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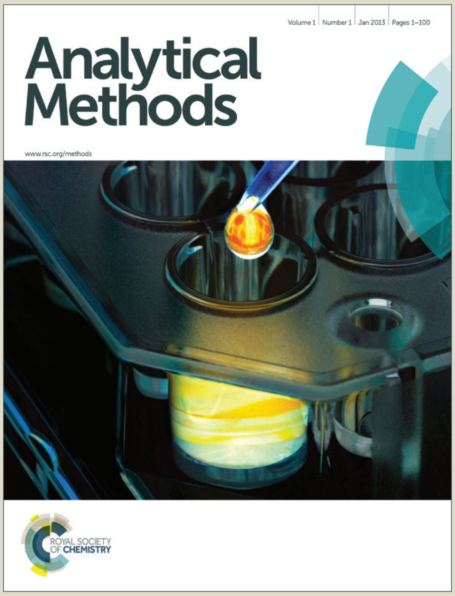

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Indium tin oxide coated PET film contactless conductivity detector for microchip capillary electrophoresis

Xingxing Yan, Wenfang Liu, Yu Yuan and Chuanpin Chen*

A conducting thin film contactless conductivity detection (CCD) sensor integrated with microchip capillary electrophoresis (CE) has been developed for the detection of metal ions. The CCD sensors were patterned on indium tin oxide (ITO) coated poly(ethylene terephthalate) (PET) film using DuPont dry film as a negative photoresist, followed by a bonding of poly(dimethylsiloxane) plate with the opposite side of the PET film. With the introduction of low-cost ITO-coated film and photosensitive film, the efficiency of prototyping and fabrication of the desired microchip CE−CCD system was significantly enhanced. Relevant experimental parameters for using CCD to analyze metal ions were optimized, including the concentration of run buffer, the gap between two sensing electrodes, and the excitation voltage and frequency. The analytical performance of the proposed device was illustrated by the separation of potassium, sodium, and lithium ions, achieving the detection limits of 5.8 μM, 8.0 μM, and 14.3 μM, respectively. Furthermore, the versatility of the device was demonstrated by the determination of a simulation sample with a mixture of heavy metal ions including zinc, cadmium, and copper ions in Xiangjiang River water. The three weak base ions were completely separated within 1 min with negative peaks due to their binding with acetate ions.

Introduction

Since the first micro total analysis system was presented by Manz in 1990,[1] microfluidic device has gained a significant development and made a creative revolution in the field of analytical chemistry.[2-5] Nowadays, researches are increasingly directing towards the development of highly integrated, miniaturized, and portable microfluidic devices in order to satisfy the growing demands for on-site analysis, especially in environmental monitoring and in process controlling.[6-8] Capillary electrophoresis (CE) is one of the most attractive techniques for separation and is inherently suitable to implement portable analytical tasks with the features of rapid analysis, low power consuming, and most importantly, without the loss of efficient separation capability in microfluidics.[9, 10]

For the construction of a truly portable device for analysis, compatible detection modes are in urgent needs to monitor the separation.[11-13] Although laser-induced fluorescence is capable of the detection, a bulky and external light source and other conventional components are generally required.[14-16] The integration of UV-absorption detection on microchip usually leads to a dramatic decrease of sensitivity since the pathlength is only several or dozens of microns.[15] For electrochemical detection mode, amperometric detection requires a high-priced potentiostat,[17-20] and potentiometric detection is based on an ion-selective electrode, which is applicable to limited ion species and difficult to be integrated into microchip as well.[21, 22] Therefore, these detection techniques are all difficult to achieve portability for wide applications. Conductivity detection has gained much attention on account of the advantages including a universal detector for all ionic species, intrinsic capability to be miniaturized, and compatibility with advanced microfabrication techniques. Such detection could be accomplished either by direct contact between sensing electrodes and solutions[23, 24] or by contactless form where electrodes are isolated from solutions by an insulating layer[25-31]. Contactless conductivity detection (CCD) is an attractive mode infinitely superior to the contact one, because it successfully avoids many problems caused by the direct contact of electrodes with solutions, such as electrode degradation, bubble generation, and limitation to low separation fields. Furthermore, CCD is characterized for simple signal electronic circuitry, unprecedented ease of cell arrangement and tremendous development of electrodes fabrication techniques, thus facilitating the integration, miniaturization and portability of devices.

The integration of CCD with microchip CE was proposed by Guijt firstly in 2001.[32] Since then, increasing approaches for the fabrication of sensing electrodes in CCD have been reported, with respect to electrodes materials and fabrication techniques.[30] Guijt RM developed CCD with the electrodes constructed on a printed circuit board using standard processing of exposure-developing-etching.[33] Lichtenberg J prepared platinum electrodes by depositing platinum layer in previously etched glass recesses in plane with a microchannel network.[34] Henderson RD successfully patterned polymer electrodes in polyaniline films by laser-welding.[35] However, these attempts have some common restrictions covering the demands for higher standard cleaning laboratory, specialized and expensive equipments and multi-step patterning/fabrication processes. Consequently, the overall time and costs are increased undesirably, which is contradictory to the requirements for fabricating portable microfluidic devices.

