Ceramic microchips for capillary electrophoresis–electrochemistry

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A capillary electrophoresis–electrochemistry chip constructed from low-temperature co-fired ceramic (LTCC) tape is presented. This is the first report of such a chip constructed in this manner using these materials. Electroosmotic flow at pH 7 is demonstrated by the migration of a neutral marker, catechol. The separation and detection of two phenolic compounds are presented.

Introduction

Capillary electrophoresis (CE) in the microchip format is a powerful separation technique[1] of considerable research interest as evidenced by the number of related publications.[2] The advantages of microchip CE include smaller size, reduced reagent volume and cost, the ability to design highly parallel systems and to perform high speed, efficient separations.[3,4] The increase in speed without loss of efficiency is a result of decreased injection and detection volumes, shorter delay times between sample loading and separation, and the use of higher field strengths. This results in less band broadening than is seen in standard CE systems.[5]

One major concern in the construction of microchip CE systems is the choice of substrate material. Because most detection systems are based on optical detection, transparent substrates such as glass, plastics, and elastomers have frequently been used for microchip applications.[4] Glass provides a rigid substrate with electroosmotic flow similar to that of fused silica. Unfortunately, high quality glass is costly, fragile, and difficult to bond.[6] Plastic and elastomer substrates are less expensive than glass and are amenable to mass production.[7,8] A limitation of polymer substrates is the lack of information on their electroosmotic properties.

It is desirable to develop microchip CE systems from materials that have properties similar to those of glass but are easier and less expensive to fabricate. The ceramic materials used in this report fit these criteria. The material is an aluminium borosilicate ceramic and should, therefore, have electroosmotic properties similar to those of fused silica. The material may be purchased in large, relatively inexpensive sheets. Fabrication can be accomplished using either milling or laser ablation. Electrical conductors and electrodes can be printed on individual layers, and interlayer hydraulic and electrical connections can be provided by vertical vias. Bonding of multiple layers (up to 80 total) is simple, requiring a lamination and firing step. Ceramic tape technology is, thus, the convergence of layered manufacturing and rapid prototyping. In contrast to standard microfabrication techniques, ceramic tape technology does not require clean rooms, which reduces the cost of fabrication.[9]

The use of electrochemical detection (EC) with microchip CE has many potential advantages.[10–12] Electrochemical instrumentation is inexpensive in comparison to the laser systems typically used for fluorescence detection. The mass sensitivity and selectivity for EC are comparable to fluorescence detection. The microchip need not be transparent. Additionally, both the detection (microelectrodes) and control (potentiostat) components of the system can easily be miniaturized onto the microchip with the CE. These benefits, combined with the range of naturally electrochemically active analytes available,[12] make the further development of microchip CE–EC attractive.

In this report, we describe the first use of ceramic substrates for the construction of a microchip CE system. Electrochemical detection was used for the characterization of electroosmotic flow. Although electrode placement limited the separation efficiency, two model analytes, catechol and dopamine, were separated in less than 1 min.

Experimental

Catechol, dopamine, sodium hydroxide, sodium chloride and *N*-tris-[hydroxymethyl]-methyl-2-aminoethanesulfonic acid (TES) were obtained from Sigma (St. Louis, MO) and used as received without further purification. All solutions were prepared from deionized water (LabConco). Platinum wire was used for the anode, cathode, working electrode and auxiliary electrode (Goodfellow, Cambridge, UK). The diameter of the working electrode was 25 μm, all other electrodes were 0.5 mm in diameter. A 1 mm Ag wire was used for the reference electrode (Goodfellow). Green Tape 951AX was obtained from DuPont (Wilmington, DE) and processed according to their specifications.

Ceramic microchips were produced using conventional milling equipment and ceramic processing procedures. This began with the patterning of two pieces of ceramic tape (Fig. 1). In one piece, a shallow capillary trench was cut with a CNC milling machine (Fadal VMC 15XT). In the second piece, which serves as the cover plate, the openings for the reservoirs were cut using the same CNC machine. These two pieces were stacked, with a third piece of unmodified tape on the bottom for extra strength, and aligned using alignment pins. The stack of materials was laminated for 10 min at 80 °C and 3000 psi† using a Labpress (Carver Inc. Wabash, Indiana) fitted with heated plates. After lamination, the three pieces formed a single unit. Final firing of the ceramic tapes was accomplished in a temperature-controlled oven with a maximum temperature of 875 °C. A standard cross-configuration (Fig. 1) was used for this work.[1] The distance between the anode and cathode was 3.0 cm and the effective distance between injection point and detection was 2.5 cm. The capillary channel was 25 μm deep and 100 μm wide.

