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Integrated Droplet Analysis System with Electrospray Ionization-Mass Spectrometry Using a Hydrophilic Tongue-Based Droplet Extraction Interface

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This paper describes a simple, robust, and integrated microchip-based system for droplet analysis with electrospray ionization-mass spectrometry (ESI-MS) detection. The microchip integrated multiple modules including a droplet generator, a droplet extraction interface, and a monolithic ESI emitter. The novel droplet extraction interface based on hydrophilic tongue structure was developed. The interface could transfer droplets from segmented phase to aqueous phase with high reliability and high controllability by coupling with a back pressure regulator. The flow injection mode was adopted to introduce the transferred droplets to the ESI emitter for minimizing the cross-contamination between droplets and achieving droplet matrix modification. The system performance was evaluated using angiotensin as a model sample, and high sensitivity (<1 μM) and a good reproducibility of 5.2% RSD ($n = 7$) were obtained. The present device was further applied in the online monitoring of droplet-based microreaction for alkylation of peptide.

Droplet-based microfluidics offers an attractive alternative to conventional microfluidic technologies based on continuous flow systems by performing chemical and biological reaction in picoliter to nanoliter volumes without dilution and cross-contamination.[1−7] Analytical techniques such as fluorescence,[8] electrochemistry,[9] and capillary electrophoresis[10−13] have been successfully applied to measure the chemical compositions of droplets. In addition, high-information-content techniques such as mass spectrometry and NMR spectroscopy, which could significantly broaden the applications of droplet microfluidics, are in great demand.[5,14]

Recently, considerable attention has been focused on analyzing the droplet composition with electrospray ionization mass spectrometry (ESI-MS) owing to its favorable ability to perform label-free and online detection.[15−17] Pioneered by Huck's group, online ESI-MS analysis of individual droplets was first realized.[15] Electrical pulse was utilized to transfer the droplets from segmented phase to aqueous phase, and the aqueous phase stream was delivered to a fused-silica capillary emitter for ESI-MS detection. Kennedy's group[16] described a simple method to achieve droplet ESI-MS analysis by directly coupling the droplet cartridge to a commercial nanospray emitter without the need of droplet extraction interface. High detection sensitivity without droplet dilution was obtained in the system. More recently, Smith's group developed an integrated poly(dimethylsiloxane) (PDMS) chip capable of generating droplets, extracting droplets to an aqueous stream, and analyzing the droplet content with ESI-MS.[17] Since the distance from the droplet extraction interface to the emitter was very short (1 mm), the droplets could be analyzed nearly without dilution.

The droplet extraction interface plays a key role in the combination of the droplet system with ESI-MS.[15,17] Several methods were proposed to transfer droplets from the segmented phase to the aqueous phase. In the method reported by Huck's

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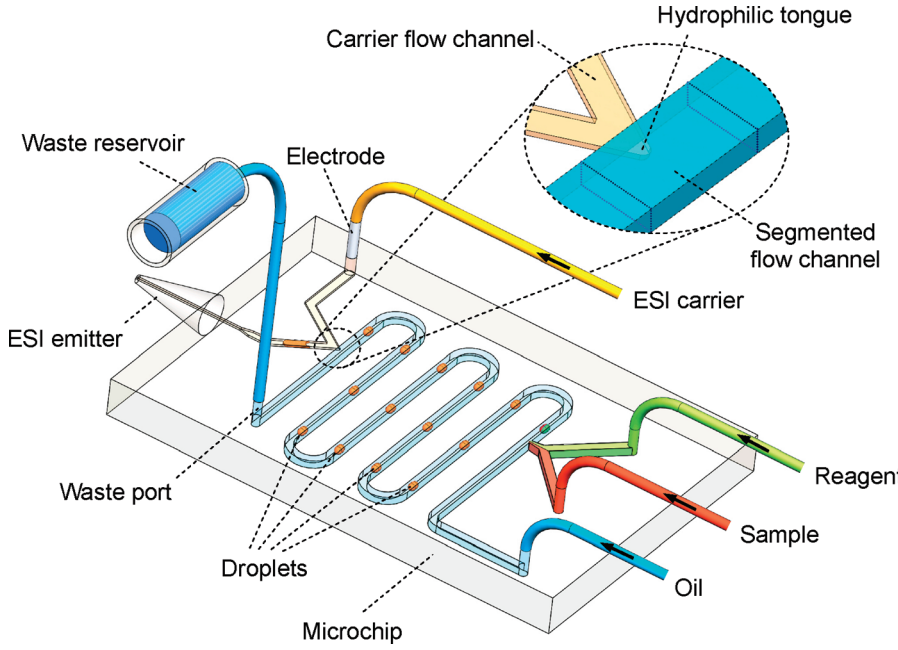

