

CONTENTS

The 5th International Symposium on Environmental Economy and Technology (ISEET-2012)

Sensitive voltammetric and amperometric responses of respiratory toxins at hemin-adsorbed carbon-felt Yasushi Hasebe, Yue Wang	1055
Destruction of 4-phenolsulfonic acid in water by anodic contact glow discharge electrolysis Haiming Yang, Baigang An, Shaoyan Wang, Lixiang Li, Wenjie Jin, Lihua Li	1063
Nitrous oxide emissions from black soils with different pH Lianfeng Wang, Huachao Du, Zuoqiang Han, Xilin Zhang	1071
Coulometric determination of dissolved hydrogen with a multielectrolytic modified carbon felt electrode-based sensor Hiroaki Matsuura, Yosuke Yamawaki, Kosuke Sasaki, Shunichi Uchiyama	1077
Palladium-phosphinous acid complexes catalyzed Suzuki cross-coupling reaction of heteroaryl bromides with phenylboronic acid in water/alcoholic solvents Ben Li, Cuiping Wang, Guang Chen, Zhiqiang Zhang	1083

Aquatic environment

Organic matter produced by algae and cyanobacteria: Quantitative and qualitative characterization Maud Leloup, Rudy Nicolau, Virginie Pallier, Claude Yéprémian, Geneviève Feuillade-Cathalifaud	1089
Effects of environmental factors on sulfamethoxazole photodegradation under simulated sunlight irradiation: Kinetics and mechanism Junfeng Niu, Lilan Zhang, Yang Li, Jinbo Zhao, Sidan Lv, Keqing Xiao	1098
Irrigation system and land use effect on surface water quality in river, at lake Dianchi, Yunnan, China Takashi Tanaka, Takahiro Sato, Kazuo Watanabe, Ying Wang, Dan Yang, Hiromo Inoue, Kunzhi Li, Tatsuya Inamura	1107
Temporal and spatial changes in nutrients and chlorophyll- <i>a</i> in a shallow lake, Lake Chaohu, China: An 11-year investigation Libiao Yang, Kun Lei, Wei Meng, Guo Fu, Weijin Yan	1117
Phosphorus speciation in the sediment profile of Lake Erhai, southwestern China: Fractionation and ³¹ P NMR Runyu Zhang, Liying Wang, Fengchang Wu, Baoan Song	1124
Effect of ammonium on nitrous oxide emission during denitrification with different electron donors Guangxue Wu, Xiaofeng Zhai, Chengai Jiang, Yuntao Guan	1131
Adsorption of 2-mercaptobenzothiazole from aqueous solution by organo-bentonite Ping Jing, Meifang Hou, Ping Zhao, Xiaoyan Tang, Hongfu Wan	1139
Differences in rheological and fractal properties of conditioned and raw sewage sludge Hui Jin, Yili Wang, Ting Li, Yujing Dong, Junqing Li	1145
Competitive sorption between 17 α -ethinyl estradiol and bisphenol A/ 4- <i>n</i> -nonylphenol by soils Jianzhong Li, Lu Jiang, Xi Xiang, Shuang Xu, Rou Wen, Xiang Liu	1154
Determination of estrogens and estrogenic activities in water from three rivers in Tianjin, China Kaifeng Rao, Bingli Lei, Na Li, Mei Ma, Zijian Wang	1164

Terrestrial environment

Adsorption and desorption characteristics of diphenylarsenicals in two contrasting soils Anan Wang, Shixin Li, Ying Teng, Wuxin Liu, Longhua Wu, Haibo Zhang, Yujuan Huang, Yongming Luo, Peter Christie	1172
Evaluation of remediation process with soapberry derived saponin for removal of heavy metals from contaminated soils in Hai-Pu, Taiwan Jyoti Prakash Maity, Yuh Ming Huang, Cheng-Wei Fan, Chien-Cheng Chen, Chun-Yi Li, Chun-Mei Hsu, Young-Fo Chang, Ching-I Wu, Chen-Yen Chen, Jiin-Shuh Jean	1180

Environmental biology

Vertical diversity of sediment bacterial communities in two different trophic states of the eutrophic

Lake Taihu, China (**Cover story**)

Keqiang Shao, Guang Gao, Yongping Wang, Xiangming Tang, Boqiang Qin 1186

Abundance and diversity of ammonia-oxidizing archaea in response to various habitats

in Pearl River Delta of China, a subtropical maritime zone

Zhixin Li, Wenbiao Jin, Zhaoyun Liang, Yangyang Yue, Junhong Lv 1195

Environmental catalysis and materials

Effect of pretreatment on Pd/Al₂O₃ catalyst for catalytic oxidation of *o*-xylene at low temperature

Shaoyong Huang, Changbin Zhang, Hong He 1206

Efficient visible light photo-Fenton-like degradation of organic pollutants using *in situ* surface-modified

BiFeO₃ as a catalyst

Junjian An, Lihua Zhu, Yingying Zhang, Heqing Tang 1213

Basic properties of sintering dust from iron and steel plant and potassium recovery

Guang Zhan, Zhancheng Guo 1226

Degradation of direct azo dye by *Cucurbita pepo* free and immobilized peroxidase

Nabila Boucherit, Mahmoud Abouseoud, Lydia Adour 1235

Environmental analytical methods

Determination of paraquat in water samples using a sensitive fluorescent probe titration method

Feihu Yao, Hailong Liu, Guangquan Wang, Liming Du, Xiaofen Yin, Yunlong Fu 1245

Chemically modified silica gel with 1-f4-[(2-hydroxy-benzylidene)amino]phenylgethanone:

Synthesis, characterization and application as an efficient and reusable solid phase extractant
for selective removal of Zn(II) from mycorrhizal treated fly-ash samples

R. K. Sharma, Aditi Puri, Anil Kumar, Alok Adholeya 1252

Serial parameter: CN 11-2629/X*1989*m*207*en*P*25*2013-6



Sensitive voltammetric and amperometric responses of respiratory toxins at hemin-adsorbed carbon-felt

Yasushi Hasebe^{1,*}, Yue Wang²

1. Department of Life Science and Green Chemistry, Faculty of Engineering, Saitama Institute of Technology, Fukaya, Saitama 369-0293, Japan

2. School of Chemical Engineering, University of Science and Technology Liaoning, Anshan 114501, China

Received 24 January 2012; revised 07 March 2013; accepted 10 March 2013

Abstract

A hemin [iron-Fe(III) protoporphyrin IX chloride] was adsorbed onto a carbon-felt (CF), which is a microelectrode ensemble of micro carbon fiber (ca. 7 μm diameter). The resulting hemin-adsorbed-CF (hemin-CF) showed a well-defined redox wave based on the hemin-Fe(III)/Fe(II) redox process with the formal potential of -0.225 V vs. Ag/AgCl in deoxygenated phosphate/citrate buffer solution (0.1 mol/L, pH 5.0). The apparent heterogeneous electron transfer rate constant was estimated to be 8.6 sec^{-1} . In air-saturated electrolyte solution, the hemin-CF exhibited an excellent electrocatalytic activity for the reduction of dioxygen (O_2). This activity was reversibly inhibited by respiratory toxins such as cyanide and azide, which bind sixth coordination position of iron active center of hemin. The electrocatalytic O_2 reduction current at the hemin-CF was modulated by the toxins in a concentration-depending manner. Based on the relationship between the %inhibition and the toxin concentration, apparent inhibition constants of cyanide and azide were evaluated to be 4.52 and 1.98 $\mu\text{mol/L}$, respectively. When the hemin-CF was used as a working electrode unit of the CF-based electrochemical flow-through detector with air-saturated carrier, the injection of the azide induced peak-shape current responses, which allowed rapid and continuous flow-amperometric determination of azide with high sensitivity.

