Liquid-junction-free system for substitutional stripping voltammetry using a closed bipolar electrode system

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Abstract

A liquid-junction-free electrochemical system has been developed for substitutional stripping voltammetry (SSV). SSV is a type of stripping analysis that changes the redox current to metal deposition. Generally, SSV requires a liquid junction to maintain electrical conductivity between separated cells. In this report, a closed bipolar electrode system (cBPES) was used to perform SSV without a liquid junction. In this system, electrical conductivity between separated cells was maintained using driving electrodes inserted into each cell. We demonstrated quantification of *p*-aminophenol (pAP) using a liquid-junction-free system for SSV. We investigated the effect of electrode area ratio on the BPE and the concentration ratio of redox species present in each cell. Then, we calibrated the pAP. A linear relationship between pAP concentration and SSV electrical charge was successfully obtained using our liquid-junction-free system, based on cBPES. This novel analytical system is a promising method for the fabrication of a compact SSV sensor for highly sensitive detections.

Keywords: closed bipolar system; electrochemical detection; substitutional stripping voltammetry

1. Introduction

Substitutional stripping voltammetry (SSV) is a highly sensitive electrochemical analytical method with pre-electrolysis step and stripping step [1-6]. The pre-electrolysis step is conducted with a galvanic cell (Fig.1A), in which the oxidation of the analyte molecular at the anode (reaction electrode; BPr) in a cell (reaction cell) occurs concurrently with the reduction of metal ions at the cathode (deposition electrode; BPd) in the other cell containing metal ions (deposition cell). The amount of the deposited metal is obtained with anodic stripping voltammetry in the stripping step. Because the concentration of the analyte molecules is related to the amount of the deposited metal, we can obtain the concentration of the analyte molecule with SSV. Taking advantages of the high sensitivity of the SSV, applications of SSV to the detection of small amount of analyte are demonstrated including an endotoxin sensor [3], a sensor to estimate the oxygen consumption rate of a small fish [4] and an immune assay chip for carcinoembryonic antigen [5]. To perform the pre-electrolysis step of the SSV, a liquid junction is required to provide ion conductivity between two electrochemical cells. However, the use of a liquid junction often causes problems such as clogging and undesirable ion leakage. In particular, for compact-type measurement systems, it is difficult to fabricate a robust liquid junction [6].

To overcome this problem, we propose a liquid-junction-free system for SSV using a couple of electrodes (driving electrode 1 and 2 in Fig. 1A) connected to the potentiostat instead of a liquid junction to provide the electrical conductivity for the two cells. Figure 1A shows a basic design of the liquid-junction-free system for SSV. Similar to conventional SSV, reaction cell and deposition

cell are connected by BPr and BPd. The electrons generated by the oxidation of the analyte on the BPr are transferred to the BPd, resulting in metal deposition on the BPd. Instead of a liquid junction, we inserted driving electrodes in each cell and connected the electrodes to a potentiostat to act as the counter electrode (CE), working electrode (WE). A reference electrode (RE) was inserted in a same cell as the CE. By applying an appropriate voltage between the RE in a cell and a driving electrode in another cell, the electrochemical reactions at two driving electrodes compensate for the reactions at the BPr and BPd, and supply the ions to maintain electrical neutrality of the cells. The cell configuration shown in Fig. 1A is the same as a closed bipolar electrode system (cBPES) proposed by Zhang's group [7,8]. The same system was also reported by Osakai's group [9] and Dryfe's group [10]. Crooks' group reported the use of a bipolar electrode (BPE) as a sensing platform using metal oxidations [11,12], as well as an alternative system to micro/nanochannel junction for ion transportation [13,14]. However, there are no reports of cBPES used for SSV.

In this study, we propose a liquid-junction-free SSV system using a cBPES. We prove the principle of our system by demonstrating the quantitative detection of *p*-aminophenol (pAP). This is for the future application of this system to the endotoxin sensor which we reported previously using conventional SSV with the combination of oxidation of pAP and reduction of Ag^+ [3]. First, we characterize the cBPES by cyclic voltammetry in order to check the necessary conditions for the quantitative detection of pAP. Then, we demonstrated the quantitative detection of pAP. The successful results indicate that our proposed system is a promising method for the fabrication of compact-type SSV sensors for highly sensitive detection.

2. Material and Methods

2.1. Chemicals and apparatus

Reaction solutions (pAP/HEPES) were prepared by dissolving pAP (Wako Pure Chemical Ind. Ltd., Japan) with 100 mM HEPES (Dojindo Molecular Technologies, Inc., Japan) solution (pH 7.0) containing 100 mM KCl as an electrolyte. Deposition solutions ($\text{AgNO}_3/\text{KNO}_3$) were prepared by dissolving AgNO_3 with 100 mM KNO_3 solution. All chemicals used were special reagent grade. For the preparation of aqueous solutions, high-purity distilled and deionized water from a Milli-Q filtration system (Millipore Corporation, USA) was used. Compact Stat (Ivium Technologies B.V., Netherlands) was used as a potentiostat to perform all electrochemical measurements.

