Colloids and Surfaces B: Biointerfaces xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Gold nanoparticles coated zinc oxide nanorods as the matrix for enhanced L-lactate sensing

Yanguang Zhao[a,1], Xiaofei Fang[a,1], Yousong Gu[a], Xiaoqin Yan[a,*], Zhuo Kang[a], Xin Zheng[a], Pei Lin[a], Leichao Zhao[a], Yue Zhang[a,b,**]

[a] State Key Laboratory for Advanced Metals and Materials, School of Materials Science and Engineering, University of Science and Technology Beijing, Beijing 100083, People's Republic of China
[b] Key Laboratory of New Energy Materials and Technologies, University of Science and Technology Beijing, Beijing 100083, People's Republic of China

ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form
24 December 2014
Accepted 30 December 2014
Available online xxx

Keywords:
Zinc oxide
Gold nanoparticles
Electrochemical biosensor
L-Lactate
Lactate oxidase

ABSTRACT

In this study, an enzymatic electrochemical biosensor for L-lactate detection was proposed. The device was developed based on gold nanoparticles (Au NPs) modified zinc oxide nanorods (ZnO NRs). The sensing performance of the device was examined by cyclic voltammetry and amperometry. Compared with pristine ZnO based biosensor, Au/ZnO based sensor exhibited higher sensitivity of 24.56 μA cm^{-2} mM^{-1}, smaller K_M^{app} of 1.58 mM, lower detection limit of 6 μM and wider linear range of 10 μM–0.6 mM for L-lactate detection. The introduction of Au NPs enhances electro-catalytic ability and electron migration, which contributes to the improvement of the sensing performance. Hence, the results confirm the essential character of Au NPs in such semiconductor based electrochemical biosensing system.

1. Introduction

L-lactate, a biochemical compound produced from pyruvate, is an important analyte in clinical analysis, food industry, etc. Consequently, measurement of lactate is of great importance [1]. Chromatography and spectrophotometry have been reported for the determination of L-lactate [2,3]. However, those methods are time-consuming, costly, nonselective or need large sample quantities. Electrochemical enzyme biosensing is an alternative candidate which is inexpensive, rapid, well selective and reliable for L-lactate detection [4].

To date, one-dimensional nanomaterials have attracted great attention due to their high surface-to-volume ratio and novel electron transport properties. Nanobiosensors based on carbon nanotubes [5,6], silicon nanowires [7,8], conducting polymer nanowires [9], gold nanowires/nanorods [10,11], copper oxide nanowires [12], have been investigated and reported previously. However, the unstable surfaces of silicon nanowires, the chirality and defects of carbon nanotubes may degrade device reliability and sensitivity, and gold nanowires/nanorods based biosensors are costly [13]. Besides, both silicon nanowires and carbon nanotubes require harsh conditions for synthesis, and conducting polymer nanowires are mechanically weak and likely to be broken easily [14].

Given the advantages of large specific surface area, high isoelectric point (9.5), excellent charge transport abilities and good biocompatibility of one-dimensional ZnO nanomaterials [4], they have been applied to the biosensing of glucose [15,16], lactic acid [4,17], uric acid [18], etc.

Noble metal nanoparticles adopted in biosensing have aroused much interest because of their excellent surface-enhanced Raman scattering, enhanced electron transfer rate and minimized overpotential, as well as high electrochemical activity [19–21]. Lu [21] has designed a palladium NPs/graphene based nonenzymatic electrode, which displays a very high electrochemical activity for electro-catalytic oxidation of glucose. Wang [22] has developed a hydrogen peroxide biosensor based on nanosilver/DNA, and the sensor exhibits marked catalytic activity in the presence of silver nanoparticles. Lamas-Ardisana [23] has introduced platinum NPs

* Corresponding author. Tel.: +86 10 62334725; fax: +86 10 62333113.
** Corresponding author at: State Key Laboratory for Advanced Metals and Materials, School of Materials Science and Engineering, University of Science and Technology Beijing, Beijing 100083, People's Republic of China; Key Laboratory of New Energy Materials and Technologies, University of Science and Technology Beijing, Beijing 100083, People's Republic of China. Tel.: +86 10 62334725; fax: +86 10 62333113.
E-mail addresses: xqyan@mater.ustb.edu.cn (X. Yan), yuezhang@ustb.edu.cn (Y. Zhang).
[1] These authors contriuted equally to the work.

