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Microchip Capillary Electrophoresis with Solid-State Electrochemiluminescence Detector

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We report microchip capillary electrophoresis (CE) coupling to a solid-state electrochemiluminescence (ECL) detector. The solid-state ECL detector was fabricated by immobilizing tris(2,2′-bipyridyl)ruthenium(II) (TBR) into an Eastman AQ55D−silica−carbon nanotube composite thin film on an indium tin oxide (ITO) electrode. After being made by a photolithographic method, the surface of the ITO electrode was coated with a thin composite film through a micromolding in capillary (MIMIC) technique using a poly(dimethylsiloxane) (PDMS) microchannel with the same pattern as an ITO electrode. Then the TBR was immobilized via ion exchange by immersing the ITO electrode containing the thin film in TBR aqueous solution. The whole system was built by reversibly sealing the TBR-modified ITO electrode plate with a PDMS layer containing electrophoresis microchannels. The results indicated that the present solid-state ECL detector displayed good durability and stability in the microchip CE-ECL system. Proline was selected to perform the microchip device with a limit of detection of 2 μM (S/N = 3) and a linear range from 25 to 1000 μM. Compared with the CE-ECL of TBR in aqueous solution, while the CE microchip with solid-state ECL detector system gave the same sensitivity of analysis, a much lower TBR consumption and a high integration of the whole system were obtained. The present system was also used for medicine analysis.

Capillary electrophoresis (CE) microchips have received considerable interest in analytical chemistry recently due to their promising features such as short analysis time, efficient separation capabilities, minute consumption of samples and reagents, and portability.[1,2] Among the detection methods employed on CE microchips the commonly used detection mode is laser-induced fluorescence (LIF)[3,4] due primarily to the lowest concentration detection limits and high sensitivity. However, a LIF detector is expensive, difficult to implement, and often requires derivatization of the sample with a fluorophore. Another alternative detection mode is mass spectrometry (MS).[5] Although MS can provide more chemical information, it is nonportable, more costly, and less sensitive than LIF.[6] During the past several years, electrochemical detection has received increased attention and has been demonstrated as an attractive detection mode, which can offer comparable sensitivity and high flexibility.

Since electrochemiluminescence (ECL) based on tris(2,2′-bipyridyl)ruthenium(II) (TBR) was first reported by Tokel and Bard,[7] TBR ECL as a sensitive detection method has received considerable attention in chemical analysis because of its excellent stability and high efficiency in aqueous phase.[8] Moreover, as TBR is regenerated during the ECL process, a reagentless ECL sensor can be constructed by immobilizing the TBR on an electrode surface.[9] Compared with the solution-phase ECL procedure, the immobilization of the TBR reduces the consumption of expensive reagent and simplifies experimental design. So far, several different methods have been used to immobilize the TBR on an solid surface, including the Langmuir−Blodgett technique,[10,11] self-assembled technique,[12−14] and electrostatic attachment.[15−17]

Although so many techniques to immobilize TBR have been developed, the application of a solid-state ECL sensor is very limited. Our group[18] first fabricated a solid-state ECL sensor for traditional capillary electrophoresis by immobilizing TBR in poly(*p*-styrenesulfonate)−silica−poly(vinyl alcohol) grafting 4-vinylpyridine copolymer films. Lee's group[19] reported that the sol−gel-immobilized TBR ECL sensor was applied to the reversed-phase

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high-performance liquid chromatography determination of phenothiazine derivatives. Recently, Chen's group[20] developed TBR−zirconia−Nafion composite films as solid−state ECL detector for CE. Ju's group[21] detected heroin by flow injection ECL with immobilized TBR in a zeolite Y-modified carbon paste electrode. To our knowledge, the immobilizing TBR used as a solid-state ECL sensor has never been applied to a CE microchip mainly because most of the electrodes presently used to attach TBR are not transparent, such as platinum,[18] graphite electrode,[20] and glassy carbon electrode,[15,16,22] which makes the operation process complex and time-consuming. Moreover, once the microchip CE is combined with solid-state ECL detection, the whole system is further integrated together and simplified.

