JOURNAL OF CHEMICAL EDUCATION

On-Line Synthesis and Analysis by Mass Spectrometry

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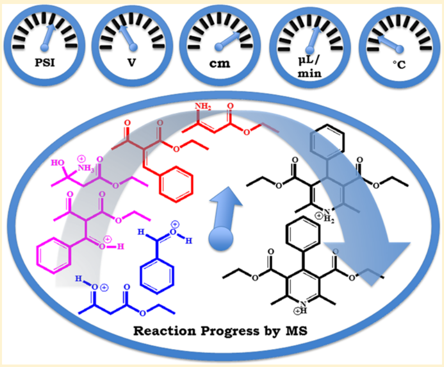
Ⓢ *Supporting Information*

ABSTRACT: In this laboratory experiment, students learn how to use ESI to accelerate chemical synthesis and to couple it with on-line mass spectrometry for structural analysis. The Hantzsch synthesis of symmetric 1,4-dihydropyridines is a classic example of a one-pot reaction in which multiple intermediates can serve to indicate the progress of the reaction as a function of the electrospray variable parameters. This reaction allows students to gauge the effects of the spray variables while observing the enhanced reaction rate through product analysis in the charged microdroplets. Soft ionization techniques used in conjunction with collision-induced dissociation provide students experience with full scan MS to infer reaction progress and collision-induced fragmentation patterns for structural analysis. The experiment as a whole combines the physical phenomena of electrospray, Hantzsch chemical reactivity, and analytical measurements involving use of an ion trap mass spectrometer.



KEYWORDS: *Upper-Division Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Instrumental Methods, Mass Spectrometry, Qualitative Analysis, Synthesis*

■ INTRODUCTION

Accelerated organic reactions in charged microdroplets produced by electrospray ionization (ESI)[1] can be studied in real time by mass spectrometry (MS). The main aim of this laboratory is to have students understand how the variables of ESI can affect reaction acceleration using spray-based ionization techniques. In the general model[2] of ESI, rapid droplet desolvation is followed by successive Coulombic fissions as the charged droplets reach the Rayleigh limit. This limit is reached when the force due to charge repulsion is greater than the surface tension holding the droplet together.[3] Furthermore, the charged species are localized near the surface of the droplet rather than spread homogeneously throughout. Many spray-based ionization methods including desorption electrospray ionization (DESI),[4] electrosonic spray ionization (ESSI),[5] and paper spray (PS) ionization[6] have been shown to yield charged droplets in which chemical reactions are accelerated.

ESSI[7] (a variant on ESI using a micro ESI source with a high velocity nebulizing gas) was chosen for this experiment because many spray parameters can be easily varied to control initial droplet size and the rate of desolvation through evaporation. The effect of changing the distance[8] between the spray source and ion transfer capillary of the MS will be explored in this experiment as illustrated in Figure 1. Increasing distance allows more time for the droplets to slowly desolvate before they rapidly and completely desolvate at the MS inlet. The degree of reaction can be investigated by spraying a reaction mixture and observing characteristic intermediates which can indicate reaction progress. The extent to which a reaction is accelerated can be optimized by changing the ESI spray variables. The objective of this study is to use accelerated reactions in electrosprayed droplets to show students (1) the chemical reactivity within droplets, and (2) the capabilities of analytical measurements for exploring reactions by MS.

