Video Article

Real-time Monitoring of Reactions Performed Using Continuous-flow Processing: The Preparation of 3-Acetylcoumarin as an Example

Trevor A. Hamlin[1], Nicholas E. Leadbeater[1]

[1]Department of Chemistry, University of Connecticut

Correspondence to: Nicholas E. Leadbeater at nicholas.leadbeater@uconn.edu

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Abstract

By using inline monitoring, it is possible to optimize reactions performed using continuous-flow processing in a simple and rapid way. It is also possible to ensure consistent product quality over time using this technique. We here show how to interface a commercially available flow unit with a Raman spectrometer. The Raman flow cell is placed after the back-pressure regulator, meaning that it can be operated at atmospheric pressure. In addition, the fact that the product stream passes through a length of tubing before entering the flow cell means that the material is at RT. It is important that the spectra are acquired under isothermal conditions since Raman signal intensity is temperature dependent. Having assembled the apparatus, we then show how to monitor a chemical reaction, the piperidine-catalyzed synthesis of 3-acetylcoumarin from salicylaldehyde and ethyl acetoacetate being used as an example. The reaction can be performed over a range of flow rates and temperatures, the *in-situ* monitoring tool being used to optimize conditions simply and easily.

Video Link

The video component of this article can be found at https://www.jove.com/video/52393/

Introduction

By using continuous-flow processing, chemists are finding that they can perform a range of chemical reactions safely, effectively, and with ease[1,2]. As a result, flow chemistry equipment is becoming an integral tool for running reactions both in industrial settings as well as research labs in academic institutions. A wide variety of synthetic chemistry transformations have been carried out in flow reactors[3,4]. In select cases, reactions that do not work in batch have been shown to proceed smoothly under continuous-flow conditions[5]. For both reaction optimization and quality control, incorporation of in-line reaction monitoring with flow processing offers significant advantages. In-line monitoring provides continuous analysis with real-time response to actual sample conditions. This is faster and, in some cases, more reliable than comparable off-line techniques. A number of in-line analytical techniques have been interfaced with flow reactors[7]. Examples include infrared[8,9], UV-visible[10,11], NMR[12,13], Raman spectroscopy[14,15], and mass spectrometry[16,17].

Our research group has interfaced a Raman spectrometer with a scientific microwave unit[18]. Using this, a range of reactions have been monitored from both a qualitative[19] and quantitative[20] standpoint. Building on this success, we have recently interfaced our Raman spectrometer with one of our continuous-flow units and employed it for in-line reaction monitoring of a number of key medicinally-relevant organic transformations.[21] In each case it was possible to monitor the reactions and also in one example, by means of a calibration curve, we could determine product conversion from Raman spectral data. In Here we describe how to set up the apparatus and use it to monitor reactions. We use the piperidine-catalyzed synthesis of 3-acetylcoumarin (**1**) from salicylaldehyde with ethyl acetoacetate (**Figure 1**) as the model reaction here.

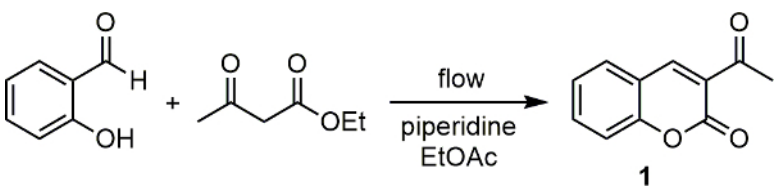

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Figure 1. Base catalyzed condensation reaction between salicylaldehyde and ethyl acetoacetate to yield 3-acetylcoumarin (1). Please click here to view a larger version of this figure.

Protocol

1. Find Suitable Signals for Reaction Monitoring

1. Obtain Raman spectra for all starting materials and the product.
2. Overlay spectra and identify an intense band that is unique to the product.
3. Use this Raman band to monitor the progress of the reaction. A band at 1,608 cm^{-1} was selected in this case.

