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Low temperature bonding of poly(methylmethacrylate) electrophoresis microchips by in situ polymerisation

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Abstract

A novel method for bonding poly(methyl methacrylate) (PMMA) electrophoresis microchips at the temperature below the glass transition temperature of PMMA based on in situ polymerization has been demonstrated. Methyl methacrylate (MMA) containing initiators was allowed to prepolymerize in an 85 °C water bath for 8 min and 15 min to produce a bonding solution and a dense molding solution, respectively. The channel plate of the PMMA microchip was fabricated by the UV-initiated polymerization of the molding solution between a nickel template and a PMMA plate at room temperature. Prior to bonding, the blank cover was coated with a thin layer of the bonding solution and was bonded to the channel plate at 95 °C for 20 min under the pressure of binder clips. The attractive performance of the PMMA chips bonded by the new approach has been demonstrated by separating and detecting dopamine, catechol, three cations, and three organic acids in connection with end-column amperometric detection and contactless conductivity detection.

Keywords: Miniaturization; Capillary electrophoresis; Amperometric detection; Conductivity detection; Poly(methyl methacrylate)

1. Introduction

During the past decade, microfluidic analytical systems fabricated on glass, silica and polymer microchips have undergone an explosive growth. Much attention has been paid to capillary electrophoresis (CE) microchips owing to their high degree of integration, portability, minimal solvent/reagent consumption, high performance and speed [1–3]. These microchip analysis systems hold considerable promise for biomedical and pharmaceutical analysis, clinical diagnostics, environmental monitoring, and forensic investigations, etc.

Most early reports on miniaturized analytical systems have relied on glass or silicon substrates in connection with the standard lithographic fabrication technology. CE microchips are mainly fabricated using glass substrates, from cheap soda lime glass to high quality quartz [4]. However, their application was limited because of high cost, harmful and complicated fabrication procedures, and the limitation on the geometric modification of the chip channel [5]. Therefore, polymers are becoming the most promising materials for the microfluidic devices because they can be produced with mass-replication technologies, such as injection molding and hot embossing. Industrial interest in utilizing plastics for the production of microanalytical systems is primarily driven by the fact that these materials are less expensive and easier to be manipulated than silica based substrates. Polymers offer attractive mechanical and chemical properties, low cost, ease of fabrication, biocompatibility, and higher flexibility [5,6]. Such plastic chips have been fabricated using in situ polymerization [7], laser ablation [8], imprinting [9], injection molding [10], etc. A wide variety of polymer materials have been evaluated for fabricating microchips instead of glass, in which poly(dimethylsiloxane) (PDMS) and poly(methyl methacrylate) (PMMA) are the two most commonly used polymers. PMMA has been particularly useful for microfluidic chips with the features of low price, excellent optic

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transparency, and excellent electric and mechanical properties [6,11,12]. It has been reported as the least hydrophobic of the commonly used plastic materials [13], and can directly generate stable electroosmotic flow (EOF) in the microchannels under the electrical field applied [8,14]. In the fabrication methods mentioned above, the imprinting approach has been commonly employed due to some attractive advantages of simple procedure, less expense, and suitableness of mass production. Usually, imprinting methods involve the commercial available hot-embossing systems and the rigid templates such as quartz [15], Si [9] and nickel [16] templates. Recently, methods based on in situ polymerization have been developed for the fabrication of PMMA chip by using Si [17,18] and metal [7] templates. The Si or quartz templates readily tend to be broken due to the different thermal expansion coefficients of the templates and the polymers in imprinting or molding process.

In comparison with glass and PDMS microchips, PMMA-based CE microchips have been developed limitedly. It is a challenge task to find a reliable sealing technique to enclose the PMMA microchannel networks without clogging. Thermal bonding techniques [9,16,19,20] under pressure are usually employed for bonding PMMA substrates. In addition, thermal lamination [10], solvent bonding [21], glue layer [12], adhesive tape [22], and PDMS films [23–25] have also been used. Among them, various thermal bonding techniques are preferable as they allow formation of microchannels with uniform surfaces composed entirely of the same polymeric materials. Prior to sealing, the PMMA substrates were heated to the temperature above its glass transition temperature (T_g, 105 °C) and positive pressures were applied on the channel plate and the cover [9]. However, slight variance of the pressures and temperature may cause microchannel deformation and affect the reproducibility and yield because of the higher bonding temperature employed. It is of high importance to decrease the bonding temperature below the T_g of PMMA. Recently, Wooley et al. have demonstrated that PMMA substrates can be bonded together to form microfluidic devices by clamping a blank piece to a channel plate and heating the assembly in a boiling water bath. Rapid, high-resolution CE separations of derivertized amino acids have been successfully carried out on devices fabricated using this method [26]. Other sealing approaches unavoidably introduce multiple materials to form microchannels with non-homogeneous internal surfaces, which leads to undesired effect on the EOF and may reduced the separation efficiency [27,28]. Solvent bonding or thermal lamination, are less satisfactory, due to possible blocking of the microchannels or swelling of the pressure-sensitive adhesive of the laminated chips, respectively.

In this paper, a novel bonding technique for the PMMA electrophoresis microchips has been developed. The channel plates of the CE chip were fabricated by ultraviolet (UV)-initiated polymerization of the prepolymerization solution of methyl methacrylate (MMA) between a nickel template and a commercially available PMMA plate under the ambient pressure. Subsequently, the PMMA channel plate and the cover were bonded together at a lower temperature (95 °C) below the T_g of PMMA with the aid of the in situ polymerization of a layer of the prepolymer of MMA coated on the cover. The ease, simplicity, versatility, and low cost of the new fabrication route thus make it extremely attractive for the mass production of PMMA electrophoresis microchips. The feasibility and performance of the PMMA chips bonded by the new method have been demonstrated by separating and detecting dopamine, catechol, three cations, and three organic acids in connection with end-column amperometric detection (AD) and contactless conductivity detection (CCD).

