Electrocatalytic Reduction and Determination of Dissolved Oxygen at a Poly(nile blue) Modified Electrode

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Abstract

A novel dissolved oxygen (DO) sensor based on poly(nile blue) modified glassy carbon electrodes is described. The modified electrodes were prepared by electrochemical cyclic sweep between -0.6 and $+1.0$ V in a nile blue solution. The electrochemical behavior of poly(nile blue) displayed a two-electron and two-proton surface-controlled quasireversible process with a charge transfer coefficient of 0.17 and electron transfer rate constant of $0.37\ \mathrm{s}^{-1}$. The modified electrodes showed an excellent electrocatalytic activity for the reduction of DO at pH 4.8–5.5. The catalytic peak current is linear with DO concentration in the range from 1.3×10^{-6} to 2.6×10^{-5} mol/L with a calculated detection limit of 7.8×10^{-7} mol/L at a signal-to-noise rate of 3 and a correlation coefficient of 0.998. The variation coefficient of electrocatalytic current for 10 determinations at 2.6×10^{-5} mol/L DO concentration was 2.6%. The voltammetric response of the modified electrodes to O_2 reduction showed a Michaelis-Menten's behavior at higher O_2 concentrations. The K_m value for the sensor was found to be 0.028 mmol/L. The low K_m value demonstrated that poly(nile blue) immobilized at glassy carbon electrodes exhibited a high affinity to dissolved oxygen.

Keywords: Electrochemical sensor, Dissolved oxygen, Poly(nile blue), Chemically modified electrodes

1. Introduction

Dissolved oxygen (DO) in environmental water is an important parameter of environment quality. Its determination has been accomplished by several methods such as the classical Winkler method [1], photometry [2], EPR [3], chemical [4, 5] and electrochemical methods [6–8]. Some of these methods are time-consuming or suffer from high limits of detection. Electrochemical methods based on chemically modified electrodes, especially polymer-coated electrodes, for DO determination have attracted considerable interest [6–11]. These methods have higher sensitivity than the initial Clark O_2 electrode, which was based on the direct reduction of O_2 at solid electrodes and showed a tailed cyclic voltammetric profile to O_2 at increasing negative potentials. In early works, most of chemically modified electrodes to be used for DO determination were prepared with the metal-porphyrin [6, 7, 12, 13] or metal-phthalocyanine [14–16] complexes as the electron transfer mediators of oxygen reduction, and the construction of chemically modified electrodes for DO determination was rather complicated and time-consuming [9]. Since the catalytic activity of poly(xylylviologen) for oxygen reduction was reported [17], the metal-free organic compound modified electrodes have been developed for oxygen determination [6, 11, 17, 18]. Recently, several oxygen microsensors fabricated by coating cellulose acetate on an etched Pt wire [10] and methylviologen [11] and/or Nafion [19] on carbon fiber microelectrodes have also been proposed for in vivo electrochemical measurements of oxygen [20–22]. However, most of those works were based on poly(viologen derivatives) modified work electrodes. Here we reported an oxygen sensor based on poly(nile blue) modified glassy carbon electrodes.

Nile blue, one of phenoxazine dyes, is a well-known electroactive molecule. It has been used as a mediator of electron transfer with a two-electron redox conversion to modify solid electrodes for electrocatalytical oxidation of the reduced form of the nicotinamide coenzymes NADH and NADPH [23–25]. In these works, nile blue molecules were immobilized at the electrode surface by dipping the electrode into its solution. As well known, the stability of the modified layer on carbon electrode materials towards desorption is mainly governed by the number of conjugated aromatic rings, so that a mediator containing a large number of ring makes a more stable chemically modified electrode than one with a low number of rings. In order to improve the stability of nile blue modified electrode, Chen et al. prepared a semiconducting poly(nile blue) modified electrode by an electrochemical polymerization process in 0.5 mol/L H_2SO_4 solution containing 1.0 mmol/L nile blue, which was used for the amperometric determination of hemoglobin [26]. In this work, we obtain a poly(nile blue) polymer modified glassy carbon electrode by electrochemical cyclic sweep between -0.6 and $+1.0$ V in 0.1 mol/L pH 5.3 PBS containing 0.5 mmol/L nile blue. The modified electrode shows a good stability and a high affinity to dissolved oxygen. It possesses excellent electrocatalytic activity for the reduction of dissolved oxygen and has been developed for the voltammetric determination of dissolved oxygen with a high sensitivity.

