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Plastified poly(ethylene terephthalate) (PET)-toner microfluidic chip by direct-printing integrated with electrochemical detection for pharmaceutical analysis

Ai-Lin Liu, Feng-Yun He, Yu-Lin Hu, Xing-Hua Xia *

Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, China

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Abstract

Rapid separation and determination of acetaminophen and its hydrolysate with end-channel electrochemical (EC) detection integrated on a plastified poly(ethylene terephthalate) (PET)-toner microchip capillary electrophoresis (CE) system was investigated. In this separation and detection system, a Pt ultramicroelectrode integrated on a three-dimensional adjustor was used as working electrode. Factors influencing the separation and detection were investigated and optimized. Results show that acetaminophen and *p*-aminophenol can be well separated within 84 s with R.S.D. < 1% for migration time and R.S.D. < 3.6% for detection current for both analytes. Detection limits for both analytes are determined to be 5.0 μM (S/N = 3). This method has been successfully applied to the detection of trace *p*-aminophenol in paracetamol tablets. The results demonstrate that the PET-toner microchips can obtain better performance than PDMS microfluidic devices but at much lower cost.

Keywords: Poly(ethylene terephthalate) (PET)-toner; Microchip capillary electrophoresis; Electrochemical detection; Acetaminophen; *p*-Aminophenol

1. Introduction

During the past decade, considerable interest has been focused on micro-total analysis system (μTAS) or so called "lab-on-a-chip", and particular attention has been paid to capillary electrophoresis microchips due to its advantages over conventional analysis methods, such as rapid separation speed, high separation efficiency, low reagent consumption, reduced production of waste and use of energy, and its potential portability and disposability [1–8]. As yet, the μTAS has been developed, refined and applied to a variety of chemical and biological problem [2–4].

The microfluidic devices developed in the early years were mostly fabricated from silicon and glass using photolithography and etching technique [9,10]. However, these fabrication processes were costly, time-consuming, labor intensive, and clean-room conditions were required. The fabricated microfluidic devices are also fragile and mass production is not easy to be achieved. Recently, polymeric microchips are of increasing interest because they can offer attractive mechanical and chemical properties, low cost, ease of fabrication, biocompatibility, and higher flexibility and so on [11–13]. Polymeric materials, including poly(dimethylsiloxane) (PDMS) [14,15], poly(methyl methacrylate) (PMMA) [16], polycarbonate [8], polystyrene (PS) [17], and PET [18], have commonly been employed in the fabrication of microfluidic devices so far. Such polymeric chips have been fabricated using laser ablation, plasma etching [19], imprinting [20], hot embossing [16], injection molding [21], and compression molding [8] techniques. Recently, Lago et al. [22,23] described a very simple microfabrication process based on direct printing for mass production of microfluidic devices at very low cost. Compared

* Corresponding author. Tel.: +86 25 83597436; fax: +86 25 83597436.
E-mail address: xhxia@nju.edu.cn (X.-H. Xia).

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to photolithography approaches, this process is an attractive alternative to other expensive, laborious, and time-consuming methods for microchannels fabrication. However, we found that such chips are not so durable during the operation procedures due to possible toner falling off the transparency in experiments. Therefore, we proposed an improved method for fabricating PET microchips with high stability and durability [24,25]. In our method, PET films with adhesive film were finally plastified on the microchips based on Lago's method.

Since Mathies et al. [26] developed a microchip-based CE–EC system for indirect electrochemical EC detection of DNA, EC detection has been offered great promise for designing self-contained and totally disposable μTAS. There are several advantages associated with EC detection such as extremely low cost, low-power requirement, high selectivity, remarkable sensitivity, inherent miniaturization, high compatibility advanced micromachining and microfabrication technologies [26–43]. EC detection has been proven to be a promising detection method and the most widely reported for microchip [44,45].

