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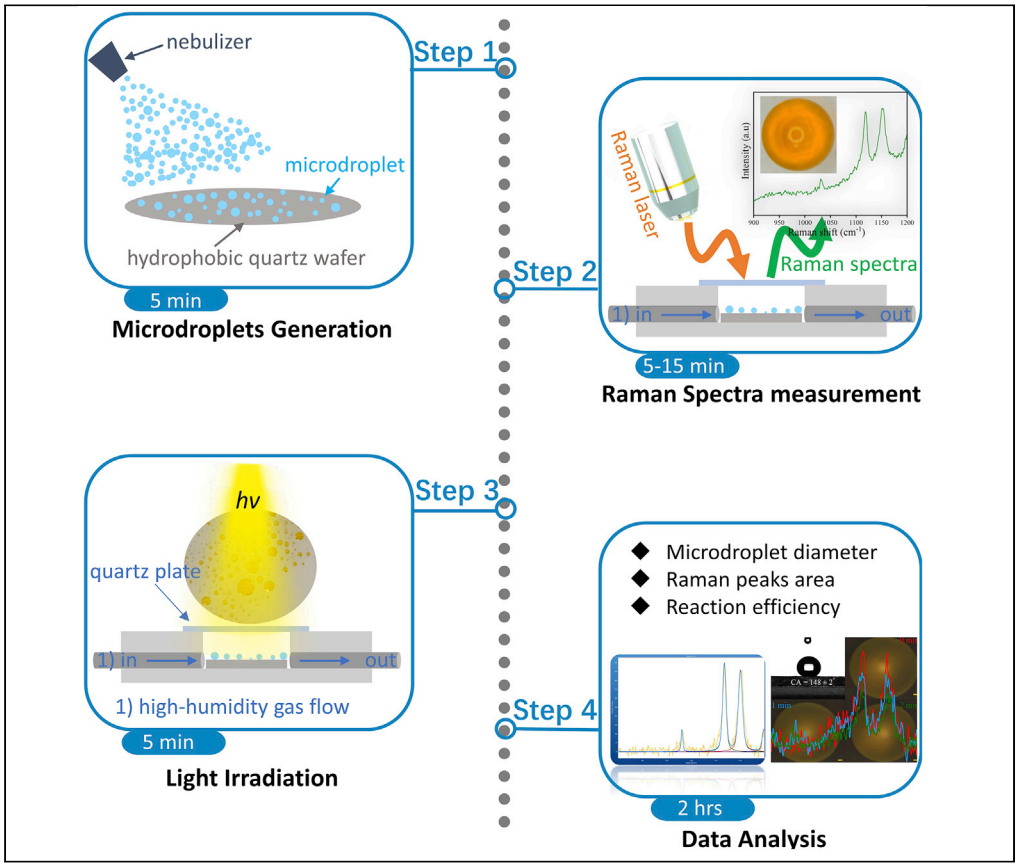
Protocol

A protocol to study microdroplet photoreaction at an individual droplet level using *in situ* micro-Raman spectroscopy



Photochemical synthesis and photocatalysis in droplet microreactors represent promising approaches to relieve the global energy and environmental crises. Here, we describe a protocol for studying microdroplet photoreaction at an individual droplet level based on *in-situ* micro-Raman spectroscopy. We provide details of superhydrophobic substrate preparation, microdroplets generation, photoreactions performing, and data analyses. In addition, we show the operational details of preliminary scale-up tests of microdroplet photoreaction for practical application.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Steps for superhydrophobic substrate preparation and microdroplet generation

Study microdroplet photoreaction using *in situ* micro-Raman spectroscopy

Large-scale application of microdroplet photochemistry and photocatalysis

Protocol is limited by high temperature and pH-dependent Raman spectra

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A protocol to study microdroplet photoreaction at an individual droplet level using *in situ* micro-Raman spectroscopy

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SUMMARY

Photochemical synthesis and photocatalysis in droplet microreactors represent promising approaches to relieve the global energy and environmental crises. Here, we describe a protocol for studying microdroplet photoreaction at an individual droplet level based on *in situ* micro-Raman spectroscopy. We provide details of superhydrophobic substrate preparation, microdroplets generation, photoreactions performing, and data analyses. In addition, we show the operational details of preliminary scale-up tests of microdroplet photoreaction for practical application.

For complete details on the use and execution of this protocol, please refer to Li et al. (2022).

BEFORE YOU BEGIN

Microdroplet, an emerging class of chemical reactor, has attracted significant interests for chemical reactions acceleration by several orders of magnitude (10–10^6), probably because of the special air-water interface physicochemical properties (Enami et al., 2014; Wei et al., 2020; Yan, 2021). In previous studies, the electrospray mass spectrometer is frequently employed for microdroplets generation, reaction and analysis (Mehrgardi et al., 2022; Müller et al., 2012; Zhao et al., 2022). However, it is important to investigate the microdroplet chemistry at an individual droplet level to understand the mechanisms. Micro-Raman spectrometer is of great advantages on the research field of microdroplet chemistry, especially at an individual microdroplet level (Fu et al., 2017; Wei et al., 2018; Xiong et al., 2020). On another hand, photochemical synthesis and photocatalysis are regarded as promising strategies to solve the global energy and environment crises, but the reaction efficiency is still far away from the practical application. Meanwhile, the photoreactions in cloud and fog droplets also play significant roles in atmospheric chemistry and global climate. With these considerations in mind, we have reported that microdroplet reactor shows great accelerations on photochemistry and photocatalysis compared to the bulk phase counterpart (Li et al., 2022). Herein, in this protocol, we detail the specific steps for investigating the microdroplet photoreaction at an individual droplet level, including the preparation of superhydrophobic substrate, microdroplets generation, *in-situ* Raman measurements, data analysis, and proof-of-concept of the large-scale application of microdroplet photoreaction. The further applications and modifications of this protocol would pave an avenue for microdroplet photochemistry and photocatalysis study.

