

Laboratory Ozonolysis Using an Integrated Batch–DIY Flow System for Renewable Material Production

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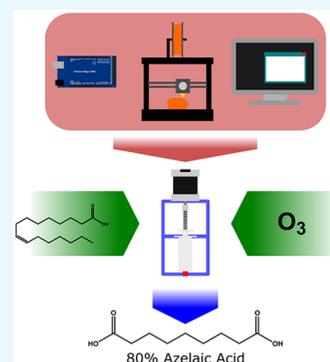


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ABSTRACT: Flow chemistry offers a solution for replacing batch methods in chemical preparation where intermediates or products may pose toxicity or instability hazards. Ozonolysis offers an ideal opportunity for flow chemistry solutions, but multiple barriers to entry exist for use of these methods, including equipment cost and performance optimization. To address these challenges, we developed a programmable DIY syringe pump system to use for a continuous flow multireactor process using 3D-printed parts, off-the-shelf stepper motors, and an Arduino microcontroller. Reaction kinetics of ozonide formation informed the use of an integrated batch–flow approach, where ozone addition to an olefin was timed to coincide with fluid movement of a single-syringe pump, followed by downstream Pinnick oxidation and reductive quench in flow. The system was demonstrated by continuous preparation of azelaic acid from ozonolysis of palmitoleic acid, a process limited to low production volumes via batch chemistry. High total production of azelaic acid with 80% yield was obtained from an algae oil sourced unsaturated fatty acid: a product with important applications in medicine, cosmetics, and polymers. This low-cost, scalable approach offers the potential for rapid prototyping and distributed chemical production.



1. INTRODUCTION

Flow chemistry—the process of using continuous flow for chemical reactions—evolved from basic laboratory techniques to industrial practices.^{1–4} Although flow chemistry offers both advantages and disadvantages at different scales,⁵ its rise opened the door for next-generation chemical reaction development and process engineering.⁶ The concept of continuous flow ozonolysis with perfect control of gas–liquid mass transfer processes appears to be highly preferred to batch ozonolysis due to its exothermic nature, thermal stability, and toxicity risk.^{7–9} A variety of flow ozonolysis technologies were recently demonstrated, including the use of semipermeable tubing for gas to liquid transfer¹⁰ and a commercially available ozonolysis flow reactor designed for lab scale by ThalesNano capable of producing up to 10 g of finished materials per day.⁷ Lonza recently designed a pilot flow ozonolysis system and demonstrated its ability to scale processes for implementation to produce 500 kg of product per day in a continuous mode.¹¹ However, not all these recent technologies are appropriate or cost-effective for an academic laboratory. Therefore, we set out to develop a simple, scalable approach to apply the powerful oxidative potential of ozone to the production of useful chemical intermediates.

With the advent of hobbyist electronics and development of open-source microcontrollers and single-board computers such as the Arduino Uno and Raspberry Pi, it is possible to create advanced projects using do-it-yourself (DIY) approaches. This advancement has been fueled by dropping prices in 3D printing, allowing complex and custom parts to be rapidly manufactured at low cost, as well as the ready availability of

precision parts such as stepper motors.¹² Science and medicine have offered creative applications, where Arduino controller setups provide basic sensors to perform diverse experiments.^{13–15} Freely available plans, open-source code, and programming flexibility also enabled healthcare applications such as acoustofluidics to lower point-of-care medical costs.¹⁶ In chemistry, microcontroller microcomputer boards combined with cost-effective sensors were applied to monitor and optimize chemical reactions for both batch and flow processes, reducing operator intervention for product purification and supervision.¹⁷ Open-source hardware can also be applied to flow chemistry systems, lowering the barrier to entry for conducting these experiments. Syringe pumps assembled using open-source 3D models and off-the-shelf parts can provide DIY solutions to challenging applications such as multistep chemical syntheses when combined with open-source code.^{18–21} While not necessarily replacing commercial flow systems, open-source and DIY technologies allow proof-of-concept flow chemistry development and troubleshooting where these simple, customizable, and inexpensive tools offer clear accessibility advantages.²²

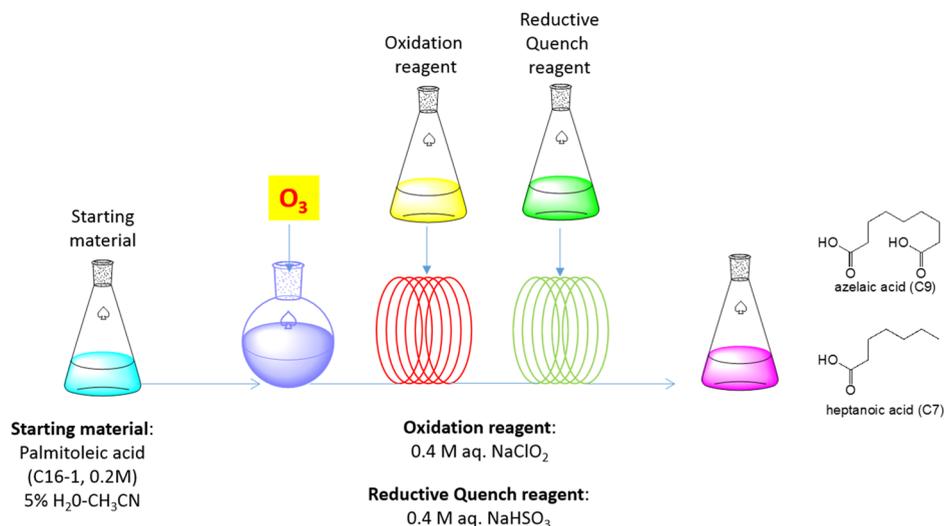
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Scheme 1. Schematic Illustrating an Integrated Batch-Flow Chemistry Process Applied to the Synthesis of AA and HA