In this work, we proposed a novel microchip CE using solid-state ECL detection based on immobilizing TBR into the Eastman AQ55D−silica−carbon nanotube composite thin film on an indium tin oxide (ITO) electrode. While the ITO electrode allowed electrochemical current, its transparency did not affect the emission detection. The thin composite film was just coated on the ITO electrode with the help of a poly(dimethylsiloxame) (PDMS) microchannel stamp. The incorporation of TBR took place only at the area of the thin film, and thus, the TBR-modified ITO microelectrode of the pattern was formed. The PDMS layer containing separation and injection channel was reversely sealed with the ITO-coated glass slide to form the whole system. Proline was selected as test analyte to validate this solid-state CE-ECL microchip system in the experiments. And the present system was also used to detect ofloxacin and 4-(dimethylamino)butyric acid (DMBA).

EXPERIMENTAL SECTION

Chemicals and Materials. Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate was purchased from Aldrich (Milwaukee, WI). Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning (Midland, MI). RZJ-390 photoresist (for TN/ STN ITO) was purchased from Suzhou Ruihong Electronic Chemicals Co., Ltd. (Suzhou, China). Proline was obtained from Shanghai Biochemical Co., Ltd. Ofloxacin and DMBA were purchased from Sigma-Aldrich (St. Louis, MO). Other reagents and chemicals were at least analytical reagent grade. ITO-coated (150 nm thick and resistance of <15 Ω/square) glass was purchased from HIVAC Technology Co., Ltd. (Shenzhen, China). All solutions were prepared with water purified by a Milli-Q system (Millipore, Bedford, MA) and stored at 4 °C in a refrigerator.

Preparation of TBR-Modified ITO Electrode with MIMIC Technique. An ITO electrode with the desired pattern was fabricated by a photolithographic method and a PDMS stamp with a microchannel was made through a micromolding procedure as previously described.[23−25] A photoresist mask to fabricate the ITO electrode was also used for preparation of the PDMS stamp for MIMIC. So the microchannel pattern of the PDMS stamp was the same as that of the ITO electrode. Thus, they overlapped when the PDMS stamp was brought into conformal contact with the ITO electrode with the help of an optical microscope. The silica sols were prepared by TEOS hydrolysis in a manner similar to the previous report.[26] After the sol was blended with Eastman AQ55D and 0.1 mg/mL carbon nanotube (1:1:1, v/v), a 5-μL aliquot of the mixture above was dripped in one of the reservoirs at the end of the PDMS microchannel and automatically filled the whole micrchannel under capillary action. The sol solution was let to dry at room temperature. A thin composite film with the desired pattern of the ITO electrode was obtained when the PDMS stamp was carefully peeled off. The incorporation of TBR into the composite film was carried out by placing the electrode plate into 1.0 mM TBR solution for 10 min. Thus, the TBR-modified ITO microelectrode was formed.

Assembly of the Chip. The configuration of the PDMS chip was a simple cross, the microchannels for CE were typically 10 μm deep, 48 μm wide at the bottom, and 60 μm wide at the top, and the efficient length of the separation channel was 45 mm. Four reservoirs at each end of the channels were created with a hole punch. The PDMS layer containing electrophoresis microchannels and reservoirs was rinsed and dried and then was reversibly bound to the electrode plate under an optical microscope. The alignment between the separation channel and the ITO electrode was easily and quickly attained. Once the alignment of 50 μm was performed, a light pressure was evenly applied to the PDMS layer in order to seal two parts tightly.

