**Microfluidic Chip Coupled with Thermal Desorption Atmospheric Pressure Ionization Mass Spectrometry**

Chia-Hsien Chang, Tsung-Yi Chen, and Yu-Chie Chen*

Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan

Microfluidic chips have been used as platforms for a diversity of research purposes such as for separation and micro-reaction. One of the suitable detectors for microfluidic chip is mass spectrometry. Because microfluidic chips are generally operated in an open air condition, mass spectrometry coupled with atmospheric pressure ion sources can suit the requirement with minimum compromise. In this study, we develop a new interface to couple a microfluidic chip with mass spectrometry. A capillary tip coated with a layer of graphite, capable of absorbing energy of near-infrared (NIR) light is used to interface microfluidic chip with mass spectrometry. An NIR laser diode (λ=808 nm) is used to irradiate the capillary tip for assisting the generation of spray from the eluent of the microfluidic chip. An electrospray is provided to fuse with the spray generated from the microfluidic chip for post-ionization. Transesterification is used as the example to demonstrate the feasibility of using this interface to couple microfluidic chip with mass spectrometry.

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**INTRODUCTION**

The concept of a micro-total analytical system and a lab-on-a-chip system has been emerged as a means of providing vital technology to enable many applications in academic researches. One of the promising systems, microfluidics, have been employed to various types of research purposes such as for sample pre-concentration, extraction, purification, separation, and micro-reaction. These applications are normally carried out in dimensions of micrometer scale by consuming only a small volume of samples. Microfluidic devices can provide a suitable environment for chemical reactions to be carried out in a faster and more efficient way inside microfluidic channels such as generating microdroplets for micro-reactions. It is of great interest to interface microfluidics with mass spectrometry (MS) for detection of chemical reactions. One of the MS techniques, i.e., electrospray ionization (ESI) MS, provides advantageous connection between a microfluidic device and a mass spectrometer, owing to not only the similar flow rate of microfluidic device to meet the requirement for ESI-MS but also the ability to directly deal with aqueous samples. Efforts have been made in interfacing microfluidic chips with MS. For example, simply applying a high voltage on the outlet of the channel on a microfluidic chip can readily generate spray from the eluent. To improve band broadening and sample dilution problems, appropriate methods were also further studied. Alternatively, interface development involves using a capillary attached to a chip as the electrospray emitter was proposed. The method offers advantages to provide stable electrospray condition over previously mentioned direct spray from the channel outlet on the chip. It has been demonstrated that microfluidic chips attached with a capillary emitter can be used to carry out tryptic digestion for complex protein samples online.

Moreover, recent miniaturization devices used in the MS application have been focused on multiplexed MS analysis; for example, hundreds of thousands of micrometer scale compartments can be fabricated for high-throughput DNA analysis. Arrays of microchannels have been used for infusion analysis of a variety of samples in connection with MS. Recent developed atmospheric pressure ionization methods such as desorption electrospray ionization (DESI), direct analysis in real time (DART), electrospay-assisted laser desorption/ionization (ELDI), an atmospheric solid analysis probe (ASAP), laser diode thermal desorption (LDTD), extractive electrospray ionization (EESI), and laser spray provide more options when coupling with microfluidic devices.

We previously demonstrated a thermal desorption ambient MS, which employed a near infrared (NIR) laser (808 nm) to irradiate the backside of a sample loading substrate coated with multilayers gold nanoparticles that are capable of absorbing NIR light and assisting thermal desorption of analytes from the substrate. The desorbed species from the sample was then fused with a stream from electrospray for post-ionization. On the basis of similar

* Correspondence to: Yu-Chie Chen, Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan, e-mail: yuchie@mail.nctu.edu.tw

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concept, we further demonstrated an interface that can be used to couple a microfluidic system for liquid samples to carry out micro-reactions inside the channels. The generated product ions can readily be detected by MS online. The setup of this approach was uncomplicated: By simply connecting a capillary tip coated with a thin layer of graphite to the microfluidic outlet, gas species were generated from the eluent on the outlet under irradiation of an NIR laser (808 nm) through thermal desorption spray. The generated spray was then fused with the other stream of electrospray for post ionization. Online detection of transesterification of vegetable oil reacting with alcohol in the presence of aqueous sodium hydroxide in microfluidic channel was investigated using this approach.

