一、 Development of microchip integrated with electrochemical sensor in conjunction with indium tin oxide electrode

We developed a microchips integrated with electrochemical sensor which uses indium tin oxide (ITO) film electrodes as a working electrode and a decoupler. There were many kinds of electrodes employed in microchip, such as carbon paste, carbon fiber, or metal evaporation. However, the difficulties of fabrication and integration between electrodes and a microchip device always exist. The ITO film (30-nm-thin) on a glass substrate was patterned as 200 and 100-μm-wide stripes using photolithography and wet etching. The microchannels (100 μm wide by 50 μm deep) were made by molding poly(dimethylsiloxane) (PDMS) from a patterned silica wafer, then the PDMS slab was bonded to the ITO-glass substrate by air plasma. This fabrication provided a way to solve the problem that the in-channel electrochemical detector integrated with microchip. The performance of this electrochemical sensor was evaluated by the separation of catecholamine. Excellent efficiency and resolution were obtained. Four catecholamines were successfully separated and detected by this microchip. The calibration curves were all linear with the coefficient of determination ($r^2$) exceeding 0.997 for target analytes. The limit of detection of norepinephrine was less than 0.89 μM. This approach has a great potential for use in the fabrication of in-channel electrochemical microscale analytical systems for chemical and biochemical separations.

**Introduction**

Microchip capillary electrophoresis (microchip-CE) is characterized by a number of analytical advantages, including its rapidity, small sample requirement, and the potential for integration.[1-3] The electrochemical detection is recently being widely use for micro-total-analysis system (μ-TAS). The most important advantage of electrochemical detection is the compounds need not to be labeled with any fluorescent dye, so long as they can be reduced or oxidized. Presently, most electrochemical detections integrated into microchip are adopted end-channel.[4-7] Although the end-channel detection design is easily integrated with microchip, the detecting signal is also easily influenced by sample diffusion and mass transfer. Therefore, the in-channel detection design is getting to put in use. However, the integration of in-channel detection with

microchip is still a difficult problem. In the former scientific literatures that used in-channel detection mostly need the decoupler to decouple the current of electrophoresis.[8-9] Therefore, the material and pattern designed of decoupler is becoming important. We choose the indium tin oxide (ITO) glass to pattern the decoupler and working electrode design.

The ITO glass is recently widely used for thin display panel industrial thus can easily to purchase the standard specification that we needed.[10] By using photolithography and wet etching, we can get the designed electrodes' pattern on a glass plate. In addition, by molding poly(dimethylsiloxane) (PDMS) from a patterned silica wafer can get the microchannels.[11-15] These two parts can be easily assembled by air plasma. The structure of completed microchip, including ITO glass part and PDMS part, was stable, lasting, and without any leaking. In the former research, the ITO glass based microchips were most used for the thermal source of polymerase chain reaction (PCR) microchip[16], chemical luminance detection[17,18], and detect the cells releasing compounds.[19] Directly applying the ITO as the detecting electrode was seldom utilized. In this experiment, according to the results of cyclic and hydrodynamic voltammetry testing, some neurotransmitters can directly be detected by ITO-base detection in the specific condition.

Neurotransmitters are indispensable components of the human body and their detection remains an important challenge. In this paper we describe a detection method that allows the presence of dopamine metabolites to be monitored on a dry-film-based microchip incorporating a copper wire electrode. This system can quickly and effectively separate and detect all of the products in the dopamine metabolic procedure, including 3,4-dihydroxyphenylalanine (DOPA), dopamine, norepinephrine, and epinephrine.

## Experimental

*Apparatus*

The photolithographic procedures involved a UV aligner (model Union EMA-400, Tokyo, Japan) for exposing, and an auto-development machine for developing. The plasma cleaner (model PDC-32G, Harrick Plasma, NY, USA) was used for microchip bonding. The detection system was an electrochemical analyzer (model 8021b, CHI, USA) coupled to the working, auxiliary, and reference electrodes through sockets. A high-voltage power supply system (model MP-5000-250P, Major Science, Taipei, Taiwan) having a programmable adding voltage system

and an adjustable voltage range from 0 to +5 kV was used to perform microchip CE separation.

*Electrophoresis*

Before performing each electrophoresis experiment, the channels of the microchip were rinsed with D.I. water (resistance: >18 MΩ/cm) and 10 mM phosphate buffer solution (pH 7.4) for 10 min each. All standard solutions of neurotransmitters (1 mM) were prepared in D.I water; the stock solutions were diluted with running buffer to the desired concentrations. The reservoirs were filled with the running buffer and the sample and then a sample injection potential of 100 V/cm was applied for 15 s. Subsequently, the separation was initiated by switching to different voltages across the separation channel.

**Results and Discussions**

*Hydrodynamic voltammetry*

The details of the procedures for fabricating the ITO-microchip are described in Section 2.3. To determine the oxidized potential of each neurotransmitter, we used hydrodynamic voltammetry to simulate oxidization at the working electrode in the ITO-microchip. A solution of each neurotransmitter was prepared in running buffer and injected as the background solution; we then recorded the oxidized current from the working electrode. Figure 3 depicts typical hydrodynamic voltammograms for oxidation of 500 μM of epinephrine, norepinephrine, DOPA, and dopamine at the ITO detector. The curves were developed point wise (in steps of 0.1 V) over the 0.0-0.8 V range using a separation voltage of +1000 V. These voltammetric profiles indicate that the ITO detector offers a oxidized performance for those compounds. For all four compounds, the amperometric detection work employed a constant potential of +0.6 V (in order to obtain sensitive detection) and offered the most favorable signal-to-noise characteristics. A dramatic increase in the baseline current, its slope, and the corresponding noise was observed at higher potentials.