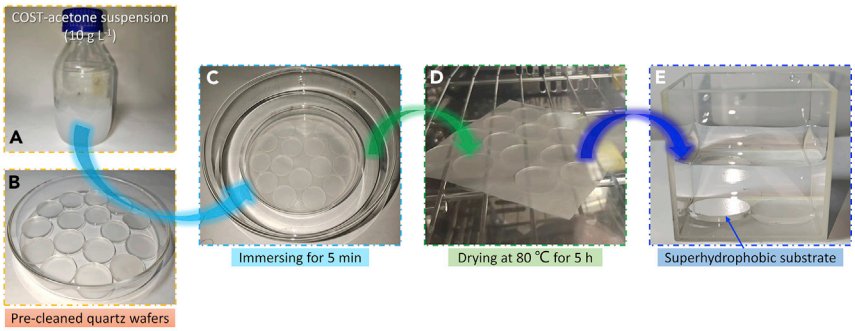


Figure 1. Preparation of superhydrophobic quartz wafer

(A) The as-prepared COST in acetone suspension with a concentration of 10 g L^{-1}.

(B) Place the precleaned quartz wafers in a petri dish.

(C) Immerse the quartz wafers in suspension (A) for about 5 min.

(D) Dry the COST coated quartz wafers at 80°C in air for at least 5 h.

(E) The as-obtained superhydrophobic quartz wafer submerged in DI water. A silver mirror-like sheen appears at the superhydrophobic quartz wafer surface while it is absent for the precleaned one, which is caused by the reflection of light from the air layer retained, indicating the super-hydrophobicity of the as-prepared quartz wafer (Xia et al., 2021).

Refer to ''key resources table'' section for a list of equipment needed for this protocol.

Preparation of superhydrophobic substrates

Timing: 10 h

Several approaches have been developed for preparing superhydrophobic substrates for micro-droplet chemistry study (Lee et al., 2019; Xiong et al., 2020). Amongst, some methods are complex and time-consuming. In this protocol, as shown in Figure 1, we use the immersion coating strategy to prepare superhydrophobic quartz wafers due to the advantages of great repeatability, excellent hydrophobicity, and easy operation (Wei et al., 2018). The superhydrophobic treatment method is also suitable for electrode preparation even under high temperature calcination.

1. Clean the as-obtained quartz substrates via ultrasonication first in acetone and then in DI water for 15 min each. Finally, dry these substrates at 80°C in air for 3 h.
2. Prepare the CAB-O-SIL TS720 (abbreviated as COST) in acetone suspension. Measure 4 g of the COST powder with a clean lab spoon on an analytical balance and then disperse them into a blue cap bottle containing 400 mL acetone under ultrasonication for at least 30 min.

⚠ CRITICAL: The acetone is toxic and hazardous because of the volatility and inflammability. Therefore, one should wear face shield, glove and protective apron and operate in a fume cupboard for safety.

3. Superhydrophobic treatment. Place the precleaned quartz substrates into a petri dish with monolayer arrangement form. Then, pour the COST in acetone suspension into the petri dish to submerge the quartz wafers. After 5 min, transfer the quartz wafers to an oven and dried at 80°C in air for at least 5 h.

Note: The COST in acetone suspension can recycle for further use, but the ultrasonic treatment for at least 10 min is needed before reuse.

Prepare photocatalyst

Timing: 5 days for g-C_3N_4 nanosheets

In order to study photocatalytic reactions in microdroplets, semiconductor photocatalysts are needed and should be prepared before experiments. Here, as an example, we describe the steps for preparing g-C_3N_4 nanosheets for microdroplet photocatalysis study (Yang et al., 2013).

4. Measure 10 g melamine and place them in a porcelain boat covered with a lid.
5. Heat the porcelain boat at 520°C in air for 4 h with a ramping rate of 2.3°C min^{-1} in a muffle furnace under static conditions and cool down to 25 $\pm$ 3°C.
6. Grind the as-obtained bulk particles into powder.
7. Calcine the powders at 550°C in air for 4 h with a ramping rate of 2.3°C min^{-1} in a muffle furnace under static conditions and cool down to 25 $\pm$ 3°C.
8. Sonicate 100 mg of the secondary calcined powders in 600 mL deionized water for 10 h.
9. Stand the suspension for 24 h to make aggregates settle down.
10. Transfer the upper highly-stable dispersion into a blue cap bottle for later use.