Figure 1. Schematic diagram of the droplet-based analysis system with ESI-MS detection (not to scale).

group, microelectrodes were embedded between the segmented stream and aqueous stream, and by applying an electric field on the electrodes, the droplets was driven to merge with the aqueous stream.[15] However, high flow rates should be maintained for the two streams to stabilize the multiphase laminar flow profile. Kelly et al. used an array of cylindrical posts to stabilize the two phase streams and to enable the droplets to pass through the apertures between the posts into the aqueous stream.[17] In the droplet extraction systems developed by Kennedy's group, selectively patterned microchannels were used to significantly increase the stability of the immiscible phases and improve the reliability of droplet extraction.[11,13] The surface of segmented phase channel was modified to be hydrophobic and the aqueous channel was retained hydrophilic. The interfacial tension between oil and aqueous phases at the hydrophobic−hydrophilic channel junction could stabilize the phase boundary during the droplet transferring process. In spite of the above-mentioned successful systems, it is still challenging to develop a highly reliable and controllable droplet extraction interface.

In this work, we developed an integrated glass chip for droplet-based analysis with ESI-MS. The microchip integrated multiple modules including a T-junction droplet generator, a droplet extraction interface, and a monolithic ESI emitter. A novel droplet extraction interface based on a hydrophilic tongue structure was produced to improve the reliability and controllability of the droplet transferring operation. With a back pressure regulator coupled, the interface could perform droplet extraction reliably without oil leaked into the aqueous phase and with high flexibility in adjusting the extraction ratio at different extraction frequencies. The droplet system was coupled with ESI-MS using the flow injection mode to minimize the cross-contamination between droplets and more importantly to provide an opportunity to modify the droplet matrixes to match the requirement of ESI-MS detection. The present system was applied to characterize the alkylation of peptides in droplets to demonstrate its feasibility and potential in online monitoring of real microreactions.

EXPERIMENTAL SECTION

Fabrication of the Microchip. Microchannels with different depths were fabricated on two glass substrates, respectively, using standard photolithographic and wet etching technique as described previously.[18] The substrate with a shallow channel (15 μm depth) for aqueous flow and the substrate with a deep channel (60 μm) for segmented flow were aligned under a stereomicroscope and then bonded together using room-temperature prebonding and high-temperature (560 °C) bonding techniques.[19] The microchannel configuration and detailed fabrication procedure are shown in Figure S1 in the Supporting Information. The on-chip monolithic electrospray emitter was fabricated at the outlet of the shallow channel using the stepped grinding method as described previously.[20] The schematic diagram of the microchip is shown in Figure 1.

Fabrication of the Droplet Extraction Interface. A two-step laminar flow treatment method was used to perform selective hydrophobic modification for the deep channel while keeping the tonguelike region in the deep channel and the shallow channel hydrophilic. The procedures are illustrated in Figure 2. Before the treatment, the chip channels and the outer surface of the emitter were treated with 1 M sodium hydroxide solution for 15 min and rinsed to neutral pH with deionized water. The microchip was dried at 120 °C under nitrogen flow for 3 h. First, all the chip channels and ports were filled with toluene by infusing it from the MS carrier port using a syringe pump (Pump11 Picoplus, Harvard, Holliston, MA) with a 500 μL syringe. In the next three steps, toluene was continuously infused with a flow rate of 2 μL/min. Second, the sample and reagent ports were blocked with plastic plugs, and the oil port was filled with 2% (v/v) octadecyltrichlorosilane (OTCS) in toluene. Another syringe pump was used to aspirate liquid from the waste port for 10 min at a flow rate of 6 μL min^{-1}. A multiphase laminar flow of the OTCS solution