Acetaminophen, *N*-acetyl-*p*-aminophenol, or paracetamol, is a commonly used analgesic and antipyretic drug, formulated in a variety of dosage forms. *p*-Aminophenol, the primary hydrolytic degradation product of acetaminophen, can present in pharmaceutical preparations of acetaminophen as a synthetic intermediate or as a degradation product of acetaminophen [46]. Because *p*-aminophenol has significant nephrotoxicity and teratogenic effects, *p*-aminophenol is limited to the low level of 50 ppm (0.005, w/w) in the drug substance by the European, United States, and Chinese Pharmacopoeias. Therefore, establishment of a simple, economical, and accurate analytical method for the simultaneous determination of *p*-aminophenol and acetaminophen would be useful to medical manufacturers, etc., for investigation of the stability of acetaminophen, for pharmaceutical analysis, and for quality control. In our previous paper, we used PDMS chip for separation and electrochemical (EC) detection of the drug and its hydrolysis product. We noticed that the separation efficiency was not high enough, e.g., for separation and EC detection of trace amount of hydrolysis product in the acetaminophen contained buffer solution, realization of base line separation was quite tough due to the low electroosmosis of PDMS chip.

In the present paper, we report on an improved fabrication procedure for PET-toner microchips on the basis of the Lago's [22] method. This method is simple and the fabrication cost is very low. The separation and measurement of acetaminophen and its hydrolysate were investigated using such PET-toner microchip CE with end-channel EC detection. Fabricated microchips based on our method showed better mechanical stability and better durability [24]. Under optimum conditions, better results for separation and EC detection of acetaminophen and *p*-aminophenol were achieved.

2. Experiment

2.1. Materials and reagents

Transparency films (PET) (100 μm thick) were used for the base material (STD Printing Materials Limited Company, Suzhou, China). The plastification PET film, 80 μm thick with a 5 μm thick adhesive polyethylene adhesive on one side, was used for final plastification of the PET-toner microchips. All solvents and reagents were of analytical grade. Acetaminophen was obtained from Shanghai No. 3 Chemical Reagent Factory (Shanghai, China). *p*-Aminophenol was obtained from Shanghai Yiyuan Chemical Reagent Limited Company (Shanghai, China). Paracetamol tablets (500 mg acetaminophen per tablet) were purchased from local drug store. All aqueous solutions were prepared from deionized water (18 MΩ, PURELAB Classic, PALL, USA).

2.2. Preparation of the stock and standard solutions

Aqueous stock solution of 10 mM acetaminophen was used for further preparation of final solutions. *p*-Aminophenol was dissolved in a 0.05 M HCl aqueous solution to reach the final concentration of 10 mM, as it is stable in strong acidic medium. Both stock solutions of acetaminophen and *p*-aminophenol were kept at 4 °C. Sample solutions were prepared by diluting stock solutions with running buffer prior to use. The running buffer used as separation medium of CE was 10 mM acetate buffer (pH 5.0).

2.3. Tablet sample preparation

Five tablets (500 mg acetaminophen per tablet) were finely pulverized and dispersed in 40 mL of 0.5% acetic acid in 50 mL volumetric flask. The flask was shaken vigorously and then ultrasonically extracted for 10 min and diluted to required volume with 0.5% acetic acid. The mixture was centrifuged to obtain a clear solution. The supernatant was adjusted to the same pH value as that of the running buffer and then filtered through a 0.22 μm membrane for analysis. The sample was then spiked with appropriate amount of *p*-aminophenol for recovery experiments.

2.4. Microfabrication process

The layouts of the CE chips were designed using standard computer design software (Adobe Illustrator 9.0, Adobe). The fabricated PET CE microchip is schematically shown in Fig. 1A. The features in Fig. 1A were printed out on an EPL 5800 Laser Printer (Epson, Japan) with toner cartridge S050010 at 1200 dots per inch (dpi).