EXPERIMENTAL

Reagents and materials
Acetonitrile, ethanol, acetic acid, hydrochloric acid, trifluoroacetic acid, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Graphite powder was purchased from Ted Pella (Redding, CA, USA). Soybean oil was purchased from Uni-President (Tainan, Taiwan). Fused silica capillaries (50 µm inner diameter (i.d.), 366 µm outer diameter (o.d.)) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Narrow bore glass capillaries (0.5 mm i.d., 1.5 mm o.d.) were purchased from Hilgenberg GmbH (Malsfeld, Germany).

Fabrication of microfluidic chip
Microchannels were molded in poly(dimethylsiloxane) (PDMS) (Dow Corning, MI, USA) by the technique of replica-molding. Initially, a silicon wafer with 2,000 µm (E&M Corp., Japan) as a replica molding master was prepared. This was followed by sputtering a chromium (Cr) layer (10 µm) as a replica molding. Initially, a silicon wafer with 2,000 µm (E&M Corp., Japan) as a replica molding master was prepared. This was followed by sputtering a chromium (Cr) layer (10 µm) as a mask for etching process. After the resist was patterned with photoresist (Ofpr 800, TOK, Japan) using deep-ultraviolet (UV) lithography followed by a wet etching process, the Bosch deep Si dry etching (Oxford Instruments, UK) was employed to engrave the patterns of microfluidic channels on the silicon wafer. A pre-polymer PDMS mixture consisting of Silpot 184 and Silpot 184 catalyst (10 : 1, v/v) was poured onto the Si mold. This was degassed for 20 min at a pressure of 0.1 MPa to remove air bubbles generated from mixing and cured at 80°C for 20 min.

Fabrication of the ESI emitter and the sample spray emitter
A silica capillary (~10 µm i.d., ~20 µm o.d.) was fabricated and used as the ESI emitter. First, a certain length of the capillary (length: 10 cm) was placed vertically and applied a small weight (50 g) at the lower end of the silica capillary. To form a bare-fused silica capillary tip, a Rekrow butane flame gun was used to heat at a point of a few centimeters above the lower end, in which the weight was served as a pull force. After cooling to room temperature, the capillary tip was immersed in a 24% hydrofluoric acid (HF) solution for 10 min. A pump (pressure: 10 mmHg) applied a pressure difference to the other end of the capillary to wash away remaining HF solution. The capillary was then conditioned by aqueous NaOH (0.1 N) for 30 min and deionized water for another 30 min. Subsequently, a short capillary was cut to 4 cm from the as-prepared capillary. The short capillary was then horizontally inserted into a hole on the side wall of a centrifuge tube (Scheme 1). The capillary was used as the ESI emitter for providing the electrospray stream.

When preparing the sample emitter, a narrow bore glass capillary (~100 µm i.d., ~110 µm o.d.) was fabricated by tapering the capillary tip with an object (10 g). The fabrication method was the same as described earlier. After rinsing the tapered capillary, layers of graphite suspension (0.1 g/mL) prepared in methanol were coated on the surface of the capillary tip and baked in an oven at 120°C for 30 min. The thickness of the coating was ~15 µm.

Configuration of coupling microfluidic chip with MS
Scheme 1 presents the experimental setup used in this study. The sample spray emitter connected to the channel outlet on the microfluidic chip was perpendicular to the inlet of an Esquire 2000 ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany). No electrical contact was applied on the outlet of the capillary tip. An NIR laser diode (808 nm, Unice E-O Services) equipped with an optical fiber was used to irradiate the sample emitter coated with graphite as a thermal desorption source. The NIR laser was placed 4 mm above the sample spray emitter. The power was ~300 mW·cm⁻². Too high or too low laser power would cause fast solvent evaporation or inefficient thermal desorption, respectively. The ESI emitter made by a tapered capillary was inserted into a centrifuge tube containing ESI solution (acetonitrile–water, 1:1 (v/v)), while a platinum electrode was placed in the solution applied with +500 V. The distance between the ESI emitter, the sample spray emitter and the stainless steel extension tube from the mass spectrometer were all kept in 4 cm apart on the same level. The heated transfer glass-capillary in the ion trap mass spectrometer was maintained at 250°C with a dry gas flow of 5 L/min. The voltage applied on the MS inlet was set at ~3,000 V for the spectra recorded in positive ion mode. Online nanospray mode was employed during MS analysis.

