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Microfabrication of Microfluidic Cartridge for Isoelectric Focusing by Screen Printing

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A novel microfabrication technique for the preparation of microfluidic cartridges for isoelectric focusing (IEF) by screen printing has been demonstrated. Double parallel strips of polymer coating with a thickness of 50 μm were printed onto a substrate of a thin plastic sheet. Closed channels were made by bonding two units of the printed double strips face to face with epoxy glue, forming a capillary channel with dimensions of $39 \times 1 \times 0.10$ mm. Cartridges suitable for whole column detection (WCD) IEF were fabricated using the printed microchannels. Small molecular mass pI markers and hemoglobins were used as model analytes to demonstrate IEF with WCD in the cartridges. Electroosmotic flow and analyte adsorption were controlled by dynamic coating of the channel with methyl cellulose solution, and good qualitative results have been demonstrated.

1. Introduction

Capillary isoelectric focusing (CIEF) is a unique steady-state type of capillary electrophoresis (CE) in which amphoteric analytes are separated according to their different isoelectric points (pIs).[1,2] CIEF that is conducted in common CE instruments usually employs a 20–60-cm-long capillary and a single-point, on-column detector. The analytes, mixed with suitable pH range and concentration of carrier ampholytes, are injected into the separation capillary and focused at a corresponding position along the capillary under a high DC electric field. After focusing, the analytes are mobilized and detected as they pass

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through the detection point. There are three main types of mobilization: electrophoretic, hydrodynamic, and electroosmotic.[2–4] However, all mobilization methods interrupt the steady state of IEF, lengthen analysis time, and lead to uneven resolution along the separation column, making it impossible for CIEF to be conducted in a short column (i.e., 5 cm long). With the development of the whole column detection CIEF technique,[5,6] the complete analysis time for an amphoteric sample can be reduced to 2–6 min without the necessity of mobilization, and the isoelectric points of the components can be directly determined from their positions along the capillary with the help of pI markers.

Micro-fabrication of analytical devices is one of the most active areas in the analytical sciences,[7–9] due to the speed, high throughput, and reduced sample and chemical consumptions that microfabricated devices can potentially provide. Most of the reported microfabrications for capillary electrophoresis are based on the use of glass or quartz substrates employing photolithography and chemical etching techniques. Capillary electrophoresis techniques like capillary zone electrophoresis (CZE),[10,11] capillary electrochromatography (CEC),[12] micellar electrokinetic chromatography (MEKC),[13] and CIEF[14] have been investigated. Polymeric materials have also been investigated because of their relatively low costs, wide selection of materials, and the development of suitable microfabrication techniques.[8,15,16] Screen printing allows the application of prepolymeric inks through a patterned screen or stencil onto a suitable substrate for the mass production of inexpensive electrodes and electrochemical cells,[17,18] and can provide a resolution of 100 μm. This method has received much attention for medical and environmental applications because it allows the reproducible mass production of sensors at competitive costs. Here, we report the novel application of screen printing techniques to the mass production of microfluidic cartridges for whole column detection (WCD) IEF.

2. Experimental

2.1 *Apparatus*

The screen printer, which was designed for printing circuit boards, was the DEK Model 248 semiautomatic screen printer from DEK USA Inc. (Flemington, NJ). A vacuum tooling plate attachment with 900 2-mm-diameter openings was custom designed by DEK to hold flexible substrates such as thin plastic sheets in place during the printing process. The screen patterns were designed in-house and were fabricated by Hybrid Integrated Service Inc. (Mississauga, ON, Canada). The size of the screen was 508×508 mm. Considering two parallel strips with a distance between them of 1 mm as a subunit, 80 units can be printed per printing. Before printing, the substrates were cleaned with ethanol and baked at 80°C for one h in a convection oven and placed into the screen printer. The polymeric ink was applied to the screen surface and printed on the substrate surface. Once the polymeric strips had been applied (with a desired thickness of 50 μm), the printed substrate was cured immediately at 93°C for 8 h.

The IEF experiments were conducted in the iCE280 (Convergent Bioscience Ltd., Toronto, Canada) with a fixed wavelength of 280 nm. The data collection and processing steps were implemented on a PC, and the electropherogram was recorded as absorbance versus the distance to the anode.