The detailed procedures used to create channels were described previously [22] except for the final plastification process that covers the final PET-toner microchip for ruggedness. The improved PET-toner microchip fabrication procedures can be briefly described as following [24,25]: the layout

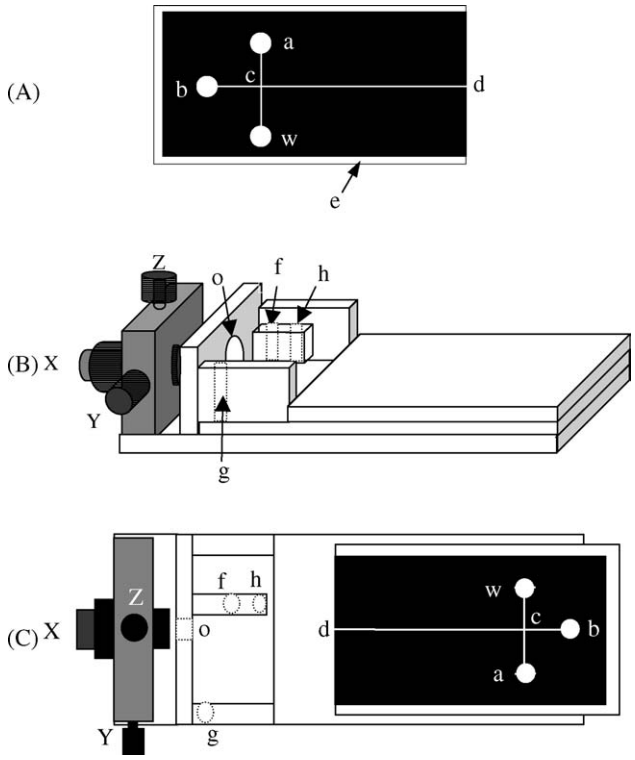

Fig. 1. Schematic layout of a PET-toner microchip and homemade plexiglass holder integrated with a three-dimensional micromanipulator. (A) Plastified PET-toner microchip: (a) sample reservoir; (b) buffer reservoir; (w) sample waste reservoir; (c) injection crossing; (d) end-channel detection point and (e) plastification film. Separation channel, total length bd = 6 cm; effective length, cd = 5 cm; injection channel, aw = 2 cm (not to scale). (B) Side three-dimensional view of the homemade plexiglass holder integrated with a three-dimensional micromanipulator: *X*-, *Y*-, *Z*-direction adjustor; (o) working electrode hole; (f) auxiliary electrode hole; (h) reference electrode hole and (g) CE ground electrode hole. (C) Top view of the holder and PET-toner microchip.

of the chip was printed on a transparency film with blank regions as the microfluidic channels. Then the two printed films with mirrored images were laminated together by heating laminator, producing the channels with access holes. Another two adhesive sheets of PET with access holes at the corresponding places were finally laminated over the PET-toner microchip. Results showed that the final plastification process could greatly improve the durability of the prepared chips. Due to the hydrophobic property of PET, no additional tips are necessary for reservoirs as reported previously [22], which further simplified the fabrication process and substantially lower the cost (e.g., ca. $ 0.2 for a PET chip and ca. $ 2 for a PDMS chip).

The best performance for the lamination steps was obtained by a laminator (Zhejiang Huada Limited Company, Zhejiang, China) at 140 °C for three times. The channel of our microchips was found to be 150 μm in width for injection channels, 200 μm in width for separation and approximately 10 μm in depth. The smaller depth compensates the width of the channel for effective separation of samples.

2.5. Electrophoresis procedures

A laboratory-made voltage power supplier has a voltage range between 0 and +5000 V. The applied voltage could be automatically controlled by a personal computer via AD/DA converter. The separation current can be real time monitored and the corresponding data can be saved in text files. Prior to use, the running buffer and analytes were all passed through a 0.22 μm filter and degassed ultrasonically. For a new microchip, channels were washed in sequence using deionized water, 50% ethanol aqueous solution (v/v), 1.0 M NaOH solution and deionized water each for 2 min. Then, they were rinsed and reconditioned with buffer solution for several minutes. The injection was carried out by applying high voltage (HV) to the sample reservoir for 5 s via Pt electrodes connected to a HV power supplier, with the sample waste reservoir grounded and the other reservoirs floating. Once sample injection was completed, separation voltage was applied to the buffer reservoir with the detection reservoir grounded and the other reservoirs floating. The change of current in the separation channels can be utilized to real time monitor the whole process.