Indium tin oxide (ITO) electrode presented an alternative promising approach for CCD. ITO is a very attractive conducting material due to its good durability and stability in analytical procedures.[36, 37] Substrates for ITO include glass and poly(ethylene terephthalate) (PET) film. ITO-coated glass has been demonstrated to produce CCD sensors patterned by silk-screen printing for the determination of aminopyrine and caffeine.[38] However, because of many limitations, for example, a slide for one chip (75 × 25 × 1.1 mm with surface resistivity of 30 – 60 Ω/square) costs $50.9, it is very hard to be applied in portable devices as a disposable chip.

Moreover, the thickness of ITO-coated glass, which normally ranges from 0.3 to 2 mm, increases the distance between ITO electrodes and analytes, and thus, significantly reduces the sensitivity of CCD. Although a 1.3 mm thick ITO glass had been reported to be used for CCD without great loss of response signal,[38] a higher excitation frequency of 120 kHz was utilized, which requires sophisticated protection of the response signal from the interference of the stray capacitance between two sensing electrodes. Conversely, thinner glass substrate contributes high signal but has the disadvantage of fragility, which is not suitable for the fabrication and manipulation of electrodes. In recent years, ITO-coated PET film, popularly applied in touch display screen and solar cell panel, has attracted our interests. It has the features over ITO-coated glass. First, the most competitive is the extremely low cost with \$62.6/square meter (about \$0.1 per piece with the same size as the previously mentioned ITO-coated glass), which is very promising for the fabrication of portable microfluidic devices. Second, the thickness of PET film is only 0.125 mm, which could drastically decrease the impedance and contribute to obtaining a sensitive CCD response if it was used as insulating layer, thus, circumvent the need for high excitation frequency and more importantly, increase measurement flexibility. The usability of PET film with a thickness of 0.25 mm as an insulating layer has been demonstrated in the previous study, which was combined with PDMS microchannels and offered a stable electroosmotic mobility of $4.25 \pm 0.04 \times 10^{-4}$ V cm^{-2} s^{-1}.[39] Third, the elasticity of PET film provides a simple operation for the electrodes and their integration with poly(dimethylsiloxane) (PDMS) chips and increases durability as opposed to a glass chip. And to our knowledge, the use of conducting thin film as a CCD sensor in microfluidics has not been reported. In addition, a low-cost dry film photoresist technique, which had been used for the fabrication of the masters of PDMS microchannels,[40] was available for the patterning of ITO electrodes. The substantial cost savings provide a great possibility for the production of portable diagnostic equipments to achieve self-diagnosis at home in time. And benefiting from good elasticity and durability of

ITO-PET film as well, the device will be very applicable for analysis in complex, dangerous and emergency actual situations, helping protect analysts from damage.

In this report, we present a simple, low-cost CCD system based on a commercial ITO-coated PET film for microchip CE. The ITO CCD sensors were patterned on PET film using DuPont dry film photoresist and were isolated from run buffer in microchannels with the PET substrate. The analytical performance of the proposed device was illustrated using a standard analyte of potassium, sodium and lithium ions. And the versatility was then demonstrated by determining a simulation sample with a mixture of Zn^{2+}, Cd^{2+} and Cu^{2+} in Xiangjiang River water that was severely polluted by heavy metal ions.

Experimental

Materials, reagents and samples

PDMS and Sylgard 184 elastomer curing agent were supplied by Dow Corning (San Diego, CA, USA). A 25-μm thick Riston FX-925 dry film was obtained from DuPont (Wilmington, DE, USA). The ITO-coated PET film with surface resistivity of 45 Ω/square and the thickness of PET film in 0.125mm was produced by Zhuhai Kaivo Optoelectronic Technology Co. Ltd. (Zhuhai, China).

Unless otherwise specified, all chemicals were of analytical grade and used without further purification. Deionized water (resistivity of 18 MΩ/cm) from a Mill-Q purification system (KL-MINI4-T, Kertone, UK), was used throughout. 2-(*N*-morpholino)ethanesulfonic acid (MES) and *L*-histidine (His) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium, sodium, lithium, zinc, copper, and cadmium used in their chloride salts and acetic acid were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

All sample solutions were freshly prepared prior to use. The simulation sample was prepared by dissolving chemicals in the solution that was prepared by mixing Xiangjiang River water with 1 M acetic acid in a volume ratio of 9:1, or directly dissolving chemicals in Xiangjiang River water. All solutions were degassed in an ultrasonic bath for 5 min and filtered through 0.22 μm nylon syringe filters, which were obtained from Jinteng Laboratory Equipment Co. Ltd. (Tianjin, China), before being injected into microchip.

