MICROFABRICATED ELECTROCHEMICAL DETECTOR FOR CAPILLARY ELECTROPHORESIS

Richard J. Reay, Rajeev Dadoo, Christopher W. Storment, Richard N. Zare, and Gregory T. A. Kovacs*

*** To whom correspondence should be addressed. G. T. A. Kovacs, Department of Electrical Engineering, Stanford University, Room CIS 130, Stanford, CA 94305-4070, email: kovacs@glacier.stanford.edu**

R. J. Reay, C. W. Storment and G. T.A. Kovacs are with the Department of Electrical Engineering, Stanford University. R. Dadoo and R. N. Zare are with the Department of Chemistry, Stanford University.

ABSTRACT

A microfabricated electrochemical detector, suitable for retrofitting existing capillary electrophoresis (CE) systems and providing them with improved analytical capabilities has been demonstrated. This approach provides the advantages of electrochemical detection and overcomes previous limitations caused by manual fabrication. Deep channels were plasma etched into a silicon substrate to form coupling channels for conventional CE capillaries. Platinum detection electrodes were lithographically patterned on a glass wafer that was aligned and anodically bonded to the silicon, sealing the capillary channels. After dicing, bond wires were attached to exposed bond pads and capillaries were epoxied into the two coupling channels of each detector. Amorphous ("black") platinum was then electrodeposited onto the detection electrodes, completing the device. When used with custom-built conductivity measurement circuitry, Li^+ and K^+ ions were detected at a concentration limit of 0.7 μmolar.

INTRODUCTION

Capillary electrophoresis (CE) is a powerful analytical separation technique for the analysis of complex mixtures. In CE, an unknown sample is introduced at the inlet of a capillary channel filled with a buffer solution, and a high voltage is applied across the length of the capillary. Different constituents of the sample migrate through the capillary at different rates depending on their electrophoretic mobilities. Mobility is a complex function of a particle's charge, mass, and shape in solution. A difference in mobilities allows separation of the sample into its components. By detecting the chemicals passing through the outlet of the capillary as a function of time, and knowing the mobilities of the possible constituents, the chemical composition of the sample can be determined.

Substantial work has already been done in the area of fabricating integrated CE systems. Our original efforts in this area were based on fabricating the entire CE column on a silicon wafer using plasma etching, subsequent growth of a dielectric, and sealing with an anodically bonded glass cover. Successful separations resulted, but without undue efforts to increase the deposited dielectric thicknesses, the high breakdown voltages desired could not be achieved. Other groups have made similar efforts [1, 2, 3] including the use of glass as a substrate to avoid breakdown problems due to thin dielectrics on conductive silicon substrates [4, 5]. We decided to pursue a different approach in which we construct the detector from silicon and use a conventional capillary. This strategy avoids breakdown problems and preserves the capability to take advantage of silicon micromachining and, potentially, on-chip circuitry.

Several detectors for CE have been developed including absorbance, fluorescence, mass-spectrometric, and electrochemical methods. Electrochemi-

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cal detection has certain advantages including sensitivity and selectivity for individual species [6]. In addition, the required electrodes can readily be implemented in a micromachined device. Several different types of electrochemical detection can be used, including conductivity measurement, impedance spectroscopy, amperometry, and voltammetry. Two key problems with electrochemical detection that need to be overcome are isolation of the detection apparatus from the 1 to 30 kV potentials present, and rejection of power supply noise. If the electrodes are not precisely perpendicular to the fluid flow, a large noise signal is coupled into the electrodes from the high voltage power supply. Another difficulty with previous electrochemical detectors is the irreproducible construction and inaccurate placement of the electrodes inside the separation column [7]. This paper describes a batch-fabricated, micromachined electrochemical detector that overcomes these problems.

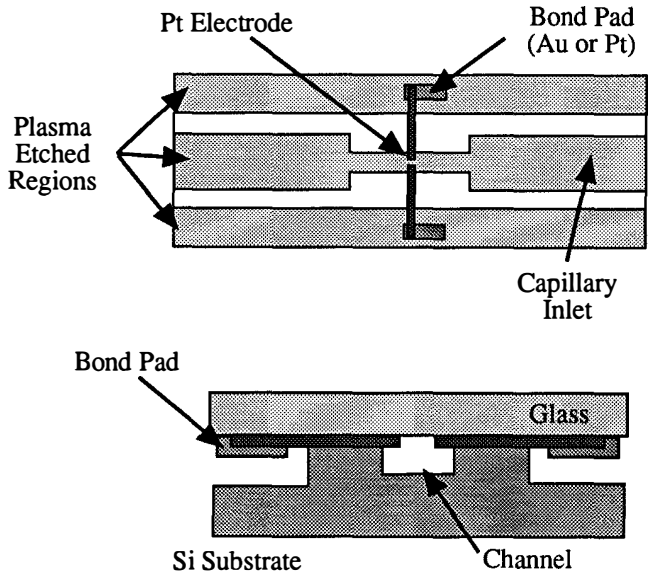

Figure 1: Illustration of the structure of the micromachined capillary electrophoresis detector. The upper view shows the device as seen through the top glass cover. The lower view is a cross-section in the electrode region. (Not to scale.)