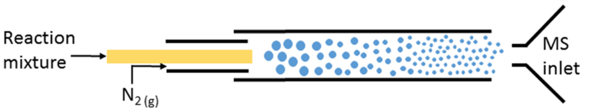
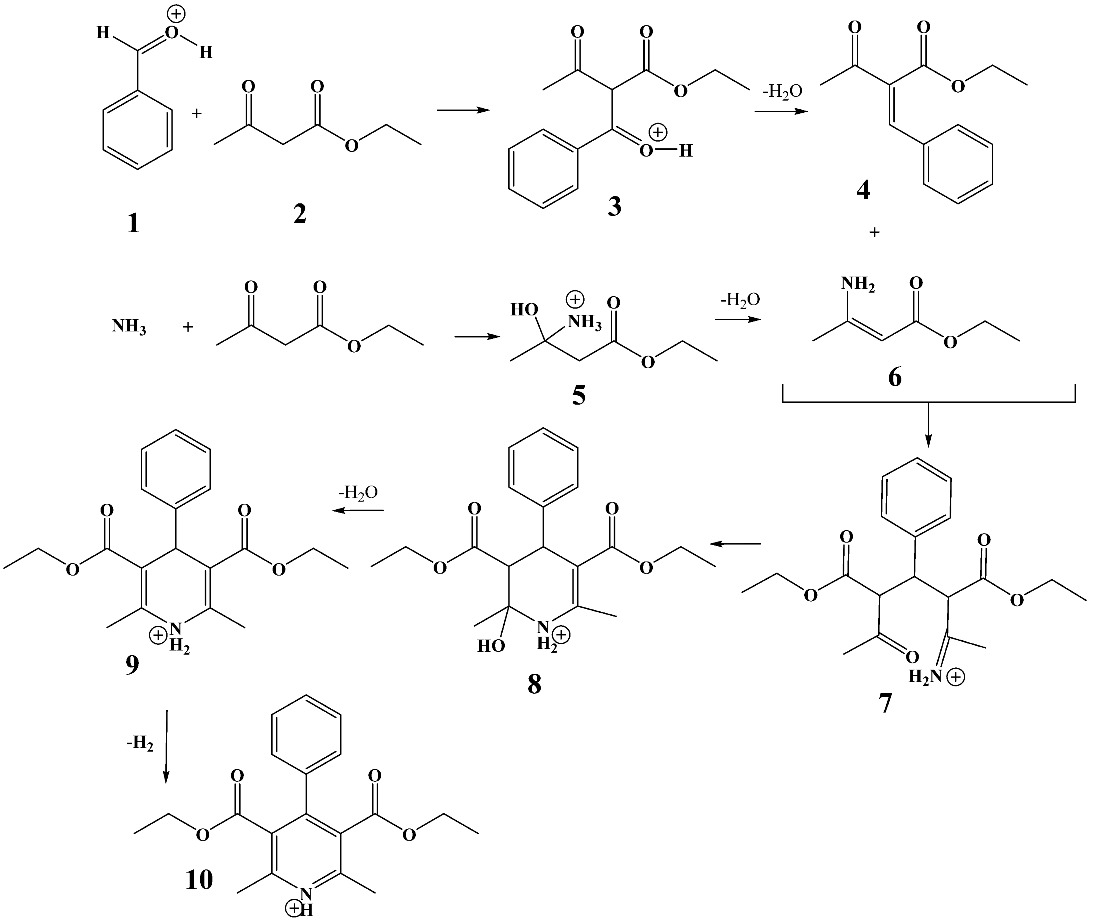


Figure 1. Representation of the ESSI source with a focusing tube coupled to a MS used in this exercise.

The open-ended nature of the laboratory session left students to explore other electrospray variables in a truly experimental manner. The distance variable was required of all groups to illustrate the two types of desolvation droplets undergo and how they reflect reaction progress. First, droplets desolvate as they move through the air approaching the mass spectrometer and then in the MS inlet they are rapidly and completely desolvated in a much faster step. These two droplet desolvation processes are different; the former accelerates the reaction as reagent concentrations increase, whereas the latter quenches further reaction. (If they had similar effects on reaction rate acceleration, reactants would always be converted to products prior to mass analysis when spraying a reaction mixture.) The other variables, in addition to flight distance, include the following: applied voltage, solvent flow rate, gas flow rate, and temperature. Other factors that influence the progress of the reaction include concentration and solvent

DOI: 10.1021/acs.jchemed.5b00165

Scheme 1. Reaction Sequence for the Hantzsch Synthesis As Performed in This Laboratory[a]



[a]One equivalent of **2** undergoes a Knovenagel condensation with **1** to form **3**, while an enamine ester (**6**) is formed from ammonia and another equivalent of **2**. Compounds **5** and **6** then undergo a Michael addition to form **7**, which undergoes proton transfer and intramolecular enamine formation to yield **9**, which can then be thermally dehydrogenated to the aromatic **10**.

