Amperometric Measurement of Nitric Oxide (NO) Using an Electrode Coated with Polydimethylsiloxane

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Dip-coating of a platinum electrode from an aqueous dispersion of polydimethylsiloxane provided an amperometric nitric oxide (NO) sensor with high performance characteristics such as rapid response (100% response time, 3 s), high sensitivity (detection limit, 20 nM; 1 M = 1 mol dm^{-3}), high stability (usable for more than a month) and high selectivity (e.g., the ratio of response for uric acid to that for the same concentration of NO, less than 10^{-4}).

Nitric oxide (NO) plays important roles as a molecular messenger in biological systems. For example, the release of NO from endothelial cells follows the relaxation of a blood vessel so as to control blood pressure.[1] The concentration of NO released from biological cells is initially (sub)micromolar level and diminishes within several minutes due to the high reactivity of NO with various molecules. Hence sensitive and rapid measuring method are requested for in situ NO monitoring. Amperometric determinations can accomplish this function; NO is oxidized on a metal[2–4] or catalyst-attached[5] electrode around 0.9 V vs Ag/AgCl. However, such anode systems usually suffer from electrochemical interference by oxidizable species (e.g., L-ascorbic acid, uric acid, L-cysteine, acetaminophen, catecholamines, active oxygens and nitrite) in a complex matrix. The electrode oxidizes these interferants as well as NO, which results in a current response with a positive error.

Although several authors[3,5] have described the use of a Nafion coating layer for the suppression of interference response, such an anionic polymer was ineffective for preventing neutral (e.g., acetaminophen) and cationic (e.g., dopamine) interferants from reaching the electrode surface. Shibuki[3] has prepared an NO-sensing electrode by replacing a PTFE membrane of a Clark-type oxygen probe with a chloroprene rubber membrane. But the Clark-type probe has a complicated structure and the miniaturization of the probe is rather difficult. Hence the development of an NO-sensing microelectrode system that can be prepared easily and cheaply is desired. Friedemann et al.[4] have prepared an NO-selective electrode by the use of electrodeposited poly(phenylenediamine) layer. However, the polymer layer was known to show a high permeability for hydrogen peroxide,[6] a kind of active oxygen, and the poly(phenylenediamine)-coated electrode gave a narrow linear range against NO.[4]

For the discrimination between the interferants and NO, the use of porous, hydrophobic coating layer would be a suitable approach: NO would easily pass through the pores to reach the electrode surface, whereas the transport of hydrophilic species (e.g., L-ascorbic acid, dopamine, hydrogen peroxide and nitrite) through the hydrophobic polymer is strongly restricted.[7] We have found that an electrode prepared by dip-coating from an aqueous dispersion of polydimethylsiloxane (PDMS) is useful for the rapid, sensitive and selective determination of NO.

A platinum disc electrode (diameter, 1.6 mm; Bioanalytical Systems, West Lafyette, IN) was dipped into an aqueous dispersion of PDMS (Dow Corning, Midland, MI) and allowed to dry with the surface facing up at room temperature for 4 h. The dispersion contained the polymer particles [4%(w/v)] having ca. 0.5 μm.[8] The thickness of the PDMS layer was ca. 5 μm. A standard solution of NO was prepared by bubbling it (99.7%, Sumutomo Seika Chemicals, Tokyo) into pure water, which was under argon atmosphere, at 25.0 ± 0.2 °C. The NO concentration of the saturated solution was reported to be 1.88 mM.[9] The test solution usually used was a 0.1 M potassium phosphate buffer (pH 7.0, 20 mL) under argon atmosphere, and its temperature was kept at 25.0 ± 0.2°C. The solution was stirred with a magnetic bar during the amperometric measurements.

Linear sweep voltammograms for the PDMS-coated and bare platinum electrodes (ν = 10 mV s^{-1}) showed that an anodic peak for the oxidation of NO was given at 0.82 V vs Ag/AgCl (saturated with KCl) for each electrode, although the polymer coating resulted in a decrease in the peak height, as described below. This suggests that NO molecules permeate through the PDMS layer to be oxidized on the electrode surface. The permeabilities of oxidizable species including NO were evaluated as follows. Amperometric responses on the PDMS-coated and bare electrodes were measured at 0.85 V vs Ag/AgCl for several electroactive species. The ratio of current response on the PDMS-coated electrode to that on the bare electrode was

Table 1. PDMS-coated electrode response-to-bare electrode response ratios for oxidizable species.

Species used	Ratio
NO	3×10^{-1}
L-Ascorbic acid	$< 10^{-4}$
Uric acid	$< 10^{-4}$
L-Cysteine	$< 10^{-4}$
Acetaminophen	4×10^{-3}
Dopamine	3×10^{-3}
Hydrogen peroxide	$< 10^{-4}$
Nitrite	$< 10^{-4}$

Anodic current responses for the PDMS-coated and bare electrodes were measured after the addition of each species at 0.85 V vs. Ag/AgCl, and the ratio of the current for the former electrode to that for the latter was calculated. The concentrations of the species were 10 μM for NO and 0.2 mM for others. Each ratio was averaged over three measurements, and was reproducible within ±40% in the measurements using three different electrodes.

recorded for each species, as summarized in Table 1. A high ratio, 0.3, was obtained for NO. In contrast, the ratios were very low for L-ascorbic acid, uric acid, L-cysteine, acetaminophen, dopamine, hydrogen peroxide and nitrite. This means that the PDMS-coated electrode can be used for the anodic detection of NO without serious error from such interferants.