2.6. Electrochemical detection

2.6.1. Electrode preparation

A 10 μm Pt microelectrode sealed with Pyrex tube (Shanghai Chenhua Instrument Company, Shanghai, China) was used as working electrode for detection. Before use, the electrode was polished with 0.05 μm alumina powder, rinsed with deionized water. Then the electrode was cleaned by potential scanning between −0.2 and 1.25 V against the Ag/AgCl (3.0 M KCl) reference electrode in 0.5 M H_2SO_4 until a stable current–potential profile was obtained.

2.6.2. Apparatus

A homemade plexiglass holder integrated with a three-dimensional micromanipulator (Fig. 1B and C) was fabricated for fixing the microchip and housing the detector and reservoirs (the total system size was 13 cm long, 6 cm wide and 5 cm high). Proper seal of the solution reservoir at the end of the microchannel was achieved by using silicone grease and fixed with double-side adhesive tape. This reservoir served as both the cathodic buffer reservoir for the CE system and the EC detection reservoir. A three-dimensional micromanipulator (Shanghai Lianyi Instrument Factory of Optical Fiber and Laser, Shanghai, China) was fixed on the plexiglass holder for precisely positioning the working electrode. Alignment of the working electrode to the microchannel was performed under a microscope (Jiangnan Optical Instrument Factory, Nanjing, China). An Ag/AgCl reference electrode, a platinum wire counter electrode and a ground Pt electrode for CE were also placed in the reservoir along with the working electrode. Amperometric detection was carried out in a three-electrode configuration. Linear sweep voltammetry and amperometric detection were carried out on an Electrochem-

ical Workstation CHI 630 (Shanghai Chenhua Instrument Company, Shanghai, China).

2.6.3. Safety considerations

The high-voltage power supplier should be handled with extreme care to avoid electrical shock. *p*-Aminophenol is a harmful compound; skin and eye contacts or ingestion should be strictly avoided.

3. Results and discussion

3.1. Characteristics of the PET-toner microchannals

The EOF characteristic has been systematically investigated by us [24], and results showed that the EOF of such microchannel was in the direction of the cathode for a phosphate buffer system. Electroosmotic mobilities of 1.71×10^{-4} to 4.35×10^{-4} cm^2 V^{-1} s^{-1} were observed on an average from pH 3 to 10. The obtained EOF demonstrates that our printed PET-toner microchannels can provide a source of electroosmotic pumping for μTAS.

3.2. Influence of detection potential

Fig. 2 shows the hydrodynamic voltammograms of *p*-aminophenol and acetaminophen on a 10 μm platinum ultramicroelectrode. When the potentials exceeded +0.3 V for *p*-aminophenol and +0.6 V for acetaminophen, respectively, the peak currents for both analytes increased rapidly. However, the oxidation current leveled off when the potentials were more positive than +0.6 and 1.0 V for *p*-aminophenol and acetaminophen, respectively. Since too much higher anodic potential will result in higher background current, the detection potential in microchip system was set at 0.9 V. In addition, we found that the working electrode showed relatively good stability and reproducibility at the detection potential of 0.9 V under the present conditions.

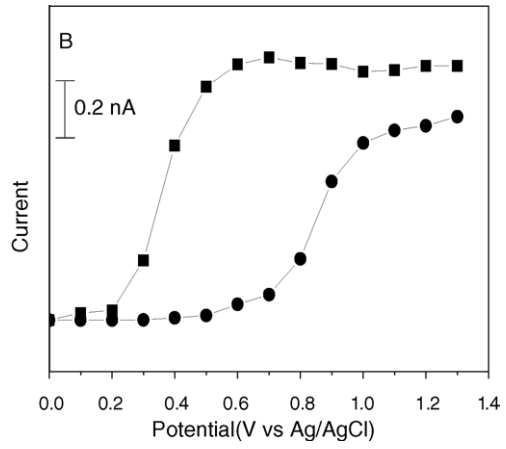

Fig. 2. Hydrodynamic voltammograms for 200 μM *p*-aminophenol (■) and 200 μM acetaminophen (●). Conditions—separation voltage: 1800 V; sample injection: at 1000 V for 5 s; working electrode: Pt ultramicroelectrode; running buffer: acetate buffer (10 mM, pH 5.0).