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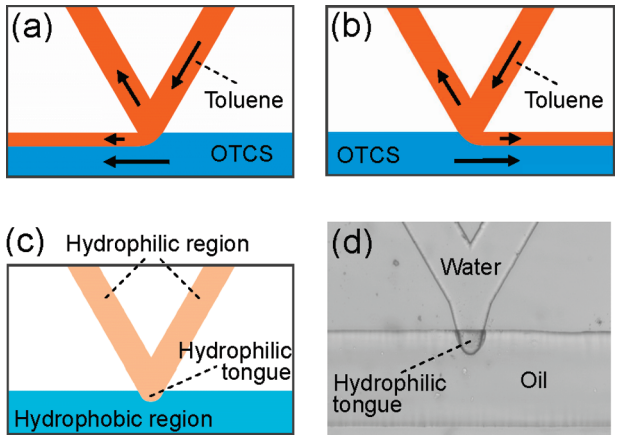

Figure 2. Illustration of the selective surface modification of the chip microchannels using a two-step laminar-flow treatment method. (a) A two-phase laminar flow of toluene and 2% OTCS solution was formed at the interface by infusing toluene from the buffer port and aspirating from the waste port. (b) The laminar flow was reversed by aspirating from the oil port. (c) The surface properties of the microchannels after selective modification. (d) CCD image showing the hydrophilic tongue region formed at the interface.

and toluene was formed at the deep channel (see Figure 2a) to protect the whole shallow channel as well as the partial deep channel with toluene flowing through from hydrophobic modification. Third, the laminar flow was reversed by removing the syringe pump from the waste port and connecting it with the oil port to aspirate liquid for 10 min, during which the OTCS solution was aspirated into the deep channel from the waste port (see Figure 2b). The unmodified sidewall of the deep channel protected by toluene in the second step was modified, leaving a small hydrophilic region (hydrophilic tongue) at the tip of the shallow channel stretching into the deep channel (Figure 2c,d). Fourth, toluene was continuously infused from the carrier port for 5 min to remove the unreacted OTCS in the channel. At the same time, the outer surface of the emitter was modified by immersing its tip into the OTCS solution. Finally, the chip channel and the emitter tip were rinsed with methanol for 2 min and dried on a heating stage (120 °C).

Setup of the System. Before use, the shallow hydrophilic channel was treated with 1 M NaOH solution for 5 min and rinsed with water. Four syringe pumps were used to deliver the oil (tetradecane), aqueous sample and reagent solutions, and the ESI carrier solution (50% methanol containing 1% acetic acid) to the microchip. Poly(tetrafluoroethylene) (PTFE) tubings (0.40 mm i.d., 0.76 mm o.d.) were used to connect the syringes with the channel ports. A waste reservoir made of a glass tubing (3 mm i.d., 5 mm o.d.) was connected to the waste port on the chip with a 80 cm long elastomeric silicon tubing (1 mm i.d., 1.7 mm o.d.) and mounted on a vertically moving platform.

An ion trap mass spectrometer (LCQ DECA XP, ThermoFisher, Waltham, MA) was employed to perform the MS experiment. The microchip was positioned horizontally at a distance of 2 mm between the chip emitter tip and the MS inlet orifice. Electrical contact for the electrospray was achieved by connecting the high-voltage source to the stainless steel tubing fixed on the ESI buffer port of the microchip (Figure 1). An inverted fluorescence microscope (ECLIPSE TE-2000-S, Nikon, Japan) equipped with a high-sensitivity charge coupled device (CCD) camera (SPOT RT-SE6Monochrome, Diagnostic Instruments, MI) was used for direct observation and recording of the droplet extraction process. A home-built confocal laser induced fluorescence (LIF) detector was used to monitor the sample zone after the droplet was extracted.

Safety Consideration. The experiments of wet etching of the glass chips as well as silanizing of the microchannels should be performed in a well-ventilated hood, while wearing protective gloves and goggles.

