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Determination of Catecholamines by an Electrochemical Flow-Through Detector with Indium-Tin Oxide Microelectrode Arrays Coated with Nafion

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An electrochemical flow-through detector with indium-tin oxide interdigitated microelectrode arrays was used for the determination of catecholamines. This detector showed amplification of the current signals by redox cycling. The undesired response due to ascorbic acid can be eliminated by coating the electrode surface with Nafion.

Electrochemical detection in flow systems becomes popular because of its high sensitivity and analyte selectivity.[1] Among various types of the detectors reported, a configuration of two disk electrodes located in series in a flow-through cell has substantial advantages.[2] For reversible or quasi-reversible substrates, the products formed at the up-flow electrode can be detected at the down-flow electrode, resulting in improving the selectivity and sensitivity. The characteristics of the series electrode configuration depends largely on the efficiency of the down-flow electrode to collect the reaction products, and thus on the inter-electrode gap. Recently, Anderson and coworkers[3-5] have demonstrated the attractive feature of an electrochemical detector with interdigitated microelectrode arrays. The microelectrode arrays have a small equal inter-electrode gap; therefore, the collection efficiency is high compared with those observed on series disk electrodes. Taking advantages of such characteristics, we have carried out the determination of catecholamines (epinephrine, norepinephrine, and dopamine) in the presence of ascorbic acid by an electrochemical flow-through cell with indium-tin oxide (ITO) interdigitated microelectrode arrays. We have also investigated the effect of a Nafion film coated on the arrays in order to eliminate the undesired response of ascorbic acid.

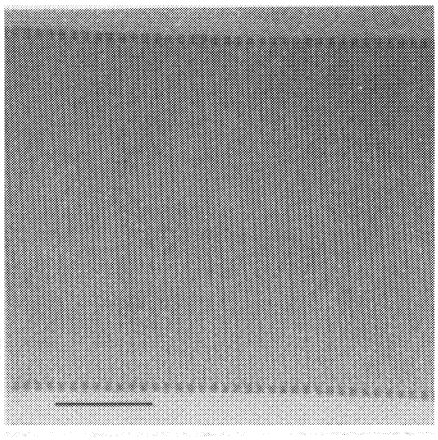

Fig. 1. A SEM image of the ITO microelectrode arrays. The bar indicates 0.5 mm.

The interdigitated microelectrode arrays were prepared by conventional microphotolithography using a Shipley photoresist (Microposit 1400-31) from an ITO plate (Seiko Electric, film resistivity; 15 ohms square^{-1}). The etching of ITO was conducted by electroreductive dissolution at

-0.9 V vs. SCE in a 1.0 M(=mol dm^{-3}) HCl solution. Figure 1 shows a SEM image of the resultant electrode. The whole electrode has two microelectrode arrays; each has 50 electrode elements. The element is 0.02 mm in width and 2 mm in length, and separated by 0.01 mm from the adjacent element. Since the ITO electrode has large overpotentials for oxidation of catecholamines, platinum was deposited[6] on the ITO electrode surface. The surface coverage by the platinum particle was less than 2%. This treatment reduced the overpotentials; at pH 5.3, the treated electrode showed an oxidation peak for dopamine at 0.69 V vs. Ag/AgCl in a voltammogram (0.1 V/s), whereas the bare electrode at 1.09 V vs. Ag/AgCl. We have also fabricated gold microelectrode arrays, but the gold showed insufficient adhesion to a glass plate. The ITO microelectrode arrays were placed in a flow-through cell with a Teflon spacer (thickness; 0.2 mm). The flow channel was 1 mm wide and 8 mm long and the total cell volume was 1.6 mm^3. The auxiliary eletrode was a glassy carbon disk mounted in the opposite wall. An Ag/AgCl reference electrode was placed in an out-flow chamber connected with the flow-through cell. The potential control and current monitoring were carried out using a self-made bipotentiostat. A detector with two GC disk (Tokai Carbon, GC-20, 3 mm in diameter) electrodes placed in series (referred to a series-disk detector) was also fabricated to make a comparison of the two detectors. All chemicals used in the present study were obtained from the commercial suppliers. The coating of the electrode surface with Nafion was carried out by a spin-coating method using a Nafion solution (Aldrich). The thickness of the Nafion film was estimated by SEM measurements and found to be ca. 100 nm. The chromatographic system was comprised of a JASCO 880-PU pump connected with a SUS column (4.0 mm x 10 cm) packed with ODS silica (Gasukuro Kogyo, 5 μm). The sample was injected with a 50 mm^3 sample loop. The mobile phase was 0.1 M sodium acetate buffer (pH 5.3) containing 1% THF, 2.3 x 10^{-4} M sodium octylsulfate, and 1.6 x 10^{-4} M EDTA. The flow rate was 1.0 cm^3 min^{-1}.

The characteristics of the array detector was compared with that of the series-disk detector by using ferrocenylmethyltrimethylammonium ion (FA^+) as a substrate. The collection efficiency, the ratio of the cathodic to anodic currents, was 0.60 for the array detector, whereas that for the series-disk detector was only 0.37. Both anodic and cathodic responses on the array detector are large compared with those on the disk detector. This difference implies the signal amplification[3,4] by redox cycling. The array detector can amplify the current response without amplification of the area-dependent, current-independent noise.[7]

