	Δ (AP) Acetylsalicylic Acid	Δ (AP) Sailic Acid	Δ (AP) Impurity
0.16	-47.57	45.71	1.86
0.38	-71.32	65.59	5.73
0.75	-85.59	79.03	6.56
1.50	-95.17	79.88	15.87

Conclusion

This application note demonstrates the use of the Agilent InfinityLab Online LC Solution in combination with a microflow reactor to optimize reaction conditions. At one sampling cycle every 3 minutes, excellent peak area and retention time precision were achieved. These results make the Agilent InfinityLab Online LC Solution an excellent choice to examine characteristic parameters of the reaction for their optimization. This can help maximize the yield of valuable products generated by flow chemistry.

References

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