2. Set up the Flow Cell

1. Obtain a suitable flow cell. Here use one with the following dimensions: width of 6.5 mm, height of 20 mm, and a path length of 5 mm (**Figure 2A**).
2. Place the flow cell in a container that provides an environment free of ambient light.
3. Connect tubing to the inlet and outlet of the flow cell (in this case 1 mm I.D. PFA tubing).

3. Interface the Raman Spectrometer with the Flow Cell

1. Obtain a suitable Raman spectrometer with a flexible optical assembly that can be placed in close proximity to the flow cell.
2. Place the optical assembly through a suitably sized aperture in the box containing the flow cell assembly (**Figure 2B**).
3. Slide the optical assembly until it touches the flow cell and then pull it back leaving a gap of ~ 2 mm.
4. Fill the flow cell with 100% acetone.
5. Turn on the Raman spectrometer and acquire spectra in continuous-scan mode.
6. Focus the laser by gently moving the light pipe a fraction at a time. Keep moving the light pipe until the signal is at its greatest intensity and the peaks are sharp and well defined.

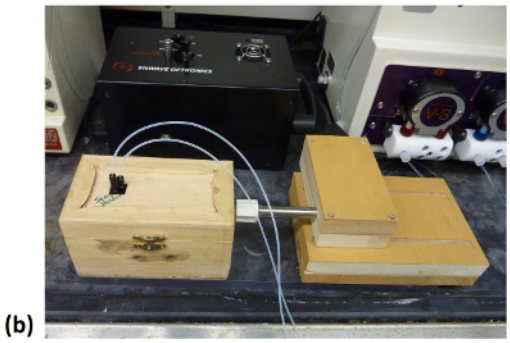

Figure 2. (**A**) Flow cell and (**B**) Raman interface used. Please click here to view a larger version of this figure.

4. Prepare Reagent and Solvent Solutions

1. Add salicylaldehyde (6.106 g, 50 mmol, 1 equiv) and ethyl acetoacetate (6.507 g, 50 mmol, 1 equiv) to a 50 ml volumetric flask.
2. Add ethyl acetate to a total volume of 50 ml and then thoroughly mix the contents.
3. Transfer a 10 ml aliquot of the stock solution to a 20 ml glass vial containing a magnetic stir bar. Label this vial “reagent.”
4. In a 100 ml bottle place 90 ml of ethyl acetate. Label this bottle “solvent”. In a 100 ml bottle place 90 ml of acetone. Label this bottle “solvent intercept”.

5. Prepare the Flow Apparatus

1. Ensure that the flow unit has at least two pumps and label them “P1” and “P2”. Identify solvent and reagent inlet lines for each pump. Place the exit lines from the “collect” and “waste” lines into two individual 100 ml bottles labeled “product” and “waste”, respectively.
2. As a reactor, use a 10 ml capacity PFA coil capable of being heated.
3. Connect the tube exiting P1 to the inlet of the PFA reactor coil.
4. Install a three-port polyether ether ketone (PEEK) tee-mixer after the reactor coil.
5. Connect the tube exiting P2 to the tee-mixer, 180° from the reactor coil exit tubing. Connect a piece of tubing to the third port of the Tee-mixer. On the other end of this tube place a back-pressure regulator.
6. Connect a line from the output of the back-pressure regulator to the input of the flow cell. Connect a line from the output of the flow cell to the “waste/collect” switch.
7. Prime the solvent lines for both P1 and P2 as well as the reagent line for P1 with solvent. Move the reagent line for P1 from the solvent bottle to the reagent bottle.
8. Using P1, pass ethyl acetate through the reactor coil at 2 ml/min until it is filled. Pass acetone through P2 at a flow rate of 2 ml/min for 2 min.
9. Adjust the solvent flow rates for both P1 and P2 to 1 ml/min. Set the back-pressure regulator to a pressure of 7 bar. Set the reactor coil temperature to the desired temperature.
10. Double-check the equipment is configured as shown in the schematic in **Figure 3**.
11. Once the system reaches constant temperature and pressure, check for leaks and then run the reaction.