2.2 *Chemicals and CIEF procedure*

Polyethylene sheet with a thickness of 0.25 mm was purchased from Cadillac Plastic (London, ON, Canada). The polymer protective silicone coating ESL 240-SB was donated by Electro-Science Laboratories, Ins. (King of Prussia, PA). Five-minute epoxy syringe glue was obtained from LePage (Brampton, ON, Canada). A capillary of 183 μm ID, 342 μm OD (TSP180350) was purchased from Polymicro Technologies Inc (Phoenix, AZ). Microporous hollow fibers with pore sizes of 0.03 μm and 383.3 μm ID were obtained from Hoechst Celanese (Frankfurt, Germany). Human hemoglobin controls A and A_2 (pIs, 7.0 and 7.4, respectively) were purchased from Helena Laboratories (Beaumont, TX). BioMark synthetic pI markers were obtained from Bio-Rad (Mississauga, ON, Canada). Methylcellulose (MC, 4000 cp for a 2% solution) and Pharmalyte of pH 3–10 were obtained from Sigma (St. Louis, MO) and were of analytical grade. Water was purified using an ultra-pure water system (Barnstead/Thermolyne, Dubuque, IA) and was used for all solutions.

The sample was prepared by dissolving the proteins in a 2% Bio-lyte (pH 3–10) and 0.25% methyl cellulose solution to the desired concentration and injected into the separation column. After sample injection, the electrolyte vials were rinsed with water three times, and were filled with catholyte (sodium hydroxide solution) or anolyte (phosphoric acid solution). DC power was applied after 30 s of sample injection for the balance of the sample inside the channel, and data were collected at 40 s intervals to monitor the focusing process. The separation channel was rinsed with 0.25% MC between analyses for better reproducibility.

3. Results and Discussions

3.1 *Micro channel fabrication*

Fabrication of micro channels for IEF by screen printing is a relatively simple process that involves channel design, substrate printing, and cartridge assembly. Of these steps, channel design is the most important. The structure of the channel can be varied due to the inherent flexibility of the design for the screen pattern, and channels with almost any structure above the resolution limit of screen printing are possible. The designs in Fig. 1 show only two possible channel structures, but more complicated structures can be designed and printed. The screen design designated A was fabricated and used in these experiments to demonstrate the feasibility of fabrication of microfluidic devices for WCD IEF by screen printing.

Materials that constitute the printed channel are also important. There are two kinds of materials present in the microchannel, the printing ink and the substrate (plastic sheet or glass plate). The paste should adhere to the surface of the substrate very well, and at the same time should be chemically and electrically inert. A mineral-filled, thermosetting modified silicone coating ESL 240-SB (Electro-Science Laboratories Inc.) was found to be superior to other polymers available in terms of insulation and adhesion to the substrate, and was thus used in these experiments. The substrate functions as a component as well as a detection window of the micro channel; therefore, its chemical inertness and optical transparency are important with respect to the focusing process and detection sensitivity of

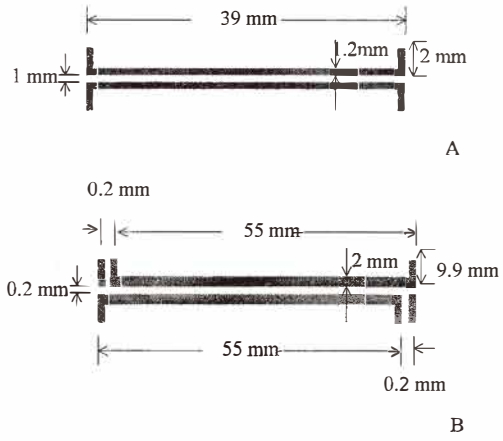

Fig. 1. Structures of the designed microchannels. A: Simple straight channel; B: Simple straight channel with side branch for sample introduction.

the channel IEF. Both glass plates and plastic sheets can be used as long as they are transparent at 280 nm and are electrically and chemically inert. In the experiment, a thin polyethylene substrate was investigated. Even though the transparency of the 250-μm-thick polyethylene sheet at 280 nm is suboptimal, it can be used to demonstrate the principle of microchannel IEF, and was used in these experiments.

Screen printing is a simple but important step, and the thickness of the printed strips must be precisely controlled. Both the size of the screen mesh and the pressure applied during printing depend on the ink specifications and viscosity. The ink viscosity is critical: if it is too low, strip widening may occur; if the viscosity is too high or if the ink contains particulates, marked edges may result.

