MICROFLUIDIC CHIP-CAPILLARY ELECTROPHORESIS WITH ADJUSTABLE ON-CHIP SAMPLE DILUTION FOR PROFILING OF URINARY MARKERS

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ABSTRACT

The paper reports the development of a microfluidic chip-capillary electrophoresis device using UV/Vis detection for profiling of urinary markers with adjustable on-chip sample dilution. A PMMA polymer microfluidic chip-CE device with on-chip ferrofluid valve control for adjustable sample dilution is developed for profiling of urinary markers related to metabolism disorders for clinical diagnosis. On-chip sample dilution up to a 100 folds can be achieved prior to separation in a running buffer containing 3mM 1,3,5-benzenetricarboxylic acid (BTA), 1.5 mM tetraethylenepentamine (TEPA) and 15mM tris(hydroxyl) aminomethane (Tris buffer) solution with pH adjusted to 8.4 by lithium hydroxide.

KEYWORDS: Ferrofluid valve, Microchip CE, Metabolite disorder, Micromixer

INTRODUCTION

The paper reports the development of a microfluidic chip – capillary electrophoresis device using UV/Vis detection for profiling of urinary markers with adjustable on-chip sample dilution (Figure 1). Joseph et al. reported that uric acid in urine can be determined utilizing microchip-CE with electrochemical detection [1]. Prest et al. reported the determination of halide anions including chloride, bromide, and iodide using PMMA microfluidic chip with conductivity detection [2]. Only a few anions in urine or in standard solution are covered by current methods. Our method differs from theirs by covering more than ten anions in one CE run for real urine sample. This method is a further development of our previous work [3] by integrating on-chip ferrofluid valve system for adjustabl sample dilution.
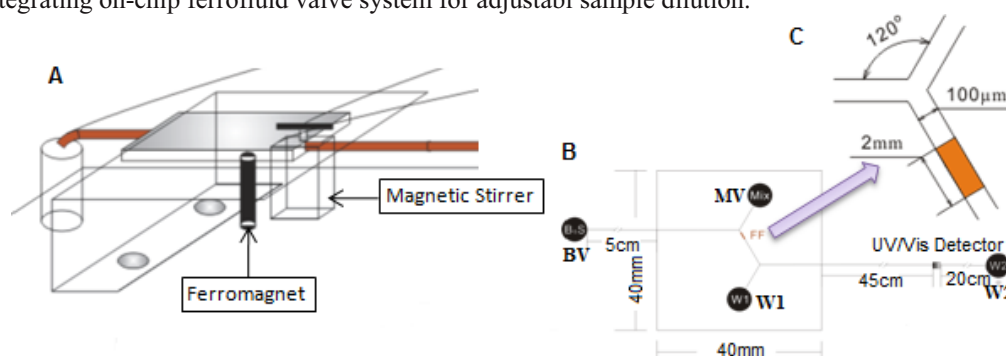

Figure 1: Schematic diagrams showing the PMMA microchip-CE device; (A) Enlarged view of the microchip-CE control unit; (B) Layout of the microchip used; (C) Enlarged view of the micro chip channel.

A rapid advance of the µTAS technologies have been made in the biomedical analysis methods since Manz et al reported a novel miniaturized total chemical analysis system for chemical sensing in 1990 [4]. Existing procedures based on high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) [5] require complicate instrumentation, high capital and operational cost.

THEORY

The coupling of the PMMA polymer microfluidic chip for on-chip sample dilution with the high separation efficient CE for separation and quantitation of urine biomarkers based on anionic inorganic and organic ions are investigated. Factors affecting both processes are studied for optimization of the overall operation procedures. The applicability of the microfluidic chip-CE device for urine anionic biomarkers is reported and assessed.

EXPERIMENTAL

All solutions were prepared in distilled deionized water and stored in a refrigerator at 4°C unless otherwise stated. Stock solutions containing 24 mM BTA buffer, 1.5 mM TEPA and 15 mM Tris were prepared by dissolving appropriate amounts in deionized distilled water. The working buffer was freshly prepared by mixing appropriate volumes of the stock solutions by 0.1M NaOH with pH adjusted to 8.4. Both the working buffer and aqueous standard solutions were filtered using a 0.45 µm Core-Parmer non-sterile syringe filter (PTFE membrane) and degassed before use. The CE running buffer was made up to the following composition: 3 mM BTA, 1.5 mM TEPA and 15mM tris(hydroxyl) aminomethane (Tris buffer) solution with pH adjusted by lithium hydroxide to 8.4 and filtered through a 0.45µm filter prior to use. The water-immiscible ferrofluid APG S12N, a stable colloidal suspension of sub-domain magnetic particles in a liquid carrier of synthetic ester oil was generously provided from Ferrotec Inc. (Nashua, NH). The ferrofluid contained 5% magnetic solid, 10% surfactant and 85% carrier by volume. The average size of the ferro-particles was about 10 nm.