http://dx.doi.org/10.1016/j.colsurfb.2014.12.053
0927-7765/

into carbon nanofibers based lactate biosensor, which results in efficient electro-catalysis towards H_2O_2 oxidation and high sensitivity of the device. Wu [24] has proposed a glucose biosensor based on multilayer films composed of multi-wall carbon nanotubes, Au NPs and glucose oxidase, and the structure demonstrated effective low-potential amperometric detection of glucose. However, systematic analysis for the modification effect of Au NPs on L-lactate biosensor has rarely been studied and reported.

In this work, Au nanoparticles were introduced to the ZnO nanorods based L-lactate biosensor due to their excellent electrocatalytic property, outstanding electron transport property and favorable bio-safety. The performance of pristine ZnO and Au/ZnO based sensor was systematically compared and investigated. Simultaneously, the effect of Au NPs was discussed accordingly.

2. Materials and methods

2.1. Reagents and chemicals

L-lactate (90% solution in water) was purchased from *Acros Organics*. Lactate oxidase (LOx, from *Pediococcus*, lyophilized powders, ≥20 units mg^{-1} solid), Nafion solution (5 wt.%) and hexamethylenetetramine (HMTA) were obtained from *Sigma*. All the reagents were of analytical grade and used without further purification. Phosphate buffer saline (PBS) of 0.1 M was prepared by dissolving appropriate quantity of Na_2HPO_4 and NaH_2PO_4 into deionized water, and the pH was adjusted to 7.4.

2.2. Preparation and characterization of Au/ZnO composites

Aligned ZnO NR arrays on fluorine-doped tin oxide (FTO) glass were grown in a 50 mL aqueous solution of equimolar (0.05 M) zinc nitrate and HMTA at 95 °C for 16 h. Au NPs were synthesized by photo-reduction method [25] and adsorbed onto ZnO via electrostatic self-assembly. First, 0.05% $HAuCl_4$ aqueous solution, 1% polyvinyl alcohol solution (dispersant) and methanol were mixed together with a suitable ratio. The pH of the mixed solution was adjusted to 8 by NaOH. FTO glasses with ZnO NR arrays were then immersed in the solution and irradiated for 15 min by an ultra-vitalux lamp (OSRAM, 300 W). After placed for 6 h, the ZnO NRs were modified with Au NPs via electrostatic adsorption. Finally, the samples were annealed at 350 °C in air for 3 h to remove the capped polyvinyl alcohol. Hereto, Au NPs/ZnO NR arrays were successfully prepared.

The synthesized Au/ZnO nanocomposites were characterized by field-emission scanning electron microscope (FE-SEM, SUPRA-55, Zeiss), transmission electron microscopy (TEM, JSM 2010, JEOL), energy dispersive spectrometer (EDS, Inca X-Max, Oxford Instruments) and X-ray photoelectron spectrum analyzer (XPS, AXIS Ultra DLD, Kratos).

2.3. Construction and performance test of L-lactate biosensor

ZnO NRs and Au NPs/ZnO NRs were then transferred to the columnar Au electrodes, respectively. Here, all the standard gold electrodes we used are with a diameter of 2 mm, and the effective working area is therefore calculated to be 3.1416 mm^2. The fabrication of the bioelectrodes was previously reported [26]. A series of electrochemical experiments were performed on an electrochemical workstation (SI 1287, Solartron) with a conventional three-electrode system at room temperature. A platinum wire behaved as the auxiliary electrode and an Ag/AgCl electrode acted as the reference electrode. The fabricated Nafion/LOx/ZnO/Au or Nafion/LOx/Au@ZnO nanocomposites/Au electrode was the working electrode.