RESULTS AND DISCUSSION

System Design. This work aimed to develop a reliable ESI-MS detection system for droplet analysis, especially for the online monitoring of droplet-based microreaction. To achieve this goal, one of the challenges was to develop a highly reliable and controllable droplet extraction interface to transfer the droplets from the segmented stream into the aqueous stream, while preventing the oil from leaking into the aqueous stream which may result in the unstable electrospray.[15,17] In addition, the buffer systems used in many actual reactions are not compatible with ESI-MS in which organic additives and low pH condition are usually required. Therefore, matrix modifications to the reaction solutions should be carried out online before the MS detection. The approaches to address the above-mentioned challenges are listed as follows: (a) Two methods including the use of the hydrophilic tongue structure in the droplet extraction interface and the use of a back pressure regulator coupled with this interface were adopted to improve the reliability and controllability of droplet extraction. (b) After the sample droplets were extracted from the segmented stream, the flow injection mode[21] was used to transfer them to the ESI emitter for MS detection employing an aqueous solution containing 50% methanol and 1% acetic acid (v/v) compatible for MS detection as the carrier. This could significantly minimize the cross-contamination between the droplets due to the continuous rinsing effect of the carrier flow. Although the sample concentration was diluted to some degree due to the mixing of the sample zone with the carrier flow under this mode, the sample matrix was modified simultaneously by mixing with the carrier, resulting in the enhancements of the ESI efficiency and stability as well as the MS detection sensitivity. (c) Multiple modules including droplet generation, the mixing of sample and reagent, online reaction within the droplets, droplet extraction, matrix modification, and electrospray ionization of the sample zones were integrated into a single glass chip (Figure 1). (d) The present system was applied in the characterization of alkylation of peptides to demonstrate its ability in actual reaction monitoring. To the best of our knowledge, this is the first application of a microfluidic droplet-based system with ESI-MS detection in online monitoring of actual microreaction.

Droplet Extraction Interface. In the preliminary study, we used the single-step laminar-flow treatment method[11] to pattern the microchannels (Figure 2a). A relatively large hydrophilic region was formed on the sidewall of the segmented flow channel, thus more aqueous solution was retained on this region when the segmented flow passed by (Figure S2a in the Supporting Information). This may lead to evident cross-contamination between the adjacent droplets (Figure S2b in the Supporting Information). On the basis of the single-step method,[11] we further

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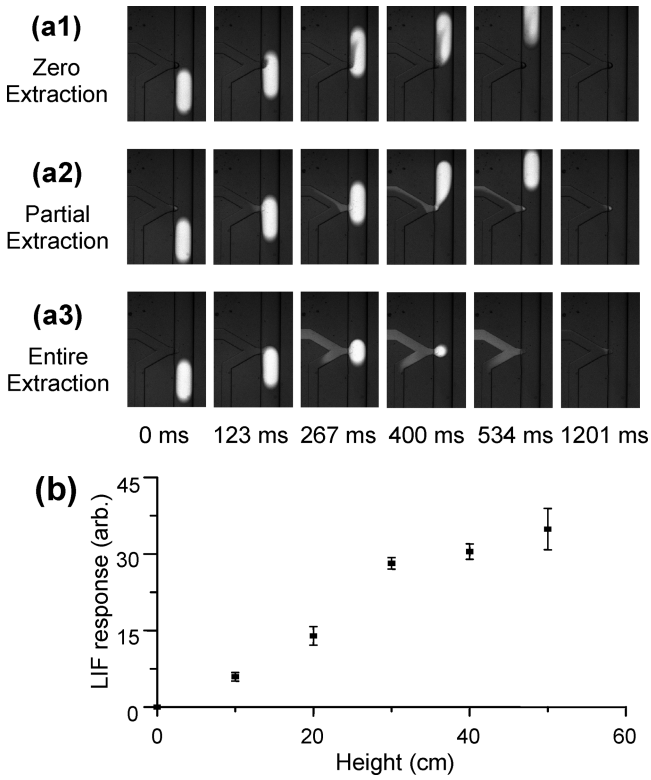

Figure 3. Fluorescence images of the extraction interface under three droplet extraction modes including zero extraction (a1), partial extraction (a2), and entire extraction (a3), corresponding to the waste reservoir heights of 0, 10, and 30 cm, respectively. (b) Effect of the waste-reservoir height on the extraction performance. Flow rates: oil (tetradecane), 1500 nL/min; sample (10 μM fluorescein solution), 500 nL/min; carrier (water), 300 nL/min.

developed the two-step laminar-flow treatment method to eliminate the hydrophilic region on the sidewall of the segmented flow channel (Figure 2b), while only retain a hydrophilic tongue region extending from the hydrophilic aqueous flow channel into the hydrophobic segmented flow channel (Figure 2c,d). This hydrophilic tongue functioned as a droplet extraction probe by first making the droplet wet on it and then drawing the droplet into the ESI carrier flow channel. Since the hydrophilic tongue extended nearly one-fourth the width of the segmented flow channel, the opportunity of the aqueous droplets contacting with it was significantly increased, resulting in the reliability improvement of the droplet extraction operation.

