Sensitive and Selective Electrochemical Detection of Carbon Monoxide in Saline at a Pt-Ru/Nafion/MnO₂-Modified Electrode

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We fabricated a sensitive and selective electrochemical carbon monoxide (CO) sensor for physiological conditions based on the Pt-Ru system. At a bare Pt-Ru electrode, a linear amperometric response to CO concentration was obtained in the range of 0.9 – 9 μM . However, significant current response to model electroactive interferents for physiological conditions, uric acid (UA), ascorbic acid (AA) and hydrogen peroxide (HP), was also recorded at the Pt-Ru electrode. The response to UA and AA was highly suppressed by coating the Pt-Ru electrode surface with a Nafion layer, and the response to HP was almost completely eliminated by the additional coating with a MnO₂/chitosan layer. Finally, at the Pt-Ru/Nafion/MnO₂ electrode, amperometric CO detection with a sensitivity of 173 nA cm⁻² μM^{-1} was obtained in the concentration range of 0.9 – 9 μM with the UA, AA and HP signal being below 1.7% at the same concentration of CO.

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Introduction

Carbon monoxide (CO) can be generated by incomplete combustion, which is often associated with silent asphyxiation if improper ventilation is not used. Additionally, CO is endogenously and physiologically generated in mammalian cells as a result of catabolism of heme in the rate-limiting step by heme oxygenase system. Recently, such CO systems have drawn attention due to their various essential roles in living bodies such as anti-inflammatory, antioxidant and antiapoptotic effects.¹⁻⁸ Researchers have employed several methods to study the effect of CO on living bodies by using heme oxygenase (HO-1) knockout mice^{9,10} and CoxA-based biosensors,^{11,12} and by monitoring CO concentration indirectly from the breath.¹³ By these methods, however, it is difficult to measure CO concentration locally, in real time and *in vivo*, which would be highly beneficial for understanding the function of CO in living bodies. In this study, we have developed a sensitive and selective electrochemical sensor for CO in physiological conditions by surface modification of a Pt-Ru electrode. The Pt-Ru system is known to be effective in the electrochemical oxidation of CO to CO₂, while in the pure Pt system CO can be adsorbed strongly on the surface, causing serious poisoning.¹⁴⁻¹⁶ For the Pt-Ru system, oxidation of CO has been considered to occur *via* reaction between CO adsorbed on Pt sites (Pt-CO) and Ru-OH species formed by discharging the adsorbed water (bifunctional mechanism).¹⁷ In addition, ruthenium can modify the electronic structure of platinum. This modification induces a weakening of the Pt-CO bond, lowering the energy required for the oxidation of the adsorbed CO on Pt (ligand effect).¹⁸ To

improve selectivity of CO detection from possible electroactive interferents which are ubiquitous in a biological fluid, such as uric acid (UA), ascorbic acid (AA) and hydrogen peroxide (HP), we modified the Pt-Ru electrode surface with Nafion and MnO₂/chitosan layers (Fig. 1). Nafion can be act as a permselective membrane, which can exclude the diffusion of UA and AA.^{19,20} MnO₂ is a well-known catalyst for HP decomposition into H₂O and O₂. Chitosan is a natural organic polymer with excellent properties for an immobilization matrix for a biosensor such as low cost, high mechanical strength, good film-forming capability, biocompatibility and nontoxicity.²¹⁻²³ The modified Pt-Ru electrode showed sensitive CO detection in the physiological concentration range (0.9 to 9 μM), while it showed almost no current response to AA, UA and HP for the same concentrations to CO.