choice; however, students were not encouraged to experiment with these variables. Charge is transferred from the solvent by the voltage applied. To some extent, the higher the amplitude of voltage applied, the greater charge the droplet carries. The greater charge should allow the Rayleigh limit to be reached faster as less desolvation would be needed before the forces due to charge−charge repulsion surpass the forces due to surface tension and the droplet becomes unstable. Solvent flow rate directly controls the initial droplet size and is directly related to the gas flow rate, which influences the rate of desolvation of droplets in flight. Temperature can be controlled in many ways, but is most easily changed by simply heating the focusing tube placed between the MS inlet and the ESI source.

The Hantzsch synthesis[9] was chosen for these particular experiments since it can be used to gauge the effect of the electrospray parameters on the extent of reaction. Multiple intermediates along the pathway from reagents to products can be detected and their contributions change as the electrospray variables are optimized. The reaction sequence for the synthesis as performed in this laboratory can be seen in Scheme 1.[10] This reaction typically takes 4−6 h to complete in the bulk phase (>80% yield) while under reflux. In the experiment done in this laboratory, the reaction mixture was combined in a syringe without any noticeable reaction progress due to bulk-phase reaction in the syringe. (Students were encouraged to monitor the syringe contents for bulk-phase reaction by electrospraying at a standard ESI distance periodically throughout their experiments.) Little to no reaction was observed unless the spray distance was increased, indicating that the evaporation of the droplets in air accelerates reaction. All ESI experiments were done using a linear ion trap mass spectrometer (LTQ, Thermo Scientific, San Jose, CA). This instrument was chosen for its MS/MS capability, which allowed students to assign chemical structures to mass-selected ions of interest.[11] Using both ESI and an ion trap gives students valuable hands-on experience, which is important to both physics and chemistry curricula as previously illustrated.[12] This experiment does not require MS/MS analysis as most of the reactive species are visible in the full scan mass spectrum and this means it should be easily adaptable to other MS instruments. As this experiment was performed in an instrumental analysis course, MS/MS analysis was chosen as an additional topic of focus.

Collision-induced dissociation (CID) is a gas phase technique used to fragment protonated molecules in MS experiments as a means of obtaining information on their structures.[13] In CID, ions are accelerated (increasing their kinetic energy) by an electrical field and collided with inert neutral gas molecules or atoms. Upon collisions with the inert atoms/molecules, the increased internal energy induces fragmentation of the protonated molecules. When using a linear ion trap, as was done in this experiment, resonance ejection is used to remove all ions in the ion trap except for