In the experiments, we observed that the controllability of the droplet extraction operation could be improved by adjusting the back pressure in the segmented flow channel with the back pressure regulator. The back pressure was adjusted by changing the liquid level difference between the waste reservoir and the chip (i.e., the waste reservoir height) (Figure 1). Three typical extraction modes including zero extraction, partial extraction, and entire extraction were achieved by simply using different waste reservoir heights (Figure 3a). A movie recording these processes is available in the Supporting Information. We further investigated the effect of the waste reservoir height in the range of 0−50 cm on the droplet extraction performance using a 10 μM fluorescein solution as a model sample. The LIF detector was used to measure the fluorescence intensity of the sample zone transferred into the ESI carrier flow channel. The results are as shown in Figure 3b; the error bars indicate the standard deviation (n = 13) of the fluorescent signals for 13 extracted droplets in each height condition. The peak height of fluorescence intensity signals increased with the waste reservoir height (Figure 3b). Such behavior could be attributed to the pressure change in the segmented flow channel generated by the back pressure regulator relative to the pressure in the ESI carrier flow channel. With a height of zero, i.e., the waste reservoir was placed at the same level as the chip, the pressure in the segmented flow channel was lower than that in the carrier flow channel, and thus the droplets passed through the droplet extraction interface without being transferred into the carrier flow channel. Actually, a few transfer of the carrier from the ESI carrier channel to the droplets could be observed under the zero extraction mode (see the movie in the Supporting Information), which implies the present interface could be used to add reagents to droplets. As the height increased, the pressures in the segmented flow channel exceeded that in the carrier flow channel, thus partial droplet solution was transferred. When the height was higher than 30 cm, entire droplet transferring was achieved. Further increasing the height to over 50 cm could lead to the leakage of oil into the carrier channel, which resulted in the large variation of the fluorescent signals (Figure 3b).

A typical droplet extraction process is described in Figure 4. When the droplet passed by the hydrophilic tongue (Figure 4a), it first wetted on the hydrophilic tongue (Figure 4b) and then was drawn into the aqueous carrier flow channel rapidly (Figure 4c). The aqueous sample solution remained at the hydrophilic tongue and the tip region of the carrier flow channel was rapidly washed away within 2 s by the continuous carrier flow benefiting from their small region areas (volumes) (Figure 4d,e), which significantly minimized the cross-contamination between droplets. By using the hydrophilic-tongue based droplet extraction interface coupled with the back pressure regulator, a stable and reliable droplet extraction operation could be achieved in a broad droplet frequency range of 0.1−10 Hz and droplet size range of 5−50 nL.

In many droplet-based microfluidic systems, surfactants are commonly used to stabilize the droplets against fusion. In the present system, no surfactant was used due to its interference to ESI-MS detection. In addition, it should be noted that the addition of surfactants may also increase the difficulty of droplet extraction in the hydrophilic tongue-based interface.

Analytical Performance. The performance of the present droplet analysis system was evaluated in the determination of a model peptide, angiotensin II. The flow rates of oil, sample, and ESI buffer were set as 600, 50, and 150 nL/min, respectively. The height of the waste reservoir was set at 30 cm to achieve entire droplet extraction. The analysis throughput of ~10 droplets per min (i.e., 0.17 Hz) was obtained with a droplet volume of ~5 nL. A further increase of the analysis throughput was limited by the dispersion of the sample zone in the ESI carrier channel and the response speed of the mass spectrometer. Figure 5a shows the extracted ion trace (m/z 524−526) for a series of droplets containing 1 μM angiotensin. A relative standard deviation of 5.2% for the peak heights was obtained in the continuous analysis of seven droplets. The double-charged angiotensin (m/z 524.8) shows a strong signal in the mass spectrum (Figure 5b), which