⚠ CRITICAL: The semiconductor photocatalysts should have great dispersity in DI water and should stably suspend in microdroplets for a long time (at least longer than the reaction time period). Or else, the photocatalyst particles will settle down quickly and it is unfavorable to study the roles of unique properties of microdroplet in photocatalysis. Notably, the homogeneous photocatalysis does not have to consider this issue.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Anhydrous ferric chloride (≥ 97.0%)	Sinopharm Chemical Reagent Co., Ltd	CAS#7705-08-0
Sodium oxalate (≥ 99.8%)	Sinopharm Chemical Reagent Co., Ltd	CAS#62-76-0
Hydrochloric acid (HCl, 36%–38%)	Sinopharm Chemical Reagent Co., Ltd	CAS#7647-01-0
Sodium hydroxide (≥ 96.0%)	Sinopharm Chemical Reagent Co., Ltd	CAS#1310-73-2
Methyl orange (96.0%)	Sinopharm Chemical Reagent Co., Ltd	CAS#547-58-0
Melamine (≥ 99.0%)	Sinopharm Chemical Reagent Co., Ltd	CAS#108-78-1
Acetone (≥ 99.0%)	Sinopharm Chemical Reagent Co., Ltd	CAS#67-64-1
Anhydrous ethanol	Sinopharm Chemical Reagent Co., Ltd	CAS#64-17-5
Gold orange II (≥ 98.0%)	Sigma-Aldrich	CAS#633-96-5
Levoglucosan (99.0%)	Sigma-Aldrich	CAS#498-07-7
p-Benzoquinone (97.0%)	Sigma-Aldrich	CAS#67-64-1
Tertiary butanol (99.9%)	Sigma-Aldrich	CAS#75-65-0
COST	Shanghai King Chemical Co., Ltd	https://www.4006787252.com/
High-purity N_2	Shanghai TOMOE Gases Co., Ltd	CAS#7727-37-9
High-purity O_2	Shanghai TOMOE Gases Co., Ltd	CAS#7782-44-7
High-purity Air	Shanghai TOMOE Gases Co., Ltd	N/A
Deionized (DI) water	Direct-Q5, Merck, Germany	N/A
Software and algorithms		
LabSpec 6	Jobin Yvon, HORIBA Gr, France	https://www.horiba.com/chn/
UVProbe 2.5	Shimadzu	https://www.shimadzu.com.cn/
ImageJ	Open source (Schneider et al., 2012)	https://imagej.net
OriginPro 2021	OriginLab Corporation	https://www.originlab.com/
Other		
Micro-Raman spectrometer	Jobin Yvon, HORIBA Gr, France	https://www.horiba.com/int/
Custom-designed reactor	Beijing Scistar Technology Co., Ltd	http://www.bjscistar.com/
High-definition camera	DSC-HX350, Sony	https://www.sonystyle.com.cn/
150 W Xe lamp	CET-TEX250, Ceaulight, China	https://www.aulight.com/
Drying oven	DHG-9070 (A) (101-1), Shanghai Yiheng Co., Ltd.	http://www.yihengchina.com/
Muffle furnace	SX2-2.5-10N, Shanghai Yiheng Co., Ltd.	http://www.yihengyt.com/cp-view/439

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Gas flow controller	SC171 CS100D, Beijing Sevenstar Flow Co., Ltd.	https://www.mfcsevenstar.com/
Centrifuge	H2050R, Cence Co., Ltd.	http://www.xiangyilxj.com/
Analytical balance	CP224C, Ohaus Co., Ltd.	http://www.aohaosiyq.com/CP224C.html
Quartz wafer	N/A (custom-designed)	https://shop107284405.taobao.com/?spm=a1z10.1c.0.0.6e705489lhNsHL
365 nm UV lamp	N/A	https://shop68885445.taobao.com/?spm=2013.1.1000126.d21.6e037099rmOXkg
UV-vis absorption spectrometer	Shimadzu UV-2600, Japan	N/A
X-ray diffractometer	Bruker D8 Advance	N/A
Transmission electron microscopy (TEM)	Hitachi H-9500	N/A
Multi 3620IDS	Xylem Analytics Co., Ltd., Germany	N/A
Optical microscope	Nikon SMZ 745T	N/A
100 mL beaker	N/A	N/A
Quartz cuvette	N/A	N/A
Glass petri dish	N/A	N/A
Moisturizer	N/A (commercial)	https://shop362525531.taobao.com/?spm=2013.1.0.0.2d6e1b15C3U4dI
Nebulizer	N/A (commercial)	https://shop155274974.taobao.com/?spm=2013.1.0.0.429b6e9bP0dJtG

MATERIALS AND EQUIPMENT

The equipment needed:

- Environmental control chamber.

The flow cell and reaction chamber shown in Figure 2A are necessary for microdroplet photoreactions and *in-situ* Raman measurements. The manufacture of the custom-designed chambers can be realized by commercial corporation as listed in "key resources table".

- Nebulizer and moisturizer for microdroplets generation.

The commercial nebulizer and moisturizer (Figure 2A) are options.

Alternatives: Other types of reaction chamber and nebulizer can be designed for microdroplets generation and photoreactions study.

⚠ CRITICAL: (i) The gas tightness of the reaction chamber should be guaranteed, or the water evaporation will be difficult to suppress. (ii) The gases flow channel should be considered in chamber fabrication for reaction atmosphere control. The airtightness can be ensured by screwing, and at the same time, the gas inlet and outlet should be plugged. (iii) A quartz lid should be applied to ensure the efficiency of Raman laser excitation, signal collection and light irradiation.

STEP-BY-STEP METHOD DETAILS

Switch on the micro-Raman spectrometer

Timing: 1 h

In this protocol, micro-Raman spectrometer is employed for measuring the target molecules concentration in an individual microdroplet. In order to avoid the influences of operational state of Raman spectrometer on the tests, it would be better to switch on the micro-Raman spectrometer before experiments to obtain a steady state for measurements.

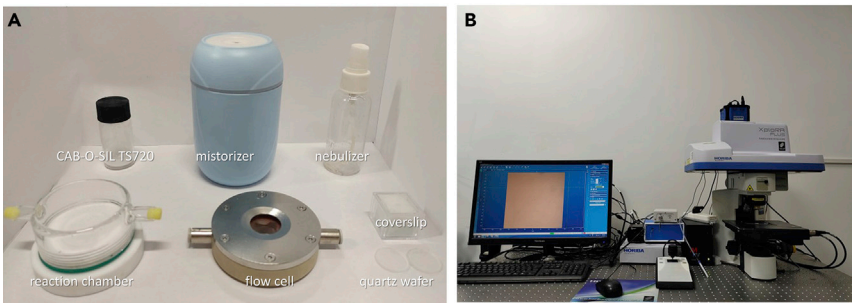


Figure 2. Laboratory equipment necessary for microdroplets photoreaction study
(A) Recommended equipment for microdroplets generation, photoreaction and collection.
(B) Raman spectrometer used for *in-situ* Raman spectra measurement.

Note: Please follow the operating manual to switch on the micro-Raman spectrometer.

