

Article

Optimization of Hydrogen Peroxide Detection for a Methyl Mercaptan Biosensor

Zhan-Hong Li ^{1,2}, Houssemeddine Guedri ², Bruno Viguiet ², Shi-Gang Sun ¹ and Jean-Louis Marty ^{2,*}

¹ State Key Laboratory of Physical Chemistry of Solid Surfaces, and Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China; E-Mails: lzh1508@gmail.com (Z.-H.L.); sgsun@xmu.edu.cn (S.-G.S.)

² Laboratoire IMAGES EA 4218, Groupe Biocapteurs, Université de Perpignan Via Domitia (UPVD), Bâtiment S, 52 av Paul Alduy 66860 Perpignan Cedex, France; E-Mails: houssemeddine.guedri@live.fr (H.G.); viguietbr@gmail.com (B.V.)

* Author to whom correspondence should be addressed; E-Mail: jlmarty@univ-perp.fr; Tel.: +33-4-6866-2254; Fax: +33-4-6866-2223.

Received: 11 March 2013; in revised form: 3 April 2013 / Accepted: 7 April 2013 /

Published: 15 April 2013

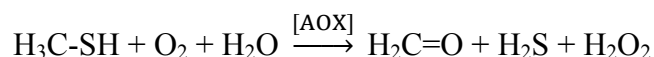
Abstract: Several kinds of modified carbon screen printed electrodes (CSPEs) for amperometric detection of hydrogen peroxide (H_2O_2) are presented in order to propose a methyl mercaptan (MM) biosensor. Unmodified, carbon nanotubes (CNTs), cobalt phthalocyanine (CoPC), Prussian blue (PB), and Os-wired HRP modified CSPE sensors were fabricated and tested to detect H_2O_2 , applying a potential of +0.6 V, +0.6 V, +0.4 V, −0.2 V and −0.1 V (*versus* Ag/AgCl), respectively. The limits of detection of these electrodes for H_2O_2 were 3.1 μ M, 1.3 μ M, 71 nM, 1.3 μ M, 13.7 nM, respectively. The results demonstrated that the Os-wired HRP modified CSPEs gives the lowest limit of detection (LOD) for H_2O_2 at a working potential as low as −0.1 V. Os-wired HRP is the optimum choice for establishment of a MM biosensor and gives a detection limit of 0.5 μ M.

Keywords: methyl mercaptan; hydrogen peroxide; amperometric sensor; screen printed electrode

1. Introduction

Methyl mercaptan (MM) is one of the volatile sulfur compounds (VSCs), which are known to be involved in halitosis (bad breath) [1,2] and periodontal diseases [2], and the predominant causative factor of noticeable oral malodor [3]. MM is also present in several other cases such as the bottle storage of wines [4]; wood-pulp mills, sewage treatment plants and factories producing jet fuel, pesticides and plastics [5]; and even in the atmosphere and on the ocean surface [6]. Consequently, MM detection is important in the dental, medical, food, environment and atmosphere fields.

A low-cost, sensitive and specific sensor for detecting MM could be an interesting alternative to conventional MM monitoring methods such as the use of a halimeter, an expensive device, in the dental field [7,8]. Biosensors to monitor MM have been described by Mitsubayashi *et al.* [9–12]. In their work, monoamine oxidase A (MAO-A) or flavin-containing monooxygenase (FMO) was used to catalytically oxidize MM, and the oxygen consumption induced by this reaction was monitored. Coupled with this system, a substrate regeneration cycle with ascorbic acid was carried out. However, a sensor for the detection of O₂ depletion, which has the initially high current background of the oxygen electrode [13], is less sensitive than one for H₂O₂ measurement. To solve this problem and seek sensitive detection methods, our objective was to develop a MM biosensor coupled with sensitive hydrogen peroxide detection. Alcohol oxidase (AOX) is known to catalytically oxidize MM with production of formaldehyde, sulfide and H₂O₂ [14] according to the reaction:



Hydrogen peroxide generated during enzyme-catalyzed reactions can be electrochemically detected on modified/unmodified carbon matrixes [15–27]. In this work, unmodified carbon nanotube (CNT), cobalt phthalocyanine (CoPC), Prussian blue (PB), and Os-wired HRP modified screen printed electrode (CSPE) sensors were fabricated and tested to detect H₂O₂. Our aim was to seek the most sensitive and optimal detection method of H₂O₂ for a MM amperometric biosensor.

