

Electrochemical Detection of Hydrogen Peroxide at the Sputtered Titanium Oxide Electrode *

ZHOU Qing-li¹, YUAN Qing², YU Jing², WU Jian³ *

1. Women's Hospital, School Of Medicine, Zhejiang University, Hangzhou 310006, China;
2. School of Biomedical Engineering and Instrument, Zhejiang University, Hangzhou 310027, China;
3. School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310029, China

Abstract : In this paper a planar titanium oxide electrode was fabricated by standard microelectronic technology and its electrochemical properties in phosphate buffer solution was investigated. According to the cyclic voltammetric results, both hydrogen peroxide and oxygen can be cathodic reduced at titanium oxide electrode, while the reduction of hydrogen peroxide is preferential. The amperometric measurement of hydrogen peroxide at the planar titanium oxide electrode in air saturated phosphate buffer solution at - 300 mV shows fast responses. The amperometric response to 0.1 mmol/L hydrogen peroxide is 0.4 μ A while the residual current due to the reduction of oxygen is only 14 nA. Glucose oxidase has been immobilized on the surface of titanium oxide film to explore the possible applications of this electrode in biosensing. The experimental results show it is difficult to immobilize enzyme on titanium oxide surface.

Key words : titanium oxide; hydrogen peroxide; sputtering; reduction
EEACC :7320J

溅射二氧化钛电极对过氧化氢电化学检测的研究 *

周庆利¹, 袁青², 于晶², 吴坚³ *

1. 浙江大学医学院附属妇产科医院, 杭州 310006;
2. 浙江大学生物医学工程与仪器科学学院, 杭州 310027;
3. 浙江大学生物系统工程与食品科学学院, 杭州 310029

摘要 : 介绍一种使用微电子技术制造的二氧化钛电极, 并且研究了该电极在磷酸缓冲液中的电化学特性。循环伏安法测量的结果显示, 过氧化氢和氧气都能在该电极上还原, 而且过氧化氢的还原更为明显。在磷酸盐缓冲液中, 以 Ag/AgCl 为参考电极, 当所施加电压为 - 300 V 时, 安培法显示二氧化钛电极对过氧化氢有很快的响应速度, 其对于 0.1 mmol/L 过氧化氢的响应电流是 0.4 μ A, 而由于溶解氧所带来的背景还原电流仅为 14 nA。为了研究这种电极在生物传感器中的应用可能性, 实验中把葡萄糖氧化酶固定在钛氧化物电极薄膜上并测试了相应特性, 发现存在的主要问题为酶在氧化钛表面的固定较为困难。

关键词 : 二氧化钛电极; 过氧化氢; 溅射; 还原

中图分类号 : TP212.3

文献标识码 : A

文章编号 : 1004-1699(2008)09-1487-06

The electrochemical detection of hydrogen peroxide (H_2O_2) plays a key role in the development of biosensors based on oxidase enzymes. Up to now, the most successful approach is the electrochemical oxidation of H_2O_2 on anodic electrodes

like platinum. The main drawback of this approach is the high anodic potential required for the oxidation of H_2O_2 (> 700 mV vs Ag/AgCl). Some common materials in physiological fluids such as ascorbic acid and uric acid can also be oxidized at this

基金项目 : 国家自然科学基金项目资助(30670535); 教育部“新世纪优秀人才支持计划”基金项目资助(NCET-07-0752)

收稿日期 : 2008-03-09 **修改日期 :** 2008-05-26

potential and it results in error signals. An alternative to detect H_2O_2 is the electrochemical reduction of H_2O_2 . In this case, the main interference will be the dissolved O_2 . Karyakin, et al developed a Prussian Blue-modified platinum electrode, which permits the cathodic reduction of H_2O_2 at 180 mV vs. Ag/AgCl reference electrode in the presence of O_2 in the buffer solution of pH = 6.0^[1-2]. However, the reduction of H_2O_2 on the Prussian Blue modified electrode is drastically decreased in neutral solution, the latter being most important in bioanalysis. Wang, et al reported that carbon-paste electrodes dispersed with ruthenium, rhodium, or iridium particles show preferential reduction of H_2O_2 ^[3-5]. The rhodium-dispersed carbon paste electrode doped with glucose oxidase offers detection of the glucose over the potential range of -200 mV ~ 900 mV vs. Ag/AgCl reference electrode. All these approaches need composite materials as electrodes to preferentially reduce H_2O_2 .