Prepare high-humidity gases

Timing: 1 h

On the one hand, the gaseous molecules are necessary for gas-involved chemical reactions. On the other hand, the high relative humidity (RH > 95%) environment is essential to inhibit the water evaporation of microdroplets (Wei et al., 2018). Therefore, the high RH condition should be maintained throughout the whole experiments, which can be realized by purging the high-humidity gases into the reaction chamber. The following steps detail the procedures for humidifying gases.

1. Fill 80 vol.% of the cylinder with DI water.
2. Establish the gas pipelines.
3. Determine the concentration and flow rate of target gases, which can be achieved by using a mass flow controller.

Note: The gases flow rate cannot be too high, or the fast gas flow will blow off microdroplets and contribute to the water evaporation. The recommended gases flow rate is less than 50 mL min^{-1}.

4. Make the gas mixture pass through the cylinder and measure RH at the end of the flow tube.

⚠ CRITICAL: The exhaust should be treated using liquid absorption or other methods before emitting into the atmosphere, especially for toxic gases.

Clean the nebulizer and moisturizer

Timing: ~1 h

For microdroplets generation, here the commercial nebulizer and moisturizer are used. The microdroplet generators should be cleaned using 0.1 M HCl and DI water through ultrasonication in sequence for at least two times, respectively.

Prepare bulk solution

Timing: 1 h

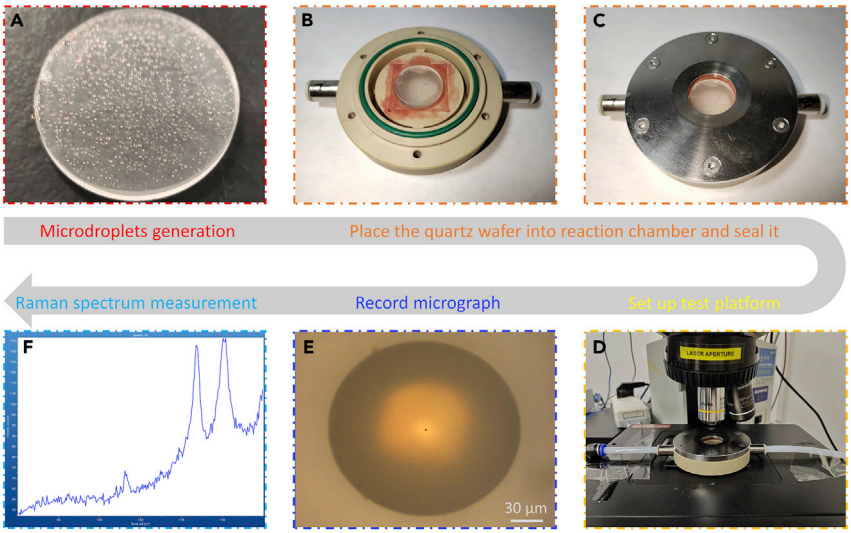


Figure 3. Processes for microdroplets generation and Raman measurement

(A) A photo of the generated microdroplets on superhydrophobic quartz wafers.

(B) Place the quartz wafer loading microdroplets into the custom-designed reaction chamber.

(C) Seal the reaction chamber tightly.

(D) Immobilize the environmental chamber on the objective table of Raman spectrometer and introduce the high-humidity gases.

(E) Record micrograph of a microdroplet. The scale bar is 30 μm.

(F) Measure the Raman spectra of target molecule. The screen is a Raman spectrum of methyl orange.

5. For homogenous photochemistry: We study Fe(III)-oxalate chelates photochemistry as an example. In detail, measure certain amount of $FeCl_3$ and $Na_2C_2O_4$ and dissolve them into DI water. Afterwards, add the target molecule (e.g., methyl orange and levoglucosan) with pre-designed concentration. Finally, adjust the solution pH to a value using 0.1 M HCl and 0.1 M NaOH.
6. For heterogeneous photocatalysis: The photocatalyst suspension containing g-C_3N_4 nanosheets and target molecule is prepared. Then, the suspension is stirred under dark for 30 min to establish adsorption-desorption equilibrium.

Note: All the homogeneous solution and heterogeneous suspension should be prepared before each experiment and the bulk solution should be stored under dark condition in a refrigerator at 4°C.

Microdroplets generation

Timing: 5 min

The following steps describe the processes of microdroplets generation and seal in the flow cell for *in-situ* Raman investigations, as shown in Figure 3.

7. Pour the as-prepared bulk solution into the cleaned nebulizer.
8. Spray the bulk solution to generate microdroplets on the superhydrophobic quartz wafer.

Note: The freshly prepared superhydrophobic substrate should be used for each experiment.

9. Place the superhydrophobic quartz wafer loading microdroplets into the custom-designed flow cell and seal the reactor tightly.
10. Fix the flow cell on the objective table of micro-Raman spectrometer.
11. Introduce the high-humidity gases into the reaction chamber.

⚠ CRITICAL: The flow cell must be fixed tightly to avoid its movement during the shift of objective table, or it will be difficult to find the same microdroplet once again.

Raman measurements (first time)

Timing: ~10 min

The micro-Raman spectrometer is used to monitor the microdroplet size and detect the target molecules concentrations before and after light irradiation, in order to estimate the microdroplet photoreaction efficiency. At first, the initial Raman spectrum of target molecule in microdroplet is measured before photoreactions following the steps shown as below.

12. Identify the laser wavelength and power energy (785 nm and 20 mW in our study, respectively).
13. Select a microdroplet for analysis via shifting the objective table.
14. Record position of the microdroplet.
15. Record micrograph of the microdroplet for measuring its diameter.

⚠ CRITICAL: The position and micrograph of every microdroplet must be recorded.

16. Measure the Raman spectrum of the target molecule, focused at the microdroplet center.

Note: The detailed Raman measurement parameters including laser wavelength and power, acquisition time, accumulation number, Raman shift range, and grating can be selected dependent on the experimental conditions.