2. Experimental

2.1. Reagents

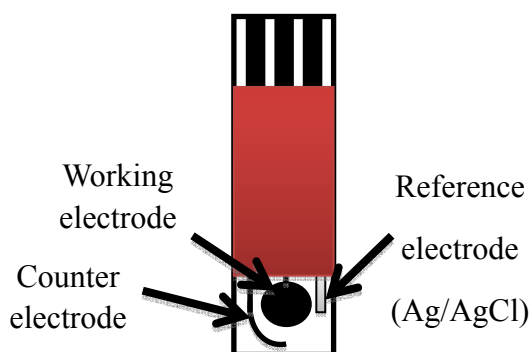
Hydrogen peroxide (H₂O₂, 30%, w/w), disodium hydrogen phosphate (Na₂HPO₄), potassium chloride (KCl), sodium chloride (NaCl), acetic acid (CH₃COOH), sodium acetate trihydrate (CH₃COONa·3H₂O), potassium hexacyanoferrate (III) (K₃Fe(CN)₆), *o*-phenylenediamine (99.5%), bovine serum albumin (BSA, ≥96%) were obtained from Sigma-Aldrich (Lyon, France). The concentration of diluted H₂O₂ solutions was determined by the classic potassium permanganate titration method. Sulfuric acid (95%) (H₂SO₄) and NaH₂PO₄·2H₂O were purchased from Prolabo (Briare, France). Hydrochloric acid (HCl) (37%) was obtained from Carlo Erba Reagenti (Milan, Italy). Peroxidase redox polymer (Os-wired HRP) was purchased from Bioanalytical Systems, Inc. (Gloucestershire, UK). Carbon pastes used for screen printed electrodes (Electrodag PE-410, 423SS and 6037SS) were obtained from Acheson (Plymouth, UK). A glycerolphtalic paint (Astral, France) was used as insulating layer. Transparent PVC sheets (200 mm × 100 mm × 0.5 mm) (SKK, Denzlingen,

Germany) were used as screen-printing substrates. All chemicals were used without any further purification. All solutions were prepared using Milli-Q water.

2.2. Instrument

CSPEs were produced in the laboratory using a semi-automatic DEK 248 screen-printing system (DEK, Weymouth, UK). The working electrode was a 4 mm diameter disk, the auxiliary electrode was a 16 mm $\times$ 1.5 mm curved wire and the Ag/AgCl reference electrode was a 5 mm $\times$ 1.5 mm straight wire (Figure 1).

Figure 1. Screen printed electrode.



Cyclic voltammetry (CV) measurements, PB electrodeposition, PPD electropolymerisation and amperometric measurements were carried out on an AUTOLAB PGSTAT100 (Metrohm, Switzerland), using GPES v4.7 (Metrohm) as informatic interface. All potential values are reported *versus* Ag/AgCl. Amperometric measurements were performed in a 10 mL glass bath cell with magnetic stirring at room temperature.

2.3. Carbon Screen Printed Electrode Modifications

2.3.1. Preparation of CNT Modified CSPEs

CNT modified CSPEs were prepared as described in the work of Silveira *et al.* [28]. Briefly, 10 μ L of 0.3 mg/mL SWCNT water dispersion were successively deposited on the CSPEs working electrode, drying each layer one by one under vacuum. The electrodes were then washed with water.

2.3.2. Preparation of CoPC Modified CSPEs

Cobalt-phthalocyanine-modified paste was purchased from Gwent Electronic Materials, Ltd. (Gwent, UK) and modifications were performed on working electrode by the DEK screen-printing system.

2.3.3. Preparation of PB/PPD Modified CSPEs

The PB film was first deposited by covering the CSPEs with a solution containing 2.5 mM FeCl₃, 2.5 mM K₃Fe(CN)₆, 0.1 M KCl and 0.1 M HCl and applying a potential +0.4 V *versus* Ag/AgCl for 40 s. Then the PB film was activated by covering the electrode by a solution containing 0.1 M KCl and 0.1 M HCl, electrochemically cycling for 20 cycles between −0.05 V and 0.35 V *versus* Ag/AgCl at a scan rate

of $50 \text{ mV}\cdot\text{s}^{-1}$. After washing with distilled water, it was dried for 1 h at 100°C in oven. To improve the stability and selectivity properties of the PB electrodes, the electropolymerisation of a poly-(*o*-phenylenediamine) (PPD) coat was formed. The PPD layer was deposited by electrochemically cycling the PB modified electrode with potential between -0.5 V and 0.7 V *versus* Ag/AgCl at a scan rate of $50 \text{ mV}\cdot\text{s}^{-1}$ in deaerated 0.1 M , pH 5.0 acetate buffer solution containing 0.5 mM *o*-phenylenediamine under a stream of nitrogen [29].