Titanium oxide is a transition-metal oxide with a variety of applications^[6-8]. In the field of electrochemistry, titanium oxide has been mainly tested as an electrode for use in photoelectrochemically induced water splitting. Only few researches focused on its electroanalytical utility. Ikeda, et al investigated the electrocatalytic reduction of dioxygen on titanium oxide electrode^[9]. Titanium oxide was prepared by anodic oxidation of the surface of a Ti wire in a dilute H_2SO_4 solution. Ulmann, et al reported that titanium oxide film may act as an electrocatalytic material for the reduction of H_2O_2 in the presence of dissolved O_2 ^[10]. Furthermore, Cosnier, et al developed a mesoporous titanium oxide film for the cathodic detection of hydrogen peroxide in air-saturated aqueous solution^[11-12]. In this case, titanium oxide film was prepared by depositing a colloidal suspension of particles, produced by the hydrolysis of titanium isopropoxide by acetic acid. In this paper, we investigated the electrochemical reduction of H_2O_2 in the presence of oxygen on sputtered titanium oxide electrode. Unlike traditional wet chemical processes, sputtering is a

standard microelectronic process. Taking the advantages of the microelectronic technology, it is possible to fabricate electrodes or arrays of electrodes with complex or fine pattern that would be very difficult to fabricate with conventional techniques. Glucose oxidase was also immobilized on the surface of titanium oxide electrode to explore the possible applications of this electrode in biosensing.

1 Materials and Methods

1.1 Reagents

Glucose oxidase (GOD), (EC 1.1.3.4, 139 U/mg, from *Aspergillus niger*) was obtained from Fluka. (3-glycidioxy-propyl-di-methy-ethoxy) silane was obtained from ABCR (Karlsruhe, Germany). Phosphate buffered solution (PBS) was prepared by dissolving 45 g NaCl, 11.8 g KH_2PO_4 and 53 g K_2HPO_4 in 10 liter DI water (pH = 7.3). All other chemicals were of analytical reagent grade.

1.2 Fabrication of Titanium Oxide Electrode

Figure 1 shows the fabrication processes. A 1 μm thick thermal silicon oxide layer was grown on the 3-inch silicon wafer for electronic isolation (a). Negative photoresist (SC180) layer was spun and patterned (b). A stack of 50nm Ti/ 100nm Pt layer was sputtered on the wafer(c). Here the Ti layer is used just as an adhesion layer between silicon oxide and Pt. Then, by subsequent dissolution of photoresist, and thereby removal of the Ti/ Pt layer on top of the photoresist, the Ti/ Pt pattern was obtained (d), which formed the Pt electrodes and bonding pads. Another negative photoresist (SC180) layer was spun and patterned (e). The wafer was covered first with a Ti film (2 minutes of sputtering) and then with 65 nm thick titanium oxide layer, sputtered from a titanium target in oxygen atmosphere (rf power: 400W; oxygen: 7 μbar) by Balzer BAE370 coating system (f). Finally, by dissolution of the photoresist, the titanium oxide electrode was patterned on the top of platinum electrode (g). The dimension of one titanium oxide electrode is 2 mm \times 7 mm.

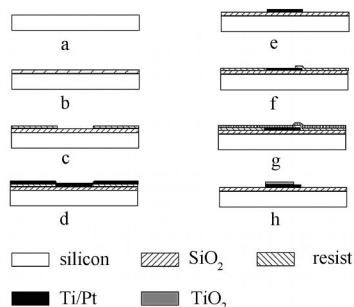


Fig. 1 Fabrication of the titanium oxide electrode on the silicon wafer.

1.3 Enzyme Immobilization on Titanium Oxide Electrode

The enzyme immobilization is based on the silanization of the surface of titanium oxide film with the bi-functional silane. The titanium oxide electrode was first hydroxylated in 5 % NaOH solution. The silanization was performed with 4 % (v/v) solution of (3-glycidoxy-propyl-di-methy-ethoxy) silane out of 95 % ethanol/ 5 % water mixture. After silane absorption, the electrode was rinsed and dry. Finally, it is dipped in GOD solution for 15 min. In this way, a monolayer of GOD was formed on the surface of titanium oxide electrode.

1.4 Measurement

Model 273 potentiostat/galvanostat from EG&G Princeton Applied Research was used for both potentiometric and amperometric measurements. The measurement control and data collection were done with a dedicated program in LabWindows® environment through the connected PC. The external Ingold Ag/AgCl reference electrode and platinum counter electrode were used. The applied voltage or bias is that of the titanium oxide electrode with respect to the reference electrode. A magnetic stirrer and a stirring bar provided convective flow during measurement. All experiments were carried out at room temperature.

