

# Chemical Synthesis Accelerated by Paper Spray: The Haloform Reaction

Ryan M. Bain,<sup>†</sup> Christopher J. Pulliam,<sup>†</sup> Shannon A. Raab,<sup>†</sup> and R. Graham Cooks<sup>\*,†,‡</sup>

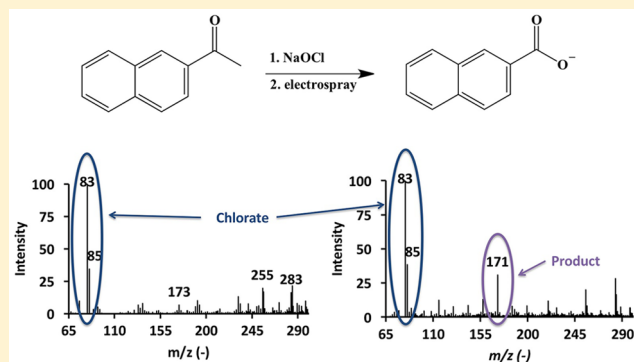
<sup>†</sup>Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, United States

<sup>‡</sup>Center for Analytical Instrumentation Development, Purdue University, West Lafayette, Indiana 47907, United States

## Supporting Information

**ABSTRACT:** In this laboratory, students perform a synthetic reaction in two ways: (i) by traditional bulk-phase reaction and (ii) in the course of reactive paper spray ionization. Mass spectrometry (MS) is used both as an analytical method and a means of accelerating organic syntheses. The main focus of this laboratory exercise is that the same ionization source can be operated to perform chemical analysis or to accelerate synthesis. More specifically, students explore the effect on reaction progress of operating in one of two modes of paper spray ionization. The modes relate to droplet size that changes as the solvent is depleted from the paper over time. The online mass spectral data for the two modes allow students to see the differences that small changes to spray conditions can have on the MS of a reaction mixture, allowing transition from traditional analysis to accelerated chemical synthesis by simply allowing the paper to partially dry. Chemical analysis and accelerated synthesis can be performed sequentially in one simple, 2 min paper spray experiment with the haloform oxidation.

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



## INTRODUCTION

Chemical analysis by MS can sometimes be facilitated by derivatization of the analyte to give a more easily ionized compound. Traditionally, derivatization is performed off-line and takes time; however, derivatization and other chemical reactions can be accelerated in electrosprayed droplets so that they occur during ionization by spray-based ionization sources.<sup>1</sup> These accelerated reactions are valuable as the reaction times are significantly reduced and the experiment is streamlined. In fact, accelerated droplet reactions can be used to prepare/collect milligram quantities of material in minutes.<sup>2</sup> Under different electrospray conditions, the constituents of a reaction mixture can be analyzed in real-time with no discernible reaction acceleration.<sup>3</sup> Analysis by MS of reaction mixtures and products is increasingly common,<sup>4</sup> and therefore, students should be exposed to the phenomenon of a particular reaction mixture giving reagent signals when analyzed by mass spectrometry or giving product ions when a reaction product is formed during MS analysis.

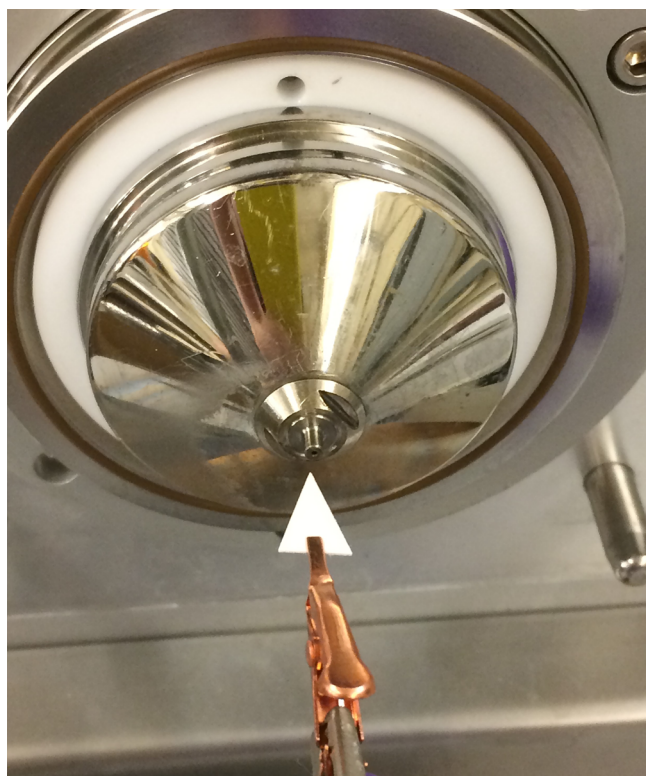
Ambient ionization is defined as ionization of samples in the ambient environment without significant prior sample preparation.<sup>5</sup> Many spray-based ionization methods including desorption electrospray ionization (DESI<sup>6</sup>), electrosonic spray ionization (ESSI),<sup>2</sup> and paper spray (PS) ionization<sup>7</sup> can be operated under conditions that facilitate the occurrence of chemical reactions between reagents present in the solution. PS

