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펩타이드 질량 분석을 위한 전기 이온화 분사기의 제작 및 성능 평가

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Mass spectrometry analysis system with integrated micro electrospray ionization emitter for peptide detection

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Abstract - This paper describes a novel microfluidic device with a microfabricated electrospray source for a sheathless electrospray ionization interface to a mass spectrometer. This electrospray ionization-mass spectrometry (ESI-MS) device consists of a triangular-shaped metal emitter, allowing the generation of an efficient electrospray for peptide detection, and microfluidic channels monolithically in a glass microchip. The performance of the proposed interface was evaluated by opimizing its experimental condition and spraying standard peptides. The spraying has high signal strength and stability, with a relative standard deviation of 2.9% and singly-charged and doubly-charged peaks of the peptides were successfully detected. The metal emitter source showed a good performance to be comparable to commercially available emitters in signal strength and stability.

1. Introduction

Miniaturized total analytical schemes have been developed in the field of proteomicsand the final detection step is performed using mass spectrometry(MS) techniques. As a result, new tools are essential for MS analysis and especially MS analysis based on electrospray ionization(ESI). The ESI technique uses strong electric fields to create ions from solution. The ESI-MS method has the ability to detect large biomolecules with great sensitivity and accuracy. Hence, it is a suitable analytical tool that has been widely applied to biomolecular structure analysis[1]. Several groups have been explored to make a chip-based ESI-MS interface[2, 3]. In common method, the interface based on a microfluidic device is coupled to conventional sprayer or micro nozzles fabricated using silicon and polymers. However, all the systems require a gold conducting coating of the tip which may lead to deterioration of the spray stability due to the poor adhesion of such a thin conducting layer. Also, they are not fully integrated with the microfluidic channels, producing a large dead volume in the interface between the chip and the capillary sprayer, which can lead to broadening of separation bands[4].

In this paper, a new microfluidic device with an integrated gold electrospray emitter, allowing the generation of an efficient nanospray for peptide detection, is presented. As a mass spectrometry electrospray source, a triangular-shaped open emitter was formed by lithography and electroplating on a glass substrate instead of inserting a separate tip. The fabrication and assembly method of open emitters is simple while still maintaining a sharp emitter and it is more robust than that recently reported for silica or polymer. The triangular emitter acts like a nozzle that prevents liquid spreading at the exit of the microfluidic channel and helps to form and fix the position of the Taylor cone by directly applying high voltage on the metal emitter. The sensitivity and analytical characteristics of the proposed interface were investigated for a standard peptide mixture.

2. Experimental

2.1 Microchip fabrication

The fabrication process for the ESI microfluidic device is shown in Fig.1. The separation channel and a metal emitter were fabricated using a glass wet etching and gold electroplating process, respectively. As a wet-etching mask, an amorphous silicon layer was deposited on both sides of the glass wafer by low pressure chemical vapor deposition at 540 °C to realize two different etch depths. Reactive ion etching was used to transfer the pattern to the amorphous silicon. The microfluidic channel, 4 cm long, 70 μm wide at half-depth, 20 μm deep, was then isotropically etched in 49% HF. The metal emitter was aligned with the channel at the end of the glass channel, so the microchannel was expanded to the end of the emitter. The emitter part, 10 μm deep with an apex angle of 30°, was isotropically etched for 40 s under the same conditions of the microchannel etching. Then, a triangular-shaped gold emitter, 2.5 mm long, was formed by lithography and an Au electroplating process on a glass substrate. Au emitter structure was released to establish a well-defined Taylor cone by a wet etching process using 49% HF solution. This was followed by removal of the electroplating mold and all etch mask. To form a closed channel network, this PDMS plate was irreversibly bonded to the glass plate containing the emitter structure after surface treatment with oxygen plasma.

2.2 ESI/MS instrumentation

ESI-MS was performed in the positive-ion mode using an LCQ Deca ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with a syringe pump. The device was mounted on the xyz stage positioner and adjusted to obtain the maximum total ion current. The electrical contact for the high voltage application was realized directly by the integrated gold emitter. The inlet reservoir was connected to the syringe pump via silica capillary tubing(Fig.2). For the mass spectrometry analysis, the Bradykinin fragment 2-9 (903 Da) was prepared as a peptide sample with the concentration of 1-10 μM. The sample and carrier solvent, i.e.

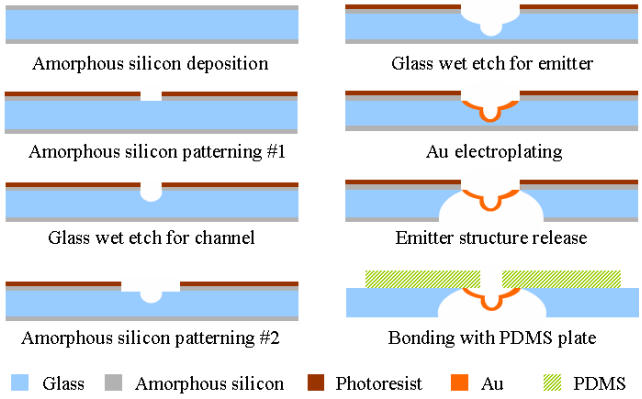

Fig.1. Schematic representation of an ESI-MS microchip fabrication

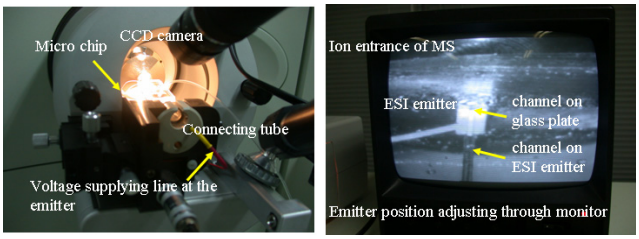

Fig.2. Corresponding ESI-MS experimental setup

water:acetonitrile (1:1) was introduced via syringe pump.