Conventional ozonolysis of olefin compounds has been conducted in aprotic solvents to form the secondary ozonide, which possesses a high energy of decomposition.^{23,24} Recent developments employed aqueous solvent mixtures to avoid secondary ozonide formation, thereby minimizing the accumulation of the high-energy secondary ozonides and increasing process safety.^{7,10,23,25,24,26} Here, water plays a critical role as an effective nucleophile during molozonide rearrangement. This results in direct formation of aldehydes or ketones, which avoids the accumulation of the decomposed ozonides.²⁶ Mild, one-pot ozonolysis–oxidation processes using aqueous solvent mixtures were recently employed to prepare carboxylic acids in excellent yields.²⁴ Here, aldehyde formation is followed by treatment with sodium chlorite to deliver the respective carboxylic acid, whereupon a reductive quench with sodium bisulfite can safely neutralize any residual oxidants.^{26,24} This procedure has been evaluated with a variety of primary alkenes to synthesize desired acids in high purity and yield.²⁴

Dicarboxylic acids such as azelaic acid play important roles in a variety of commercial applications as key components of fragrances, adhesives, paints, coatings, plasticizers, textiles, lubricants, and hydraulic fluids.²⁷ Algae biomass offers one of the most sustainable and productive photosynthetic resources for petroleum replacement, and we have an ongoing program to scale these processes for eventual commercial application.^{28–33}

A recent report from Ley demonstrated the potential to integrate batch and flow reactions in one process and inspired us to explore the use of a pulsed batch reactor to complete the ozonolysis, oxidation, and reductive quench in a single automated process.³⁴ Flow chemistry is proved as a viable alternative approach to handle the ozonolysis process's safety;³⁵ however, this method required equipment valued at upward of \$200,000 USD. Here we aim to further leverage algae-sourced unsaturated fatty acids for sustainable production of azelaic acid at the lab scale. We demonstrate the adaptation of DIY flow chemistry with batch ozonolysis for an integrated batch–flow method using open-source hardware and software technologies to create a scalable, cost-effective solution to renewable azelaic acid preparation (Scheme 1).

2. DISCUSSION

2.1. Ozonolysis Methodology and UV–Vis Monitoring.

Ozonolysis—a method to oxidatively cleave hydrocarbons that contain a carbon–carbon double or triple bonds using ozone—was discovered in the mid-18th century by Christian Friedrich Schönbein. Ozonolysis can provide an alternative to heavy metal oxidation reactions and is considered a green and sustainable oxidizing agent providing the potential for green chemistry methods.⁹ This method has been developed for organic chemical reactions of alkenes or alkynes to prepare alcohols, aldehydes, ketones, and carboxylic acids.^{36–38} Achievable products from ozonolysis depend upon the substrate identity and the workup conditions. For instance, reductive work up of 1,2-dialkyl-substituted olefins results in alcohols or aldehydes, while oxidative work up leads to carboxylic acids.^{39,40} The mechanism of ozonolysis was first proposed by Criegee⁴⁰ and more recently confirmed by Berger using ¹⁷O NMR spectroscopy.⁴¹ In the ozonolysis mechanism, the electrophilic addition of ozone to the π -bond first forms a 5-membered molozonide intermediate (prime ozonide), which rapidly rearranges to create the secondary ozonide in organic solvents.³⁸ The secondary ozonide is an unstable, potentially explosive intermediate that requires low-temperature handling and immediately converts into the desired carbonyl products via oxidative or reductive workup.³⁸ Conventional ozonolysis of unsaturated fatty acids has been studied for decades^{42–46} and currently offers an important route to industrially produce azelaic acid.⁴⁷

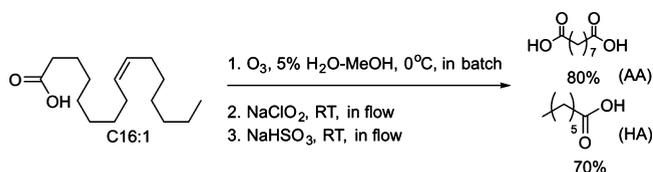
The synthesis of azelaic acid and heptanoic acid by batch ozonolysis of palmitoleic acid originating from microalgae oil waste streams was accomplished in our previous work.¹⁸ In that study, a water-based ozonolysis methodology from Dussalt was applied to avoid safety concerns from accumulation of peroxide intermediates.^{26,48} Our ozonolysis studies in aqueous methanol suggested that polar organic solvents miscible in water improved the water nucleophilicity for carbonyl oxides, resulting in the direct formation of aldehydes,²⁶ avoiding the formation of energetic intermediates,^{48,49} which are further oxidized with sodium chlorite to produce the desired carboxylic acid in high yield and purity.^{18,24} However, our attempts to scale up these reactions faced several challenges.

Beyond the simple setup and ease of the process, batch ozonolysis offered several limitations for increasing scale. Beyond the anticipated concerns of reactor acquisition and setup, some practical challenges included the requirement of a fourth equivalent of oxidant (sodium chlorite) and the necessity to age the reaction overnight to fully convert all intermediates into carboxylic acids.²⁴ In addition, increasing reaction vessel size in a laboratory environment escalated safety concerns for ozone formation.^{7,8,10} Flow chemistry had the potential to address all these limitations by conducting a continuous reaction in a small volume, which would avoid the complexities of large volume reactions and reduce the high ratio of oxidant. Flow methods offer high surface area-to-volume ratios, which in turn enhance heat and mass transfer rates and lead to more precisely controllable reaction conditions. Therefore, we chose to adapt palmitoleic acid ozonolysis to a flow chemistry approach in order to safely scale the production of azelaic acid in a laboratory setting. Evaluation of commercial flow chemistry systems did not meet our requirements for cost and adaptability, so we chose to develop a DIY flow chemistry setup to meet our needs within a very reasonable budget (Table S1).