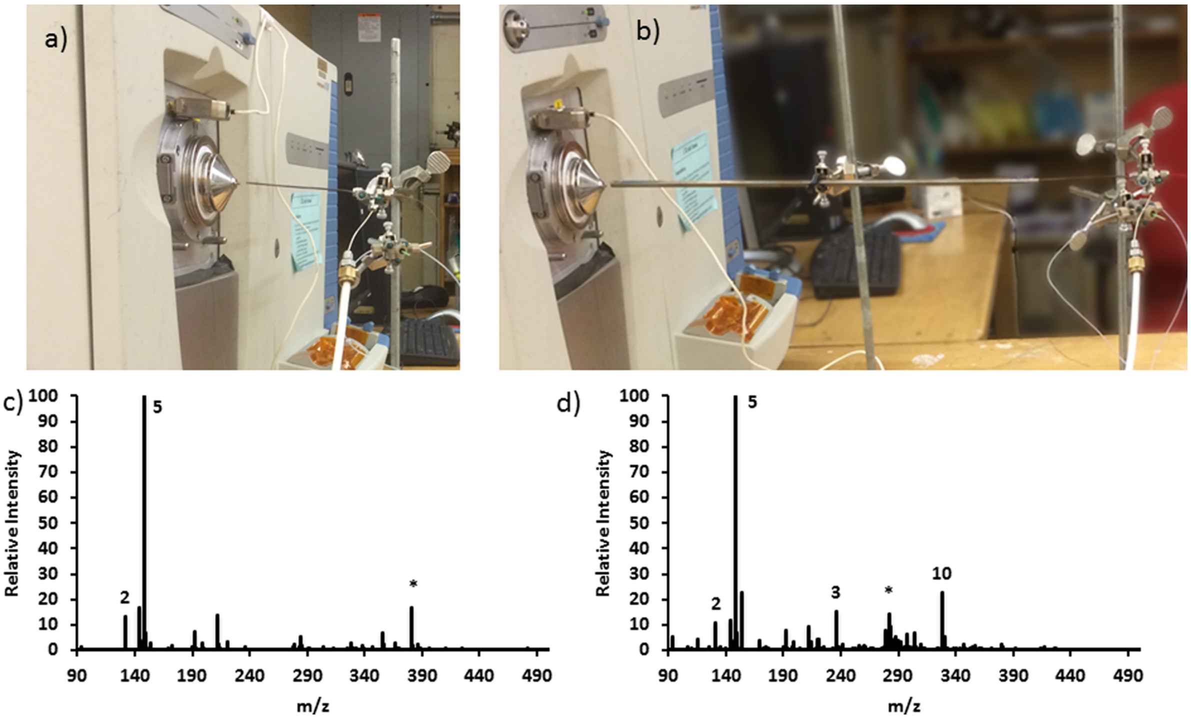


Figure 2. (a) Top left picture is a student setup of a standard spray distance (ca. 5 mm) and the corresponding mass spectrum is shown in (c) below. (b) Top right picture is the same group's experiment with a focusing tube of 50 cm placed between the sprayer and MS inlet and the mass spectrum is below in (d). The main spectral differences are the presence of **10** and **3**, the final product and a later stage intermediate, at the longer distances. Asterisk (*) denotes a contaminant and an impurity in the focusing tube for the left and right spectra, respectively.

those with the desired mass/charge ratio. (Parenthetically, we note that using this technique with soft ionization methods simplifies analysis of complex mixtures.[14])

EXPERIMENTAL OVERVIEW

The laboratory experiment was conducted as one station in a three-station instrumental analysis laboratory session at Purdue University (West Lafayette, IN). Students (in groups of two or three) rotated through all three stations in the course of this three lab period set of experiments, the other two being scanning tunneling microscopy (STM) and Fourier transform-infrared spectroscopy (FT-IR) experiments. This rotation was set up to allow students the opportunity for more time with the instrumentation and software associated with each experiment. If this were to be implemented in larger laboratory settings, groups of students could rotate between using the MS (as done here), performing the bulk synthesis with catalyst, and spraying/collecting the product (off-line) for other forms of analysis including NMR spectroscopy. The experiment as discussed was completed in 1−2 h.

Temporal Resolution

Increasing the distance between the MS inlet and the ionization source increases the time the constituents of the droplet have to react in flight, and varying this parameter is useful in exploring reaction acceleration in the droplets as seen in Figure 2. The characteristic intermediates of the reaction (steps **1** to **10** in Scheme 1) were analyzed in order to gauge the extent of reaction. Increasing the distance between the MS inlet and the spray source comes at a cost of lower ion signal. To help maintain signal at larger distances, an ion focusing tube was used for this experiment. This experiment was performed by all eight groups of students with varied success. All groups showed a change in reaction progress and observed product ions in at least 5% relative intensity in the mass scan at the 50 cm distance compared to no product at 5 mm. Some students went as far as to use MS/MS to search for the presence of all of the known intermediates species and reported all to be present at the 50 cm distance even when they were not clearly evident in the full scan mass spectrum.

Experiments with Spray Variables

Students were asked to deviate from the standard spray variables found in Table 1. Six out of the eight groups chose to use an increase in temperature (of the focusing tube) to increase the progress of the reaction. The other two groups altered the reagent flow rate and the gas flow rate. The increase in temperature was accompanied by a change in the mass spectrum to include more late stage intermediates and products. The other two groups saw similar trends, but both of the variables greatly influence the ion signal that reaches the MS; therefore, the students found it challenging to interpret these results. These experiments were left completely open for the students to determine the construction of the experiment and interpret the results.