Experimental

Preparation of modified electrodes

The Pt-Ru electrode was prepared by electrodeposition. A ruthenium thin film was first deposited onto a gold electrode (BAS, electrode diameter was 1.6 mm) surface by applying a potential of -500 mV vs. Ag/AgCl in 20 mM RuCl₃ at 40°C for 180 s. The Ru-covered electrode was then subjected to platinum deposition in an aqueous solution containing 1 mM H₂PtCl₆ at a potential of -200 mV vs. Ag/AgCl at 40°C for 14 s. The Ru/Pt ratio of the electrode was determined by X-ray photoelectron spectroscopy (XPS; AXIS-NOVA, Kratos). For XPS analysis, the Pt-Ru samples were prepared on a gold wire. The binding energy was calibrated with the position of a C 1s peak to be 284.8 eV. The Nafion-coated Pt-Ru electrode was prepared by casting a 7.1 $\mu\text{L cm}^{-2}$ of 2 wt% Nafion solution on the Pt-Ru electrode. The 2 wt% Nafion solution was prepared by diluting

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Fig. 1 Schematic illustration of the fabrication of a Pt-Ru/Nafion/MnO₂ electrode.

5 wt% Nafion solution (Aldrich) with a mixture of 1-propanol (80 vol%) and distilled water (20 vol%). The thickness of the Nafion layer was estimated by scanning electron microscopy (SEM) observation of the cross-section of a silicon wafer coated with Nafion with the same condition to be *ca.* 1.5 μm .

MnO₂ particles were prepared as follows: 1.35 g of MnSO₄·H₂O (Aldrich) and 0.3 g of Pluronic F-88 (ADEKA) were added to 20 mL of distilled water, and the solution was magnetically stirred for 10 min. The mixture was transferred into a flask and heated up to 90°C. Twelve milliliters of aqueous solution containing 1.83 g of (NH₄)₂S₂O₈ (Wako) were then slowly added into the flask, and the mixture was heated at 90°C for 15 min under a stirred condition. The synthesized MnO₂ particles were filtered to collect fine particles. A MnO₂ suspension was prepared by adding MnO₂ (48 mg) and chitosan (30 mg), as a binder, into a mixture of distilled water (4.0 mL), 1-propanol (5.9 mL) and acetic acid (0.1 mL).

A Pt-Ru/Nafion/MnO₂ electrode was prepared first by casting a 7.1 $\mu\text{L cm}^{-2}$ of 1 wt% Nafion solution on a Pt-Ru electrode. The Nafion-coated Pt-Ru electrode was soaked in 10 μM NaOH to exchange protons with the sulfonyl groups of Nafion, creating -SO₃⁻ groups. Then a 35.4 $\mu\text{L cm}^{-2}$ MnO₂ suspension was cast on the Nafion-coated Pt-Ru electrode to obtain a Pt-Ru/Nafion/MnO₂ electrode after drying. From the cross-sectional SEM image of Nafion/MnO₂-coated silicon wafer, the thickness of the Nafion and the MnO₂ layers was estimated to be *ca.* 1 and 3 μm , respectively.

Amperometric detection of CO

Amperometric detection of CO and other model interferents (UA, AA and HP) was carried out using a three-electrode electrochemical cell equipped with a Pt wire and a Ag/AgCl electrode as counter and reference electrodes, respectively, connected to a potentiostat (HZ-5000, Hokuto Denko). For amperometric measurement, the electrode was held at +520 mV vs. Ag/AgCl in saline (Otsuka Pharmaceutical): after the current stabilized, CO-containing argon (CO/Ar) gas (1000, 3000, 5000 or 10000 ppm) was bubbled into the saline or an aliquot of a model interferent solution was added into the saline using a microsyringe. The CO/Ar gas was purchased from Takachiho Trading Co., Ltd. According to Henry's law, if one considers the vapor pressure of water to be 23.8 Torr and the solubility of CO to be 2.3 mL/100 mL in water at 25°C, the saturating concentrations of CO in the bubbled saline were calculated to be 0.9, 2.7, 4.5 and 9.0 μM for the 1000, 3000, 5000 and 10000 ppm CO/Ar gases, respectively.

Fig. 2 Relationship between atomic concentration ratio of Pt on the Pt-Ru electrode surface and electrodeposition time of Pt on the Ru electrode.