3. Results and discussion

3.1. Characterization of synthesized products

Fig. 1a–c show the morphology of synthesized ZnO NR arrays and Au NPs/ZnO NR arrays. The ZnO NRs have uniform morphology

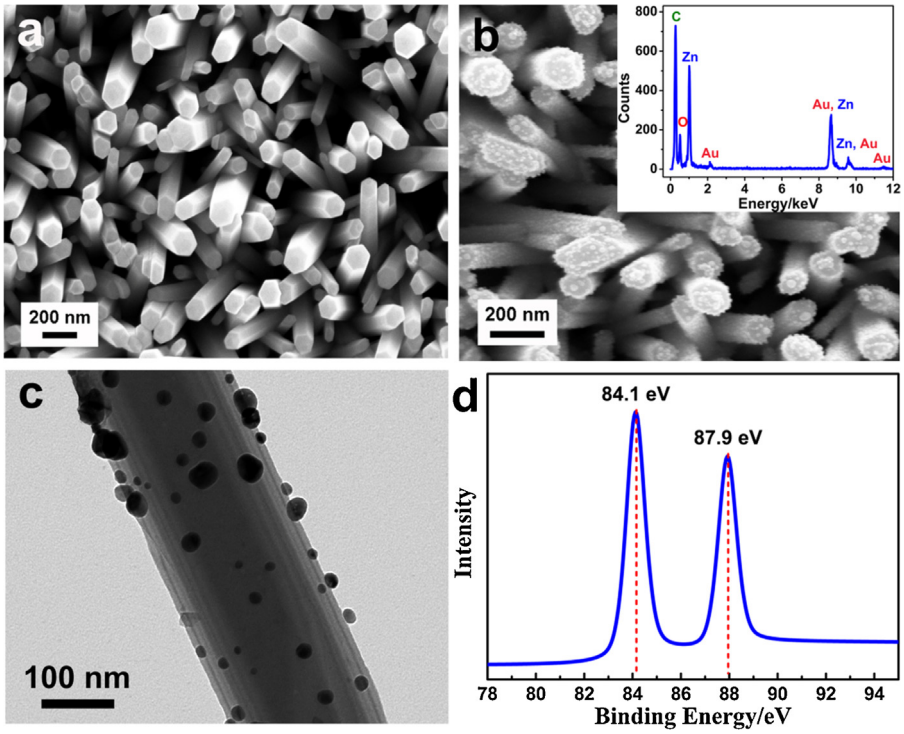

Fig. 1. (a) FE-SEM image of ZnO NR arrays grown on FTO glass; FE-SEM image (b) and EDS pattern (inset of b) of Au NPs/ZnO NR arrays on FTO; (c) TEM image of Au NPs/ZnO NR; (d) XPS spectrum of Au 4f region.

Please cite this article in press as: Y. Zhao, et al., Gold nanoparticles coated zinc oxide nanorods as the matrix for enhanced L-lactate sensing, Colloids Surf. B: Biointerfaces (2015), http://dx.doi.org/10.1016/j.colsurfb.2014.12.053