Table 1. Spray Variables

Variable		Value
Capillary diameter (i.d.)	*Silica (inner)*	250 μm
	Stainless (outer)	~500 μm
Flow rate	*Reagent*	100 μL/min
	Gas (N_2)	80 psi
Voltage		5 kV
Concentration sprayed		3 mM
Spray distance		Varied

Collision-Induced Dissociation

Fragmentation experiments were performed by each group of students for peaks of interest and for all the intense peaks in the collected spectra. Students were expected to interpret the CID spectra that they reported and were encouraged to do multiple stages of dissociation rather than simply MS/MS. Nearly all students were able to discover and recognize the common losses associated with dissociation of these protonated molecules. Most understood the weakest bonding points of the molecules and proposed fragmentation patterns accordingly. Two groups were successful at collecting and partially interpreting MS^3 (MS/MS/MS) data for most of the analytes of interest. Most groups had performed a subsequent stage of dissociation on the ions seeing the loss of CH_3CH_2OH from isolated **10** at *m*/*z* 328. To perform these measurements, the students had to explore values associated with the ion isolation width and the normalized collision energy.

HAZARDS

Students should review all safety data sheets (SDS) for chemicals utilized in this experiment to ensure safe laboratory practices including handling and disposal. When working with the ion trap mass spectrometer (a Thermo LTQ in these experiments), it is important to note that the applied source potential is 5.0 kV (DC although low current) when the instrument is scanning. This can always be checked visually on the LTQ front panel when the scan light is blue and the system light is green (rather than yellow) as well as in the LTQ Tune software package.

EVALUATION AND ASSESSMENT

The pedagogical aims for this laboratory were focused on the connections between analytical and organic chemistry. This instrumental analysis course requires prior organic chemistry coursework. The first aim of this experiment was for students to understand the fundamentals of ESI and how spray variables can influence the signal in the mass spectrum. Second, the students were to understand the chemical nature of the droplets and how chemical reactions can be facilitated in these droplets under appropriate conditions. Last, it was intended that students see how structural elucidation can be conducted using MS/MS. The laboratory experiment can be found in the Supporting Information (S1).

Pre-Experimental Questions

Questions were asked prior to the laboratory session (see Supporting Information, S1). These questions explored students' understanding of the process of electrospray based on what they had learned in lecture. Other questions took a more practical approach having students anticipate *m*/*z* values for the species in the supplied reaction mechanism. About half of the students did not understand how the ions were created and simply assumed molecular weight was equivalent to the mass/charge ratio whether the species were illustrated as ions or not. Nearly all students who initially made this mistake had learned from the mistake before writing their laboratory reports. Some groups were fortunate enough to observe ions due to ammoniated and sodiated molecules as well as typical protonated molecules in their mass spectra. The abundance of these ions varied from day-to-day as is common with adducts in spray ionization. An increase in ammonium salt increased the $[M + NH_4]^+$ species. Other groups saw some fragmentation of the product (**10**) in the main scan as well. A full table of ions found during the experiment as they relate to the species in Scheme 1 can be found in the Supporting Information (S2).

Other pre-experimental questions ensured that students understood the analytical figures of merit involved in both MS and NMR spectroscopy. These questions covered the processes of CID used in this experiment and practical comparisons of MS with NMR spectroscopy when looking at reaction mechanisms. All students successfully explained and illustrated the processes of ESI and CID. These questions ensured that a prepared student would come ready with questions to help them understand both electrospray ionization for chemical synthesis and structural elucidation using an ion trap MS.

Laboratory Reports

Most students understood that the process of ESI involves desolvation of charged microdroplets followed by Coulombic fission events and accurately described an acceptable model of ESI from lecture material. Most went on to note that the greater the extent of desolvation, the further the reaction progresses when spraying a reactive mixture. Fewer students grasped the fact that the ions are solvent-free during mass analysis. The rapid and discontinuous desolvation that the droplets undergo at the MS inlet ensures the ions can properly undergo mass analysis, quenching all further solution-phase reaction. This is something that very few students discussed in their reports (this point was not necessarily intuitive and it was not expected that the students explain this without prior instruction). Some students seemed confused on aspects of electrospray relating to the role the applied potential plays in the process of electrospray at a molecular level although the same students did accurately describe the Rayleigh limit in previous sections.

