Biosensors and Bioelectronics 26 (2011) 2579–2584

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Nonenzymatic glucose sensor based on over-oxidized polypyrrole modified Pd/Si microchannel plate electrode

Jing Shi[a], Pengliang Ci[a], Fei Wang[a], Hui Peng[a], Pingxiong Yang[a], Lianwei Wang[a,*],
Shuli Ge[b], Qingjiang Wang[b], Paul K. Chu[c]

[a] Laboratory of Polar Materials and Devices, Ministry of Education, and Department of Electronic Engineering, East China Normal University, Shanghai 200241, China
[b] Department of Chemistry, East China Normal University, Shanghai 200241, China
[c] Department of Physics and Material Sciences, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

ARTICLE INFO

*Article history:*
Received 20 July 2010
Received in revised form 4 November 2010
Accepted 9 November 2010
Available online 18 November 2010

*Keywords:*
Nonenzymatic glucose sensor
Silicon microchannel plate
Palladium nanoparticle
Over-oxidized polypyrrole
Three dimensional array

ABSTRACT

A silicon microchannel plate (MCP) array electrode modified with over-oxidized polypyrrole (OPPy) has been fabricated to detect glucose. The morphology and structure of the electrode are characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The OPPy modified silicon MCP array electrode exhibits high electrocatalytic activity as well as excellent selectivity to the electrochemical oxidation of glucose. At a potential of +0.08 V, good sensitivity of 0.37 mA $\text{mM}^{-1}$ $\text{cm}^{-2}$ and detection limit of 2.06 μM are attained. The linear range is up to 24 mM with a linear correlation coefficient of 0.997. Furthermore, the electrode is highly resistant to interfering substances because the effects of common coexisting substances can be effectively eliminated by the OPPy film and the response in the current to interferences on the electrode surface is almost negligible. This novel electrode has high potential in nonenzymatic detection of glucose.

## 1. Introduction

Since the concentration of glucose is an important index in clinical diagnoses, regulation of metabolism, and biochemical analysis, determination of glucose has attracted considerable interest (Lee et al., 2008; Musameh et al., 2002; Newman and Turner, 2005; Wu et al., 2007). Various amperometric biosensors based on glucose oxidase (GOx) which catalyzes the oxidation of glucose to gluconolactone with hydrogen peroxide as the reaction product are extensively used for glucose detection due to the high chemical specificity and rapid response (Du et al., 2008; Kandimalla et al., 2006; Wang et al., 2009a,b; Zhang et al., 2007; Zhao and Ju, 2006; Zhao et al., 2009). However, the enzyme-modified electrodes have some common and serious disadvantages such as complicated immobilization techniques, rigorous operating conditions, and inherent instability (Hrapovic et al., 2004; Yang et al., 2006a,b). Many ambient factors such as temperature, humidity, pH value, and toxic chemicals can impact the activity of GOx resulting in functional inactivation (Wilson and Turner, 1992). To circumvent these problems, several nonenzymatic glucose sensors with metal nanoparticles utilizing direct electrochemical oxidation of glucose on the electrode surface have been proposed (Farrell and Breslin, 2004; Salimi and Roushani, 2005; Sun et al., 2001; Zhao et al., 2006). They are attractive alternatives on account of the stability, reproducibility, and catalytic properties. Nevertheless, these glucose sensors still suffer from drawbacks such as hyposensitivity and poor selectivity caused by adsorption of interfering substances like uric acid (UA) and ascorbic acid (AA) under physiological conditions (Li et al., 2007; Shen et al., 2008). Therefore, a novel nonenzymatic sensor with high sensitivity, quick response, and good selectivity is needed.

Several three dimensional (3D) structures have recently been adopted in the construction of glucose biosensors in order to increase the sensitivity (Gavalas et al., 2004; Lin et al., 2005; Merkoci et al., 2005). For instance, silicon microchannel plate (MCP) is a promising substrate with excellent characteristics including large surface area, relatively high mechanical stability, low cost, and compatibility to commercial microfabrication processes in the microelectronics technology. Moreover, the suitable spatial dimension and high porosity in this 3D array improves mass transport and catalytic reactions consequently accelerating fast electron transfer and enabling sensitive detection of glucose. Polypyrrole (PPy) and its derivatives are superior polymeric materials used in the preparation of membranes in electrochemical analytical applications, chemical sensors, as well as biosensors (Gerard et al., 2002; Majidi et al., 2006). The PPy film can be easily electrodeposited on the electrode surface and a permselective over-oxidized polypyrrole (OPPy) film can be formed by expelling the doping ions from the

---

* Corresponding author. Tel.: +86 21 54345160; fax: +86 21 54345119.
*E-mail address:* lwwang@ee.ecnu.edu.cn (L. Wang).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.11.010

polymer film. Experimental results have demonstrated that electrodes with OPPy are able to eliminate the negative effects induced by interfering species thus improving the selectivity of the sensors (Li and Lin, 2007; Tian et al., 2001; Vidal et al., 1999).