Apparatus and Electrophoresis Procedure. A model 800 electrochemical analyzer (CHInstruments, Co., Austin, TX) was used to perform cyclic voltammetry and provide potentials for the oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$. The detection reservoir was filled with 30 μL of 50 mM PBS. A conventional three-electrode system consisted of a TBR-modified ITO working electrode developed on the electrode plate, Ag/AgCl reference electrode (in saturated KCl solution), and a platinum wire counter electrode. ECL signals were monitored by a MCFL-A multifunctional chemi- and bioluminescent analytical system (Remax Electronic Co., Ltd., Xi'an, China) with the voltage of the photomultiplier tube (PMT) set at 800 V. A model GY-4 programmable high-voltage power source (Research Center of Analytical Science, Northeast University, China) was applied to perform the electrokinetic sample injection and electrophoretic separation. The electrophoresis buffer was 10 mM PBS. The separation channel was treated before use by rinsing with 0.1 M NaOH, Milli-Q water, and running buffer for 15, 5, and 30 min, respectively. All these were carried out with the aid of a vacuum pump. In the experiments, external platinum wires were used to provide electrical contact from the high voltage to the solutions in the reservoirs. One of the three reservoirs was not used, but running buffer was added for balance. The injection was performed by applying high voltage 1 (HV_1) to the sample reservoir and high voltage 2 (HV_2) to the buffer reservoir for 5 s with detection reservoir at ground potential, in which HV_2 was equal to the partial value of HV_1 at the cross-connection point. During the separation, HV_1 and HV_2 were reversed with the detection reservoir maintained at ground all times.

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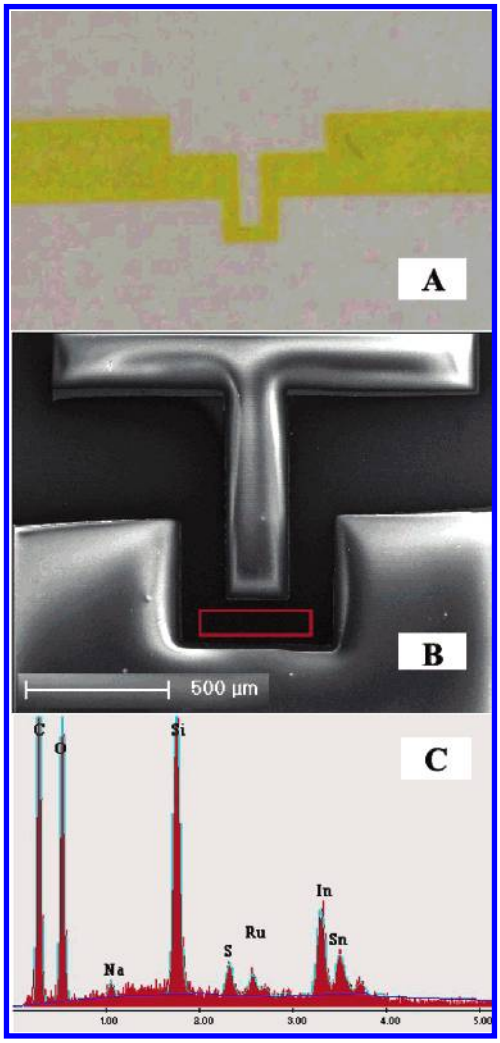

Figure 1. Photograph of TBR-modified ITO electrode by digital camera (A) and SEM (B). (C) EDS of rectangular part of composite film in (B).

Safety Considerations. The high-voltage power supply should be handled with extreme care to avoid electrical shock.

RESULTS AND DISCUSSION

Fabrication of the TBR-Modified ITO Electrode. The fabrication of the TBR-modified ITO electrode was given in Experimental Section. To characterize the modified electrode, SEM measurements were made on a XL30 ESEM FEG scanning electron microscopy at an accelerating voltage of 20 kV. Panels A and B of Figure 1 show typical TBR-modified ITO electrode images with good agreement to the electrode pattern of our original design (that of the ITO substrate). The effective width of the ITO working electrode is 200 μm. The chemical composition of the sectional rectangular part of prepared working electrode was determined by an energy-dispersed spectrum (EDS) (Figure 1C). The EDS spectrum shows the peaks corresponding to Ru, S, C, In, and Sn elements (other peaks originated from the substrate). Based on the observations, the TBR has been successfully assembled to the composite film after immersing in TBR aqueous solution. For measurement of the film thickness, the film was fabricated on a cover slide under the same conditions, and then the cover slide was cut to get a cross section of the film.

