Electropolymerization of Azure A and pH Sensing Using Poly(azure A)-modified Electrodes

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A modified electrode was developed by immobilizing poly(azure A) (pAA) onto the surface of a glassy carbon electrode *via* the electropolymerization of azure A (AA). The pAA immobilized on the electrode exhibited redox response during cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The redox reaction obeyed the Nernst equation because of the involvement of H^+ ions. In addition, the peak potential was shifted according to the solution pH. The shifts of the oxidation peak potential could be more easily observed using DPV than when using CV, indicating that the developed electrode could be useful as a pH sensor. This pH measurement method can be successfully applied in the pH range of 1 to 10 and can be successfully repeated more than 50 times.

Keywords pH sensor, electropolymerization, azure A, methylene blue

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Introduction

The pH level is one of the most important properties associated with an aqueous solution. There are various methods for measuring the pH depending on the type of the sample and the purpose of use, including using a glass membrane electrode, an ion-sensitive field-effect transistor (ISFET), pH indicator dyes, and a fluorescent probe. Among those methods, the currentmeasuring electrochemical method is characterized by its low cost, simple operation, and ease of developing an electrode. Therefore, it can be applied to develop simple sensors. $1-4$ Because of the aforementioned advantages, various studies have investigated methods to measure pH based on electric current.5–10

Therefore, we decided to use azure A (AA), which is considered an analogue of methylene blue (MB) (Fig. 1). The peak potential associated with the redox reaction of MB shifts according to the pH changes. $11,12$ In addition, another study has investigated intracellular pH measurements using a MB solution.13 We attempted to develop an electrode *via* the electropolymerization of AA. AA can be easily immobilized as poly(azure A) (pAA) on an electrode surface *via* electropolyme rization.14–17 MB immobilized electrodes can be prepared by electrochemical polymerization. However, we decided to use AA as a pH probe to compare with the method of immobilization

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on a multilayer film *via* a primary amine of AA.10 The AA immobilized on the electrode surface showed a pH-dependent redox response similar to that exhibited by MB, as well as a good response in the pH range of 1 to 10.

Materials and Methods

A pAA-modified electrode was prepared *via* the electropolymerization of AA as described below. A glassy carbon (GC) electrode having a diameter of 3 mm was polished using 1-μm alumina and then immersed in 1 mM AA solution (pH 3), which was prepared by dissolving AA (Tokyo Chemical Industry Co.) in 100 mM phosphate buffer solution. Thereafter, the AA was polymerized on the electrode *via* CV (sweep potential: approximately 0.8 – 1.2 V, sweep speed: 50 mV/s, and number of cycles: 2).

CV and DPV were conducted using an electrochemical analyzer (ALS Model 660B; BAS, Tokyo, Japan) in a conventional three-electrode cell comprising a pAA-modified electrode as the working electrode, a platinum wire as the counter electrode, and Ag/AgCl (3 mol L^{-1} KCl) as the reference electrode. The DPV parameters applied included a step potential of 4 mV, pulse amplitude of 50 mV, pulse width of 60 ms, pulse period of 200 ms, sample period of 20 ms, and voltage range of –0.6 – 0.4 V. All measurements were performed multiple times, and the average values were plotted.

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Fig. 1 Structure and redox reaction of methylene blue (MB).

Fig. 2 (a) Cyclic voltammogram of electropolymerization of azure A (1 mM, pH 3.0) with a glassy carbon (GC) electrode (3-mm diameter). (b) Cyclic voltammograms of poly(azure A)-modified electrode (3-mm diameter in a 100 mM phosphate buffer solution (pH 3.0) at different scan rates of 10, 20, 50, 75, 100, 150, and 200 m V s⁻¹. Insert: Plots of the anodic peak current (I_{pa}) and the cathodic peak current (I_{pc}) *vs.* the scan rate.

Results and Discussion

The probe was immobilized onto the electrode to ensure easy application as an electrochemical sensor. Various methods have been proposed for the immobilization of a probe on a solid surface, including the bindinf of the pendant groups on a polyelectrolyte *via* covalent bonds^{18,19} and the self-assembled monolayer method.^{20,21} In this study, we attempted to immobilize pAA onto an electrode *via* the electrochemical oxidation of AA.14–17 Figure 2a shows the voltammogram of polymerization in the case of 1 mM AA (prepared with 100 mM phosphate buffer solution) at pH 3. A reversible redox reaction of the AA was observed with a reduction peak of 0.031 V and oxidation peaks of approximately 0.082 and 1 V. The oxidation peak was

observed at 1 V, indicating that the polymerization of AA progressed at the electrode surface and that pAA was immobilized (Fig. 3). The CV scans of the pAA-modified electrode at various scan rates in PBS at pH 3.0 were performed and the results are shown in Fig. 2(b). The results indicated that the anodic and cathodic peak currents are directly proportional to the scan rate, and that AA was immobilized on the electrodes surface by electrolytic polymerization. Similar results have been obtained in previous works.¹¹ Furthermore, the polymerization of AA was observed even if polymerization was similarly conducted in a phosphate buffer at pH 5, 7, and 9. In the following experiments, based on the good reproducibility of the resultant voltammograms, we performed the electrolytic polymerization of AA for performing pH measurements using 1 mM AA solution prepared in a 100 mM phosphate buffer solution.