2. Experimental

2.1. Reagents

Methyl methacrylate (MMA), benzoin ethyl ether (BEE), and 2-2′-azo-bis-isobutyronitrile (AIBN), sodium oxalate, sodium potassium tartrate, sodium acetate, methyl ammonium, ammonium chloride, sodium chloride were all purchased from Shanghai Chemical Reagent Company (SinoPharm, Shanghai, China). The commercial MMA and AIBN should be purified prior to use. The MMA was purified by the following steps as conventional method: removing hydroqunone (a inhibitor) with 5% sodium hydroxide aqueous solution, washing with deionized water, dehydration with anhydrous magnesium sulfate, and distillation under vacuum. High-purity AIBN was prepared by recrystallization using hot methanol as solvent. Dopamine, catechol, histidine (His), and 2-(*N*-morpholino)-ethanesulfonic acid (MES), were all obtained from Sigma (St. Louis, MO, USA). Stocking solutions (10 mM) of dopamine and catechol were both prepared in doubly distilled water (Medical Center of Fudan University, Shanghai, China). Appropriate amounts of methyl ammonium, ammonium chloride, sodium chloride, sodium oxalate, sodium potassium tartrate, and sodium acetate were all dissolved in doubly distilled water to reach the final concentration of 100 mM. Other chemicals were all analytical grade. The analysis of dopamine and catechol was performed with a 10 mM phosphate buffer (pH 6.5). The run buffer for the separation of cations and organic acids was a MES/His buffer (20 mM each, pH 6.1). Samples solutions were prepared by diluting the stock solutions in the corresponding running buffer solutions. Microscopic glass slide (75 mm × 25 mm × 1 mm) were received from Shanghai Jinglun Industrial Glass Co. Ltd. (Shanghai, China).

2.2. Microfabrication procedure

The PMMA microchips (16 mm × 70 mm) had simple cross layouts, with the four-way injection cross-connected to the three reservoirs and the separation channel. The PMMA chip consisted of a 60 mm-long separation channel (between the injection cross and the detection reservoirs) and a 5 mm-

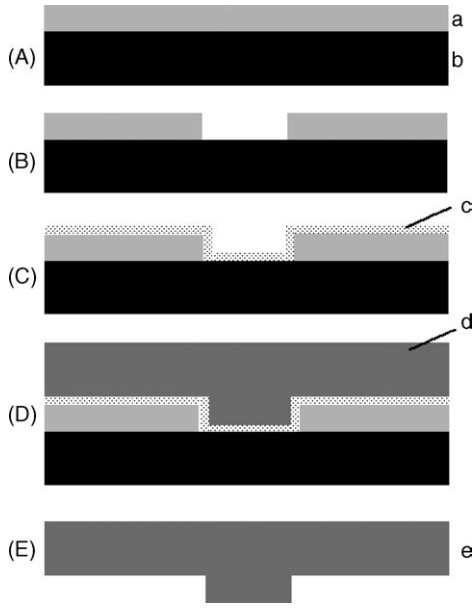

Fig. 1. Metal template fabrication using UV-LIGA: (A) a layer of SU-8 photoresist (a) was spin-coated on a silicon wafer (b); (B) exposure and development of the photoresist (a) provided the surface pattern on (b); (C) seed layer (c) was sputtered on the surfaces; (D) electrodeposition of nickel (d); and (E) the SU-8 photoresist was removed to release the nickel template (e). Dimensions are not in scale.

long injection channel. The two channels crossed each other halfway between the sample and the unused reservoirs, at 5 mm from the run buffer reservoir. The nickel master template composed of a positive relief structure of nickel for the channels was made at the Research Institute of Micro/Nano Science and Technology in Shanghai Jiaotong University (Shanghai, China) by using a SU-8 (a negative photoresistor) based UV-LIGA (in German, Lithographie, Galvanoformung, Abformung) technology [29].

The fabrication processes of the nickel template were illustrated in Fig. 1. The designs for the templates were drawn using a standard CAD software to generate a chrome mask. The channel network was represented by 50-μm-wide chrome lines on a transparent background. A 4-inch diameter silicon wafer (Fig. 1(b)) was oxidized to create a 0.6-μm-thick SiO_2 layer. The wafer was rinsed with acetone, ethanol, and deionized water in sequence, and then dried at 200 °C for 4 h. A negative tone photoresist (SU-8 50, Microchem Corp., Newton, MA) layer (Fig. 1(a)) was spin-coated onto the wafer. The rotation speed was raised to 500 rpm with an accelerating ramp of 100 rpm/s and hold at this speed for 5 s to allow the resist to cover the entire surface (the spread cycle). And then, the speed was ramped to 2000 rpm at an acceleration of 100 rpm/s and hold at this speed for a total of 30 s (the spin cycle). The preexposure bake of the photoresist was performed in a programmable convection oven. The temperature was kept at 65 and 95 °C for 10 min and 15 min, respectively, and was allowed to decrease to room temperature slowly. After bake, the mask was placed on the photoresist-coated silicon wafer and exposed to UV-light for 100 s using a Karl Suss MA6/BA6 mask aligner (Karl Suss, Germany). A post-exposure bake was carried out at 65 °C (for 3 min) and 95 °C (for 10 min) to cross-link the exposed photoresist that was allowed to cool down gradually. Subsequently, the wafer was developed using XP SU-8 developer (Microchem Corp., Newton, MA) for 90 s. After the substrate was rinsed briefly with isopropyl alcohol and dried with a gentle stream of air, a seed layer (0.1-μm-thick copper, Fig. 1(c)) was deposited on the surface of the photoresist layer (bearing the negative relief of the designed microstructure) by using sputtering technology. Then nickel (Fig. 1(d)) was electrodeposited on the copper-coated photoresist in a bath containing 330 g/L nickel(II) sulfate heptahydrate, 45 g/L nickel(II) chloride hexahydrate, and 30 g/L boric acid at pH of 4.3–4.5. The temperature of the bath was kept at ∼50 °C. The electrodeposition was carried out continuously at the current density of 10 mA/cm^2 for 120 h when the thickness of the nickel template reached ∼1 mm. After the electroplating, the photoresist mold was removed to release the replica nickel template (Fig. 1(e)).