2.3.4. Preparation of Os Wired HRP (Os-HRP) Modified CSPEs

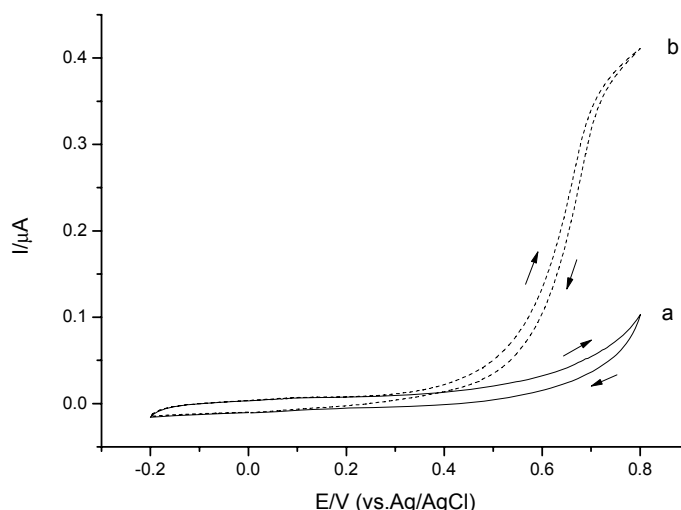
For the Os-HRP modified CSPEs, $10 \mu\text{L}$ 0.1 M phosphate buffer solution, pH 7.5, containing 10% (v/v) Os-HRP was deposited on the surface of CSPEs. It was allowed to dry at room temperature for 2 h. It was thoroughly washed with buffer before use.

3. Results and Discussion

3.1. H_2O_2 Detection with Unmodified CSPEs

The cyclic voltammetry studies were performed in 0.1 M phosphate buffer solution, pH 7.5 to investigate the CSPEs' electrochemical behavior (Figure 2). CSPEs showed no obvious peak in the absence of H_2O_2 in the potential range from -0.2 V ~ 0.8 V *versus* Ag/AgCl. In the presence of H_2O_2 , CSPEs started to perform current response at potential around $+0.3 \text{ V}$ *versus* Ag/AgCl, indicating the onset potential of the H_2O_2 electrooxidation.

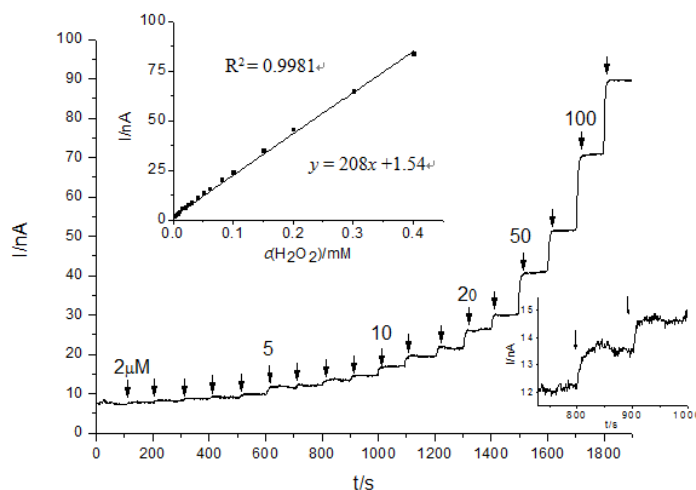
Figure 2. Cyclic voltammograms of CSPEs in 0.1 M phosphate buffer solution, pH 7.5 (a); in presence of 1 mM H_2O_2 (b). Scan rate $20 \text{ mV}\cdot\text{s}^{-1}$.



For the investigation of H_2O_2 limit detection, chronoamperometry experiments were carried out with several concentrations of H_2O_2 injected into the stirred bath cell (Figure 3). For unmodified CSPEs, the H_2O_2 detection limit was $3.1 \mu\text{M}$ ($\text{S/N} = 3$) applying a $+0.6 \text{ V}$ potential *versus* Ag/AgCl, and the current response slope of the calibration curve was $0.208 \mu\text{A}/\text{mM}$. To investigate the reproducibility, three parallel measurements with 0.1 mM H_2O_2 revealed a relative standard deviation (RSD) of 12.1%. The

high RSD observed of unmodified CPSEs is likely related to the marked differences in the real active electrode area, which is difficult to handle and adjust.

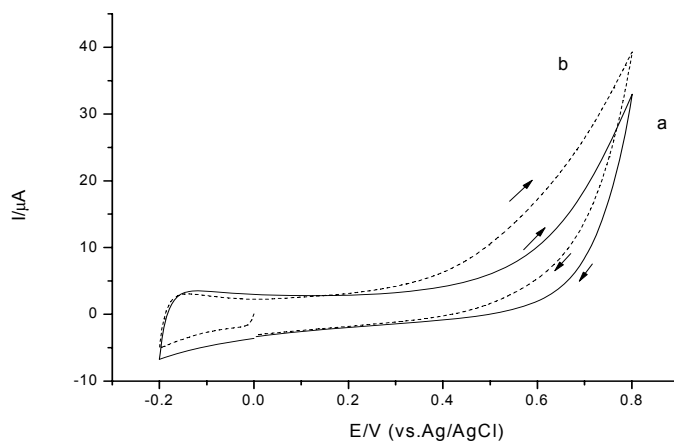
Figure 3. Amperometric performance of CSPEs for detection of H_2O_2 at +0.6 V in a stirred 0.1 M phosphate buffer solution, pH 7.5. The upper left inset shows the calibration curve of CSPEs for H_2O_2 concentration; the lower right inset zooms in the part of the amperometric response of 5 μM H_2O_2 injected.