After the preparation of the pAA-modified electrode (3-mm diameter), CV measurements were conducted in a phosphate buffer solution (100 mM) at pH 1 to 13 (Fig. 4a). During CV measurement, the peak oxidation potentials were 0.25 V at pH 1 and –0.22 V at pH 13, suggesting a negative potential shift with increasing basicity. The peak reduction potentials were 0.17 V at pH 1 and –0.20 V at pH 8, which also indicated a negative potential shift with increasing basicity. However, the peak potential was not clear at pH 8.5 – 13.5 because of the reduction current of oxygen. By plotting the peak potentials observed in case of oxidation and reduction against the pH, linear changes can be observed with respect to the peak potentials depending on the pH change in the pH range of 1 – 10 (Fig. 4b). Based on the calibration curves obtained from the potential peaks during oxidation and reduction, the potential shifts per unit of pH change in the oxidation and reduction peaks were 46 and 59 mV, respectively, and the correlation coefficients were 0.995 for both the cases.

For DPV using a pAA-immobilized electrode, pH measurements were conducted based on the peak shifts in the oxidation potential. As a result, similar to CV, the peak potentials were 0.24 V at pH 1 and –0.38 V at pH 13, indicating a negative shift of the peak potential with increasing basicity (Fig. 5a). The current values increased under strong base and strong acidic pH conditions. The same trend was observed in the DPV measurement of methylene blue,¹⁰ although the cause was unknown. The peak potentials in DPV could be more easily determined when compared with those in CV. Therefore, the measurement can be easily performed using DPV. Figure 5b presents a plot of the peak potentials *vs.* pH, suggesting that DPV can be used for pH sensing similar to CV. Based on the slope in Fig. 5b, the peak potential shift was 55 mV when the pH value was changed by 1 within the pH range of 1 – 10 (*R* = 0.995). pH sensing based on DPV measurements was investigated through the following experiments.

Then, the durability of the modified electrode as a pH sensor was evaluated. The microfabricated electrochemical sensors, which can be repeatedly used, can be applied to various fields, including real-time monitoring and imaging.4,19,20 The durability

Fig. 3 Structure and electropolymerization of azure A.

a)

pH 13

 2.5

 2.0 Current / µA 1.5 1.0 0.5 $\overline{0}$ -0.6 -0.4 -0.2 $\mathbf 0$ 0.2 0.4 Potential / V $b)$ 0.3 ARANTHE AVENUE 0.2 0.1 C Lip / V -0.1 -0.2 -0.3 -0.4 -0.5 $\mathsf 0$ \overline{c} $\overline{4}$ 6 8 10 12 14 pH

Fig. 4 (a) Cyclic voltammograms of the poly(azure A)-modified glassy carbon (GC) electrode (3-mm diameter) in a 100 mM phosphate buffer solution at pH 1.1 – 13.5. (b) Plots of oxidation and reduction peak potentials *vs.* pH.

Fig. 5 (a) Differential pulse voltammetry (DPV) measurement using a poly(azure A)-modified glassy carbon (GC) electrode (3-mm diameter) in 100 mM phosphate buffer solution at pH 1.1 – 13.5. (b) Plot of peak potential *vs.* pH.

of the pAA-modified GC electrode was evaluated by repeating the DPV measurements in a phosphate buffer solution (100 mM) at pH 3, 7.4, and 9. The peak potential did not change after 50 measurements, indicating that the pAA-modified GC electrode can be repeatedly used (Fig. 6). The pAA current decreased with each measurement because the pAA on the electrode gradually became electrochemically inactive after each measurement. Therefore, almost no oxidation current was observed after the 50th measurement, and it became difficult to detect any peak potential.

We also studied the influence of additives on the pAAimmobilized electrode. DPV was conducted in a phosphate buffer solution (100 mM) containing glucose, glutamic acid, ascorbic acid (10 mM), or NaCl (1 M), and the peak potentials were plotted against the pH (Fig. 7). The peak oxidation potential of the pAA-modified electrode was –0.165 V at pH 7.4 condition (control). The oxidation potential of the pAA-

modified electrode was shifted to –0.110 and –0.105 V by the addition of glucose and glutamate, respectively. Perhaps the interaction of these molecules with the secondary amines in the pAA structure shifted the oxidation potential. This pH measurement was also slightly affected by the ionic strength. A strong influence could be observed when ascorbic acid was added, *i.e.*, the oxidation potential shifted by approximately 200 mV at pH 5. This can be attributed to the reduction ability of ascorbic acid. We speculate that the reduction effect of the ascorbic acid affected peak potential shift more than anion doping. Therefore, pretreatment with ascorbate oxidase or similar materials may be required for analyzing samples containing ascorbic acid.

pH₁

Fig. 6 Peak potential changes associated with the repeated differential pulse voltammetry (DPV) measurements in a phosphate buffer solution (100 mM) at pH 3, 7.4 and 9 using the poly(azure A) modified glassy carbon (GC) electrode (3-mm diameter).

Number of trials

Fig. 7 Plot of peak potentials *vs.* pH for differential pulse voltammetry (DPV) measurements in a phosphoric acid solution (100 mM) containing either glucose, glutamic acid, ascorbic acid (10 mM), or NaCl (1 M) using a poly(azure A)-modified electrode (3-mm diameter).

Conclusions

A pAA-modified electrode was successfully developed *via* the electropolymerization of AA. Based on the CV and DPV measurements, the peak potential of the pAA-immobilized electrode shifted with the change in pH. Although the measurement was slightly influenced by additives, the electrode exhibited durability for 50 or more measurements. We immobilized pAA on a multilayer film *via* a primary amine of AA and performed similar pH measurements.²³ In comparison, this method could be applied measure pH in a wide range, although it was slightly affected by coexisting substances. In addition, the apparatus required to develop the electrode was the same as that required for performing the pH measurements. Furthermore, the polymerization and measurement of AA was

completed within minutes and seconds, respectively. Therefore, pH can be easily measured using this type of pAA-modified electrode.

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