Higher current density will generate the form of hydrogen that may be entrapped in the nickel substrate. In this work, a low current of 10 mA/cm^2 was employed for the electrodeposition considering the time and the quality of the nickel template. In addition, continuous agitation was necessary to enhance the mass transfer in the bath so that high-quality template could be produced.

2.3. Molding procedure

The microchip substrate has been templated by UV-induced polymerization micromolding of prepolymer solutions of MMA between the nickel template and a PMMA plate. The molding solution was prepared by the prepolymerization of MMA. BEE (0.15%, w/v, the UV-initiator) and AIBN (0.15%, w/v, the thermal initiator) were dissolved in MMA and the clear mixed solution in a conical flask was allowed to prepolymerize to generate a dense prepolymer molding solution in an 85 °C water bath for ∼15 min under nitrogen flow. Because the mixture solution contained the UV-initiator, the conical flask was immersed in the water bath except the bottleneck was left outside to minimize the effect of light. At the prepolymerization time of 8 min, a partial solution was taken out for bonding the chip and will be referred to as the bonding solution. The viscosity of the molding solution was directly affected by the prepolymerization time. Upon increasing the prepolymerization time above 16 min, the molding became difficult because the viscosity of the molding solution was too high. The bonding solution and the molding solution can further polymerize with the aid of heat or UV-light because they contain not only the thermal initiator, but also the UV-initiator. The containers for both solutions should be wrapped with aluminum foils or dark papers. Both bonding and molding solutions were stored in a 4 °C refrigerator and could be used for at least one month. The molding solution could polymerize under UV-light or

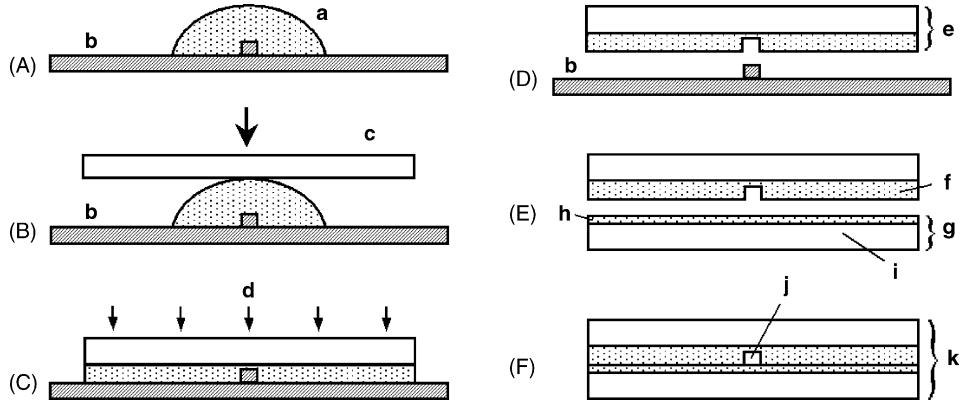

Fig. 2. Schematic of the microfabrication process: (A) casting the MMA prepolymer molding solution (a) on a nickel template (b); (B) sandwiching (a) between a commercially available PMMA plate (c) and (b); (C) exposing the sandwich mold to the UV-light (d); (D) demolding of the PMMA channel plate (e); (E) covering the cover plate (g) on (e); and (F) thermal sealing of (e) and (g) to form the complete microchip (k). (f) the synthesized PMMA layer with microchannels replicated inside; (h) the synthesized PMMA layer of (g); (i) a commercially available PMMA plate. Dimensions are not in scale.

sunlight. Prior to use, the solutions were taken out from the refrigerator and put inside a dark box, allowing to be warmed up to the room temperature.

Fig. 2 shows the schematic of the microfabrication process. To fabricate the PMMA channel plate (Fig. 2(e)), adequate amount of the molding solution (about 1.5 ml, Fig. 2(a)) was cast directly on the nickel template (Fig. 2(b)) along the raised separation channel (see Fig. 2A). Note that the molding solution should be cast slowly to avoid bubbles be entrapped. Subsequently, a piece of commercially avaliavle PMMA plate (70 mm × 16 mm × 1 mm, Fig. 2(c)) was carefully covered on the prepolymerized molding solution and pressed slightly by hand (Fig. 2B). The molding solution spread in the interspaces between the nickel template and the PMMA plate. The pressure was left until that all the interspaces were filled by the molding solution. The excess molding solution could flow out and agglomerated along the edge of the PMMA plate on the nickel template and could prevent the ingress of air bubbles as a result of the shrinkage during in situ polyperization. As shown in Fig. 2C, the molding solution sandwiched between the PMMA plate and the nickel template was exposed to UV-light (Fig. 2(d), 365 nm lamp, 20 W, Shanghai Jinguang Lamp Co. Ltd., Shanghai, China) through the PMMA plate. Complete polymerization of the solution was accomplished within 1 h under ambient conditions. During the polymerization, the images of microchannels on the nickel template were precisely replicated into the synthesized PMMA layer (Fig. 2(f)). Demolding was accomplished by sonicating the mold in a 40 °C water bath for 10 min (see Fig. 2D). The scanning electron micrograph of the cross sections of an unsealed microchannel in PMMA substrate was shown in Fig. 3. The channel was approximately 40 μm deep and 50 μm wide.

