Design and Fabrication of 3D-Structured Contactless Capacitive-Type Detector for Capillary Electrophoresis Microchip

CHIA-YEN LEE
Department of Mechanical and Automation Engineering, Da-Yeh University, Changhua, 515, Taiwan

CHE-HSIN LIN
Department of Mechanical and Electro-Mechanical Engineering, National Sun Yat-Sen University, 800, Taiwan

LUNG-MING FU*
Graduate Institute of Materials Engineering, National Pingtung University of Science and Technology, 912, Taiwan
loudyfu@mail.npust.edu.tw

Abstract. Using simple and reliable microfabrication techniques, this study develops a capillary electrophoresis (CE) microchip with 3-dimensional-structured (3D-structured) contactless capacitive detector electrodes mounted parallel to the separation channel. The off-channel electrodes are deposited by Au sputtering and patterned using a standard ‘lift-off’ process. A vacuum fusion bonding process is employed to seal the lower substrate containing the microchannels and electrodes to an upper glass cover plate. The variation in the capacitance between the electrodes in the side channels is measured as different samples and ions pass through the detection region of the CE separation channel. Samples of Rhodamine B and a commercial sports drink are mixed in different buffer solutions and successfully separated and detected using the developed device. The 3D-structured contactless capacitive-type detection device has microscale dimensions and provides a valuable contribution to the realization of the lab-on-a-chip concept.

1. Introduction
Lab-on-a-chip devices have the potential to revolutionize chemical and biological analysis procedures. The capillary electrophoresis (CE) technique is commonly employed to separate ions in a sample since it has a low sample consumption and a good detection resolution.1 Traditional CE systems generally incorporate some form of optical detection system. However, integrating the optic fibers with the on-chip CE system can be challenging, and hence interest in electrochemical detection arrangements is growing. Three basic approaches have been proposed for electrochemical detection systems, namely end-channel,2 in-channel3 and off-channel4 detection. As microfabrication technology has evolved, off-channel detection systems, in which two or more electrodes are positioned at a micro-distance from the separation channel, have attracted particular attention. Such systems permit electrical
measurement to be carried out without the need for special transducers (e.g. photomultipliers). Contactless electrical detectors are universal detectors suitable for all manner of compounds.4

Over the past decade, many different approaches5-7 have been proposed for integrating electrodes within microfluidic devices in such a way that they do not make contact with the solution, but are located at a very small distance from the capillary such that their detection sensitivity is enhanced. Recently, various optical detection methods have been proposed in which optical waveguides such as Su-8/spin-on-glass (SOG)8 or optical fibers9 are buried beside the separation channel. The results have shown that these coupled waveguides enable a successful detection of samples in the separation channels.

The present study designs and fabricates a 3D-structured contactless capacitive-type detector for a capillary electrophoresis microchip. Using simple fabrication processes, 3D-structured capacitor electrodes are deposited in buried side channels located either side of the separation channel. The experimental results show that the device is capable of separating and detecting various samples with a high detection sensitivity. The proposed 3D-structured contactless capacitive-type detector provides a further crucial step towards the realization of the “Lab-on-a-Chip” concept.

2. Experimental Section

2.1. Design

Figure 1(a) presents a schematic illustration of the current cross-form separation and detection CE-chip. In contrast to conventional CE microchips, in which planar electrodes are deposited on just one side of the channel wall, the present design incorporates two buried shielding electrodes located on either side of the channel. In operation, a field effect is generated between these two electrodes and the separated samples are detected by measuring the variation in capacitance as the sample flows through the detection region between the 3D-structured electrodes. As shown in Figure 1(b), the electrodes have a length of 30 µm and are offset from the separation channel by a distance of just 40 µm, thereby enhancing the detection sensitivity of the device. Although not shown in Figure 1(b), the microchannels have a width of 80 µm and the reservoirs have a diameter of 1.5 mm.

Fig.1 Schematic of: (a) cross-shaped microchannel with two short shielding electrodes designed to detect the capacitance variation of the sample flow, and (b) shielding electrodes.