2. Experimental

2.1. Chemicals and Materials

Nile blue was purchased from British Drug Houses LTD (UK). 0.1 mol/L phosphate buffer solutions (PBS) of various pHs were prepared by mixing the stock solutions of NaH_2PO_4, and Na_2HPO_4, and then adjusting the pH with 0.1 mol/L H_3PO_4 or NaOH. Other chemicals were of analytical reagent grade. All solutions were prepared in double distilled water. The O_2-saturated standard solution was produced by bubbling double distilled water with pure O_2 at room temperature for 1 hour and O_2 content in the O_2 saturated water was 2.6×10^{-4} mol/L calculated from its saturated solubility.

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2.2. Apparatus

Electrochemical measurements were performed with a BAS 100B electrochemical analyzer (Bioanalytical Systems Inc., USA) using a standard three-electrode system. A SCE was used as reference electrode, a platinum wire as counter electrode and a modified glassy carbon electrode ($0.071\,\mathrm{cm}^2$) as working electrode. All potentials are referenced to the SCE.

All electrochemical measurements were carried out in a 20 mL measuring cell with 5 mL solutions which were deaerated by purging with pure nitrogen and kept under nitrogen atmosphere at room temperature. The determination of O_2 concentration was performed after a short stirring upon addition of an aliquot of O_2-saturated standard solution was added to 5 mL PBS using a microliter syringe.

2.3. Preparation of Poly(nile blue) Modified Electrodes

Prior to electrochemical polymerization, the glassy carbon electrodes were polished successively with 0.3 μm and 0.05 μm Al_2O_3 slurry on microcloth pads (Buehler), followed by rinsing with distilled water and removal of traces of alumina from the surface by brief sonicating in double distilled water. The electrodes were then pretreated electrochemically by continuous cyclic sweep between −0.4 and +1.2 V at 50 mV/s in pH 6.3 PBS until a constant background was observed. Electrochemical polymerization of nile blue at the pretreated electrodes was achieved in nitrogen atmosphere by using cyclic voltammetric sweep between −0.6 and +1.0 V in pH 5.3 0.1 mol/L PBS containing 0.5 mmol/L nile blue at 50 mV/s for 8 cycles. Experimental results indicated that the polymers yielded with cyclic voltammetry were more stable than a potentiostatic mode.

3. Results and Discussion

3.1. Electrochemical Polymerization of Nile Blue

The cyclic voltammogram of nile blue in pH 5.3 PBS at 50 mV/s showed a couple of redox peaks when the sweep potential was between −0.65 and +0.65 V. The anodic and cathodic peak potentials were at −0.282 and −0.359 V, respectively. With a continuous cyclic sweep, the voltammogram did not change. However, when the sweep potential is more than +0.9 V, for example between −0.6 and +1.1 V shown in Figure 1, the cyclic voltammogram appeared in another irreversible oxidation peak at +0.82 V. With the continuous cyclic sweep, the current of this irreversible oxidation peak decreased and its peak potential shifted in a positive direction. At the same time, the redox peak currents around −0.3 V also decreased greatly. The oxidation peak potential moved from −0.282 V to a more positive value and the reduction peak potential transferred in a negative direction. These features show the behaviors of an obvious electrochemical polymerization process. The irreversible oxidation peak at +0.82 V is attributed to the formation of radical cation by the one-electron oxidation of nile blue monomer [26]. With the potential cycling, more radicals are formed. Delocalization of unpaired electron in the radical cations produces the electrophilic attacking site with high probability on either amine group or at its ortho position [27]. Radical dimerization can occur via carbon-nitrogen coupling route to form a mixture of linkage species, as proposed by Bauldreay and Archer for the electropolymerization mechanism of thionine [28]. With the continuous cyclic sweep, the radical cations of the formed dimers (or oligomers) and other monomers were formed, they further react each other or with other monomers to form a polymer. The poly(nile blue) film formed at electrode surface blocks the electrode reaction of nile blue monomer, resulting in the changes in peak currents and potentials till the disappearance of these peaks. Considering the stability and electrochemical activity of the resulting polymer, a polymer obtained after 8 complete potential cycles between −0.6 and +1.0 V was used for our purpose.