The array detector has been used for selective determination of catecholamines in the presence of ascorbic acid. Ascorbic acid is widely distributed in biological samples. The concentration of ascorbic acid in the biological sample is usually by two or three orders of magnitude higher than that of catecholamines. Therefore, the elimination of undesired response due to ascorbic acid is necessary for rapid analysis of catecholamines. Figure 2 shows the chromatograms observed by the array detectors, where the up-flow electrode was set at 0.80 V and the down-flow electrode at 0.0 V vs. Ag/AgCl (A) or disconnected from the potentiostat (B). The comparison of the peak heights on (A) with those on (B) shows the

amplification of current by redox cycling. Collection efficiencies for the catecholamines are around 0.5, similar to the value observed for FA^+. Ascorbic acid gave a large oxidation peak, while the peak in the cathodic chromatogram was small. This makes it easy to recognize the peaks for catecholamines. Catecholamines are known to show an ECE-type reaction with intramolecular cyclization of the quinone intermediates on electrooxidation.[8] Using the rate constant reported,[8] one can calculate the half-lives of the quinone derivatives at pH 5.3 to be 350 ms, 30 s, and 53 s, respectively, for epinephrine, norepinephrine, and dopamine. The electrochemical oxidation of ascorbic acid produces dehydroascorbic acid which is rapidly hydrated and converted to an electro-inactive form.[9] Since the rate constant for the hydration is 1.3×10^3 s^{-1},[9] the half-life of the dehydroascorbic acid is calculated to be ca. 0.5 ms. Under the present conditions, the average time required for the solution to travel the gap spacing between the electrode elements (0.01 mm) is 0.24 ms. Thus, the down-flow electrode is capable of reducing the quinone derivatives, although the flow rate near the electrode surface is slow compared with the average value. On the other hand, most of dehydroascorbic acid formed at the up-flow electrode is hydrated before it reaches the down-flow electrode. The undesired peak of ascorbic acid, however, still appears on the cathodic chromatogram.

The peak of ascorbic acid could be wiped out from the cathodic chromatogram when a detector with Nafion-coated arrays was used. Figure 3 shows the chromatograms obtained with the Nafion-coated array detector. The relative peak of

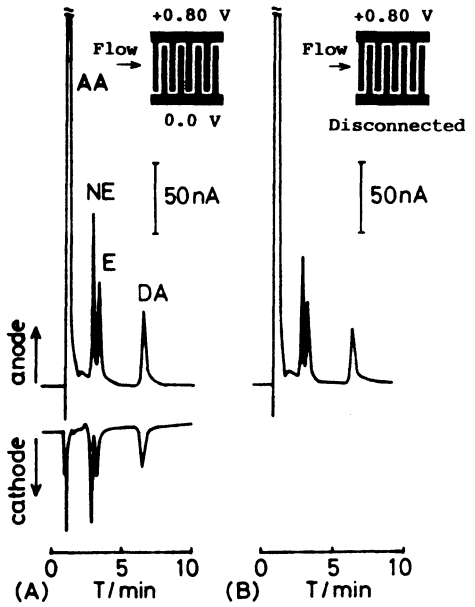

Fig. 2. Chromatograms for 2.0×10^{-5} M catecholamines and 1.0×10^{-3} M ascorbic acid at the bare ITO microelectrode arrays. Applied potential: up-flow electrode, 0.80 V vs. Ag/AgCl; down-flow electrode, 0.0 V vs. Ag/AgCl (A), disconnected from the potentiostat (B). AA; Ascorbic acid. NE; Norepinephrine. E; Epinephrine. DA; Dopamine. Mobile phase; 0.1 M acetate buffer (pH 5.3). Flow rate; 1.0 cm^3 min^{-1}.

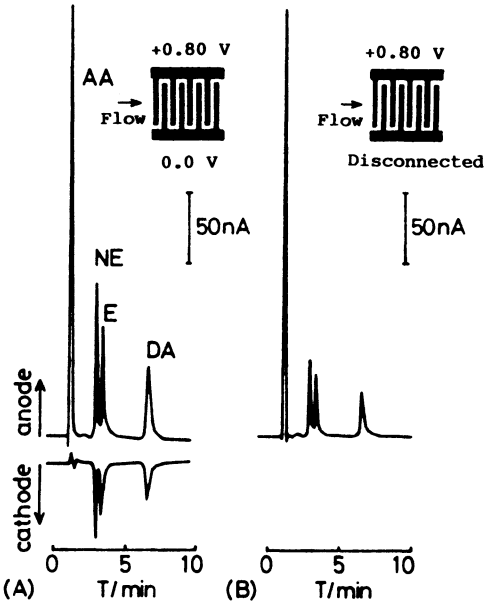

Fig. 3. Chromatograms for 2.0×10^{-5} M catecholamines and 1.0×10^{-3} M ascorbic acid at the Nafion-coated ITO microelectrode arrays. Experimental conditions are same as those in Fig. 2.

ascorbic acid to those for catecholamines was much smaller than that observed with the bare array detector and no response was observed for ascorbic acid in the cathodic chromatogram. At pH 5.3 catecholamines are present in the cation forms, whereas ascorbic acid in the anion form. Nafion could recognize this difference in charge and reject anionic ascorbic acid.[10-14] The shapes of the peaks were greatly dependent on the thickness of the Nafion film on the detector. The coating with thick Nafion film resulted in effective rejection of ascorbic acid but in broadening the peaks for catecholamines. We have used the film thickness of ca. 100 nm. Under this condition, dopamine in the solution needs only ca. 30 ms for penetration of the Nafion film to reach the electrode surface, since the apparent diffusion coefficient of dopamine in Nafion was 2×10^{-9} cm^2 s^{-1}.[10] This situation would be practically same for other two cathecholamines. The coating with the Nafion film of ca. 100 nm thickness had no large influence on the shape or height of the peaks for catecholamines. On the other hand, the peak of ascorbic acid reduced remarkably by the coating. The plots for catecholamines were linear in the concentration range, 10^{-6} - 10^{-4} M.

The above results demonstrate that interdigitated microelectrode arrays coated with Nafion is applicable to the selective determination of catecholamines. Further investigation including digital simulation is now being undertaken.

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