Communications to the Editor

Online Analysis of Flowing Streams Using Microflow HPLC

Christopher J. Welch,* Xiaoyi Gong,* James Cuff, Sarah Dolman, Jason Nyrop, Fiona Lin, and Hallena Rogers

Separation and Purification Center of Excellence, Department of Process Research, Merck & Co., Inc., Rahway, New Jersey, U.S.A.

Abstract:

The application of a recently developed online HPLC reaction sampling instrument for monitoring flow chemistry reactions is described. The system was found to work well for online analysis of flowing streams at or near atmospheric pressure, allowing for convenient time-based withdrawal, dilution, and HPLC analysis of the output of flow reactors. A general study of the capability of the instrument to sample from flowing streams is presented, along with a detailed study in which the instrument is used to characterize a thermal isomerization reaction carried out using a hot-zone flow reactor.

Introduction

In recent years, there has been a growing interest in the use of flow chemistry for carrying out chemical reactions and processes.[1-7] Flow chemistry reactions present some unique and special analytical challenges that differ from those encountered in typical batch reactions. This difference stems from the fact that, with flow systems, the chemical reaction is continually being initiated over the entire period of the run as new starting materials are pumped into the reactor. In an ideal flow reaction system operating at constant flow rates and temperature, product exiting the flow reactor early in a run will be identical in composition to product produced at a later time. However, relatively minor changes in flow rates or reactor temperature, or the presence of air bubbles or clogging will alter the residence time and reaction conditions of the system, and therefore may lead to significant changes in the quality of product being produced. Consequently, flow chemistry is particularly in need of analytical technologies that can afford continuous assessment of product purity over time. At this time, analysis of most laboratory-scale flow chemistry reactions is typically handled by manual collection of effluent and offline HPLC analysis.

However, a number of online analytical technologies including FTIR,[8] NMR,[9] UV,[10] Raman,[11] visible,[12] mass spectrometry,[13] and HPLC[14,15] have been used or have shown promise for monitoring of flow chemistry reactions.

We recently reported the development of a mobile online microflow HPLC system and its use for kinetic profiling of a variety of batch reactions.[16] In this study we investigate the utility and limitations of this now commercially available instrument for online monitoring of continuous flow reactors.

Results and Discussion

At the outset of this study we already knew that the Express RT instrument (Figure 1) performed well for withdrawing samples from a reaction flask, diluting and analyzing the resulting aliquots by HPLC.[16] We anticipated that the system would also work for sampling from a flowing stream. The Express RT instrument is composed of a compact sampling unit capable of performing automated sampling and dilution, a microbore HPLC system capable of executing rapid gradients and quick turnaround times, and a computer system and software for data acquisition and analysis.

The Express RT system does not have the ability to accurately sample from a pressurized reaction vessel. We had originally considered this capability during the initial design discussions with Eksigent, reasoning that a high-pressure sampling feature would be useful for monitoring reaction progress for hydrogenations or other reactions taking place within a pressurized vessel. We ultimately opted for a simple

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* Author for correspondence. E-mail: christopher_welch@merck.com.

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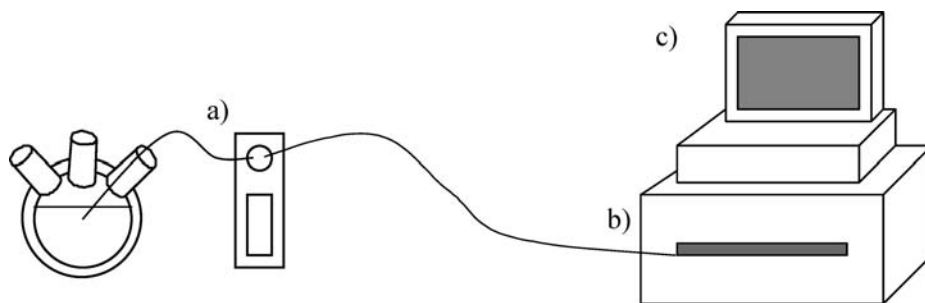