† 1 psi ≈ 6.894 757 × 10^3 Pa.

Electrochemical detection was accomplished using a standard three-electrode setup. End-column detection was employed as first reported by Gavin and Ewing[10] for conventional CE and later modified for the microchip format by Mathies and co-workers.[11] A Pt wire was manually mounted at the exit of the capillary channel into the waste reservoir and held in place using epoxy (Miller–Stephenson 907). Ag wire and Pt auxiliary electrodes were mounted in the waste reservoir. The Ag/AgCl reference electrode was generated by oxidizing the Ag wire in the presence of Cl^- ions for 5 min. NaCl was added to the waste reservoir to a final concentration of 0.1 M to ensure stability of the reference potential. The oxidation of catechol and dopamine was monitored using a computer-controlled potentiostat (LC-4C, BioAnalytical Systems, West Lafayette, IN) and data collection system (DA-5, BioAnalytical Systems).

Results and discussion

To date, two classes of substrate materials have been evaluated for microchip CE systems—glass and polymers. These substrate materials were selected based on the need for optical transparency due to the use of fluorescence detection.[4] When electrochemical detection is employed, the need for transparency is eliminated. This led us to evaluate ceramic tape, which has several advantages for microchip CE. One benefit is reduced cost of production, as the base material is less expensive than glass and the same price as many plastics. Additionally, the chips need not be manufactured in a clean room setting. The system described in this communication was constructed totally outside of a clean room. Finally, multiple layers may be integrated into a single device, permitting an increased functionality per unit surface area.

The first parameter considered in testing the ceramic microchips was the wall roughness. The use of a milling tool for formation of the channels should result in walls that are more uneven than those generated using photolithography. The increased roughness may result in band broadening and a decrease in peak efficiency. A micrograph of a channel cut with the milling tool is shown in Fig. 2. The observed roughness is approximately 0.2 μm, which is similar to the roughness measured in channels fabricated by laser ablation of polymers.[13]

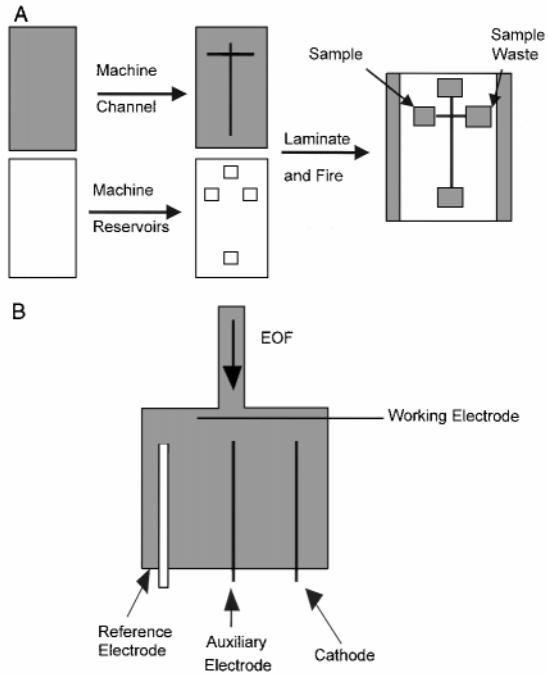

Fig. 1 Schematic depicting the design and construction of the ceramic microchips.

The basic material in the ceramic tape is aluminium borosilicate, which should generate an electroosmotic flow similar to that of fused silica capillaries. The migration of catechol, an electrochemically active molecule that is neutral at pH 7.0, was investigated. The capillary channel was treated initially with 0.1 M NaOH for 1 h. Catechol (25 mM) was injected by applying 600 V between the sample and sample waste reservoirs (Fig. 1) for 5 s. During this time, the intersection of the separation and sample channels became filled with sample solution. The shape of the plug is not well characterized at this time because the top plate is opaque; however, it should resemble that seen by others using a similar geometry and injection technique.[14] The electrophoretic mobility of catechol was measured at pH 7.0 with a field strength of 200 V cm^{-1}. Fig. 3 shows a representative electropherogram. The electroosmotic flow was calculated to be $3.00 \pm 0.05 \times 10^{-4}$ cm^2 V^{-1} s^{-1} (n = 4), which is very similar to that of fused silica (4×10^{-4} cm^2 V^{-1} s^{-1}) at the same pH.[15,16] As expected, the linear velocity increased with increasing voltage, going from 0.053 cm s^{-1} at 167 V cm^{-1} to 0.066 cm s^{-1} at 225 V cm^{-1}. The use of higher voltages with ceramic chips was not tested during this initial phase; however, no problems are foreseen as the breakdown voltage and thermal conductivity of this material are similar to those of glass.