To fabricate the PMMA cover plate (Fig. 2(g)), about 1.0 ml of the molding solution mentioned above was cast on a piece of microscopic glass slide (75 mm × 25 mm × 1 mm) along the midline from one side to another side. Subsequently, another PMMA plate (70 mm × 16 mm × 1 mm, Fig. 2i) was put on the molding solution and squeezed until a film without bubble was formed between the PMMA plate and the glass side and was exposed to UV-light (365 nm) for 1 h to produce the PMMA cover plate. The PMMA cover sheets were produced by in situ polymerization of the molding solution between two glass slides (75 mm × 25 mm × 1 mm) with 100 μm thick plastic film spacers to define the thickness. The bonding between the PMMA and the glass slide was very strong after in situ polymerization. A demolding method based on expansion and contraction was employed in the work. Prior to demolding, the PMMA covers bonded to the glass slide was immersed in a 70 °C water bath for 1 min. And then, it was flush with tap water for 1 min. Due to the different thermal expansion coefficients of glass and PMMA, the PMMA covers could be easily separated from the glass

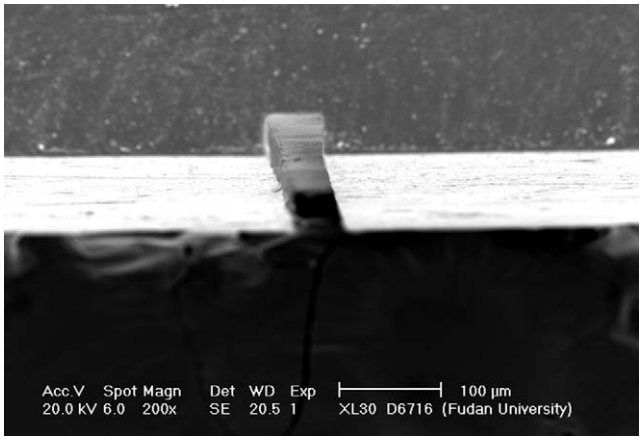

Fig. 3. Scanning electron micrograph of the cross section of an unsealed microchannel in the PMMA substrate. Conditions: accelerating voltage, 20 kV; magnification, ×200.

slide. Note that the molding solution for the fabrication of the channel plate and the blank cover should be the same.

2.4. Thermal sealing

As shown in Fig. 2E, the PMMA cover plate were bonded with the channel plate to fabricate the complete CE microchips (Fig. 2(k)) that were coupled with the amperometric detector in this work. Prior to sealing, the edges of the channel plate (Fig. 2(e)) were cut off (with a saw) and 2 mm-diameter access holes were drilled at the ends of channels to create reservoir ports. The channel plate (Fig. 2(e)) and the cover plate (Fig. 2(g)) were cleaned by sonicating in water and isopropanol for 1 min and were dried under a stream of nitrogen. About 100 μl of the bonding solution was dropped on a microscopic glass slide (75 mm × 25 mm × 1 mm), and then the synthesized layer of the cover plate (Fig. 2h) was covered on it for 30 s. The bonding solution can spread very fast to form a liquid film between the glass slide and the PMMA cover. And then, the cover plate was pulled away in the parallel direction to the microscopic glass slide and the coating was allowed to expose in the air for about 15 s at room temperature. This so-called "sandwich coating" provided a simple and convenient way to form uniform coatings without a spin-coater. Subsequently, the channel plate and the cover plate were put together with the newly synthesized surfaces touched face-to-face and sandwiched between two glass slides (75 mm × 25 mm × 1 mm), clamped together using six binder clips (25 mm, Shanghai Stationery Co. Ltd., Shanghai, China) beside both sides of the separation channel of the microchip and were placed in a 95 °C convection oven for 20 min. Each side was clamped with three clips. The bonded chip was then allowed to cool slowly to the room temperature and was removed from the glass slides. The microchips were stored dry at laboratory temperature until further use. The cover plate could be replaced by the 100 μm cover sheet to fabricated the microchip for the contactless conductivity detection.

2.5. Apparatus

Details of the microchip CE system coupled with amperometric detection were illustrated in Fig. 4. Plexiglas holders (h) were fabricated for housing the separation chip (a) and the detection reservoir (m) allowing their convenient replacement and reproducible positioning, with silicone grease providing proper sealing. A self-positioning electrode system designed previously [30] for miniaturized CE system has been modified and integrated in the detection reservoir. A three-electrode amperometric detection system was fabricated in the detection reservoir (at the channel outlet side, see Fig. 4) and consisted of a platinum wire auxiliary (n), an Ag/AgCl wire reference (o), and a carbon disc (q) electrode. The 320 μm diameter carbon disc electrode (q) was placed opposite the channel outlet (g) via the stainless-steel guiding tube (p, 500 μm I.D. × 800 μm O.D.). The detection reservoir of the PMMA microchip was cut off to facilitate the end-column electrochemical detection. The effective length of the separation channel was 50 mm (from cross-section to the channel outlet). Short pipette tips were inserted into the holes of the various reservoirs. Platinum wires (l), inserted into the individual reservoirs on the holders, served as contacts to the high-voltage power supply. The end of the guiding tube (p) outside the detection cell (m) was sealed by silicone grease not only to immobilize the detection electrode (q), but also to prevent solutions from leaking. The gap distance between the disc electrode (q) and the channel outlet (g) was adjusted to 50 μm approximately by comparison with the width of the channel (50 μm) while being viewed under a microscope [31]. The width of the groove in the PMMA holders (h) is a little bigger than that of the microchip (a), allowing the adjustment of the position of the microchip slightly to accomplish a good alignment with the detection electrode because the channel width (50 μm) to the diameter (320 μm) of the disc working electrode was at a ratio of ∼1:6. A 2 mm thick high-elasticity silicon rubber plate (16 mm × 15 mm) was attached to the bottom of the microchip and subsequently sandwiched

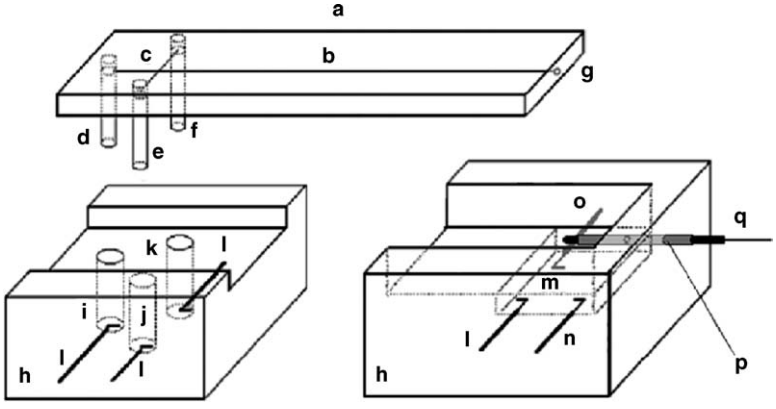

Fig. 4. Schematic diagram of the CE microchip coupled with end-column amperometric detection. (a) PMMA microchip, (b) separation channel, (c) injection channel, (d) pipette tip for buffer reservoir, (e) pipette tip for buffer reservoir (not used), (f) pipette tip for sample reservoir, (g) channel outlet, (h) Plexi glass bodies, (i) buffer reservoir, (j) reservoir not used, (k) sample reservoir, (l) high voltage power electrodes, (m) detection reservoir, (n) auxiliary electrode, (o) Ag/AgCl wire reference electrode, (p) stainless-steel guiding tube, (q) working electrode. Dimensions are not in scale.