Microchip CE–CCD system

The microchip CE−CCD system was comprised of a high voltage supplier, a contactless conductivity detector and a PDMS/PET microchip integrated with ITO electrodes. The high voltage supplier was purchased from Spellman High Voltage Electronics Corporation (New York, America), which provided a potential of 0 – 5 kV. The homemade contactless conductivity detector provided an oscillation frequency of 0 – 80 kHz and an oscillation voltage of 0 – 175 V_{pp}. The detector was connected to a computer by a USB signal acquisition card (MPS-110001) obtained from Morpheus Electronics Technology Co. Ltd. (Beijing, China).

Device fabrication

The procedures and instruments used to fabricate the device were referred to the previous work.[40] Photomasks of desired electrodes and microchannel networks were designed by Adobe illustrator CS5 software, and then printed on a transparency film in a professional graphic service for later use.

Fabrication of sensing electrodes

ITO electrodes were manufactured using DuPont dry film as a negative photoresist (Fig. 1A). ITO-coated PET film was firstly cut into small pieces, followed by the removing of protective film. A layer of dry film was then laminated on the ITO film in an office laminator, and exposed to an irradiation for 40 s through an electrodes' photomask in a UV-exposure box. After removing the photomask and protective layer on dry film, ITO-coated dry film was immersed into 0.85% (m/v) sodium carbonate (Na_2CO_3) solution for developing and a subsequent treatment in 5% (m/m) hydrochloric acid for chemical etching. Then, the exposed dry film was cleaned away using 1.5% (m/v) sodium hydroxide (NaOH) solution. During the process, the ITO-coated PET film was rinsed in deionized water thoroughly before another solution was used. Finally, it was cleaned and dried with an electric drier, where the desired ITO electrodes were acquired.

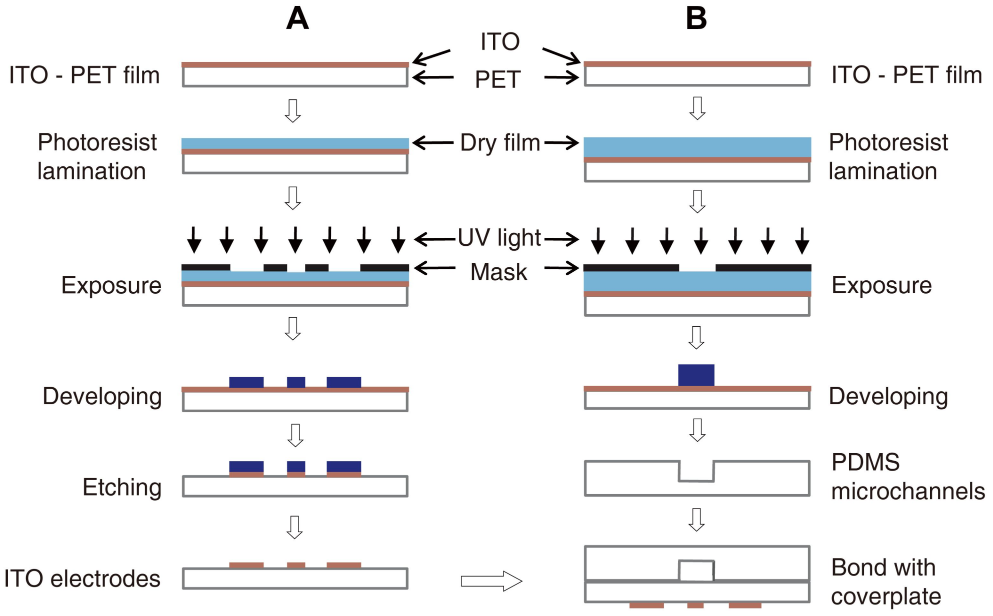

Fig. 1 Schematic illustration of the fabrication process of (A) the electrodes on ITO-coated PET film, and (B) the PDMS replica and bond with PET film for the assembled microchip.

Fabrication of PDMS microchannels

The master of a microchip was fabricated using DuPont dry film, which is schematically depicted in Fig. 1B. To start, two layers of dry film were sequentially laminated on the substrate, an ITO-coated PET film, followed by the exposure to UV radiation for 100 s using a microchannels' mask. Then the ITO-coated dry film was developed by immersing in 0.85% Na_2CO_3 solution. After a rinse in deionized water and dried at 60 °C in an oven for 4 hours, an ideal master was obtained. A 10:1 mixture of PDMS oligomer and crosslinking agent was poured onto the master, degassed in a vacuum chamber and cured at 60 °C for 2 hours. Finally, a PDMS replica with a 50 μm depth and a 50 μm width for both separation and injection channels was peeled off. Access reservoirs were then drilled for later fluid introduction.