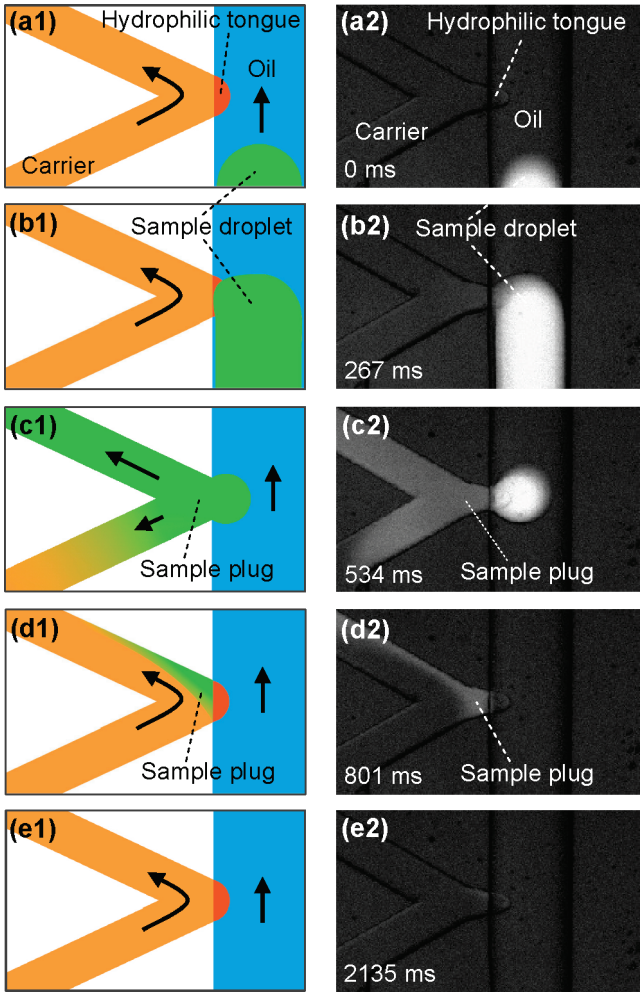

Figure 4. (a1−e1) Schematic diagram of the droplet extraction process using hydrophilic tongue. (a2−e2) Fluorescence images of the extraction interface corresponding to (a1−e1). Flow rates: oil (tetradecane), 1000 nL/min; sample (10 μM fluorescein solution), 50 nL/min; carrier (water), 150 nL/min. Waste-reservoir height: 30 cm.

is comparable to that reported by Smith's group.[17] Figure 5c shows the mass spectrum recorded at the interval between the droplets. No angiotensin signal (m/z 524.8) was observed, which indicated that there was no evident cross contamination between the droplets due to the continuous washing effect of the carrier flow under the flow-injection mode. The present system could continuously analyze 1000 droplets within 3 h without significant performance deterioration, demonstrating its ability in long-term monitoring.

MS Characterization for the Alkylation Reaction of Peptide. The droplet-based system has been proven to be a promising platform to perform microscale reactions due to its extremely low sample and reagent consumptions and high throughput.[3,5] In this work, a preliminary study on applying the droplet analysis system with MS detection to the characterization of alkylation of peptide was performed to demonstrate its potential in online reaction monitoring. The alkylation of peptides is a widespread method in proteomics to increase the identification score of peptide mass fingerprinting.[22,23]

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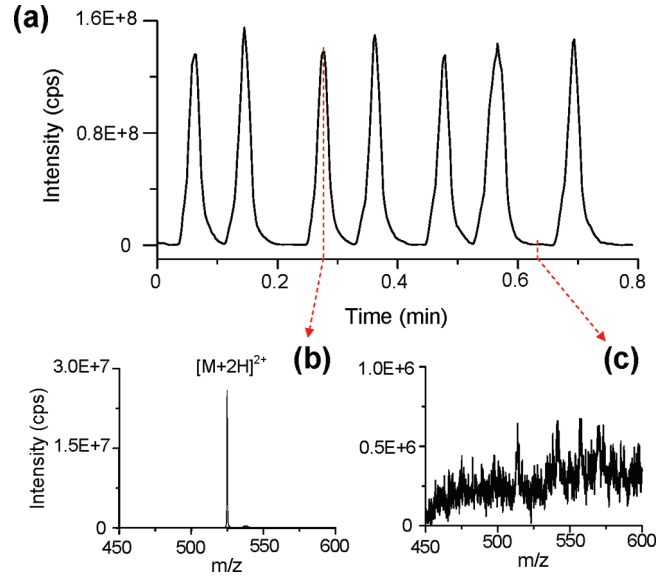