between a 3 mm thick PMMA plate and the right holder (h) in Fig. 4 with the aid of two screws (not shown). It facilitated the movement of the microchip up and down to align the channel outlet to the detection electrode. Amperometric detection was performed with a CHI 830B electrochemical analyzer (Shanghai Chen-Hua Instruments Co., Shanghai, China) using the "amperometric *i–t* curve" mode.

A capacitively coupled contactless conductivity detector (CCD) was used for the detection of ionic species [32]. The circuit contained a RC filter (time constant, 0.01 s), followed by a voltage follower (LF 356) to the circuit output, and allowed convenient data reading. A VC 2002 function generator (Shenzhen Victor Electronics Co. Ltd., Shenzhen, China) was used for generating the sinusoidal signal that was applied in the CCD detection electrode pairs on the cover sheet of the PMMA electrophoresis microchip. The electronic circuit was placed in a shielding box to protect the electronics from external electric fields. The open side of the box was placed as close as possible to the detection reservoir, to act also as a shield for the sensing electrode system. A N2000 chromatographic workstation (Zhejiang University Star. Information Technology Co. Ltd., China) connected to a P4 2.5 GHz personal computer with 256 MB RAM was used to record electropherograms. The electronic components were purchased from local suppliers. Scanning electron micrograph (SEM) of the cross section of the unsealed microchannel in the PMMA substrate was obtained with a PHILIPS XL 30 scanning electron microscope (Netherlands). Microscopic images were obtained with a Motic video microscope (DS300, Motic-Optic Group, Xiamen, China), which was connected to a computer and controlled by Motic software.

2.6. Electrode fabrication

A piece of copper wire (10 cm long, 150 μm diameter) was inserted into a 3.0 cm length fused silica capillary (320 μm I.D. × 435 μm O.D., Polymicro Technologies, Phoenix, AZ, USA) and a 2 mm opening was left in the capillary for the subsequent filling of the graphite-epoxy composite. The other end of the capillary was sealed together with copper wire by quick epoxy. Epoxy resin and hardener (Zhejian Cixi Tiandong Adhesive Co. Ltd., Ningbo, China) was mixed thoroughly at a weight ratio of 2:1. The graphite powder and epoxy resin/hardener were hand-mixed in a ratio of 1:1 (w/w). The graphite-epoxy composite was subsequently packed into the capillary by pressing the opening end of the capillary (to a depth of ∼2.5 mm) into a sample of the composite. The graphite-epoxy composite should touch the end of the copper wire inside the capillary tightly for conductive contact. The composite was then allowed to cure at room temperature for at least 3 h.

Details of the fabrication of the CCD electrodes were reported earlier [32]. The rectangular-shaped electrodes (0.8 mm × 10 mm) were fabricated from two 10 μm thick aluminium-foil strips. The end side of the electrode was widened to 4 mm to facilitate the electrical connection. The electrodes were fixed on the top of the 100 μm thick PMMA cover sheet using a common epoxy with a distance of 800 μm between them. The electrode pairs were located at the end of the separation channel and the effective length (from the injection cross to the CCD electrodes) of separation channel is 50 mm. The thin copper wires (20 mm long) were attached to the electrodes using a conducing epoxy (Chemtronics, Kennesaw, GA, USA) and were connected to the detector electronics. The electrodes were placed in an 'anti-parallel' orientation to minimize the stray capacitance between them and to enhance the *S*/*N* ratio. The CCD electrode pair above the separation channel could detect the variance of the conductivity in the channel through the thin cover sheet.

2.7. Electrophoretic procedures

The channels of the PMMA chip were treated before use by rinsing with deionized water for 10 min. The "buffer" reservoirs were filled with the corresponding CE run buffer, while the "sample" reservoir with the sample mixture. A ±3000 V high-voltage dc power supply (Tianjin Lansilai Electronic Tech Co. Ltd., Tianjin, China) provided a voltage for the electrophoretic separation and the electrokinetic sample introduction. The injections were performed by applying the desired potential between the "sample buffer" and the "grounded-detection" reservoirs for 3 s (for dopamine and catechol) and 1 s (for cations and organic acids) while all other reservoirs floating. Separations were performed by switching the high-voltage contacts and applying the corresponding separation voltages to the "running buffer" reservoir with the "detection" reservoir grounded and all other reservoir floating.

3. Results and discussion

In the typical manufacturing procedures for PMMA microchannels such as imprinting [9], commercially available PMMA plates are usually used. The variance of the properties of the commercial PMMA plates may affect the microchip fabrication including bonding parameters (such temperature, pressure, time, etc.). In this work, PMMA microchannels were fabricated by in situ polymerization on the surface of commercially available PMMA plates, this will provide a potential method to control the material characteristics of PMMA microchannels by adding some functional compounds to the molding solution. The ease, simplicity, versatility, and low cost of the new fabrication route make it extremely attractive for the mass production of PMMA electrophoresis microchips. The fresh layers of PMMA channel plates and cover plates (Fig. 2(f) and (h)) can obviate the extensive cleaning and allow high-quality bonding. As shown in Fig. 3, a high quality microchannel can be achieved by the surface molding. The replica of the nickel template results in high-quality structures without observable changes in the channel geometry.