In our previous work, a glucose sensor based on 3D Pd-Ni/MCP was successfully fabricated by electroless plating (Miao et al., 2009). Nickel and trace palladium nanoparticles act as the sensing materials and this sensor shows good electrocatalytic activity. In order to further improve the sensitivity and selectivity, we propose a nonenzymatic glucose sensor based on the OPPy modified Pd/Silicon MCP electrode here. The vertically aligned silicon MCP is fabricated by photo-assisted electrochemical etching (Chen et al., 2008; Yuan et al., 2009) and palladium nanoparticles which are widely used in catalysis are synthesized on the surface of the silicon MCP by electrodepositing. The OPPy film is prepared using a three-electrode electrochemical workstation. Our results demonstrate advantages such as better sensitivity to glucose, fast current response, stability, and insensitivity to interferences.

## 2. Experimental details

### 2.1. Reagents and materials

A single side polished p-type silicon wafer with a resistivity of 2–9 Ω cm and thickness of 525 μm was used to fabricate the MCP. Hydrofluoric acid, dimethylformamide, $PdCl_2$, pyrrole, glucose, AA, UA and other chemical reagents were analytical grade and used without further purification. All of serum samples were kindly supplied by School of Life Science, East China Normal University. The solutions were prepared using >18 MΩ de-ionized water and all experiments were performed at room temperature in a clean environment.

### 2.2. Fabrication of OPPy modified palladium/silicon MCP electrode

#### 2.2.1. Preparation of silicon MCP

The silicon wafer was cleaned using a three-step wet chemical process before fabrication. A 300 nm thick thermally grown $SiO_2$ layer was generated as the masking layer. Photolithography was used to define 3 μm × 3 μm square open regions aligned on the front side and then wet etching in a buffered hydrofluoric acid (BHF) was used to open the etching windows. The BHF consisted of hydrofluoric acid (40%), ammonium fluoride and de-ionized water with the mass ratio of 1:2:3. Pyramidal notches serving as hole nucleation centers for anodizing were subsequently created by tetramethyl ammonium hydroxide (TMAH) (25 wt%) etching at 85 °C for 3 minutes. The silicon MCP was formed by photo-assisted electrochemical etching in the HF solution diluted with DMF and de-ionized water (HF (40%):$H_2O$:DMF = 1:4:5). The pH value adjusted by a small amount of hydrochloric acid was around 3. The current density used during etching was 4 mA cm$^{-2}$. After etching of 8 hours, the silicon microchannel plate layer was automatically removed from the substrate. Pores about 5 μm × 5 μm in size were distributed in a square lattice, and the spacing between two pores was about 1 μm. The thickness of the silicon MCP was about 200 μm. Afterwards, a thin nickel film was coated on the surface of MCP by electroless deposition and served as the electrically conductive layer. The thickness of the Ni layer was controlled by electroless deposition time. Details concerning the electroless deposition can be found elsewhere (Miao et al., 2009). After immersed in the plating bath for 20 minutes, the Ni thin films were coated onto the whole surface including transverse surface and inside wall of each channel homogenously.

**Table 1**
Materials and the conditions used in the electrodepositing palladium process.

| Chemicals name | Concentration (M) | Main function |
|---|---|---|
| Palladium dichloride | 1.5 m | Pd source |
| Ammonium chloride | 0.38 | Conductive salt |
| Ethylene diamine tetraacetic acid | 0.25 | Complexing agent |
| Ammonia | *X* | Adjust pH 7–9 |

#### 2.2.2. Electrodeposition of palladium nanoparticles

The silicon MCP with the nickel layer inside the wall was cut into 0.5 cm × 0.5 cm pieces. Prior to electrodeposition, the silicon MCP was dipped in a solution of Triton X-100 for at least 3 minutes to enhance its hydrophilicity. A uniform distribution of palladium nanoparticles was obtained on the sidewall of silicon MCP by electrodepositing. The chemical ingredients in the plating solution and reaction conditions are shown in Table 1. The temperature of electrodepositing was kept at 55 °C in thermostatically controlled water bath and the current density was 50 mA cm$^{-2}$. The palladium/silicon MCP was then annealed at 400 °C for 60 s under nitrogen. Copper wires were connected to the MCP by silver conductive adhesive to construct the electrode for subsequent electrochemical experiments.

