Talanta 81 (2010) 1069–1075

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Fabrication of low-melting-point alloy microelectrode and monolithic spray tip for integration of glass chip with electrospray ionization mass spectrometry

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ARTICLE INFO

Article history:
Received 23 November 2009
Received in revised form 25 January 2010
Accepted 28 January 2010
Available online 6 February 2010

Keywords:
Low-melting-point alloy
Electrospray ionization mass spectrometry
Glass chip
Monolithic emitter

ABSTRACT

In this paper, a glass microchip-based emitter with a low-melting-point alloy (LMA) microelectrode and a monolithic tip for electrospray ionization mass spectrometry (ESI-MS) was described. So far, the fabrication of metal microelectrode achieving direct electrical contact in the microchannel of glass chip is still a challenge. A novel fabrication approach for LMA microelectrode in the glass chip was developed to achieve direct electrode–solution electrical contact in the microchannel. An electrode channel and a sample channel were firstly fabricated on a glass chip with a micropore connecting the two channels. The melted LMA was filled into the electrode channel under a pressure of *ca.* 100 kPa, forming a stable and nicely fitted interface at the micropore between the sample and the electrode channels due to surface tension effect. The melted LMA filled in the electrode channel was then allowed to solidify at room temperature. The channel geometries including the distance between the sample and the electrode channels on the mask and the turning angle of the electrode channel were optimized for fabricating the LMA electrode. In this work, an improved fabrication approach for monolithic emitter tip based on pyramid-shaped tip configuration and stepped grinding method was also developed to fabricate well-defined sharp tips with a smallest tip end size of *ca.* 15 μm × 50 μm. Two types of emitter tip end including puncher-shaped tip and fork-shaped tip were produced. The emitter with the fork-shaped tip showed better working stability (4.4% RSD, TIC) at nanoliter-scale flow rate of 50 nL/min. The fabrication approaches for the LMA microelectrode and emitter tip are simple and robust, and could be carried out in most of routine laboratories without the need of complicated and expensive instruments. The performance of the emitter was evaluated in the analysis of reserpine, angiotensin II and myoglobin. A continuous experiment over 6 h demonstrated good stability of the present system in long-term analysis.

1. Introduction

The past decade has witnessed the rapid development of microfluidic chip-based analytical systems [1,2]. Various detection techniques, including absorbance and fluorescence detection [3,4], electrochemistry [5] and mass spectrometry (MS) [6,7] have been applied in these systems. MS detection technique has advantages of good versatility for different types of analytes, relatively high sensitivity and strong capability in molecule structural interpretation. In recent years, electrospray ionization mass spectrometry (ESI-MS) has become a major MS detection technique used in microfluidic chips due to its favorable ability in performing on-line detection [6,7].

Various approaches have been developed to achieve the combination of microchips and ESI-MS. Pioneered by Karger's group and Ramsey's group [8,9], the coupling of microchips with ESI-MS was firstly realized in a simple manner, by which the high voltage for electrospray was applied between the sample reservoir and the MS inlet orifice, and the electrospray was directly generated at the microchannel outlet on the flat edge of the chip. Later, microchip-based emitters for ESI-MS using a capillary emitter connected with the chip microchannel were developed [10–20]. In these emitters, the fabrication of spray tips and the electrical contact were easily achieved using the well-established technique for conventional capillary emitters. However, the relatively large dead volume existing at the junction of the chip microchannel and the capillary inlet in this type of emitter may lead to evident sample plug broadening when performing capillary electrophoresis separation on the microchip [6,21]. Thus, the development of on-chip monolithic emitters without dead volume has attracted more attention.

Currently, most of the on-chip monolithic electrospray emitters were fabricated based on plastic materials [22–30]. The electrical

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doi:10.1016/j.talanta.2010.01.064

contact in these emitters was realized using microfabricated electrode [23], conductive material coating [25], or embedded platinum wire [29]. Due to the simplicity in fabrication of plastic materials, well-defined monolithic spray tips could be easily produced using blade cutting [22,24], injection molding [26], laser ablation [27] or micromilling [25,28] techniques. Compared with plastic materials, glass has good chemical inertness and thermostability, and thus the ESI-MS emitters made from glass could provide much lower mass background level and higher detection sensitivity [30]. However, the fabrication of monolithic glass emitters for ESI-MS was quite difficult in both construction of electrical contact and fabrication of emitter tip [7,31], owing to the high hardness and brittleness properties of glass. Recently, several approaches have been proposed to address this challenge [31–33]. Yue et al. [32] used an etched glass membrane (20–50 μm in thickness) to conduct high voltage to sample solution. Hoffman et al. [31] described an approach using a computerized numerical control machine and a home-built heating puller to produce monolithic glass spray tips. During the preparation of this manuscript, Mellors et al. [33] developed a 300-μm thick glass chip for CE-ESI-MS, in which the potential was applied to the end of separation channel via a sheath flow channel and electrospray was generated directly at the corner of the rectangular chip.