RESULTS AND DISCUSSION

The interface designed for coupling a microfluidic chip with MS herein is combining thermal spray from the eluent of a microfluidic chip fused with a stream of electrospray. The generation of charged species was expected. We employed an NIR laser to irradiate the capillary outlet coated...
with graphite from the microfluidic chip. Graphite was used as energy absorber for thermal spray to take place. Figure 1(a) shows the photograph of the capillary outlet from the microfluidic channel. The eluent was pushed out from the microfluidic channel by a syringe pump (Scheme 1), so the droplets retained around the tip. As the laser irradiated on the capillary tip, a stream of spray was observed (Fig. 1(b)). When the NIR laser irradiated on the graphite free capillary tip, no spray was observed (Fig. 1(c)), indicating the graphite coating was essential for thermal spray to take place.

To demonstrate the feasibility of the current approach in interfacing microfluidic chip with MS, taurodeoxycholic acid (MW = 498.7) was used as the model sample. Figure 2 shows the resultant mass spectrum obtained in negative ion mode. Deprotonated taurodeoxycholic acid at m/z 498 dominated the mass spectrum. This result indicated this approach can be readily used to generate gas phase ions for MS analysis.

Transesterification of triacylglyceride (TAG) was selected as the model reaction to demonstrate the feasibility of using this approach for detecting reaction species online. TAG is an ester derived from glycerol and three fatty acids. Fatty acids such as linolenic acid (Ln), linoleic acid (L), oleic acid (O), and palmitic acid (P) are basic compositions in TAG (Scheme 2). TAG is a common compound in vegetable oils and can carry out transesterification in the presence of NaOH (catalyst) and alcohol. Under catalysis of sodium methoxide, the main transesterification products of TAG are methyl esters. We selected soybean oil as the model sample for conducting transesterification of TAG. Figure 3 shows the conventional ESI mass spectrum of soybean oil. Sodiated TAG species appear at m/z above 800. The details of the compositions of these peaks are marked on the mass spectrum as the inset.

In the microfluidic chip (Scheme 1), two sample channels were fabricated on the chip: one channel was used to introduce TAG containing samples, i.e., soybean oil, while the other channel was used to transport a solution of sodium methoxide. When introducing the two solutions into the channels simultaneously, an oil droplet was formed on the intersection of the two channels. The generated micrometer-sized droplet encapsulated reactants and acted as a microreactor. When the oil droplet flowed through the outlet, thermal spray was readily occurred under irradiation of an NIR laser. The spray was followed by fusion with ESI spray for MS detection. Certain length (∼28 mm) of S-shape channel was fabricated on the chip. This provided the advantage to carry out transesterification reactions for a sufficient period of reaction time. When conducting online monitoring of transesterification of soybean oil, three different flow rates (162 µL/h, 41 µL/h, and 21 µL/h) were used to infuse the liquid in the channel on the microfluidics chip. Figure 4 shows the resulting mass spectrum of the generated product species obtained at the flow rate of 162 µL/h. The mass spectra were dominated by the ions at m/z 293.2, corresponding to linolenic acid methyl ester. The result is similar to what we obtained from a thermal desorption MS approach.24) The ion intensity was affected slightly by the flow rate because a lower volume of samples was eluted from the sample emitter at a lower flow rate, leading lower ion intensity. That is, the reaction product was readily generated when running through the microfluidic channel, taking ∼10 min. The results indicate that this proposed interface can be readily used to couple a microfluidic chip with MS and suitable for being used to detect reaction species eluted from the microfluidic channel.

**CONCLUSION**

We have successfully demonstrated a new interface for coupling MS with a microfluidic chip based on thermal spray and fused droplets for post-ionization. The results show the interface is capable of carrying out online detection of transesterification reactions of TAG. In the microfluidic channel, rapid emulsion mixing phenomenon occurred when the reactants flow into the interaction region as a
Scheme 2. Transesterification reactions of TAG.

Fig. 3. TDA mass spectrum of soybean oil.
reaction starting point. The microdroplets work as suitable micro-reactors for the transesterification reactions to be taken place. Additionally, the length of the channel can be varied; reaction times can be adjusted by the flow rate and the length of the channel for completion of reactions. The interface setup is straightforward and easy to be operated. This approach can be potentially used for extensive reactions to carry out a diversity of microreactions in a micro-fluidic channel.

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