

Simultaneous Electroanalysis of Nitric Oxide and Nitrite

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Abstract. The simultaneous sensing of nitric oxide (NO) and its metabolite, nitrite (NO_2^-) has been studied by Osteryoung square-wave voltammetry (OSWV) in physiological pH solution (0.1 M phosphate buffer solution, pH 7.2). Using an electrochemically pretreated glassy carbon (GC) electrode, OSWV was successfully applied to observe the well-separated oxidation peaks at ca. 0.58 and 0.80 V vs. Ag/AgCl for NO and NO_2^- , respectively. This clear separation between the NO and NO_2^- oxidation peaks may be due to the formation of surface oxides (e.g., quinone ($\text{C}=\text{O}$) or carboxylic (COO^-) group) and surface defects introduced by the electrochemical pretreatment of GC electrodes.

Key words : Nitric oxide, Nitrite, Electrochemical pretreatment, Osteryoung square-wave voltammetry, Single detection system

Nitric oxide (NO) has been known to act as the endothelium-derived relaxing factor (EDRF) in the vasodilatory system¹⁾. NO has also been identified as a neurotransmitter in the nervous system and has many other physiological roles²⁾. The determination of concentrations of NO and its metabolite, nitrite (NO_2^-) is of considerable significance because the change in their concentrations is symptom of several diseases. Therefore, an accurate measurement of NO and NO_2^- concentrations in complex biological matrices has been extremely needed³⁾. In order to detect NO and its metabolites directly, an electrochemical technique is the best approach which is presently available. Many electrochemical techniques have been utilized to measure the transient concentrations of NO and its metabolites with sufficient spatial and temporal resolution. The oxidation potentials of NO and NO_2^- are so close at the conventional electrodes and thus a special strategy is needed for their simultaneous sensing. For simultaneous measurements of NO and NO_2^- in rat brain Mao *et al.*⁴⁾ used the chemically modified ring-disc electrode which was simultaneously sensitive to NO and NO_2^- by modifying its inner disc with electropolymerized film of cobalt(II) tetraaminophthalocyanine / Nafion and its outer ring with poly(vinylpyridine), respectively. Recently, Zen *et al.*⁵⁾ have reported the construction of so-called *single detection system* based on the electrocatalytic oxidation of NO_2^- and reduction of NO at the Nafion/lead-ruthenate pyrochlore electrode in acidic media (pH 1.65) in which the disproportionation of NO_2^- into NO_3^- and NO occurs. Cserey *et al.*⁶⁾ have observed the voltammogram with well-separated oxidation peaks of NO and NO_2^- at cellulose acetate-immobilized platinum electrode in acidic solution.

Here we will report a more convenient *single detection system* to detect NO and NO_2^- simultaneously in physiological

pH solution (0.1 M phosphate buffer solution (PBS), pH 7.2). Using an electrochemically pretreated glassy carbon (GC) electrode^{6,7)}, Osteryoung square-wave voltammetry (OSWV) was successfully applied to observe the well-separated oxidation peaks for NO and NO_2^- . Nafion membrane does not need to be employed unlike conventional NO electrode sensors^{8,9)}.

NO sample solutions of various concentrations were prepared by mixing NO gas (99.0%, Toho Sanaso Kogyo) and Ar gas at the appropriate ratio through a gas mixture apparatus (Gas Blender SECB-2, STEC Co. Inc.) and then introducing the mixed gas into deoxygenated solutions for 20 min. The NO concentration in each solution was calculated from the corresponding gas mixture ratio using the saturated concentration of NO (ca. 1.9 mM)¹⁰⁾, e.g., it is 95 μM in the case of a NO (10%)-Ar (90%) mixture. The following instrumental parameters were used to record the square-wave voltammograms: square wave amplitude: 25 mV; step height: 5 mV, and square wave frequency: 4 Hz.

Figure 1 shows the typical Osteryoung square-wave voltammograms (OSWVs) of 95 μM NO in PBS (pH 7.2). At the GC electrode pretreated by continuously scanning the potential between 0 and 1.5 V vs. Ag/AgCl at 0.1 Vs^{-1} 50 times in PBS, two clear oxidation peaks (E_{p1}^{a} and E_{p2}^{a}) were observed at ca. 0.58 and 0.80 V (Fig. 1A(a) and Fig. 1(B)), while at the untreated GC electrode the oxidation peak at E_{p1}^{a} was not clearly observed (Fig. 1A(b)). After Ar gas was sufficiently purged into the solution used in (A), the peak at E_{p1}^{a} completely disappeared and one oxidation peak was only observed at 0.8 V. When NaNO_2 solution was added into the solution used in Fig. 1(A), the oxidation peak current at 0.8 V was increased with increasing the concentration of NaNO_2 added, while the oxidation peak at E_{p1}^{a} remained essentially unchanged (Fig. 2). Thus, the data in Figs. 1 and 2 indicate that E_{p1}^{a} and E_{p2}^{a} correspond to the oxidation of NO and NO_2^- , respectively. In this case, the concentration of NO_2^- was esti-

