Surface-Modified Electrochemical Detector for Flow Injection Analysis. Poly(*N,N*-dimethylaniline)-Coated Glassy Carbon Electrodes

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An electrochemical thin-layer flow detector comprised of a glassy carbon electrode coated with a thin film of electropolymerized poly(*N,N*-dimethylaniline) (PDMA) with the net positive charge has been constructed and its performance has been examined. Substantial improvement in the selectivity of amperometric detection for flow injection system was observed as a result of an electrostatic interaction between the positively charged PDMA film and analytes, that is, cationic species (e.g., dopamine, 1-norepinephrine and DL-3,4-dihydroxyphenylalanine) exhibited little response, while the response of neutral and anionic species (e.g., 3,4-dihydroxyphenylacetic acid, 4-hydroxy-3-methoxyphenylacetic acid and catechol) was only slightly changed at the PDMA-coated electrode compared with the bare electrode. The film permeability toward analytes, defined as the ratio of the peak current at the film-coated electrode to that at the bare electrode, was found to depend on the preparation conditions of the PDMA film (electrolysis potential and amount of charge passed). The flow injection response at both bare and coated electrodes increased with increasing the flow rate. In addition, the PDMA coating was found to function, to some extent, as a barrier against the electrode poisoning due to adsorption of surface-active compounds (e.g., bovine serum albumin).

1 INTRODUCTION

Electrochemical detection (in particular, amperometric detection) in a flow system such as flow injection analysis or liquid chromatography has attracted considerable interest in recent years ^{1, 2)}, because excellent sensitivity and selectivity toward electroactive analytes can be expected to be achieved by proper functionalization of the detector surface, e.g., by coating the detector surface with polymeric films with

discriminative properties ³⁻¹⁴⁾ (based on size exclusion or charge exclusion). Wang et al. ⁵⁾ have demonstrated that electrodes coated with negatively charged Nafion films offer highly selective response for cationic neurotransmitters (dopamine, epinephrine, etc.) in the presence of other interfering anionic or neutral oxidizable species. They have also reported that the permselective, positively charged film of a protonated poly(4-vinylpyridine) offers significant selectivity improvements in a manner (charge exclusion) analogous to that of Nafion coatings ⁶⁾. Similar studies have also been done by other researchers ³⁻¹⁶⁾. The amperometric detectors of this type in flowing streams may eliminate the need for a sample pretreatment or separation column, commonly required when mixtures of oxidizable compounds (e.g., dopamine and ascorbic acid) are assayed, because they

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possess the "in situ separation property" ⁵⁾ based on the analyte charge.

In this paper, we describe an electrochemical detector comprised of glassy carbon electrode coated with poly(*N,N*-dimethylaniline) (abbreviated as PDMA) film, which can be prepared by electro-oxidative polymerization of *N,N*-dimethylaniline with the glassy carbon electrode in an acidic aqueous solution ^{17,18)}. We have previously demonstrated that the PDMA film with quarternary ammonium groups as permanently charged positive sites has an anion-exchange character irrespective of pH and strongly binds multiply charged negative ions via an electrostatic interaction ¹⁷⁻²⁰⁾. A molecular-charge-based, excellent permselectivity of the PDMA film toward dissolved species as well as its strong adhesion to electrode surface and reasonable chemical stability are the main reason for its use as an electrode modifier. The selectivity and the analytical application of the PDMA film-coated glassy carbon electrode for different kinds of molecular charge were examined.

