Int. J. Electrochem. Sci., 14 **(2019)** 1310 – 1317, doi: 10.20964/2019.02.16

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Construction of Nafion/Hb/Au/ZIF-8/CILE and its application as electrochemical sensor for determination of bromate and nitrite

Juan Liu[1,2], *Wenju Weng*[2], *Chunxiao Yin*[2], *Guiling Luo*[1], *Hui Xie*[1], *Yanyan Niu*[1], *Xiaoyan Li*[1], *Guangjiu Li*[2],*Yaru Xi*[1], *Yutong Gong*[1], *Shuyao Zhang*[1], *Wei Sun*[1*]

[1] Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou 571158, China;
[2] Key Laboratory of Optic-electric Sensing and Analytical Chemistry for Life Science of Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China;
[*]E-mail: sunwei@hainnu.edu.cn

Received: 10 October 2018/ *Accepted:* 17 November 2018 / *Published:* 5 January 2019

In this paper zinc-based metal organic framework material (ZIF-8) modified carbon ionic liquid electrode (CILE) was prepared and electrodeposited with gold nanoparticles to get a composite modified electrode, which was used as the sensing interface for the immobilization of hemoglobin (Hb) with Nafion to prepare a modified electrode (Nafion/Hb/Au/ZIF-8/CILE). Electrochemical behaviors of Hb on the modified electrode were checked by cyclic voltammetry with a pair of well-defined redox peaks appeared. The effects of pH and scan rate on direct electrochemistry of Hb were studied with electrochemical parameters calculated. The prepared electrochemical enzyme sensor showed good catalytic activity to the electroreduction of bromate and nitrite with the linear ranges from 0.5 to 10.0 mmol/L and from 0.1 to 0.8 mmol/L, respectively. The detection limits were calculated as 0.83 mmol/L and 0.03 mmol/L, and the water samples were analyzed to prove the practical application.

Keywords: carbon ionic liquid electrode, zinc-based metal organic framework, gold nanoparticle, hemoglobin, electrochemistry, bromate

1. INTRODUCTION

Hemoglobin (Hb) plays a very important role in biological, which includes four electroactive iron hemes and acts to transport oxygen [1]. Because of its commercial availability, cheap cost and well-reported structure with heme as the electroactive center, Hb is offer used as model for electrochemistry of heme proteins. However it usually has a slow electron transfer rate between redox proteins and bare solid electrodes with irreversibly denaturation on the electrode surface. To fasten the electron transfer rate from proteins to electrodes and fabricate a stable biosensor, various immobilization procedures including adsorption [2], cross-linking [3] and layer-by-layer assembly [4] have been devised with the usage of different materials such as hydrogel, polymer [5], surfactant [6], nanomaterial [7-9] for the enzyme modified electrodes construction.

Metal organic frameworks (MOFs) belong to structured hybrid materials which are consisted of inorganic linkers and organic linking molecules. Due to their large surface areas, high porosity, adjustable pore sizes, ordered crystalline structures and wonderful mechanical stability [10-12], MOFs have been reported in sensors, gas storage, catalysis, magnetism and luminescence [13-17]. Zeolitic imidazolate frameworks (ZIFs) combine the properties of zeolites and MOFs, which have acted as crystalline porous materials [18,19]. Among them, ZIF-8 is composed of inorganic metal nodes linked by imidazole or imidazolate bridging ligands, which has exhibited the characteristics including large pore size, big surface area, high thermal stability and remarkable chemical stability [20]. However, there is a shortcoming that the conductivity of ZIF-8 is poor and it has been proposed to introduce conductive materials into the structure of MOF [21,22]. Metal nanoparticles are commonly used sensing materials in modified electrodes due to its wonderful conductivity, electrocatalytic activity and chemical stability [23-27]. In this paper, gold nanoparticles (AuNPs) were used for the further decoration of ZIF-8 modified electrode to improve the bulk conductivity.