Key words: respiratory toxin; hemin; carbon-felt; cyanide; azide; voltammetry; amperometry

DOI: 10.1016/S1001-0742(12)60128-4

Introduction

Cyanide is commonly used in industrial applications (e.g., metal-plating, metal mining, plastic manufacture, extracting gold and silver from ores) and in agricultural sectors (e.g., fumigating fruit trees). Accidental release and leakage of cyanide in wastewater or rivers may lead rapidly serious contamination of ground water and drinking water. Because cyanide is extremely toxic to humans, in particular its role in the suppression of O_2 transport and respiration system, the presence of cyanide in drinking water causes serious human diseases. For example, uptake of cyanide results in the cellular hypoxia, with severe damage to the medullar respiratory center and vasomotor center, by inhibiting enzymes in the terminal respiratory chain. From this point the World Health Organization fixes the maximum acceptable level of cyanide in drinking water at 1.9 $\mu\text{mol/L}$.

On the other hand, sodium azide is a toxic chemical widely used in hospitals and laboratories as a chemical preservative, in agriculture for pest control, detonators, and

other explosives. In addition, sodium azide is the starting materials of a variety of medicines. Moreover, sodium azide is used daily in the air-bags of vehicles with large tonnage. The azide poisoning depends on amount, route, and length of time of exposure, as well as, the age and pre-existing medical conditions of the person exposed. It prevents the cells of the body from using O_2 , and is thus very harmful to organs, especially the heart and the brain, because the heart and the brain use a lot of O_2 .

Therefore, the quantitative determination of cyanide and azide is quite important. Several conventional methods such as titrimetric, colorimetric, and chromatographic methods have been established for the determination of these respiratory toxins (Miki et al., 2000; Tsuge et al., 2001). However, these methods usually need relatively expensive equipments, complicated sample treatments and time-consuming analytical procedures. In contrast, electrochemical methods are attractive tools for simple and highly sensitive determination of these toxins

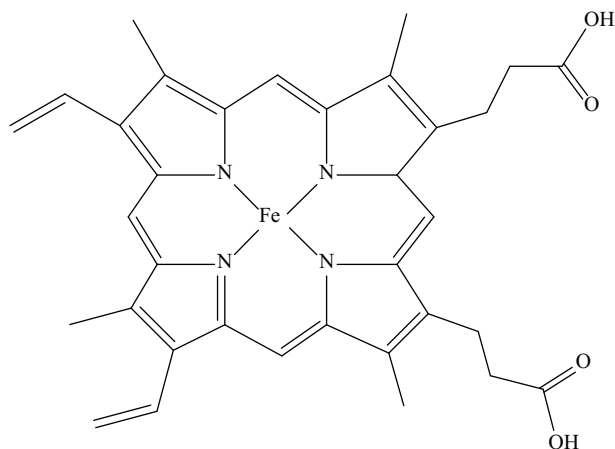
Cyanide and azide are known to bind to the active-metal center of heme-proteins (e.g., peroxidase, catalase, hemoglobin and myoglobin), and inhibit their ordinary

* Corresponding author. E-mail: hasebe@sit.ac.jp

functions and catalytic activities. Based on these features, various types of inhibition-based enzyme and/or protein-modified electrodes have been proposed for the electrochemical determination of cyanide and azide. For example, peroxidase (Smit and Cass, 1990; Tatsuma and Oyama, 1996; Wang and Hasebe, 2011) catalase (Sezgintürk et al., 2005; Singh et al., 2009) cytochrome oxidase (Amine et al., 1995) hemoglobin (Hasebe and Wang, 2012a; Hosono et al., 2012) and myoglobin (Hasebe and Wang, 2012b) have been successfully used as biocatalysts for the determination of these toxins. However, serious drawbacks of these biomolecules-based systems are lesser stabilities originating from the structural change of proteins during the operational and storage periods.

Hemin (**Scheme 1**) is a protoporphyrin IX Fe(III) complex, which is the active part of heme-proteins. The hemin is known to strongly adsorb onto the carbon materials such as pyrolytic graphite (Chen et al., 2000; Duca et al., 2010; Tao et al., 1995) and carbon nanotube (Ye et al., 2004) based on hydrophobic and π - π stacking interactions. The hemin-modified electrodes have been used in wide potential electrochemical applications as the electrocatalyst in addressing the electrochemical reductions of nitrite (Duca et al., 2010; Turdean et al., 2006), dioxygen (Antoniadou et al., 1989; Arifuku et al., 1992; Feng et al., 2012; Ye et al., 2004), hydrogen peroxide (Feng et al., 2012; Turdean et al., 2006), and superoxide anion (Chen et al., 2000), and the electrochemical oxidations of tryptophan (Nan et al., 2002) and L-tyrosine (Ma et al., 2010). Because of an excellent stability, small size and cost-efficiency, the hemin is attractive candidate as a stable electro-catalyst for the electrochemical sensing. However, the application of hemin-modified electrode for the detection of cyanide and azide based on its inhibitory event has not been reported so far, from our best knowledge.

A carbon-felt (CF) is a microelectrode ensemble of micro carbon fiber (ca. 7 mm diameter), and possesses a random three-dimensional structure. CF has high surface area (estimated to be 0.1–20 m²/g), which allows large



Scheme 1 Molecular structure of hemin.

measurable current density and high electric efficiency. Additionally, high porosity (> 90%) permits low diffusion barrier of solution flow. Therefore, CF is useful for a working electrode unit of electrochemical flow-through detector. As compared with other porous electrode materials (e.g., gold and platinum mesh and reticulated vitreous carbon), CF has many advantages (i.e., inexpensive; physically and mechanically stable; easily handled; and easily be manufactured to be arbitrary shapes). Physical and chemical properties of the CF used in this study are summarized in **Table 1**. Based on these features, recently we are engaging in the studies on the functionalization of the CF surface with catalytic molecules and have developed the CF-based electrochemical flow-sensing systems (Hasebe et al., 2011) and flow-biosensors (Hosono et al., 2012; Hasebe and Wang, 2012b; Wang and Hasebe, 2009, 2011, 2012).