PDMA

2 EXPERIMENTAL

2.1 Reagents

N,N-dimethylaniline (DMA) of reagent grade was obtained from Kanto Chemical Co. and was purified by distillation using ordinary techniques. Sodium sulfate of reagent grade was used as a supporting electrolyte in the electrolysis of DMA for the preparation of poly(*N,N*-dimethylaniline)-coated electrodes. Stock solutions of dopamine (DA), 4-hydroxy-3-methoxyphenylacetic acid (HVA) (Kanto

Chemical Co. Ltd.), norepinephrine(NE), ascorbic acid (AA) (Wako Pure Chemical Co.), catechol (CA), 3,4-dihydroxyphenylalanine(DOPA) (Tokyo Kasei Co., Ltd.) and 3,4-dihydroxyphenylacetic acid (DOPAC) (Aldrich Chemical Co.), which are of reagent grade, were prepared fresh before each experiment. A 0.05 M (1M=1 mol dm⁻³) phosphate buffer (Na₂HPO₄/NaH₂PO₄·2H₂O, adjusted to pH 4.0 with phosphoric acid) was used as electrolyte and carrier in the flow-injection experiments. Aqueous solutions were prepared from doubly distilled water. All other chemicals were of reagent grade and were used without further purification.

2.2 Apparatus and procedures

A standard three-electrode electrochemical cell was used for the electrochemical experiments. The electrode assembly consisted of a glassy carbon (GC, Bioanalytical System Inc.) or a poly(*N,N*-dimethylaniline) film-coated GC as the working electrode, an Ag/AgCl electrode (HS-205C, TOA Electronics Ltd.) as the reference electrode and a spiral platinum electrode as the counter electrode. For cyclic voltammetry, a potentiostat (HAB-151, Hokuto Denko Co., Ltd.) was employed along with an X-Y recorder (Graphtec Co.). All electrochemical experiments were run under nitrogen atmosphere at laboratory temperature.

The poly(*N,N*-dimethylaniline) (PDMA) film-coated GC electrode was prepared by the same procedure as previously described ^{17,18)}. Prior to the coating, the amperometric response of the bare surface was evaluated. For this purpose, the electrode was polished with alumina particles (No. 2000, Marumoto Kogyo Co., Ltd.), rinsed with double-distilled water, and allowed to air-dry. The electro-oxidative polymerization to coat the same surface with the thin film of PDMA was potentiostatically carried out by holding the working electrode at 0.8-1.2 V vs. Ag/AgCl in 0.5 M Na₂SO₄ aqueous solution (pH1.0) containing 0.1 M DMA and H₂SO₄. Then, the amount (*Q*) of charge passed, measured with a coulometer (Toho Technical Co., Model 3320), was 0.5-3.0 C cm⁻². Previously, it was found that the thickness of the

film prepared on electrodes was controlled by Q and typically the films of PDMA (0.48 μm thick) were grown on an electrode by passing 1 C cm^{-2} of charge 18).

The BAS-200 electrochemical analyzer (Bioanalytical Systems, Inc. (BAS)) along with a DM2M-1024 Solution Delivery Pump of the dual plunger type and injector (SV1-507, Sanuki Kogyo Co., Ltd.) was used in the flow-injection experiments 21). A sample loop of 100 μl was used. The flow cell used for electrochemical detection was of the thin-layer design (LC-17A, BAS). The Ag/AgCl reference electrode (RE-4, BAS) was positioned downstream of the working electrode, and the auxiliary electrode was positioned directly across the thin-layer channel (the gap: ca. 50 μm) from the working electrode. The working electrode was bare or PDMA-coated glassy carbon (electrode area: $7.1 \times 10^{-2} \text{ cm}^2$, MF-1000, BAS) and the auxiliary electrode was stainless steel. The PDMA film-coated GC detector was equilibrated with the flowing supporting electrolyte (or mobile phase) solution, while the working potential was applied (typically at 0.7 V vs. Ag/AgCl). Amperometric measurements were made after the background current decayed to a steady-state value. The flow rate was varied in the range 0.25–2.0 ml min^{-1} . Injected volume of sample solutions was 10 μl . The current-time output was recorded with a survocorder (SR6342, Graphtec Co.).