Bromate is reported as a genotoxic carcinogen and a byproduct of various natural water sources such as mineral water and mountain spring after ozone disinfection [28,29]. Moreover, due to its oxidizing properties it can also be used as a food additive for flour ripening and producing fish sauce [30]. However, studies have shown that large amount of bromate can cause renal cell tumors and thyroid follicular cell tumors in rats [31]. Nitrite is often used as a preservative in meat production, and the higher nitrite concentrations in the human body can result in the loss of oxygen turnover capacity of Hb. Therefore sensitive determinations of bromate and nitrite are of great importance in analytical chemistry.

In this paper, an electrochemical sensor was prepared with ZIF-8, AuNPs and Hb as the modifier. By selecting CILE as the base electrode, a modified electrode (Nafion/Hb/Au/ZIF-8/CILE) was prepared by the combination of dropping cast and electrodeposition technique. The modifier was present on the surface of electrode with good stability and direct electrochemistry of Hb on the electrode was studied by cyclic voltammetry. Well-defined redox peaks on cyclic voltammetric curves indicated that direct electron transfer of Hb was achieved. Therefore, the above electrode was used as a sensitive sensing device for the third generation electrochemical sensor, which was used in water sample detection.

2. EXPERIMENTAL

2.1. Reagents and instruments

Hb (MW. 64500, Sinopharm Chemical Reagent Co., China), 1-hexylpyridinium hexafluorophosphate ($HPPF_6$, Lanzhou Yulu Fine Chem. Co., China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Co., China), ZIF-8 (Nanjing XFNANO Materials Tech Co., China), $HAuCl_4$ (Tianjin Biochemical Reagent Co., China), Nafion (5.0% ethanol solution, Sigma), $KBrO_3$ (Shanghai Aladdin Bio-Chem. Technology Co., China) and $NaNO_2$ (Shanghai Chem. Plant, China) were used as received. 0.1 mol/L phosphate buffer solution (PBS) was used as supporting electrolyte. All the chemicals were of analytical reagent with double-distilled water used throughout the experiment.

Voltammetric experiments were performed on a CHI 750B electrochemical analyzer (Shanghai CH Instruments, China). A traditional three-electrode system was used with a Nafion/Hb/Au/ZIF-8/CILE as working electrode, a Ag/AgCl (sat. KCl) as reference electrode and a platinum wire as the auxiliary electrode. Scanning electron microscopy (SEM) images were obtained on a JSM-7100F scanning electron microscope (JEOL, Japan).

2.2. Electrode Preparation

According to previous reports [32], CILE was hand-made by mixing $HPPF_6$ and graphite powder with a glassy electrode tube (Φ=4mm), which was smoothed on a piece of polishing paper just before use. Then 8.0 μL of 1.0 mg/mL ZIF-8 dispersion was directly dropped onto the surface of CILE and the electrode was dried to obtain ZIF-8/CILE. Then AuNPs were electrodeposited on the surface of ZIF-8/CILE by imposing a potential of -0.3 V for 100 s in a 1.0 mmol/L $HAuCl_4$ solution to get Au/ZIF-8/CILE, which was rinsed with water and dried for further usage. Electrochemical biosensor was prepared by dropping 8.0 μL of 15.0 mg/mL Hb solution directly Au/ZIF-8/CILE and dried naturally. Finally, 6.0 μL of 0.5% Nafion solution was casted on Hb/Au/ZIF-8/CILE and dried to obtain the working electrode, which was stored in a 4 °C refrigerator. Other modified electrodes such as Nafion/CILE and Nafion/Hb/CILE were constructed with the similar procedure.

3. RESULTS AND DISCUSSION

3.1. SEM results

SEM images of ZIF-8/CILE, Au/CILE and Au/ZIF-8/CILE were exhibited in Fig.1. ZIF-8 was aggregated with hexagonal morphology (Fig.1A) and AuNPs were evenly distributed on the electrode surface (Fig.1B). SEM image of Au/ZIF-8/CILE (Fig.1C) indicated that ZIF-8 and AuNPs were present on CILE simultaneously with clear appearance.