To monitor ozonolysis completion during the process, we implemented online monitoring of the batch reactor output. While some studies have utilized FT-IR or ATR to monitor ozonolysis completion, we reasoned that an online UV monitor would also function in this role.^{23,25} We integrated an online UV/vis cell behind the ozonolysis batch reactor 2, which allowed us to modify the single-syringe pump governing removal of aldehyde products from the reactor. This way, aldehyde product removal could be precisely timed to match reaction kinetics and allow for complete conversion. This design offered the benefits of both batch and flow methods into a single automated process. For optimizing the reaction parameters, the reaction was monitored by taking aliquots and measured via NMR for quantifying the amount of reactant left. However, the UV-vis system provided a rapid qualitative solution for detecting deviations in the reaction as the flow system was operated for extended periods of time up to 8 h a day continuously.

The UV-vis online monitoring system was implemented using a Waters 996 photodiode-array-detector (PDA) with a Waters 510 solvent delivery pump to connect to monitor to the batch reactor in real time (Figures S38 and S39). Ozonolysis completion (Step 1 of Scheme 2) was monitored

Scheme 2. Oxidative Cleavage Ozonolysis That Forms Azelaic Acid and Heptanoic Acid from C16-1



by measuring absorption at 280 nm (Figure 1). As shown in Figure 1, the UV-vis spectrum of the C16-1 solution shows absorption bands in the 265–300 nm range for the n- π and π - π transitions for the π bonds in palmitoleic acid,^{50–55} while the aldehyde products exhibit no absorption in this range. For testing this analytical method, a Waters 510 HPLC Pump Solvent pumped C16-1, ozonide, and blank solvent solutions

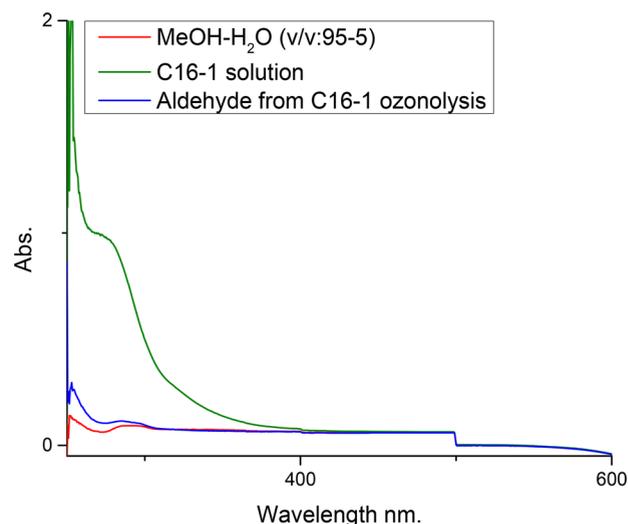


Figure 1. UV-vis spectrum of the C16-1 solution and its intermediates. Online UV monitoring at 280 nm (see Figure 4 and S26, S38 and S39) was used to time the reaction kinetics to set the batch reactor timing.

in order. As shown in Figure S39, the resulting chromatogram of C16-1 and ozonide solutions could be evaluated for intensity change across the analyzed spectrum throughout the reaction. This simple PDA implementation demonstrates the potential for online testing to inform volume and reaction time selection qualitatively and immediately during the development of an integrated batch-flow ozonolysis system.

2.2. Flow Chemistry Design via an Integrated Batch-DIY Flow Chemistry Setup. Before attempting to study reaction kinetics, we encountered several mechanical challenges in our flow chemistry setup. Despite our attempts to optimize reactor volume, flow rate, and ozone concentration, most ozonolysis flow reactor variations failed to completely oxidize the starting material due to inefficient mixing of the gaseous ozone phase with the continuous liquid stream of reactants in the coil reactor. We determined that the pressure limits on the syringe pump system prevented sufficient mixing between ozone and reagents. Comparisons to batch reactions indicated that batch methodologies can accommodate lower ozone concentrations and ensure reaction completion more rapidly. We concluded that, although continuous flow chemistry could be achieved in flow with more sophisticated reactor design and pump improvements, an alternative strategy could utilize small batch processes to ensure reaction completion while keeping the latter steps in continuous flow. This led to an integrated batch-flow strategy.

For an integrated batch-DIY flow system, we reasoned that a single-syringe pump could be used to fill an ozonolysis reactor with the starting material in aqueous methanol with regular ozone addition, and a second single-syringe pump could serve to empty that reactor into a holding vessel at a rate that could be timed with the ozonolysis reaction kinetics. Excess ozone gas was converted into oxygen by passing it through ozone destroyer solution (NaHSO₃) to prevent ozone flooding. Continual injection of the aldehyde product into a flow system could complete Pinnick oxidation and subsequent quench reaction using continual dual-syringe systems. The comparison of a single-syringe system and a dual-syringe system can be seen in Figure 2 and Figures S1–S6.