3 RESULTS AND DISCUSSION

Figure 1 shows typical cyclic voltammograms for DA and DOPAC at bare and PDMA film-coated GC electrodes in 0.05 M phosphate buffer solution (pH4.0). The current response (e.g., anodic peak current) for DOPAC is almost the same at the bare and coated electrodes, while the response for DA at the coated electrode is significantly smaller than that at the bare electrode. According to their pK_a values (4.22(DOPAC) and 8.92(DA))²², DOPAC and DA are considered to be present as anionic (and/or neutral) and cationic species, respectively, in the buffer solution used (pH4.0). As a result of

Fig. 1 Cyclic voltammograms of (A) DOPAC and (B) DA at bare and PDMA-coated GC electrodes in 0.05M phosphate buffer solution (pH4.0) containing $1.0 \times 10^{-3} \text{ M}$ DOPAC or DA. (A-1, B-1): at PDMA-coated electrode (area: $8.0 \times 10^{-2} \text{ cm}^2$), (A-2, B-2): at bare electrode (area: $8.0 \times 10^{-2} \text{ cm}^2$). Potential scan rate: 50 mV s^{-1} . The PDMA film-coated electrode was prepared by electro-oxidative polymerization of DMA at 1.0V vs. Ag/AgCl in 0.5M Na_2SO_4 solution (pH1.0) containing 0.1M DMA and H_2SO_4 (amount of charge passed: 1.0 C cm^{-2}).

electrostatic rejection of PDMA film (thickness: ca. 0.5 μm), DA exhibited little response. The same situation was the case for NE. On the other hand, the response of anionic and/or neutral DOPAC (at pH4.0) was only slightly changed at the coated electrode compared with the bare

Fig. 2 Flow-injection current/time profiles for $2 \times 10^{-5} \text{ M}$ DOPAC, DA and NE at (A) PDMA-coated and (B) bare GC electrodes. Applied potential: 0.7 V vs. Ag/AgCl. Flow rate: 1.0 ml min^{-1} . Injected volume of each sample solution: 10 μl . Electrolyte and carrier: 0.05M phosphate buffer solution (pH4.0). Other experimental conditions are the same as in Fig. 1.

Table 1 Selective permeability of the PDMA coating toward various solutes under flow injection conditions^{a)}.

Solute	net charge of solute at pH4.0	$i_{p,f}/i_{p,b}^{c)}$
3,4-dihydroxyphenyl acetic acid (DOPAC)	-1	0.96
catechol (CA)	0	0.74
ascorbic acid (AA)	-1	0.64
4-hydroxy-3-methylphenylacetic acid (HVA)	-1	0.56
DL-3,4-dihydroxyphenylalanine (DOPA)	+1, -1 ^{b)}	0.26
dopamine (DA)	+1	0.13
1-norepinephrine (NE)	+1	0.12

a) Experimental conditions: flow rate, 1.0 ml min⁻¹; injection volume, 10 μ l; electrolyte and carrier, 0.05 M phosphate buffer (pH4.0); applied potential, 0.7 V vs. Ag/AgCl; solute concentration, 20 μ M; thickness of PDMA film, ca. 0.5 μ m.

b) DOPA is present as a zwitter ion in the buffer solution used.

c) $i_{p,f}$ and $i_{p,b}$ correspond to the peak currents obtained at the film-coated electrode and at the bare electrode, respectively.

electrode due to the ion-exchange behavior of PDMA film 17-20).