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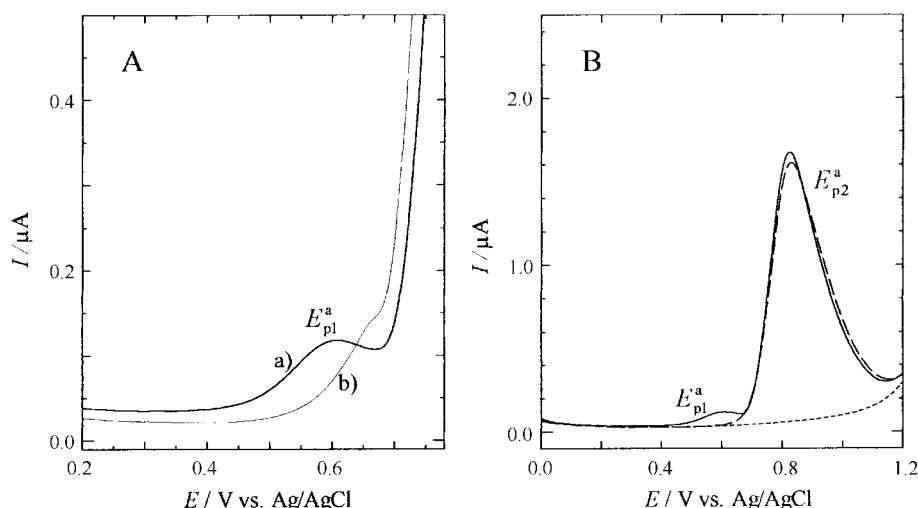
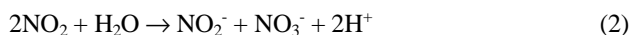
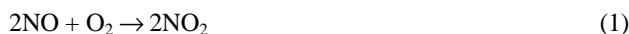


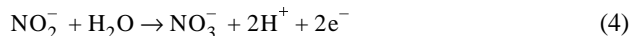
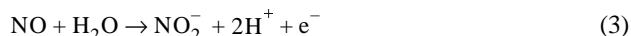
Fig. 1. (A) OSWVs obtained at (a) pretreated and (b) untreated GC electrodes ($d = 1.0$ mm) in 0.1 M phosphate buffer solution (pH 7.2) containing $95 \mu M$ NO. (B) OSWVs obtained at pretreated GC electrode ($d = 1.0$ mm) in 0.1 M phosphate buffer solution (pH 7.2) before (solid) and after (dashed) Ar gas was sufficiently (for ca. 10 min) purged into the solution used in (A). Dotted line represents the background response in 0.1 M phosphate buffer solution (pH 7.2).

mated to be 0.8 mM. Here it should be noted that NO reacts readily with traces of O_2 to produce NO_2 , which then forms NO_2^- and NO_3^- in neutral aqueous solution according to the following reactions¹¹:



NO_3^- is electroinactive in the potential range examined here. Thus, NO_2^- is the only available electroactive species after Ar gas was sufficiently purged into the NO gas-containing solution. About 0.1 mM NO_2^- is expected in biological systems produced from the NO oxidation by dissolved oxygen¹².

The electro-oxidation reactions of NO and NO_2^- in neutral pH solutions are expressed as follows^{1,13}:



The oxidation peak potential of NO at the electrochemically pretreated GC electrode was shifted by ca. 100 mV toward less positive potential compared with that at the untreated GC electrode, while that of NO_2^- remained almost unchanged at both electrodes. As a result, we could separate the two oxidation peaks of NO and NO_2^- at the pretreated GC electrode. At the present stage, we have no satisfactory explanation for the observed different effects of the relatively strong electrochemical pretreatment of the GC electrode on the oxidation of NO and NO_2^- . However, the formation of surface oxides such as quinone (C=O) or carboxylic (COO⁻) group^{14,15} and surface defects^{6,7} introduced by the electrochemical pretreatment process may contribute to the clear separation of the oxidation peaks of NO and NO_2^- .

In conclusion, the electrochemically pretreated GC electrode

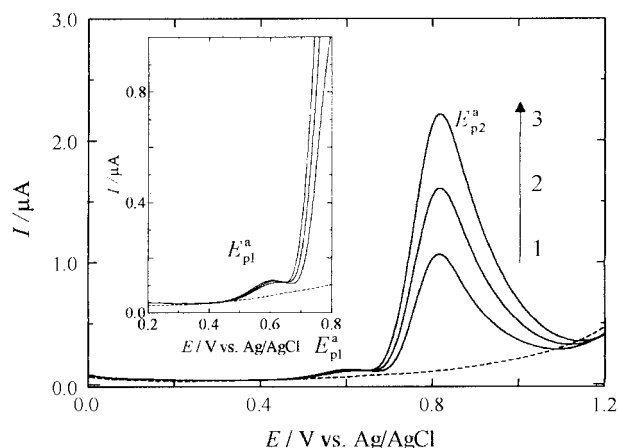


Fig. 2. OSWVs obtained at pretreated GC electrode ($d = 1.0$ mm) in 0.1 M phosphate buffer solution (pH 7.2) containing (1) $95 \mu M$ NO + 0.8 mM NO_2^- , (2) (1) + 0.4 mM $NaNO_2$ and (3) (1) + 0.8 mM $NaNO_2$. Inset shows the partially enlarged voltammograms. Dotted line represents the background response in 0.1 M phosphate buffer solution (pH 7.2).

allows us to simultaneously electroanalyze NO and NO_2^- in neutral aqueous media based on their well-separated OSWV oxidation peaks.

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