Scanning electron microscopy of the PDMS layer indicated a porous structure with cracks and micropores.[8,10] The PDMS particles in the dispersion were piled up on the based electrode surface and fused partially to form a hydrophobic, porous layer, which was useful for the selective permeation of NO.

Figure 1 shows a current-time curve for the PDMS-coated electrode upon the successive addition of NO in 10 μM steps. The steady-state current increase, which was obtained within 3 s after the addition of NO, was proportional to the analyte concentration up to 50 μM. The inset in Figure 1 shows a current-time curve for the addition of 20 nM NO, which suggested that such a low concentration can be measured on the electrode (signal-to-noise ratio, 5). The relative standard deviation for ten successive measurements of 2 μM NO was 1.5%. Thus the PDMS-coated electrode has proved to show high performance characteristics, in terms of sensitivity and response time, enough for monitoring NO-releasing processes in biological

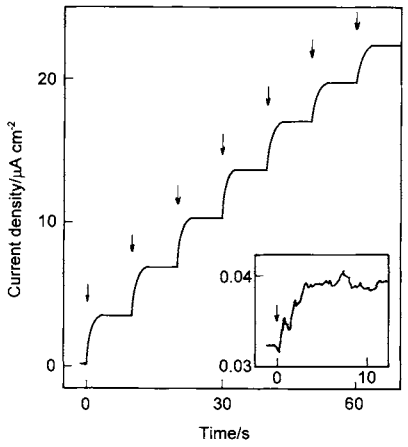

Figure 1. Current-time curve for the PDMS-coated electrode obtained on increasing NO concentration in 10 μM steps, at 0.85 V vs. Ag/AgCl. The inset shows current-time curve obtained after the addition of 20 nM NO.

systems including endogenous NO release.[1]

The long-term stability of the PDMS-coated electrode was examined; measurements of the current response for 2 μM NO and 0.1 mM uric acid were respectively carried out three times a day, each day, for a month. Average values of the electrode response for both compounds in the respective three measurements, as well as the baseline, did not change for a month, indicating that the PDMS-coated electrode could be used for the selective determination of NO for a long time.

The dip-coating of PDMS was useful as a simple and reproducible procedure for preparing NO-sensing electrodes with low interference levels (see Table 1). Furthermore, the dip-coating procedure is of particular interest because it is highly suitable for preparing microsensors. We have already prepared an NO-sensing microelectrode with the use of a platinum microdisc electrode (diameter, 5 μm) as the base transducer. The determination of NO in biological systems with the PDMS-coated microelectrode is in progress.

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References and Notes

1 a) R. F. Furchgott and J. V. Zawadski, *Nature*, **299**, 373 (1980). b) T. Malinski, Z. Taha, G. Grunfeld, S. Patton, M. Kapturczak, and P. Tomboulian, *Biochem. Biophys. Res. Commun.*, **193**, 1076 (1993).
2 K. Shibuki, *Neurosci. Res.*, **9**, 69 (1990).
3 F. Pariente, J. L. Alonso, and H. D. Abruna, *J. Electroanal. Chem.*, **379**, 191 (1994).
4 M. N. Friedemann, S. W. Robinson, and G. A. Gerhardt, *Anal. Chem.*, **68**, 2621 (1996).
5 a) T. Malinski and Z. Taha, *Nature*, **358**, 676 (1992). b) F. Lantione, S. Trevin, F. Bedioui, and J. Devynck, *J. Electroanal. Chem.*, **392**, 85 (1995). c) F. Bedioui, S. Trevin, J. Devynck, F. Lantione, A. Brunet, and M.-A. Devynck, *Biosens. Bioelectron.*, **12**, 205 (1997). d) K. M. Mitchell and E. K. Michaelis, *Electroanalysis*, **10**, 81 (1998). e) T. Haruyama, S. Shiino, Y. Yanagida, E. Kobatake, and M. Aizawa, *Biosens. Bioelectron.*, **13**, 763 (1998). f) J. Jin, T. Miwa, L. Mao, H. Tu, and L. Jin, *Talanta*, **48**, 1005 (1999). g) L. Mao, K. Yamamoto, W. Zhou, and L. Jin, *Electroanalysis*, **12**, 72 (2000).
6 S. V. Sasso, R. J. Piece, R. Walla, and A. M. Yacynych, *Anal. Chem.*, **62**, 1111 (1990).
7 F. Mizutani, T. Sawaguchi, S. Yabuki, and S. Iijima, *Electrochemistry*, **67**, 1138 (1999).
8 F. Mizutani, S. Yabuki, and S. Iijima, *Trans. IEE Jpn.*, in press.
9 "Lange's Handbook of Chemistry," 12th ed., ed. by J. A. Dean, McGraw-Hill, New York (1978), p.10-5, .
10 F. Mizutani, S. Yabuki, and S. Iijima, *Electroanalysis*, submitted.