The flow-injection responses for DOPAC, DA and NE obtained with the bare and PDMA film-coated GC detectors, typically shown in Fig. 2, reflected the above-mentioned cyclic voltammetric behavior. For DOPAC, the response at the coated electrode is almost the same as that at the bare electrode. In contrast, the response for DA and NE is largely decreased at the coated electrode. This basis for the practical utility of the PDMA-coated detector in flow injection analysis is its discrimination against cationic electroactive species. Such a discrimination is attributed to the charge repulsion, which is associated with the net positive charge of the PDMA film. The degree of this discrimination depends on the film permeability toward various oxidizable analytes under flow injection

conditions. The ratio of the peak current at the film-coated electrode to that at the bare electrode, $i_{p,f}/i_{p,b}$, can be used as a measure of the permeability. Table 1 summarizes the data concerning the selective permeability of the PDMA coating toward various oxidizable solutes. We can see distinct changes in the permeability based on solute charge. Cationic species such as DOPA, DA and NE yield very low $i_{p,f}/i_{p,b}$ values, 0.26, 0.13 and 0.12, respectively. On the other hand, facile transport through the PDMA film is observed for anionic (counter ions) or neutral species $i_{p,f}/i_{p,b}$ values ranging from 0.56 to 0.96). Thus, contributions from easily oxidizable cationic species can be minimized without lowering the operational potential and high sensitivity is maintained for numerous analytes. The fact that the $i_{p,f}/i_{p,b}$ values for cationic species are not zero may suggest that the PDMA coating is sufficiently porous to allow slow diffusion of cations 17-20). In addition, the clearly different permeability for CA and the structurally similar catecholamines (e.g., DA and NE), being attributed to the protonated amine moiety, should be noted.

Fig. 3 Dependence of the film permeability ($i_{p,f}/i_{p,b}$) on the amount (Q) of charge passed during the electropolymerization of DMA. (○): DOPAC, (○): DA, (●): NE. Flow-injection experimental conditions are the same as those in Fig. 2.

Fig. 4 Dependence of the film permeability ($i_{p,f}/i_{p,b}$) on the potential ($E_{\text{preparation}}$) at which the constant-potential electrolysis of DMA was carried out to prepare the PDMA-coated electrodes. (○): DOPAC, (○): DA, (●): NE. Flow-injection experimental conditions are the same as those in Fig. 2.

The film permeability was found to depend on the amount (Q) of charge passed during the electrolysis of DMA, i.e., the film thickness (Fig. 3). The $i_{p,f}/i_{p,b}$ values decreased with increasing Q irrespective of the net charge of solute, being in agreement with the expectation that the transport through the PDMA film is diffusional for all the solutes examined: The thicker the film is, the resistance for physical diffusion is the larger and thus the $i_{p,f}/i_{p,b}$ response is the smaller. The relatively large permeability observed for DA and NE at $Q < 1 \text{ C cm}^{-2}$ is probably due to the pin-hole of the thin films. For the film of $Q = 1.5 \text{ C cm}^{-2}$, the largest differences in permeability between DOPAC and DA (or NE) were obtained; The ratios of $i_{p,f}/i_{p,b}$ for DOPAC to those for DA and NE are 5.7 and 8.5, respectively. Further, we observed that the selective permeability of the film largely depends on the

applied potential ($E_{\text{preparation}}$) at which the electropolymerization by a constant-potential electrolysis was conducted (see Fig. 4)*1. The direct reason for this fact has not been clarified yet, but this may be probably concerned with the different morphology, porosity, etc. of the films prepared at different $E_{\text{preparation}}$ values. A further study concerning this is now in progress. At any rate, we can see from the results in Fig. 4 that the film prepared at $E_{\text{preparation}} = 1.0 \text{ V vs. Ag/AgCl}$ has the most effective permselectivity.

To examine the flow-rate dependence, flow injection detection peaks for DOPAC were recorded at different flow rates, ranging from 0.25 to 2.0 ml min^{-1} . The results are shown in Fig. 5. Both the bare and the PDMA-coated GC detectors

Fig. 5 Flow rate dependence of peak current (i_p) for DOPAC. (○): at bare GC electrode, (●) at PDMA-coated GC electrode. Flow-injection experiments were carried out at different flow rates in the same way as in Fig. 2.

exhibited an increase of signal with increased flow rate. For the bare electrode, the increased current response with increasing the flow rate can be explained as a result of larger flux of DOPAC to the electrode surface at higher flow rate. On the other hand, the current response observed