In this work, both UV- and thermal-initiators have been mixed with MMA to form a highly efficient initiate system. The thermal initiator, AIBN, allowed MMA to prepolymerize to generate a thin bonding solution and a viscous molding solution in a water bath. The thermal initiator in the bonding solution allowed the bonding solution to polymerize completely during the thermal bonding, while the UV-initiator in the molding solution could initiate the further polymerization of the molding solution to form the channel plate and the blank covers. Because the image of the relief on the nickel template was precisely replicated into just a thin layer of synthesized PMMA (Fig. 2(f)) on the surface of a commercially available PMMA plate (Fig. 2(c)), the heat released from the UV-initiated polymerization was minimized and the molding procedure was greatly simplified. Although the volume shrinkage of the molding solution was inevitable during molding, no bubble formation was found because no rigid space or frame was sandwiched between the nickel template (Fig. 2(b)) and the PMMA plate (Fig. 2(c)) in this work. As shown in Fig. 2F, the microchip was composed of four layers. The synthesized layers (Fig. 2(f) and (h)) that formed the microchannel (Fig. 2(j)) were sandwiched between two commercial PMMA plates (Fig. 2(c) and (i)).

In order to form capillaries, the microchannels, which are open after the fabrication steps have to be closed, without clogging the channels, changing their physical parameters or altering their dimension. This often represents a big challenge for the microchip fabrication [5]. In the case of PMMA microchips, thermal bonding techniques are commonly used because homogeneous internal surfaces can be obtained. The PMMA substrates were heated above its T_g (105 °C) and pressures were applied on them for bonding, the higher bonding temperature (for example, 108 °C, [9]) employed may cause microchannel deformation and affect the yield. It is a challenging task to decrease the bonding temperature below the T_g of PMMA substrate to alleviate the microchannel deformation. In the present study, in situ polymerization has been introduced to the thermal bonding of PMMA substrates to decrease the bonding temperature to 95 °C. After the PMMA cover was coated with the bonding solution, the coatings had to be allowed to dry in the air for a short time (typically 15 s at room temperature) before bonding. In this step, partial MMA evaporated to form a very thin layer of semi-solid coating. Because the coating contained both low molecular weight PMMA and MMA directly touched the synthesized PMMA channel plate (Fig. 2(e)), the T_g of the surface layer of the channel plate could be decreased to some extent, allowing a low temperature bonding below 105 °C. When the channel plate and the cover were clamped together, the low-fluidity of the coating could alleviate blocking of the microchannels. When the bonding device was put in a 95 °C convection oven, the layer of prepolymer coating containing low molecular weight PMMA, MMA and initiators became softer and interacted with the internal surface of the channel plate under the applied pressure. The synthesized layers of the channel plate and the cover (Fig. 2(f) and (h)) contained the thermal initiator, some unpolymerized groups could further cross-linked with the thin layer of coating on the blank cover under heat and pressure. Meanwhile, the layer of prepolymer containing MMA will further polymerize in situ to realize the final bonding. The channel cross sections, after thermal bonding, are shown in Fig. 5.

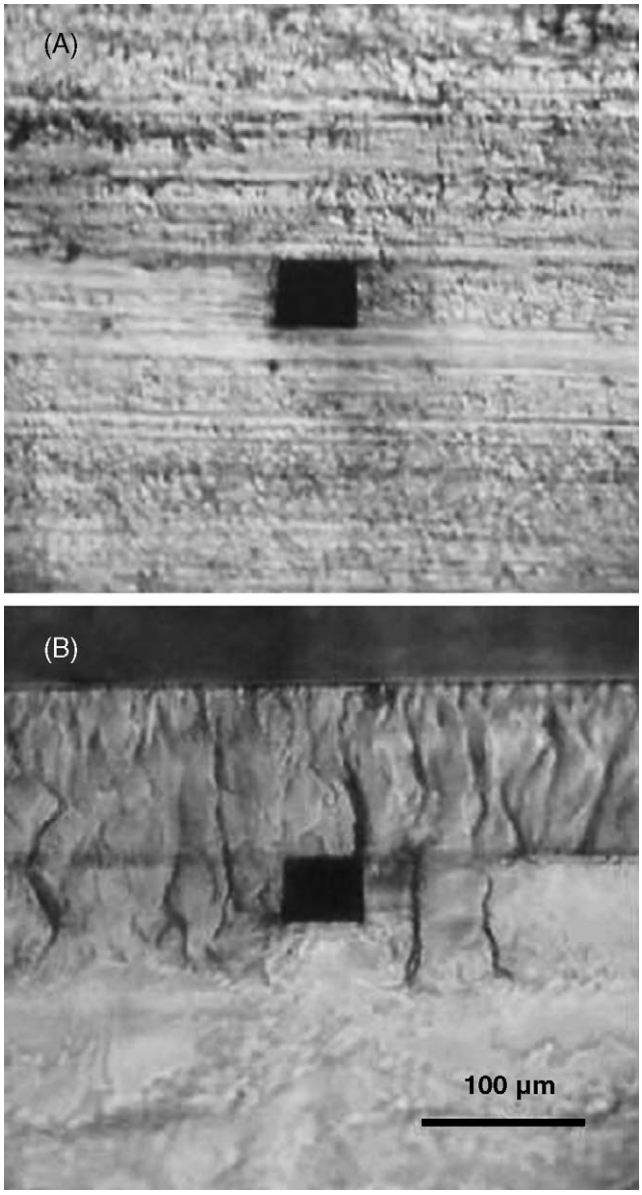

Fig. 5. Microscopic images of the cross sections of the channels in the complete PMMA electrophoresis microchips bonding with (A) a cover plate and (B) a 100 μm cover sheet. Magnification, ×100.