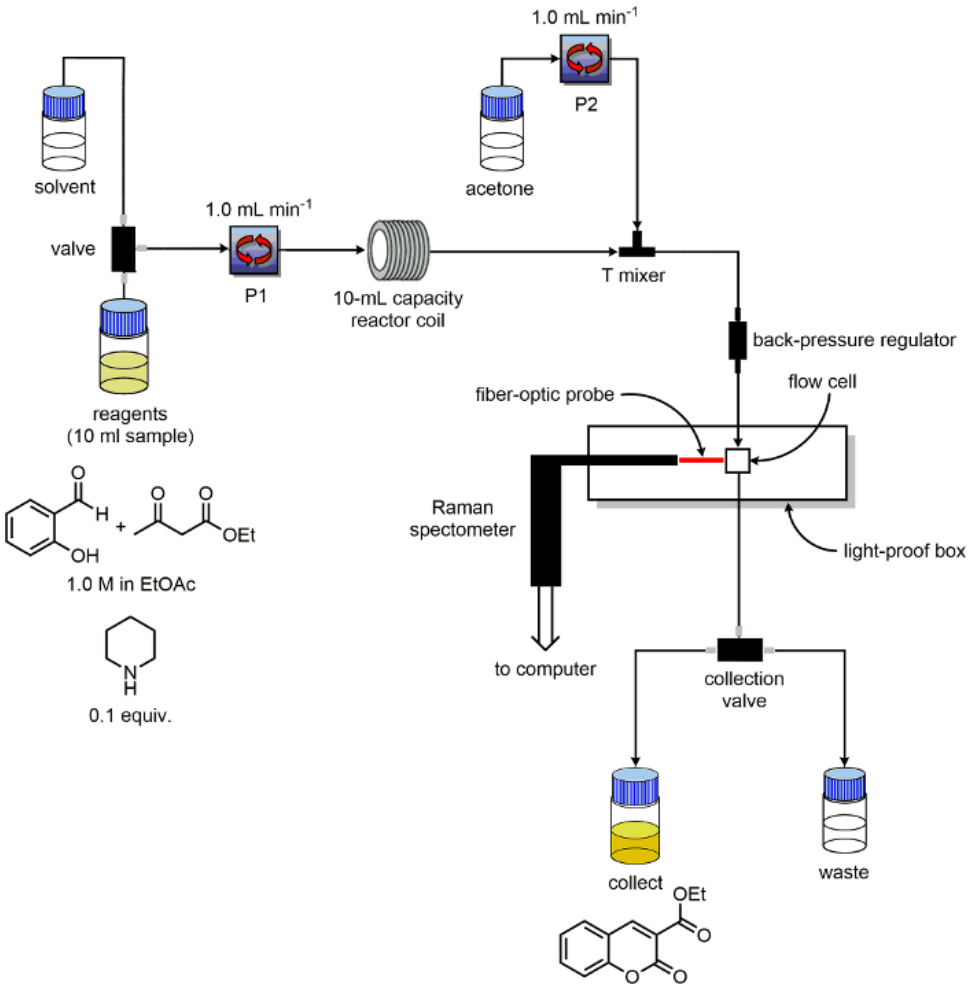

Figure 3. Schematic of the equipment configuration used for reaction monitoring experiments. Please click here to view a larger version of this figure.

6. Monitor the Reaction

1. Take a background scan of the ethyl acetate/acetone solvent system as it passed through the flow cell. This will be automatically subtracted from all subsequent scans.
2. Configure the spectrometer to take scans every 15 sec (in this case the Raman spectrometer was set to a 10 sec integration time, boxcar = 3, and average = 1).
3. Inject piperidine (0.05 ml, 0.05 mmol, 0.1 equiv) all at once into the glass vial labeled “reagent”.
4. After thoroughly mixing, switch P1 from “solvent” to “reagent.” Set the exit stream to “collect.”