Fabrication of the PDMS/PET microchip integrated with ITO electrodes

The PDMS plate with microchannels was treated with corona discharge for 2 min, and then brought into contact with PET film, the side without ITO electrodes, resulting in an irreversible covalent bonding. The CE–CCD microchip (Fig. 2) consisted of a standard cross-configuration with a 50 mm long separation channel and three 10 mm long channels for injection. And a high voltage electric filed was applied to the microchip through four 1 mm wide reservoirs. The two ITO sensing electrodes with a width of 1 mm for each were positioned 46 mm away from the cross-over. In the middle of the two electrodes, a 0.2 mm wide shielding electrode was grounded to decrease noise.

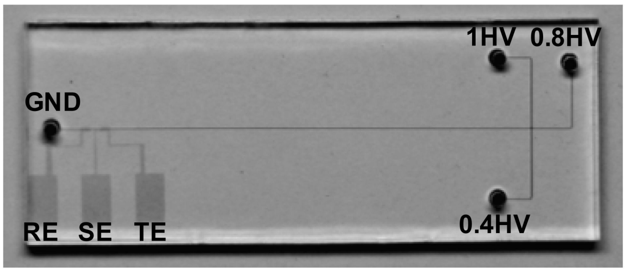

Fig. 2 Photograph of the PDMS/PET microchip integrated with ITO electrodes. The PDMS substrate was composed of a cross-over microfluidic structure and reservoirs that were full of black ink for visualization. High voltages in a ratio of 0.8:1:0.4 were applied to the sample reservoir, buffer reservoir, and sample waste reservoir, while the buffer waste reservoir was grounded. CCD sensor was shown with the transmitter electrode (TE), the shielding electrode (SE), and the receiver electrode (RE).

Electrophoresis procedure

Prior to running CE, the microchannels were sequentially flushed with 0.1 M NaOH, deionized water, and run buffer for 30 min, respectively. After flushing, all the reservoirs and channels were filled with run buffer, and the sample reservoir was replaced with sample solution finally. Sample loading and injection processes were performed electrokinetically with a gated injection method, in which the injection volume is dependent on the injection time. For sample loading, positive potentials of 1280, 1600 and 640 V were applied to sample reservoir, buffer reservoir (BR), and sample waste reservoir, respectively, whilst buffer waste reservoir was grounded. Injection occurred by electrically floating the potential at BR for 1 s and the sample migrated into the separation channel. Separation proceeded by reapplying the potential at BR. All experiments were performed at room temperature (25 °C) with a humidity of 50%.

Results and discussion

Optimization of the operating parameters

For successfully implementing ITO electrodes in PDMS/PET microchip CE–CCD system, operating conditions are required to be optimized in terms of the concentration of run buffer, the gap between two sensing electrodes, the excitation frequency and the excitation voltage. A mixture of K^+, Na^+ and Li^+ (1 mM) was used as standard analyte to evaluate the performance of CCD detector.

The concentration of run buffer

A chip with 1 mm gap between two sensing electrodes was utilized to evaluate the effect of different concentrations of run buffer (5, 10, 15, 20 and 25 mM MES-His (1:1)) on the separation efficiency, peak height, and signal-to-noise ratio (S/N) with an excitation voltage of 160 V_{pp} and an excitation frequency of 60 kHz. Since the three ions (K^+, Na^+ and Li^+) shared an identical trend, only the data of K^+ was chosen for display in Fig. 3. The separation efficiency, the peak height, and S/N all profiled an increase to the maximum and a subsequent rapid drop. The maximum separation efficiency was obtained at the concentration of 15 mM，slightly higher than that at 10 mM (Fig. 3A). However, the maximum of the peak height and S/N both appeared at 10 mM (Fig. 3B). Moreover, the stability of CCD signal at 10 mM was better than that at other concentrations. Therefore, the concentration of run buffer at 10 mM was chosen for further experiments.