2.2. Electrochemical cells

A conventional three-electrode measurement cell was constructed with a Au disk electrode having a diameter of 1.6 mm (BAS Inc., Japan), a Au-sputtered electrode and a Pd sputtered electrode used as WE, CE and RE, respectively. A cBPES for a liquid-junction-free SSV was constructed as shown in Fig. 1A. BPr and BPd were Au disk electrodes. A Au-sputtered glass

substrate (2 mm×10 mm) and a Pd-sputtered glass substrate (2 mm×10 mm) in the reaction cell were used as a driving electrode connected to the CE connector of a potentiostat and a RE, respectively. In a deposition cell, a Ag plate (5 mm×15 mm) was inserted as another driving electrode which connected to the WE connector on the potentiostat.

3. Results and Discussion

3.1. Voltammetric response of liquid-junction-free system for SSV

First, we characterized the electrochemical properties of the liquid-junction-free SSV system using cyclic voltammetry. The reaction cell shown in Fig. 1A, was filled with 1.0 mM pAP/HEPES, and the deposition cell was filled with 10 mM AgNO₃/KNO₃. The concentration of AgNO₃ was set to 10 times higher than the concentration of pAP, so that the pAP redox reaction at the BPr was rate limiting. We scanned the voltage of the Ag driving electrode in the deposition cell versus the Pd RE in the reaction cell and recorded the current response. As a comparison, a conventional three-electrode measurement was performed in 1.0 mM pAP/HEPES. Figure 1B shows typical voltammograms obtained in the cBPES and the three-electrode system at a scan rate of 20 mV/s. When the potential was scanned to the positive direction from -0.2 V, an increase in the oxidation current was observed, making an oxidation peak at 0.14 V. When the oxidation current was observed, the oxidation of silver occurred at the driving electrode (used as a WE) in the deposition cell, whereas the reductive reaction occurred at the other driving electrode (used as a CE) in the reaction cell. At the same time, reactions (1) and (2) were considered to occur at the BPr and BPd, respectively.

Because of these reactions, electrons passed through the BPES, as shown in Fig. 1A. After the potential application was reversed to the negative direction at 0.6 V, the oxidation current gradually decreased and converted to a reduction current, leading to a reduction peak at -0.015 V. When the reduction current was observed, a reversed reaction occurred at each electrode. The voltammogram of the cBPES showed a very similar curve to that of the conventional three-electrode measurement ~~in 1.0 mM pAP~~. This indicates that the redox of pAP at BPr was actually the rate-limiting process ~~in this condition~~. The wider peak separation in the voltammogram of BPES compared with that of the three-electrode system showed the same trend as those reported for cBPES [7,8]. ~~We consider that the reason why the mid-point of the voltammograms appeared at the same potential as the formal redox potential of pAP is that the reaction at the BPd was the redox of metal/metal-ion proceeding without polarization, according to the reports discussed about the potential shift of the voltammograms obtained in the cBPS [7,8,10]. But further studies are required to discuss about this phenomenon.~~ The ~~obtained voltammogram~~ indicate that we can proceed with the SSV deposition

process without a liquid junction using a cBPES by applying the appropriate voltage (e.g. 0.2 V) between the RE in the reaction cell and the driving electrode in the deposition cell. Note that the system shown in Fig 1A has an advantage to the conventional SSV at the point that the electrode potential of the BPr is freely changeable by the potentiostat, regardless of the redox potential of the reaction at the BPd.

We also investigated the effect of electrode area ratio on the BPE and the concentration ratio of redox species in the two cells, in order to check the necessary conditions to limit the overall reaction by pAP oxidation at BPr when ~1.0 mM pAP is a reaction solution. First, we conducted voltammetric experiments fixing the concentration of the redox species (1.0 mM pAP and 10 mM AgNO₃) and varied the BPr diameter from 1.0 to 5.0 mm. The diameter of BPd was fixed to 1.0 mm. Then, we varied the AgNO₃ concentration from 0.1 mM to 10 mM, with fixing the concentration of pAP at 1.0 mM and using symmetrical BPE (1.6-mm-diameter). The blue dots in Fig. 2A and 2B indicate the oxidation peak currents obtained from voltammograms at a scan rate of 20 mV/s. The red line and the black dashed lines are the calculated oxidation peak currents from Eq. (3) if the oxidation of pAP on BPr and the reduction of Ag⁺ on BPd limit the overall reaction of the cBPES, respectively.