5. When all material is completely loaded, switch P1 from “reagent” back to “solvent.” Continue flowing solvent through the reactor coil for another 30 min. Once this time has elapsed, turn off the heating.
6. Turn pumps P1 and P2 off when the reactor coil temperature has cooled to below 50 °C.

7. Analyze the Data

1. Export the Raman spectrometer data to a spreadsheet and plot Raman intensity at 1,608 cm^{-1} vs. time.
2. To optimize conditions, perform the reaction across a number of flow rates and reactor temperatures in an iterative manner.
3. Overlay plots of Raman intensity at 1,608 cm^{-1} vs. time.
   Note: Higher Raman intensity correlates with higher product conversion.

8. Run the Reaction Using Optimized Conditions

1. Having screened various conditions (varying flow rates / reactor temperatures), run the reaction using the optimized conditions to afford the highest product conversion.

9. Isolate the Product

1. Take the contents of the product flask and pour it into a beaker containing 100 ml of ice and 20 ml of 2 M HCl.
2. Rinse the product flask with a minimal amount of ethyl acetate (2 ml) and transfer to the beaker.
3. Stir the icy mixture until all the ice is completely melted.
4. Set up a filtration system with a Hirsch funnel, side-arm flask, rubber collar and a length of rubber vacuum tubing.
5. Filter the resulting precipitate under vacuum, rinse with cold diethyl ether (10 ml) and allow it to dry under a heat lamp (2-3 hr) or dry O/N under vacuum.
6. Confirm the identity of the product by ^{1}H nuclear magnetic resonance (NMR) spectroscopy using $CDCl_3$ as the solvent. For a 500 MHz NMR spectrometer, the ^{1}H NMR data of 3-acetylcoumarin is as follows: δ = 2.73 (s, 3 H) 7.31 - 7.40 (m, 2 H) 7.65 (ddd, J = 7.53, 4.37, 2.60 Hz, 2 H) 8.51 (s, 1 H) ppm, ^{13}C NMR data: δ = 30.84 (CH_3) 117.00 (CH) 118.56 (C) 124.86 (CH) 125.27 (CH) 130.51 (CH) 134.68 (C) 147.74 (CH) 155.64 (C) 159.52 (C) 195.77 (C) ppm.

Representative Results

The continuous-flow preparation of 3-acetylcoumarin was chosen as a representative reaction for in-line monitoring. In batch, the reaction proceeds well when using ethyl acetate as the solvent. However, the product (**1**) is not completely soluble at RT. To prevent potential clogging of the back-pressure regulator, as well as mitigate the risk of having solid particles in the flow cell which would perturb signal acquisition, we used a technique we developed previously for this and other reactions[22]. We intercepted the product stream after the reaction coil with acetone to solubilize the product and allow it to pass through the flow cell and back-pressure regulator unimpeded.

To identify a suitable Raman signal to monitor we predicted the Raman spectra of **1** and of the two starting materials (salicylaldehyde and ethyl acetoacetate) using the computer program Gaussian 09 (**Figure 4A**, **B** and **c**)[23]. It should be noted that experimentally derived Raman spectra of starting materials and the product can also be used if one does not have access to Gaussian 09. An overlay of the three spectra (**Figure 4D**) indicated that, while **1** exhibits strong Raman-active stretching modes at 1,608 cm^{-1} and 1,563 cm^{-1}, the starting materials exhibit minimal Raman activity in this area. As a result, we chose to monitor the signal at 1,608 cm^{-1}.