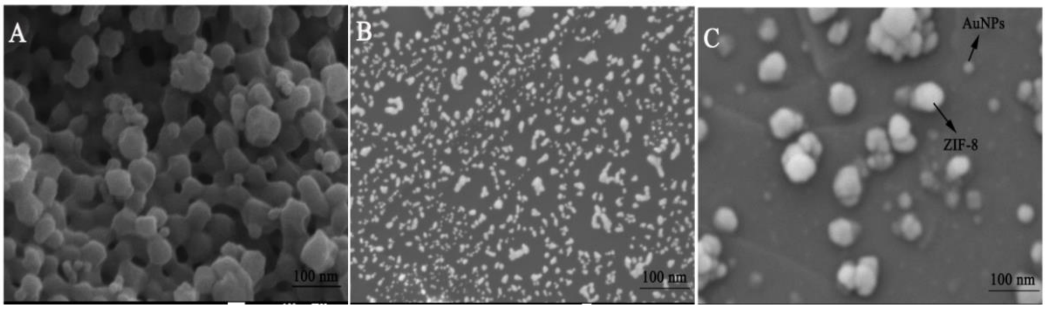

Figure 1. SEM images of (A) ZIF-8/CILE, (B) Au/CILE, (C) Au/ZIF-8/CILE.

3.2. Direct electrochemistry of Hb

Cyclic voltammograms of different modified electrodes in 0.1 mol/L PBS (pH 3.0) at the scan rate of 100 mV/s were overlapped in Figure 2. On Nafion/CILE (curve a) no redox peaks appeared with stable background. On Nafion/Hb/CILE (curve b) a pair of redox peaks appeared, which revealed the realization of direct electron transfer between Hb and CILE. While On Nafion/Hb/Au/ZIF-8/CILE (curve c), the redox peak currents increased obviously, manifesting the synergistic effects of ZIF-8 and AuNPs to the Hb electron transfer. The presence of ZIF-8 can provide a rough interface and electrodeposited AuNPs can enhance the interfacial conductivity. Therefore the electron communication from Hb to electrode was easily realized with enhanced rate. The values of peak potential were located at -0.227 V (Epc) and -0.117 V (Epa) with the peak-to-peak separation (ΔEp) as 110 mV. The formal peak potential ($E^{0'}$) was calculated -0.172 V, which was the typical feature of heme Fe(III)/Fe(II) in redox protein. Also the ratio of redox peak currents was got as 0.98, which was close to 1. All the results revealed a quasi-reversible electrochemical process of Hb heme Fe(III)/Fe(II) redox center on the electrode.

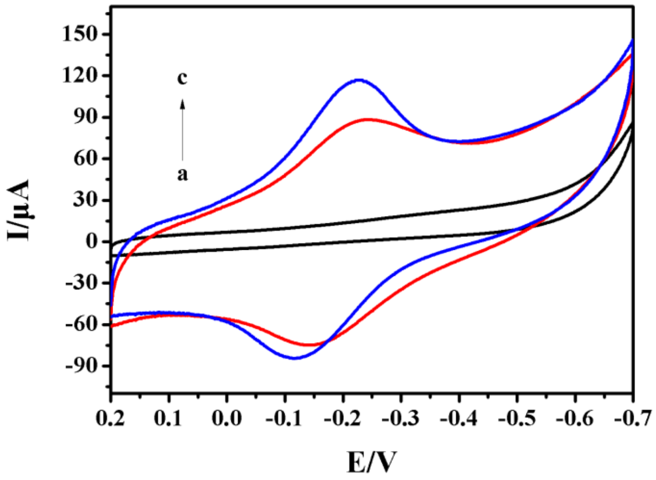

Figure 2. Cyclic voltammograms of (a)Nafion/CILE, (b) Nafion/Hb/CILE and (c) Nafion/Hb/Au/ZIF-8/CILE in pH 3.0 PBS with the scan rate as 100 mV/s.