$$I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} \nu^{1/2} C^* \quad (3)$$

where I_p is the oxidation peak current (A), n is the numbers of reacting electrons per molecule (2 for pAP and 1 for Ag⁺), A is the electrode area (cm²), D is the diffusion coefficient (6.80×10^{-6} cm²/s for pAP [16] and 1.65×10^{-5} cm²/s for Ag⁺ [17]), ν is the scan rate (0.02 V/s), C^* is the bulk concentration redox species (mol/cm³). In Fig 2A, the calculated lines predict that the area ratio of BPr/BPd should be smaller than 7.8. As predicted, the peak currents of the experimental voltammograms (blue dot) increase with increasing area ratio along with the red line when the ratio is smaller than 4 and tends to be saturated to follow the black dashed line after the ratio is greater than 9. This result shows that the 1:1 area ratio of BPr to BPd is sufficient when 10 mM AgNO₃/KNO₃ is used as the deposition solution to detect ~1.0 mM pAP. In Fig 2B, the oxidation peak currents increased as the AgNO₃ concentrations increased along with the black dashed line in the range of 0.1–2 mM and then follow the red line in the range over 2 mM. This indicates that when symmetrical BPE is used, it is safe to use more than three times higher concentration of AgNO₃ than the concentration of pAP. As a result, we employed 1:1 area ratio of BPE and 10 mM AgNO₃/KNO₃ for a deposition solution to quantify ~1.0 mM pAP.

3.2. Quantification of pAP with SSV

We demonstrated the quantification of pAP with SSV using cBPES. The reaction cell was filled with 0–1.0 mM pAP/HEPES solution and the deposition cell was filled with 10 mM AgNO₃/KNO₃ solution. In the deposition step, 0.2 V was applied for 10 min between the Pd RE in the reaction cell

and the Ag driving electrode in the deposition cell. After the deposition step, the BPd was disconnected from the bipolar system and washed with Milli-Q water. The amount of Ag deposited on the BPd was quantified with anodic sweep voltammetry (ASV) by sweeping the potential of BPd from 0.0 to 1.0 V vs. Ag/AgCl in 100 mM KNO₃ solution at a scan rate of 20 mV/s. Figure 5A shows the voltammetric responses obtained with ASV. The oxidation current of Ag increased with increasing pAP concentration. Figure 5B shows the electrical charge calculated using Eq. (4), plotted against pAP concentration.

$$Q = \int Idt \quad (4)$$

Electrical charge increases linearly with pAP concentration ($R = 0.997$). This result indicates that pAP can be quantified with a junction-free SSV using the amount of electrical charge generated by the oxidation of Ag as the signal. The detection limit (adapting 3σ of the blank) was 30 μ M calculated using the IUPAC-recommended method [18].

4. Conclusion

We have demonstrated a liquid-junction-free SSV system using a cBPES. In this system, the oxidation of the analyte was coupled with the reduction of metal ions on the poles of BPE by applying an appropriate voltage between the driving electrodes. Owing to the ion supply from the electrochemical reactions on the driving electrodes, this system does not require a liquid junction to maintain the electrical conductivity between separated cells. First, we checked the effects of the electrode area ratio of BPE and the concentration ratio of pAP to AgNO₃ on the overall voltammetric response of the cBPES, to find conditions suitable to detect pAP. As a result, we employed 1:1 area ratio of BPE and 10 mM AgNO₃ for a deposition solution to detect ~1.0 mM pAP. Then, we demonstrated the quantification of pAP with the system. A linear relationship between pAP concentration and oxidation charge of Ag deposited on the PBd was obtained. This approach is a promising method for fabrication of a compact SSV sensor for highly sensitive detection applications without fabrication of a liquid junction. For example, by applying this system to an SSV device used in our previous study [3], detection of endotoxin would be possible with a more compact device.

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Figure captions

Figure 1 (A) Schematic illustration showing the basic design of the liquid-junction-free system for SSV. (B) Typical voltammograms obtained in the cBPES (red) and the three-electrode system (black). Au electrodes with 1.6 mm diameter were used for BPr, PBd, and the WE in the three-electrode system. 1.0 mM pAP/100 mM HEPES solution containing 100 mM KCl was used as the reaction solution of BPES and the three-electrode system. 10 mM AgNO₃/100 mM KNO₃ solution was used as the deposition solution for the BPES. Scan rate was 20 mV/s.

Figure 2 Oxidation peak currents plotted against the electrode area ratios of BPr/BPd with fixing the concentration of the redox species (1.0 mM pAP and 10 mM AgNO₃) and a diameter of PBd (1.0 mm) (A), and against AgNO₃ concentration with fixing the concentration of pAP (1.0 mM) using symmetrical BPE (BPr=PBd=1.6-mm-diameter) (B). Scan rate was 20 mV/s. Blue dots indicate mean values and error bars indicate standard deviations obtained by three repetitive measurements. Red line and black dashed lines are the calculated oxidation peak currents if the oxidation of pAP on BPr and the reduction of Ag⁺ on BPd limit the overall reaction of the cBPES, respectively.

Figure 3 (A) Voltammograms obtained with ASV. Potential of BPd was scanned from 0.0 to 1.0 V vs. Ag/AgCl at 20 mV/s in 100 mM KNO₃ solution after a 10-min deposition step. (B) Electrical charges plotted against pAP concentration. Error bars indicate standard deviations (n=3).

Fig. 1A

Liquid junction in conventional SSV

Fig. 1B

Fig. 2A

Fig. 2B

Fig. 3A

Fig. 3B

Graphical abstract