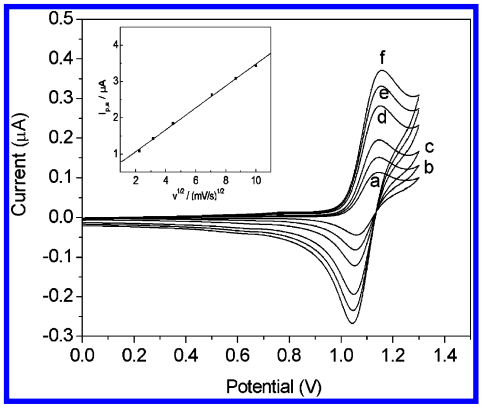

Figure 2. CVs of the TBR-modified ITO electrode at various scan rates in 50 mM PBS (pH 9.0). (a) 5, (b) 10, (c) 20, (d) 50, (e) 75, and (f) 100 mV/s. Inset: relationship between the anodic peak current and the square root of the scan rate.

The thickness of the cross section film was ~350 nm measured by SEM. We did not find any leakage with the present thickness of solid-state electrode.

Electrochemistry and ECL of Immobilized TBR on ITO Electrode. To check the electroactivity of TBR after entrapment in the AQ55D−silica−carbon nanotube composite thin film, the cyclic voltammograms (CVs) were performed in a detection reservoir containing 50 mM PBS (pH 9.0). Figure 2 shows the CVs of the TBR immobilized in composite film at various scan rates. A pair of redox waves appeared at ~1.1 V, which was the characteristic redox wave of TBR. The difference between the anodic and cathodic peak potential (ΔE_p) was 90 mV. Moreover, as shown in the inset of Figure 2, the oxidation peak current was proportional to the square root of scan rate, $v^{1/2}$, which indicates the immobilized TBR underwent a diffusion process within the film.

Figure 3A depicts the CVs of TBR-modified ITO working electrode in phosphate buffer (pH 9.0) with and without 0.5 mM proline at a scan rate of 50 mV/s. In the presence of proline, the anodic current increased while the cathodic current decreased due to the reaction of TBR with proline. The corresponding ECL intensity−potential curves obtained with PMT biased at 800 V are shown in Figure 3B. The onset of luminescence occurred near 1.0 V and then the ECL intensity arose steeply, which was consistent with the oxidation of the immobilized TBR. The ECL peak intensity occurred at 1.25 V, 1.06 V with and without proline in buffer solution, respectively. The shift of ECL peak potential may be attributed to the diffusion of proline in the solid composite film on electrode.

Determination of Proline by Microchip CE with a Solid-State ECL Detector. The analytical performance of the microchip CE with solid-state ECL detector was characterized by the detection of proline. Proline is a secondary amine and the most efficient amino acid for TBR ECL reaction. Both injection and separation were performed under electric field strength of 200 V/cm. With a solid-state ECL detector in the end-column detection mode, no significant effect of high separation electric field on ECL signals was observed at a distance of 50 μm between the microchannel outlet and working electrode. The effect of detection potential on the peak height for proline was investigated by altering the detection potential from 0.8 to 1.3 V (Figure 4). It