2 Results and Discussions

2.1 Electrochemical Examination of the Sputtered Titanium Oxide Electrode

Figure 2 is SEM picture of the sputtered titanium oxide film. It is shown that titanium oxide film is amorphous and also quite porous. The porous structure can enhance the surface active area of

the film and it is beneficial to electrochemical detection. Figure 3 show the cyclic voltammogram of the sputtered titanium oxide electrode under the positive bias in 0.1 mmol/L H_2O_2 PBS solution. It is shown that there is no anodic current in this potential range and titanium oxide electrode functions as a capacitor. Titanium oxide is an *n*-type wide bandgap (ca. 3.0 eV) semiconductor^[13]. On the contact of titanium oxide with the electrolyte, some electrons in the conduction band of titanium oxide go into the electrolyte and a depletion layer of electrons is formed on the surface of titanium oxide. Due to the separation of positive and negative charge, a potential barrier forms between titanium oxide and the electrolyte, which prevents the further movement of electrons between semiconductor and electrolyte. In the absence of UV illumination, the hole concentration in the valence band of titanium oxide is very low. Therefore, there is no appreciable anodic current flow for the oxidation of H_2O_2 .

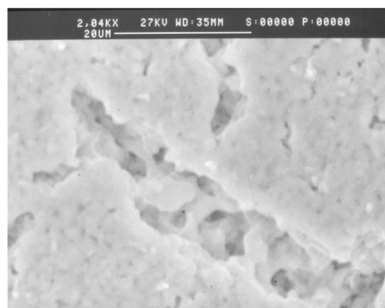


Fig. 2 The SEM picture of the sputtered titanium dioxide film.

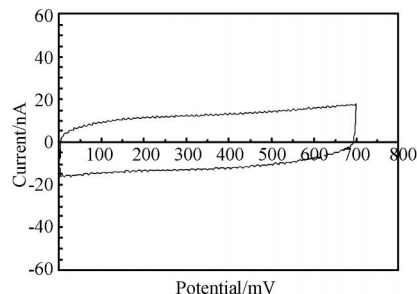


Fig. 3 Cyclic voltammogram of the sputtered titanium oxide electrode in 0.1mmol/L H_2O_2 PBS solution in the positive potential range. Reference electrode: Ag/AgCl; scan rate: 35mV/s.

Figure 4 shows the cyclic voltammograms of the sputtered titanium oxide electrode under the negative bias in N_2 purged, air-saturated and O_2

purged PBS solution respectively. Under the negative bias, the potential barrier between titanium oxide and electrolyte is reduced and electrons in the conduction band of titanium oxide may move into the electrolyte. In this way, the oxidants in the electrolyte can be reduced. It is shown in Figure 3 that O_2 starts to be reduced at titanium oxide electrode when the potential is more negative than -300 mV. So the sputtered titanium oxide electrode is capable to be used as cathode in electrochemical analysis, which is exactly we need for H_2O_2 measurement.

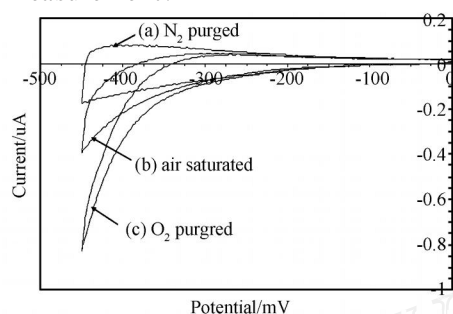


Fig. 4 Cyclic voltammograms of the sputtered titanium oxide electrode in PBS solution in negative potential range. Reference electrode: Ag/AgCl; scan rate: 22.5 mV/s.

2.2 Detection of H_2O_2 at the Sputtered Titanium Oxide Electrode

Figure 5 shows the cyclic voltammograms of sputtered titanium oxide electrode under negative bias in O_2 purged PBS solution and 0.1 mmol/L H_2O_2 PBS solution, respectively. Titanium oxide electrode shows the preferential reduction of H_2O_2 in the presence of O_2 . The reduction of H_2O_2 starts at more positive potential than the reduction of O_2 and meanwhile the response of H_2O_2 is also much larger than that of O_2 at the same potential. So it should be possible to detect H_2O_2 at sputtered titanium oxide electrode in the presence of O_2 . Figure 6 shows the amperometric response of titanium oxide electrode on the addition of H_2O_2 at the potential of -300 mV in air saturated PBS solution. The inset is the calibration curve. The residual current due to the reduction of dissolved O_2 is around 14 nA. On the addition of 0.2 mmol/L H_2O_2 , the current is increased to 0.4 μ A immediately. At low concentrations of H_2O_2 , the responses are larger,