3.2. H_2O_2 Detection with CNT/CSPEs

Both CSPEs (Figure 2) and CNT/CSPEs (Figure 4) showed a H_2O_2 oxidation peak in the cyclic voltammetry experiments for the studied potential range. The onset potential of the H_2O_2 electrooxidation for CNT/CSPEs was around +0.2 V *versus* Ag/AgCl, the detection limit was 1.3 μM ($\text{S/N} = 3$) applying a positive potential of +0.6 V *versus* Ag/AgCl, and the current response slope of CNT/CSPEs for H_2O_2 was 32.1 $\mu\text{A}/\text{mM}$. To investigate the reproducibility, three parallel measurements with 0.1 mM H_2O_2 revealed a RSD of 19.7%.

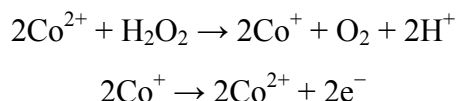
Figure 4. Cyclic voltammograms of CNT/CSPEs in 0.1 M phosphate buffer solution, pH 7.5 (a); in presence of 1 mM H_2O_2 (b). Scan rate 20 $\text{mV}\cdot\text{s}^{-1}$.



Both unmodified CSPEs and CNT are intrinsically carbon. Compared to unmodified CSPEs, detections of H_2O_2 for CNT/CSPEs need lower oxidation potential (CSPEs, +0.3 V; CNT/CSPEs, +0.2 V), and have lower detection limit (CSPEs, 3.1 μM ; CNT/CSPEs, 1.3 μM) with higher current response (CSPEs, 0.208 $\mu\text{A}/\text{mM}$; CNT/CSPEs, 32.1 $\mu\text{A}/\text{mM}$). The increased current response may arise from the large electric active area and a thin, porous diffusion layer [30]; the reasons of lower onset oxidation potential and lower detection limit are still controversial [31], because CNT may contain metal impurities derived from the catalysts used for their growth [32,33]. In a sense, CNT/CSPEs could be more favorable than unmodified CSPEs for H_2O_2 detection.

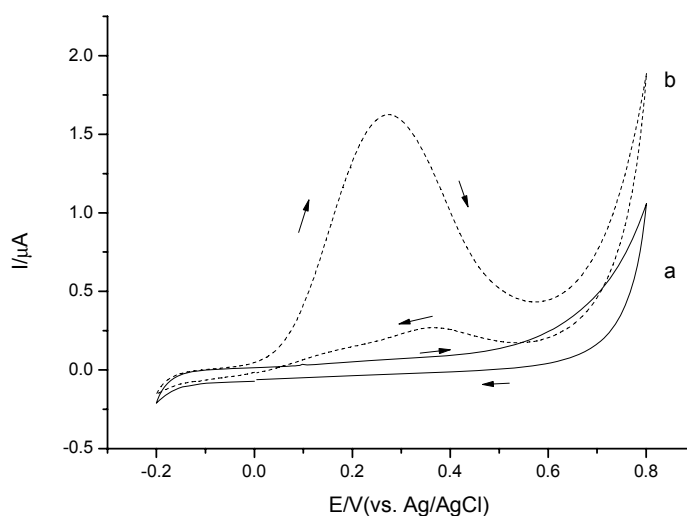
3.3. H_2O_2 Detection with CoPC/CSPEs

To investigate the electrochemical behavior of CoPC/CSPEs in phosphate buffer solution, cyclic voltammetry experiments were carried out in the potential range of -0.2 V~ 0.8 V *versus* Ag/AgCl (Figure 5). The presence of a well-defined oxidation current peak at around +0.3 V *versus* Ag/AgCl is consistent with the following reaction [21]:



The reaction can be described by a chemical-electrochemical (CE) mechanism [21]: H_2O_2 chemically reduces Co^{2+} to Co^+ and its subsequent electrochemical re-oxidation is observed as an oxidation peak. Consequently, this peak is used for the quantification of H_2O_2 . For CoPC/CSPEs, the H_2O_2 detection limit was calculated as 71 nM ($\text{S/N} = 3$) applying a positive potential of +0.4 V *versus* Ag/AgCl in chronoamperometry experiments, and the slope of the calibration curve was 3.7 $\mu\text{A}/\text{mM}$ by. Three parallel measurements with 10 μM H_2O_2 reveal a RSD of 2.4%, indicating a good reproducibility for this sensor.