17. Repeat steps 13–16 to measure at most 4 microdroplets with varying diameter.

⚠ CRITICAL: Be careful about the position of the reaction flow cell. If the position has been changed and cannot return to the initial state, the experiments should restart from the microdroplet generation step (step 7).

Light irradiation

Timing: ~5 min

In order to initiate the photoreaction, light irradiation is necessary. The following steps describe the processes to perform light irradiation for inducing microdroplet photoreactions. The influences of light irradiation intensity and irradiation wavelength can be studied according to the scope of the study.

18. Shift the objective table to make the light irradiation vertical.
19. After pre-designed time, end up the light irradiation.
20. Shift the objective table back for Raman measurements.

Note: (i) The light irradiation time is dependent on the experimental design. (ii) An electric fan can be used to suppress the photothermal effects of light irradiation and spare no efforts to suppress the water evaporation.

Raman measurements (second time)

Timing: ~10 min

Here, the Raman spectra of target molecules in microdroplets after light irradiation are detected.

21. Move the objective table to return to the initial position of the first time detected microdroplet.

Note: The testing order should be the same as that in the first time Raman spectra measurements.

22. Record micrograph of the microdroplet and measure its diameter.
23. Collect Raman spectrum of the target molecule, focused at the center of the microdroplet.
24. Repeat steps 21–23 to detect the molecules concentration and size of other microdroplets after photoreaction.

Note: The Raman measurement parameters must be kept the same as that in the first-time measurements.

Bulk phase reaction

Timing: ~5 min

In order to show the superiority of droplet microreactor, bulk phase reactions should be performed for comparison. The following steps detail the operations of bulk phase photoreactions.

25. Choose the reactor for bulk phase photoreaction and clean it.
26. Determine the volume of bulk solution and transfer the solution into the reactor.
27. Measure the initial concentration of target molecule using suitable equipment.
28. Switch on the light irradiation source to initiate photoreaction.
29. After pre-designed reaction time, switch off the light irradiation.
30. Withdraw the liquid sample and filter using 0.22 μm-PTFE filter membrane.
31. Detect the concentration of target molecule after photoreaction.

Note: For comparison, the light irradiation time for bulk phase reaction should be the same as that for microdroplet photoreaction.

Scale-up application of homogeneous microdroplet photoreaction

Timing: dependent on the experimental design

The previously described protocols are mainly for investigating the photoreactions in a single microdroplet and studying the microdroplet size effects. From the practical aspects, the large-scale application of microdroplet photoreaction should be tested to promote the process of industrialization of microdroplet photoreaction. The following steps give some tentative guidelines on the large-scale application of homogeneous microdroplet photochemistry (Figure 4).

32. Prepare bulk solution and pour it into a commercial mistorizer (Gong et al., 2022).
33. Detect the initial concentration of target molecules.
34. Clean a quartz container for microdroplets suspension and collection.
35. Place the moisturizer into the quartz container and cover it with a quartz lid.
36. Place this setup underneath the light irradiation source.
37. Switch on the moisturizer and light irradiation source simultaneously. Cooling effect can be supplied by an electric fan.
38. After a certain time of reaction, switch off the moisturizer and light irradiation simultaneously.
39. Let the device stand still for about 5 min.
40. Collect the liquid for concentration determination.

Note: This protocol is also suitable for other homogeneous microdroplet chemistry study.

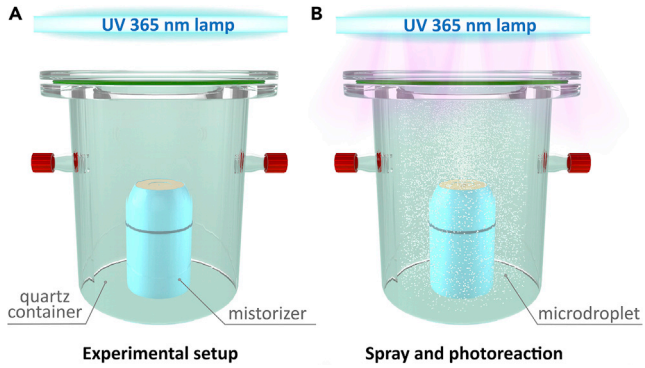


Figure 4. Schematic illustration of large-scale application of homogeneous microdroplets photoreaction
(A) Experimental setup for scale-up tests.
(B) The operations of homogeneous microdroplets photoreactions.

Large-scale tests of heterogeneous microdroplet photocatalysis

Timing: dependent on the experimental design

Owing to the limitations of commercial moisturizer, here the scale-up tests of microdroplets heterogeneous photocatalysis are performed using microdroplet generation, reaction and collection method. The detailed steps are listed as below (Figure 5).

41. Prepare bulk solution and stir the solution to establish adsorption-desorption equilibrium for at least 30 min.
42. Measure the initial concentration of target molecules.
43. Pour a certain volume of the solution (about 50 vol.%) into a nebulizer.
44. Generate microdroplets of varying diameter on superhydrophobic quartz wafer via nebulizer spraying.
45. Take micrographs of microdroplets to measure the average diameter.
46. Place the quartz wafer into a custom-designed reactor.
47. Seal the reactor under high relative humidity condition.
48. Switch on the light irradiation to initiate the photocatalytic reaction. The environmental temperature (25 $\pm$ 3°C) was controlled by using an electric fan and placing ice bags around the reactor.

Note: The ice bag should be replaced with a fresh one every hour.

49. Switch off the light irradiation to end up the photoreaction after pre-designed time.
50. Take micrographs of the microdroplets again for investigating the diameter change during photocatalysis.
51. Collect the microdroplets using a coverslip.
52. Transfer the collected sample into a centrifuge tube using a pipette and dilute within a reasonable range.
53. Measure the concentration of target molecules to calculate the reaction efficiency.