#### 2.2.3. Preparation of OPPy modified palladium/silicon MCP

Electrodeposition of OPPy was performed on a conventional three-electrode electrochemical workstation. A platinum wire was used as the counter electrode whereas a saturated calomel electrode (SCE) served as the reference electrode. The palladium/silicon MCP was immersed in a solution containing 0.05 M pyrrole, 0.02 M $K_3Fe(CN)_6$, and 0.02 M $H_2SO_4$. The PPy film was formed electrochemically on the surface of the channels by cyclic voltammetry. The potential ranged from −0.1 to 0.7 V and the scanning rate was 100 mV s$^{-1}$. The scanning circles which determined the thickness of the PPy film were three. Afterwards, the PPy film was over-oxidized by a potential scanned from −0.3 to 0.95 V at a scanning rate of 30 mV s$^{-1}$ for seven circles in a 0.1 M $Na_2HPO_4$ solution. Finally, the OPPy modified palladium/silicon MCP was used for non-enzymatic determination of glucose. In addition, a bare palladium/silicon MCP electrode and an OPPy modified palladium/silicon planar electrode were prepared in a similar way as controls.

#### 2.2.4. Characterization

The images of prepared OPPy modified palladium/silicon MCP arrays were acquired using a scanning electron microscope (SEM, JSM 5610) at 10 kV. XRD patterns were obtained on an X-ray diffractometer with Cu $K_\alpha$ radiation. Electrochemical measurements were carried out on a CHI 830B electrochemical workstation (Shanghai Chenhua, China) consisting of an SCE reference electrode, a platinum wire as the counter electrode, and a modified MCP as the working electrode.

#### 2.2.5. The detection of glucose in serum samples

0.10 mL of serum samples (newborn bovine serum and horse serum) were diluted with 10.0 mL of 0.10 M KOH solution, and the current response was measured at +0.08 V by the prepared sensor. At the same time, glucose in the serum sample was also detected by using a blood glucose monitor (SmartScan$^{TM}$, LifeScan).

## 3. Results and discussion

### 3.1. Characterization of the OPPy modified Pd/silicon MCP electrode

The porous array of silicon MCP has a uniform and highly interconnected network of micropores with a pore diameter of around

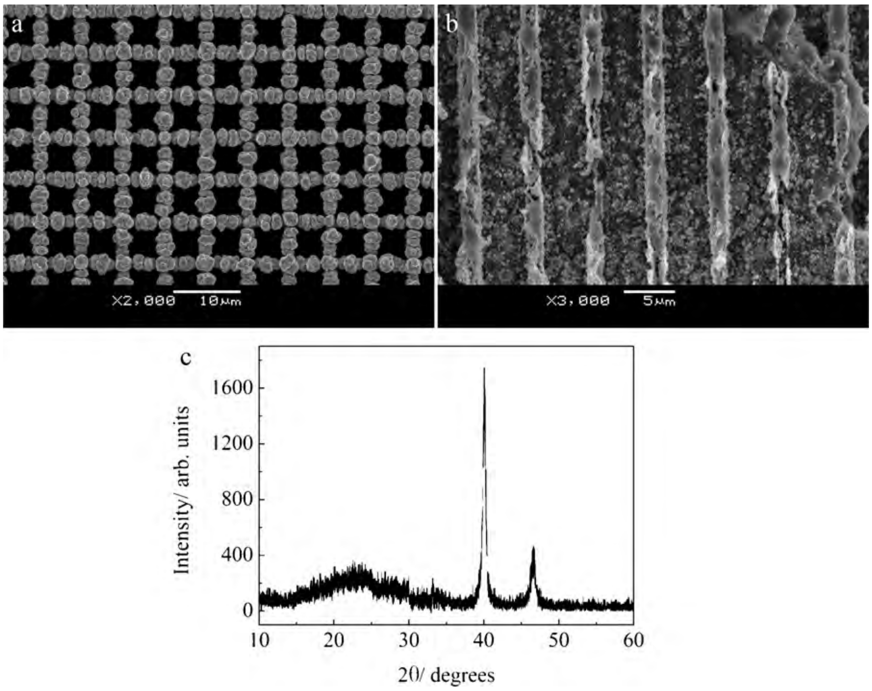

**Fig. 1.** SEM image of OPPy modified Pd/Si MCP electrode: (a) top view, (b) cross section, and (c) X-ray diffraction pattern of the OPPy modified Pd/Si MCP electrode.