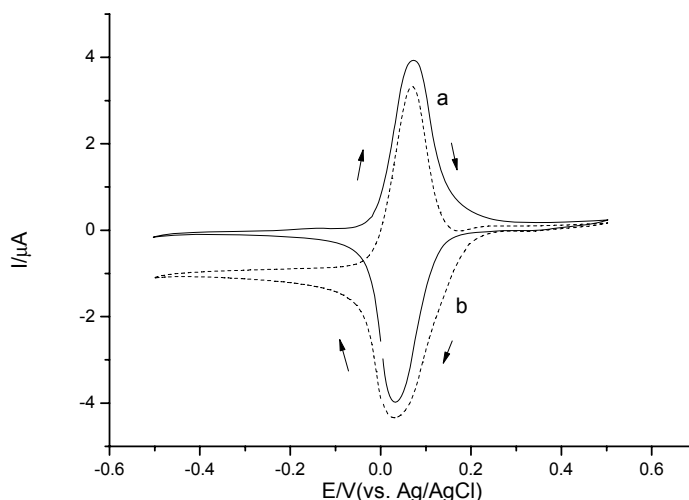
Figure 5. Cyclic voltammograms of CoPC/CSPEs in 0.1 M phosphate buffer solution, pH 7.5 (a); in presence of 0.1 mM H_2O_2 (b). Scan rate 20 $\text{mV}\cdot\text{s}^{-1}$.



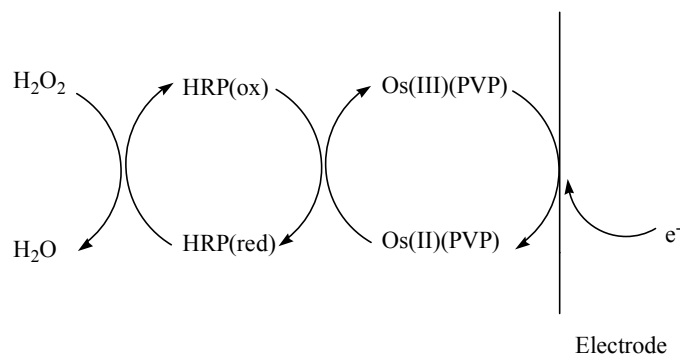
3.5. H_2O_2 Detection with Os-HRP/CSPEs

The Os-HRP/CSPEs show a couple of stable and well-defined redox peaks at around +30 mV and +70 mV at a scan rate of $20 \text{ mV}\cdot\text{s}^{-1}$ (Figure 7 curve a). In the presence of H_2O_2 (Figure 7 curve b), the electrocatalytical reductive reaction process of Os-HRP towards to H_2O_2 can be described by Scheme 1.

Figure 7. Cyclic voltammograms of Os-HRP/CSPEs in 0.1 M phosphate buffer solution, pH7.5 (a—); in presence of 0.1 mM H_2O_2 (b-----). Scan rate $20 \text{ mV}\cdot\text{s}^{-1}$.

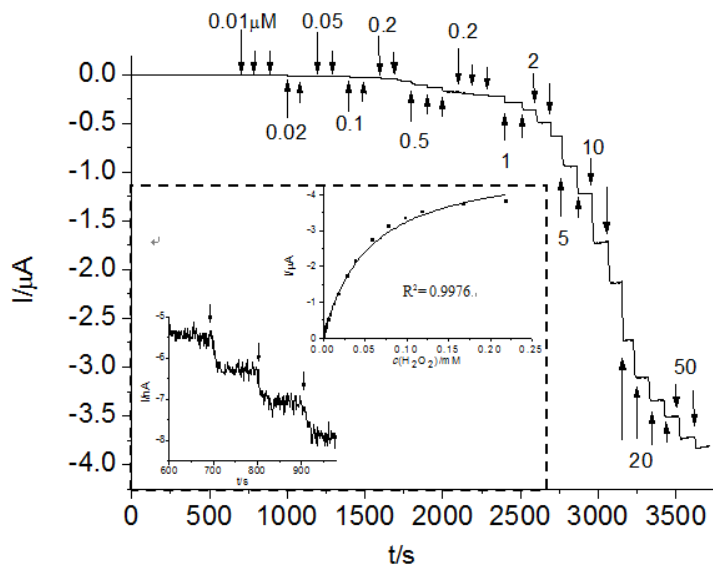


Scheme 1. The electrocatalytical reductive reaction process of Os-HRP towards H_2O_2 .