As a starting point, the reaction was run at 25 °C and a reagent flow rate of 1 ml/min and the Raman intensity at 1,608 cm^{-1} was recorded (**Figure 5**). With the goal of obtaining the highest possible conversion, we next performed the reaction at higher temperatures. Operating at a flow rate of 1 ml/min, increasing the reaction temperature first to 65 °C and then 130 °C resulted in a concomitant increase in product conversion as evidenced by the steady increase in Raman intensity at 1,608 cm^{-1}. At a reactor coil temperature of 130 °C, decreasing the flow rate from 1.0 to 0.5 ml/min did not significantly increase the Raman intensity at 1,608 cm^{-1}. With optimized conditions in hand, we performed the reaction one more time, isolating the product in 72% yield.

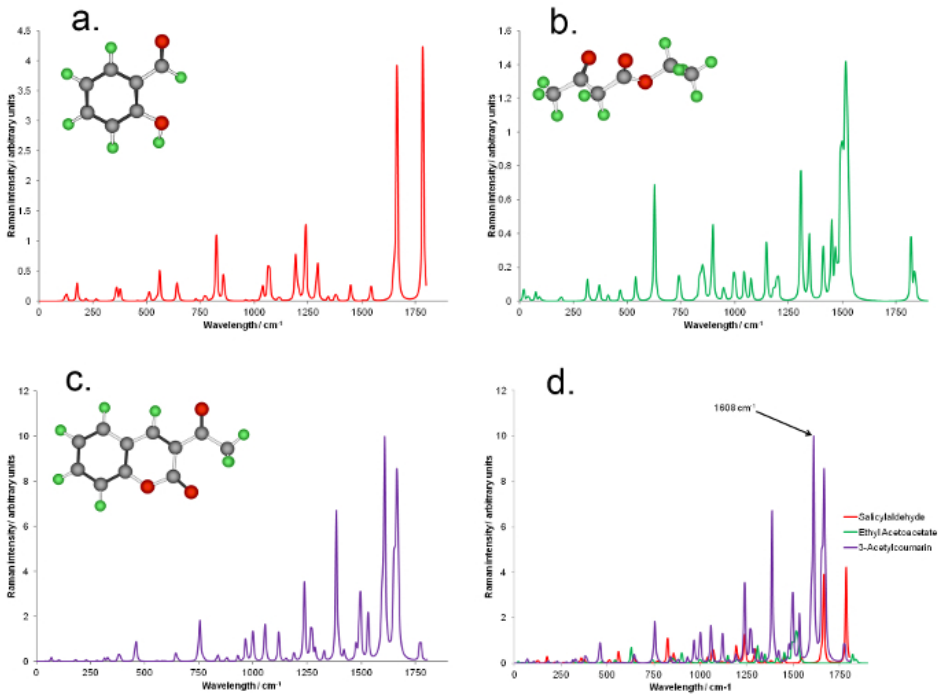

Figure 4. Raman spectra of (A) 3-acetylcoumarin, (B) salicylaldehyde, (Cc) ethyl acetoacetate, and (D) an overlay of the three spectra.
Please click here to view a larger version of this figure.