Cartridge assembly, shown in Fig. 2A, used two printed units that were bound together, face to face, by epoxy glue to form a closed micro-channel. A suitable amount of epoxy glue was applied from the outside of the strip to prevent leaking into the separation channel which would change the physical properties or dimensions of the closed channel. The bounding was conducted by applying mild pressure with the aid of a microscope to ensure precise alignment. The thickness of the printed polymer was 50 μm, and the closed channel was 100 μm thick. The channel made of thin plastic film substrate was sufficiently strong, and therefore it was attached to a glass microscope slide for support with the transparent channel side being aligned with the detection window of the IEF instrument. Two pieces of plastic tubing each with an inner diameter of 0.5 cm and a length of 1 cm were mounted with epoxy glue on both sides of the channel with the opening of the channel in the middle as an electrolyte vial. A piece of hollow fibre membrane connected to a piece of capillary was flattened and inserted into the separation channel to facilitate sample injection and eliminate possible mixing of the sample with electrolyte. However, the other side of the

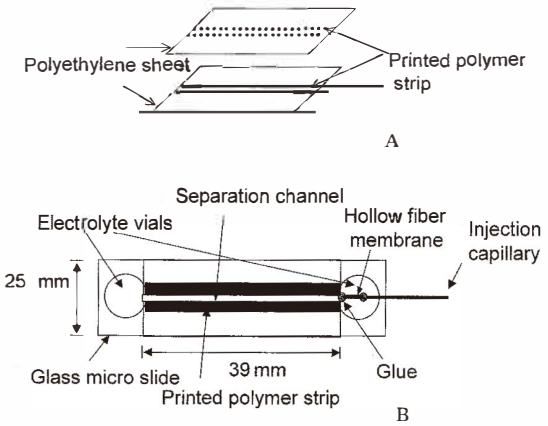

Fig. 2. Schematic of the cartridge made of a microchannel for whole column detection IEF. A: Channel closing; B: Top view of cartridge made of microchannel.

channel had to connect with the electrolyte vial directly, because the flattened hollow fibre membrane would block the channel when the sample solution came out. Figure 2 illustrates the schematic of the printed channel for IEF.

3.2 *Isoelectric focusing*

Surface modification is very important in IEF to eliminate EOF and interaction between analytes and the surface of the separation channel.[19–21] Channels that are made of fused silica or quartz will have significant EOF because of the hydrolysis of surface silane groups. On the other hand, channels that are fabricated from plastic may have a significant interaction with analytes owing to their hydrophobicities. There are two kinds of surface modification methods, chemical modification and dynamic coating. In the former, hydrophilic molecules are chemically bound to the surface of the fused silica or plastic channel, while in the latter, surfactant additives are added to the sample solution and are dynamically bound to the surface of the capillary. Dynamic coating is commonly used in CIEF when plastic materials are used as columns or column coatings, and therefore 0.25% methyl cellulose was added to the sample solution. Figure 3 presents the dynamic focusing of four small-molecular-mass pI markers (pIs: 5.3, 6.4, 7.4, and 8.4). Upon application of the electric field, ampholytes that are uniformly distributed in the whole separation channel migrate from both sides and converge into four well-separated peaks within 4 min. The dynamic focusing process displayed in Fig. 3 verifies that EOF was virtually eliminated by the methyl cellulose additive for the 6 min duration of the experiment, since no cathodic drift can be seen. Table 1 lists the reproducibilities of peak positions for the four pI markers in six consecutive separations.

The data in Table 1 show that RSD values within 1.8% were obtained for all four pI markers. Considering that this research is in the preliminary stage, the channel design,

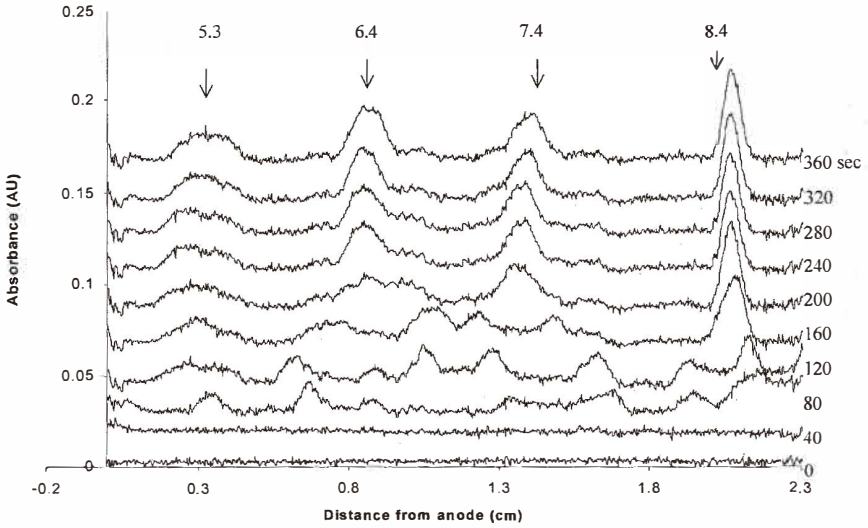

Fig. 3. Dynamic focusing of four pI markers (pI: 5.3, 6.4, 7.4, 8.4; all at the concentration of 20 μg/ml) under electric field of 385 V/cm. The x-axis shows the position on the separation column, and the y-axis shows the relative absorbance in the separation column at various time points (compensated as focusing time increases to provide a clearer view). The right y-axis shows the time interval for which the absorbance profile of the column was measured during focusing. 2% Bio-Lyte pH 3–10 was present in the sample mixture. Catholyte was 20 mM NaOH, and anolyte was 20 mM H_3PO_4. The separation channel was conditioned by passing 0.25% methyl cellulose through it for 30 min.