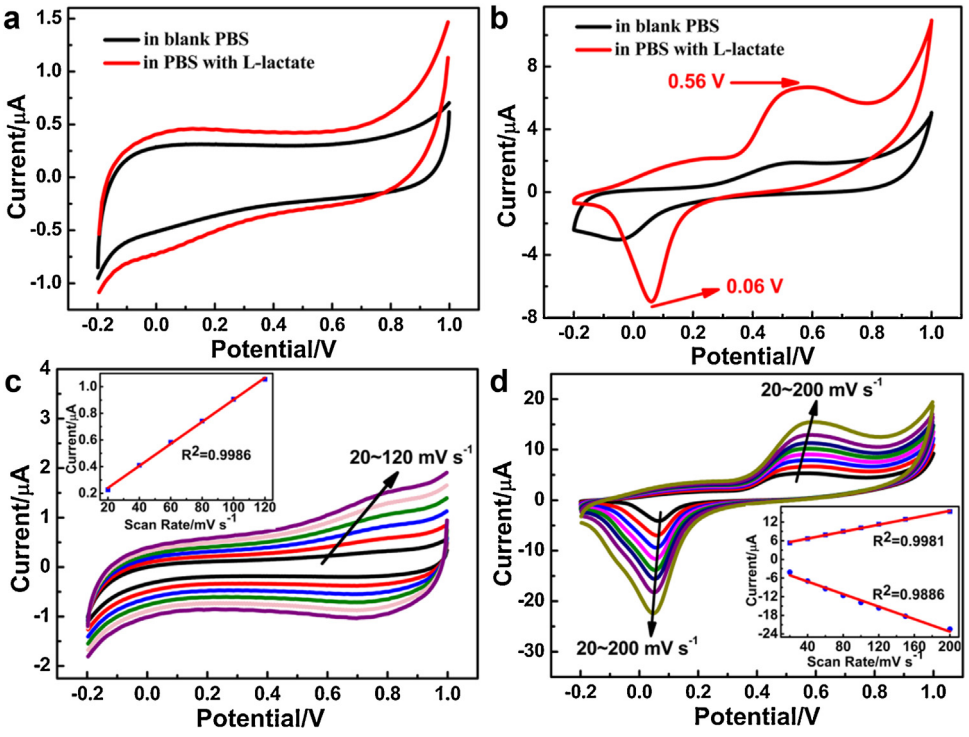

Fig. 2. Cyclic voltammetry characteristics of pristine ZnO NRs (a) and Au NPs/ZnO NRs (b) based electrode in PBS (black line) and 0.3 mM L-lactate (red line) at a scan rate of 50 mV s^{-1}; Cyclic voltammograms of pristine ZnO (c) and Au/ZnO (d) based electrode at various scan rates (with an interval of 20 mV s^{-1}) in 0.3 mM L-lactate, and insets of (c) and (d) depict corresponding curves of peak current *vs.* scan rate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with diameter of 100–200 nm, and Au NPs with average diameter of 10–18 nm were homogeneously absorbed onto the ZnO NRs. The EDS pattern in the inset of Fig. 1b demonstrates the existence of Au element except for Zn and O elements. The XPS spectrum of Au 4f region is shown in Fig. 1d, and the peaks of 84.1 and 87.9 eV are ascribed to metallic Au [27].

3.2. Cyclic voltammetry measurement

Cyclic voltammetry was firstly used to characterize the performance of ZnO nanorods based biosensors with and without Au modification. Fig. 2a displays the voltammetric curves of pristine ZnO NRs based electrode in PBS (0.1 M, pH = 7.4) in the absence and presence of 0.3 mM L-lactate. The response currents were larger for L-lactate than that for PBS, due to the oxidation reaction of L-lactate catalyzed by LOx [26]. For Au NPs/ZnO NRs based electrode, the current response to L-lactate increased dramatically in comparison to that of blank PBS, and a pair of redox peaks appeared at 0.56 V and 0.06 V, respectively (Fig. 2b). This can be attributed to the enhanced electro-catalytic ability of the bioelectrode facilitated by Au.

Cyclic voltammograms of the pristine ZnO and Au/ZnO based electrodes in 0.3 mM L-lactate were collected at different scan rates (Fig. 2c and d). The peak currents were enhanced and peak potentials shifted positively with the increase of scan rates. Moreover, peak currents were linear versus scan rates for both of pristine ZnO and Au/ZnO electrodes, demonstrating a surface-controlled process [28]. This suggests the Au/ZnO nanocomposite can be applied as an friendly immobilization matrix for LOx and effective electron transfer channel between the LOx and Au electrode [28].

3.3. Amperometric response

Fig. 3a displays the amperometric response of pristine ZnO NRs based biosensor to L-lactate with varying concentrations at +0.6 V. The response current step appeared for L-lactate of 10 μM (inset of Fig. 3a). Hence, the concentration of 10 μM is considered as the detection limit. The corresponding calibration curve is shown in Fig. 3b. The linear range is 0.02–0.6 mM with $R^2 = 0.9976$. The slope of the fitted line indicates that the sensitivity was 13.1 μA cm^{-2} mM^{-1}.