MATERIALS AND METHODS

The electrochemical detector discussed herein is a micromachined channel that is inserted into the flow path of a conventional polyimide-coated silica capillary. The detector consists of a deep plasma-etched trench in a 10 mm x 4 mm silicon die that is sealed with anodically bonded glass. The trench in the silicon has two widths. One region matches the 150 μm outer diameter of the external capillary and provides structural support; the other region matches the 50 μm inner diameter of the capillary and acts as part of the flow channel. Platinum microelectrodes patterned on the glass are aligned to the trench to allow electrochemical sensing in the channel (Figure 1). Standard CE capillaries are inserted into the channel and sealed with epoxy, placing the detector into the flow path (Figure 2).

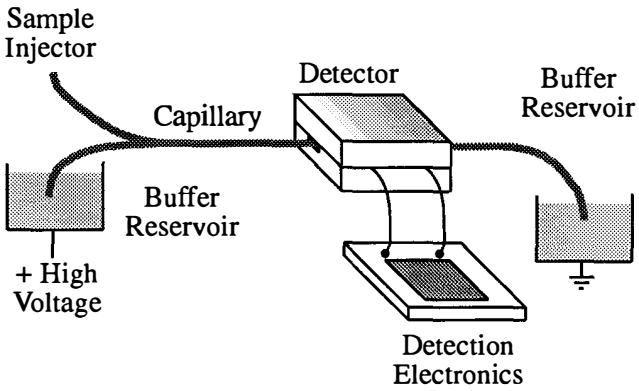

Figure 2: Diagram illustrating the use of the micromachined detector in a typical CE system.

The silicon trenches were etched using a SF_6/C_2ClF_5 plasma to a depth of 150 μm using a two-layer AZP4620 photoresist mask. A 0.5 μm silicon dioxide layer was then thermally grown to electrically isolate the silicon substrate. Platinum electrodes and bond-pads were patterned using lift-off onto a Tempax™ glass wafer. The glass was then aligned and anodically bonded to the silicon wafer at 350°C and 2000 V at atmospheric pressure. For access to the bond pads, trenches were etched below the bond pads during the capillary trench etch step. The resulting overhanging silicon covering the bond pads was removed during dicing. Gold bond wires were therm-sonically bonded to the platinum pads and onto matching copper traces on a glass supporting substrate. To improve the sensitivity of conductivity measurements, amorphous ("black") platinum was electrodeposited on the platinum electrodes [8]. This lowered the total electrode impedance by more than an order of magnitude. Photographs of the resulting structure are shown in Figures 3 and 4.

The electrochemical detection instrumentation has been realized with a precision battery-powered circuit. A.C. conductivity was measured with a custom designed analog lock-in amplifier and 18-bit A/D converter (Figure 5). A microcontroller chip controls the analysis and transmits the resulting data over an infrared serial link to a personal computer running custom analysis software. Floating the instrumentation at the capillary potential allows high voltage isolation and improves power supply noise rejection.

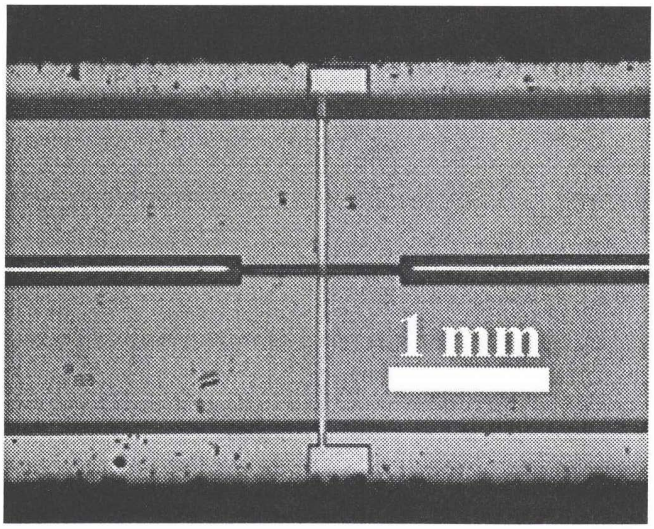

Figure 3: Optical micrograph of a completed detector, viewed through the top glass.