In this study, the hemin was physically adsorbed onto the CF surface, and the electrochemical properties of the hemin-adsorbed CF (hemin-CF) were evaluated. The resulting hemin-CF exhibited an excellent electrocatalytic activity for the reduction of O₂, and this activity was reversibly inhibited by cyanide and azide, which enabled voltammetric and flow-amperometric determination of the respiratory toxins with high sensitivity. As compared with previous works with peroxidase (Wang and Hasebe, 2011), hemoglobin (Hasebe and Wang, 2012a; Hosono et al., 2012) and myoglobin (Hasebe and Wang, 2012b), the present hemin-CF-based sensor showed excellent stabilities. This would be a first report on the application of hemin-modified porous electrode for voltammetric and flow-amperometric sensing of respiratory toxins.

Table 1 Physical and chemical properties of the CF used in this study

Property	Value
Thickness (mm)	3
Carbon fiber diameter (μm)	7
Treatment temperature ($^{\circ}\text{C}$)	2000
Standard weight (g/m^2)	400
Density (g/cm^3)	0.13
Carbon content (%)	99
Specific surface area (N ₂) by BET method (m^2/g)	0.29
Electric resistivity ($\Omega \text{ cm}$)	0.5

1 Experimental

1.1 Reagents and materials

Hemin [iron(III)protoporphyrin IX chloride] was purchased from Tokyo Kasei Co., Japan and used as received. Sodium cyanide (NaCN) and sodium azide (NaN₃) were obtained from Wako Pure Chemicals Industry, Japan. All other chemicals were of the highest grade available, and were used without further purifications. Millipore Milli-Q water (resistivity > 18 M Ω cm, TOC, 3 ppb) was used

for preparation of all solutions throughout the experiments. Phosphate/citrate buffer (0.1 mol/L, pH 5.0) prepared by K_2HPO_4 (Wako) and citric acid monohydrate (Wako) was used as an electrolyte and carrier solutions. A carbon-felt (CF) sheet was obtained from Nippon Carbon Ltd., Japan. Hemin-dissolving solution was prepared just before the adsorption experiment. Standard solutions of cyanide and azide were prepared just before each experiment daily by dissolving NaCN and NaN_3 in 0.1 mol/L phosphate/citrate buffer (pH 5.0). All the experiments were performed at ambient temperature ($22 \pm 2^\circ C$).

1.2 Preparation of hemin-adsorbed CF (hemin-CF)

The CF sheet was cut into $10 \text{ mm} \times 3 \text{ mm} \times 3 \text{ mm}$ in size (weight, ca. 12 mg), and washed with pure water under ultra-sonication for 10 min (40 kHz, AS ONE, US-1R, Japan). The hemin was adsorbed onto the CF surface by immersing bare-CF into hemin-dissolving 0.1 mol/L NaOH solution (0.3 mmol/L, 2 mL) for 5 min at ($22 \pm 2^\circ C$). To remove weakly adsorbed species, the hemin-adsorbed CF (roughly rinsed with Milli-Q water) was placed on the flow-cell and 0.1 mol/L phosphate/citrate buffer (pH 5.0) was constantly flowed at 2.0 mL/min for 1000 sec with a double-plunger intelligent pump (Al-12, FLOM Co., Japan). It has been reported that hemin adsorbs on graphite surface from alkaline aqueous solution, and a monolayer of hemin has formed within 5 min and during further adsorption time, aggregate begins to form on the monolayer (Tao et al., 1995). In this study, to avoid the negative effect of the covered aggregate, we selected adsorption time of 5 min. The resulting hemin-adsorbed CF is denoted here as hemin-CF. The hemin-CF was stored in air-saturated phosphate/citrate buffer (0.1 mol/L, pH 5.0) in refrigerator at $4^\circ C$ when not in use.

1.3 Electrochemical measurements

Cyclic voltammetry (CV) was carried out to evaluate electron transfer characteristics and electrocatalytic activity of the hemin-CF. The CV measurements were performed with ALS 611B electrochemical analyzer (BSA Co. Ltd., USA). An one-compartment electrochemical glass cell with an electrolyte volume of 15 mL was used. The hemin-CF (or bare-CF) with platinum lead wire (0.5 mm diameter, 6 mm length) was used as a working electrode. A platinum wire (1 mm diameter, 50 mm length) and Ag/AgCl (BAS, RE-1B, 3 mol/L NaCl) electrode were used as the counter and the reference electrode, respectively. All the potentials reported in this work were versus Ag/AgCl. Deoxygenated, air-saturated and O_2 -saturated 0.1 mol/L phosphate/citrate buffers (pH 5.0) were used as electrolyte solutions. Deoxygenated buffer was prepared by purging high-purity nitrogen gas into the buffer at least for 30 min, and the nitrogen atmosphere was kept over the solutions during the CV measurement to prevent O_2 from reaching the solution. O_2 -saturated buffer was prepared by purging

pure O_2 gas into the buffer for 20 min.

1.4 Flow-amperometry

Flow amperometry was carried out by using the electrochemical flow-injection analysis system previously reported by us (Hasebe et al., 2011; Hosono et al., 2012; Wang and Hasebe, 2009, 2011, 2012). The system is composed of a double-plunger intelligent pump (Al-12, FLOM Co., Japan) with a six way injection valve (V17, 200 μL injection loop, FLOM Co., Japan) and the hemin-CF-based electrochemical flow-through detector connected to the electrochemical analyzer (ALS 611B, USA). All flow-amperometric measurements were carried out at room temperature. Air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0) was used as a carrier solution. Prior to the measurements, the carrier solution was flowed at 2.0 mL/min for 1000 sec under the applied potential of -0.05 V vs. Ag/AgCl to remove weakly adsorbed hemin from the CF surface and to obtain stable background currents. After the background current, originated from the electrocatalytic reduction of O_2 , had reached to the steady-state value (ca. 35–39 μA), the standard solutions of toxins (i.e., cyanide and azide) were injected through disposable syringe filter unit (0.45 μm pore size, Advantec, Dismic 3cp, Japan) at regular time intervals (100 or 200 sec), and the peak-shape current changes, based on the inhibitory effect upon the electrocatalytic activity of hemin-CF were recorded.