3. Results and Discussion

3.1 Electrospray performance and stability of the total ion current (TIC)

The spraying stability performance was evaluated by adjusting sample infusing flow rate with the concentration of 5 μM in water: acetonitrile (1:1, v/v) and the buffer solution of sodium bicarbonate(10 mM, pH 8.3). However liquid injection to the emitter interface by capillary action is also applicable, steady flow rate by external pressure is needed to get distinguished stability of the electrospray ion generation. Important parameters for stable electrospray were sample flow rate, electrostatic potential and distance of the chip from a mass spectrometer orifice. In our experiment, the applied ESI potential and the distance between the edge of the emitter and the entrance of the MS orifice was fixed at 2 kV and 2 mm, respectively. To determine the optimal sample infusing flow rate, the sample flow rate was decreased gradually from 5 μl/min to 0.5 μl/min adjusted via syringe pump of the MS equipment. As the flow rate was decreased, a stable electrospray was maintained with a current of 10 μA. Fig. 3 shows the long-term stability of the total ion current for a 20 min continuous infusion MS run with an acquisition speed of 0.6 spectrum/s. The scan range of the mass-to-charge ratio (m/z) was from 150 to 2000. The spraying, with the flow rate of 0.5 μl/min, was stable with a relative standard deviation (RSD) of 2.9%. Typically, electrospray ionization was stable for five hours at least, and the electrospray performance in other chips with the same design was not significantly different without any sign of degradation of the ESI stability after several performance tests. The proposed electrospray source disperses the liquid sample purely by electrostatic means, and no assistance such as sheath flow or nebulizing gas is used. Nevertheless, it is a very stable source that has high tolerance due to the unique characteristics of gold material.

3.2 Electrospray mass spectrometry using fabricated emitters

The electrospray signal was recorded with the experimental method. The sample was introduced via syringe pump and carrier solvent, i.e. wateracetonitrile (1:1), at a flow rate of 0.5 µl/min. High voltages of 26 V and 1.5 kV directly were applied to the MS orifice and integrated gold emitter, respectively. Fig.4 shows the corresponding mass spectrum obtained for a Bradykinin 2-9 sample, 5 μM, in acetonitrile

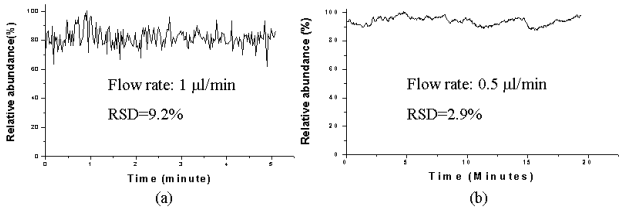

Fig.3. Total ion current of a Bradykinin 2-9 sample with the concentration to 5 μM in water and flow rate of (a) 1 μl/min and (b) 0.5 μl/min

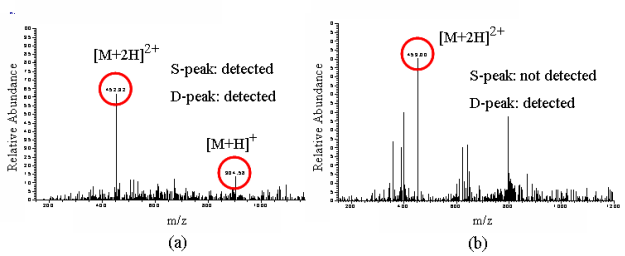

Fig.4. Mass spectrum of Bradykinin 2-9 sample with the concentration to 5 μM for 20 min. with (a) a microfabricated emitter and (b) a conventional tip

5% (v/v) mixture (1:1) and the buffer solution of sodium bicarbonate (10 mM, pH 8.3) using the microemitter and conventional emitter, respectively. Singly-charged ($[M+H]^+$, m/z 904) and doubly-charged peaks ($[M+2H]^{2+}$, m/z 453) of Bradykinin 2-9 were detected using microfabricated emitter, but singly-charged peak was not detected in conventional ESI analysis system. The mass spectrum obtained with the sample has a good intensity (4.72×10^8). The resulting mass spectrum aquired using microfabricated ESI emitter presented several peaks having comparable intensities. With the help of the released emitter structure and the characteristics of the gold material, which avoids solution spreading at the outer walls, the device has shown an efficient ionization performance constantly providing a stable MS signal. The detected fragmentation spectrums provide an excellent signal-to-noise ratio and they were seen to be the same as with conventional glass or fused silica emitters with respect to matching peaks and their intensities.

4. Conclusions

We have demonstrated that a sheathless electrospray interface with a conducting metal emitter is a promising method for MS analysis based on ESI. This approach is less involved than applying a conductive coating to the exit end to establish electrical contact. This ESI-MS setup offers the possibility of a direct application of the ionization voltage on the gold emitter without using a Pt wire or additional coating with conductive material on electrosprayer. Furthermore, the interface is less dependent upon the longevity or durability of such a coating, factors that have been concerns in the sheathless interfaces. The performance of the metal emitter sources was seen to be comparable to that of commercial emitters in signal strength and stability.

Acknowledgements

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