In this work, a glass chip-based monolithic emitter for ESI-MS was developed. A novel fabrication approach for direct electrical-contact microelectrode on microchip was proposed using low-melting-point alloy (LMA). The fabrication technique of LMA microelectrode on microchip was firstly described by Whitesides' group to fabricate on-chip contactless electrodes, which functioned as electromagnets for the manipulation of magnetic microspheres [34]. In the present system, this technique was further explored, and for the first time applied to fabricate microelectrode for achieving direct electrode–solution contact in the chip-based emitter for ESI-MS. For the fabrication of the chip-based emitter tip, an improved fabrication approach was developed to fabricate well-defined sharp tips with a smallest tip end size of *ca.* 15 μm × 50 μm. The fabrication approaches for microelectrode and emitter tip are simple and robust, and could be carried out in most of routine laboratories. The performance of the chip-based emitter was evaluated in the analysis of reserpine, human angiotensin and myoglobin.

2. Experimental

2.1. Reagents and materials

Deionized water prepared with Millipore Ultrapure Water Systems (Billerica, USA) was used throughout. HPLC-grade methanol was purchased from Merck (Darmstadt, Germany), and glacial acetic acid was from TEDIA (Fairfield, USA). Reserpine, human angiotensin II, and horse myoglobin were products of Sigma–Aldrich (St. Louis, USA). Octadecyltrichlorosilane (ODS) was bought from Acros (Fair Lawn, USA).

2.2. Fabrication of glass chips

The mask design for the microchannels is illustrated in Figs. 1A and 2A, including a straight sample channel (10 μm width) and a V-shaped electrode channel (50 μm width). The distance *D* between the two channels was 30 μm unless mentioned otherwise. The turning angle of the V-shaped channel was 160°. Standard photolithographic and wet chemical etching techniques were used to fabricate microchannels on glass plate as described elsewhere [35]. After a 15-min wet etching process, the tip end of the V-shaped electrode channel (20 μm depth, 90 μm width) was connected to the sample channel (20 μm depth, 50 μm width) via a micropore

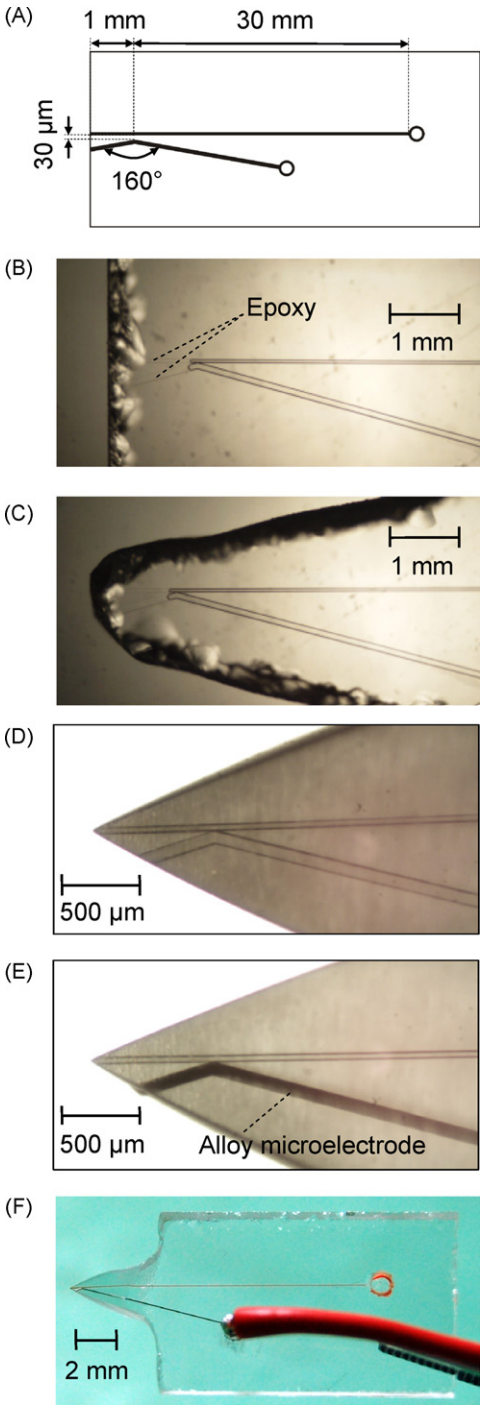

Fig. 1. Mask design for the microchannels on chip (A), CCD images of the ESI emitter chip during fabrication process (B–E) and the final appearance of the chip (F).

(Fig. 2B). Room-temperature pre-bonding and high-temperature (560 °C) bonding techniques were used to achieve the bonding of the cover and etched plates [36].

2.3. Fabrication of monolithic tips

Fig. 1B–D shows the fabrication process of the monolithic tip. Before fabrication, the outlet end of the channels was preblocked with epoxy to avoid clogging during the fabrication process (Fig. 1B). A relatively large tip (Fig. 1C) with a diameter of about 1 mm was first produced using an emery drill to grind the glass chips [37]. The tip was further ground into a pyramid shape by sequen-