Establish the standard curves

Timing: ~2 h

In this protocol, *in-situ* Raman measurements are used to determine the photoreactions efficiency in microdroplets. Thus, the standard curves should be established for calculating the actual

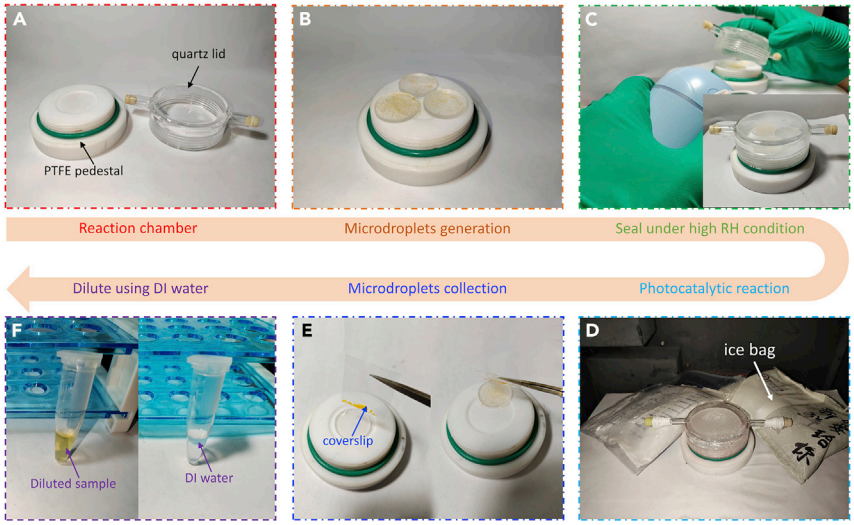


Figure 5. Experimental processes for scale-up tests of heterogeneous microdroplets photoreaction

(A) The custom-designed environmental chamber for microdroplet photoreaction study.

(B) Photo of the generated microdroplets.

(C) Seal the microdroplets into reaction chamber under high-humidity condition.

(D) Operational environment for photocatalytic reaction.

(E) Details about microdroplets collection using coverslip.

(F) Transfer the collected sample into a centrifuge tube containing DI water for analyses.

concentration of target molecules, as well as for bulk phase photoreactions. Here, the following steps describe the methods to establish standard curves.

54. Prepare several solutions of target molecule with gradient concentrations.
55. Generate a droplet (diameter < 1 mm) using pipette from one solution.
56. Measure the Raman spectrum of the target molecule in droplet at least 3 times.
57. Repeat steps 55–56 to collect the Raman spectrum of other solutions.
58. Use suitable equipment to establish the standard curves for bulk phase reaction.
59. Plot the standard curves using average value.

EXPECTED OUTCOMES

This protocol provides the procedures for investigating the microdroplet photoreaction using micro-Raman spectrometer, which is also suitable for studying the physicochemical properties of microdroplet. In addition, the details about the proof-of-concept of the large-scale application of microdroplet photoreaction are also described. Regarding the organics oxidation by Fe(III)-oxalate photochemistry and by g-C_3N_4 photocatalysis in microdroplets, the expected results would be almost consistent with our previous report on *Cell Reports Physical Science* (Li et al., 2022), and some expected outcomes list below.

Expected hydrophobicity

The water contact angle of as-prepared superhydrophobic quartz wafer will be larger than 140°, which is the basic to the formation of microdroplets (Figure 6A).

Expected water evaporation

If 785 nm laser excitation and simulated sunlight irradiation are used, the water evaporation of microdroplets will be inevitable owing to the photothermal effects of light irradiation, especially for smaller microdroplets. Even though, the observed average decrement on the microdroplet diameter is about 10% with 1–2 min irradiation (Figures 6A and 7F).

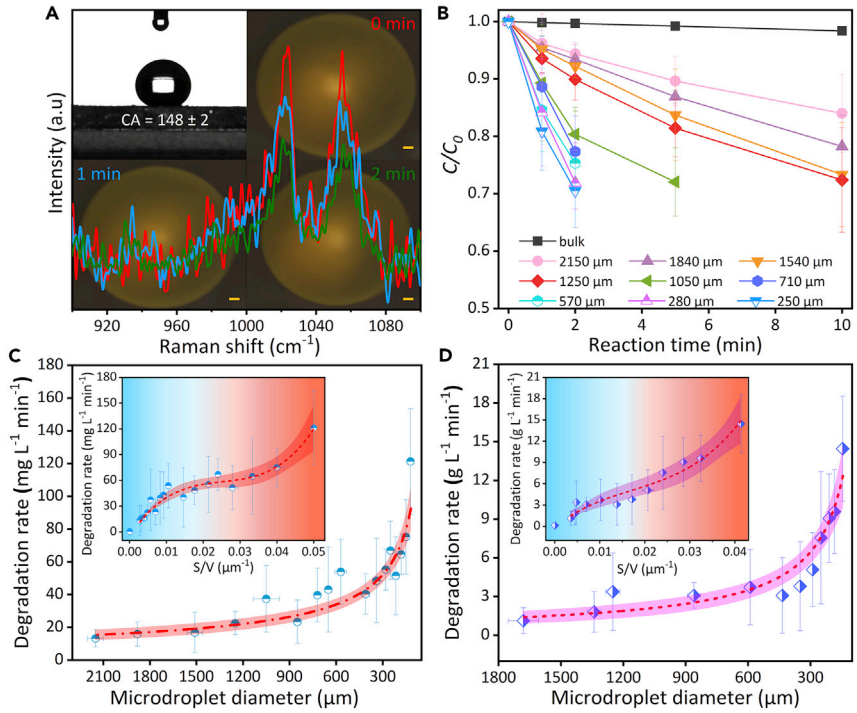


Figure 6. Expected organics degradation efficiency in microdroplets by Fe(III)-oxalate photochemistry

(A) Raman spectra of methyl orange (MO) in a microdroplet and micrographs of the microdroplet (scale bar: 20 μm) recorded as reaction proceeded. The inset in top-left is photograph of a water droplet on an as-prepared superhydrophobic substrate.