but the response is saturated when the concentration of H_2O_2 is higher than 0.5 mmol/L. Working at more negative potentials may extend the linear range more or less but the price of larger residual current due to the reduction of O_2 has to be paid. At this moment, the mechanism of H_2O_2 reduction on the titanium oxide electrode is not clear. According to the report of Guerin, et al^[14], the film sputtered from titanium target in atmosphere of oxygen and argon is composed of combinations of metallic titanium and three oxides. We suppose that metallic titanium element and titanium element with the low valence values ($< +4$) are responsible for the preferential reduction of H_2O_2 . The linearity range of H_2O_2 reduction at the titanium oxide may also related to the concentration of those elements in the sputtered film.

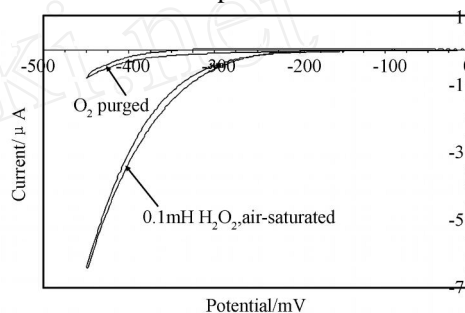


Fig. 5 Cyclic voltammograms of the sputtered titanium oxide electrode in PBS solution in negative potential range. Reference electrode: Ag/AgCl; scan rate: 22.5 mV/s.

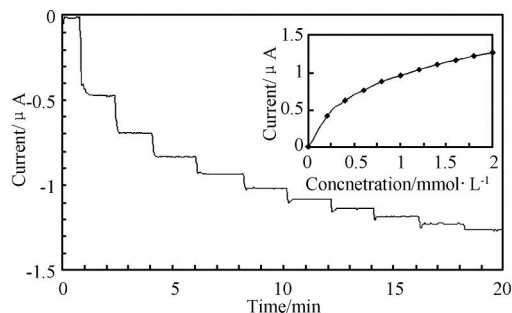


Fig. 6 Current vs. time curve on the successive addition of 0.2 mmol/L H_2O_2 in PBS solution on the sputtered titanium oxide electrode. Reference electrode: Ag/AgCl; operating potential: -300 mV. Inset: calibration curve.

2.3 Application of the Sputtered Titanium Oxide Electrode as Biosensors

One of main applications of H_2O_2 electrode is the detection of H_2O_2 released during enzyme cata-

lytic reactions for biosensing. Figure 7 shows the amperometric response of GOD immobilized Titanium oxide electrode to the glucose in PBS solution. The responses on the addition of glucose are not large. Since sputtered titanium oxide electrode is quite sensitive to H_2O_2 , it suggests that there is no enough enzyme on the electrode. In our case, the enzyme immobilization is based on the silanization of the surface of titanium oxide film with the bi-functional silane. For a good silanization, there must be plenty of hydroxyl groups on the surface. However, titanium oxide is a hydrophobic material itself. So for the applications in bioanalysis, other immobilization method should be developed.

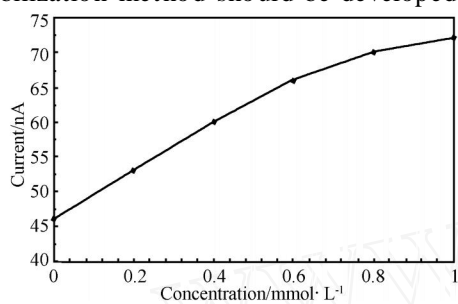


Fig. 7 The calibration curve of the amperometric response of enzyme immobilized the sputtered titanium oxide to glucose at -300 mV vs Ag/AgCl reference electrode.

3 Conclusions

In this paper we fabricated a planar titanium oxide electrode by sputtering process and investigated its electrochemical properties in phosphate buffer solution by cyclic voltammetry and amperometry. Under the positive potential, there is no anodic current at titanium oxide electrode. Under the negative potential, both hydrogen peroxide and oxygen can be cathodic reduced at titanium electrode, while the reduction of hydrogen peroxide is preferential. The amperometric measurement of hydrogen peroxide in air saturated phosphate buffer solution shows fast response. The amperometric response to 0.1 mmol/L hydrogen peroxide is 0.4 μ A while the residual current due to the reduction of oxygen is only 14 nA. Glucose oxidase was immobilized on the surface of titanium oxide film to explore the possible application of this electrode in biosensing.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (projects No. 30670535) and by Program for New Century Excellent Talents in University (NCET-07-0752).