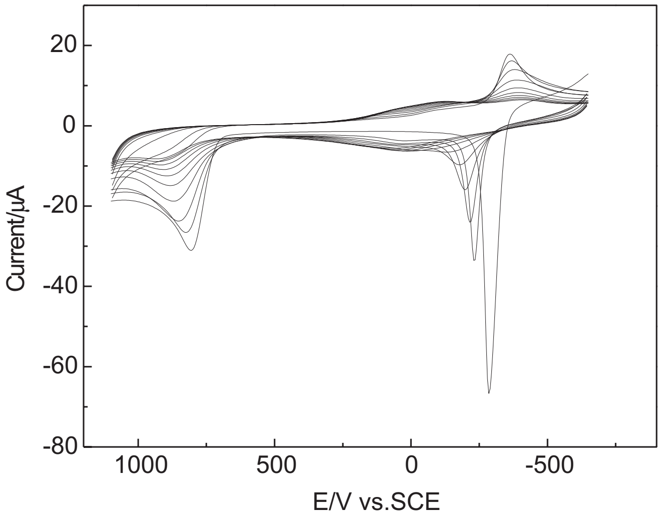


Fig. 1. Cyclic voltammogram of 5.0×10^{-4} mol/L nile blue in 0.1 mol/L pH 5.3 PBS at a scan rate of 50 mV/s.

3.2. Electrochemical Behavior of Poly(nile blue)

With the continuous cyclic sweep between −0.6 and −0.1 V in pH 6.3 PBS, the cyclic voltammogram of the poly(nile blue)

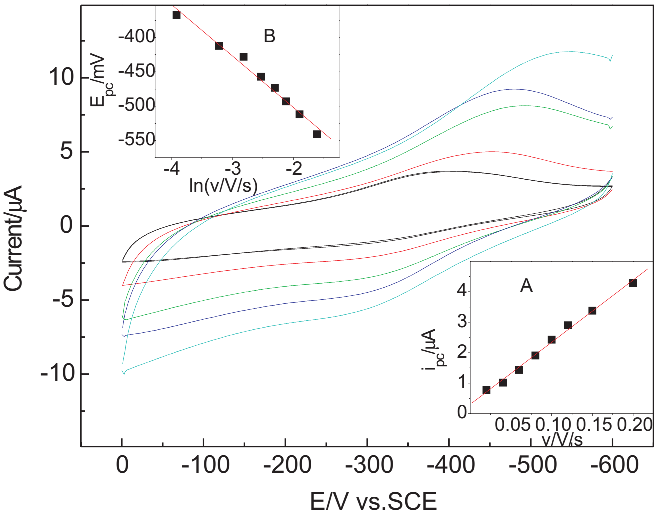


Fig. 2. Cyclic voltammograms of poly(nile blue) modified electrode in 0.1 mol/L pH 6.3 PBS at the scan rates of 150, 120, 100, 60, 40 and 20 mV/s (from highest to lowest peak currents). Inset A: plot of cathodic peak current vs. scan rate. Inset B: plot of cathodic peak potential vs. logarithm of scan rate.