Figure 1. Schematic illustrating components of the Express RT online HPLC reaction monitoring tool. (a) Sampling and dilution unit, (b) Eksigent ExpressLC 100 microbore HPLC, (c) computer and control software.
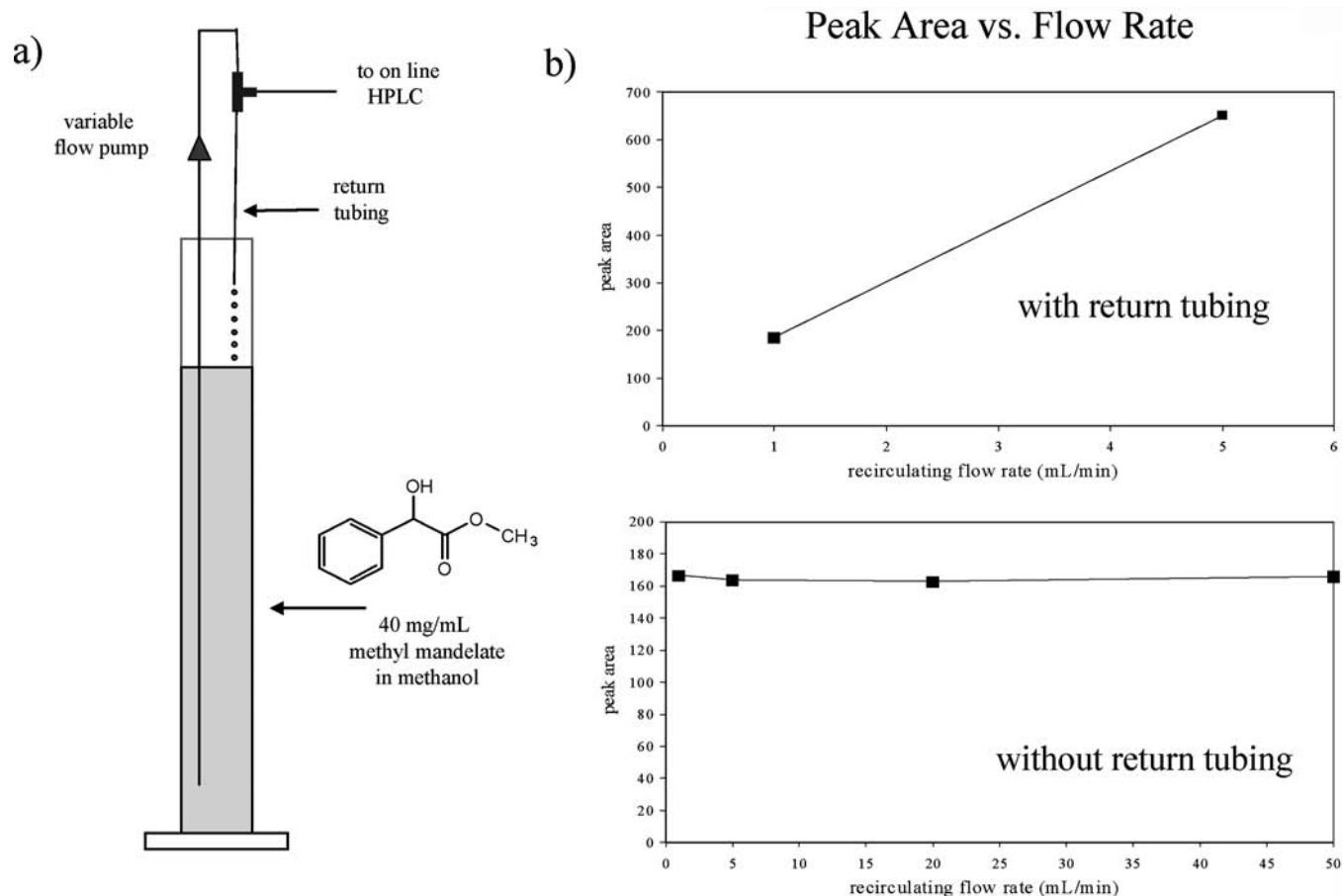

Figure 2. (a) Circulating flow experimental setup used to investigate ability of Express RT to sample from a flowing stream under modest backpressure. Conditions: Solution of methyl mandelate in methanol (40 mg/mL) with recirculating flow between 1 and 50 mL/min (Knauer 501 pump). Express RT conditions: Wakosil II C18 column (300 μ i.d. × 5 cm), 0.3 μ particle size, 60% ACN/0.1% aqueous phosphoric acid, 8 μL/min flow rate, 100× dilution, 30 nL injection. (b) UV peak area of methyl mandelate under moderate backpressure from return tubing to pump and under very low backpressure in absence of return tubing at varied flow rates.

instrument design which would be suitable for sampling at atmospheric pressure, where the vast majority of organic reactions are carried out. We were, however, uncertain of the ability of the system to accurately sample from a flowing stream under modest backpressure, and designed the experimental setup, shown in Figure 2a, to investigate this issue.