2 Results and discussion

2.1 Electrochemical behavior of hemin-CF

Figure 1a and **b** shows the results of CVs of hemin-CF in deoxygenated 0.1 mol/L phosphate/citrate buffer (pH 5.0) at different scan rates. At the potential scan rate of 5 mV/sec, the hemin-CF showed a pair of well-defined, almost reversible CV peaks located at -0.226 V and -0.223 V vs. Ag/AgCl. In contrast, bare-CF showed no apparent redox peaks at the same potential region (data not shown). The midpoint potential ($E_{1/2}$) obtained from CV, $E_{1/2} = (E_{pa} + E_{pc})/2$, is -0.225 V vs. Ag/AgCl at pH 5.0, where, (E_{pa} (V) is anodic peak potential and E_{pc} (V) is cathodic peak potential). The peak separation (ΔE) between the anodic and cathodic peaks is 3 mV at the scan rate of 5 mV/sec. This redox wave of hemin-CF is assigned to the Fe(III)/Fe(II) redox process of hemin (Ma et al., 2010; Ye et al., 2004). In the scan rate range of 5 to 900 mV/sec (**Fig. 1a, b**), the redox peak currents are proportional to the scan rates (**Fig. 1c**). Thus, the electrochemical reactions of the hemin-CF are surface-controlled quasi-reversible process. As shown in **Fig. 1d**, the slope of the plot of $\log(\text{anodic peak current})$ versus $\log(\text{scan rate})$ is 0.987 with a correlation coefficient of 0.9994, which is close to the theoretical slope of 1 for thin layer voltammetry (Murray, 1984).

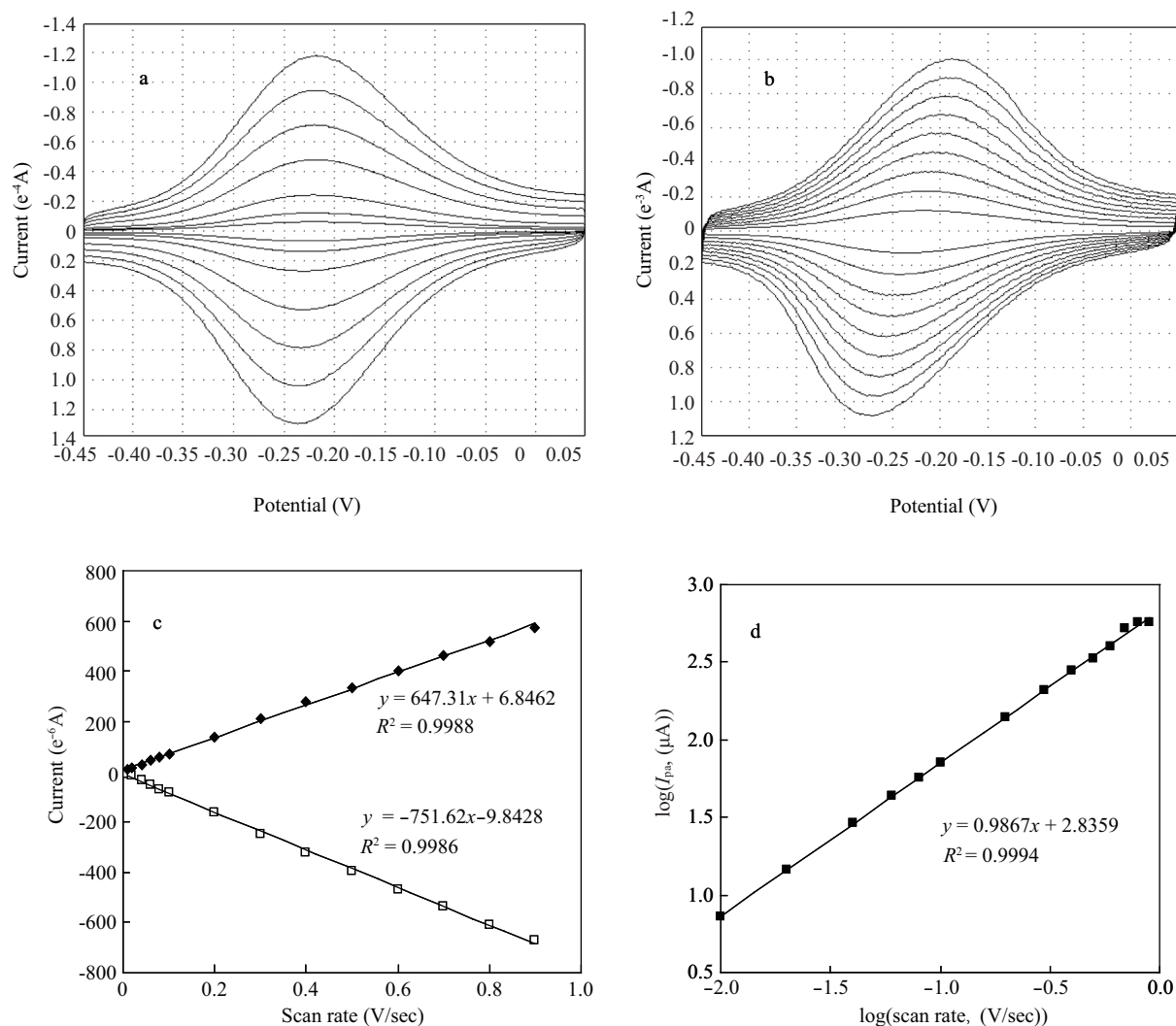


Fig. 1 (a) Cyclic voltammograms (CVs) of hemin-CF in deoxygenated 0.1 mol/L phosphate/citrate buffer (pH 5.0) at different potential scan rates, from inner to outer: 5, 10, 20, 40, 60, 80 and 100 mV/sec. Starting potential was 0.05 V vs. Ag/AgCl. (b) CVs of hemin-CF in deoxygenated 0.1 mol/L phosphate/citrate buffer (pH 5.0) at different potential scan rates, from inner to outer: 100, 200, 300, 400, 500, 600, 700, 800 and 900 mV/sec. Other CV measurement conditions are same in panel a. (c) Plot of anodic and cathodic peak currents vs. potential scan rates. (d) Plot of logarithm of anodic peak current vs. $\log(\text{scan rate})$.

Based on the assumption that all hemin molecules adsorbed on the CF are electrochemically active, the surface coverage (Γ) of the hemin on the CF surface was estimated from integration of the reduction peak in the CV (at 5 mV/sec), according to $\Gamma = Q/nFA$ (where Q is the charge (110.2 μ C), n is the number of electron transferred (in this case, 1), F is faraday constant and A is the electrode surface area (37.7 cm^2)). The estimated Γ of the adsorbed hemin on the CF was 3.03×10^{-11} mol/ cm^2 . This value is about 2.3 times smaller than hemin monolayer (7×10^{-11} mol/ cm^2) assuming that the area of hemin molecule, horizontally oriented to the electrode surface, is about 2.38 nm^2 (Kolpin and Swofford, 1978). Thus the adsorbed hemin on the CF surface is sub-monolayer. The surface coverage of the hemin on the CF (3.03×10^{-11} mol/ cm^2) is much larger than those of adsorbed hemoglobin (2.27×10^{-12} mol/ cm^2 , Hasebe and Wang, 2012a) and adsorbed

myoglobin on the CF (3.04×10^{-12} mol/ cm^2 , Hasebe and Wang, 2012b). This is reasonable because the size of hemin is much smaller than hemoglobin and myoglobin and lacks bulky polypeptide backbones.

