Determination of Saturated Concentration and Diffusion Coefficient of Nitric Oxide in Physiological pH Solution Using Hydrodynamic Chronocoulometry

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Electro-oxidation reaction of NO in phosphate buffer solution (0.1 M (1 M = 1 mol dm⁻³), pH 7.2) containing its oxidized species, electroactive NO₂⁻, has been investigated using cyclic and linear sweep voltammetry with electrochemically pretreated Au, Pt, and glassy carbon electrodes. Well-separated oxidation peaks for NO and NO₂⁻ could be observed at these pretreated electrodes. Thus the values of saturated concentration (*C*_{NO}) and diffusion coefficient (*D*_{NO}) of NO were simultaneously determined to be 1.7 ± 0.1 mM and (1.3 ± 0.1) × 10⁻⁵ cm² s⁻¹, respectively, using hydrodynamic chronocoulometry. The values obtained are briefly discussed on the basis of comparison with those previously reported.

Key Words : Nitric Oxide, Saturated Concentration, Diffusion Coefficient, Hydrodynamic Chronocoulometry

1 Introduction

In this communication, we report the determination of the diffusion coefficient (*D*_{NO}) and saturated concentration (*C*_{NO}) of nitric oxide (NO) in physiological pH solution, to the study in biological systems of which a great deal of attention has been devoted because of its important biological role as a potent endogeneous vasodilator^{1,2} as well as in inflammation, thrombosis, immunity and neurotransmission.^{1,3} Knowledge of *D*_{NO} and *C*_{NO} is essential to the analysis of an electrochemical system of NO from the viewpoints of electrode kinetics and electroanalytical chemistry. We have applied hydrodynamic chronocoulometry (HCC) to estimate *D*_{NO} and *C*_{NO}, because it allows simultaneous determination of *D*_{NO} and *nC*_{NO} (*n*: the number of electrons involved in the electrode reaction of NO and in this case *n* = 1) without previous knowledge of either of them from a single experiment. The precise formulation of HCC has been reported by our group, and successfully applied to determine the diffusion coefficients and the saturated concentrations of molecular oxygen (O₂) and superoxide ion in various media.²⁻⁴ The half-life of NO is very short, *e.g.*, *ca.* 6 s in physiological solutions,⁵ but NO was found to be stable in the present experimental time scale (*ca.* 15 min) and NO is liable to be oxidized by O₂ to nitrite (NO₂⁻) and nitrate (NO₃⁻).^{1,5-7} This is the reason why HCC was preferably employed for determination of *D*_{NO} and *C*_{NO} in this study.

2 Experimental

NO-saturated solution was prepared by bubbling NO gas (99.0%, TOHO SANSO KOGYO, Japan) through deoxygenated phosphate buffer (pH 7.2) for 20 min. 0.1 M Phosphate buffer (pH 7.2) solutions were prepared from

deionized water (Milli-Q system, Millipore, Japan) using Na₂HPO₄ and NaH₂PO₄ salts (KANTO Chemical Co.). All other chemicals used in this study were of analytical grade and used as received.

The electrochemical measurements were performed in a conventional three-electrode system with Au, Pt and glassy carbon (GC) working electrodes, a spiral Pt wire auxiliary electrode, and a potassium chloride saturated silver/silver chloride (Ag/AgCl) reference electrode. Au (diameter, *d* = 1.6 mm) and Pt (*d* = 2.0 mm) disk electrodes were polished with alumina powder and then were electrochemically cleaned. GC disk electrodes of 3.0 mm diameter were also polished with alumina powder and then were electrochemically pretreated.⁸ Cyclic voltammetry and hydrodynamic chronocoulometry were carried out using a computer-controlled electrochemical analyzer (BAS 100 B/W) and a rotating disk electrode system from Nikko Keisoku, Japan according to the same procedure as previously reported.²⁻⁴ During all the electrochemical measurements, Ar gas (99.9999%, Nippon SANSO Co.) was flushed over the NO-saturated cell solution.