In this experimental setup, HPLC sampling of a recirculating methanol solution of methyl mandelate is made possible by a "T" connection, into which the sampling capillary of the Express RT system is plumbed. When a 1-ft section of 170 μM i.d. HPLC tubing is placed downstream of the sampling T, a dramatic influence of flow rate on measured peak area is
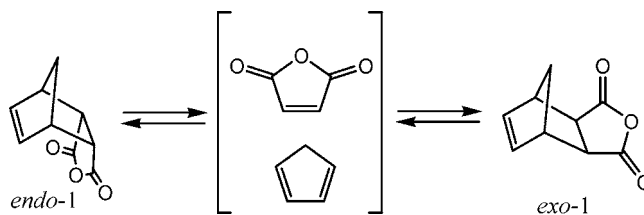

Figure 3. Thermal isomerization of endo-1 to exo-1 used as a model system.

observed, with a 3-fold increase in peak area seen as the flow rate is varied from 1.0 mL/min to 5.0 mL/min, as shown in Figure 2b. These data clearly indicate that the HPLC sampling
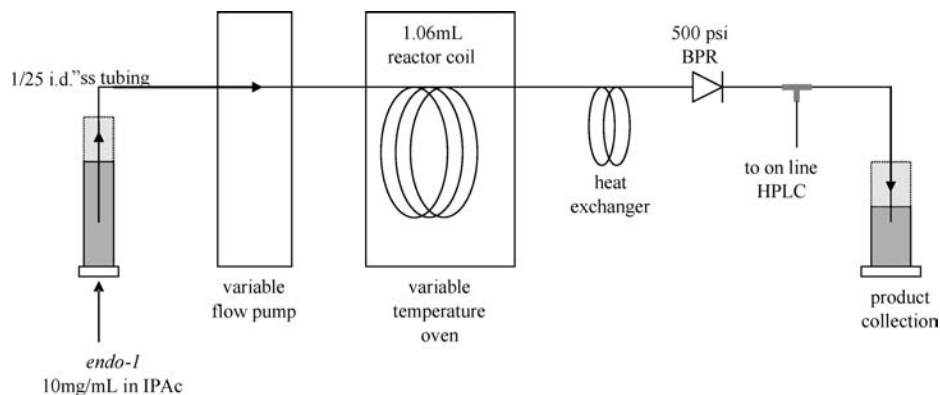
Figure 4. Schematic diagram of the flow reactor setup used to investigate the thermal isomerization of 1.
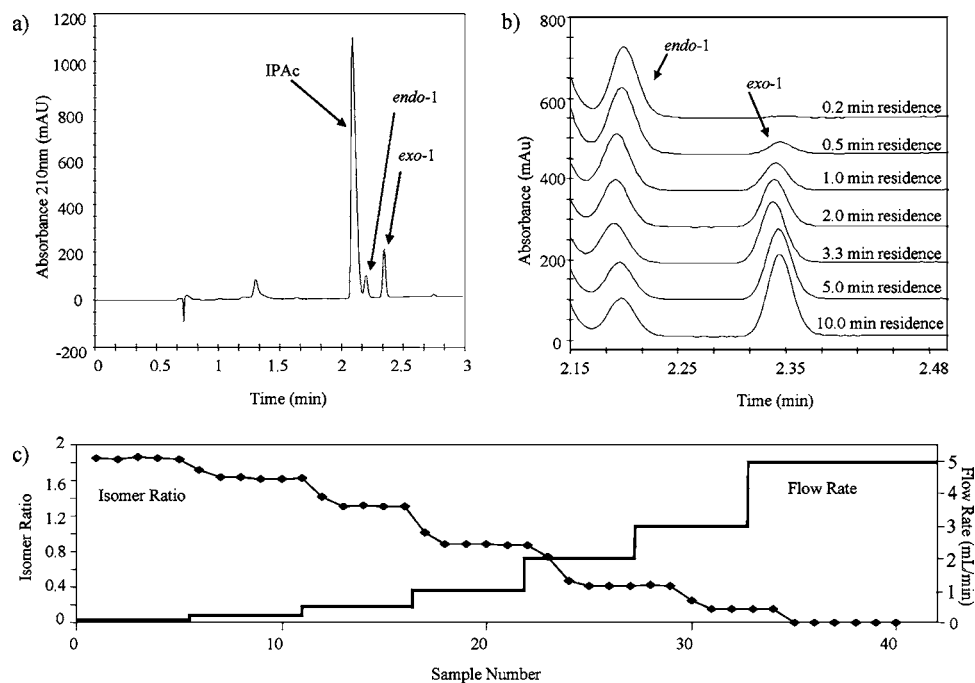