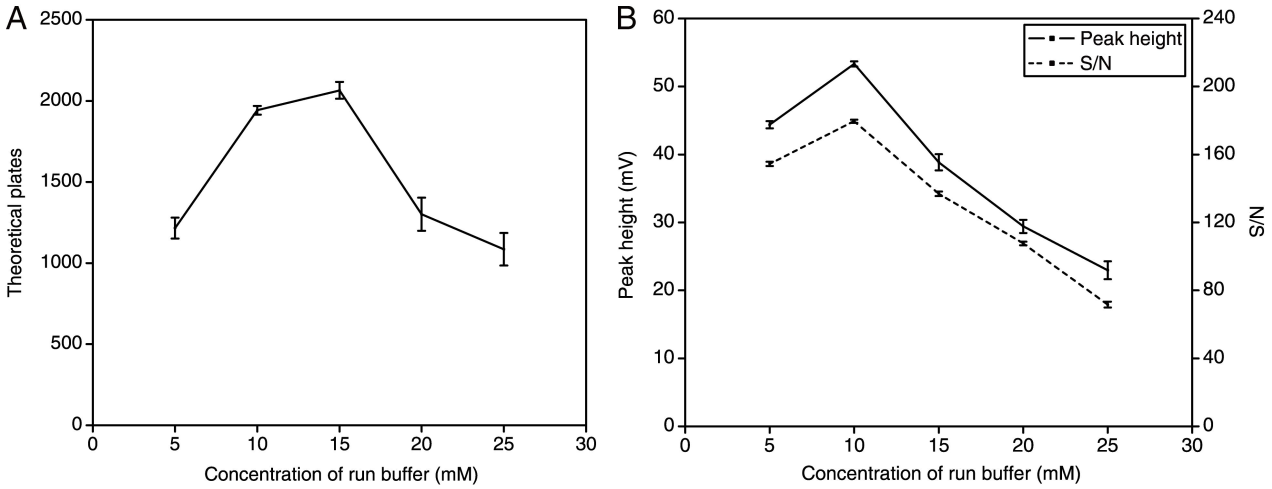

Fig. 3 Effect of the concentration of run buffer on (A) the separation efficiency, and (B) the peak height and S/N of K^+ (1 mM). Operating conditions: gap between two sensing electrodes, 1 mm; excitation frequency, 60 kHz; excitation voltage, 160 V_{pp}.

The gap between two sensing electrodes

The gap between two sensing electrodes from 0.5 to 2.5 mm was employed to evaluate the effect on the performance of the microchip CE–CCD system at a fixed excitation frequency of 60 kHz and voltage of 160 V_{pp} (Fig. 4). As the gap increased from 0.5 to 1 mm, both the peak height and S/N initially increased, and then decreased when the gap continued to increase (Fig. 4A). The maximum of peak height and S/N were obtained at the gap of 1 mm. This variation could be explained as the following. In microchannel, CCD signal is response to sample solution and/or buffer solution between the two electrodes. The buffer solution contributes as a background. At a definite excitation signal, if the gap is smaller than sample plug, an increase in the gap until to the sample plug of 1 mm can cover longer sample plug and increase its contribution to the CCD signal, thus resulting in an increase of the peak height. However, a gap, which is longer than sample plug, would not increase the amount of the sample further, and results in a decrease of the peak height because the gap covers the sample solution and more run buffer. Besides, a wide gap that covers more solution leads to low separation efficiency (Fig. 4B). Though, in general, the separation efficiencies of the chip were lower than the reported before[30], which might be due to the inhomogeneous microchannels formed by PDMS and PET. Considering that the alkali metal ions were both completely separated at the gap of 0.5 mm and 1 mm, 1 mm was selected as the optimal gap and was employed for further experiments because of its higher S/N.

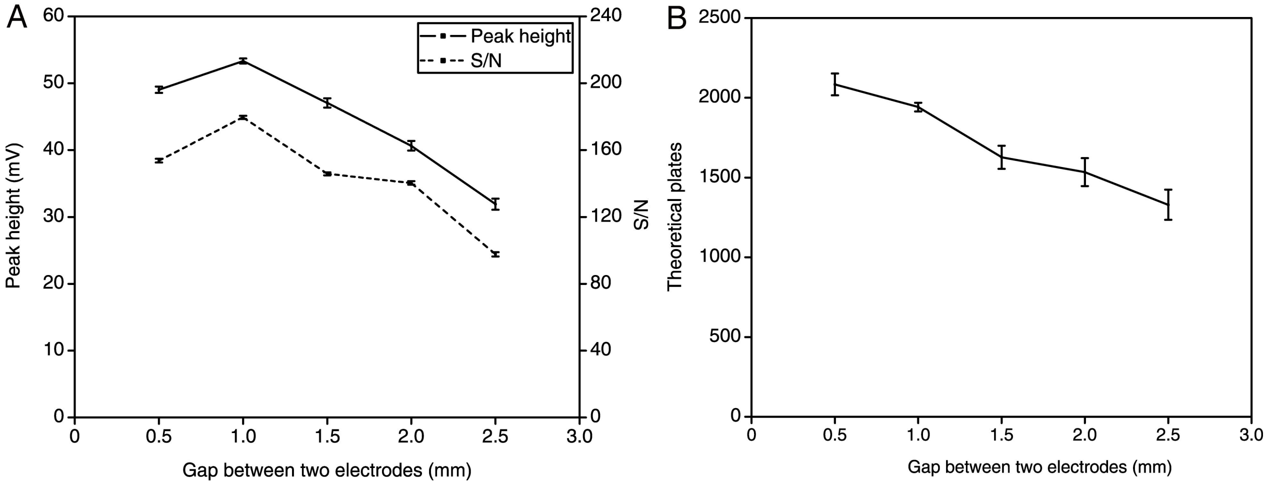

Fig. 4 Effect of the gap between two sensing electrodes on (A) the peak height and S/N, and (B) the separation efficiency of K^+ (1 mM). The other separation and detection conditions are same to Fig. 3.