The value of kinematic viscosity (*ν*) of the NO-saturated phosphate buffer solution was measured at 25.0 ± 0.1°C in a thermostated water bath using an Ubbelohde viscometer. The *ν* value was (9.23 ± 0.10) × 10⁻³ cm² s⁻¹.

3 Results and Discussion

NO-saturated solutions are prepared under anaerobic conditions using phosphate buffer solution deoxygenated with Ar gas. However, water-soluble oxidation products of NO can form even if trace quantities of O₂ are present in the solution. It has been reported that NO reacts with

O₂ to produce nitrogen dioxide (NO₂) (Eq. 1), and NO₂ immediately reacts again with NO at an almost diffusion-controlled rate ($k_2 > 10^9 \text{ M}^{-1} \text{ s}^{-1}$) to produce NO₂⁻ (Eq. 2) or disproportionates to NO₂⁻ and NO₃⁻ (Eq. 3) in physiological pH solution.^{6, 7, 9}

Therefore, NO, NO₂⁻ and NO₃⁻ are considered to coexist in the NO-saturated solution used in this work.

Figure 1A shows the typical voltammograms of NO obtained at a bare Au electrode in 0.1 M phosphate buffer solution. Two well-separated oxidation peaks, E_{p1}^{a} and E_{p2}^{a} , were observed at 0.65 and 0.82 V vs. Ag/AgCl, respectively. These well-separated oxidation peaks could not be obtained when the electrochemically unpretreated (*i.e.*, uncleaned) electrodes were used. This is because the oxidations of NO and NO₂⁻ occur at a close potential and they overlap each other at uncleaned electrodes.¹⁰ In order to identify these two oxidation reactions, Ar gas was purged into the solution immediately after the voltammogram shown in Fig. 1A(a) was recorded. The oxidation peak at E_{p1}^{a} totally disappeared after Ar gas was sufficiently purged (Fig. 1A(b)). This fact suggests that the oxidation peak at E_{p1}^{a} would be due to the oxidation of dissolved NO gas (Eq. 4¹¹).

Fig. 1 (A) Typical voltammograms recorded at Au electrode (diameter, $d = 1.6 \text{ mm}$) in NO-saturated 0.1 M phosphate buffer solution (pH 7.2) before (a, solid line) and after (b, dashed line) Ar gas was sufficiently (for *ca.* 10 min) purged into the solution. Dotted line shows the voltammogram corresponding to the background response recorded in Ar gas-saturated 0.1 M phosphate buffer solution (pH 7.2). Potential scan rate was 100 mVs^{-1} . Inset shows the entire voltammograms in the potential range between 0.4 and 1.0 V. (B) Typical voltammograms obtained at Au ($d = 1.6 \text{ mm}$) electrode in NO-saturated 0.1 M phosphate buffer solution (pH 7.2) in the absence (a) and presence (b) of 0.3 mM NaNO₂. Potential scan rate was 100 mVs^{-1} . Inset shows partially enlarged voltammograms. Dotted line represents the background response obtained in Ar gas-saturated 0.1 M phosphate buffer solution (pH 7.2).

However, the oxidation peak at E_{p2}^{a} (Eq. 5,¹²) was still observed on the voltammogram (see the inset in Fig. 1A). Addition of NO₂⁻ into the NO-saturated solution led to the increase of the oxidation peak current at E_{p2}^{a} as shown in Fig. 1B. In the potential region from 0.65 to 1.00 V, the oxide layer formation on gold as well as platinum electrode surfaces takes place as shown by the dotted line in Fig. 1B.⁸ Therefore, the oxidation peak E_{p2}^{a} is considered to be based on the overlapping of both the oxidation of NO₂⁻ produced in the above-mentioned pathway (Eqs. 1-3) and the oxide layer formation. That is why GC electrode has been employed for the simultaneous quantitative detection of NO and NO₂⁻ in our previous work.⁸ On the other hand, the oxidation peak E_{p1}^{a} remained unchanged before and after the addition of NO₂⁻ anion (see the inset in Fig. 1B). Meanwhile NO₃⁻ is an electroinactive species in the examined potential region. Thus the electrode pretreatment and the appropriate selection of potential region allowed us to apply HCC to the estimation of C_{NO} and D_{NO} of NO dissolved in 0.1 M phosphate buffer solution as mentioned below.