Results and Discussion

Preparation and CO detection properties of Pt-Ru electrode

Initially, a ruthenium thin film was formed on a gold electrode by electrodeposition using 20 mM RuCl₃ at a potential of -500 mV vs. Ag/AgCl at 40°C for 180 s. With this procedure, the gold surface was covered fully by the ruthenium thin film: the coverage was confirmed by the Au 4f peak disappearing in the XPS of the gold wire after ruthenium deposition with the same condition (result not shown). Then, platinum particles were deposited on the ruthenium thin film by electrodeposition. We then investigated the relationship between the Pt/Ru ratio and the electrodeposition time for Ru deposition. The ratio was calculated from XPS quantitative analysis using peak areas for Pt 4f and Ru 3p. The Pt/Ru ratio was found to show a linear increase as the deposition time increased (Fig. 2). Based on this result, we determined the deposition time of the Pt deposition to be 14 s for fabrication of a Pt-Ru electrode with a Pt/Ru ratio of unity, which had been reported to be the most active for methanol oxidation and hence for CO oxidation at Pt-Ru alloy catalysts.^{14,15} Figure 3 shows XPS Pt 4f and Ru 3p spectra of a Pt-Ru electrode (Pt/Ru = 1). The peak position of the Pt 4f spectrum indicated a binding energy more positive by 0.4 - 0.5 eV than that of pure platinum. This should be due to an increased 5d vacancy of Pt by Ru on the Pt-Ru electrode, which is considered to decrease the back donation from Pt 5d

Fig. 3 XPS (a) Pt 4f and (b) Ru 3p core level spectra of the Pt-Ru electrode. Electrodeposition time for Pt deposition was 14 s.

Table 1 Current response to CO and model interferents (ΔI) at Pt-Ru, Pt-Ru/Nafion and Pt-Ru/Nafion/MnO₂ electrodes, and selectivity indexes (S_{CO})

Electrode	$\Delta I / \text{nA cm}^{-2 \text{ a}}$				S_{CO}^{b}		
	CO	UA	AA	HP	UA	AA	HP
Pt-Ru	4650	660	1115	165	0.88	0.81	0.76
Pt-Ru/Nafion	3130	60	30	190	0.98	0.99	0.62
Pt-Ru/Nafion/MnO ₂	1760	25	30	0	0.99	0.98	1.00

a. Concentration of CO, UA and AA was 9 μM , and concentration of HP was 1 μM .

b. Definition of S_{CO} is indicated in Eq. (1) in the text.

Fig. 4 (a) Amperometric current response to CO bubbling in saline at the Pt-Ru electrode. The arrow sign indicates the time when the bubbling started. (b) Calibration curve for amperometric CO detection at the Pt-Ru electrode.

Fig. 5 (a) Amperometric current response to CO bubbling in saline at the Pt-Ru/Nafion electrode. The arrow sign indicates the time when the bubbling started. (b) Calibration curve for amperometric CO detection at the Pt-Ru/Nafion electrode.

orbital to CO $2\pi^*$ orbital, contributing to the chemisorption of CO onto the Pt surface.^{24,25}

Amperometric detection of CO with various concentrations in saline is shown in Fig. 4a. The response current started to increase immediately after the CO gas bubbling started, followed by saturation of the current after 50 – 250 s. The lag between the times when the bubbling started and when the current reached its saturation should be because of the time needed for saturation of concentration of the dissolving CO in the solution, which is inevitable in this study. The current was rather stable after the saturation, confirming that the Pt-Ru system was

tolerant to CO poisoning. The current increment at the saturation (ΔI_{CO}) was found to be directly proportional to the calculated CO concentration in the solution from 0.9 to 9 μM with a slope of 520 $\text{nA cm}^{-2} \mu\text{M}^{-1}$ (Fig. 4b). Vreman has determined the CO concentration in rodent tissues to be in the range of 1 – 10 pmol mg^{-1} (or 1 – 10 μM), and *ca.* 45 pmol mg^{-1} (or 45 μM) in rodent blood.²⁶ Thus, this result indicates that the Pt-Ru electrode can be applied to the electrochemical CO detection, and should be useful for quantitative determination of CO at physiological concentrations *in vivo*. In order to estimate the selectivity of the Pt-Ru electrode of CO to other possible interferents *in vivo*, we also carried out amperometric detection