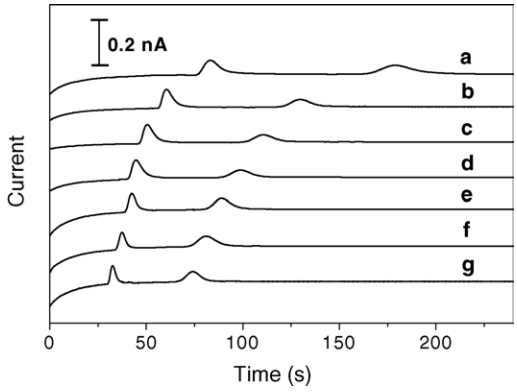

Fig. 3. Influence of separation voltage on the electropherograms. Separation performed at (a) 800 V; (b) 1000 V; (c) 1200 V; (d) 1400 V; (e) 1600 V; (f) 1800 V and (g) 2000 V. Other conditions as in Fig. 2.

3.3. Influence of separation voltage

Fig. 3 shows the effect of separation voltage on the amperometric response and separation efficiency. As expected, with the increasing of separation voltage from 800 to 2000 V (in 200 V increments, curves a–g), the migration time decreased significantly from 83.5 to 32.3 s and from 178.9 to 73.8 s for *p*-aminophenol and acetaminophen, respectively. The half-height peak width decreased from 8.9 s at 800 V to 3.1 s at 2000 V for acetaminophen and 17.5 s at 800 V to 8.0 s at 2000 V for *p*-aminophenol. As shown in Fig. 3, the separation voltage had negligible effect on the background noise level from 800 to 1400 V, but the background noise level would increase with the separation voltage from 1400 to 2000 V. Taking consideration of separation speed, separation efficiency, signal/noise ratio and baseline, a separation voltage of 1800 V was employed for further experiments. Theoretical plate number of 12 651 and 7195 m^{-1} was obtained at separation voltage of 1800 V for acetaminophen and *p*-aminophenol, respectively. At separation voltage of 1800 V, acetaminophen and *p*-aminophenol on a PET-toner-CE microchip integrated EC detection could be effectively separated and sensitively detected electrochemically as shown in Fig. 3, curve f.

3.4. Linearity, detection limit and reproducibility

The oxidation peak currents for acetaminophen and *p*-aminophenol on a Pt ultramicroelectrode at 0.90 V showed a linear relationship in the concentration range from 10 to 500 μM with sensitivities of 2.59 and 1.07 pA/μM and intercepts of 6.39 and 6.59 pA (correlation coefficients 0.9993 and 0.9989 for *p*-aminophenol and acetaminophen, respectively). Detection limits for both analytes were 5 μM (based