The amperometric curve of Au/ZnO based biosensor is shown in Fig. 3c. The detection limit is 6 μM (inset of Fig. 3c). The linear range is 0.01–0.6 mM ($R^2 = 0.9925$) with a sensitivity of 24.56 μA cm^{-2} mM^{-1} (Fig. 3d). Compared with prisitine ZnO based biosensors, Au/ZnO based devices exhibited higher sensitivity and lower detection limit. This is related to the excellent electrocatalytic property introduced by Au, which can facilitate the enzymatic reaction [29]. Moreover, the interfacial interactions between Au and ZnO lead to deficient electron population on Au, which facilitates the electron transfer from Au to ZnO and further enhances the electron migration between the enzymatic active sites and the electrode [30].

The apparent Michaelis–Menten constant (K_M^{app}, reflection of enzymatic affinity) can be calculated from the Lineweaver–Burk equation:

$$\frac{1}{i} = \frac{K_M^{app}}{i_{max}} \frac{1}{C} + \frac{1}{i_{max}}$$

where i is the current, i_{max} is the maximum current, and C is the L-lactate concentration. The calculated K_M^{app} values of ZnO and Au/ZnO based electrode are 1.82 and 1.58 mM, respectively. This implies the better affinity of the LOx to L-lactate in the presence of Au NPs.

3.4. Interference, stability and reproducibility study

Furthermore, electroactive species like glucose, uric acid (UA) and ascorbic acid (AA) were adopted in the interference

Please cite this article in press as: Y. Zhao, et al., Gold nanoparticles coated zinc oxide nanorods as the matrix for enhanced L-lactate sensing, Colloids Surf. B: Biointerfaces (2015), http://dx.doi.org/10.1016/j.colsurfb.2014.12.053

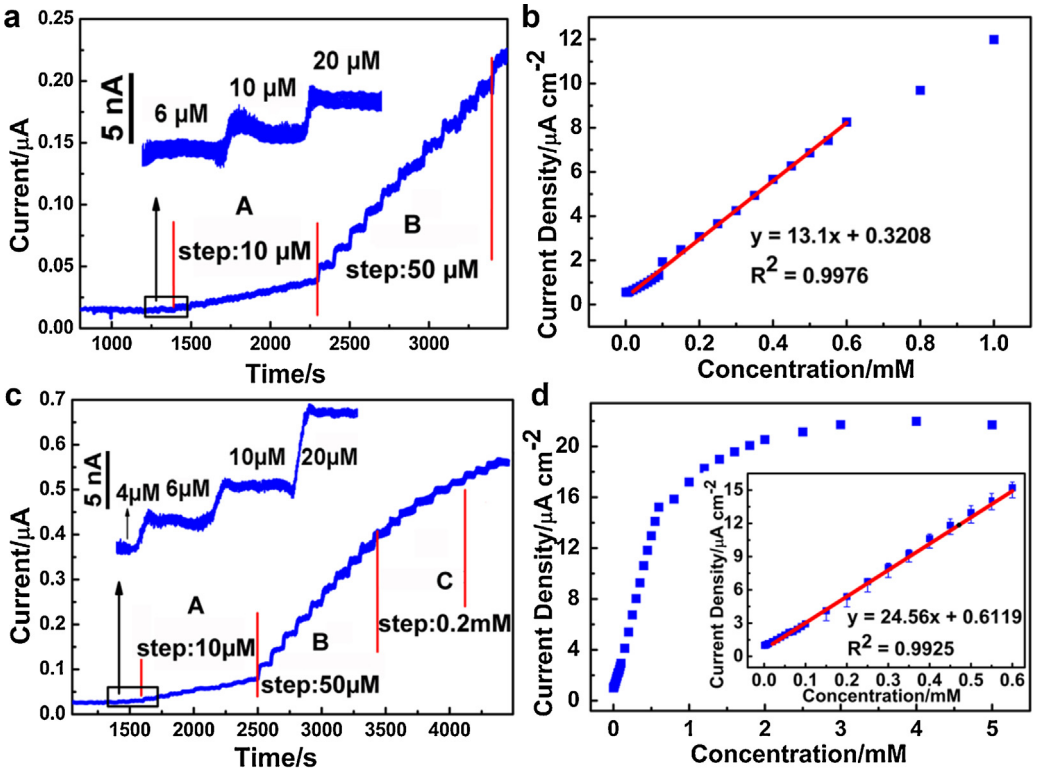

Fig. 3. The amperometric response of ZnO NRs (a) and Au NPs/ZnO NRs (c) based biosensor in PBS for L-lactate of different concentrations at an applied potential of +0.6 V, and the response current *vs.* concentration (insets); corresponding calibration curve and linear fitting curve of pristine ZnO (b) and Au/ZnO (d) based biosensor.