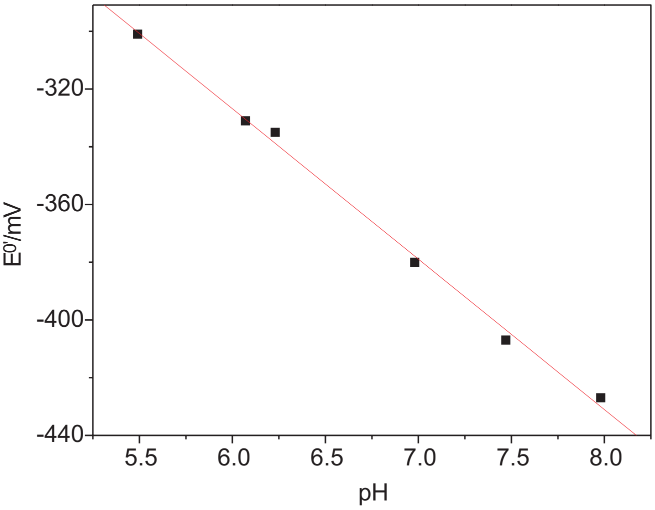
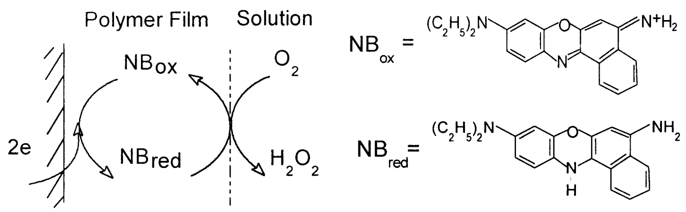


Fig. 3. Plot of formal potential of nile blue unit in polymer film vs. pH value of supporting electrolyte solution.



Scheme 1.

showed a couple of redox peaks at −0.325 and −0.416 V at 50 mV/s. Their peak currents initially decreased and trended to constant values after three cycles. The film showed a good stability after the continuous potential sweep cycles. The peak potential values were close to those of nile blue monomer at a bare glassy carbon electrode at the same pH, suggesting that these peaks were attributed to the oxidation and reduction of nile blue unit in the polymer film. The initial decrease in the peak current resulted from the desorption of a few nile blue monomers adsorbed at the polymer film.

Figure 2 shows the cyclic voltammograms of the poly(nile blue) at various scan rates. With increasing scan rate, the peak currents increase, the cathodic peak potential shifts in a negative direction and the anodic peak potential moves to a more positive value. The peak currents are proportional to the scan rate (inset A in Fig. 2 for cathodic current), indicating a surface-controlled electrode process. The plot of cathodic potential vs. the logarithm of scan rate shows a good linear relation with a slope of −75 mV (inset B in Fig. 2), producing a charge transfer coefficient of 0.17 from the electron transfer number of 2 and the equation of Laviron [29]. The peak-to-peak separation at 100 mV/s is 140 mV. Thus the electron transfer rate constant is calculated to be $0.37\,\mathrm{s}^{-1}$ with the equation of $\log k = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\left(\frac{RT}{nFv}\right) - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT}$ [29].

Surface coverage of nile blue unit in the polymer can be obtained from the peak area S of cyclic voltammogram and the equation of $\Gamma = Q/nFA = S/nFAv$, where A is the electrode area. The surface coverage of nile blue unit is $1.39 \times 10^{-10}\,\mathrm{mol/cm^2}$ determined at the scan rate of 30 mV/s.

Generally, the electrode process of phenoxazine dyes involves the participation of proton. Figure 3 shows a linear plot of formal potential of nile blue unit in the polymer vs. pH value of PBS. Here the formal potentials are obtained from the equation of $E^{0'} = E_{pa} - \alpha(E_{pa} - E_{pc})$. The slope is −52 mV/pH and the number of electrons transferred in the electron reaction process is 2, thus, the number of proton participating in the process is 2. The result is close to that reported in [26].

3.3. Electrocatalytic Reduction of Dissolved Oxygen at Poly(nile blue) Modified Electrode

The cyclic voltammogram (not shown) of dissolved oxygen in pH 5.3 PBS at a bare glassy carbon electrode displayed an irreversible continuous tail at the potentials more negative than −0.35 V. No peak could be observed. There was also no linear