Figure 5. Online HPLC sampling of thermal isomerization flow reactor. (a) Representative chromatogram showing separation of endo-1, exo-1 and IPAc solvent. Reaction conditions: 230 °C, 500 psi, 5 min residence time. Chromatographic conditions: Zorbax Eclipse Plus C-18 column (3.5 μ, 10 cm × 300 μ i.d), mobile phase A: 2 mM ammonium formate, adjusted to pH 8.5 with ammonium hydroxide (aq) mobile phase B: acetonitrile, 10%−90%B over 3 min, 1 min post time, 8 μL/min flow rate, 40 °C, 210 nm UV detection, 10× dilution in acetonitrile, 30 nL injection volume. (b) Sampling of reactor exit flow at different flow rates at 230 °C affords an understanding of the effect of reactor residence time on isomer ratio. (c) Combination of automated online HPLC sampling with programmed flow control allows for convenient collection of reaction isotherm data.

device is in some way being 'force fed' when backpressure is applied to the system. In contrast, when the return tubing downstream of the sampling T is removed, measured peak area is not influenced by recirculation flow rate (Figure 2b), and is identical with the measurement obtained by sampling directly from the sample reservoir. These results clearly show that the Express RT system, in its current configuration, is poorly suited to the task of sampling from a flowing stream under pressure, but performs quite well when sampling from a flowing stream at atmospheric pressure.

With this knowledge in hand, we next turned to the study of a simple flow chemistry reaction. The model system that we chose involves the thermal isomerization of the Diels−Alder adduct of cyclopentadiene and maleic anhydride, endo-1, to afford the thermodynamically more stable exo-1 isomer (Figure 3). This isomerization reaction is of some historical note, having played an important role in the early controversy on whether

the Diels−Alder reaction proceeded via a stepwise or concerted mechanism.[17]

Hamper has shown that an effective hot zone flow reactor can be easily constructed from HPLC pumps, stainless steel tubing, and an electrical resistance heater.[18] By using this simple and easily programmable system it is also possible to heat solvents above their boiling points, provided that a backpressure regulator at the exit of the system maintains the flow reactor under sufficient pressure. We opted for a similar experimental setup, illustrated in Figure 4, in which a programmable GC oven is used to heat the flow reactor coil. The flow rate for the system is controlled by the programmable reagent delivery pump, allowing convenient adjustment of the time required for the sample stream to pass through the heated reaction zone. The

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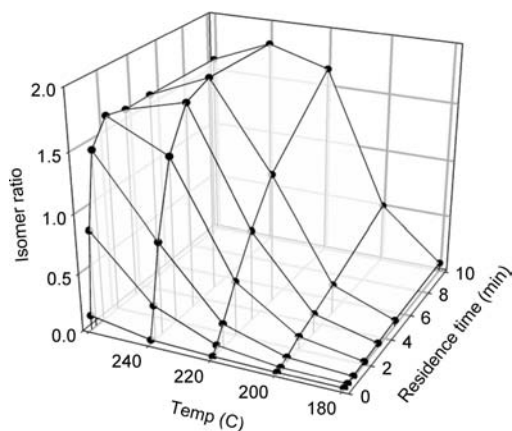(18) Hamper, B. C.; Tesfu, E. *Synlett* **2007**, *14*, 2257–2261.

Figure 6. Response surface showing the effect of temperature and residence time on the thermal isomerization of *endo*-**1**. Data points were obtained by averaging three points from the equilibrated linear regions of single temperature, variable-flow rate experiments such as that depicted in Figure 5c.

volume of the reactor coil used in these studies was about 1.0 mL, meaning that at a flow rate of 1 mL/min, the sample would spend about 1 min in the heated reaction zone. The use of a programmable GC oven to heat the reaction tubing allows for timed temperature changes that can be coordinated with the residence time and LC sampling protocols to obtain data for multiple isotherms within a single experimental run.