Figure 5. (a) Extractive ion traces (m/z 524−526) for angiotensin (1 μM). (b) Mass spectra obtained from the apex of the peak indicated by the dashed line. (c) Mass spectra obtained from the baseline showing the cross-contamination between adjacent droplets. Flow rates: oil (tetradecane), 600 nL/min; sample (1 μM angiotensin), 50 nL/min; carrier (50% methanol containing 1% acetic acid), 150 nL/min. Waste-reservoir height: 30 cm. ESI voltage: 2.5 kV.

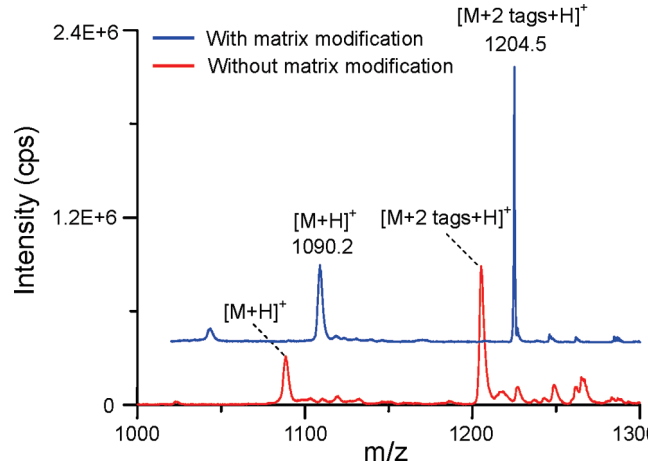

Figure 6. Mass spectra obtained using the standard capillary emitters for the mixtures of peptide Lys-Cys-Asp-Ile-Cys-Thr-Asp-Glu-Tyr (50 μM) and iodoacetamide (10 mM) with and without sample matrix modification. Flow rate: 150 nL/min. ESI voltage: 1.6 kV.

One of the main challenges in online monitoring of a reaction using ESI-MS is to adjust the reaction matrix to match the requirement of ESI, since the buffer solutions used in many reactions are not compatible with ESI-MS. The ideal buffer solution for positive ESI should be a low-pH aqueous solution containing at least 50% methanol or acetonitrile.[24] However, the alkylation reaction of peptides with iodoacetamide should be performed under weak alkaline condition (pH 8.0). We examined the ESI response of the alkylation reaction solutions of peptide Lys-Cys-Asp-Ile-Cys-Thr-Asp-Glu-Tyr (KCDICTDEY) with and without matrix modification using a standard pulled capillary emitter in an offline experiment. As shown in Figure 6, although the reaction product concentration in the modified reaction solution was diluted to 50% with methanol containing 1% acetic acid (v/v), its signal