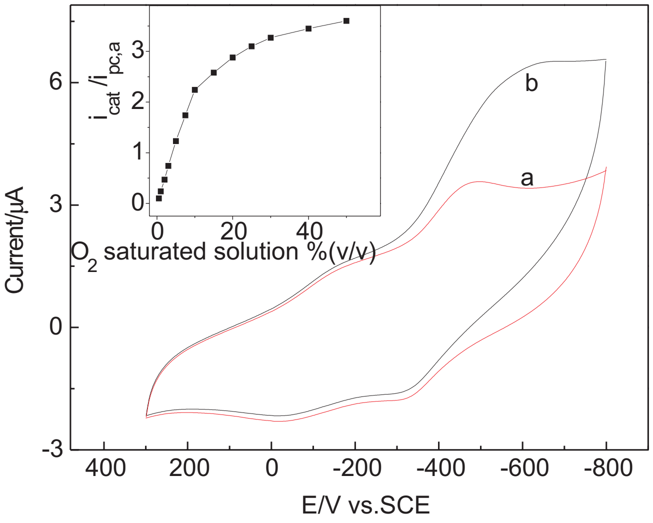


Fig. 4. Cyclic voltammograms of poly(nile blue) modified electrode in 0.1 mol/L pH 5.3 PBS (a) and containing 10 % O_2 saturated solution (v/v) (b) at 50 mV/s. Inset: plot of electrocatalytic efficiency obtained at pH 5.3 at 50 mV/s vs. dissolved oxygen content.

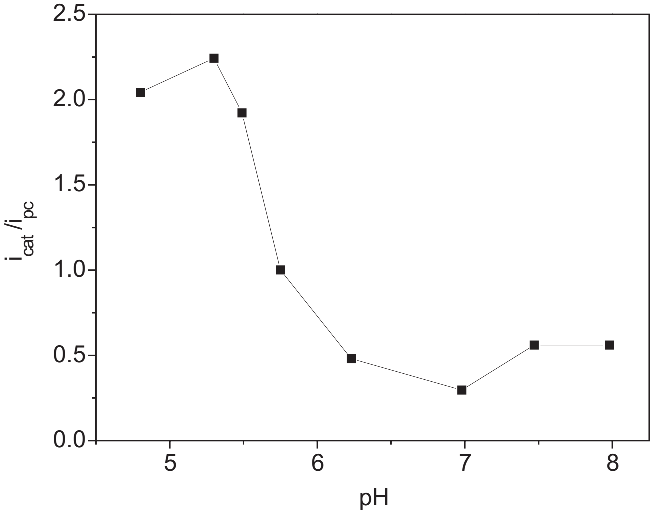


Fig. 5. Influence of pH on electrocatalytic efficiency in the sample solution containing 10 % O_2 saturated solution (v/v) at a scan rate of 50 mV/s.

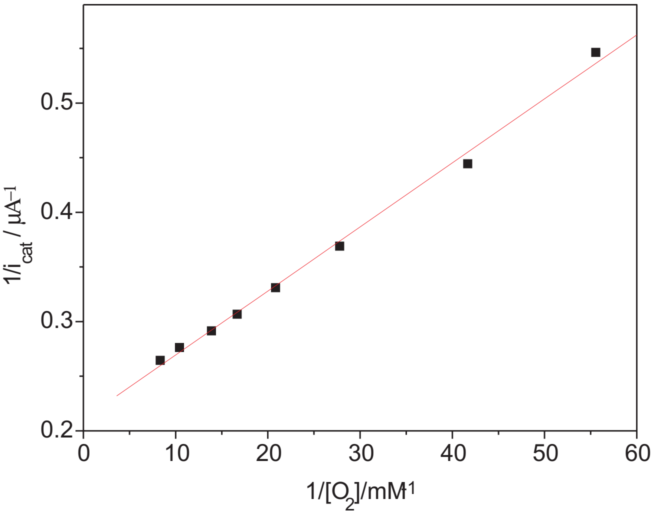


Fig. 6. Data analysis of electrocatalytic current vs. dissolved oxygen content. The experimental conditions were the same as in the Figure 4.

relation between the cathodic current at a fixed potential and the dissolved oxygen concentration. At a poly(nile blue) modified glassy carbon electrode, upon addition of dissolved oxygen standard solution to the electrochemical cell containing 0.1 mol/L pH 5.3 PBS, the cathodic peak current of poly(nile blue) increased and the cathodic wave became a sigmoid shape, showing a characteristic of the electrocatalytic electrode process. At the dissolved oxygen concentration of 10 % saturated solution the cathodic peak current was triple the value of poly(nile blue) in PBS without oxygen (Fig. 4b). The increase in reduction peak current could be attributed to the electrocatalytic action of nile blue unit to the reduction of oxygen, which could be described as shown in Scheme 1.