2.2. Microfabrication

The present contactless capacitive-type detector system was fabricated on glass substrates (Assistant Inc., Germany). Prior to fabrication, the substrates were annealed at 400 °C for 4 hours to relieve any internal residual stresses. Figure 2 presents a schematic illustration of the current fabrication process.10 Following annealing, the glass substrates were cleaned in a boiling Piranha solution (H2SO4 (%):H2O2 (%)= 3:1) at 120°C for 10 min. Traditionally, a time-consuming vacuum deposition process is used to fabricate the mask for glass etching in a HF-based etchant. However, this study used a 3-µm thick AZ4620 (Clariant Corp., USA) photoresist layer. The photoresist was spun
coated on the glass substrate and then etched using a wet chemical etching process (Figure 2(a)). A standard UV lithography process (Figure 2(b)) was then used to generate the required configuration of microchannels. Note that the microchannels were designed with a width of 30 µm. The PR layer was then developed by immersing the exposed substrate in a developer solution (AZ400k:DI water=1:3) for 70 seconds (Figure 2(c)). The patterned substrates were then etched in a 6:1 BOE (buffered oxide etchant, J. T. Baker, USA) bath aided by ultrasonic agitation for 30 min to form microfluidic trenches of depth 25 µm following the stripping of the PR layer (Figure 2(d)). The microchannels were formed using a modified glass etching technique with an etching rate of 0.9 µm/min. Importantly, the average surface roughness (Ra) of the etched surface was controlled such that it was less than 45 Å to ensure a high quality of the microchannel surface. The fabrication steps shown in Figures 2(a) – 2(c) were then repeated, as shown in Figures 2(e) – 2(g), to form the off-channel detector electrodes adjacent to the downstream region of the separation channel. After the photoresist stripping process, a thin layer of Cr (0.05 µm) was sputtered into the shielding electrode channels to form an adhesion layer for the subsequent deposition of a 0.4-µm Au layer. (Figure 2(h)). A “lift-off” method was then used to pattern the Au layer to form the two electrical electrodes (Figure 2(i)). Meanwhile, via holes with a diameter of 1.5 mm were drilled in a second glass plate to form sample inlets and outlets (Figure 2(j)). The base plate and the cover plate were then fusion-bonded in a furnace at 580°C for 20 min to form the completed microfluidic device (Figure 2(k)).

Figure 2 Overview of fabrication process used to form 3D-structured contactless capacitive-type detector for microchip capillary electrophoresis

Figure 3 shows a close-up SEM photo of the detection region of the separation channel. Please note that the distance between the separation channels and the electrode channels is 40 µm. The shielding electrode channels let these detection electrodes could be 3D “buried” inside the microchip so that it could measure the capacitance values between the two electrodes beside the channels (Figure 4(a)). Thus measured capacitance values varied as separated ions passing through the detection area of the microchannels.

The electrodes were separated away from the microfluidic channel at a distance of 40 µm. After a photoresist stripping process, the 3D-structured electrodes were formed by using a sputtering process to deposit a 0.4-µm Au layer within the shielding electrode channels. Subsequently, a “lift-off” method was used to form electrical electrodes. Prior to Au deposition, a thin layer of Cr (0.05 µm) was deposited as an adhesion layer. After drilling the cover plate for fluid inlet/outlet holes, a vacuum fusion bonding process was used to seal the microstructures (Figure 4(b)).
3. Results and Discussion

A high voltage programmable power supply (MP-3500, Major Science, Taiwan) was used to generate the electrokinetic force required to inject the sample and drive it through the microchip. The injection step (reservoir 2 → reservoir 3) was driven by a electrical field of 200 V/cm applied over a 0.5-minute loading time, while the separation step (reservoir 1 → reservoir 4) was performed using a electrical field of 300 V/cm applied over a 1.5-minute separation time. Two different test samples were considered in this study, namely: (1) fluorescent dye (Rhodamine B) with a concentration of $3 \times 10^{-4}$ M in a buffer solution of Na$_2$B$_4$O$_7$•10H$_2$O (1 mM, pH = 9.2), and (2) a commercial sports drink (Supau,
Taiwan) in a buffer fluid of 10 mM 2-(N-morpholino)ethanesulfonic acid (MES)/10 mM histidine (His) (pH 6.1), respectively. The reported cation concentrations of the sports drink were 448.5 ppm (Na⁺), 191.1 ppm (K⁺), 24.0 ppm (Ca²⁺) and 6.075 ppm (Mg²⁺). A LCR meter (LCR-821, Good Will, Taiwan) was used to detect the instantaneous capacitance and impedance variations as the separated samples were driven through the detection region of the separation channel. The capacitance values measured during the course of the separation experiments were taken to represent the sample concentration by its velocities induced by the electrokinetic force (EOF) of the different cations, respectively. In order to prevent the sample leakage effect from degrading the detection performance of the microfluidic device, this study employed the double-L injection method shown in Figure 5.