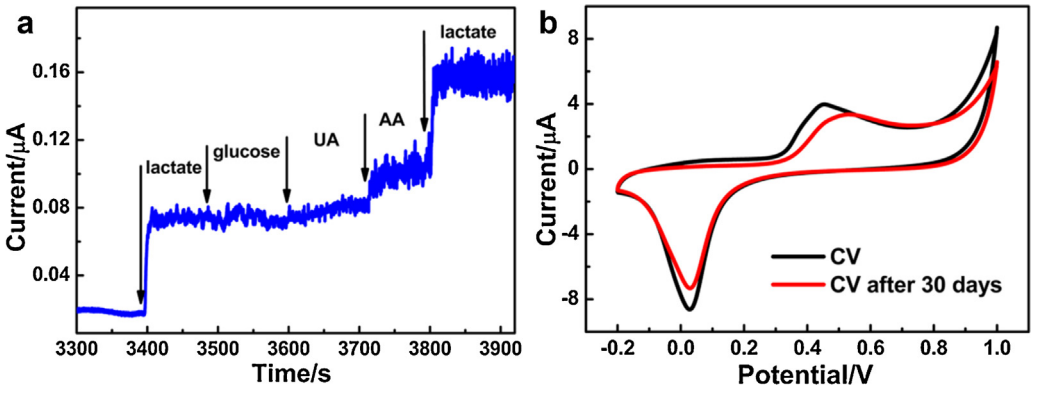

Fig. 4. (a) Selectivity study of Au NPs/ZnO NRs based L-lactate biosensor under the interference of 1 mM glucose, 0.1 mM UA and 0.02 mM AA. (b) Stability study of Au NPs/ZnO NRs based L-lactate biosensor after stored at 4 °C for a month.

experiments. As shown in Fig. 4a, the Au/ZnO based biosensor was insensitive to 1 mM glucose, 0.1 mM UA and 0.02 mM AA. However, the response current showed pronounced increase for 0.1 mM L-lactate, indicating acceptable selectivity of the biosensor for L-lactate detection.

The storage stability of LOx/Au@ZnO based electrode was also evaluated by testing the cyclic voltammogram in 0.3 mM L-lactate after stored at 4 °C for a month. 86% of the original peak current remained, illustrating good long-term stability of the electrode (Fig. 4b). Besides, Nafion/LOx/Au@ZnO/Au electrodes displayed

Table 1
Comparison of the L-lactate biosensor with those reported earlier.

Materials	Limit of detection	Linear range	Sensitivity	Reference
Polysulfone/carbon nanotubes	3.4 μM	5–500 μM	10.47 μA cm^{-2} mM^{-1}	[31]
Polyaniline-co-fluoroaniline fllms	0.1 mM	0.1–5.5 mM	1.18 μA mM^{-1}	[32]
(3-Mercaptopropyl)-trimethoxysilane	4 μM	50–250 μM	3.4 μA mM^{-1}	[33]
Hydroxymethylferrocene	10 μM	10–300 μM	0.77 ± 0.08 μA mM^{-1}	[34]
Meldola's Blue-Reinecke Salt	0.55 mM	0.55–10 mM	4.2092 nA mM^{-1}	[35]
Hydrogen titanate nanotubes	200 μM	0.5–14 mM	0.24 μA cm^{-2} mM^{-1}	[36]
Multi-walled CNT/Pt nanoparticles	0.3 μM	0.2–2 mM	6.36 μA cm^{-2} mM^{-1}	[37]
Hydrogel microstructures	–	0–10 mM	1.1 μA cm^{-2} mM^{-1}	[38]
Au NPs/ZnO NRs	6 μM	10–600 μM	24.56 μA cm^{-2} mM^{-1}	This work

Please cite this article in press as: Y. Zhao, et al., Gold nanoparticles coated zinc oxide nanorods as the matrix for enhanced L-lactate sensing, Colloids Surf. B: Biointerfaces (2015), http://dx.doi.org/10.1016/j.colsurfb.2014.12.053

ideal reproducibility with a relative standard deviation of 4.23% ($n = 5$).