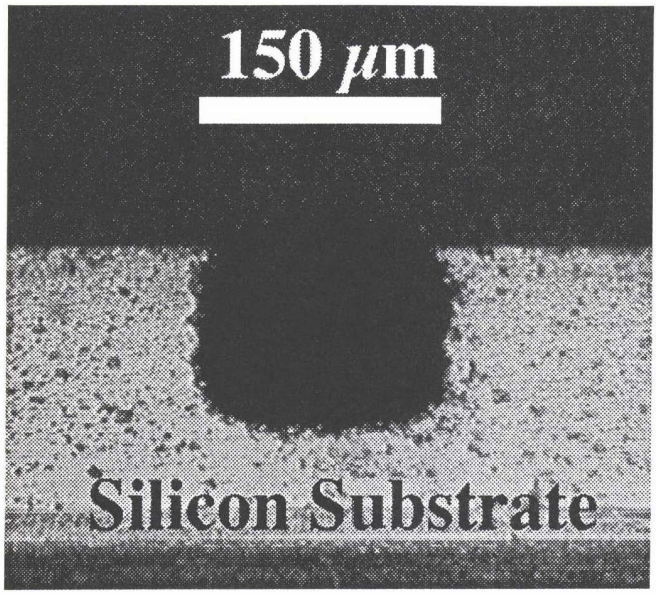

Figure 4: Cross-sectional view of the inlet of a detector.

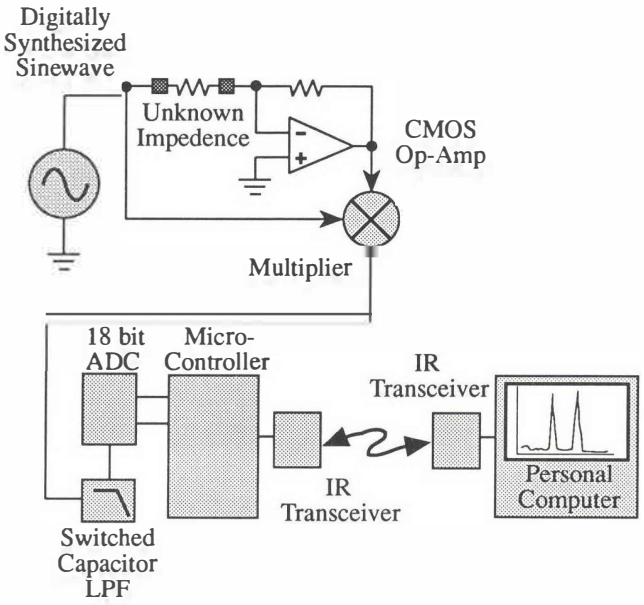

Figure 5: Block diagram of experimental conductivity-based, optically-isolated CE detection system.

EXPERIMENTAL RESULTS

Preliminary electrophoresis experiments performed with the system show clear separation of peaks and a good signal-to-noise ratio. An analysis of a solution containing K^+, Na^+ and Li^+ ions is shown in Figure 5. The detection limit for these ions is better than 7 x 10^{-7} M, which is comparable to the best reported detection limits for conductivity measurement [7]. Improvement of the sensitivity is expected by using lower conductivity buffer solutions.

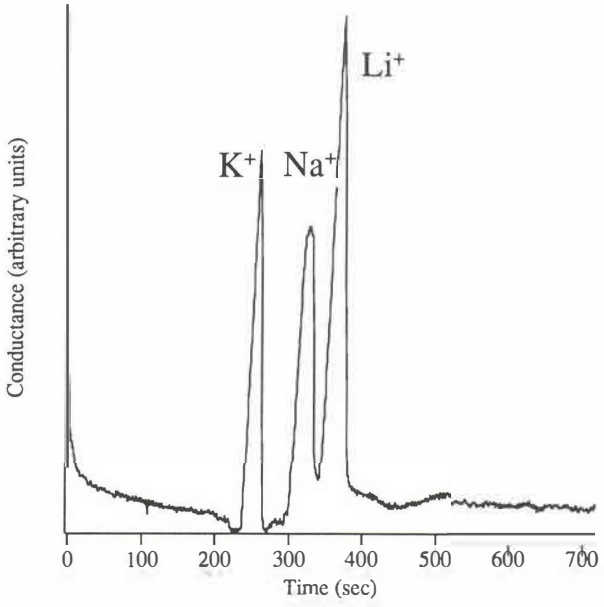

Figure 6: Typical detection results using 4 kV applied voltage and 0.7 millimolar concentrations of K^+, Na^+ and Li^+. The capillary used in this case was 20 cm in length.

CONCLUSION

A microfabricated electrochemical CE detector system has been developed. This process allows accurate, reproducible construction of a detector that is compatible with existing electrophoresis systems. Improved detection electronics have reduced the noise that is common in electrochemical CE detection. The overall system performance was comparable to the best reported conductivity detection systems for CE.

ACKNOWLEDGMENTS

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