Talanta 117 (2013) 333–337

Contents lists available at ScienceDirect

Talanta

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

Gold nanoparticles/polyaniline Langmuir–Blodgett Film modified glassy carbon electrode as voltammetric sensor for detection of epinephrine and uric acid

Lina Zou [a], Yinfeng Li [a,b], Shaokui Cao [a,*], Baoxian Ye [a,**]

[a] *College of Chemistry and Molecular Engineering and School of Material Science & Engineering, Zhengzhou University, 450001, PR China*
[b] *School of Chemical and material Engineering, Henan University of Urban Construction, Pingdingshan 467036, PR China*

ARTICLE INFO

Article history:
Received 10 July 2013
Received in revised form
16 September 2013
Accepted 19 September 2013
Available online 25 September 2013

Keywords:
Gold nanoparticles
Langmuir–Blodgett Film
Polyaniline
Voltammetric sensor
Epinephrine
Uric acid

ABSTRACT

Gold nanoparticles/polyaniline nanocomposite thin film was deposited onto the surface of glassy carbon electrode(GCE) by Langmuir–Blodgett(LB) technology to fabricate a new voltammetric sensor (GNPs/PAn-LB GCE) for epinephrine(EP) and uric acid(UA) detection. Cyclic voltammetry, electrochemical impedance spectroscopy and atomic force microscopy were employed to study the characteristic of the GNPs/PAn-LB film. Electrochemical behaviors of EP and UA at the modified electrode were investigated in pH 6.6 phosphate buffer solutions. A sensitive electroanalytical method for simultaneous determination of EP and UA was proposed with high precision and good reproducibility.

1. Introduction

Over the past decade gold nanoparticles (GNPs) modified electrodes have received extensive research interest due to their biological compatibility, excellent conducting capability, high surface-to-volume ratio and attractive catalytic property [1–3]. Assembled process of GNPs on electrode is the critical first step. Generally, self-assembly technology [4], electrochemical deposition [5] and seed mediated growth method [6] are widely used. Another effective approach is Langmuir–Blodgett (LB) technique, by which order monolayer or multilayer of nanoparticles can be formed and the interparticle distance can be precisely controlled with the change of surface pressure.

At present, most researches of GNPs LB film are focused on surface modification of GNPs, for the reason that GNPs' surface is hydrophilic, which cannot build LB film directly. For example, Bourgoin [7] and Sbrana et al. [8] investigated how to use dodecanethiol modified GNPs to fabricate LB film. Hanwell et al. [9] researched LB film-forming properties of three kind of thiol encapsulated GNPs, which had response characteristics to NO_2 gas and toluene vapor. Chen et al. [10] used LB technique along with dithiols as bifunctional linkers on the air/water interface to achieve a 2D crosslinked gold nanoparticle network. In these researches, the surface properties of GNPs are mostly changed after modified by organic molecule, particularly the weakening of conductivity and catalytic property. Thereby, it is necessary to employ new method to fabricate GNPs LB film without surface modified.

In this approach, we take advantage of electrostatic interaction between the negatively charged GNPs in the subphase and the positively charged polyaniline(PAn) at the air-water interface to assemble GNPs/PAn-LB film. In this kind of nanocomposite LB film, GNPs could hold its original morphology and appear more stable because PAn allow stacking of the particles [11–13]. Furthermore, to our knowledge, there has been no report regarding the application of GNPs-LB modified electrode as voltammetric sensors. So we deposited this GNPs/PAn-LB film on the surface of glassy carbon electrode to fabricate a new voltammetric sensor (GNPs/PAn-LB/GCE). The electrochemical behavior of epinephrine(EP) and uric acid(UA) at this modified electrode was investigated in detail. The experimental results showed that this modified electrode displayed a significant electrocatalytic response to EP and UA, and could readily achieve simultaneous determination with high sensitivity and stability, as well as a wider linear range. The goal of this work is to preliminarily investigate the response property of GNPs-LB modified electrode as voltammetric sensors.

---

[*] Corresponding author. Tel.: +86 371 67763561; fax: +86 371 67763523.
[**] Corresponding author. Tel.: +86 371 67781757; fax: +86 371 67763654.
E-mail addresses: caoshaokui@zzu.edu.cn (S. Cao), yebx@zzu.edu.cn (B. Ye).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.09.035

2. Experimental

2.1. Apparatus and reagents

LB films were performed with a JML-04 LB trough (Shanghai Zhongchen Co., Shanghai, China). Electrochemical experiments were carried out using RST 5000 Electrochemical analyzer (Zhengzhou Shiruisi Instrument Technology Co., Ltd., China) and CHI 650 Electrochemical analyzer (CH Instruments Company, USA). A standard three-electrode electrochemical cell was used for all electrochemical experiments with a bare GCE or modified electrode ($d=3$ mm) as working electrode, a platinum (Pt) wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode.

All reagents were of analytical grade. Polyaniline (PAn) (Sigma Aldrich; Mw ca. 10,000) was doped with p-toluene sulfonic acid (PTSA) using methanol as the solvent [14,15]. Epinephrine (EP) and Uric acid (UA) was purchased from Sigma. Chloroauric acid was obtained from Aladdin Chemistry Company. Gold nanoparticles were prepared as the previous report [16] and characterized using TEM which provided the diameter of 17 nm GNPs. Human blood samples were obtained from healthy volunteers and treated prior to usage.