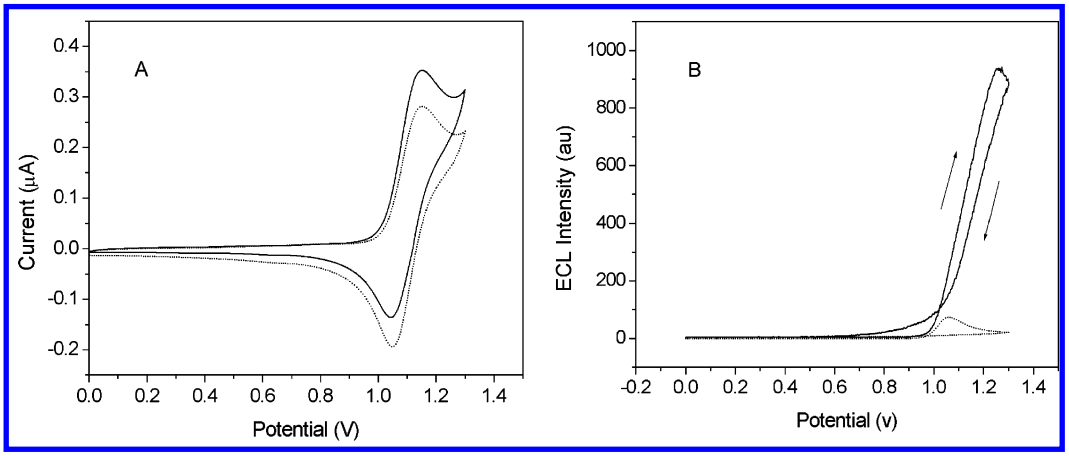

Figure 3. CVs (A) and corresponding ECL intensity−potential curves (B) of TBR-modified ITO electrode in the presence (solid line) and absence (dotted line) of 0.5 mM proline in 50 mM PBS (pH 9.0). Scan rate, 50 mV/s. PMT biased at 800 V.

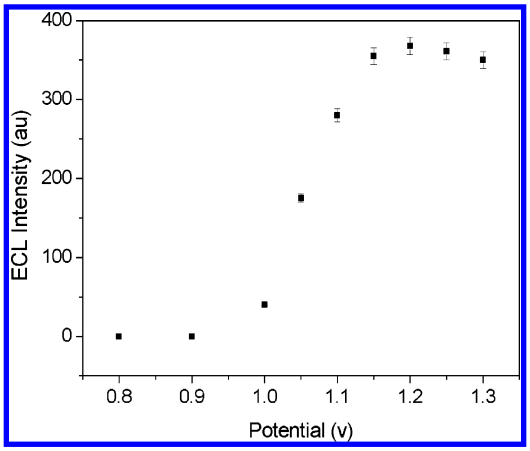

Figure 4. Dependence of the peak height on detection potential. Injection time, 5 s; $E_{injection} = E_{separation} = 200$ V/cm; running buffer, 10 mM PBS (pH 9.0); detection buffer, 50 mM PBS (pH 9.0); proline, 0.4 mM.

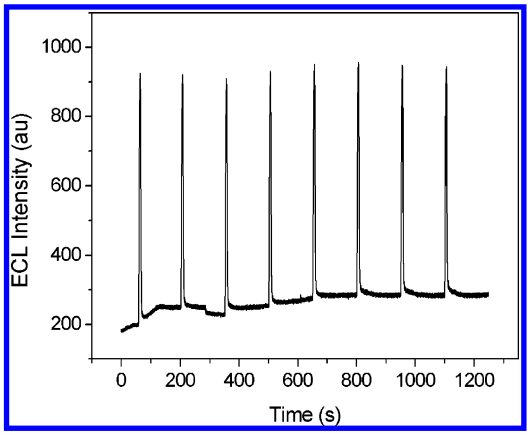

Figure 5. Electropherogram of eight repeated injections of 0.75 mM proline. Detection potential, 1.2 V (vs Ag/AgCl); injection time, 5 s; $E_{injection} = E_{separation} = 200$ V/cm; running buffer, 10 mM PBS (pH 9.0); detection buffer, 50 mM PBS (pH 9.0).