(B) C/C_0 of MO as a function of irradiation time.

(C) MO photooxidation rates and fitting curves in microdroplets of different size.

(D) Levoglucosan photochemical oxidation efficiency and fitting curves in microdroplets of various diameter. Data are represented as mean ± SEM. Unless otherwise stated, the experimental conditions were: $FeCl_3$, 1 mM; $Na_2C_2O_4$, 7 mM; MO, 350 mg L^{-1}; initial pH, 4.75; Xe lamp irradiation, ~35.7 mW cm^{-2}. Data were adopted with changes from (Li et al., 2022).

Expected performances of microdroplet photoreactions

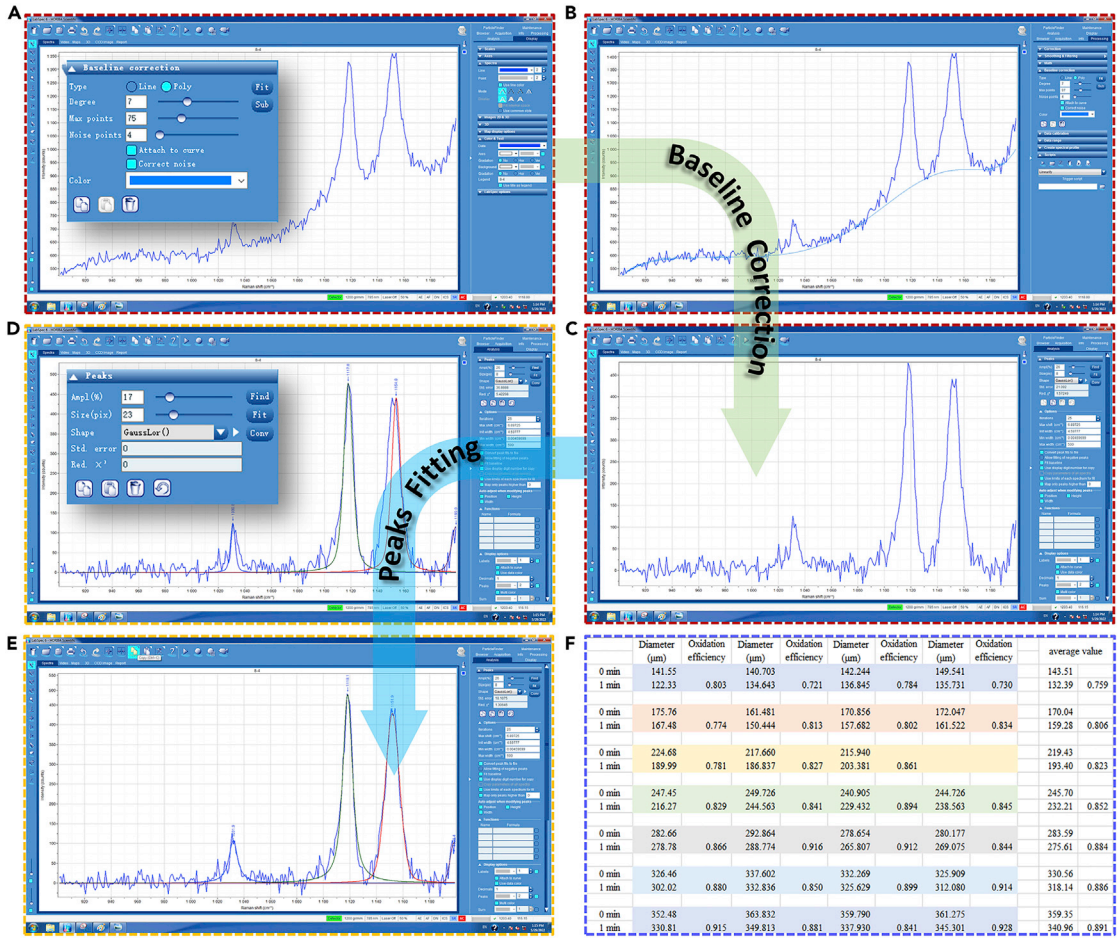
Compared to the bulk phase photoreaction, the organics (methyl orange and levoglucosan) oxidation rates by Fe(III)-oxalate photochemistry and by g-C_3N_4 photocatalysis in microdroplets are significantly accelerated. In addition, the photoreaction efficiency will increase with decreasing the microdroplet diameter, with an exponential variation trend. The acceleration effects will reach two orders of magnitude in microdroplet with a diameter of about 150 μm (shown in Figures 6C and 6D).

QUANTIFICATION AND STATISTICAL ANALYSIS

Raman spectra treatment

Owing to the fluorescence interferences of organics, the Raman spectra should be treated via baseline correction by polynomial equation. The peaks intensity and area are obtained from Raman spectra fitting with Gauss-Lorenz function, which can be used as indicator to establish standard curves and calculate concentrations of target molecule. All these treatments are performed using the Raman software (Labspec 6, Figure 7).

> ***Note:*** The parameters for baseline correction and peaks fitting should keep consistent for the same target molecule.



	Diameter (μm)	Oxidation efficiency	Diameter (μm)	Oxidation efficiency	Diameter (μm)	Oxidation efficiency	Diameter (μm)	Oxidation efficiency	average value	
0 min	141.55		140.703		142.244		149.541		143.51	
1 min	122.33	0.803	134.643	0.721	136.845	0.784	135.731	0.730	132.39	0.759
0 min	175.76		161.481		170.856		172.047		170.04	
1 min	167.48	0.774	150.444	0.813	157.682	0.802	161.522	0.834	159.28	0.806
0 min	224.68		217.660		215.940				219.43	
1 min	189.99	0.781	186.837	0.827	203.381	0.861			193.40	0.823
0 min	247.45		249.726		240.905		244.726		245.70	
1 min	216.27	0.829	244.563	0.841	229.432	0.894	238.563	0.845	232.21	0.852
0 min	282.66		292.864		278.654		280.177		283.59	
1 min	278.78	0.866	288.774	0.916	265.807	0.912	269.075	0.844	275.61	0.884
0 min	326.46		337.602		332.269		325.909		330.56	
1 min	302.02	0.880	332.836	0.850	325.629	0.899	312.080	0.914	318.14	0.886
0 min	352.48		363.832		359.790		361.275		359.35	
1 min	330.81	0.915	349.813	0.881	337.930	0.841	345.301	0.928	340.96	0.891

Figure 7. Data analyses

(A-E) Processes of Raman spectra analyses including baseline correction and peaks fitting.