A CO_2 laser engraver with a wavelength of 10.6 μm (V-series, Pinnacle, USA) was used to fabricate the designed chip pattern onto a PMMA polymer base plate under control of a computer software (CorelDraw 10.0 edition). The hot press-bonding machine (up to 500°C and 1 MPa air pressure) was purchased from Guangju Machinery Co. (Dongguan, China).

The PMMA microchip (Figure 1) was made up by a base plate (40 mm × 40 mm × 1.5 mm) with fabricated micro-fluidic channel pattern under computer aided design (CAD) based on CorelDRAW. The PMMA chip design and fabrication can be easily modified and realized with little cost and time using this technique. Two triple cross microchannels and two vials were laser ablated under the control of the CAD software. The ablated channel showed a reverse triangular shape in the cross section. Both the length of the base and the height of the triangle were 100 μm. For the triple cross microchannels, the distance from MV and W1 vial to the proximate channel crossing point was 5mm. The distance between two crossing points was also at 5 mm to allow loading with about 17.5 nL sample. The mixing vial was a circular channel fabricated on-chip with a 3mm diameter and 1.5mm height with loading of about 11 μL samples.

Two capillaries were sandwiched between two PMMA plates. One with 50 μm i.d and 45 cm length was used for separation and the other with 100 μm i.d. and 5cm was used for sample introduction. A thermally controlled hot plate press machine was used to bond the two PMMA plates under constant pressure and temperature at 0.6 MPa and 92°C respectively for 15 min. After cooling to room temperature, the bonded microchip was cleaned in distilled water inside an ultra-sonic bath and dried prior to use.

RESULTS AND DISCUSSION

The sequence for the operation of the ferrofluid valves and the corresponding microfluid movement are shown in Figure 2. The use of a 5 second mixing was found to give satisfactory mixing.
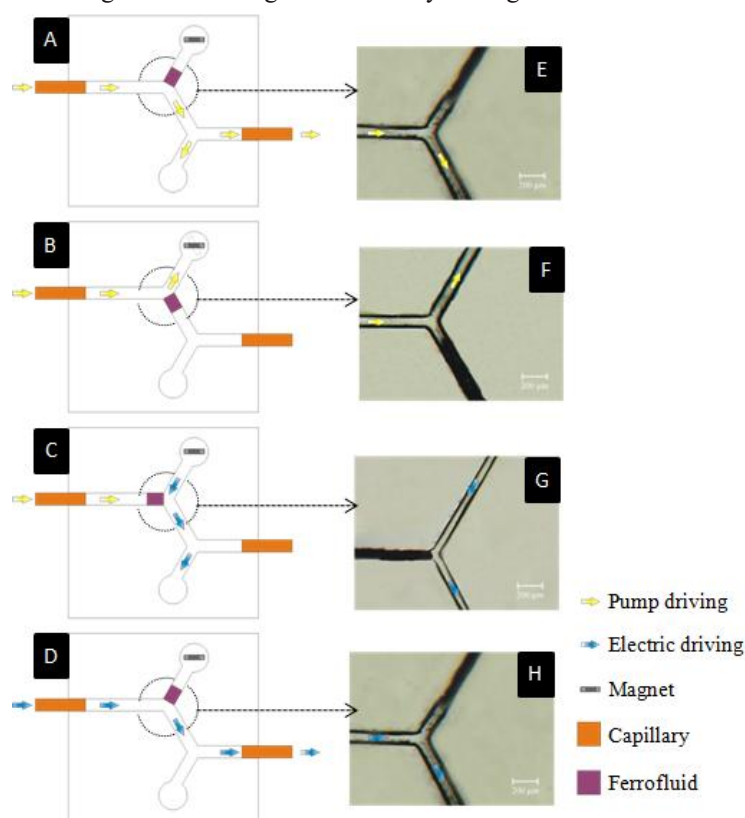

Figure 2. The sequence of ferrofluid valve operation (A-D) and the corresponding microfluid movement (E-H).