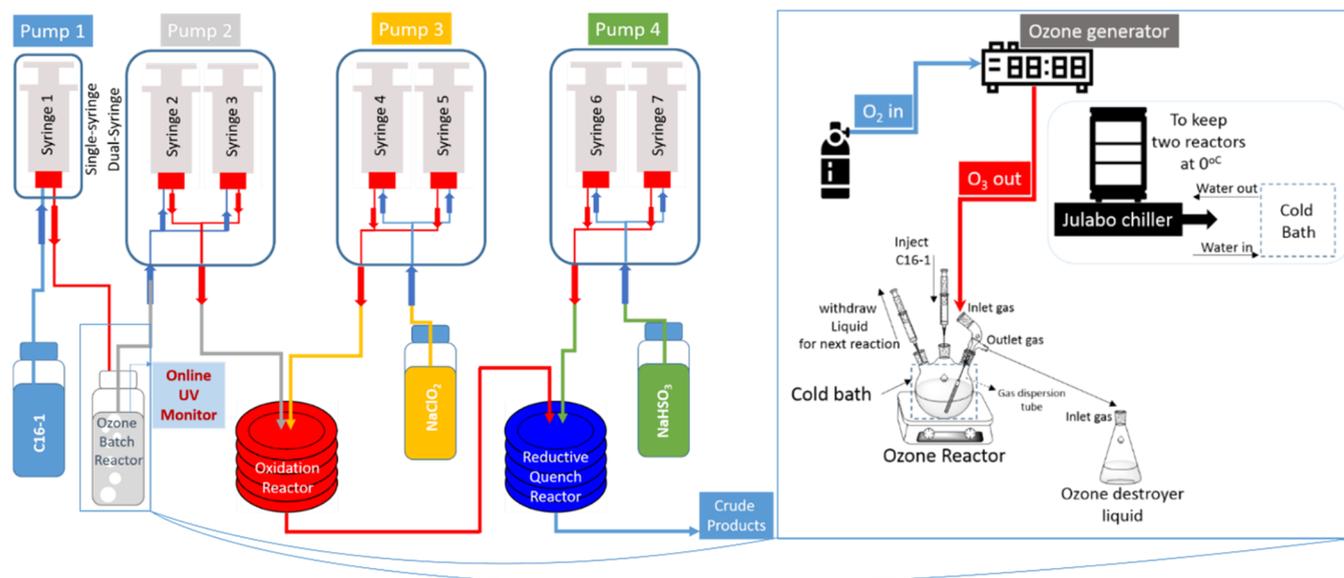


Figure 2. 3D-printed system setup flow diagrams for continuous ozonolysis in lab scale in the single ozone reactor variant. On the right side is the corresponding cutout detailing the setup of the ozone reactor. One single syringe in pump 1 handles delivery of C16-1 in and out of the ozone batch reactor where the aldehyde forms. Pump 2 transports the collected aldehyde for Pinnick oxidation with NaClO_2 added by pump 3 and reduction by NaHSO_3 via pump 4 for conversion into the desired carboxylic acid and reduction of the remaining oxidants.

The single-syringe-pump setup uses only one syringe, which relies on a servo motor to deliver fluid through the lines. Although noncontinuous, the advantage of this system is that it provides a reaction time equal to the time needed to refill the syringe. Applications of this pulsed pumping design in flow chemistry include batch steps that require time to bring the reaction to completion before injection into the flow system. The pause can be adjusted to match the reaction kinetics by decreasing or increasing the volume that the syringe must refill in the Arduino code. The challenge of using this single-syringe pump in combination with a continuous flow process is the requirement for a dispensing reservoir that also accounts for dead volume, so that a single-syringe pump with an intermittent dispensing rate can replenish the reservoir rapidly enough to serve as a continuous flow system further downstream in the process. One solution would be to utilize two separate batch systems pumping alternatively which would eliminate the need for a reservoir, but we did not employ this method to save on costs further. The same strategy also applies if a continuous step were to be used before a semibatch single-syringe pump.

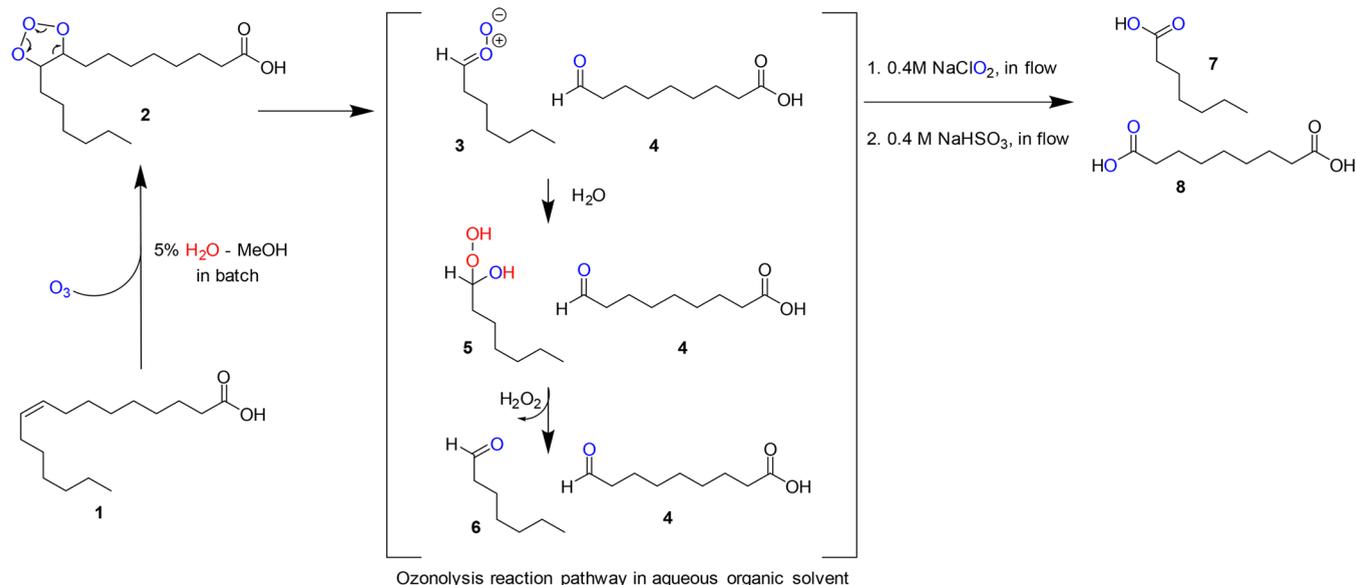
The dual-syringe setup works best for continuous flow chemistry, where continuous flow delivers fluid for each reaction step. This setup consists of two syringe pumps with two sets of two chemically resistant powered valves, which allows the syringes to work with continuous infusion, where one pump dispenses as the other withdraws, to deliver liquids continuously. The DIY syringe pumps in this setup possess significantly lower pressure limits compared to professional grade HPLC pumps and, therefore, limit the reactor coil tube lengths and the volume delivered into a single coil, although this can be partially addressed by increasing the tubing diameter. Attempts were made to use conventional check valves made for microfluidics by IDEX, but the gold coated springs inside the valves quickly deteriorated and broke, proving the valves to be too costly for practical implementation. Hence, a new set of valves was created taking advantage of a 3D-printed coupler between a servo and a luer-lock 3-way