Figure 2 shows the typical steady-state voltammogram obtained for NO oxidation at the rotating GC disk electrode at 200 rpm. The RDE voltammetric measurements were performed to determine the range of potential step and of the electrode rotation rate for HCC. At that time, it was found that NO was stable in this RDE voltammetric time scale (*ca.* 15 min). At electrode rotation rates of more than 450 rpm, the voltammogram became ill-defined because of the overlapping of the oxidation waves of NO and NO₂⁻. Thus, HCC for the oxidation of NO was carried out at electrode rotation rates from 250 to 400 rpm, and in this case the potential was stepped from 0 to 0.62 V. The obtained HCC curves are shown in Fig. 3, in which it took about 1 min to obtain these all curves. For each curve, a linear regression was con-

Fig. 2 Typical steady-state voltammograms recorded for the oxidation of NO at rotating GC disk electrode ($d = 3.0$ mm) in NO-saturated 0.1 M phosphate buffer solution (pH 7.2) before (a, solid line) and after (b, dashed line) Ar gas was sufficiently (for *ca.* 10 min) purged into the solution. Electrode rotation rate was 200 rpm. Potential scan rate was 1 mVs^{-1} .

Fig. 3 Hydrodynamic chronocoulometric data of NO oxidation at rotating GC disk electrode ($d = 3.0$ mm) in NO-saturated 0.1 M phosphate buffer solution (pH 7.2). Electrode rotation rates: (1) 250, (2) 300, (3) 350, and (4) 400 rpm. The potential of the working electrode was stepped from 0 to 0.62 V vs. Ag/AgCl. All the measurements were performed under Ar gas atmosphere. The dotted line shows the typical regression line obtained over the electrolysis time 1.2 and 1.5 s for the Q vs. t curve at 350 rpm.

ducted over the electrolysis time between 1.2 and 1.5 s. The straight line obtained by such a linear regression (typically shown by the dotted line for the Q vs. t curve at 350 rpm in Fig. 3) can be expressed by the following charge (Q)-time (t) relationship:

$$Q = Q_{\delta} + I_L t \quad (6)$$

with

$$Q_{\delta} = 0.3764 n F A C_{\text{NO}} \delta, I_L = (n F A D_{\text{NO}} C_{\text{NO}} / \delta) \quad (7)$$

where δ is the thickness of the hydrodynamic boundary layer and is given by

Fig. 4 (A) Q_{δ} vs. $\omega^{-1/2}$ plot and (B) I_L vs. $\omega^{1/2}$ plot for the oxidation of NO in NO-saturated 0.1 M phosphate buffer solution (pH 7.2). Data were taken from Fig. 3.

$$\delta = 1.610 D_{\text{NO}}^{1/3} \nu^{1/6} \omega^{-1/2} (1 + 0.2980 Sc^{-1/3} + 0.14514 Sc^{-2/3}) \quad (8)$$

In Eqs. 7 and 8, n is the number of electrons involved in the electrode reaction, F is the Faraday constant, A is the electrode surface area, ν is the kinematic viscosity of the solution, $Sc (= \nu / D_{\text{NO}})$ is the Schmidt number, and ω is the electrode angular velocity ($\omega = 2 \pi f$, where f is the frequency of the electrode rotation). Thus the values of slope (I_L) and intercept (Q_{δ}) estimated from Fig. 3 were plotted against $\omega^{1/2}$ and $\omega^{-1/2}$, respectively, as shown in Fig. 4A and 4B. Both plots of I_L vs. $\omega^{1/2}$ and Q_{δ} vs. $\omega^{-1/2}$ were found to be satisfactorily linear and to pass through the origin, as expected from Eqs. 7 and 8. Then, assuming that NO undergoes a one-electron oxidation reaction (Eq. 4),¹¹⁾ we could estimate the values of D_{NO} and C_{NO} using Eq. 7. The similar experiments were performed with the pretreated Au and Pt electrodes. All the obtained results are summarized in Table 1, together with those previously reported.