The electron transfer rate constant (k_s) between the adsorbed hemin and the CF can be estimated by the method of Laviron (1979). When $n\Delta E_p < 200$ mV, the electron transfer rate constant k_s of heme-protein on the modified electrode can be obtained by the following equation (Eq. (1)) (Laviron, 1979),

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log(RT/nFv) - [\alpha(1-\alpha)nF\Delta E_p]/2.3RT \quad (1)$$

where, v (V/sec) is scan rate. Taking a charge transfer coefficient α of 0.5, and a scan rate of 2 V/sec, $\Delta E = 155$ mV, and then k_s was calculated to be 8.6 sec^{-1} . This value is slightly larger than those for hemin-adsorbed multi-

walled carbon nanotube (2.9 sec^{-1}) (Ye et al., 2004) and for hemin/poly(amidoamine)/multi-walled carbon nanotube-modified glassy carbon electrode (3.8 sec^{-1}) (Ma et al., 2010) and slightly smaller than those for hemin covalently bound Fe_3O_4 microparticle-modified glassy carbon electrode (14.5 sec^{-1}) (Feng et al., 2012) and hemin-adsorbed pyrolytic graphite electrode (15 sec^{-1}) (Chen et al., 2010).

These voltammetric responses of the hemin-CF were quite stable, and in deoxygenated 0.1 mol/L phosphate/citrate buffer (pH 5.0) with a scan rate of 50 mV/sec, the potential and peak currents remained almost unchanged after 600 CV cycles (between +0.05 and -0.45 V vs. Ag/AgCl). Furthermore, even after 21 days storage in air-saturated phosphate/citrate buffer (0.1 mol/L, pH 5.0) at 4°C , the hemin-CF showed same redox waves. The insolubility of hemin under neutral and acidic conditions (Ye et al., 2004), and the strong hydrophobic interaction and π - π interaction between hemin and the basal plane of the graphite (Tao et al., 1995) would contribute the excellent stability of the hemin-CF.

2.2 Electrocatalytic activity of hemin-CF for the reduction of O_2 and its inhibition by respiratory toxins.

Figure 2A shows CVs of hemin-CF and bare-CF in air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0). As compared with bare-CF, the hemin-CF showed much larger cathodic current responses at the potential region from +0.1 to -0.1 V with a peak at around -0.05 V vs. Ag/AgCl. When same experiment was carried out in O_2 -saturated buffer, the reduction current was significantly enlarged (data not shown). These results clearly indicate that the adsorbed hemin on the CF exhibited sufficient electrocatalytic activity for the reduction of O_2 .

The mechanism of electrocatalytic reduction of O_2 by hemin has been extensively studied in previous works

(Antoniadou et al., 1989; Arifuku et al., 1992). The O_2 molecule is reduced to water via one-step reduction accompanying four-electron and four proton transfer at pH < 11, according to the following Eqs. (2)–(3).

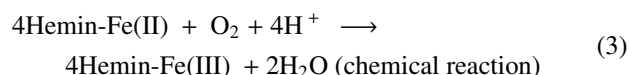
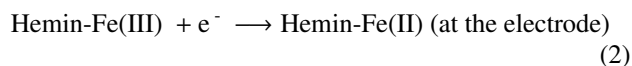


Figure 2B shows the change of the catalytic currents of the hemin-CF in air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0) in the presence of various concentration of cyanide (NaCN). The electrocatalytic cathodic current for the reduction of O_2 decreased in the presence of cyanide in a concentration-depending manner. These results imply that cyanide-binding to the iron-active center of hemin (sixth coordination position) prohibits the reaction of O_2 with the ferrous-hemin, resulting in the change in the CV response. Essentially similar inhibitory effect was observed for azide (data not shown). Here, it should be emphasized that clear inhibitory effect was observed even in the concentration of 0.1 $\mu\text{mol/L}$ toxins. These results suggest the potential ability of the hemin-CF for highly sensitive voltammetric determination of the respiratory toxins.

Figure 3 shows the relationship between the percent inhibition (%In) and total cyanide (panel a) and azide (panel b) concentrations. The %In was calculated from the following Eq. (4) for various cyanide and azide concentrations

$$\% \text{In} = [(I_0 - I_{\text{in}})/I_0] \times 100 \quad (4)$$

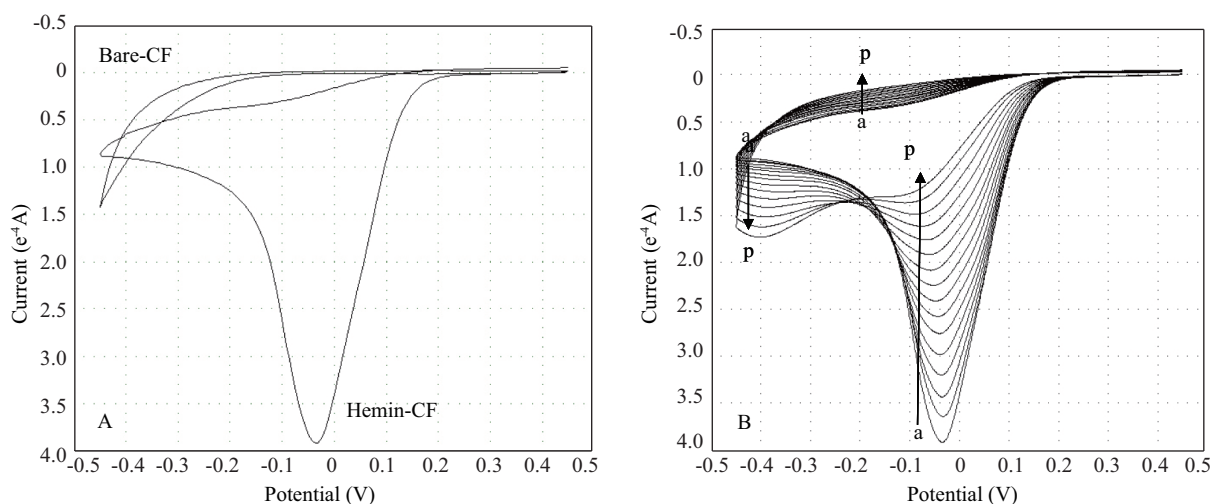


Fig. 2 (A) CVs of hemin-CF and bare-CF in air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0). Starting potential was +0.45 V vs. Ag/AgCl. Potential scan rate was 5 mV/sec. (B) CVs of hemin-CF in air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0) in the absence of and in the presence of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4 and 1.5 $\mu\text{mol/L}$ (lines a–p) cyanide (NaCN). The CV measurement conditions are same in panel (A).