ionization was chosen as the ionization method in this laboratory experiment for its simplicity. PS ionization (Figure 1) is performed by simply applying sample and solvent to a piece of sharply pointed (usually triangular) filter paper with an applied voltage that produces an electric field at the tip of the paper large enough to cause field desorption of charged droplets in the form of an electrospray plume.<sup>8</sup> As the solvent is depleted the spray characteristics change and a different spray mode occurs. Optical measurements have shown that the second (low solvent) spray mode of PS yields significantly smaller (~15×) average droplet sizes when compared to the first spray mode. This difference in droplet size between the two spray modes can influence reaction rates.<sup>9</sup> The initial droplet size in other techniques has also been shown to have a significant effect on reaction rates.<sup>10</sup> Note that accelerated reaction rates are associated with droplet desolvation, which in turn leads to higher concentrations of reagents and pH extremes.

The haloform reaction (Scheme 1) was chosen for this experiment because it is often part of the curriculum for undergraduate laboratory courses and is synthetically unusual in that oxidation occurs without affecting carbon–carbon double

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**Figure 1.** Photo of paper triangle used in PS ionization and positioned in front of the vacuum inlet of an ion trap mass spectrometer as used in this laboratory experiment. The paper is ca. 5 mm long.

bonds.<sup>11</sup> The mechanism of the haloform reaction is considered to involve two steps: (1) trihalogenation of the methyl ketone followed by (2) base attack at the carbonyl carbon. Once the first halogen is added, each successive halogen addition is more favorable due to the electron withdrawing nature of the newly added substituent. Therefore, no intermediates were expected to be observed in this experiment as the first step is rate limiting. The procedure for the haloform reaction was adapted from the course textbook.<sup>12</sup>

An ion trap MS (LTQ, Thermo Scientific, San Jose, CA) was chosen for this experiment for its ease of use. The many capabilities of an ion trap MS,<sup>13</sup> especially the option to perform tandem MS (MS/MS) analysis, allows considerable

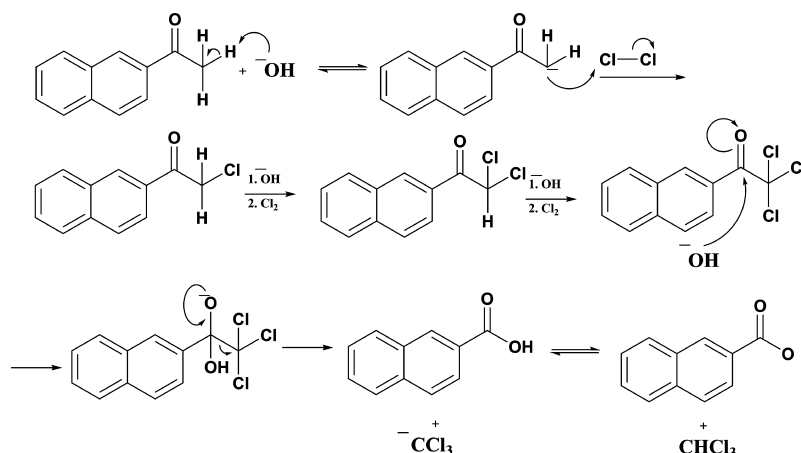
student freedom in performing this experiment (e.g., students were encouraged to use MS/MS to attempt structural identification of the product to accompany their typical NMR and IR spectroscopic findings). The experiments were performed with the source/probe housing removed and a high voltage lead attached. With the source housing removed, the students were not left unattended with the instrument as the high voltage (albeit with low currents (microamperes)), lead is exposed. Commercially available PS sources can be purchased or a simple housing can be built to protect students from the risk of shock. Recent laboratory exercises using MS have proven to be worthwhile for students, including hands-on vacuum chamber construction,<sup>14</sup> ESI techniques for analysis,<sup>15</sup> and exploration of negative mode ESI-MS for detection of NADH-boronic acid complexes.<sup>16</sup>