For Os-HRP/CSPEs, the H_2O_2 detection limit was 13.7 nM ($S/N = 3$) at a cathode potential -0.1 V versus Ag/AgCl (Figure 8). According to the Michaelis-Menten equation, the calculated apparent $K_{M,p}$, from the curve fitting is $53.5 \mu\text{M}$. This low $K_{M,p}$ value indicates Os-HRP's high affinity and high effective conversion for the H_2O_2 substrate and a favorable electron-transfer rate with the osmium mediator. A linear range was obtained until $25 \mu\text{M}$. Three parallel measurements with $1 \mu\text{M}$ H_2O_2 revealed a RSD of 1.3%, which indicates a good reproducibility. This may be ascribed to the application of a low cathode potential to avoid the interferences with electro-active species. In addition, Os-HRP/CSPEs can be the most specific in detection of H_2O_2 because of the specificity of the reaction between HRP and H_2O_2 .

Figure 8. Amperometric performance of Os-HRP/CSPEs sensor in detection of H_2O_2 at -0.1 V in a stirred 0.1 M phosphate buffer solution, pH 7.5. The upper right inset shows the calibration curve of Os-HRP/CSPEs for H_2O_2 concentration; the lower left inset zooms in on the part of the amperometric response of $0.01 \mu\text{M}$ H_2O_2 injected.



The comparison of the amperometric analytical behavior to H_2O_2 of the five kinds of electrodes developed in this study is summarized in Table 1. It shows that the Os-HRP/CSPEs are the most sensitive electrodes with a low reduction potential applied for H_2O_2 detection. This is due to the specific, sensitive and rapid turnover of Os-HRP to H_2O_2 .

Table 1. Comparison of the amperometric analytical performances for H_2O_2 detection with the five kinds of electrodes prepared in the present work.

Type of Electrodes	Potential Applied (V)	LOD (S/N = 3)	RSD (Tested Concentration of H_2O_2)
CSPEs	+0.6	$3.1 \mu\text{M}$	12.1% (0.1 mM)
CNT/CSPEs	+0.6	$1.3 \mu\text{M}$	19.7% (0.1 mM)
CoPC/CSPEs	+0.4	71 nM	2.4% (10 μM)
PPD/PB/CSPEs	-0.2	$1.3 \mu\text{M}$	4.1% (10 μM)
Os-HRP/CSPEs	-0.1	13.7 nM	1.3% (1 μM)

Interference of electro-active species [25,37–39] is often encountered when using amperometric biosensors and applying a high potential in real samples. The decrease of the applied potential can be effective to avoid a lot of electrochemical interferences. With this consideration, PPD/PB/CSPEs and Os-HRP/CSPEs are used to combine with alcohol oxidase (AOX) in bovine serum albumin matrix to detect methyl mercaptan (MM) applying a low potential in the aqueous phase. The limit of detection of AOX/PPD/PB/CSPEs to MM is $10 \mu\text{M}$; of AOX/Os-HRP/CSPEs to MM is $0.5 \mu\text{M}$. For AOX/Os-HRP/CSPEs, the calibration curve of the response to MM is linear in the concentration range $0\sim 15 \mu\text{M}$ with a good correlation with the classical analytical method. We are working on the stability of the biosensor which is the crucial point to improve its accuracy and reliability. Consequently, Os-HRP/CSPEs are combined with alcohol oxidase an optimum method which gives the best sensitivity in methyl mercaptan detection.

4. Conclusions

Five kinds of modified carbon screen printed electrodes applied for H₂O₂ amperometric detection for MM biosensors were presented in this work. In comparison, and despite a worse reproducibility, CNT/CSPEs are a better choice than unmodified CPSEs in H₂O₂ detection resulting from their lowest detection limit, lowest onset oxidation potential and highest current response of CNT/CSPEs. However, the applied potential of +0.6 V *versus* Ag/AgCl is too positive to avoid the interference of electro-active species. CoPC/CSPEs and PPD/PB/CSPEs are also a good choice in H₂O₂ detection because of their low applied potential, low detection limit and good reproducibility. Os-HRP/CSPEs display the lowest detection limit and the best operational reproducibility towards H₂O₂. With the cathode potential applied and the use of HRP, Os-HRP/CSPEs can avoid the interference of electro-active species and be specific for H₂O₂ detection. The Os-wired HRP modified screen printed electrode is the optimum method we used to combine with alcohol oxidase in a methyl mercaptan biosensor, usable in both aqueous and gaseous phase detection.

Acknowledgements

Zhan-Hong Li thanks China Scholarship Council (CSC) for his financial support.