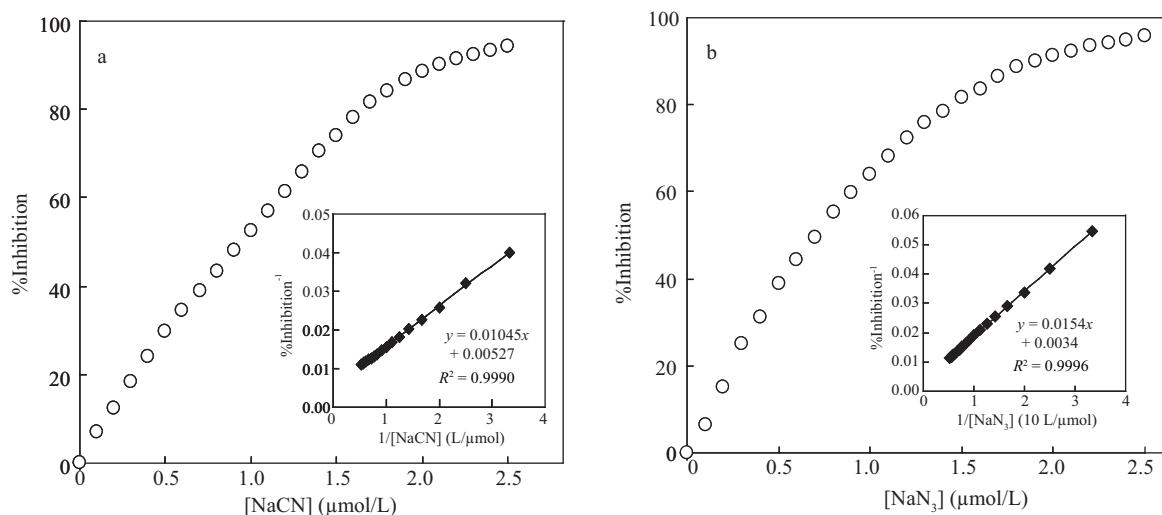


Fig. 3 (a) Relationship between the %Inhibition and the total concentration of cyanide. Inset: Hughes-Klotz plot for the estimation of apparent inhibition constant (K_i'). The experimental conditions are same as **Fig. 2**. (b) The relationship between the % inhibition and the total concentration of azide. Inset: Hughes-Klotz plot. The experimental conditions are same as panel (a).

where, I_0 is a current value at -0.035 V without toxins, I_{in} is a current value in the presence of toxins. The apparent inhibition constant (K_i') was evaluated using Hughes-Klotz equation (Eq. (5)) (Chang, 1990).

$$1/\%In = 1/\%In_{max} + K_i' / (\%In_{max} \cdot [\text{inhibitor}]) \quad (5)$$

Inset graphs in **Fig. 3a** and **b** are the Hughes-Klotz plots for the estimation of K_i' values. The K_i' values for cyanide and azide were calculated to be 4.52 and 1.98 $\mu\text{mol/L}$, respectively. These values are smaller than those of adsorbed myoglobin (8.71 $\mu\text{mol/L}$ for cyanide and 5.57 $\mu\text{mol/L}$ for azide; Hasebe and Wang, 2012b) and adsorbed hemoglobin on CF (5.45 $\mu\text{mol/L}$ for azide; Hasebe and Wang, 2012a).

In order to evaluate the intra-day precision (between-lot variation) of hemin-CF, three hemin-CFs were prepared in the same manner and in the same day, and the voltammetric response of hemin-Fe(III)/Fe(II) in deoxygenated buffer, electrocatalytic current in air-saturated buffer were measured. The relative standard deviation values ($n = 3$) for Fe(III)/Fe(II) redox peaks and electrocatalytic currents obtained by different hemin-CFs were 1.5% and 3.6%, respectively, indicating that the present physical adsorption protocol of the hemin-CF has acceptable reliability. Thus, judging from the obtained results, it can be safe to conclude that the hemin-CF is useful electrocatalytic-sensing element for highly sensitive voltammetric determination of cyanide and azide.

2.3 Flow-amperometry of respiratory toxins using hemin-CF-based electrochemical flow-through detector

From practical viewpoint, as compared to chemical sensors with batch-type mode, sensors with flow-injection mode (i.e., FIA-based sensor) have many advantages; (1) poten-

tial applicability for on-line analysis (automated system); (2) possible high sample through-put; (3) the effect of sample dilution is almost negligible; and (4) the detectable concentration range and sensitivity can be modulated by changing the sample injection volume and carrier flow rate.

If the binding of cyanide and azide to active iron center of hemin is reversible, continuous monitoring of these respiratory toxins would be possible with hemin-CF-based electrochemical flow-through-detector. In this experiment, we selected the applied potential of -0.05 V vs. Ag/AgCl to minimize the direct electro-reduction of O_2 at the CF surface (**Fig. 2A**). When air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0) was used as a carrier, the cathodic base-current was ca. 35–39 μA , which is originated from the hemin-CF-catalyzed electro-reduction of dissolved O_2 in carrier and much higher than that with bare-CF (ca. 0.8–1.1 μA).

Figure 4a shows the typical inhibition peak current (I_p^{in}) responses of cyanide (10 $\mu\text{mol/L}$) by hemin-CF-based electrochemical flow-through detector. Although the inhibition peak current was observed, the recovery was not complete (ca. 70% at 124 sec after the peak top), and unfortunately, the repetitive injections of same concentrations of cyanide samples resulted in gradual decrease in peak height of the inhibition peaks (data not shown). This would be because of the strong binding affinity of cyanide toward iron center of hemin.

Because cyanide dissociation is assisted by protonation of bound CN^- , the cyanide dissociation process tends to be faster at acidic pH (Bellelli et al., 1990). In addition, the reduction of heme-Fe(III)-cyanide complex by dithionite and $Na_2S_2O_4$ facilitates the cyanide dissociation (Bellelli et al 1990; Milani et al 2004). Therefore, the use of acidic carrier or dithionite and/or $Na_2S_2O_4$ -containing carrier may be useful for fast recovery of baseline of the present hemin-CF-based system.