Excitation frequency and excitation voltage

It is well known that excitation frequency has a decisive influence on the response signal of CCD. The effect of frequency on the peak height and S/N was evaluated on microchip at a given excitation voltage of 160 V_{pp} and 1 mm gap between two electrodes (Fig. 5A). It was shown that the peak height progressively increased when frequency increased from 20 kHz to 80 kHz, while the S/N experienced a tendency of increasing to the maximum, and then dramatically decreased. Thus, the maximum of S/N was found at 60 kHz. An increase of frequency can reduce a relatively high impedance of the coupling capacitance between the electrodes and the solution in microchannel, contributing to an increase of response signal. In our case, the capacitive reactance is close to the resistance of the run buffer between CCD electrodes. However, when a frequency was higher than 60 kHz, the response was simultaneously impacted by the terrible augment of the stray capacitance between two electrodes, which could lead to an increase of leakage current on the detector. Therefore, a significant deterioration of S/N was observed. In conclusion, the optimal excitation frequency was set as 60 kHz for the microchip CE–CCD system.

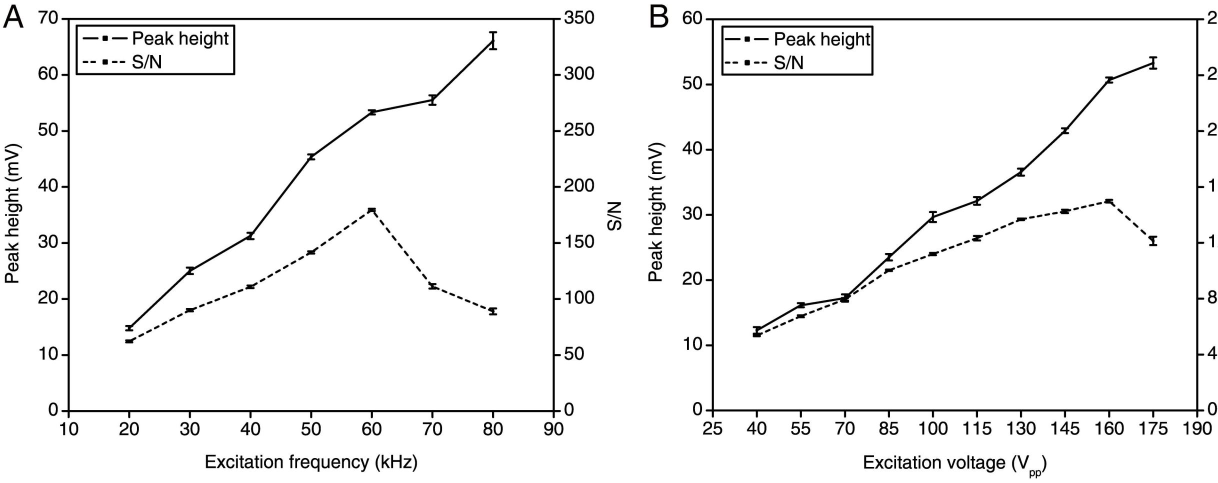

Fig. 5 (A) Effect of the excitation frequency on the peak height and S/N of K^+ (1 mM) at an excitation voltage of 160 V_{pp}. (B) Effect of the excitation voltage on the peak height and S/N of K^+ (1 mM) at the excitation frequency of 60 kHz. The other separation and detection conditions are same to Fig. 3.

Some researchers have reported that it is conducive to improve the analytical performance of the microfluidic CCD system by increasing excitation voltage.[34] However, too high voltage may significantly increase the leakage current between electrodes. It was demonstrated that the peak height increased with increasing excitation voltage from 40 to 160 V_{pp} under the frequency of 60 kHz (Fig. 5B). But when an excitation voltage was higher than 160 V_{pp}, the S/N was severely lost. In addition, the baseline drift appeared during high excitation voltage. Hence, the excitation voltage of 160 V_{pp} could provide most beneficial response characteristics.