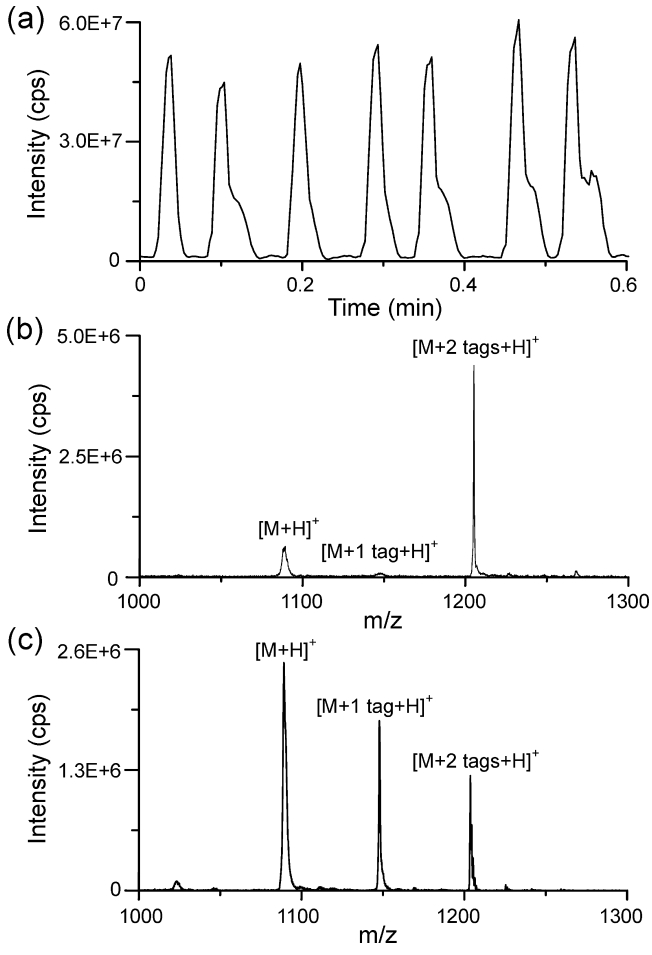

Figure 7. (a) Extractive ion traces (*m/z* 1203−1205) of the sample from the online alkylation of peptide KCDICTDEY (50 μM) with a sample−reagent ratio of 1:1. (b) Mass spectra obtained from the apex of the peak indicated by dashed line. (c) Mass spectra of the sample from the alkylation of peptide KCDICTDEY with a changed sample−reagent ratio of 8:1. Flow rates: oil, 1000 nL/min; sample (100 μM KCDICTDEY), 30 nL/min for parts a and b, 240 nL/min for part c; reagent (20 mM iodoacetamide), 30 nL/min; carrier, 150 nL/min. Other conditions were the same as in Figure 5.

(M + 2 tags, *m/z* 1204.2) was still significantly higher than that of the unmodified one, which demonstrated the necessity of the matrix modification.

The present system was also applied in the characterization of the alkylation reaction of peptide KCDICTDEY. The flow rates of oil (tetradecane), iodoacetamide (20 mM), peptide solution (100 μM), and buffer solution were set at 1 μL/min, 30 nL/min, 30 nL/min, and 150 nL/min, respectively. The frequency of droplet generation was ~10 droplets per min and the reaction time was ~2.5 min. We used the LIF detector to evaluate the dispersion of the extracted sample zone in the ESI carrier channel under flow injection mode using a 10 μM fluorescein solution as a model sample. The dispersion coefficient of the sample zone in the ESI buffer solution was 2.1 under entire extraction mode.[21] Figure 6 displays the mass spectrum of the reaction product in a series of droplet microreactors. The RSD of the peak heights from the extracted ion current trace was 9.8% (n = 7) (Figure 7a). The signal intensity of the reaction product (M + 2 tags, 1204.2) was comparable to that obtained in the offline experiment with a pulled capillary emitter, indicating the favorable efficiencies of the reaction and the matrix modification (Figure 7b). We also attempted to change the reaction ratio of the sample and reagent from 1:1 to 8:1 by increasing the flow rate of the peptide solution to 240 nL/min with the other conditions fixed. The signal intensity of KCDICTDEY and single-tagged product was significant increased while the signal intensity of double-tagged product was decreased (Figure 7c). Although no accurate quantitative analysis was performed, this result could still demonstrate the potential of the present system to be applied as a promising tool in study of reaction kinetics.

CONCLUSIONS

The integrated device described here proved to be an efficient means for online droplet analysis with ESI-MS detection. The successful application in the actual reaction monitoring demonstrated its potential in the studies of combinatorial chemistry, reaction dynamics, intermediate product identification, as well as in high-throughput screening based on chemical reactions. The system performance could be further improved by integrating sample pretreatment operations before MS analysis, such as liquid−liquid extraction, solid-phase extraction, desalting, or chemical derivation, which could be used in the direct analysis of real biological samples such as body fluids or cells.

The hydrophilic tongue-based droplet extraction interface proved to be robust and flexible. We envision this interface could be a versatile platform for droplet extraction in the combination of droplet-based systems with other analytical systems, such as electrochemical detection, capillary electrophoresis, and liquid chromatography.

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SUPPORTING INFORMATION AVAILABLE

Reagents and sample preparation procedures, comparison experiment of matrix improvement using standard nanospray emitters, and a movie recording the droplet extraction process. This material is available free of charge via the Internet at http://pubs.acs.org.

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