5.0 µm and the thickness of sidewall is less than 1.0 µm. This structure has the apparent advantage of a large real surface area which provides more surface sites for electrocatalytic reactions. Compared with the planar electrode, MCP can increase surface area 56 times more. Fig. 1(a) and (b) depicts the top view and cross section of the modified silicon MCP, respectively. A uniform coating can be observed on the sidewalls. The XRD pattern of the layer in Fig. 1(c) shows a broad diffraction peak around 22° which can be attributed to the PPy in an amorphous state and the peaks with $2\theta$ of 40.12° and 46.66° correspond to diffraction from the {1 1 1} and {2 0 0} planes of Pd, respectively. The results clearly indicate that the layer deposited on the surface of the silicon MCP is PPy and palladium and the crystallinity of the palladium nanoparticles is not influenced by the PPy.

### 3.2. Electrocatalytic oxidation of glucose

Oxidation of glucose on the OPPy modified palladium/silicon and palladium/silicon MCP electrodes is investigated in an alkaline medium by CV. Fig. 2(a) shows the cyclic voltammograms (CVs) obtained from the OPPy modified palladium/silicon MCP electrode in the absence (the dash curve) and presence (the solid curve) of 10.0 mM glucose in 0.10 M KOH solution at a scan rate of 10 mV s$^{-1}$. In the blank alkaline solution, there is a broad oxidation peak in the range −0.2 V to 0.4 V which corresponds to the palladium oxide formation region. Two reduction peaks at +0.04 V and −0.45 V are observed from the cathodic scan. The peaks may correspond to the reduction of palladium oxide. After adding 10.0 mM glucose, the changes in the CV are quite pronounced exhibiting a complicated electrochemical behavior consistent with the nonenzymatic oxidation of glucose reported previously (Cui et al., 2007; Li et al., 2009; Myung et al., 2009). There are two anodic peaks in the positive scan due to oxidation of glucose catalyzed by palladium nanoparticles at potentials of −0.22 V and +0.08 V, respectively. The first oxidation peak arises from intermediates formed by electrochemical adsorption of glucose. Each glucose molecule releases a proton during the adsorption process and generates an electrochemical adsorption current. As decomposition of glucose proceeds, more adsorbed intermediates accumulate on the surface of the OPPy modified palladium/silicon MCP electrode and occupy active sites on the electrode surface, thereby hampering further electrochemical adsorption of glucose and subsequent direct oxidation. This

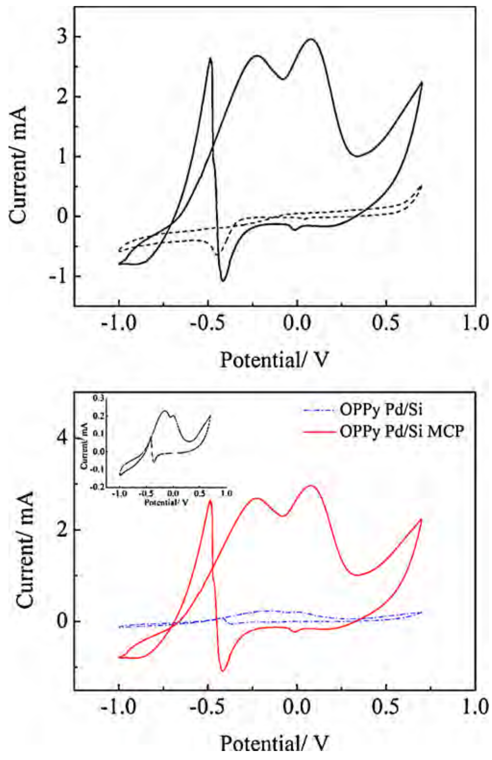

**Fig. 2.** (a) Cyclic voltammograms of OPPy modified Pd/Si MCP electrode in the absence (the dash curve) and presence (the solid curve) of 10.0 mM glucose in 0.10 M KOH solution at a scan rate of 10 mV s$^{-1}$. (b) Cyclic voltammograms of OPPy modified Pd/Silicon MCP electrode and planar Pd/Silicon electrode in the presence of 10.0 mM glucose in 0.10 M KOH solution at scanning rate of 10 mV s$^{-1}$. The inset shows the amplified CV curve obtained by using a planar electrode.

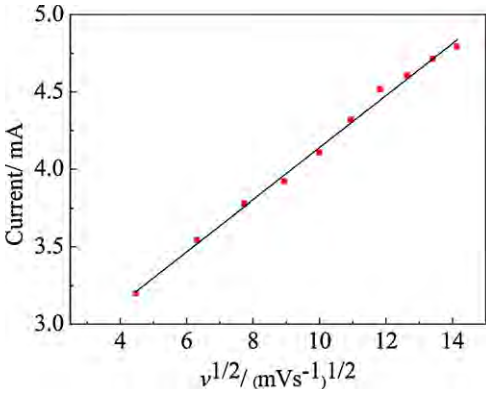

**Fig. 3.** The dependence of oxidation peak current on the potential scan rates for OPPy modified Pd/Si MCP electrode in 0.10 M KOH solution containing 10.0 mM glucose.