3.3. Kinetic investigations

Cyclic voltammograms of Nafion/Hb/Au/ZIF-8/CILE in pH 3.0 PBS at different scan rates (from 50 to 1000 mV/s) were investigated. As shown in Fig.3A, a pair of symmetric redox peak appeared and the redox peak currents increased gradually with scan rate. Good linear relationships were calculated as Ipc (μA) = 261.40 υ (V/s) + 14.35 (n=10, γ=0.992) and Ipa (μA) = -379.28 υ (V/s) + 5.89 (n=10, γ=0.996), indicating a typical surface-controlled process of Hb electrochemistry. Also two linear relationships of peak potential and lnυ were calculated as Epc (V) = -0.055 lnυ (V/s) - 0.32 (n=10, γ = 0.992) and Epa (V) = 0.044 lnυ (V/s) - 0.032 (n=10, γ=0.991). According to Laviron’s model [33], the values of electron transferred number (n), electron transfer coefficient (α) and the apparent heterogeneous electron transfer rate constant (k_s) were calculated as 1.06, 0.44 and 1.13 s^{-1}.

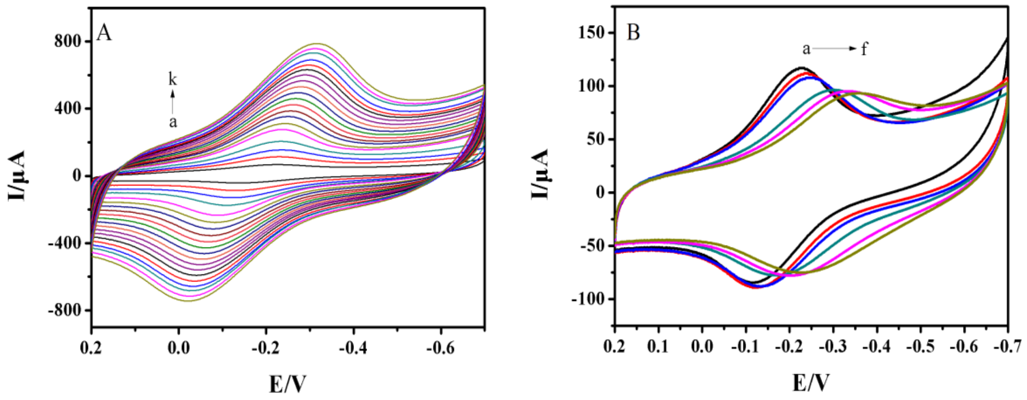

Figure 3.(A) Cyclic voltammograms of Nafion/Hb/Au/ZIF-8/CILE with different scan rates (from a to k as 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 mV/s) in pH 3.0 PBS; (B) Cyclic voltammograms of Nafion/Hb/Au/ZIF-8/CILE in different pH PBS (from a to f as 3, 4, 5, 6, 7, 8) with scan rate of 100 mV/s.

Electrochemistry of Hb also depends on the buffer pH, which was studied with cyclic voltammograms shown in Fig.3B. A symmetrical and well-defined redox peaks could be observed with the largest current value appeared at pH 3.0, which was used as the optimal pH in this experiment. With pH increased from 3.0 to 8.0, the redox peak potentials moved negatively, manifesting that protons took part in the electrochemical reaction. The formal peak potential ($E^{0'}$) was linear with pH and the regression equation as $E^{0'}$ (V) = 0.005 - 0.0421 pH (n=6, γ=0.993). The slope value (-42.1 mV/pH) was less than the theoretical value (-59.0 mV/pH) for a net one-proton coupled reversible one-electron transfer, which was due to the effect on the protonation states of transligands to the heme iron and amino acids around the heme, or the protonation of the water molecule coordinated to the central iron.