The most obvious feature of the electropherogram (Fig. 3) is the large peak width, which corresponds to an efficiency of ~2500 plates m^{-1} and was the same at all three applied potentials. This poor efficiency can be caused by a number of factors. For example, manual placement of the working electrode restricted the distance of closest approach for the electrode and led to significant band broadening. The effect of electrode placement on separation efficiency has been previously investigated for fused silica capillaries.[17] The current

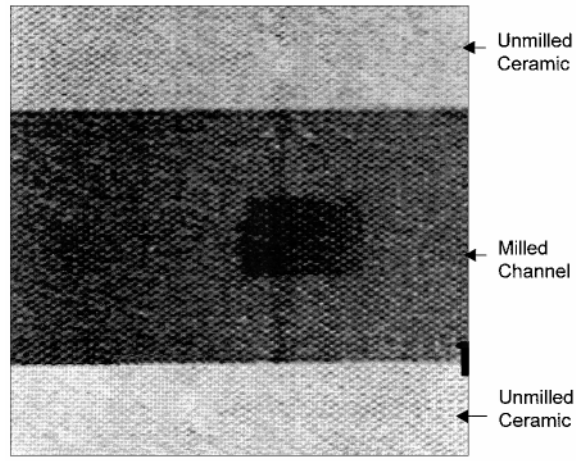

Fig. 2 Micrograph of a 100 μm wide channel in processed ceramic tape. The dark area is the recessed channel.

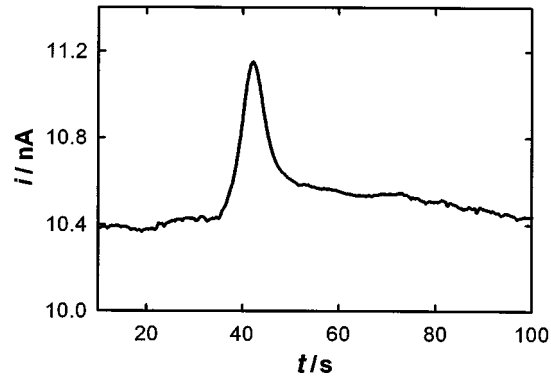

Fig. 3 Representative electropherogram of catechol: 25 μM, 200 V cm^{-1} separation potential.

work focused on the evaluation of ceramic tape as a substrate for microfabricated separation systems. In the future, the system will be designed with screen-printed electrodes that are integrated into the system. This should significantly reduce or eliminate band broadening due to the detector. A second factor that can decrease the efficiency is the plug size. No attempt was made here to use "pinched flow" injection as has been described previously.[14] The result was a large sample plug and decreased efficiency.

Despite the poor efficiency, separation of two model analytes, dopamine and catechol, was accomplished using the ceramic microchip system. A representative electropherogram for the separation is shown in Fig. 4. The average migration time for dopamine in these separations was 25.3 ± 1.9 s. Dopamine is positive at pH 7 and, therefore, migrates faster than catechol (t_m = 41.9 s). Again, the peaks are broad; however, the two peaks are clearly resolved, and the separation occurs in less than 1 min even at this low applied voltage.

Conclusions

The goal of this report is to establish the use of ceramic substrates for microchip CE systems. Figs. 3 and 4 clearly show the potential of the material for use in CE applications. The current limitation to the use of this system is the poor separation efficiency. Work is currently underway to construct and characterize a system that uses integrated screen-printed electrodes. These electrodes should permit much better separation efficiency, as they will be aligned much closer to the end of the capillary; this should substantially decrease the extracolumn effects. In addition to the use of screen-printed electrodes, work is underway to construct a system with a glass cover plate. This will allow better characterization of the plug shape and the use of optical detection.

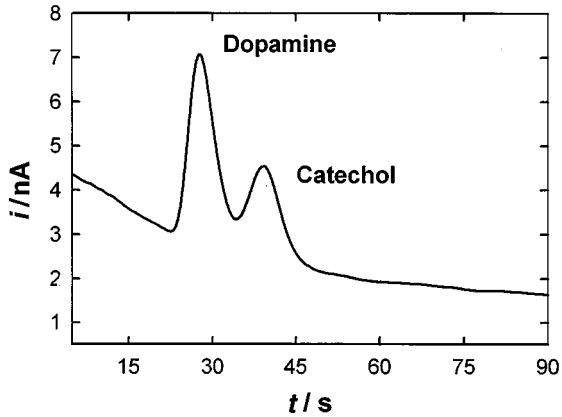

Fig. 4 Representative electropherogram of catechol and dopamine: 25 μM each, 200 V cm^{-1}.

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