Evaluation of the microchip CE–CCD system

To evaluate the proposed method, an equimolar mixture of K^+, Na^+ and Li^+ dissolved in the run buffer of 10 mM MES-His was tested in the PDMS/PET microchip CE–CCD system. In the electropherograms, 1 mM, 0.2 mM and 0.02 mM cations were completely defined and resolved within 40 s in the order of K^+, Na^+, and Li^+ (Fig. 6). And at 1 mM, the peak shapes of three ions all presented very well. Meanwhile, a good reproducibility was obtained through 10 consecutive runs of the standard mixture. The RSD% of migrated time of K^+, Na^+, and Li^+ was 0.45%, 0.63%, and 0.53%, respectively, and the RSD% of peak height was 0.73%, 0.92%, and 1.42%, respectively. Furthermore, a linear range was found from 10 to 200 μM for K^+ and Na^+, and 20 to 500 μM for Li^+ with correlation coefficients (R^2) of 0.999, 0.998, and 0.999, respectively. Based on the calibration curves, the detection limits for K^+, Na^+, and Li^+ at S/N = 3 were calculated to be 5.8 μM, 8.0 μM, and 14.3 μM, respectively, which were a little worse comparing with the previously reported 1.2 μM and 2.0 μM for K^+ and Na^+.[31] Overall, all the above results indicated a sensitive and reproducible detection method for quantitative analysis based on the ITO-coated PET film sensor.

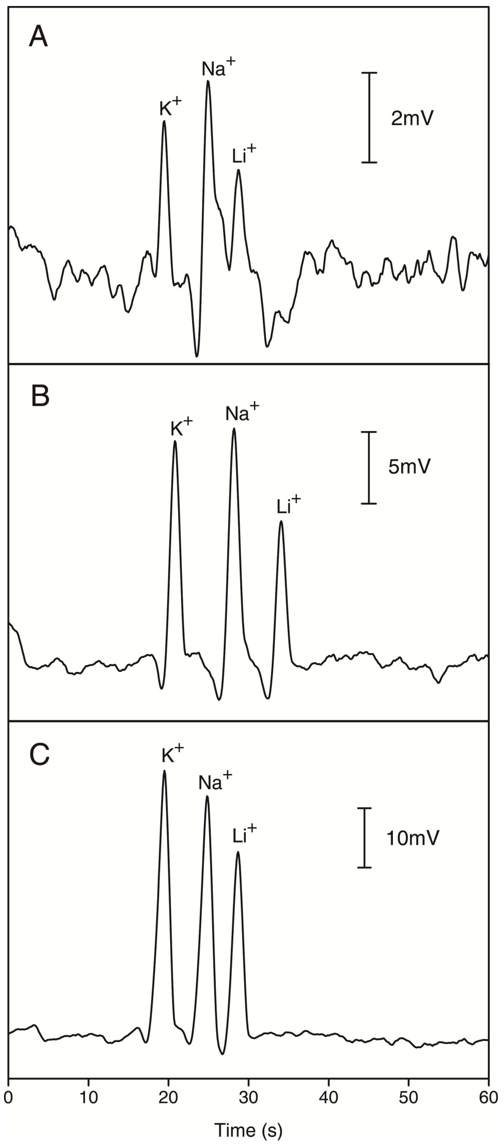

Fig. 6 Electropherograms of (A) 0.02 mM, (B) 0.2 mM and (C) 1 mM K^+, Na^+, and Li^+ based on ITO-coated PET film CCD sensor integrated with PDMS microchip. Separation and detection conditions are same to Fig. 3.

Determination of heavy metal ions

The pollution in Xiangjiang River (Changsha, China), especially heavy metals, has been paid more and more attention for the hypertoxicity and carcinogenicity. It is an extraordinary urgency to monitor the concentration of heavy metal ions on site and in real time. The electrophoretic separation of a standard mixture of Zn^{2+}, Cd^{2+}, Cu^{2+} (1 mM) was performed in the run buffer of 0.1 M acetic acid (pH 4.0). The other separation and detection parameters were set as the above-mentioned optimized value. Line a in Fig. 7 showed that the three ions were completely separated within 1 min in the order of Zn^{2+}, Cd^{2+}, and Cu^{2+} with negative peaks, which were resulted from the decrease of the run buffer's conductivity. During CE, the weak bases of the analytes including Zn^{2+}, Cd^{2+}, and Cu^{2+} were liable to combine with acetate ions, thereby, the total ionic strength was decreased. Accordingly, the conductivity of run buffer was reduced while the weak base ions migrated through the channel, and it was reflected as a negative peak. The migration time of these base ions was proportional to their alkalinity, and the peak height was related to their combination capacity with acetate ions. And the phenomenon of reversed signal strength comparing with alkali metal ions further confirmed the explanation. Then, a simulation sample of Zn^{2+}, Cd^{2+}, Cu^{2+} (1 mM) in the solution prepared by Xiangjiang River water was separated and detected in the PDMS/PET microchip CE–CCD system (line c in Fig. 7), with the electropherogram of a blank Xiangjiang River water sample to eliminate the interference from Xiangjiang River water (line b). It was displayed that there were no detectable ions in the blank water, and a complete and well-shaped separation and equivalent peak intensity with that of the standard analytes were achieved. To further enhance the response sensitivity, an effective way was used by preparing Zn^{2+}, Cd^{2+}, Cu^{2+} (1 mM) directly in Xiangjiang River water (line e in Fig. 7) and an obvious increase of the intensity of all peaks was observed. This was resulted from the stacking effect of field-amplified sample injection (FASI) caused by the low conductivity of Xiangjiang River water, in which the analyte was dissolved, compared to the run buffer. And FASI is also responsible for the detection availability of K^+ and Ca^{2+} in Xiangjiang River water (line d in Fig. 7), which had no effect on the separation and detection of Zn^{2+}, Cd^{2+} and Cu^{2+}. In a conclusion, if there is an illegal heavy metal ions wastewater discharged into Xiangjiang River, our detection system should be possible to monitor it.