References

1. Tonzetich, J.; Carpenter, P.A. Production of volatile sulphur compounds from cysteine, cystine and methionine by human dental plaque. *Arch. Oral Biol.* **1971**, *16*, 599–607.
2. Tonzetich, J. Production and origin of oral malodor: A review of mechanisms and methods of analysis. *J. Periodontol.* **1977**, *48*, 13–20.
3. Awano, S.; Koshimune, S.; Kurihara, E.; Gohara, K.; Sakai, A.; Soh, I.; Hamasaki, T.; Ansai, T.; Takehara, T. The assessment of methyl mercaptan, an important clinical marker for the diagnosis of oral malodor. *J. Dent.* **2004**, *32*, 555–559.
4. Ugliano, M.; Kwiatkowski, M.; Vidal, S.P.; Capone, D.; Siebert, T.; Dieval, J.-B.; Aagaard, O.; Waters, E.J. Evolution of 3-Mercaptohexanol, Hydrogen Sulfide, and Methyl Mercaptan during bottle storage of sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. *J. Agric. Food Chem.* **2011**, *59*, 2564–2572.
5. ATSDR. *Toxicological Profile for Methyl Mercaptan*; Agency for Toxic Substances and Disease Registry (ATSDR) US Public Health Service: Atlanta, GA, USA, 1992.
6. Bates, T.S.; Lamb, B.K.; Guenther, A.; Dignon, J.; Stoiber, R.E. Sulfur emissions to the atmosphere from natural sources. *J. Atmos. Chem.* **1992**, *14*, 315–337.
7. Rosenberg, M.; Kulkarni, G.V.; Bosy, A.; McCulloch, C.A. Reproducibility and sensitivity of oral malodor measurements with a portable sulphide monitor. *J. Dent. Res.* **1991**, *70*, 1436–1440.
8. Rosenberg, M.; Septon, I.; Eli, I.; Bar-Ness, R.; Gelernter, I.; Brenner, S.; Gabbay, J. Halitosis measurement by an industrial sulphide monitor. *J. Periodontol.* **1991**, *62*, 487–489.
9. Mitsubayashi, K.; Hashimoto, Y. Bioelectronic nose for methyl mercaptan vapor using xenobiotic metabolizing enzyme: Flavin-containing monooxygenase. *Sens. Actuators B Chem.* **2002**, *83*, 35–40.

10. Minamide, T.; Mitsubayashi, K.; Jaffrezic-Renault, N.; Hibi, K.; Endo, H.; Saito, H. Bioelectronic detector with monoamine oxidase for halitosis monitoring. *Analyst* **2005**, *130*, 1490–1494.
11. Minamide, T.; Mitsubayashi, K.; Saito, H. Bioelectronic sniffer with monoamine oxidase for methyl mercaptan vapor. *Sens. Actuators B Chem.* **2005**, *108*, 639–645.
12. Mitsubayashi, K.; Minamide, T.; Otsuka, K.; Kudo, H.; Saito, H. Optical bio-sniffer for methyl mercaptan in halitosis. *Anal. Chim. Acta* **2006**, *573*, 75–80.
13. Blum, L.J.; Coulet, P.R. *Biosensor Principles and Applications*; Marcel Dekker, Inc.: New York, NY, USA, 1991.
14. Suylen, G.M.H.; Large, P.J.; Dijken, J.P.V.; Kuenen, J.G. Methyl mercaptan oxidase, a key enzyme in the metabolism of methylated sulphur compounds by hyphomicrobium EG. *J. Gen. Microbiol.* **1987**, *133*, 2989–2997.
15. Wang, J.; Musameh, M.; Lin, Y. Solubilization of carbon nanotubes by nafion toward the preparation of amperometric biosensors. *J. Am. Chem. Soc.* **2003**, *125*, 2408–2409.
16. Trojanowicz, M. Analytical applications of carbon nanotubes: A review, trac-trend. *Anal. Chem.* **2006**, *25*, 480–489.
17. Wang, J.; Musameh, M. Carbon nanotube/teflon composite electrochemical sensors and biosensors. *Anal. Chem.* **2003**, *75*, 2075–2079.
18. Wang, J. Carbon-nanotube based electrochemical biosensors: A review. *Electroanal* **2005**, *17*, 7–14.
19. Wang, J.; Golden, T.; Li, R. Cobalt phthalocyanine/cellulose acetate chemically modified electrodes for electrochemical detection in flowing streams. Multifunctional operation based upon the coupling of electrocatalysis and permselectivity. *Anal. Chem.* **1988**, *60*, 1642–1645.
20. Salimi, A.; Hallaj, R.; Soltanian, S.; Mamkhezri, H. Nanomolar detection of hydrogen peroxide on glassy carbon electrode modified with electrodeposited cobalt oxide nanoparticles. *Anal. Chim. Acta* **2007**, *594*, 24–31.
21. Gilmartin, M.A.T.; Hart, J.P.; Birch, B.J. Development of amperometric sensors for uric acid based on chemically modified graphite-epoxy resin and screen-printed electrodes containing cobalt phthalocyanine. *Analyst* **1994**, *119*, 243–252.
22. Gilmartin, M.A.T.; Ewen, R.J.; Hart, J.P.; Honeybourne, C.L. Voltammetric and photoelectron spectral elucidation of the electrocatalytic oxidation of hydrogen peroxide at screen-printed carbon electrodes chemically modified with cobalt phthalocyanine. *Electroanal* **1995**, *7*, 547–555.
23. Ricci, F. Prussian Blue and enzyme bulk-modified screen-printed electrodes for hydrogen peroxide and glucose determination with improved storage and operational stability. *Anal. Chim. Acta* **2003**, *485*, 111–120.
24. Ricci, F.; Amine, A.; Palleschi, G.; Moscone, D. Prussian Blue based screen printed biosensors with improved characteristics of long-term lifetime and pH stability. *Biosens. Bioelectron.* **2003**, *18*, 165–174.
25. Ricci, F.; Palleschi, G. Sensor and biosensor preparation, optimisation and applications of Prussian Blue modified electrodes. *Biosens. Bioelectron.* **2005**, *21*, 389–407.
26. Garguilo, M.G.; Nhan, H.; Proctor, A.; Michael, A.C. Amperometric sensors for peroxide, choline, and acetylcholine based on electron transfer between horseradish peroxidase and a redox polymer. *Anal. Chem.* **1993**, *65*, 523–528.