Figure 6 shows the peak capacitance values observed at different times after the initial injection of the sports drink sample over 5 injection/separation cycles with a separation electrical field of 300 V/cm. As the sample travels through the microfluidic device, capillary electrophoresis effects cause the cations to be separated in the microchannel. It is observed that each sample separation has four capacitance peaks, corresponding to the four fragments of the K⁺, Mg²⁺, Ca²⁺ and Na⁺ cations, respectively. Figure 8 confirms the ability of the proposed 3D-structured contactless capacitive-type detector to separate and detect sample fluids.

4. Conclusions
This study has presented an innovative design and fabrication method for a 3D-structured contactless capacitive-type detector designed for capillary electrophoresis microchips. The developed detector comprises microchannels for sample injection and separation, and shielding channels for two buried detection electrodes. The electrokinetically driven sample is separated in the CE channel and the separated cations are detected as they pass through the detection region. The experimental results have shown that the proposed 3D-structured contactless capacitive-type detector is capable of detecting separated samples in the CE channel. The developed device provides a simultaneous sample separation and detection capability, and therefore provides a viable basis for the future development of micro-TAS devices.

Acknowledgments
The current authors gratefully acknowledge the financial support provided to this project by the National Science Council of Taiwan under Grant No's. NSC-94-2320-B-020-001, NSC-94-2320-B-110-003 and NSC-94-2211-E-212-009.

References
[1] W. R. Vanadveer IV, S. A. Pasas-Farmer, D. J. Fischer, C. N. Frankenfeld, S. M. Lunte, Recent Developments in Electrochemical Detection for Microchip Capillary Electrophoresis, Electrophoresis 25 (2004) 3528-3549.

[2] J. Wang, B. Tian, E. Sahlin, Micromachined Electrophoresis Chips with Thick-Film Electrochemical Detectors, Analytical Chemistry 71 (1999) 5436-5440.

[3] R. S. Martin, K. L. Ratzlaff, B. H. Huynh, S. M. Lunte, In-Channel Electrochemical Detection for Microchip Capillary Electrophoresis Using an Electrically Isolated Potentiostat, Analytical Chemistry 74 (2002) 1136-1143.

[4] P. S. Vuorinen, M. Jussila, H. Sirén, S. Palonen, M. Riekkola, Integration of a Contactless Conductivity Detector into a Commercial Capillary Cassette: Detection of Inorganic Cations and Catecholamines, Journal of Chromatography A 990 (2003) 45-52.

[5] J. Tanyanyiwa, P. C. Hauser, High-Voltage Capacitively Coupled Contactless Conductivity Detection for Microchip Capillary Electrophoresis, Analytical Chemistry 74 (2002) 6378-6382.

[6] J. Wang, G. Chen, A. Muck, Movable Contactless-Conductivity Detector for Microchip Capillary Electrophoresis, Analytical Chemistry 75 (2003) 4475-4479.

[7] J. Wang, M. Pumera, Dual Conductivity/Amperometric Detection System for Microchip Capillary Electrophoresis, Analytical Chemistry 74 (2002) 5919-5923.

[8] G. B. Lee, C. H. Lin, G. L. Chang, Micro Flow Cytometers with Buried SU-8/SOG Optical Waveguides, Sensors and Actuators A 103 (2003) 165-170.

[9] L. M. Fu, R. J. Yang, C. H. Lin, Y. J. Pen, G. B. Lee, Electrokinetically Driven Micro Flow Cytometers with Integrated Fiber Optics for On-Line Cell/Particle Detection, Analytica Chimica Acta 507 (2004) 163-169.

[10] L. M. Fu, R. J. Yang, G. B. Lee, H. H. Liu, Electrokinetic Injection Techniques in Microfluidic Chips, Analytical Chemistry 74 (2002) 5084-5091.

[11] L. M. Fu, C. H. Lin, High-Resolution DNA Separation in Microcapillary Electrophoresis Chips Utilizing Double-L Injection Techniques, Electrophoresis 25 (2004) 3652-3659.