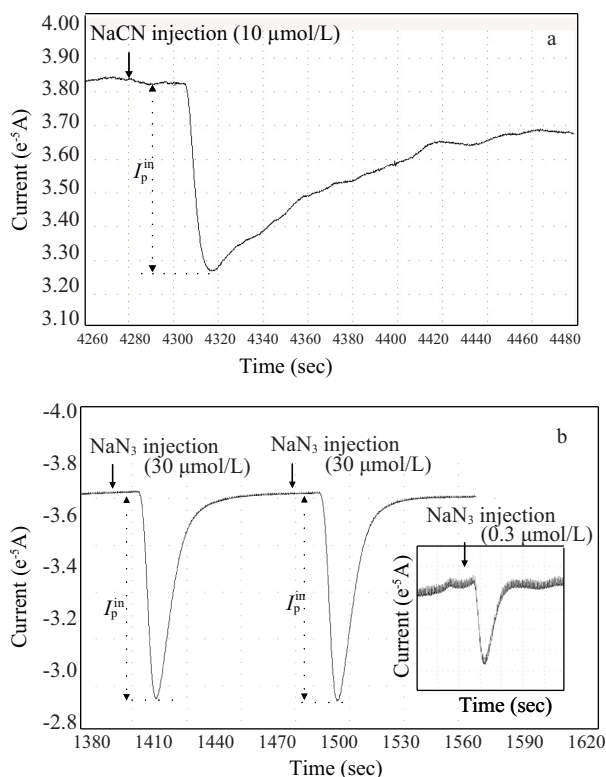


Fig. 4 (a) Typical inhibition peak current (I_p^{in}) response toward 10 $\mu\text{mol/L}$ cyanide injection obtained by the hemin-CF-based electrochemical flow-through detector. Sample injection volume is 200 μL . Applied potential is 0.05 V vs. Ag/AgCl. Air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0) was used as a carrier at flow rate of 2.0 mL/min. (b) Typical inhibition peak current (I_p^{in}) responses toward 30 $\mu\text{mol/L}$ azide injection. Inset: Peak current response toward 0.3 $\mu\text{mol/L}$ azide. Experimental conditions are same as panel (a).

In contrast, as illustrated in **Fig. 4b**, the repetitive injections of azide (30 $\mu\text{mol/L}$) resulted in quite reproducible peak-shape current responses with almost 100% recovery in spite of higher concentration (30 $\mu\text{mol/L}$), suggesting that the binding of azide to the iron-active center of the adsorbed hemin is almost completely reversible. As shown in inset graph of **Fig. 4b**, the hemin-CF-based detector showed apparent response to 0.3 $\mu\text{mol/L}$ azide, indicating the possibility of highly sensitive flow-determination of azide.

Under the present experimental conditions, the time from the injection to azide peak was ca. 23 sec and the duration time of the azide-inhibition peak was 35 sec for 0.3 $\mu\text{mol/L}$ azide (96% recovery) and 5 sec for 30 $\mu\text{mol/L}$ azide (96% recovery), respectively. Therefore, the sample throughput for continuous detection of azide is ca. 70–100 samples/hr, which is superior to our recent work using myoglobin-adsorbed CF (ca. 45–60 samples/hr, Hasebe and Wang, 2012b). The relative standard deviation of inhibition peak current (I_p^{in}) to 0.3 $\mu\text{mol/L}$ azide was 2.56% ($n = 7$). These results indicate the potential usability of the hemin-CF as an inhibition-based flow-amperometric sensor for continuous determination of azide.

Operational stability is an important parameter of flow-

analysis, especially on-line analysis application. During the repetitive injections of standard samples of 0.3 to 30 $\mu\text{mol/L}$ azide (up to 3 hrs), no serious decrease in the steady-state catalytic current were observed, suggesting that the electrocatalytic activity of the adsorbed hemin did not change during the repetitive measurements. Storage stability was finally checked. When not in use, the hemin-CF was stored in air-saturated 0.1 mol/L phosphate/citrate buffer (pH 5.0) at 4°C. Even after 21 days storage, the hemin-CF showed the steady-state catalytic background current (94% of the initial day), and the I_p^{in} to 1 $\mu\text{mol/L}$ azide was almost no change. These excellent stabilities of the hemin-CF-based system are notable advantage which overcomes biomolecules-based systems.

3 Conclusions

The hemin-adsorbed CF exhibited stable and excellent electrocatalytic activity for the O_2 reduction. This electrocatalytic activity was inhibited by the respiratory toxins such as cyanide and azide, and voltammetric determination of these toxins was possible with high sensitivity. Because the binding of azide to the iron-active center of hemin was almost completely reversible, quite reproducible inhibition peak currents were obtained when the hemin-CF was adopted in electrochemical flow-detector. Evaluation of more-detailed analytical performance characteristics of the hemin-CF-based flow-sensing system is now underway in our laboratory.

Acknowledgments

This work was financially supported in part by the NEXT-supported Program for the Strategic Research Foundation at Private Universities, 2011–2015.

References

- Amine A, Alafandy M, Kauffmann J M, Pekli M N, 1995. Cyanide determination using an amperometric biosensor based on cytochrome oxidase inhibition. *Analytical Chemistry*, 67(17): 2822–2827.
- Antoniadou S, Jannakoudakis A D, Theodoridou E, 1989. Electrocatalytic reactions on carbon fibre electrodes modified by hemine I. Electroreduction of oxygen. *Synthetic Metals*, 30(3): 283–294.
- Arifuku F, Mori K, Muratani T, Kurihara H, 1992. The catalytic electroreduction of dioxygen on iron protoporphyrin IX modified glassy carbon electrodes. *Bulletin of the Chemical Society of Japan*, 65(6): 1491–1495.
- Bellelli A, Antonini G, Bruntori M, Springer B A, Silgar S G, 1990. Transient spectroscopy of the reaction of cyanide with ferrous myoglobin. Effect of distal side residues. *Journal of Biological Chemistry*, 265(31) 18898–18901.
- Chang R, 1990. *Physical Chemistry with Applications to Biological Systems* (2nd ed.). MacMillan Publishing, New York. 248–254.
- Chen J, Wollenberger U, Lisdat F, Ge B, Scheller F W, 2000.