27. Vreeke, M.; Maidan, R.; Heller, A. Hydrogen peroxide and .beta.-nicotinamide adenine dinucleotide sensing amperometric electrodes based on electrical connection of horseradish peroxidase redox centers to electrodes through a three-dimensional electron relaying polymer network. *Anal. Chem.* **1992**, *64*, 3084–3090.
28. Silveira, C.M.; Baur, J.; Holzinger, M.; Moura, J.J.G.; Cosnier, S.; Almeida, M.G. Enhanced direct electron transfer of a multihemic nitrite reductase on single-walled carbon nanotube modified electrodes. *Electroanal* **2010**, *22*, 2973–2978.
29. Lobo, M.J.; Miranda, A.J.; Tipez-Fonseca, J.M.; Tuih, P. Electrocatalytic detection of nicotinamide coenzymes by poly(o-aminophenol)- and poly(o-phenylenediamine)-modified carbon paste electrodes. *Anal. Chim. Acta* **1996**, *325*, 33–42.
30. Scott, C.L.; Pumera, M. Carbon nanotubes can exhibit negative effects in electroanalysis due to presence of nanographite impurities. *Electrochem. Commun.* **2011**, *13*, 426–428.
31. Yang, W.; Ratinac, K.R.; Ringer, S.P.; Thordarson, P.; Gooding, J.J.; Braet, F. Carbon nanomaterials in biosensors: Should you use nanotubes or graphene? *Angew. Chem. Int. Edit.* **2010**, *49*, 2114–2138.
32. Šljukić, B.; Banks, C.E.; Compton, R.G. Iron oxide particles are the active sites for hydrogen peroxide sensing at multiwalled carbon nanotube modified electrodes. *Nano Lett.* **2006**, *6*, 1556–1558.
33. Banks, C.E.; Crossley, A.; Salter, C.; Wilkins, S.J.; Compton, R.G. Carbon nanotubes contain metal impurities which are responsible for the “electrocatalysis” seen at some nanotube-modified electrodes. *Angew. Chem. Int. Edit.* **2006**, *45*, 2533–2537.
34. Feldman, B.J.; Murray, R.W. Electron diffusion in wet and dry Prussian blue films on interdigitated array electrodes. *Inorg. Chem.* **1987**, *26*, 1702–1708.
35. Stilwell, D.E.; Park, K.H.; Miles, M.H. Electrochemical studies of the factors influencing the cycle stability of Prussian Blue films. *J. Appl. Electrochem.* **1992**, *22*, 325–331.
36. Haghighi, B.; Hamidi, H.; Gorton, L. Electrochemical behavior and application of Prussian Blue nanoparticle modified graphite electrode. *Sens. Actuators B Chem.* **2010**, *147*, 270–276.
37. Palmisano, F.; Zambonin, P.G. Ascorbic acid interferences in hydrogen peroxide detecting biosensors based on electrochemically immobilized enzymes. *Anal. Chem.* **1993**, *65*, 2690–2692.
38. Yamamoto, K.; Ohgaru, T.; Torimura, M.; Kinoshita, H.; Kano, K.; Ikeda, T. Highly-sensitive flow injection determination of hydrogen peroxide with a peroxidase-immobilized electrode and its application to clinical chemistry. *Anal. Chim. Acta* **2000**, *406*, 201–207.
39. Palleschi, G.; Ali Nabi Rahni, M.; Lubrano, G.J.; Ngwainbi, J.N.; Guilbault, G.G. A study of interferences in glucose measurements in blood by hydrogen peroxide based glucose probes. *Anal. Biochem.* **1986**, *159*, 114–121.

Copyright of Sensors (14248220) is the property of MDPI Publishing and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.