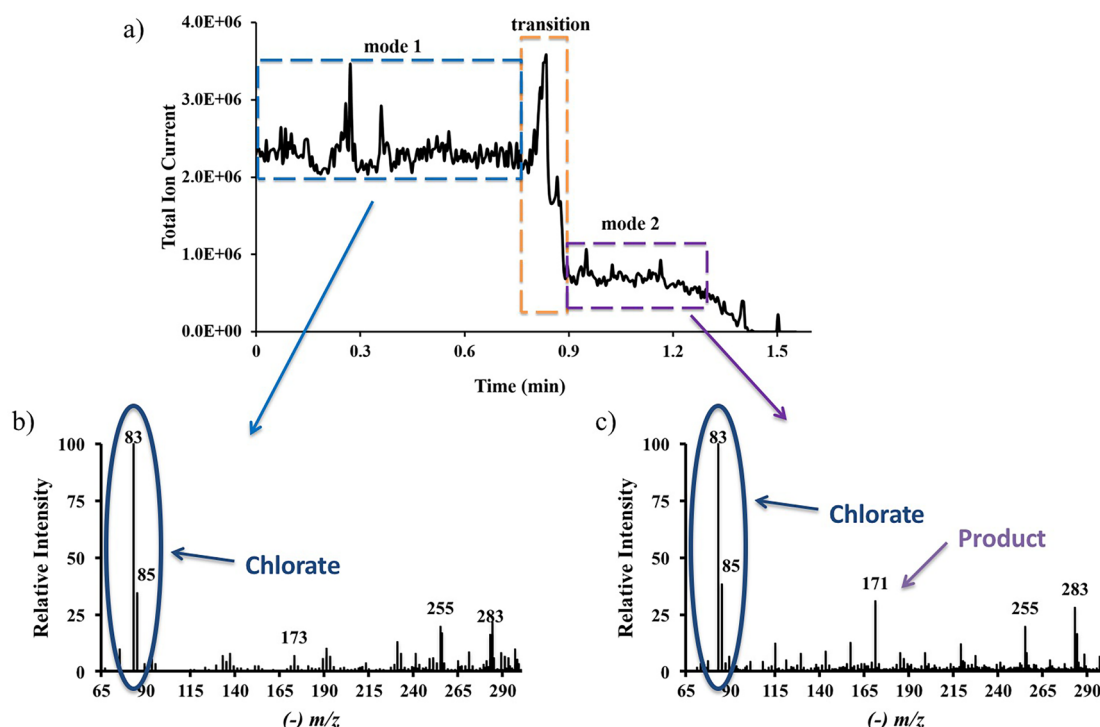
This laboratory experiment was conducted during the second semester of an honors organic chemistry course for chemistry majors. Students were introduced to the haloform oxidation in the lecture component of the course so that they could focus on techniques and instrumentation in this laboratory. As instrumental chemical analysis is a critical component of organic synthesis experiments, it is important that students understand that the analytical method itself can affect results in unforeseen and (as here) valuable ways. In this particular example, students see that accelerated reaction between the reagents occurs in the second mode of PS as is apparent from TLC analysis. Reaction mixture analysis in spray mode 1 allows accurate assessment of the reaction constituents. Furthermore, students are reminded of the role concentration plays in chemical kinetics as they work to understand the cause of the accelerated reaction kinetics in mode 2.

## EXPERIMENTAL OVERVIEW

Students worked in pairs for this 3 h experiment. All groups ( $n = 6$ ) started their bulk-phase synthesis after a short prelaboratory lecture by a graduate student and performed the paper spray experiment after their bulk-phase reaction had started. Traditional analytical techniques used in the organic teaching laboratory were performed on the recovered product (see [Supporting Information](#) for full procedures for both experimental sections). Sodium hypochlorite solution (8% available chlorine), reagent grade methanol, diethyl ether, and 2-acetonaphthone were all purchased from Sigma-Aldrich (Milwaukee, WI). Filter paper (Whatman 1 analytical) was

**Scheme 1.** Reaction of 2-Acetonaphthone with Sodium Hypochlorite to Form the Carboxylate and Chloroform





**Figure 2.** (a) Ion chromatogram produced when paper spray ionization is performed on the reaction mixture. Negative ion mass spectra are shown in (b) spray mode 1 and (c) in spray mode 2. Spray mode 1 displays predominantly chlorate ions at  $m/z$  83 and 85, whereas spray mode 2 still contains these species with the notable addition of ions due to the reaction product (2-naphthoic acid) at  $m/z$  171. Ions of palmitic acid and stearic acid at  $m/z$  255 and 283 are contaminants (most likely from handling the paper without gloves).

purchased from GE Healthcare Bio-Sciences (Pittsburgh, PA) and 3 M HCl was prepared from DI water and 37% HCl (Mallinckrodt, St. Louis, MO).