(F) Depiction of the recorded partial data.

Photoreaction efficiency calculation

First, according to the Raman spectra fitting and standard curves, we can obtain the concentrations of target molecule at beginning and the reaction time t. Next, it needs to measure the microdroplet diameter before and after photoreactions based on the recorded micrographs.

If the microdroplet diameter is negligibly changed after photoreaction, the photoreaction efficiency (RE) can be obtained from equation (1). This formula is also suitable to calculate the reaction efficiency of the bulk phase counterpart.

$$RE = 1 - C_t/C_0 \quad \text{(Equation 1)}$$

If the size of microdroplet is decreased after photoreaction, the influences of microdroplet volume on reactants concentration should be considered. In this case, the RE in microdroplet can be calculated using equation (2).

$$RE = 1 - (C_t \times V_t) \div (C_0 \times V_0) \quad \text{(Equation 2)}$$

Where C_0 and C_t respectively represent the measured target molecules concentration at the beginning and sampling time t, V_0 and V_t mean the initial and final volume of microdroplets respectively.

Note: The photoreaction efficiency obtained from microdroplets with similar diameter (± 10 μm) can be averaged for data analysis.

LIMITATIONS

Although this protocol has been successfully applied for studying microdroplet photochemistry and photocatalysis, there are still some limitations in its application. Some cases list below: (i) The chemical reactions that can occur under dark condition are not suitable to use Raman spectrometer for investigation, because the reaction time is difficult to keep identical between different microdroplets owing to the asynchronous *in-situ* Raman measurements. (ii) If the solution pH will change as reaction processes and the Raman spectra of target molecules can be affected by pH, it will not be feasible to study using *in-situ* Raman spectrometer. Even though, it can be investigated using microdroplet generation and collection method as described in the section of large-scale tests of heterogeneous microdroplet photocatalysis. (iii) The chemical reactions that must use high concentration organic solvents like acetonitrile, methanol, and others cannot be studied using this protocol, because it is not possible to generate microdroplets on hydrophobic substrate and the organics will evaporate rapidly in microdroplets. (iv) It is difficult to study chemical reactions in microdroplets at high temperature due to the fast water evaporation. Meanwhile, it is also unfavorable to investigate the influences of reaction temperature on microdroplet photoreaction due to the difficulties on controlling water evaporation and reactants volatilization.

TROUBLESHOOTING

Problem 1

The water evaporation of microdroplet caused by photothermal effects of 785 nm laser excitation and sunlight irradiation (steps 16 and 18; Figure 6A).

Potential solution

From the aspects of laboratory investigation, the 532 and 633 nm laser could use for *in-situ* Raman measurements if the target molecules have outstanding signal-to-noise ratio under the corresponding conditions. If only the 785 nm laser could work successfully, in order to inhibit the photothermal effect of laser irradiation, the target molecules concentration could increase and the laser power, excitation time and accumulation number could decrease. Besides, UV lamp can be applied as an alternative for light irradiation. Meanwhile, the near-infrared and infrared light emitted from the simulated sunlight irradiation source should be filtered using an optical filter.

Problem 2

For large-scale application of microdroplet heterogeneous photocatalysis, the commercial mistorizer will be blocked and not operate (step 37).

Potential solution

We notice that some other kinds of nebulizers probably can be used for microdroplets generation from heterogeneous suspension. However, only the upper suspension can be sprayed and it needs further technical improvements. Even though, the nebulizer provides a road for promoting the commercial feasibility of heterogeneous microdroplet photocatalysis for energy and environment applications.

Problem 3

In the scale-up application of microdroplet photoreaction, a large number of the suspended microdroplets would inhibit light penetration and cause optical attenuation as shown in (Figure 4B), thus suppressing the light absorption and decrease the photoreaction efficiency (step 37).

Potential solution

To solve this problem, on the one hand, a large container could be used to decrease the spatial density of microdroplets and weaken the photoshielding effect; on the other hand, several light lamps could be vertically placed in the container to increase the light irradiation intensity.

Problem 4

It is difficult to study the microdroplet photochemistry and photocatalysis in microdroplets with diameters lower than 20 μm due to the fast water evaporation (steps 13–16; Figures 6 and 7F).

Potential solution

To investigate the microdroplet photoreaction in extremely small microdroplets, the photothermal effects of laser excitation and light irradiation should be minimized through high-humidity control and lowering the power energy. Besides, it would be better to measure an individual microdroplet at a time.

Problem 5

The large error bars are obtained from *in-situ* micro-Raman measurements and data analyses (steps 17 and 24; Figure 6).

Potential solution

To reduce the experimental error, the water evaporation of microdroplet should be carefully controlled between independent repetitive experiments. Meanwhile, it would be better to carry out the repetitive experiments within two or three days continuously when the equipment state is almost similar.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Liwu Zhang (zhanglw@fudan.edu.cn).

Materials availability

This study does not generate any new unique reagent.

Data and code availability

Any additional information required to reanalyze the data reported in this article is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, L.Z. and K.L.; investigation, K.L., L.W., J.L., W.W., K.G., Q.G., and Y.L.; data curation, K.L.; writing – original draft, K.L.; writing – review and editing, L.Z.; funding acquisition, L.Z.; and supervision, L.Z.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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