Table 1
Peak positions (pixel) of the four pI markers from the anode in six consecutive separations.

Run \ pI	5.3	6.4	7.4	8.4
1	694	894	1125	1399
2	673	911	1139	1410
3	690	895	1128	1415
4	688	900	1130	1414
5	678	906	1125	1407
6	665	885	1084	1384
Average	681.3	898.5	1122	1405
SD	11.2	9.3	19.2	11.7
RSD(%)	1.6	1.0	1.7	0.8

surface modification, and cartridge assembly have not yet been optimized; better results are expected following these optimizations.

Having proven good separation and reproducibility for the low-mass pI markers, the printed channel cartridge was used to demonstrate the separation of protein samples.

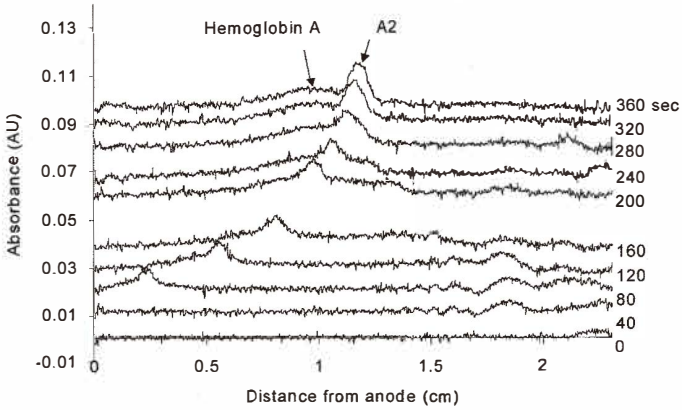

Fig. 4. Dynamic focusing of hemoglobins A and A_2 (pI: 7.0 and 7.4, respectively; at a concentration of 250 μg/ml) under electric field of 385 V/cm. Other conditions were the same as in Fig. 3.

Figure 4 shows the dynamic focusing of hemoglobins A and A_2 under an electric field of 385 V/cm. It can be seen from Fig. 4 that the hemoglobins were focused and separated within 5 min, although overlap is apparent because of peak broadening. The reduced resolution due to protein-surface interaction prevents the complete resolution of these proteins (pI difference of 0.4), suggesting the importance of optimization of the surface modification in later research. To confirm reproducibility and the quantitative determination capability of channel IEF, samples with different concentrations of hemoglobins A and A_2 were analyzed. Fig. 5 illustrates the electropherograms obtained by focusing 250 and 500 μg/mL of hemoglobins A and A_2 under an electric field of 385 V/cm. The electropherogram in Fig. 5 shows that the peak positions of the hemoglobins are reproducible, and the peak areas are proportionally higher for the higher concentration sample.

Printed channel IEF has inherent advantages, such as low cost (less than $10 per unit) and ease of fabrication. The materials used to fabricate the printed channels, such as polyethylene, cellulose acetate butyrate, and Teflon, are inexpensive and easily obtainable. The procedures for channel printing and fabrication are simple. Other than epoxy glue bonding, heat sealing, lamination and laser welding are also possible methods for channel closing.

Peak broadening in printed channel IEF can be reduced by the optimizations of channel design, material selection, and surface modification. One possible improvement in the separation channel is illustrated in Fig. 1B, where the cross section is much smaller than that of A. In this design, the micro channel can be connected with the electrolyte vials directly with a hollow membrane, and the sample can be injected into the channel through side injection channels. In this design, the risk of mixing the sample in the separation channel with electrolytes in electrolyte vials is reduced, and higher electric fields can be applied. Other materials such as acrylic and cellulose acetate butyrate plastic could be