The evaluated values of C_{NO} in 0.1 M phosphate buffer solution (pH 7.2) by HCC and Griess method are in good agreement with literature values in pure water¹³⁾ and phosphate buffer solution¹⁴⁾ which were determined by a conventional manometric-volumetric method and potential-step chronoamperometry, respectively.

The value of D_{NO} in 0.1 M phosphate buffer solution was estimated to be $(1.2 \sim 1.3) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, which is smaller than the values obtained by other researchers: $2.60 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ in pure water, $3.30 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 37°C in 10 mM phosphate buffer solution and $(4.5 \pm 0.3) \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 25°C in 50 mM phosphate buffer solution, probably due to differences in experimental conditions such as temperature and concentration of buffer solution. We also simultaneously evaluated the values of D_{NO} and C_{NO} in acidic electrolytic solution in which NO is more stable than in neutral or alkaline solutions.^{15,16)} The values of D_{NO} and C_{NO} in 4 M H_2SO_4 were comparable to the values previously reported.^{16,17)}

In conclusion, a simultaneous determination of C_{NO} and

Table 1 The values of diffusion coefficient (D_{NO}) and concentration (C_{NO}) of NO in NO-saturated solutions.

Electrodes	Electrolytes	$10^5 D_{\text{NO}}/\text{cm}^2 \text{ s}^{-1}$	$10^3 C_{\text{NO}}/\text{M}$	Temperature / $^{\circ}\text{C}$	Measurement Methods
GC	0.1 M phosphate buffer (pH 7.2)	1.20 ± 0.17	1.54 ± 0.19	25	this work
Au	0.1 M phosphate buffer (pH 7.2)	1.29 ± 0.17	1.72 ± 0.13	25	this work
Pt	0.1 M phosphate buffer (pH 7.2)	1.22 ± 0.15	1.68 ± 0.19	25	this work
—	0.1 M phosphate buffer (pH 7.2)	—	1.80 ± 0.10	25	this work ^{a)}
Pt	4 M H_2SO_4	0.59 ± 0.25	0.92 ± 0.16	25	this work
Pt	2 M H_2SO_4	0.90	1.92	25	RDE voltammetry ^{b)}
Pt	4 M H_2SO_4	0.90	1.29	23	RDE voltammetry ^{c)}
—	pure water	—	1.9	25	manometric-volumetric method ^{d)}
—	acidified saline (9 mg ml^{-1} NaCl, 0.1 mM HCl, pH 4)	—	3	25	Griess method ^{e)}
—	pure water	2.60	—	25	microscopic observation ^{f)}
carbon fiber	buffer solution (10 mM phosphate, 2.7 mM KCl, 137 mM NaCl, 1 mM L-arginine, pH 7.4)	3.30	—	37	amperometry ^{g)}
carbon fiber	phosphate buffer (pH 7.4)	—	1.74 ± 0.03	37	potential-step chronoamperometry ^{h)}
—	0.05 M phosphate buffer (pH 7.4)	4.5 ± 0.3	—	20	fluorophotometric analysis ⁱ⁾

a) The estimation of C_{NO} was performed by a combined use of Griess method and electroanalytical method.⁸⁾

b) Ref. 15), the C_{NO} was evaluated using the D_{NO} taken from Ref. 16).

c) Ref. 16), the D_{NO} was evaluated using the C_{NO} which was previously obtained by a potentiometric titration.

d) Ref. 13). e) Refs. 1) and 18). f) Ref. 19). g) Ref. 20). h) Ref. 14). i) Ref. 21).

D_{NO} in physiological pH solution has been for the first time carried out using hydrodynamic chronocoulometry with electrochemically pretreated GC, Pt and Au electrodes under the experimental condition that NO and its oxidized species (electroactive NO_2^-) coexist.

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