### Bulk-Phase Synthesis

Traditional semimicroscale synthesis was conducted by each pair of students. The synthesis took 45 min after mixing excess sodium hypochlorite with 2-acetonaphthone and methanol in a round-bottom flask with an air condenser attached. After completion of the reaction, students quenched any excess hypochlorite by the addition of sodium sulfite. The basic reaction mixture was then extracted using diethyl ether and the crude product was precipitated from the aqueous phase by the dropwise addition of 3 M HCl. The solid product was collected, washed with chloroform, and dried by vacuum filtration.

### Analysis of Bulk-Phase Reaction Products

The completeness of the bulk-phase reaction was monitored using thin-layer chromatography (TLC) with lanes for the reaction product (2-naphthoic acid), the starting material (2-acetonaphthone), and the reaction mixture. Students reported product to be visible by TLC analysis after ca. 20 min and to be complete after ca. 45 min. Bulk-phase reaction product was analyzed by chemical analysis techniques typically available to students in this laboratory, including  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectroscopy, IR spectroscopy, and melting point analysis. From these analyses, students were able to comment on the structure and purity of the collected product. Groups of students also performed percent yield calculations by mass and reported a variety of yields ranging from 28% to 80%. Most groups reported NMR chemical shifts in agreement with that of the expected product and reported little to no residual starting material after purification as evidenced by the lack of signal correlating to the  $\alpha$ -methyl of the ketone at ca. 2.6 ppm in  $^1\text{H}$

NMR spectra. A characteristic NMR spectrum can be found in the [Supporting Information](#). In performing these experiments, students were encouraged to choose their analytical techniques for characterization, a point of emphasis in this second semester organic laboratory. Students learn the techniques involved in sample preparation and instrumental analysis as it relates to reaction monitoring and analysis of reaction products.

### MS Analysis and Synthesis by Reactive Paper Spray Ionization

In a PS experiment covering both spray modes, students were able to observe by online MS analysis a transition from simple analysis of the reaction mixture to accelerated reaction during the analysis step. Freshly prepared reaction mixture (20  $\mu\text{L}$ ) was spotted onto the paper spray triangle using a micropipette, aligned orthogonally to the inlet to the MS, and data acquisition was started immediately. Students took data as the droplets changed from mode 1, through the transition region, and to mode 2 as the paper dried. Spray mode 2 has a significantly lower signal intensity accompanying the smaller droplet size compared to mode 1, as seen in the ion chromatogram recorded by students in the laboratory ([Figure 2](#)). Typical mass spectra were taken during spray mode 1 and mode 2 ([Figure 2](#)), and they show the generation of the carboxylate product ion ( $m/z$  171) only during mode 2. Positive ion mode and MS/MS data were also consistent, as shown in the [Supporting Information](#). It is also worth noting that this reaction can be of interest in converting ketones, which ionize only moderately well in the positive ion mode, into readily ionized carboxylic acids which are best observed in the negative ion mode.

## HAZARDS

Participants should review all safety data sheets (SDS) for chemicals associated with this experiment to ensure safe laboratory practice. When working with the LTQ ion trap MS, it is important to note that a potential of 4.0 kV (albeit at low current) is applied to the copper lead when the instrument is scanning. The scan indicator will be illuminated when the instrument is scanning and the status of the instrument in the software will indicate “scanning”. The inlet to the MS is also kept at 200 °C and therefore should not be touched.

## EVALUATION AND ASSESSMENT

Students were asked to answer specific questions prior to the experiment to ensure their understanding of the laboratory material. These questions were primarily based on the mechanistic aspects of the haloform reaction so that the prelaboratory lecture could cover and answer questions on the PS–MS components of the exercise. For the written laboratory reports, students (in their pairs) were left to organize and report as they saw fit with a few required components, including mass spectral interpretation, percent yield, and NMR spectra.