3.4. Electrocatalytic behavior of Hb

Electrocatalytic activity of Nafion/Hb/Au/ZIF-8/CILE toward the reduction of bromate was measured with cyclic voltammetry with curves in Fig.4A. The reduction peak appeared at -0.199 V and currents increased gradually with bromate concentration in 0.1 mol/L PBS (pH 3.0). Reduction peak current had a good linear equation with bromate concentration from 0.5 to 10.0 mmol/L. The equation was I (μA) = 9.209 C (mmol/L) + 57.988 (n=8, γ=0.993) with the detection limit as 0.16 mmol/L (3σ). When the bromate concentration was more than 10.0 mmol/L, the response remained steady, indicating a Michaelis-Menten kinetic process. The Lineweaver-Burk equation is employed to analyse the enzymatic reaction: $1/I_{ss} = 1/I_{max} + K_M^{app}/(I_{max}\cdot C)$ [34], and the value of apparent Michaelis-Menten constant (K_M^{app}) was 0.83 mmol/L.

Electrocatalytic reduction of Nafion/Hb/Au/ZIF-8/CILE towards nitrite was studied with results depicted in Fig.4B. A new reduction peak was observed at -0.624 V and the reduction peak increased with nitrite concentration from 0.1 to 0.8 mmol/L. The linear regression equation was displayed as

I_{ss}(μA) = 152.806 C (mmol/L) + 3.034 (n=7, γ=0.995) with the detection limit and K_M^{app} as 0.03 mmol/L (3σ) and 4.96 mmol/L, respectively. A comparison of this method with other reported results were compared in table 1, which indicated a relative wider linear range and lower detection limit. Therefore the combination of ZIF-8 and AuNPs could result in a sensing platform for nitrite detection.

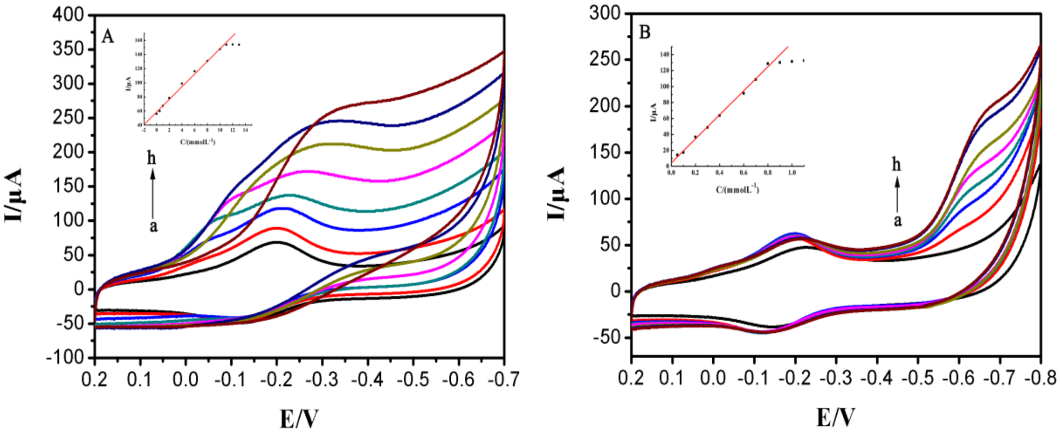

Figure 4. Cyclic voltammograms of Nafion/Hb/Au/ZIF-8/CILE (A) with different concentrations of bromate (from a to h 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mmol/L, inset is the linear relationship of bromate concentration versus reduction peak current); (B) with different concentrations of nitrate (from a to h 0.0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8 mmol/L, inset is the linear relationship of nitrite concentration versus reduction peak current).

Table 1. Comparison of analytical performances of various electrochemical methods for nitrite.

Electrodes	Linear range (μmol/L)	LOD (μmol/L)	Refs
Nafion/Hb/AuNPs-rGO/GCE[a]	0.5~100	0.1	35
CR–GO[b]/GCE	8.9~167	1	36
Hb/Au/GCE	4~350	1.2	37
CTS/TiO_2-Hb/CILE	800~20000	260	38
Mb/MoS_2-graphene/Nafion	1250~12500	125	39
GH-CS[c]/Fc-NH_2[d]/Cytc[e]/GCE	0.1~150	0.04	40
Nafion/Hb/Au/ZIF-8/CILE	100~800	30	This work

[a] glassy carbon electrode, [b] chemically reduced graphene oxide, [c] graphene-chitosan, [d] ferrocene-terminated phenylethynyaniline, [e] cytochrome c

3.5 Application

The analysis of bromate and nitrite content in laboratory water samples were studied and the results were shown in table 2 and table 3, respectively. No residue of bromate and nitrite in tap water sample could be found with the recovery in the range of 98.20%~99.75% and 98.80%~101.50%, proving the real application of this method.