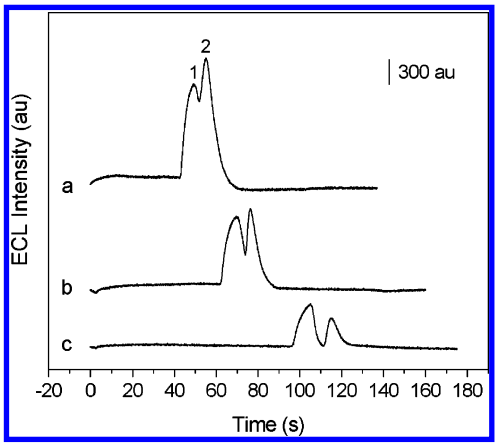

Figure 6. Influence of the electric field on the electropherograms for 0.5 mM DMBA (1) and 0.83 mM ofloxacin (2). $E_{injection} = E_{separation} = 200$ (a), 150 (b), and 100 (c) V/cm. Detection potential, 1.2 V (vs Ag/AgCl), injection time, 5 s; running buffer, 10 mM PBS (pH 7.5); detection buffer, 50 mM PBS (pH 7.5).

can be seen that, when the detection potential was less than 1.0 V, no light emission was observed. Whereas, when the potential was above 1.0 V, the ECL intensity increased with increasing potential and reached a peak at 1.2 V. So the optimum detection potential of 1.2 V was adopted to give an appropriate signal/noise ratio. Figure 5 is the electropherogram for eight consecutive injections of 0.75 mM proline. The relative standard deviation of the peak height was 3.11%, which reflects the good stability of the detection electrode. The ECL intensity for detection of proline was found to be linear between 25 and 1000 μM with a correlation coefficient of 0.997. The limit of detection was 2 μM (S/N = 3), which was similar to the previous report[24] where 5 mM TBR was added to the detection reservior. Compared with the conventional CE with solid-state ECL detector system,[18,20] the present system presented the same sensitivity.

Stability of the CE with a Solid-State ECL Detector System. The stability of the CE microchip with a solid-state ECL detector system was investigated under two storage conditions. When the TBR-modified ITO electrode was stored in dry state at room temperature over 2 months, the peak potential of TBR was essentially unchanged and no evident decrease in the ECL response for the determination of 0.5 mM proline was found. When the whole microchip CE-ECL system was under electrophoresis conditions all the time, the electrode had a lifetime ~4 h with reproducible analytical results obtained during this period. When the great reduction of ECL response of the proline occurred, the electrode was broken and the exposed area of the composite film in the detection cell come off, but the ITO substrate was in good condition. This provided the possibility to rebuild the electrode. After peeling off the PDMS layer, the ITO electrode

was cleaned by sonicating in 10% KOH in ethanol and distilled water. A subsequent MIMIC and ion-exchange procedure was used to refabricate the TBR-modified electrode. Often, using the same PDMS stamp and procedure, the same TBR-modified ITO electrode was gotten. The whole microchip CE with solid-state ECL system could be rebuilt in 1 or 2 h.

Application. The present microchip CE with solid-state ECL detector system was used to separate and detect DMBA and ofloxacin. Figure 6 displays the influence of the injection and separation electric fields on the electropherograms for 0.5 mM DMBA and 0.83 mM ofloxacin. As expected, the lower injection electric field allowed less samples to be injected in the separation channel and resulted in lower ECL intensity with the same injection time. Meanwhile, with the lower separation electric field, longer migration time and better separation efficiency were achieved. It is noted that the relative ECL intensity of ofloxacin and DMBA became weaker with lower electric field. This phenomenon may be explained by the change of electric field resulting in a larger influence on the injection of ofloxacin.

CONCLUSIONS

We have demonstrated the integrated microchip CE with solid-state ECL detector for the first time. The solid-state ECL detector was fabricated by TBR ion-exchanged AQ55D−silica−carbon nanotube composite thin film on ITO electrode by the MIMIC technique. The microchip CE with solid-state ECL detector has been successfully employed to detect proline with low detection limit and good reproducibility. Compared with other microchip CE-ECL system where TBR was added to the detection cell, this solid-state mode was a cost-effective ECL detector with the same sensitivity.

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