valve to create an automated valve to switch outlets in sync with syringe pump motion (Figures S2–S3).

2.3. Batch-Flow Oxidative Ozonolysis Process.

2.3.1. Determining Ozonolysis Parameters. One of the most important factors in flow chemistry is the residence time of the reaction within the coil reactor, which regulates reaction completion. According to Omonov et al., the optimum time to complete ozonolysis of 100 g of monounsaturated fatty acid is 50 min at a 0.35 M concentration, regardless of the solvent used.⁴⁴ In their study, a gas chromatographic (GC) time course analysis of ozonolysis of 0.35 M oleic acid in 5% H_2O –MeOH showed initial formation of the two intermediate ozonolysis products within 10 min at 0 °C.^{40,44,56} This result was also supported by their SEC-RI chromatograms, which showed a decrease in the peak intensity characteristic of 0.35 M monounsaturated fatty acid after 10 min of ozonolysis at 0 °C.⁴⁴ In addition, with a sufficient ozone/unsaturated hydrocarbon ratio of around 1.4, ozonolysis proceeds with complete ozonolysis with a residence time of 5 min in the liquid phase in flow.²³ Higher concentrations of unsaturated fatty acids required a longer reaction time. Prior studies indicate that the higher viscosities of higher concentrations, including neat oil, slowed down ozone diffusion and required significantly longer reaction times.⁵⁷ Concentrations of ozonolysis reagents are commonly kept under 0.25 M,^{7,10,24,46} and an aqueous solution of 5% H_2O in organic solvents such as acetone, methanol, or acetonitrile is commonly the preferred solvent for full conversion into carboxylic acid via a telescoped ozonolysis–oxidation process.^{18,24} Although conducting ozonolysis of unsaturated hydrocarbon aqueous solutions helps avoid the formation of high-energy intermediates,²⁶ ozonide stability in organic solvents was worth considering to understand any safety concerns.

The thermochemistry and stability of ozonide intermediates, as well as their thermal decomposition paths, have been extensively studied.^{58–63} Differential scanning calorimetry (DSC) analysis of the ozonide formed from unsaturated fatty

Scheme 3. Reaction Pathways Present in the Ozone Batch Reactor^a

^aOxidation and reductive quench reactors convert the remaining aldehydes into carboxylic acids.