reduces the current showing a positive shift in the potential. As the potential becomes more positive, hydroxide ions partially discharge on the electrode surface and catalyze oxidation of the intermediates. Therefore, the active sites on the palladium are exposed resulting in direct oxidation of glucose oxidation followed by a larger current when the electrode potential is increased. The anodic peak occurring at about +0.08 V can be ascribed to the direct oxidation process. Palladium oxide is formed on the surface of the palladium nanoparticles at a more positive electrode potential. Formation of this oxide degrades the catalytic properties of the palladium nanoparticles which hinders electrooxidation of glucose. In the negative potential scan, sufficient active sites are released with the reduction of the surface oxide enabling direct oxidation of glucose. A sharp oxidation current peak is formed in the vicinity of −0.48 V. These results indicate that the OPPy modified palladium/silicon MCP electrode has good electrocatalytic activity for oxidation of glucose (Meng et al., 2009).

To investigate the effectiveness of the silicon MCP structure in the OPPy modified palladium/silicon MCP electrode on the catalysis of glucose electrooxidation, a control experiment has been done by using OPPy modified planar palladium/silicon electrode. The obtained CV in the presence of glucose is shown in Fig. 2(b) (dot line). For comparison, The CV obtained by OPPy-Pd/silicon MCP electrode is also shown in Fig. 2(b). It can be clearly seen that the oxidation behavior of glucose on the OPPy modified planar Pd/silicon electrode is similar to that on the OPPy-Pd/silicon MCP electrode, but the oxidation current diminishes drastically. The results reveal that the microchannel structure plays an important role in the enhanced current response to glucose. This 3D structure offers a larger surface area and more active sites for glucose oxidation facilitating electron transfer.

The dependence of the peak current at potential of +0.08 V on the potential scan rate for OPPy modified Pd/Si MCP electrode in 0.10 M KOH solution containing 10.0 mM glucose is shown in Fig. 3. The current is proportional to the square root of the scan rate, indicating that electrocatalytic oxidation of glucose is mainly diffusion-controlled at the modified electrode. However, the decay after the peak is due to an inhibition effect as we mentioned before. The observation of an anodic peak in the reverse scan also indicates this inhibition effect. Therefore, more detailed investigation will conduct to elucidate the electrode reaction mechanism of the glucose oxidation.

### 3.3. Sensitivity and linearity

The current response is ideal for quantitative determination of glucose and so the steady state amperometric response of the OPPy-Pd/silicon MCP electrode to glucose is measured by successive addition of 1.0 mM glucose in 0.1 M KOH solution at a fixed potential of +0.08 V. The current versus time response of the OPPy-Pd/silicon MCP electrode to glucose is shown in Fig. 4. The time required to reach 95% of the steady state value is less than 5 s after addition of glucose. The inset in Fig. 4 is the calibration curve for glucose. In a concentration range from 1 mM to 24 mM, the current is linear to the glucose concentration and the regression equation is $y\,(\mathrm{mA}) = 8.56 \times 10^{-3} + 9.186 \times 10^{-2}x\,(\mathrm{mM})$ with a correlation coefficient of 0.997. The linear range of the OPPy-Pd/silicon MCP electrode is up to 24 mM and the detection limit is estimated to be 2.06 μM at a signal-to-noise ratio of 3. A performance comparison of our sensor with other enzymatic and nonenzymatic glucose sensors is summarized in Table 2. The OPPy modified Pd/Si MCP electrode has obviously higher sensitivity and wider linear range than those reported previously.

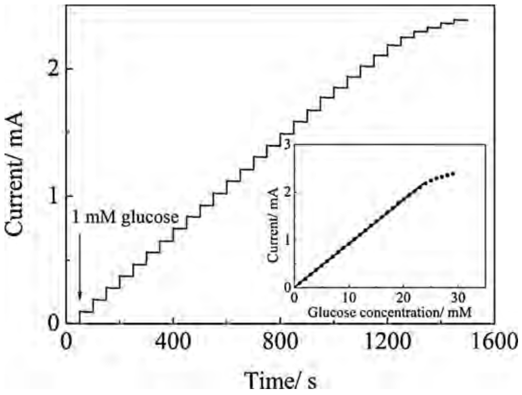

**Fig. 4.** Steady state amperometric response of the OPPy modified Pd/Si MCP electrode to successive addition of glucose at an applied potential of +0.08 V. The inset: the calibration curve of the response current as a function of the glucose concentration.