Table 1 illustrates the performance of L-lactate related biosensors. Most of the analytical parameters in our work are comparable or even better than those of others, implying its more promising prospects for L-lactate determination.

4. Conclusions

In summary, Au NPs/ZnO NRs were successfully prepared through facile synthesis and applied to fabricate L-lactate biosensors. In comparison to pristine ZnO based biosensor, the one with Au NPs displayed higher sensitivity of 24.56 $\mu A\,cm^{-2}\,mM^{-1}$ and lower detection limit of 6 μM for L-lactate detection. The improved sensing performance can be ascribed to the enhanced electrocatalytic ability and higher conductivity facilitated by Au NPs. Au/ZnO based L-lactate biosensor also exhibited good selectivity, storage stability and reproducibility. All the results implies that Au NPs coated ZnO NRs can be employed as an effective matrix to fabricate biosensors for the detection of L-lactate and other biological and chemical species.

Acknowledgments

This work was supported by the National Major Research Program of China (2013CB932602), the Major Project of International Cooperation and Exchanges (2012DFA50990), the Program of Introducing Talents of Discipline to Universities, the National Natural Science Foundation of China (51232001, 51172022, 51372023, 31371203), the Research Fund of Co-construction Program from Beijing Municipal Commission of Education, the Fundamental Research Funds for the Central Universities, the Program for Changjiang Scholars and Innovative Research Team in University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.12.053.