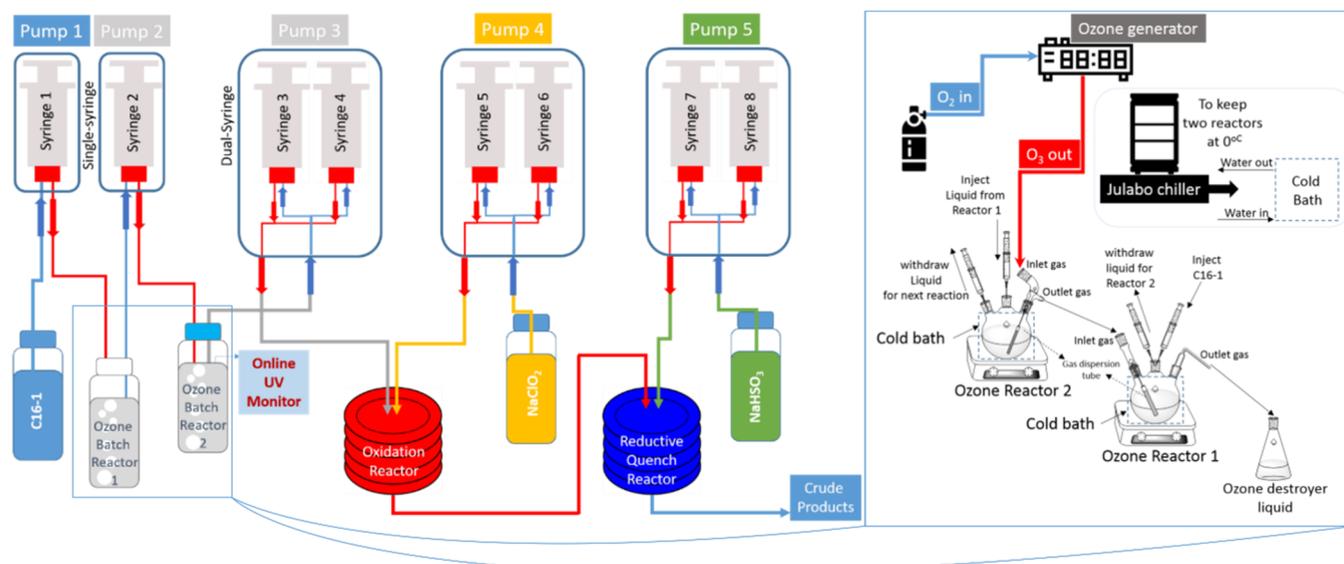


Figure 3. 3D-printed system setup flow diagrams for continuous ozonolysis in lab scale in the series reactor setup. On the right side is the corresponding cutout detailing the setups of the ozone reactors. Two single syringes in pumps 1 and 2 handle delivery of C16-1 in and out of the ozone batch reactor where the aldehyde forms. Pump 3 transports the collected aldehyde for Pinnick oxidation with NaClO_2 added by pump 4 and reduction by NaHSO_3 via pump 5 for conversion into the desired carboxylic acid and reduction of remaining oxidants.

acids showed an exothermic peak around $150\text{ }^\circ\text{C}$, indicating decomposition.^{58–61,64} The decomposition enthalpy is proportional to the amount of ozonide, and higher ozonide concentrations increased the heat liberated from the thermal decomposition.⁶⁴ Although there is a considerable amount of heat released from the decomposition of primary and secondary ozonides based on DSC data, ozonides formed from saturated fatty acids are not considered explosive at room temperature, a property attributed to the stability imparted by their long aliphatic chains.^{58–60} However, at temperatures around $150\text{ }^\circ\text{C}$ the experimental heat release of these ozonides during the decomposition is 243.5 kJ mol^{-1} , a value in reasonable agreement with the thermochemical calculation value of 278.5 kJ mol^{-1} .⁶⁰ This high exothermal property of

ozonide at high temperature requires cooling the reaction mixture and retaining only small amounts of ozonide in the unit at a time. These conclusions further support the use of aqueous ozonolysis methods for scaling carboxylic acid production from alkenes.²⁶

2.3.2. NMR Analysis of the Ozonolysis Reaction. In our study, NMR samples were taken at given time intervals to monitor the rate of ozonolysis in the ozone reactor where reaction of the C16:1 unsaturated bond and ozone gas occurred. Monitoring the ozonolysis reactions of 0.1 and 0.2 M palmitoleic acid in the batch ozone reactor (withdrawing aliquots at 10–20 min intervals) demonstrated that the double bond at position C9 in the palmitoleic acid (δ 5.2 ppm) is significantly decreased and the aldehyde product (peak at 8.6

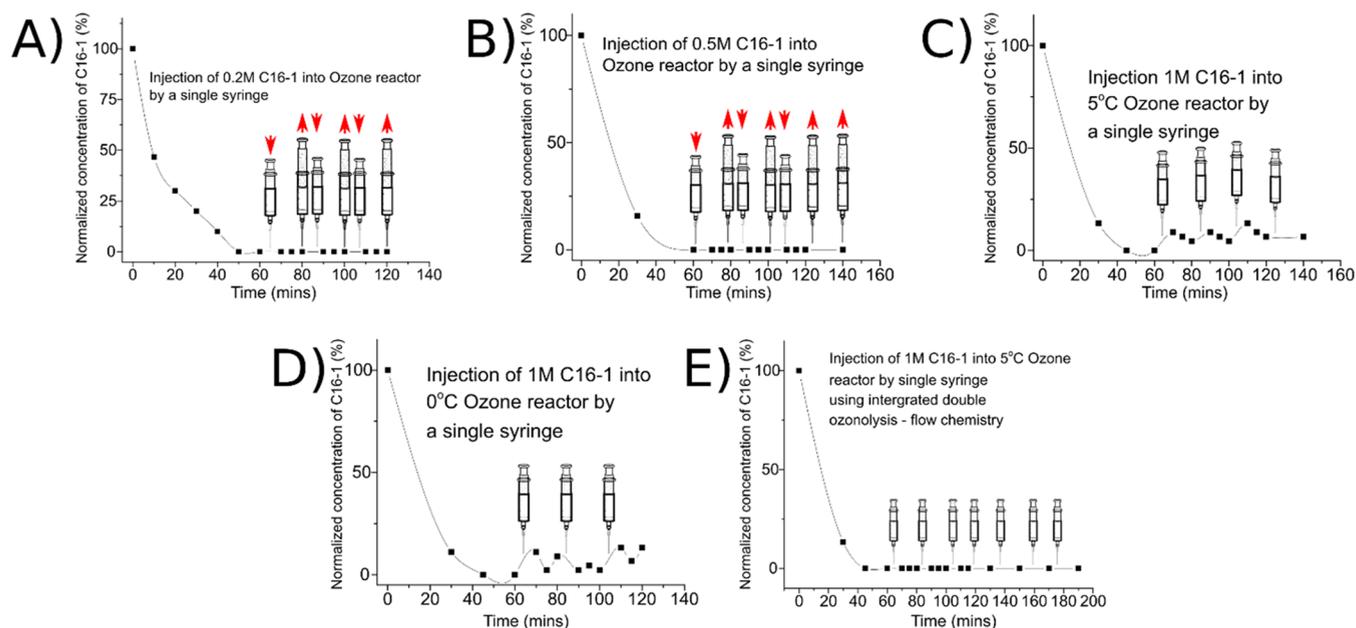


Figure 4. (A) Relative proportion of monounsaturated fatty acids to palmitoleic acid when injecting 0.2 M palmitoleic acid into a 5 °C ozone reactor. (B) Relative proportion of monounsaturated fatty acids to palmitoleic acid when injecting 0.5 M palmitoleic acid into a single 5 °C ozone reactor. (C) Relative proportion of monounsaturated fatty acids to palmitoleic acid when injecting 1 M palmitoleic acid into a 5 °C ozone reactor. (D) Relative proportion of monounsaturated fatty acids to palmitoleic acid when injecting 1 M palmitoleic acid into a 0 °C ozone reactor. (E) Relative proportion of monounsaturated fatty acids to palmitoleic acid in the second ozone batch reactor when injecting 1 M palmitoleic acid into the first 5 °C ozone reactor after using two batch ozonolysis reactors in series.