Groups that used a heat gun to heat the focusing tube saw excellent results in one of two ways. Two groups of students took an intermediate distance (~25 cm, acquired by simply cutting the supplied tube in half) and heated it; this provided spectra similar to those recorded at 50 cm but with higher overall signal intensity. The higher signal observed at higher temperatures is most simply due to more efficient ion transmission through the shorter tube. Other groups chose to heat the long tube to increase the extent of reaction. Although this did not help them to see any additional intermediates, it did allow them to see greater formation of products **9** and **10**. The findings of one of the five groups that had chosen to do the latter temperature experiment can be seen in Figure 3. The increase in product/late stage intermediates with respect to starting materials/early stage intermediates has two causes: (1) increase in temperature accelerates the reaction kinetics and (2) droplet desolvation is increased with increased temperature of the air surrounding the droplets.

Many students understood the pros and cons of MS and NMR for reaction monitoring experiments. Many commented on the ease of interpretation of pure NMR spectra while pointing out that MS can easily monitor product as well as short-lived intermediates in crude reaction mixtures. Students were able to appreciate the utility of MS/MS in experiments where NMR on crude mixtures would be nearly impossible to interpret although crude NMR spectra were not collected due to time constraints.

Exit Interviews

Table 2 provides questions and findings from the transcripts of an optional exit interview. These optional interviews were conducted with the laboratory groups by the teaching assistant

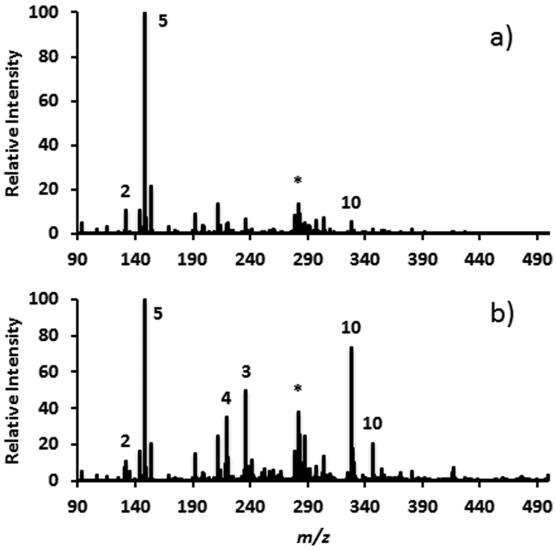


Figure 3. Influence of temperature on the droplets when the 50 cm focusing tube is heated with a heat gun at 0 and 90 s of direct heating on high in spectra a and b, respectively. **10** is the product and **5** is an intermediate. **10** exists as both the protonated species and as the ammonium adduct. Asterisk (*) denotes an impurity in the focusing tube.

immediately following the laboratory (Supporting Information S1, Appendix C). Six out of eight groups agreed to participate in the interviews.

When asked if this was a useful exercise, many students commented on how exciting it was to be given the opportunity to work with recent scientific methods in their undergraduate coursework. A student commented, “Yes, I didn’t know that was even something you could do. That was a new concept to me, so it was interesting and helpful to be able to see and do the process.” A strong understanding of the processes occurring in electrospray ionization was reinforced by the execution of the laboratory since the majority of student explanations of electrospray had been improved in the exit interviews and the laboratory reports compared to the pre-experiment questions.

Understanding of ion motion was consistent throughout the laboratory. Some students did mention that exploring fragmentation first hand in the laboratory made them better understand how the information can be of use and gave them an appreciation for the ease of performing CID. Some even commented on the proper pairing of ambient ionization to CID to elucidate the chemical information most effectively. Multiple groups saw benefit to using accelerated reactions in electrospray droplets to explore reactions. They pointed out the ease of data acquisition and the time saved using MS. About half of the students in both the interviews and the laboratory reports saw the value of the structural information acquired by NMR spectroscopy compared to MS, but acknowledged the requirement for pure samples for this technique was a major setback.