*1 In this case, the amount of charge passed was 1 C cm^{-2} in each preparation.

with the coated electrode may suggest that under the experimental conditions used here the diffusional flux of DOPAC through the film is almost comparable to its flux to the film/solution interface controlled by the flow rates employed. A similar flow rate dependence has been also reported for the Nafion-coated detector by Wang et al.⁵⁾, who observed a slight increase in peak current for injections of 50 ng NE over the 0.2-0.6 ml min⁻¹ range and a flow-rate independence over the 0.6-1.2 ml min⁻¹ range.

The potential of the permselective transport characteristics of PDMA-coated GC detectors for selective amperometric detection in flow injection system is typically demonstrated in Fig. 6. An

Fig. 6 Flow injection detection of DOPAC in the presence of NE at (A) PDMA-coated and (B) bare GC electrodes. (a) 20 μ M DOPAC, (b) 20 μ M DOPAC + 20 μ M NE, (c) 20 μ M DOPAC + 40 μ M NE. Experimental conditions are the same as in Fig. 2.

additive response is observed at the bare electrode (B) when mixtures of oxidizable compounds (DOPAC and NE) are concerned. Thus, selective detection of DOPAC in the presence of NE is not feasible (e.g., without a separating column). On the other hand, the PDMA-coated electrode (A) effectively excludes the positively charged NE from reaching the surface. As a result, DOPAC is not affected by the presence of NE (even at excess levels). Only certain concentration ratios of the two mixture components can be tolerated, since co-ions do transport through the PDMA film at some extent. These results indicate that the "in situ

separation" based on the analyte charge at the PDMA-coated detector surface enhances the selectivity of flow-injection measurements and this may eliminate the need for a sample pretreatment or separator column, usually required when such mixture of oxidizable compounds is assayed. Thus, this type of amperometric detectors with "in situ separation" function have become of great interest in recent years³⁻¹⁶⁾.

Another important function of polymeric coatings is to minimize electrode poisoning caused by adsorption of surface-active compounds (e.g., protein)^{3-7, 13)}. As can be seen from Fig. 7, the

Fig. 7 Flow injection response obtained at (A) bare and (B) PDMA-coated GC electrodes for repetitive injections of 20 μ M DOPAC solution containing 400 mgdl⁻¹ bovine serum albumin. The figures indicate the number of injections. Other experimental conditions are the same in Fig. 2.

PDMA film functions, to some extent, as a barrier against the adsorption of bovine serum albumin (BSA); a gradual decrease in the DOPAC response was observed in the presence of 400 mg dl⁻¹ BSA. The response after the 40th repetitive injection was 94% of the response for the 1st injection at the PDMA-coated electrode. Hence, BSA appears to interact with the PDMA coating, gradually blocking access of DOPAC to the carbon/film

interface. A further effective protection could be achieved by using a bilayer electrode assembly, with a cellulose acetate film over the PDMA coating, since cellulosic films have been used as effective barriers against surfactant adsorption in flow injection analysis 6, 7, 13).

In conclusion, the present study clearly demonstrates that the introduction of the permselective polymeric films of PDMA with the net positive charge onto the GC electrode surface can significantly enhance the power of amperometric detection (especially for anionic solutes) in flow injection analysis. This type of amperometric detector also possesses an "in situ separation" function based on the charge exclusion phenomenon, and thus in this case it permits effective exclusion of cationic interferences. As a result, substantial improvements in the selectivity can be achieved in detecting primary neurotransmitters in flow-injection systems. The observed permselectivity of the PDMA coating toward various solutes is almost comparable to that reported for poly(4-vinylpyridine)-coated glassy carbon flow detectors 6). A further improvement in the detector specificity could be achieved by coupling the discriminative properties of polymeric films with other functionalities such as catalytic and protective properties. Work along this line is in progress in this laboratory.

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