- Superoxide sensor based on hemin modified electrode. *Sensors and Actuators B: Chemical*, 70(1-3): 115–120.
- Duca M, Khamseh S, Lai S C S, Koper M T M, 2010. The influence of solution-phase HNO_2 decomposition on the electrocatalytic nitrite reduction at a hemin-pyrolitic graphite electrode. *Langmuir*, 26(14): 12418–12424.
- Feng J J, Li Z H, Li Y F, Wang A J, Zhang P P, 2012. Electrochemical determination of dioxygen and hydrogen peroxide using $\text{Fe}_3\text{O}_4@/\text{SiO}_2@/\text{hemin}$ microparticles. *Microchimica Acta*, 176(1-2): 176–201.
- Hasebe Y, Wang Y, 2012a. Bioelectrocatalytic reduction of oxygen by hemoglobin-adsorbed carbon-felt, and its inhibition by azide. *Electrochemistry*, 80(5): 358–362.
- Hasebe Y, Wang Y, 2012b. Flow-amperometric biosensor for respiratory toxins using myoglobin-adsorbed carbon-felt, based on an inhibitory effect on bioelectrocatalytic reduction of oxygen. *Electrochimica Acta*, 82: 26–34.
- Hasebe Y, Wang Y, Fukuoka K, 2011. Electropolymerized poly(Toluidine blue)-modified carbon felt for highly sensitive amperometric determination of NADH in flow injection analysis. *Journal of Environmental Sciences*, 23(6): 1050–1056.
- Hosono T, Wang Y, Hasebe Y, 2012. FIA with electrochemical detection of azide using hemoglobin-adsorbed carbon-felt based on an inhibitory effect of bioelectrocatalytic reduction of oxygen. *Bunseki Kagaku*, 61(8): 691–698.
- Kolpin C F, Swofford H S Jr, 1978. Adsorption preconcentration for the direct analytical determination of heme. *Analytical Chemistry*, 50(7): 916–920.
- Laviron E, 1979. General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 101(1): 19–28.
- Ma Q, Ai S Y, Yin H S, Chen Q P, Tang T T, 2010. Towards the conception of an amperometric sensor of L-tyrosine based on hemin/PAMAM/MWCNT modified glassy carbon electrode. *Electrochimica Acta*, 55(22): 6687–6694.
- Miki A, Nishikawa M, Tsuchihashi H, 2000. Simultaneous GC-MS determination of azide, cyanide and thiocyanate via phase-transfer-catalyzed pentafluorobenzoylation. *Journal of Health Science*, 46(2): 81–88.
- Milani M, Ouellet Y, Oullet H, Guertin M, Boffi A, Antonini G et al., 2004. Cyanide binding to truncated hemoglobins: A crystallographic and kinetic study. *Biochemistry*, 43(18): 5213–5221.
- Murray R W, 1984. Chemically modified electrodes. In: *Electroanalytical Chemistry* (Vol. 13) (Bard A J ed.). Marcel Dekker, New York. 191–368.
- Nan C G, Feng Z Z, Li W X, Ping D J, Qin C H, 2002. Electrochemical behavior of tryptophan and its derivatives at a glassy carbon electrode modified with hemin. *Analytica Chimica Acta*, 452(2): 245–254.
- Sezgintürk M K, Göktu T, Dinçkaya E, 2005. A biosensor based on catalase for determination of highly toxic chemical azide in fruit juices. *Biosensors and Bioelectronics*, 21(4): 684–688.
- Singh R P, Kang D Y, Oh B K, Choi J W, 2009. Polyaniline based catalase biosensor for the detection of hydrogen peroxide and azide. *Biotechnology and Bioprocess Engineering*, 14(4): 443–449.
- Smit M H, Cass A E G, 1990. Cyanide detection using a substrate-regenerating peroxidase-based biosensor. *Analytical Chemistry*, 62(22): 2429–2436.
- Tao N J, Cardenas G, Cunha F, Shi Z, 1995. *In situ* STM and AFM study of protoporphyrin and iron(III) and zinc(II) protoporphyrins adsorbed on graphite in aqueous solutions. *Langmuir*, 11(11): 4445–4448.
- Tatsuma T, Oyama N, 1996. H_2O_2 -generating peroxidase electrodes as reagentless cyanide sensors. *Analytical Chemistry*, 68(9): 1612–1615.
- Tsuge K, Kataoka M, Seto Y, 2001. Rapid determination of cyanide and azide in beverages by microdiffusion spectrophotometric method. *Journal of Analytical Toxicology*, 25(4): 228–236.
- Turdean G L, Papescu I C, Curulli A, Paleschi G, 2006. Iron(III)protoporphyrin IX-single-wall carbon nanotubes modified electrodes for hydrogen peroxide and nitrite detection. *Electrochimica Acta*, 51(28) 6435–6441.
- Wang Y, Hasebe Y, 2009. Carbon felt-based biocatalytic enzymatic flow-through detectors: Chemical modification of tyrosinase onto amino-functionalized carbon felt using various coupling reagents. *Talanta*, 79(4): 1135–1141.
- Wang Y, Hasebe Y, 2011. Amperometric flow-biosensor for cyanide based on an inhibitory effect upon bioelectrocatalytic reduction of oxygen by peroxidase-modified carbon-felt. *Electroanalysis*, 23(7): 1631–1637.
- Wang Y, Hasebe Y, 2012. Glucose oxidase-modified carbon-felt-reactor coupled with peroxidase-modified carbon-felt-detector for amperometric flow determination of glucose. *Materials Science and Engineering: C*, 32(3): 432–439.
- Ye J S, Wen Y, Zhang W D, Cui H F, Gan L M, Xu G Q, 2004. Application of multi-walled carbon nanotubes functionalized with hemin for oxygen detection in neutral solution. *Journal of Electroanalytical Chemistry*, 562(2): 241–246.

JOURNAL OF ENVIRONMENTAL SCIENCES

环境科学学报(英文版)
(<http://www.jesc.ac.cn>)

Aims and scope

Journal of Environmental Sciences is an international academic journal supervised by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The journal publishes original, peer-reviewed innovative research and valuable findings in environmental sciences. The types of articles published are research article, critical review, rapid communications, and special issues.

The scope of the journal embraces the treatment processes for natural groundwater, municipal, agricultural and industrial water and wastewaters; physical and chemical methods for limitation of pollutants emission into the atmospheric environment; chemical and biological and phytoremediation of contaminated soil; fate and transport of pollutants in environments; toxicological effects of terrorist chemical release on the natural environment and human health; development of environmental catalysts and materials.

For subscription to electronic edition

Elsevier is responsible for subscription of the journal. Please subscribe to the journal via <http://www.elsevier.com/locate/jes>.

For subscription to print edition

China: Please contact the customer service, Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China. Tel: +86-10-64017032; E-mail: journal@mail.sciencep.com, or the local post office throughout China (domestic postcode: 2-580).

Outside China: Please order the journal from the Elsevier Customer Service Department at the Regional Sales Office nearest you.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The submission should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Submission declaration

Submission of the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The publication should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Editorial

Authors should submit manuscript online at <http://www.jesc.ac.cn>. In case of queries, please contact editorial office, Tel: +86-10-62920553, E-mail: jesc@263.net, jesc@rcees.ac.cn. Instruction to authors is available at <http://www.jesc.ac.cn>.

Journal of Environmental Sciences (Established in 1989)

Vol. 25 No. 6 2013

Supervised by	Chinese Academy of Sciences	Published by	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences	Distributed by	Elsevier Limited, The Netherlands
Edited by	Editorial Office of Journal of Environmental Sciences P. O. Box 2871, Beijing 100085, China Tel: 86-10-62920553; http://www.jesc.ac.cn E-mail: jesc@263.net , jesc@rcees.ac.cn	Domestic	Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China Local Post Offices through China
Editor-in-chief	Hongxiao Tang	Foreign	Elsevier Limited http://www.elsevier.com/locate/jes
CN 11-2629/X	Domestic postcode: 2-580	Printed by	Beijing Beilin Printing House, 100083, China
		Domestic price per issue	RMB ¥ 110.00

ISSN 1001-0742