### 3.4. Elimination of interferences

The selectivity of nonenzymatic glucose detection is affected by several interfering substances such as AA, UA, etc. They can be oxidized on the electrode surface producing interfering electrochemical signals. In this work, the OPPy film is introduced to eliminate interferences caused by these organic species while ensuring normal current response during glucose oxidation. After addition of various concentrations of UA and AA, the interfering amperometric responses relative to the current at a fixed potential of 1.0 mM glucose are determined from the OPPy-Pd/silicon MCP electrode and bare palladium/silicon MCP electrode and compared, as shown in Fig. 5. Each value is the average of three independent measurements. The results indicate that the current response of UA measured from OPPy-Pd/silicon MCP electrode is only 0.14–1.66% of that of glucose. The current response of AA determined from the OPPy-Pd/silicon MCP electrode is also much lower than that measured from the bare palladium/silicon MCP electrode. The overall absolute currents for glucose and for the interferents are both reduced by the generation of the OPPy film. This has some influence on the sensitivity of sensor. But the reduction of response for interferents is more obvious relative to the overall absolute currents. The selectivity is greatly improved due to the permselectivity of the OPPy film and consequently, these interferences are not substantial. The over oxidized PPy film loses its electronic conductivity because the doped anions are expelled from the membrane during peroxidation and leave the porous structure. The electrolyte and electroactive substances in the solution can penetrate the OPPy film and diffuse to the electrode surface and so the OPPy film still

**Table 2**
Comparison of some basic parameters for glucose sensors.

| Working electrode | Detection potential (V) | Sensitivity ($\mu$A mM$^{-1}$ cm$^{-2}$) | Detection limit ($\mu$M) | Linear range (mM) | References |
|---|---|---|---|---|---|
| Pd-SMNT[a] | −0.35 | ~160 | 0.2 ± 0.05 | 0.5–17 | Meng et al. (2009) |
| PtPb-CNT[b]s | −0.15 | 18 | 7 | Up to 5 mM | Qiu et al. (2007) |
| CuO nanowires | +0.33 | 0.49 | 0.049 | $4 \times 10^{-4}$–2 | Zhuang et al. (2008) |
| Porous Au | +0.35 | 11.8 | 5 | 2–10 | Li et al. (2007) |
| Pb NC[c]-CNT[b] | +0.50 | 280 | 0.2 | 0.2–12 | Myung et al. (2009) |
| GOx-ZnO/MWNTs[d] | −0.10 | 50.2 | 0.25 | 0.1–16 | Wang et al. (2009a,b) |
| GOx-HRP[e]/SBA-15[f] | −0.40 | 90 | 0.27 | $3 \times 10^{-3}$–34 | Dai et al. (2008) |
| GOx-MWNTs[d] | 0.35 | 25 | 3.6 | $1.2 \times 10^{-2}$–3.8 | Qiu et al. (2009) |
| OPPy Pd/Si MCP | +0.08 | 367 | 2.06 | Up to 24 mM | This work |

[a] SWNTs: single-walled carbon nanotubes.
[b] CNT: carbon nanotube.
[c] NC: nanocrystal.
[d] MWNTs: multi-walled carbon nanotubes.
[e] HRP: horseradish peroxidase.
[f] SBA-15: mesoporous silica structures.

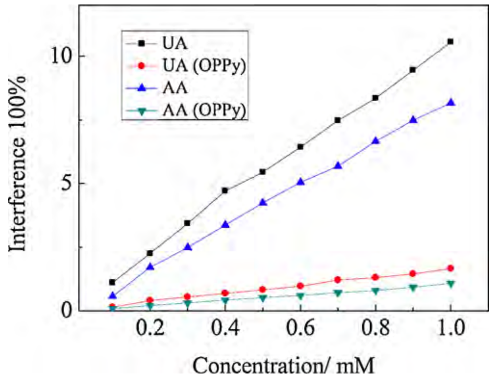

**Fig. 5.** Selectivity profile of the electrode over interfering agents of UA and AA obtained at different concentrations.

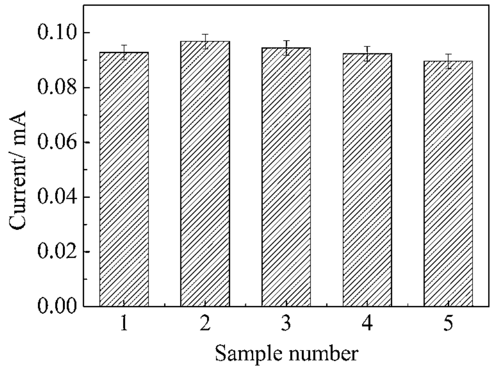

**Fig. 6.** Current response to 1.0 mM glucose measured from 5 different electrodes at a fixed potential of +0.08 V.

has ionic conductivity. The OPPy film has a number of negatively charged carboxyl groups produced in the over oxidation process and hence, it is permeable to cations and neutral molecules but impedes the diffusion of anions. In the alkali medium, UA and AA exist as anions but glucose exists mainly in the molecular form (Woolley et al., 1972). Therefore, the OPPy-Pd/silicon MCP electrode shows excellent selectivity for glucose detection in spite of the presence of interferences.