References :

- [1] Karyakin A A, Gtelmacher O V, Karyakina E E. Prussian Blue Based First-Generation Biosensor-A Sensitive Amperometric Electrode for Glucose[J], *Analytical Chemistry*, 1995, 67:2419-2423.
- [2] Karyakin AA, Puganova EA, Budashov IA, et al. Prussian Blue Based Nanoelectrode Arrays for H_2O_2 Detection[J]. *Analytical Chemistry*, 2004, 76:474-478.
- [3] Wang J, Lu F, Lopez D, and Tobias H. Highly Selective and Sensitive Amperometric Biosensing of Glucose at Ruthenium-Dispersed Carbon Paste Enzyme Electrodes [J], *Analytical Letters*, 1993, 26:1819-1830.
- [4] Wang J, Liu J, Chen L and Lu F, Highly Selective Membrane-Free, Mediator-Free Glucose Biosensor [J]. *Anal. Chem.*, 1994, 66:3600-3603.
- [5] Wang J, Rivas G and Chicharro M, Iridium-Dispersed Carbon Paste Enzyme Electrodes [J]. *Electroanalysis*, 1996, 8: 434-437.
- [6] Islam M R, Kumazawa N. and Takeuchi M. Titaniumdioxide Chemical Sensor Working with AC Voltage[J]. *Sensors and Actuators B*, 1998, 46:114-119.
- [7] Sberveglieri G, Comini E, Faglia G, Atashbar M Z, and Wlodarski W. Titanium Dioxide Thin Films Prepared for Alcohol Microsensor applications[J]. *Sensors and Actuators B*, 2000, 66:139-141.
- [8] Casta?eda L, Effects of Palladium Coatings on Oxygen Sensors of Titanium Dioxide Thin Films[J]. *Materials Science and Engineering B*, 2007, 139:149-154.
- [9] Ikada S, Hara M, Hishida Y, Kimura M, and Ito K. Development of a Needle-Type Glucose Sensor Based on a Titanium Dioxide Oxygen Electrode for the Artificial Pancreas[J]. *Sensors and Actuators B*, 1993, 13-14, 315-318.
- [10] Ulmann M, Tacconi N, and Augustynski J. Behavior of Surface Peroxo Species in the Photoreactions at Titanium Dioxide [J]. *Journal of Physical Chemistry*, 1986, 90:6523-6530.
- [11] Cosnier S, Gondran C, Senillou A, Gratzel M, and Vlachopoulos N. Mesoporous TiO_2 Films: New Catalytic Electrode Materials for Fabricating Amperometric Biosensors Based on Oxidases[J]. *Electroanalysis*, 1997, 9:1387-1392.
- [12] Cosnier S, Senillou A, Gratzel M, Comte P, Vlachopoulos N, Renault N. and Martelet C. A Glucose Biosensor Based on Enzyme Entrapment Within Polypyrrole Films Electrodeposited on Mesoporous Titanium Dioxide [J]. *Journal of Electrochemical Analysis*, 1999, 469:176-181.
- [13] Bocarsly A, Tachikawa H, and Faulkner L. Photonic Elec-

trochemistry in Laboratory Techniques in Electroanalytical Chemistry P. Kissinger and W. Heineman eds. Marcel Dekker, 1995, New York.

[14] Guerin D. and Shah S. Reactive-Sputtering of Titanium Oxide Thin Films[J]. Journal of Vacuum Science & Technology A, 1997,15:712.



周庆利(1968-),男,硕士,高级工程师。1991年毕业于浙江大学医学院,学士学位,2003年获浙江大学工程硕士学位。毕业后一直在浙江大学医学院附属妇产科医院工作,从事医疗设备的技术引进、技术创新和医疗设备的质量控制工作,从事临床医学工程研究和医学信息工程研究,主持和参与国家级、省厅级课题6项,主持省科技厅新产品项目1项,获得国家实用新型专利1项,在各级杂志上发表论文20余篇。



吴坚(1969-),男,博士,教授。2002年毕业于比利时鲁汶大学,获工学博士学位。先后在香港科技大学、丹麦技术大学和加拿大阿尔伯特大学工作。2005年起在浙江大学工作,主要从事电化学传感器、生物传感器、MEMS及相关仪器开发,wujian69@zju.edu.cn

www.cnki.net