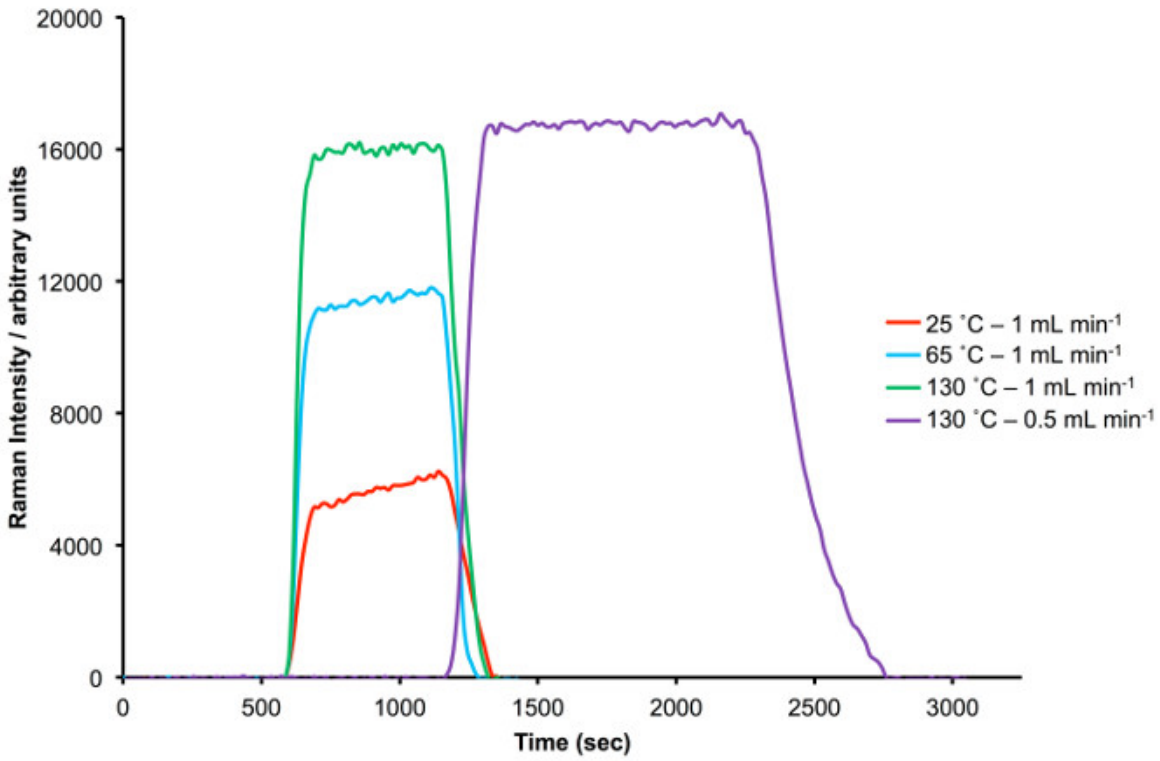

Figure 5. **Monitoring the conversion to 3-acetylcoumarin across a range of reaction conditions.** Please click here to view a larger version of this figure.

Discussion

The ease in which the Raman spectrometer can be interfaced with the flow unit makes this inline technique valuable for reaction monitoring. A number of reaction variables can be probed in an expedited manner, allowing the user to arrive at optimized reaction conditions faster than when using offline methods. Application of the techniques described herein also allows for monitoring of the formation of side products, assuming a suitable band can be found. Conditions can be screened and selected, which allow both for the highest conversion of product, and also the lowest amount of impurity. The quantitative monitoring of reactions is also possible. Since Raman signal intensity is proportional to concentration, a calibration curve can be derived by recording the Raman spectra of samples of known concentration of product. Using this, it is possible to convert units of Raman intensity to units of concentration in standard terms.

Critical steps within the protocol include the correct assembly of the reactor tubing and the interfacing of the Raman cell. It is advised that the configuration be leak tested using either water or acetone prior to performing the reaction. In addition, focusing the Raman laser by correctly positioning the quartz light pipe is essential to the success of the protocol. Poor signal strength is a sign that either the laser is not appropriately focused or there is some particulate matter in the reaction mixture.

The apparatus described here has been used successfully to monitor three other reactions, all involving formation of products bearing α,β-unsaturated carbonyl moieties, namely Knovenagel and Claisen-Schmidt condensations, and a Biginelli reaction[20]. The Raman spectrometer serves as a complementary tool to other *in-situ* monitoring probes. For example, it can be used in cases where IR spectroscopy does not prove satisfactory such as when the reaction is performed in aqueous media or when placing the spectrometer probe in physical contact with the reaction mixture is not desired[24,25]. Limitations to the application of Raman spectroscopy include the fact that the reaction mixture must be completely homogeneous to avoid signal scattering. In addition, since the probability of a Raman event is relatively low, samples have to be relatively concentrated in order to obtain satisfactory signal-to-noise ratios. In our experience, this requires working at concentrations at or above 0.25 M.

Disclosures

The authors have nothing to disclose.

Acknowledgements

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