■ MATERIALS AND METHODS

A home-built ESI source was constructed from a 1/16 in. Swagelok tee and 250 μm i.d. and 355 μm o.d. fused silica capillary (Polymicro Technologies) connected to a 1.0 mL Hamilton gastight syringe with Upchurch fittings and a nitrogen tank for sheath gas. The fused silica was placed through the tee and positioned so it was just visible out of an outer stainless steel capillary with i.d. of 500 μm. An external syringe pump (Hamilton PHD series 2000 Infusion) was used rather that the built-in syringe pump in order to allow the use of the 1.0 mL syringe, although a smaller syringe could easily be used. A copper clip was attached to the exposed metal of the syringe to supply the potential from the LTQ source potential. Experiments without the focusing tube used this ESI setup with ca. 5 mm between the MS inlet and the tip of the source. When using the focusing tube (stainless steel 4 mm i.d. and 6 mm o.d.), the ESI tip was placed just inside the focusing tube and ca. 5 mm was left between the other end of the focusing tube and the MS inlet. The focusing tube was allowed to float rather than being grounded. A standard laboratory heat gun (Johnson Electric, 1500 W, 125 V) was used to heat the outside of the tube. Temperatures on the exterior of the tube were in the range of 85 to 120 °C as measured using an IR thermometer by the students after 90 s of heating.

Reagents (10 mM ethyl acetoacetate (Fluka Analytical), 10 mM ammonium acetate (Fischer Scientific, ACS grade), and 5

Table 2. Student Exit Interview Questions and Responses[a]

Question 1: This is probably the first time that electrospray synthesis with temporal resolution has been explored in an undergraduate teaching laboratory. How would you or could you explain whether or not you think this was a worthwhile laboratory experiment overall? What would you say you learned in an organic sense? What have you learned in an analytical sense?
6 groups said that they thought the experiment was a worthwhile exercise
3 groups stated that they appreciated the exposure to the mass spectrometer as more than a method of analysis
4 groups appreciated the hands-on experience with the commercial mass spectrometer
5 groups saw the potential of the technique in both screening reactions and exploring reaction mechanisms
Question 2: Explain, as best you can, the process of electrospray ionization.
5 groups properly explained the processes occurring in electrospray ionization
4 groups thought there was a relation between desolvation time and reaction progress
Question 3: How was MS/MS carried out in this laboratory? Explain the ion motion and fragmentation in the ion trap in comparison to a triple quadrupole MS that was discussed in lecture.
4 groups showed an understanding of the stages of isolation and fragmentation
2 groups compared ion motion and fragmentation in comparison to what occurs in a standard triple quadrupole experiment
Question 4: Give the main considerations of reaction monitoring by NMR compared to MS if you were to choose between the two instruments.
5 groups noted that a crude mass spectrum gives more information than a crude NMR spectrum
3 groups discussed potential applications of both NMR spectroscopy and MS
2 groups thought that pure NMR spectra give more structural information compared to MS/MS experiments

[a] Six pairs of students were interviewed.

mM benzaldehyde (Sigma-Aldrich, ≥99.5%)) were all made fresh using 200 proof ethanol (Koptec) as the diluent. Immediately prior to the experiment, the reagents were combined 1:1:1 and mixed. Experimentation setups were cleaned with ethyl acetate and ethanol. Diluent blanks between groups and between experiments showed little to no carryover.

■ CONCLUSIONS

Reactive ESI proved to be an effective means to teach students spray-based ionization techniques. Understanding the role ESI parameters play in promoting chemical reactions in electrosprayed reaction mixtures helped students to explore electrospray variables in an informative way using relative reaction progress as a gauge. CID experiments provided students with in depth experience with an ion trap mass spectrometer and MS/MS data acquisition on a commercial instrument, while relating the fundamentals of ion motion discussed in class to laboratory experience.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00165.

> Laboratory handout and mass/charge ratios for all relevant species (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support provided by the National Science Foundation (CHE-1307264). Authors acknowledge Kinsey Bain and Kasi Onyejekwe for continuing support.

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