Fig. 6 SEM image of the MnO₂ particles. (b) Magnified image of (a).

examinations for UA, AA and HP. The current increment (ΔI) for 9 μM UA, 9 μM AA and 1 μM HP is compared with ΔI_{CO} at the corresponding concentrations in Table 1. A selectivity index (S_{CO}) defined as Eq. (1) is also listed in Table 1.

$$S_{\text{CO}} = \Delta I_{\text{CO}} / (\Delta I_{\text{CO}} + \Delta I_i) \quad (1)$$

where ΔI_i is ΔI for the interferent (UA, AA or HP) at the same concentration to CO. Although the ΔI_i values for these model interferents were smaller than the ΔI_{CO} , results revealed that the electrode should be further modified to improve the selectivity to CO.

Nafion-coated Pt-Ru electrode

In order to improve the selectivity to electrochemical detection of CO, we coated a Nafion thin film onto the Pt-Ru electrode surface. By the investigation with different concentrations of Nafion cast solution from 0.5 to 5 wt%, good selectivity to CO detection was found to be obtained at the concentration of 1–2 wt%. Figure 5a shows current response to CO bubbling in saline at a Nafion-covered Pt-Ru electrode using 2 wt% Nafion solution (Pt-Ru/Nafion electrode). Although the ΔI_{CO} was somewhat smaller than that obtained at a Pt-Ru electrode, the current response still showed good linearity to CO concentration up to 9 μM with a slope of 347 $\text{nA cm}^{-2} \mu\text{M}^{-1}$ (Fig. 5b). ΔI_{UA} and ΔI_{AA} were found to decrease remarkably by the coating with Nafion from 660 and 1115 to 60 and 30 nA cm^{-2} at 9 μM ,

Fig. 7 XPS (a) Mn 2p, (b) Mn 3s and (c) O 1s core level spectra of the MnO₂ particle.

respectively (Table 1). It should be due to the electrostatic repulsion between the anionic $-\text{SO}_3^-$ groups of Nafion and the anionic molecules (UA and AA).^{19,20} Although modification with Nafion could improve the selectivity of CO at the Pt-Ru electrode from UA and AA, it was not effective for minimizing the current response due to HP.

Preparation of MnO₂ particles and its application to selective CO sensing

In order to suppress the current response to HP, we immobilized MnO₂ onto the modified Pt-Ru electrode surface. MnO₂ particles were characterized by SEM, X-ray diffraction (XRD) and XPS. From the SEM observation of the MnO₂ particles in the suspension, the particle diameter was found to be around 1 μm (Fig. 6). Because no XRD pattern for MnO₂ crystal was observed (result not shown), the particle was considered to consist of amorphous MnO₂. Figure 7 shows XPS Mn 2p, Mn 3s

Table 2 XPS data of the MnO₂ particle

Sample	Mn 2p _{3/2}		Mn 3s		O 1s		E_b (Mn 3s) – E_b (O 1s)/ eV
	E_b /eV	$E_{b,1}$ /eV	$E_{b,2}$ /eV	ΔE_b /eV	E_b /eV	Area, %	
MnO ₂ ^a	642.30	84.19	899.08	4.89	529.9 531.5 533.0	55.9 28.8 15.3	112.39
MnO ₂ ^b	642.40	83.80	88.72	4.92	529.7 531.0 532.4	64.8 20.7 14.5	112.7
MnO ^c	641.12	83.37	89.16	5.79	529.83		111.29
Mn ₃ O ₄ ^c	641.62	83.36	88.86	5.50	530.09		111.53
Mn ₂ O ₃ ^c	641.40	83.36	88.77	5.41	529.67		111.73
MnO ₂ ^c	641.85	83.92	88.70	4.78	529.48		112.37

a. This study. b. Data from Ref. 24. c. Data of authentic samples from Ref. 25.