### 3.5. *Reproducibility and stability*

To investigate the reproducibility of the OPPy-Pd/silicon MCP electrode, the response to 1.0 mM glucose was measured from 5 electrodes at a fixed potential of +0.08 V, as shown in Fig. 6. The relative standard deviation (RSD) is 2.87% which confirms good reproducibility. The stability of the electrode is verified by measuring the current in ten successive measurements and the RSD is 1.48% for these ten measurements. The long-term stability of the sensor is also assessed by measuring the current after 30 days storage at room temperature and it is approximately 92% of its initial value which is reasonably good. The decrease of current response is probably due to the swelling of OPPy film or partly lost of Pd catalytic property during the storage. Compared to enzyme glucose sensor, the storage condition of our sensor is much more convenient and costless. These results suggest that the OPPy-Pd/silicon MCP electrode is a reliable and stable nonenzymatic sensor for detection of glucose.

### 3.6. *Glucose detection in serum samples*

To verify the practical application of the OPPy-Pd/silicon MCP electrode, the sensor was used for determination of glucose in real serum samples. The measured values were compared with those determined by a commercial blood glucose monitoring system. The results are given in Table 3. It can be clearly seen that the results obtained by the proposed OPPy-Pd/silicon MCP electrode are in good agreement with those obtained by the commercial monitor, indicating that this nonenzymatic glucose sensor has potential applications for the analysis of glucose in real biological samples.

**Table 3**
Determination of glucose concentration in the real samples by the OPPy-Pd/Si MCP electrode.

| Sample | Blood glucose monitor (mM) | OPPy-Pd/Si MCP electrode (mM) | RSD[c] (%) | Added (mM) | Recovery (%) |
|---|---|---|---|---|---|
| 1[a] | 5.3 | 5.15 | 3.62 | 0.1 | 96 |
| 2[a] | 5.1 | 5.04 | 5.13 | 0.1 | 97 |
| 3[b] | 6.5 | 6.28 | 3.84 | 0.1 | 103 |

[a] Newborn bovine serum.
[b] Horse serum.
[c] RSD calculated from five measurements for each sample.

## 4. Conclusion

The OPPy-Pd/silicon MCP electrode fabricated electrochemically boasts a number of advantages such as high sensitivity, good stability, reproducibility, and quick response. Excellent selectivity is rendered by the OPPy film and signals from oxidation of common interfering species such as UA and AA can be effectively suppressed. The excellent performance is attributed to a combination of the larger electroactive surface area resulting from 3D structure of silicon MCP and permselectivity due to the OPPy film. Our results show that the OPPy-Pd/silicon MCP electrode is promising in nonenzymatic glucose determination.

## Acknowledgements

This work was supported by Shanghai Fundamental Key Project under contract number 08JC1408900, 10JC1404600 and international collaboration project under grant 10520704400. This work was also financially supported by the China NSFC support under the contract of Grant No 50672027 and No. 60990312, Hong Kong Research Grants Council (RGC) General Research Funds (GRF) No. CityU 112608, and City University of Hong Kong Strategic Research Grant (SRG) No. 7008009. The authors would like to extend sincere thanks to Prof. Chonggang Yuan (School of Life Science, East China Normal University) for providing the serum samples.

## References

Chen, X.M., Lin, J.L., Yuan, D., Ci, P.L., Xin, P.S., Xu, S.H., Wang, L.W., 2008. J. Micromech. Microeng. 18, 037003.

Cui, H.F., Ye, J.S., Zhang, W.D., Li, C.M., Luong, J.H.T., Sheu, F.S., 2007. Anal. Chim. Acta 594, 175–183.

Dai, Z.H., Bao, J.C., Yang, X.D., Ju, H.X., 2008. Biosens. Bioelectron. 23, 1070–1076.

Du, P., Zhou, B., Cai, C.X., 2008. J. Electroanal. Chem. 614, 149–156.

Farrell, S.T., Breslin, C.B., 2004. Electrochim. Acta 49, 4497–4503.

Gavalas, V.G., Law, S.A., Ball, J.C., Andrews, R., Bachas, L.G., 2004. Anal. Biochem. 329, 247–252.

Gerard, M., Chaubey, A., Malhotra, B.D., 2002. Biosens. Bioelectron. 17, 345–359.