References

[1] N. Nikolaus, B. Strehlitz, Microchim. Acta 160 (2008) 15.
[2] B. Bleiberg, J.J. Steinberg, S.D. Katz, J. Wexler, T. LeJemtel, J. Chromatogr. B 568 (1991) 301.
[3] F.Q. Wu, Y.M. Huang, C.Z. Huang, Biosens. Bioelectron. 21 (2005) 518.
[4] Y. Lei, N. Luo, X.Q. Yan, Y.G. Zhao, G. Zhang, Y. Zhang, Nanoscale 4 (2012) 3438.
[5] S. Perez, E. Fabregas, Analyst 137 (2012) 3854.
[6] J.M. Goran, J.L. Lyon, K.J. Stevenson, Anal. Chem. 83 (2011) 8123.
[7] X.P.A. Gao, G.F. Zheng, C.M. Lieber, Nano Lett. 10 (2010) 547.
[8] G.J. Zhang, Z.H.H. Luo, M.J. Huang, J.A.J. Ang, T.G. Kang, H.M. Ji, Biosens. Bioelectron. 28 (2011) 459.
[9] M.A. Bangar, D.J. Shirale, W. Chen, N.V. Myung, Anal. Chem. 81 (2009) 2168.
[10] Q.P. Wang, F.F. Min, J.B. Zhu, Mater. Lett. 91 (2013) 9.
[11] X.L. Ren, D. Chen, X.W. Meng, F.Q. Tang, A.M. Du, L. Zhang, Colloids Surf. B (2009) 188.
[12] Y.C. Zhang, Y.X. Liu, L. Su, Z.H. Zhang, D.Q. Huo, C.J. Hou, Y. Lei, Sens. Actuators B 191 (2014) 86.
[13] Y.G. Zhao, X.Q. Yan, Z. Kang, P. Lin, X.F. Fang, Y. Lei, S.W. Ma, Y. Zhang, Microchim. Acta 180 (2013) 759.
[14] A.K. Wanekaya, W. Chen, N.V. Myung, A. Mulchandani, Electroanalysis 18 (2006) 533.
[15] J.X. Wang, X.W. Sun, A. Wei, Y. Lei, X.P. Cai, C.M. Li, Z.L. Dong, Appl. Phys. Lett. 88 (2006) 233106.
[16] Y. Lei, X.Q. Yan, J. Zhao, X. Liu, Y. Song, N. Luo, Y. Zhang, Colloids Surf. B (2011) 168.
[17] S.W. Ma, Q.L. Liao, H.S. Liu, Y. Song, P. Li, Y.H. Huang, Y. Zhang, Nanoscale 4 (2012) 6415.
[18] X. Liu, P. Lin, X.Q. Yan, Z. Kang, Y.G. Zhao, Y. Lei, C.B. Li, H.W. Du, Y. Zhang, Sens. Actuators B 176 (2013) 22.
[19] W. Yuan, H.P. Ho, R.K. Lee, S.K. Kong, Appl. Opt. 48 (2009) 4329.
[20] J.M. You, Y.N. Jeong, M.S. Ahmed, S.K. Kim, H.C. Choi, S. Jeon, Biosens. Bioelectron. 26 (2011) 2287.
[21] L.M. Lu, H.B. Li, F.L. Qu, X.B. Zhang, G.L. Shen, R.Q. Yu, Biosens. Bioelectron. 26 (2011) 3500.
[22] F.C. Wang, R. Yuan, Y.Q. Chai, D.P. Tang, Anal. Bioanal. Chem. 387 (2007) 709.
[23] P.J. Lamas-Ardisana, O.A. Loaiza, L. Añorga, E. Jubete, M. Borghei, V. Ruiz, E. Ochoteco, G. Cabañero, H.J. Grande, Biosens. Bioelectron. 56 (2014) 345.
[24] B.Y. Wu, S.H. Hou, F. Yin, Z.X. Zhao, Y.Y. Wang, X.S. Wang, Q. Chen, Biosens. Bioelectron. 22 (2007) 2854.
[25] T.K. Sau, A. Pal, N.R. Jana, Z.L. Wang, T. Pal, J. Nanopart. Res. 3 (2001) 257.
[26] Y.G. Zhao, X.Q. Yan, Z. Kang, X.F. Fang, X. Zheng, L.Q. Zhao, H.W. Du, Y. Zhang, J. Nanopart. Res. 16 (2014) 2398.
[27] A. Zwijnenburg, A. Goossens, W.G. Sloof, M.W.J. Crajeí, A.M. van der Kraan, L. Jos de Jongh, M. Makkee, J.A. Moulijn, J. Phys. Chem. B 106 (2002) 9853.
[28] N. Nesakumar, K. Thandavan, S. Sethuraman, U.M. Krishnan, J.B.B. Rayappan, J. Colloid Interface Sci. 414 (2014) 90.
[29] Y.Z. Xian, Y. Hu, F. Liu, Y. Xian, H.T. Wang, L.T. Jin, Biosens. Bioelectron. 21 (2006) 1996.
[30] Y.Y. Wei, Y. Li, X.Q. Liu, Y.Z. Xian, G.Y. Shi, L.T. Jin, Biosens. Bioelectron. 26 (2010) 275.
[31] S. Perez, S. Sanchez, E. Fabregas, Electroanalysis 24 (2012) 967.
[32] S. Suman, R. Singhal, A.L. Sharma, B.D. Malthotra, C.S. Pundir, Sens. Actuators B 107 (2005) 768.
[33] A.M. Parra-Alfambra, E. Casero, M.D. Petit-Domınguez, M. Barbadillo, F. Pariente, L. Vázquez, E. Lorenzo, Analyst 136 (2011) 340.
[34] A. Parra, E. Casero, L. Vázquez, F. Pariente, E. Lorenzo, Anal. Chim. Acta 555 (2006) 308.
[35] M. Piano, S. Serban, R. Pittson, G.A. Drago, J.P. Hart, Talanta 82 (2010) 34.
[36] M. Yang, J. Wang, H. Li, J.G. Zheng, N.N. Wu, Nanotechnology 19 (2008) 75502.
[37] J. Huang, J. Li, Y. Yang, X. Wang, B. Wu, J. Anzai, T. Osa, Q. Chen, Mater. Sci. Eng. C 28 (2008) 1070.
[38] J. Yan, V.A. Pedrosa, A.L. Simonian, A. Revzin, ACS Appl. Mater. Interfaces 2 (2010) 748.