and O 1s spectra of the MnO₂ particles. The splitting of the Mn 3s peak (Fig. 7b) is known to be useful for determining the oxidation state of manganese. The peak separation of electrons in the core level with unpaired electrons in the valence band level is caused by the electron exchange interaction upon photoelectron ejection.²⁷ The difference of the peak position (ΔE_b) varies among types of manganese oxides (MnO, Mn₃O₄, Mn₂O₃ and MnO₂). ΔE_b of the MnO₂ particles prepared in this study was 4.89 eV and this value was consistent with that in the literature (4.78 eV) (Table 2).^{27,28} Differences in the peak positions between the Mn 2p_{3/2} peak and the O 1s peak (the component with the lowest binding energy) were also consistent with that for MnO₂ in the literature. In addition, Mn/O atomic concentration ratio was estimated to be ~0.5 by quantitative analysis with XPS. Thus we conclude that the product was MnO₂ particles. The O 1s spectra could be deconvoluted into three bands (Fig. 7c). These bands were assigned to be Mn–O–Mn (529.9 eV), Mn–O–H (531.5 eV, surface hydroxide) and H–O–H (533.0 eV, water molecule) components, and were also consistent with the result in Chigane's report.²⁷

Figure 8a shows amperometric current response for CO detection at a MnO₂-modified Pt–Ru/Nafion electrode (Pt–Ru/Nafion/MnO₂ electrode). The detection current for CO was found to decrease after the MnO₂ modification. This should be due to a decrease of the mass-transfer rate of the dissolving CO at the electrode/electrolyte interface due to the modification. Beyond that, however, the current response to HP was suppressed almost completely (Table 1). MnO₂ is a well-known catalyst for the decomposition of HP into H₂O and O₂:

Since MnO₂ itself is an insulating material, loading too much of it should cause a small current response to CO due to a decrease in the mass-transfer rate. Thus, the amount of MnO₂ particles loaded is important as regards the selectivity and sensitivity of CO detection. The amount of MnO₂ in this study was optimized as the lowest loading, with almost no current observed for 1 μM HP. The current response to CO concentration was still linear in the range up to 9 μM with the slope of 173 nA cm^{–2} μM^{-1} (Fig. 8b). Finally, sensitive electrochemical detection of CO was achieved at a Pt–Ru/Nafion/MnO₂ electrode, at which current response for model interferents: UA, AA and HP was highly suppressed to below 1.7% at the same concentration of CO.

Fig. 8 (a) Amperometric current response to CO bubbling in saline at the Pt–Ru/Nafion/MnO₂ electrode. The arrow sign indicates the time when the bubbling started. (b) Calibration curve for amperometric CO detection at the Pt–Ru/Nafion/MnO₂ electrode.

Conclusions

A Pt–Ru/Nafion/MnO₂ electrode was fabricated for selective electrochemical detection of CO at physiological concentrations. By choosing the duration time for the electrodeposition of platinum on a ruthenium electrode surface, an electrode material for sensitive detection of CO was fabricated. In addition, modifying the Pt–Ru electrode surface with a Nafion layer

followed by loading a MnO₂ particle layer led to improvement of selectivity to CO detection against model interferents: UA, AA and HP. At the Pt-Ru/Nafion/MnO₂ electrode, the sensitivity of CO detection was 173 nA cm⁻² μM⁻¹ in the range up to 9 μM, and current response to UA and AA was reduced to 3.8 and 2.7% of that at the unmodified electrode, respectively, and that to HP was suppressed below noise level. Thus, the Pt-Ru/Nafion/MnO₂ electrode should be useful for quantitative and real-time analysis of CO *in vivo*, which should be of significant benefit to physiological and therapeutic studies of CO.

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