ppm) is generated immediately during reaction (Figures S27 and S29). The signal at δ 1.5 ppm is assigned to the methyl group at position C7 of the palmitoleic acid. Based on a comparison of the peak area of the double bond at C9 with the methyl proton peak at C7 of palmitoleic acid, the rate of ozonolysis can be calculated; the results are shown in Figures S28 and S30. The lower the concentration of palmitoleic acid is, the faster the ozonolysis was completed (Scheme 3 and Figures S28 and S30).

After this study, we set up an integrated batch-flow methodology with an initial concentration of 0.1 M palmitoleic acid in the ozone reactor. Once the ozonolysis in the ozone reactor is completed, palmitoleic acid with various concentrations of 0.2, 0.5, and 1 M was injected into the ozone reactor with an average flow rate of 2 mL min⁻¹ (Figure 2). For batch ozonolysis, we created an ozone tank reactor where a precisely injected flow rate controlled C16-1 solution was reacted with bubbled ozone gas with a single-syringe pump (Figures 2 and 3). Although noncontinuous, the advantage of this batch step in the system was that it provided a reaction time required for ozonolysis completion equal to the time needed to refill the syringe. At 2 mL min⁻¹, the resulting reaction time was equal to 10 min. In this study, the gas flow was delivered at a controlled rate of 500 mL/min at an ozone concentration of 40%, which provides sufficient ozone (around 2 g of ozone per hour) to convert reagents with the reaction time of 10 min.

To monitor the consumption of the double bond during ozonolysis with an injection of the solution of palmitoleic acid, samples were withdrawn at 10 min intervals. The ¹H NMR and relative proportions of palmitoleic acid during ozonolysis are shown in Figures S31–S33 and Figure 4A–C, respectively. As shown in those figures, once 0.2 and 0.5 M palmitoleic acid solutions were pumped into the ozone reactor, no double bond was detected at any point in the reaction, meaning all

quantities of palmitoleic acid dropped were immediately consumed once pumped in (Figure 4A and B). However, traces of double bonds are identified in the case of 1 M palmitoleic acid (Figure 4C), indicating that a flow rate of 2 mL min⁻¹ was insufficient for 1 M concentrations of monounsaturated fatty acids.

The solubility of ozone also depends on the temperature of the solution: as temperature decreases, ozone solubility increases.^{65,66} The temperature for ozonolysis in experiments for 0.2 and 0.5 M palmitoleic acid was set to 5 °C. We hypothesized that decreasing the temperature of the ozone reactor from 5 to 0 °C to increase the ozone saturation when injecting 1 M palmitoleic acid into the reactor would help complete the ozonolysis reaction. However, as Figures 4D and S34 show, a carbon–carbon double-bond peak from palmitoleic acid remained regardless of the temperature used. The low temperature was able to increase the ozone solubility but decreased the rate of ozonolysis.

To keep a flow rate of 2 mL min⁻¹ and complete the ozonolysis of 1 M palmitoleic acid, an integrated double ozonolysis-flow set up was proposed (Figure 3). In this setup, there are two ozone reactors: the first reactor was initially charged with 1 M C16-1, and the second reactor was charged with 0.1 M C16-1. To initiate the reaction, the two reactors were filled with 0.1 M C16-1 and 1 M C16-1 in 5% aqueous solvent, respectively, and saturated with bubbling ozone gas with stirring for 1 h. This initialization procedure is meant to bring about full conversion in the substrate during the 1 h period, supported by the concentration studies done in Figure 4. According to this procedure, syringe 1 is controlled to withdraw reagent solution 1 M C16-1 from reactant supply to reactor 1 at an average flow rate of 2 mL min⁻¹. Syringe 1 subsequently pumps the reactants to ozone reactor 1 at an overall flow rate of 2 mL min⁻¹. Syringe 1 delivers the solution

of 1 M C16-1 to the ozone batch reactor 1, where the bubbled ozone gas in an aqueous solvent reacts with an monounsaturated fatty acid to generate the molozonide, which rapidly decomposes into two aldehydes from reaction with water.^{24,26,44,46} Syringe 2 subsequently transports the resulting solution from ozone batch reactor 1 to ozone batch reactor 2 for ozonolysis of the remaining palmitoleic acid, which is now ready to enter the flow system. Since the syringe movement does not pump continuously, it provides a pause for a reaction time of 10 min that meets the kinetic requirements of ozone addition to C16-1 outlined earlier in this study.

To summarize the double-reactor setup seen in Figure 3, after initially charging each reactor with the previously mentioned stock solutions, a 1 M stock solution of palmitoleic acid is pumped at a flow rate of 2 mL min⁻¹ to the first reactor by pump 1, and after 10 min in reactor 1, it is pumped into reactor 2 by pump 2 for the next 10 min of reaction time. NMR spectra of samples were tested to observe the consumption of palmitoleic acid in the two reactors. In the first reactor, although the presence of a double bond is detected, its relative proportion slowly decreased throughout the reaction (Figures S36 and S37). However, in the second reactor, all the NMR samples after injection of the reagents from first reactor indicated the disappearance of the double bond at any time in the reaction, which confirmed completed ozonolysis (Figures 4E and S35). This reaction time as well as double-batch ozonolysis completed the reaction between the palmitoleic acid and ozone in the second batch reactor, allowing the reaction to go to completion before being withdrawn to the consequent flow reactor.