### Pre-Experiment Questions

Questions at the start of the laboratory session helped ensure students understood the haloform reaction. Students demonstrated a strong understanding of the reaction mechanism and the chemistry occurring in the semimicroscale synthesis component. For example, students discussed how seemingly small changes like solvent could influence the reaction. Overall understanding of traditional means of analysis, specifically IR and NMR spectroscopy, and how it would relate to the analysis of this reaction was shown by most groups. Examples of haloform reactions including the iodoform test were discussed in great detail in the pre-experiment questions and they illustrated the students understanding of the chemical reactivity and the applicability of the reaction.

### Laboratory Reports

All groups of students were able to record spray mode 1 and 2 data that showed a significant increase in product when using the second spray mode compared to the first. The data of the last student group can be seen in [Figure 2](#) and, like all MS data, were recorded in the negative ion mode. The last group was chosen to illustrate that there was no sample carryover in the mass spectrometer from group to group. Note that in both modes the bleach-associated ions, including chlorate at  $m/z$  83 and 85, are dominant as the reaction is run in excess sodium hypochlorite. The MS data for structural identification of ions  $m/z$  83 and 85 are in the [Supporting Information](#).

### Postlaboratory Assessments

Along with providing written laboratory reports, student groups were prompted to answer postlaboratory questions. The four questions or statements for response by the six pairs of lab partners are presented here (and are also provided in [Table S1](#) in the [Supporting Information](#)).

1. What are the benefits and limitations of synthesis by mass spectrometry?
2. Hypothesize why the reaction is accelerated in spray mode 2 of the paper spray experiment.
3. How would different solvents (polar vs nonpolar) affect the microscale bulk experiment and the mass spectrometry synthesis experiment?

4. Suggest an explanation for the difference in droplet size when comparing the two modes of paper spray ionization.

For the first question, all six groups said that they saw that the product can be created significantly faster by PS ionization than bulk-phase synthesis. Five groups made a point to note the simplicity of PS ionization and the ease of using an LTQ MS for chemical analysis. Four groups appreciated the ability to analyze crude reaction product without separation or sample cleanup. Another four of the six groups thought that the experiment could not be done in a way that product could be collected even though an experiment was explained in the prelaboratory in which authors did in fact collect the product. This work, in which the authors collected reaction product from the spray, was also a required reading. Finally, three groups commented on the potential for carryover when performing high concentration reactions.

In response to the second statement, five groups understood the role that concentration of reactants in the droplets play in the kinetics of the reaction, and three groups noted that this acceleration in spray mode 2 is due to desolvation or initial droplet size.

Responses to the third question were distributed as follows: five groups thought that the effect on the bulk-phase synthesis would be the same as the PS synthesis, four groups noted reaction would not be likely to occur if the reaction were switched to a more nonpolar solvent, and one group noted that the paper spray ionization may not work with a nonpolar spray solvent.

Responding to the fourth statement, five groups described spray mode 2 as being due to depletion of solvent compared to spray mode 1. Four groups noted that spray mode 1 gives an accurate measure of the reaction mixture where spray mode 2 shows acceleration of the reaction rate, and two groups thought that pure NMR spectra give more structural information compared to MS/MS experiments.

Nearly all students had a strong grasp on the processes of paper spray ionization and MS as used in this experiment. They had a good understanding of the spray modes of paper spray ionization and how they relate to reaction monitoring compared to causing and accelerating reactions. One student concluded when discussing droplet size differences between the spray modes by stating, “[Spray] mode 1 provides an indication of the overall reaction mixture whereas [spray] mode 2 provides information about the reaction’s progress due to solvent evaporation and increased reaction rates.”

## CONCLUSIONS

The ability to use the two distinctive modes of paper spray ionization to determine whether or not reaction rates are accelerated has been demonstrated in an undergraduate organic laboratory. Spray mode 2 showed significant reaction rate acceleration compared to spray mode 1, which simply shows the constituents of the reaction mixture. The ease of distinguishing between the two spray modes was illustrated by the interpretation of data in the laboratory reports. Students clearly understood the difference between analysis of a reaction mixture and accelerating reactions of the reagents in the same reaction mixture by simply allowing solvent to evaporate from the paper, which influences initial droplet size.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: [10.1021/acs.jchemed.5b00263](https://doi.org/10.1021/acs.jchemed.5b00263).

Supporting Information includes the laboratory handout, procedure and experimental preparation instructions, NMR spectrum of the product, positive mode results, and MS/MS spectra. (DOCX)

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## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: [cooks@purdue.edu](mailto:cooks@purdue.edu).

### Notes

The authors declare no competing financial interest.

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