The content of the solid contents in the bonding solution was determined to be 11.2% (w/w) based on the weight variance of a certain amount the bonding solution that was exposed in the air until the weight became constant. Prepolymer of MMA is the main content of the left solid. Its content in the bonding solution increases with the prepolymerization time. The optimal prepolymerization time for bonding solution is in the range of 7–9 min. Upon raising the time above

9 min, the bonding solution becomes too dense to be coated on the PMMA covers. The prepolymer of MMA serves as a host for the free MMA and can reduce its volatilization. The thickness of the prepolymerized MMA layer coated on the blank cover (Fig. 2(g)) is important as channel filling is known to depend on adhesive layer thickness [33,34]. In this work, a thin layer of prepolymer of MMA was coated by the novel "sandwich coating" method mentioned above. The thickness of the coating was measured to be approximately 4 μm. Although it was found that the adhesive layer protruded inside the channel for about 1–2 μm during bonding (see Fig. 5), the low temperature thermal bonding of the channel plate and the blank cover by in situ polymerization resulted in good sealing without any deleterious effects. The channels were complete and intact, indicating the low microchannel deformation during the low temperature bonding.

Because PMMA chip are transparent, microscopic images were used to check the bonding quality. The PMMA electrophoresis microchip with a thick PMMA cover plate was sawed and scraped to obtain the channel cross-section (as shown in Fig. 5A) while the channel was flushed with water to prevent the PMMA scrapings from entering the channel. The novel bonding method provided no observable boundary between the two plates indicating a high quality sealing. The PMMA microchip with a thin PMMA cover sheet was about 1.1 cm thick and could be broken along the notch (perpendicular to the separation channel) on the external surface of the channel plate to prepare the channel cross-section (Fig. 5B). The fact that the furrows on the cross section are continuous demonstrated that the PMMA cover sheet and the PMMA channel plate have merged during the in situ polyperization bonding process. Fig. 6. Illustrates the microscopic images of the injection-cross section of the PMMA channel plate before (A) and after (B) bonded with the PMMA cover plate. The replication quality of the injection cross section in the PMMA substrate is satisfactory. After carefully examining the final PMMA microchips by an optical microscope, it was observed that the main channel body and cross section were complete and intact after the bonding process, as shown in Fig. 6B. Such a bonding process provided no observable changes between the two plates along with the absence of voids. The crude microchips after bonding were polished with emery sand papers to acquire the final chip with uniform edges.

In this work, the six binder clips (25 mm) applied pressures just beside both sides of the separation channel of the microchip. The average pressure of each binder clip applied on the bonding devices was measured to be approximately 2.47 kg. Although the 1 mm thick glass slides were rigid, they would deform a little bit resulting in a negative pressure gradient from the middle to the sides of the PMMA substrates so that air bubbles could be squeezed out during the thermal bonding process. By using this simple and effective bonding device, the bubbles between the channel plate and the blank cover were greatly reduced so that well-sealed microchips could be obtained. Bubbles were seldom found beside the separation channel because the pressure for bonding was directly applied there. Because the bonding temperature (95 °C) is below the glass transition temperature (T_g, 105 °C) of PMMA, the deformation of the channel in the PMMA substrate was minimized to some extent so that a higher yield of great than 90% ($n = 120$) could be achieved for the production of the complete microchips in comparison with the glue bonding (60%) [12]. Recently, Chen et al. have demonstrated the PMMA channel plate and the cover plate could be sealed by vacuum-assisted thermal bonding with high yield up to 90% [35]. The bonding was performed in a vacuum-heating oven for 60 min at 112 °C. The bonding yield in our study is comparable to Chen's work [35], while both the time (20 min) and temperature (95 °C) for bonding have been significantly reduced. The bonding between the blank cover and the channel plate is fair strong, allowing the microchip to be sawed, scraped, polished, filed, and broken without debonding. The temperature is a key parameter for bonding the PMMA substrates based on the in situ polymerization. It has been found that satisfactory bonding could be achieved over the temperature range of 90–100 °C. Upon raising the temperature above 105 °C, the microchannel readily tends to be blocked due to the deformation of the PMMA substrates. In addition, the bonding is poor at temperatures below 90 °C.

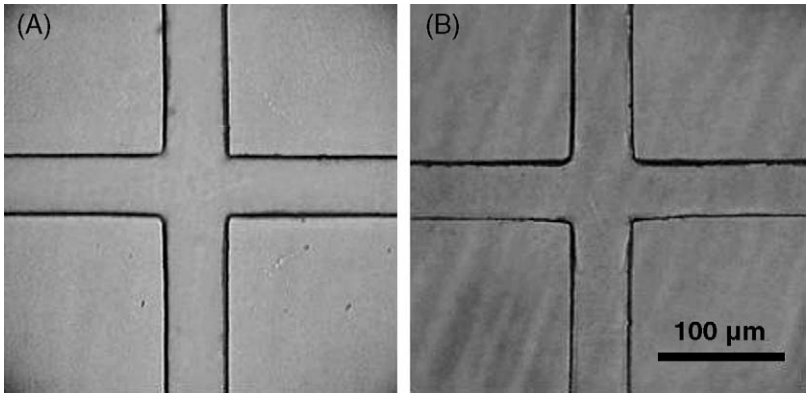

Fig. 6. Microscopic images of the injection-cross section of the PMMA channel plate before (A) and after (B) bonded with the PMMA cover plate. Magnification, ×100.

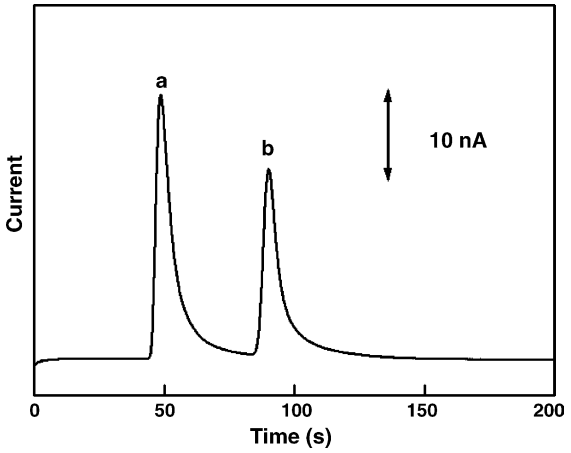

Fig. 7. Electropherograms for a mixture solution containing 0.2 mM dopamine (a) and catechol (b) at the PMMA microchip. Operation conditions: run buffer, 10 mM phosphate buffer (pH 6.5); separation and injection voltage, +1500 V; injection time, 3 s; working electrode, 320 μm diameter carbon disc electrode; detection potential, +0.8 V (vs. Ag/AgCl wire).