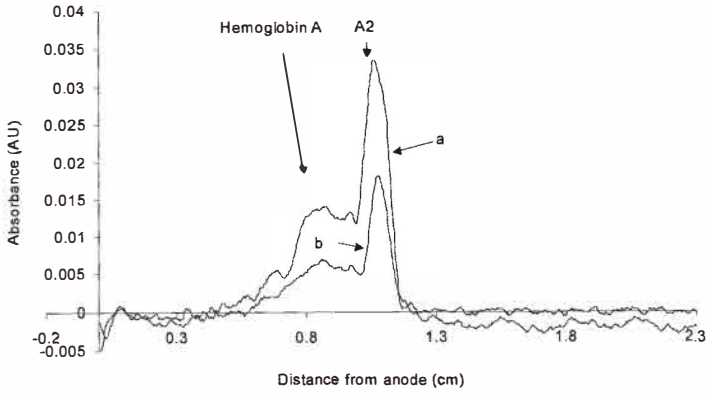

Fig. 5. Electropherogram of hemoglobin A and A_2 at different concentrations (a: 500 μg/ml; b: 250 μg/ml) under electric field of 385 V/cm. Other conditions were the same as in Fig. 3.

selected to fabricate the channels to provide better surface inertness to ampholytes and analytes. Chemical modification of the surface may also provide better modification than that of dynamic coatings.

The sensitivity for analyte detection in printed channel IEF may be improved by the selection of a better transparency channel material, an increase in the light path, and the use of fluorescence detection. The inherent transparency of the channel material at the detection wavelength and the thickness of the plastic substrate are important variables. Regarding the increase of the light path, the dimensions of the channel could be redesigned so that the larger dimension is the light path (i.e., the 1 mm dimension instead of the 0.10 mm one) to improve the detection sensitivity.

4. Conclusions

The fabrication of a microfluidic device for IEF by screen printing has been demonstrated. The fabrication procedure is simple, the materials are inexpensive, and good qualitative determination is demonstrated for the low-molecular-weight pI marker as well as protein samples. Applications of micro channels by screen printing to capillary zone electrophoresis and capillary electrochromatography will be explored.

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References

1 P. G. Righetti: Isoelectric focusing: theory, methodology and applications (Elsevier Biomedical Press, Amsterdam, The Netherlands, 1983) Chap. 1.
2 R. Rodriguez-Diaz, M. Zhu and T. Wehr: J. Chromatogr. A **772** (1997) 145.
3 P. G. Righetti and A. Bossi: J. Chromatogr. B **699** (1997) 77.
4 R. Kuhn and S. Hoffstetter-Kuhn: Capillary Electrophoresis: Principles and Practice (Springer Laboratory, Berlin Heidelberg, Germany, 1993) Chap. 5.
5 J. Wu and J. Pawliszyn: Anal. Chem. **64** (1992) 2934.
6 J. Wu and J. Pawliszyn: Analyst **120** (1995) 1567.
7 D. Qin, Y. Xia, J. A. Rogers, R. J. Jackman, X. M. Zhao and G. W. Whitesides: Topics in Current Chemistry **194** (1998) 1.
8 H. Becker and C. Gärtner: Electrophoresis **21** (2000) 12.
9 V. Dolnik, S. Liu and S. Jovanovich: Electrophoresis **21** (2000) 41.
10 D. J. Harrison, A. Manz, Z. Fan, H. Ludi and H. M. Widmer: Anal. Chem. **64** (1992) 1926.
11 S. C. Jacobson, R. Hergenroder, A. W. Moore and J. M. Ramsey: Anal. Chem. **66** (1994) 4127.
12 C. S. Effenhauser, A. Paulus, A. Manz and H. M. Widmer: Anal. Chem. **66** (1994) 2949.
13 S. C. Jacobson, R. Hergenroder, L. B. Koutny and J. M. Ramsey: Anal. Chem. **66** (1994) 2369.
14 Q. Mao and J. Pawliszyn: Analyst **124** (1999) 637.
15 J. Wen, Y. Lin, F. Xiang, D. W. Matson, H. R. Udseth and R. D. Smith: Electrophoresis **21** (2000) 191.
16 D. Kaniansky, M. Masar, J. Bielcikova, F. Ivanyi, F. Eisenbeiss, B. Stanislawski, B. Grass, A. Neyer and M. Johnck: Anal. Chem. **72** (2000) 3596.
17 S. A. Wring, J. P. Hart, L. Bracey and B. J. Birch: Anal. Chim. Acta **231** (1990) 203.
18 M. Alvarez-Icaza and U. Bilitewski: Anal. Chem. **65** (1993) 525A.
19 X. Ren, P. Z. Liu, A. Malik and M. L. Lee: J. Micro. Sep. **8** (1996) 535.
20 J. Horvath and V. Dolnik: Electrophoresis **22** (2001) 644.
21 T. Huang and J. Pawliszyn: Analyst **125** (2000) 1231.