A typical chromatogram from the Express RT system showing the separation of the *exo*-**1** and *endo*-**1** isomers is shown in Figure 5a. The separation uses a Zorbax Eclipse Plus C-18 column (3.5 μ, 10 cm × 300 μ i.d) with a gradient of 10% to 90% acetonitrile and has a run time of only 3 min, meaning that sampling of the flowing stream with a minimum cycle time of about 4 min could be possible, using a look-ahead injection protocol. Chromatograms collected from the reactor outlet at different flow rates are shown in Figure 5b. These results clearly show the increased formation of the *exo*-**1** isomer at prolonged reaction times, up to an equilibrium ratio of about 1.8:1 at 230 °C. The ability of the Express RT system to perform sampling from the exit stream of the flow reactor at discrete time intervals, combined with the ability to alter the flow rate of the reagent delivery pump and reactor temperature, affords a convenient ability to survey the effect of reactor temperature and residence time on product yield and isomer ratio. Figure 5c illustrates one such run, in which the flow rate is varied between 0.1 and 5 mL/min (residence time varied between 10 and 0.2 min) at a reaction temperature of 230 °C.

Compiled data from similar runs conducted at several different temperatures enable the construction of a response surface plot, which clearly illustrates the role of temperature and reaction time on the thermal isomerization reaction (Figure 6). In this particular case, the best reaction conditions were found to involve a temperature of 240 °C and a residence time of 5 min.

Such surface plots can be used to quickly understand and optimize reaction conditions to maximize yield and throughput. Furthermore, the approach used in this study should be adaptable to the study of many different flow chemistry examples, provided that the stream being sampled is not under pressure

and is relatively free from precipitates and particles, which have a tendency to clog the sampling capillary of the Express RT instrument.

In the current system, multiple timed events that occur in synchrony are independently controlled. Ideally, the control of flow rate, reactor temperature, and HPLC sampling would all be coordinated by a single computer. One can readily imagine an experimental setup using artificial intelligence that could iteratively assess and score the result of an online analysis, then adjust the flow rate, temperature, or other experimental parameters to quickly drive to the optimal reaction conditions.

Conclusion

In summary, these studies clearly show the value of the Express RT online HPLC system for characterizing flow chemistry reactions at ambient pressure. Data obtained from automated sampling of flow reactor exit streams under different reaction conditions can lead to rapid understanding of the critical factors influencing yield and purity. By combining time-based online HPLC sampling with programmable pumps and temperature controls, three-dimensional response surface plots can easily be assembled to provide useful data for reaction profiling.

Experimental Section

Instrumentation. Online HPLC analysis was performed using an Eksigent Express RT online microflow HPLC system (Eksigent Technologies, Dublin, CA). Initial studies investigating the feasibility of HPLC sampling from a pressurized stream utilized a Knauer 501 HPLC pump (Knauer USA, Gaithersburg, MD). The hot-zone flow reactor used for the study of the isomerization of *endo*-**1** was performed using an Agilent 1100 series G1311A quaternary pump, while the reactor coil was heated using an Agilent 6850 series G2630A gas chromatograph oven (Agilent Technologies, Palo Alto, CA). Chromatographic and reaction conditions are as reported in figure captions.

Reactor Coil. The reactor coil consisted of 0.1588 cm (1/16 in.) outer diameter, 0.1016 cm (1/25 in.) inner diameter stainless steel tubing (Fischer Scientific, Pittsburgh, PA). The heated portion of the coil was a total of 1.25 m, with a measured internal volume of 1.06 mL. The heat exchanger was composed of similar diameter tubing and had an internal volume of 0.35 mL, held in air at ambient temperature. The total dead volume of the system from pump to sampling T was 1.8 mL. A 0.2 mL volume of the stainless steel tubing was fed from the sample T and allowed to drip freely into the product collection container. The backpressure regulator was a P-765 500PSI cartridge in a stainless steel P-465 backpressure regulator holder (Upchurch Scientific, Oak Harbor, WA).

Columns. The Zorbax Eclipse Plus C-18 (10 cm × 300 μ i.d, 3.5 μ particles) column was custom-packed by Agilent Technologies (Palo Alto, CA). The Wakosil II C18 column (5 cm × 300 μ i.d, 3 μ particles) was custom-packed by Eksigent Technologies (Dublin, CA).