Table 2. Test results of bromate concentration in water sample (n=3)

Sample	Detected (mmol/L)	Added (mmol/L)	Total (mmol/L)	Recovery (%)
Tap water	0	3.00	2.96	98.67
		4.00	3.99	99.75
		5.00	4.91	98.20

Table 3. Test results of nitrite concentration in water sample (n=3)

Sample	Detected (mmol/L)	Added (mmol/L)	Total (mmol/L)	Recovery (%)
Tap water	0	3.00	3.01	100.33
		4.00	4.06	101.50
		5.00	4.94	98.80

4. CONCLUSION

By using ZIF-8 and AuNPs as the electrode modifier, Nafion/Hb/Au/ZIF-8/CILE was prepared and used as the electrochemical sensor. Combining the specific properties of ZIF-8 and AuNPs such as large surface area, high thermal stability, excellent conductivity and good electrocatalytic activity, direct electron transfer of Hb on electrode surface was enhanced obviously. Nafion/Hb/Au/ZIF-8/CILE showed good electrocatalytic effects to the reduction of bromate and nitrite with a relative wider linear range and lower detection limit. Thus the ZIF-8 and AuNPs composite was a proper candidate for the construction of third-generation electrochemical enzyme sensors.

ACKNOWLEDGMENTS
This work was supported by the financial support of the National Natural Science Foundation of Hainan Province of China (2017CXTD007), the Program for Innovative Research Team in University (IRT-16R19), the Key Science and Technology Program of Haikou City (2017042) and Graduate Student Innovation Research Project of Hainan Province (Hys2018-212).

References

1. G. Wang, X. Deng and W. Zhang, *Ann. Chim-Sci. Mat.,* 96 (2006) 247.
2. L. Zhang, X. Jiang, E. Wang and S. Dong, *Biosens. Bioelectron.,* 21 (2005) 337.
3. L. Qian and X. Yang, *Talanta,* 68 (2006) 721.
4. G. Wang, H. Lu and N. Hu, *J. Electroanal. Chem.,* 599 (2007) 91.
5. L. Shen, R. Huang and N. Hu, *Talanta,* 56 (2002) 1131.
6. Z. Lu, Q. Huang and J.F. Rusling, *J. Electroanal. Chem.,* 423 (1997) 59.
7. C.X. Lei, S.Q. Hu, N. Gao, G.L. Shen and R.Q. Yu, *Bioelectrochemistry,* 65 (2004) 33.
8. Z. Song, J.D. Huang, B.Y. Wu, H.B. Shi, J.I. Anzai and Q. Chen, *Sensor. Actuat. B-Chem.,* 15 (2006) 626.
9. L. Zhao, H. Liu and N. Hu, *Anal. Bioanal. Chem.,* 384 (2006) 414.
10. J.R. Li, J. Sculley and H.C. Zhou, *Chem. Rev.,* 112 (2011) 869.
11. M. Yaghi, M. O'Keeffe, N.W. Ockwig, H.K. Chae, M. Eddaoudi and J. Kim, *Nature,* 423 (2003) 705.