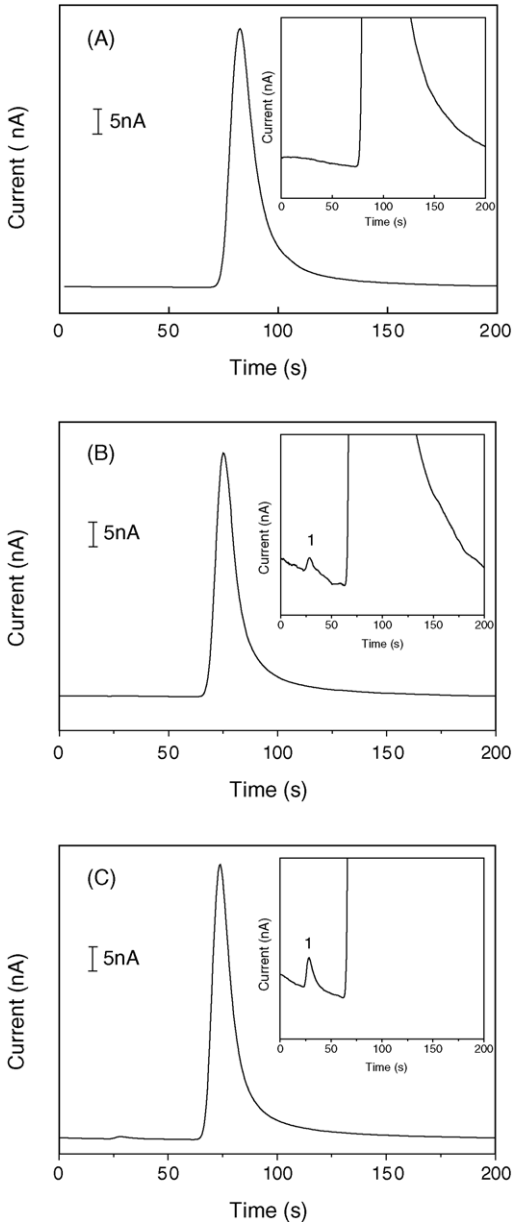

Fig. 4. Typical electropherograms obtained from extracts of tablets (A), sample spiked 0.5 μg mL^{-1} *p*-aminophenol (detection limit concentration with the present method) (B), and sample spiked 2.5 μg mL^{-1} *p*-aminophenol (the admissible concentration) (C) under the same conditions as in Fig. 2. For a better resolution of the current peak for *p*-aminophenol (peak 1), insets of local enlargements are included.

on S/N = 3). Nine repetitive injections of a mixture containing 0.2 mM acetaminophen and *p*-aminophenol resulted in a R.S.D. of 3.54% and 3.06% for the current response and of 0.92% and 0.66% for the migration times for *p*-aminophenol and acetaminophen, respectively, showing good reproducibility. These results are somewhat better than those obtained on PDMS microchips in our previous work [47]. But the PET-toner microchip has some advantages over PDMS microchip, such as simpler fabrication procedures and much lower cost.

Table 1
The recovery experiments

p-Aminophenol added (μg mL^{-1})	Result (μg mL^{-1})	Recovery (%)
0.5	0.506	101.2
2.5	2.57	102.8
5.0	4.93	98.6
7.5	7.28	97.1

3.5. Sample determination and recovery experiments

Fig. 4 shows electropherograms of the extracts of the tablet sample (Fig. 4A), the sample spiked 0.5 μg mL^{-1} (the same amount as the detection limit of our method, Fig. 4B) and the sample spiked 2.5 μg mL^{-1} (which is the admissible limit of pharmacopoeia, Fig. 4C). From the electropherograms, no signal from *p*-aminophenol in the real sample could be observed (Fig. 4A), demonstrating that *p*-aminophenol content in the tested paracetamol tablets is below the detection limit of the present method and the admissible limit. These results demonstrate that trace amount of *p*-aminophenol as low as 2.5 μg mL^{-1} can be distinguished from large amount of the acetaminophen and confirm that the amount of *p*-aminophenol in paracetamol tablets tested meets the pharmacopoeia requirements.

Standard addition recoveries experiments were performed by adding known amounts of standard to a blank sample. Results are shown in Table 1. The errors less than 3% in four experiments were in the acceptable range. Therefore, the present method could be used for qualitative detection of trace *p*-aminophenol in real sample such as paracetamol tablet and can be extended to other system.

4. Conclusion

A PET-toner microchip capillary electrophoresis system with end-channel electrochemical detection was established for effective separation and sensitive detection of acetaminophen and its hydrolysate using a Pt ultramicroelectrode. Our results demonstrate that the PET-toner microchips possess better performance than PDMS microfluidic devices. The simple fabrication procedures, low cost and mass production of the PET-toner-CE microchip device offers a great promise for bioanalysis, clinical analysis and medicine quality control.

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