The electrocatalytic activity of the modified electrode did not change upon cyclic scanning in pH 5.3 PBS for 2 h or when stored in PBS for one week. However, the electrocatalytic current ($i_{cat} = i_{pc,b} - i_{pc,a}$, here subscript a and b are for curve a and b, respectively) was dependent on the pH of buffer solutions. The influence of pH on the DO electrocatalytic efficiency ($i_{cat}/i_{pc,a}$) is shown in Figure 5. Reduction of O_2 is a two-electron and two-proton process [7]. Variations in the electrolyte pH will result in variation in the formal potential of O_2. Therefore, the thermodynamic driving force for the catalysis will vary with pH, making the peak current different at different pH values. The optimum pH for O_2 determination is in the range of 4.8–5.5 and pH 5.3 was chosen for subsequent determination.

3.4. Electrochemical Determination of Dissolved Oxygen Level with Poly(nile blue) Modified Electrode

The determination of electrocatalytic current of poly(nile blue) modified electrode to DO reduction was carried out by adding an aliquot of O_2 saturated standard solution to 5 mL PBS. The catalytic current obtained after a short stirring increased with increasing DO concentration (inset in Fig. 4). There was a linear relation between the catalytic current and DO concentration in the range of 0.5 % to 10 % O_2 concentration in saturated solution, corresponding to the range from 1.3×10^{-6} to 2.6×10^{-5} mol/L, with a correlation coefficient of 0.9986. The calculated detection limit was 7.8×10^{-7} mol/L at a signal-to-noise ratio of 3. The variation coefficient of electrocatalytic current for 10 determinations at 2.6×10^{-5} mol/L was 2.6 %. When the concentration increased further, the line curved and a Michaelis-Menten-shaped curve was observed. The calibration range of O_2 concentration could be extended to 1.3×10^{-4} mol/L. The shape of plot of catalytic current vs. DO concentration exhibited an electrode reaction mechanism observed at the classical electron transfer mediator modified electrodes.

The sensor showed good reproducibility for the determination of O_2 concentration in its linear range. The variation coefficients were 2.8 % and 4.3 % for 10 parallel DO assays at 8 % and 2 % O_2 concentration in saturated solution, respectively. By a calibration plot method and diluting the sample with 0.1 mol/L pH 5.3 PBS for twenty times, the concentration of O_2 in a water sample was obtained to be 1.48×10^{-4} mol/L. The concentration of DO obtained by a classic iodimetry in the same sample was 1.54×10^{-4} mol/L, giving a small relative deviation of −4.0 %.

3.5. Apparent Michaelis-Menten Constant

The voltammetric response of the modified electrodes to O_2 reduction showed a Michaelis-Menten's behavior at higher O_2 concentrations. The apparent Michaelis-Menten constant can be obtained from the equation of $i_{cat} = \frac{i_m c_{O_2}}{K_m + c_{O_2}}$, where K_m is the apparent Michaelis-Menten constant, i_m, the maximum catalytic current, is equal to $nFA\Gamma k_{+2}$ [30]. The linear regression analysis of the corresponding plot is shown in Figure 6. From the slope and intercept of the plot, an average K_m of (0.028 ± 0.001) mmol/L and i_m of 4.74 µA were obtained. The low K_m value demonstrated that poly(nile blue) immobilized at glassy carbon electrodes exhibited a high affinity to dissolved oxygen [31]. From the maximum electrocatalytic current value, the k_{+2} value in Michaelis-Menten's model was found to be 2.5 s^{-1}.

4. Conclusions

Nile blue units were conveniently immobilized at a glassy carbon electrode surface by cyclic sweep between −0.6 and +1.0 V in pH 5.3 PBS. The formed poly(nile blue) displayed high affinity to dissolved oxygen and could sensitively electrocatalyze its reduction at the electrode, which has been used for the determination of dissolved oxygen. The modified electrode showed a good stability, sensitivity, precision and reproducibility enough for the purpose of dissolved oxygen detection.

5. Acknowledgements

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6. References

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