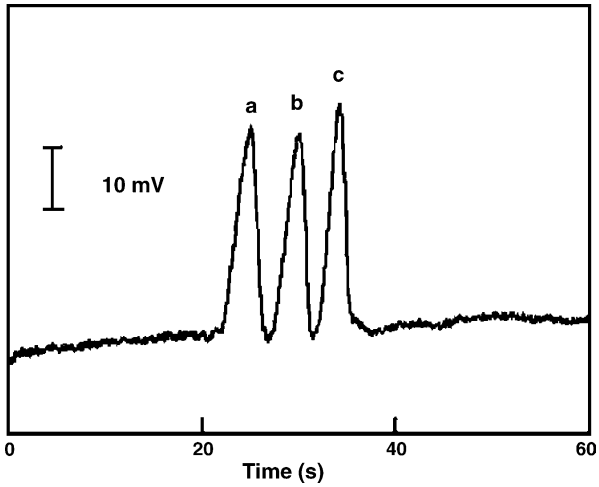

Fig. 8. Electropherograms for a mixture containing 1 mM ammonium (a), methyl ammonium (b), and sodium (c) using contactless conductivity detector. Operation conditions: separation voltage, +1500 V; injection voltage, +1500 V; injection time, 1 s; running buffer, 20 mM MES-20 mM His (pH 6.1); sinus waveform with a frequency of 200 kHz; and a peak-to-peak voltage of 10 V; electrode distance, 0.8 mm; electrode width, 0.8 mm.

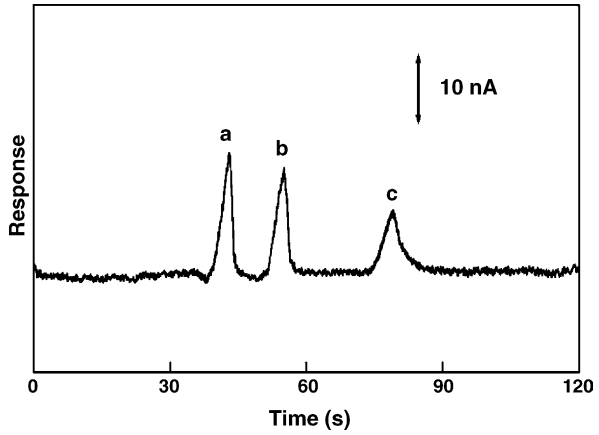

Fig. 9. Electropherograms for a mixture containing 2 mM oxalate (a); tartrate (b); and acetate (c) ions using contactless conductivity detector. Operation conditions: separation voltage, −1500 V; injection voltage, −1500 V. Other conditions as in Fig. 8.

The analytical performance of the molded PMMA electrophoresis microchips was demonstrated by the separation of organic molecules and ionic species coupled to end-column amperometric and contactless-conductivity detection. Catecholamine analytes are commonly used for assessing the separation in microfluidic devices in connection with amperometric detection [17,18]. As indicated from Fig. 7, the PMMA electrophoresis microchip provides baseline-resolved well-defined peaks for 200 μM dopamine and catechol within 100 s. The number of theoretical plates for dopamine was determined to be greater than 6780 plates/m. The half peak widths of dopamine and catechol are 6.2, and 5.9 s, respectively, with the corresponding sensitivities of 143.5 and 102.1 nA/mM. The detection limits ($3 \times S/N$) of dopamine and catechol are 0.21, and 0.29 μM, respectively. Note that the amperometric detector of chip was placed outside the separation channel, leading to an additional band broadening and hence to lower the plate numbers. To demonstrate the performance of the PMMA electrophoresis microchip with a thin cover sheet, it was coupled with the contactless conductivity detector for the separation and detection of ammonium, methyl ammonium, and sodium, three cations of environmental concern, simultaneously. These cations are baseline separated within 40 s (Fig. 8). The microchips fabricated by the new method provided also a well-defined peak shape and high plate numbers (14765, 28390, and 38683 plates/m for ammonium, methylammonium and sodium, respectively) under the selected conditions.

To further illustrate the applicability of the PMMA electrophoresis microchip with CCD, three organic acids have been separated. Fig. 9 shows the electropherograms of three small organic anions, oxalate, tartrate, and acetate at the PMMA chip coupled with a CCD detector. All the three organic acids can be baseline separated within 90 s with well-defined peak shapes. The precision was examined from a series of nine repetitive injections of a sample mixture containing 2 mM oxalate and tartrate. Reproducible signals were obtained with relative standard derivation (RSD) values of 3.7% (oxalate) and 4.1% (tartrate) for the peak heights ($n = 9$). Determination of organic acids is of high importance for the food analysis. This efficient CE-CCD microsystem should also find more application in determination of these organic acids in the biological matrices owing to their clinical and biotechnological significance.

4. Conclusions

It has been demonstrated that PMMA electrophoresis microchips can be fabricated by molding the PMMA chan-

nel plates with the aid of the nickel templates on the surface of commercially available PMMA plates. A novel approach based on in situ polymerization has been significantly developed for the thermal bonding of PMMA microfluidic chips at a lower temperature (95 °C) than the glass transition temperature of PMMA. This simple and efficient bonding method holds great promise for the mass production of PMMA microfluidic chips. Rapid CE separations of dopamine, catechol, cations, and organic acids were successfully performed on the prepared microdevices with satisfactory results. The nickel template employed in this work can be repeatedly used to mold hundreds of thousands polymer substrates without the fear of fracture. The thermal bonding procedure with the aid of the in situ polymerization ensures high yield for the enclosure of PMMA microchannels. In addition, this entire fabrication methodology may also find more application for the fabrication of other polymer microfluidic systems.

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