Hrapovic, S., Liu, Y., Male, K.B., Luong, J.H.T., 2004. Anal. Chem. 76, 1083–1088.

Kandimalla, V.B., Tripathi, V.S., Ju, H., 2006. Biomaterials 27, 1167–1174.

Lee, S.R., Lee, Y.T., Sawada, K., Takao, H., Ishida, M., 2008. Biosens. Bioelectron. 24, 410–414.

Li, J., Lin, X., 2007. Sens. Actuators B 124, 486–493.

Li, X., Zhu, Q., Tong, S., Wang, W., Song, W., 2009. Sens. Actuators B: Chem. 136, 444–450.

Li, Y., Song, Y.Y., Yang, C., Xia, X.H., 2007. Electrochem. Commun. 9, 981–988.

Lin, Y., Cui, X., Ye, X., 2005. Electrochem. Commun. 7, 267–274.

Majidi, M.R., Jouyban, A., Asadpour-Zeynali, K., 2006. J. Electroanal. Chem. 589, 32–37.

Meng, L., Jin, J., Yang, G.X., Lu, T.H., Zhang, H., Cai, C.X., 2009. Anal. Chem. 81, 7271–7280.

Merkoci, A., Pumera, M., Llopis, X., Perez, B., del Valle, M., Alegret, S., 2005. Trac-Trends Anal. Chem. 24, 826–838.

Miao, F.J., Tao, B.R., Sun, L., Liu, T., You, J.C., Wang, L.W., Chu, P.K., 2009. Sens. Actuators B: Chem. 141, 338–342.

Musameh, M., Wang, J., Merkoci, A., Lin, Y., 2002. Electrochem. Commun. 4, 743–746.

Myung, Y., Jang, D.M., Cho, Y.J., Kim, H.S., Park, J., Kim, J.U., Choi, Y., Lee, C.J., 2009. J. Phys. Chem. C 113, 1251–1259.

Newman, J.D., Turner, A.P.F., 2005. Biosens. Bioelectron. 20, 2435–2453.

Qiu, J.D., Zhou, W.M., Guo, J., Wang, R., Liang, R.P., 2009. Anal. Biochem. 385, 264–269.

Qiu, R., Zhang, X.L., Qiao, R., Li, Y., Kim, Y., Kang, Y.S., 2007. Chem. Mater. 19, 4174–4180.

Salimi, A., Roushani, M., 2005. Electrochem. Commun. 7, 879–887.

Shen, Q., Jiang, L., Zhang, H., Min, Q., Hou, W., Zhu, J.J., 2008. J. Phys. Chem. C 112, 16385–16392.

Sun, Y., Buck, H., Mallouk, T.E., 2001. Anal. Chem. 73, 1599–1604.

Tian, F.M., Zhu, L.D., Xu, B., Zhu, G.Y., 2001. Chin. Chem. Lett. 12, 1011–1022.

Vidal, J.C., Garcia, E., Castillo, J.R., 1999. Sens. Actuators B 57, 219–226.

Wang, Y.T., Yu, L., Zhu, Z.Q., Zhang, J., Zhu, J.Z., Fan, C.H., 2009a. Sens. Actuators B: Chem. 136, 332–337.

Wang, Z., Liu, S., Wu, P., Cai, C.X., 2009b. Anal. Chem. 81, 1638–1645.

Wilson, R., Turner, A.P.F., 1992. Biosens. Bioelectron. 7, 165–185.

Woolley, E.M., Tomkins, J., Hepler, L.G., 1972. J. So1ution Chem. 1, 341–351.

Wu, L., Zhang, X., Ju, H., 2007. Biosens. Bioelectron. 19, 141–147.

Yang, M., Yang, Y., Liu, Y., Shen, G., Yu, R., 2006a. Biosens. Bioelectron. 21, 1125–1131.

Yang, M., Yang, Y., Yang, H., Shen, G., Yu, R., 2006b. Biomaterials 27, 246–255.

Yuan, D., Ci, P.L., Tian, F., Shi, J., Xin, P.S., Xu, S.H., Wang, L.W., 2009. J. Micro/Nanolith. MEMS MOEMS 8, 033012.

Zhang, N., Wilkop, T., Lee, S., Cheng, Q., 2007. Analyst 132, 164–172.

Zhao, H.T., Ju, H.X., 2006. Anal. Biochem. 350, 138–144.

Zhao, J., Wang, F., Yu, J., Hu, S., 2006. Talanta 70, 449–454.

Zhao, M., Wu, X., Cai, C.X., 2009. J. Phys. Chem. C 113, 4987–4996.

Zhuang, Z.J., Su, X.D., Yuan, H.Y., Sun, Q., Xiao, D., Choi, M.M.F., 2008. Analyst 133, 126–132.