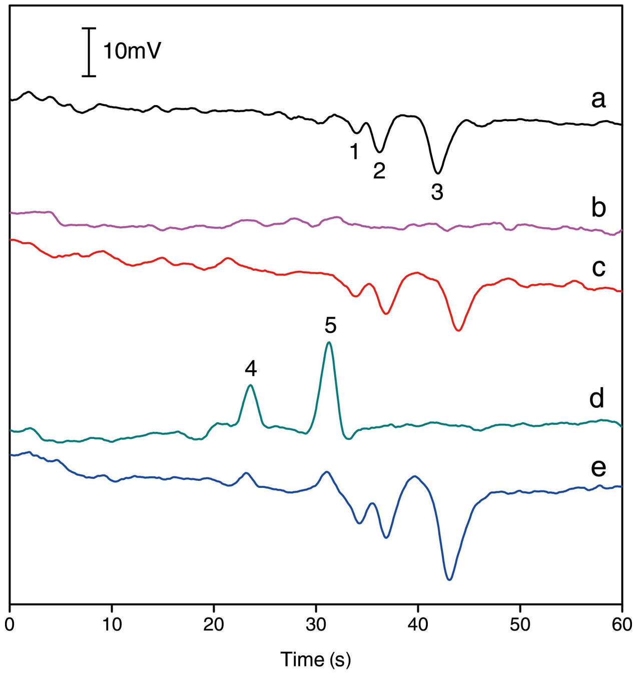

Fig. 7 Electropherograms of the samples based on the PDMS/PET microchip ITO CCD sensor with (a) standard mixture solution of Zn^{2+}, Cd^{2+}, and Cu^{2+} (1 mM), (b) a blank sample with 0.1 M acetic acid buffer in Xiangjiang River water, (c) a simulation analyte of Zn^{2+}, Cd^{2+}, and Cu^{2+} (1 mM) dissolved in 0.1 M acetic acid buffer in Xiangjiang River water, (d) Xiangjiang River water, and (e) a simulation analyte of Zn^{2+}, Cd^{2+}, and Cu^{2+} (1 mM) prepared directly in Xiangjiang River water. Peak assignments: 1, Zn^{2+}; 2, Cd^{2+}; 3, Cu^{2+}; 4, K^+; 5, Ca^{2+}. Run buffer: 0.1 M acetic acid adjusted to pH 4.0. The other separation and detection conditions are same to Fig. 3.

Conclusions

Our PDMS/PET microchip with ITO electrodes has been proved to be a low-budget and durable microchip CE–CCD system. By using a commercial ITO-coated PET film, the cost of CCD sensor is reduced to about $0.1, which is 500 times lower than an ITO-coated glass. Integrated with a DuPont dry film photoresist technique,[40] the fabrication of the PDMS/PET chip is unprecedentedly simple, rapid, and cost-effective without the requirement of a cleaning room. It has been demonstrated that the ITO CCD sensor PDMS/PET microchip has a high sensitivity (detection limit of 5.8 μM for K^+) and potential ability for the on-site detection of heavy metal pollutants including Zn^{2+}, Cd^{2+}, and Cu^{2+}. Moreover, the occurrence of negative peaks for the weak base ions in microchip CE–CCD system was elucidated. Although the detection limit is not good enough, it is hopeful to obtain a higher sensitive response based on improvement of a newly homemade contactless conductivity detector and use of thinner ITO-coated PET film. Meanwhile, with the development of ITO-coated PET film CCD sensor for microchip CE system, it could be expected that the ITO electrode fabricating technique will be employed as a standard format for CCD sensor in microchip CE, and thus, greatly expand the applications of microchip CE such as the separation of environmentally, pharmaceutically and clinically relevant compounds.

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Notes and references

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