According to data from Figures 2–4, the single-batch ozonolysis integrated with the DIY flow system only completes ozonolysis at 0.5 M C16-1, while the double-batch ozonolysis integrated with the DIY flow system can finish the ozonolysis of 1 M C16-1. Another detail to consider is the amount of C16-1 that can be processed by each variant of batch-flow setups. With a flow rate of 2 mL min⁻¹, 8 h of operation, and 0.5 M C16-1 in 943.4 mL of solvent, single-batch ozonolysis can process 120 g of C16-1 while with 1 M C16-1 in the same amount of solvent, double-batch ozonolysis can process 240 g of C16-1.

To produce at least 500 g of AA per week/2 kg per month for our progress report deadlines, we needed to process 240 g of C16-1 per day (ozonolysis of 240 g of C16-1 is supposed to produce 142 g of AA at an 80% yield). Therefore, we preferred to include the intermittent 2 stage ozonolysis setup to improve throughput for our target.

In summary, the fully assembled DIY integrated batch-flow system has three main sections: ozonolysis, oxidation, and reductive quench as shown in Figure 2. To ensure complete reactions in the ozone batch reactor, ozonolysis using a series of ozone reactors was proven in this section.

2.3.3. Oxidative and Reductive Workup. For the oxidative and reductive workup, dual-syringe pumps were used to create the effect of a continuous flow system. As shown in Figure 2, there are six syringe pumps utilized to make this pump system. These syringes are split into three pairs that pump alternatively to ensure continuous flow. Each pair served as a pump for one reactant or stream. The assembly of each dual-syringe pump is illustrated in Figure 2. The flow rate of the syringes in pumps 1–3 (Figure 2) was set at 2 mL min⁻¹, resulting in a total reaction time of approximately 20 min for both the oxidation and reductive quench reactors. The sizing of the coil reactors

can be found in the Supporting Information section under Table S2.

These pumps were employed to continuously complete the aldehyde oxidation and reductive quench to synthesize the desired carboxylic acid products. For the Pinnick oxidation to make a complete conversion into carboxylic acids,²⁴ the aldehydes were delivered into the oxidation reactor by pump 3 to react with a 0.4 M NaClO₂ solution transported by pump 4 (Figure 2). The resulting solution was neutralized with the subsequent reductive quench reactor using 0.4 M NaHSO₃ carried by pump 5 to quench all residual oxidants and resulting in crude product.²⁴ The collected crude solution was extracted by hand with ethyl acetate and dried over Na₂SO₄, followed by removal of organic solvents using a rotary evaporator to obtain a crude mixture of heptanoic and azelaic acids. The azelaic acid was isolated by extraction with hot water and crystallized upon cooling to provide an 80% yield (NMR spectrum of azelaic acid is shown in Figure S40).

2.4. Scaling up the Reaction and High-Throughput Operation. To summarize the integrated batch-flow reaction process for continuous ozonolysis of palmitoleic acid, there are two major working parts. The single-syringe system (syringes 1 and 2) comprising part 1, with intermittent liquid pumping into two batch reactors, provided a pause for a reaction time of 10 min for each reactor needed to complete the ozone addition, followed by continuous pumping of the resulting aldehydes downstream at 2 mL min⁻¹. The dual-syringe system in part 2 used continuous liquid pumping at 2 mL min⁻¹ to perform the downstream chemical reaction of aldehyde oxidation to produce the azelaic and heptanoic acids, followed by reductive quench completely in flow. The dual-syringe system is also suitable for other organic chemistry applications such as Curtius rearrangement, as described in a previous report.⁶⁷

With these optimized conditions in hand, we scaled up the process to 240 g of C16-1 (Figure S41). For this purpose, we utilized the two-reactor ozonolysis version of the system (Figure 3) and carried out the experiments for over 8 h per day to produce a total of ~140 g of azelaic acid and ~90 g of heptanoic acid, which extrapolates to a value of ~1.5–1.8 kg of AA in one month or 18 g of AA/h. The system had advantages of being run for extended periods of time by minimizing down time for initializing and finishing the reaction. For example, our single-reactor system, in practice, only yielded an average of 2.5–3.0 g of AA/h after 3 h of operation even though the theoretical output should have been closer to 9 g of AA/h, half of the output reached on the series setup which was operated for 8 h at a time.

3. CONCLUSIONS

In this report, we detailed the design of a batch-flow continuous system for the ozonolysis of palmitoleic acid for the scaled preparation of azelaic acid and heptanoic acid. Here we used 3D-printed parts combined with an Arduino microcontroller to develop this continuous system. Having selected aqueous ozonolysis for safety and efficiency, reaction kinetics dictated that we develop a continuous batch method for ozonolysis, followed by flow reactors for Pinnick oxidation and the final reductive quench steps. The system was demonstrated to conduct ozonolysis of palmitoleic acid for synthesis of azelaic acid with an 80% yield, producing up to 3.0 g per hour. Online UV–vis monitoring offered a convenient method to optimize and monitor the ozonolysis process

qualitatively. This system is robust and flexible enough to be assembled and modified for many reactions and offers the ability to conduct challenging and potentially hazardous processes for moderate to large lab scale chemical transformations.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.1c06823>.

Origin of chemicals, types of equipment, documentation for purchased and 3D-printed system parts, flow system assembly and operation guidelines, reaction preparation guidelines, reaction guidelines, and Arduino code (PDF)

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Notes

The authors declare the following competing financial interest(s): M.D.B. is a founder and advisor to Algenesis Materials, a biotechnology company interested in developing renewable materials.

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