

# 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<sup>+</sup> 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 current-measuring 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.<sup>1-4</sup> Because of the aforementioned advantages, various studies have investigated methods to measure pH based on electric current.<sup>5-10</sup>

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.<sup>11,12</sup> In addition, another study has investigated intracellular pH measurements using a MB solution.<sup>13</sup> 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* electropolymerization.<sup>14-17</sup> 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

on a multilayer film *via* a primary amine of AA.<sup>10</sup> 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- $\mu$ m 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<sup>-1</sup> 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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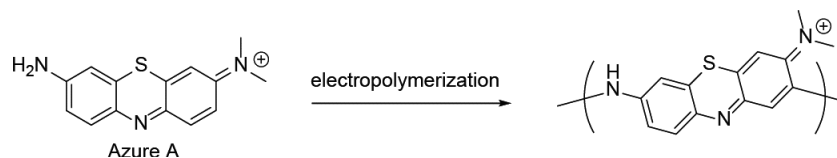


Fig. 3 Structure and electropolymerization of azure A.

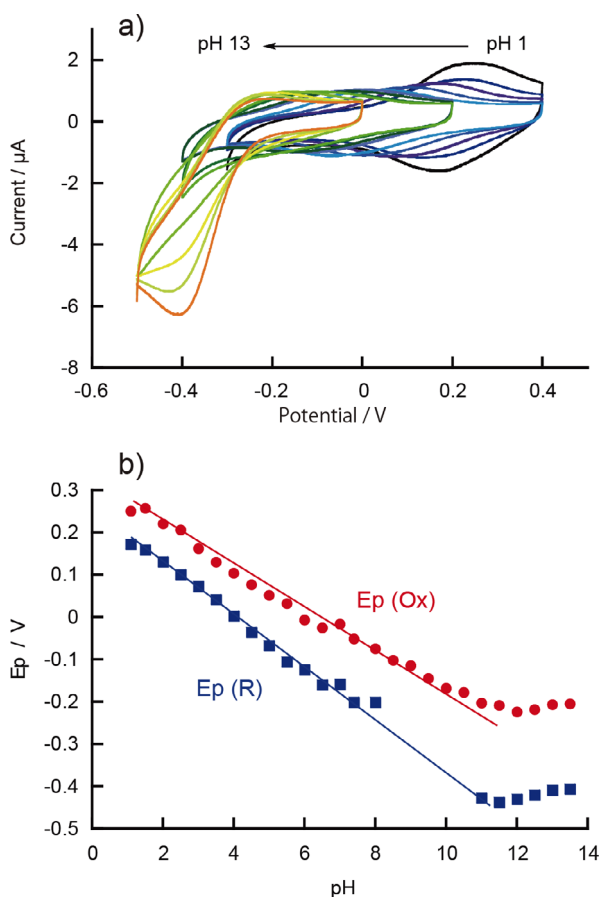


Fig. 4 (a) Cyclic voltammograms of the poly(azure A)-modified glassy carbon (GC) electrode (3-mm diameter) in a 100 mM phosphate buffer solution at pH 1.1 – 13.5. (b) Plots of oxidation and reduction peak potentials vs. pH.

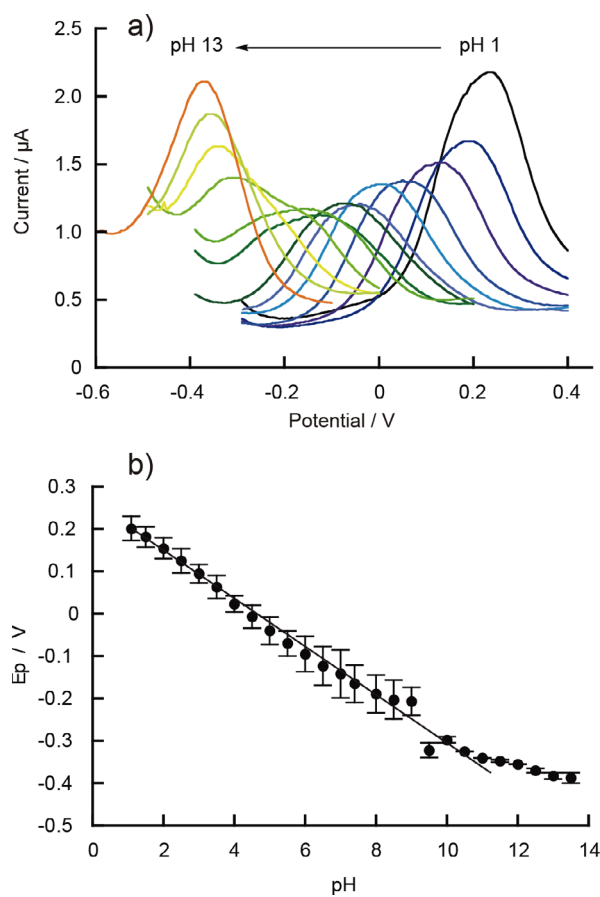


Fig. 5 (a) Differential pulse voltammetry (DPV) measurement using a poly(azure A)-modified glassy carbon (GC) electrode (3-mm diameter) in 100 mM phosphate buffer solution at pH 1.1 – 13.5. (b) Plot of peak potential vs. pH.

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 pAA-immobilized 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.

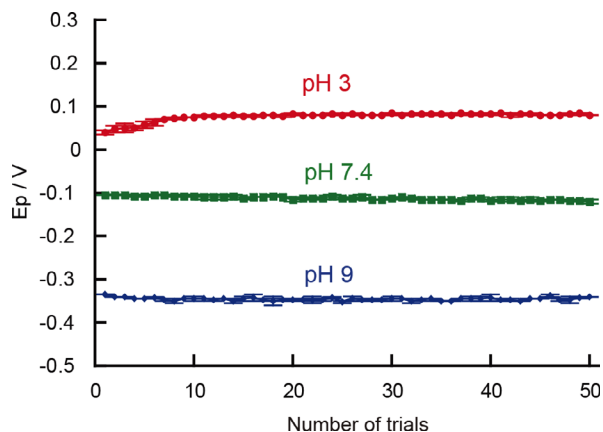


Fig. 6 Peak potential changes associated with the repeated differential pulse voltammetry (DPV) measurements in a phosphate buffer solution (100 mM) at pH 3, 7.4 and 9 using the poly(azure A)-modified glassy carbon (GC) electrode (3-mm diameter).

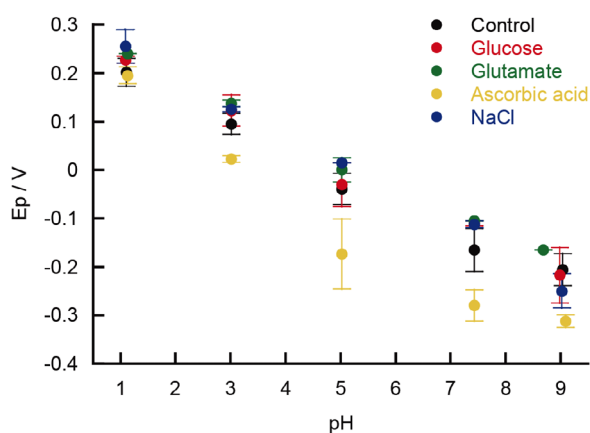


Fig. 7 Plot of peak potentials vs. pH for differential pulse voltammetry (DPV) measurements in a phosphoric acid solution (100 mM) containing either glucose, glutamic acid, ascorbic acid (10 mM), or NaCl (1 M) using a poly(azure A)-modified electrode (3-mm diameter).

## 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.<sup>23</sup> 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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