The effect of different pH on the electrophoretic mobility of closely migrated anions was studied under 3mM BTA, 1.5 mM TEPA and 15 mM Tris buffer and pH value at 8.4 was chosen as the final pH value. Under the optimized condition, a total of 27 anions were successfully separated as shown in Figure 3. On-chip sample dilution could be easily adjusted by changing the volume ratio between analyte and buffer using the device developed. Using a minimum injected sample volume at 0.1 μL, up to a 100 fold dilution can be achieved. Satisfactory repeatability from 2.13 to 5.19% RSD (n=5) for peak height measurement of 0.2 μL 2 mM oxalate was obtained. Results for comparison of calculated to experimentally determined dilution ratio are shown in Table 1, showing agreeable results within experimental variation.
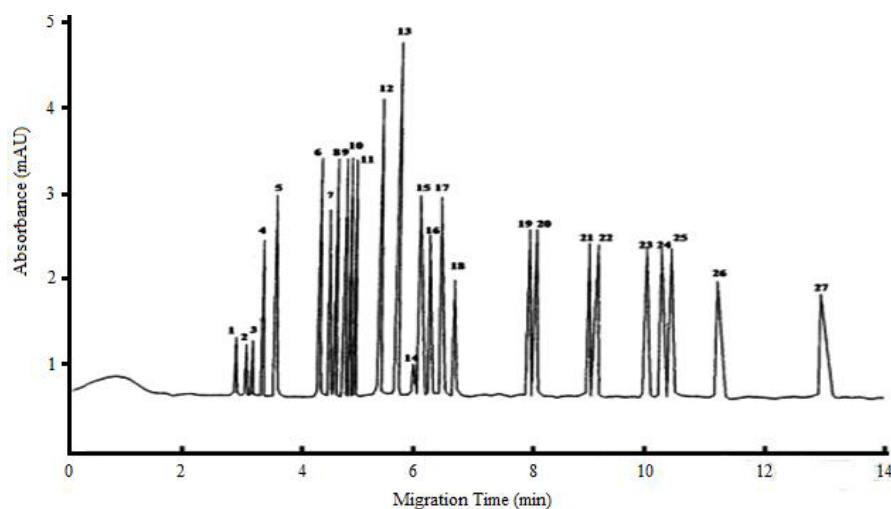
Figure 3: The electropherogram of a standard anion mixture. Buffer: 3mM BTA, 1.5mM TEPA and 15mM Tris at pH = 8.4; Anion standards: 1.0mM each; Capillary: 50µm i.d. x 65 cm total length and 4 cm to the detector. Sample introduced by pressure at 0.5 p.s.i. for 4 sec; Buffer introduced by pressure at 1 p.s.i. for 40 sec; Magnet rotated for mixing at 50 rpm for 5 sec.; Injection of 20 fold diluted anion standard mixture in MV, WV = -3 kV, W1 = ground, 30 sec; Separation: BV = -25 kV, W2 = ground, 15 min; UV/Vis detection at 240nm. Migration order: 1= chloride, 2= nitrite, 3= nitrate, 4= sulphate, 5= oxalate, 6= malonate, 7= formate, 8= fumarate, 9= tartrate, 10= malate, 11= succinate, 12=glutarte, 13= adepate, 14= phosphate, 15= carbonate, 16= citrate, 17= acetate, 18= pyruvate, 19= propionate, 20= lactate, 21= 2-hydroxyisobutyrate, 22= n-butyrate, 23= asparate, 24= n-caproate, 25= glutamate, 26= L-ascorbate and 27= glucuronate.

Table 1: Comparison of calculated and experimentally determined dilution ratio.

Sampling volume (0.5 p.s.i., 4 sec)	Buffer injecting volume (1 p.s.i.)	Dilution Ratio		Repeatability (n=5)
		Calculated	Experimental	
0.2 µL	2.0 µL (2 sec)	10	8.36	2.13
	4.0 µL (4 sec)	20	18.70	2.87
	6.0 µL (6 sec)	30	28.98	3.54
	8.0 µL (8 sec)	40	36.19	5.26
	10 µL (10 sec)	50	42.58	5.79

CONCLUSION

For clinical urine samples such as those from patient with metabolism disorders, a large variation of inorganic and organic acids are often found, requiring frequent dilution at microscale level, producing difficulties for device automation and operation by clinical staff for bedside monitoring for patients under critical care. The results obtained in the present work demonstrate the capability of the microchip-CE device for making up to a 100 fold adjustable on-chip dilution. As commercially available CE/UV instrumentation are used for profiling of organic acids in urine, the device developed is shown capable to satisfy the need for onsite monitoring of metabolites in urine to obtain urgently needed information for medical intervention.

ACKNOWLEDGEMENTS

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