*The properties of microchip*

The measurement precision is quite comparable to that obtained with the traditional injection formats discussed earlier. The sequence of separations in Figure 4 also shows that successive injections can be repeated on a rapid time scale. Injection time was controlled in 5 s

(controlled by high-voltage power supply system) and resulted in the reproducible separations. With a 1000 V separation potential, each separation was completed in 90 s. This result showed that the stability and reproductivity were good enough and suitable to use for routine analysis. The increase of baseline was due to Joule heating, which might have been apparently dominated after a long detection time.

Certainly, the bubble problem is always influence the microchip-CE. Especially in the in-channel detection design, the bubble would block the current of electrophoresis with the result that experimental failed. In this experimental, we use the ITO as the decoupler. In the practical operation result, the electric field can apply to 230 V/cm at most. When the electric field held on 230 V/cm, the bubble will produce at around 150~200 seconds. When the electric field lowered than 200 V/cm, this ITO-microchip system can keep up more than 500 seconds without bring any bubble. Therefore, we set the electrophoresis electric field lower than 200 V/cm in this experimental.

*Analysis of catecholamines*

As an application of this ITO-microchip, we demonstrated the detection of neurotransmitters using the patterned ITO film as a working electrode. Due to the structures of epinephrine and norepinephrine are very similar, and the charge ability and polarity are also pretty nearly. To be obvious that is not easily to separate them. The resolution between norepinephrine and epinephrine was not larger than 1.0 at any separation voltage (from 100 V/cm to 250 V/cm); thus, to change the separation voltage was not a useful way to improve this problem. Therefore, we chose the SDS to improve the running buffer to expect the effect of partition between the micelle and analytes. Figure 5 shows the effect of different concentration of SDS to the analytes. When the concentration of SDS higher than critical micelle concentration (8.2 mM), the migration time and peak of each analyte is getting more and broadening. However, when the concentration of SDS was at 10 mM, the peaks' resolution between the epinephrine and norepinephrine is more than 1.5 that both of them were completely separated. Although the peak's tip of four analytes were separated between 10 to 30 seconds when the concentration of SDS more than 15 mM, the band broadening was to influence the resolutions. Therefore, we chose to add 10 mM of SDS into the running buffer as the one of separating conditions.

To quantify each sample, we detected the signal at the working electrode over the

concentration range and calculated the limits of detection (LOD), the results was shown as Table 1. The correlation coefficient of each analytes was all larger than 0.997, and the LOD of norepinephrine was as low as 0.89 μM. In these experiments, the plate numbers were quite large because of adding SDS. As a general rule in capillary electrophoresis systems, higher separations occur at larger plate numbers; in our experiments, however, the plate numbers maximized (~$10^5$) at adding 10 mM SDS for detecting 200 μM L-DOPA.

Figure 6 shows the optimum conditions to separate the neurotransmitters in serum (fetal calf serum). The source of serum was provided from National Health Research Institutes (NHRI, Taiwan). The standards of analytes can be clearly identified. Although there were two unknowns in serum sample that can be detected by ITO detector, their migration times were entirely different from the standards. The unknowns were excluded from ethylenediaminetetraacetic acid (EDTA, use as anticoagulant) by standard addition, and the NHRI also promised that the treatments of serum without adding any EDTA. However, the matrixes of serum were not influence the analytical results; thus, this detection system had high potential for biological and medical researches.

**Conclusion**

The ITO-based electrochemical detector was successfully integrated with PDMS microchip. This approach that has great potential for use in the fabrication of in-channel electrochemical microscale analytical systems has completely sealed to avoid the leakage from the part of detector. The performance of this electrochemical sensor was evaluated by the separation of catecholamine. Excellent efficiency and resolution were obtained. Four neurotransmitters were successfully separated and detected by this microchip. The ITO-based detector has good linear range and long life time for detection. Otherwise, according to the analytical result of serum, this detection system that will not be influenced by the matrixes of serum, and have high potential for biological and medical researches.

二、 Determination of selective serotonin reuptake inhibitors by cation selective exhaustive injection-sweeping-micellar electrokinetic chromatography

We have investigated a rapid and highly efficient on-line preconcentration method using in

cation selective exhaustive injection-sweeping-micellar electrokinetic chromatography (CSEI-sweeping-MEKC) for the analysis of selective serotonin reuptake inhibitors (SSRIs) of antidepressant drugs. Several of the CSEI-sweeping-MEKC parameters to effect successful separations, such as the pH, concentrations of buffer, sodium dodecyl sulfate (SDS), and organic modifier, the injection length of high conductivity buffer (HCB), and the injection time of sample were optimized. The optimized background electrolyte was a pH 2.2, 50 mM citric acid/disodium hydrogen phosphate buffer containing 100 mM SDS and 22% isopropanol. The sensitive enhancement of five SSRIs, including sertraline, fluoxetine, paroxetine, fluvoxamine and citalopram ranged from $5.68\times10^4$ to$1.19\times10^5$-fold. The coefficients of determination exceeded 0.994 and the relatively standard deviation of peak height was less than 3.22%. The detection limits were in the range of 0.056 ng/mL to 0.221 ng/mL. The optimized method was employed to analyze five SSRIs in plasma sample prepared via solid phase extraction (SPE) for minimizing the matrix effect. The recovery of SPE extraction efficiency had satisfactory achieved up to 89%. The experimental results indicate the optimized conditions of CSEI-sweeping-MEKC method could successfully determine five SSRIs in human plasma.