12. P. Chowdhury, C. Bikkina, D. Meister, F. Dreisbach and S. Gumma, *Micropor. Mesopor. Mat.,* 117 (2009) 406.
13. G. Lu and J.T. Hupp, *J. Am. Chem. Soc.,* 132 (2010) 7832.
14. Y. Fu, D. Sun, Y. Chen, R. Huang, Z. Ding, X. Fu and Z. Li, *Angew. Chem. Int. Edit.,* 124 (2012) 3420.
15. M. Jahan, Q. Bao and K.P. Loh, *J. Am. Chem. Soc.,* 134 (2012) 6707.
16. F. Luo, Y. Che and J. Zheng, *Cryst. Growth. Des.,* 9 (2009) 1066.
17. H. Bux, F. Liang, Y. Li, J. Cravillon, M. Wiebcke and J. Caro, *J. Am. Chem. Soc.,* 131 (2009) 16000.
18. X.C. Huang, Y.Y. Lin, J.P. Zhang and X.M. Chen, *Angew. Chem. Int. Edit.,* 45 (2010) 1557.
19. J.B. Lin, R.B. Lin, X.N. Cheng, J.P. Zhang and X.M. Chen, *Chem. Commun.,* 47 (2011) 9185.
20. K.S. Park, Z. Ni, A.P. Cote, J.Y. Choi, R. Huang, F.J. Uribe-Romo, H.K. Chae, M. O'Keeffe and O.M. Yaghi, *Proc. Natl. Acad. Sci. U. S. A.,* 103 (2006) 10186.
21. X. Wang, Q.X. Wang, Q.H. Wang, F. Gao, Y.Z. Yang and H.X. Guo, *ACS. Appl. Mater. Inter.,* 6 (2014) 11573.
22. Y. Wang, H.L. Ge, G.Q. Ye, H.H. Chen and X.Y. Hu, *J. Mater. Chem. B.,* 3 (2015) 3747.
23. N.F. Atta and M.F. El-Kady, *Sensor. Actuat. B-Chem.,* 145 (2010) 299.
24. T.H. Tsai, K.C. Lin and S.M. Chen, *Int. J. Electrochem. Sc.,* 6 (2011) 2672.
25. Y.Y. Zhang, X.Y. Bai, X.M. Wang, K.K. Shiu, Y.L. Zhu and H. Jiang, *Anal. Chem.,* 86 (2014) 9459.
26. Y. Zhang, G.M. Zeng, L. Tang, J. Chen, Y. Zhu, X.X. He and Y. He, *Anal. Chem.,* 87 (2015) 989.
27. A. Aziz, H.N. Lim, S.H. Girei, M.H. Yaacob, M.A. Mahdi, N.M. Huang and A. Pandikumar, *Sensor. Actuat. B-Chem.,* 206 (2015) 119.
28. U.V. Gunten and J. Hoigne, *Environ. Sci. Technol.,* 28 (1994) 1234.
29. W.R. Haag and J. Hoigne, *Environ. Sci. Technol.,* 17 (1983) 261.
30. H.S. Weinberg, C.A. Delcomyn and V. Unnam, *Environ. Sci. Technol.,* 37 (2003) 3104.
31. Y. Kurokawa, S. Aoki, Y. Matsushima, N. Takamura, T. Imazawa and Y. Hayashi, *J. Natl. Cancer. I.,* 77 (1986) 977.
32. W. Sun, Y. Li, M. Yang, S. Liu and K. Jiao, *Electrochem. Commun.,* 10 (2008) 298.
33. W. Sun, L. Cao, Y. Deng, S. Gong, F. Shi, G. Li and Z. Sun, *Anal. Chim. Acta.,* 781 (2013) 41.
34. R.A. Kamin and G.S. Wilson, *Anal. Chem.,* 52 (1980) 1198.
35. H.C. Xu, S. Ding, X.D. Cao, *Modern Food Sci. Tech.,* 2015.
36. V. Mani, A.P. Periasamy, S.M. Chen, *Electrochem. Commun.,* 17 (2012) 75.
37. W. Yang, Y. Bai, Y. Li, C. Sun, *Anal. Bioanal. Chem.,* 382 (2005) 44.
38. F. Shi, W. Wang, S. Gong, *J. Chin. Chem. Soc-Taip.,* 62 (2015) 554.
39. K. Zhang, H. Sun, S. Hou, *Anal. Methods-UK.,* 8 (2016) 3780.
40. N. Huang, M. L. Liu, J.H. Deng, *Chinese J. Anal. Chem.,* 325 (2015) 43.