2.2. The GNPs/PAn-LB film modified procedure

GCE was pretreated by the following process: GCE was polished progressively with finer emery-paper, 0.5 μm Al_2O_3 on a polishing cloth, cleaned in an ultrasonic bath for 1 min, and then the electrode was treated in a 0.20 mol/L phosphate buffer solution (pH 5.8) by applying a potential of +1.75 V for 300 s under constant stirring. Finally, cyclic voltammetry (CV) was carried out in the same solution with scan potential window between 0.30 V and 1.3 V at a scan rate 100 mV s^{-1}, until a stable CV profile was obtained.

For the preparation of GNPs/PAn-LB films, 1200 μL doped PAn solution was spread on the subphase (pH=2) in the Langmuir trough, which contained 5–9 mL of GNPs. After at least 2 h, when the equilibrium between GNPs and PAn monolayer was established, the pressure–area (π–A) isotherm was recorded with barrier speed of 10 mm min^{-1}. Films were transferred onto pretreated GCE or Silicon under constants pressure (35 mN m^{-1}) using vertical withdrawal method. This fabricated electrode was named GNPs/PAn-LB/GCE. For comparison, a PAn modified GCE (denoted as PAn-LB/GCE) was prepared using the same method without spreading GNPs over the subphase. Prior to use, the modified electrode was thoroughly rinsed with pure water, and stored in 0.01 mol L^{-1} phosphate buffer (pH=7.0) at 4 °C when not in use.

3. Results and discussion

3.1. π–A isotherms of GNPs/PAn-LB films

Fig. 1 shows the surface pressure–mean molecular area (π–A) isotherm of polyaniline monolayer at subphase (pH=2) in the absence and presence of GNPs. As the curve a shown, the limiting mean molecular area (A_{lim}) of PAn observed in our study was calculated to be 0.68 nm^2, the collapse pressure was nearly to be 40 mN m^{-1}. When certain amount of GNPs was added to the subphase(curve b–d), the isotherm of PAn shifted to larger areas per molecule. This was the evidence of that GNPs interacted with the PAn Langmuir film. However, the isotherm did not change dramatically with the increased concentration of GNPs in the subphase. When the amount of GNPs added was 9 ml(curve d), A_{lim} of PAn was calculated to be 0.78 nm^2. Such a small change proved that the GNPs did not penetrate into the PAn layer. In order to achieve compact, ordered and more stable GNPs/PAn-LB film, transfer pressure was chosen as 35 mN m^{-1} and the amount of added GNPs was 7 ml.

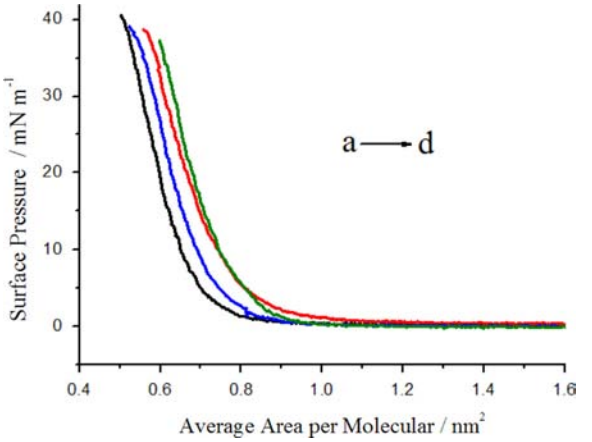

Fig. 1. π–A isotherms of PAn monolayer on subphase (pH=2) in the (a) absence and presence of 5 mL (b), 7 mL (c) and 9 mL (d) of GNPs.

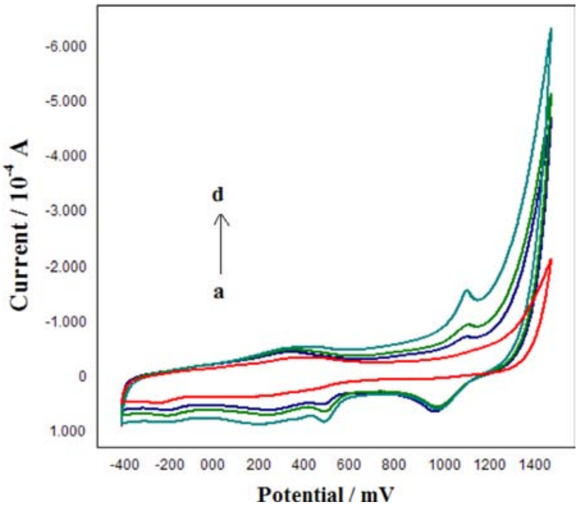

Fig. 2. Cyclic voltammograms of PAn-LB/GCE(a) and one(b), two(c), three(d) layers of GNPs/PAn-LB/GCE in 0.01 mol L^{-1} KCl.

3.2. Electrochemical characterization of the GNPs/PAn-LB/GCE

Fig. 2 shows the cyclic voltammograms of PAn-LB/GCE and different layers of GNPs/PAn-LB/GCE in 0.01 mol L^{-1} KCl. It can be seen that there was an anodic peak at 1.1 V on GNPs/PAn-LB/GCE (curve b–d), and the currents increased with the increasing modified layer number. However, this redox peak cannot be observed on PAn-LB/GCE(curve a). This anodic peak was corresponding to the oxidation and dissolution of gold in a chloride medium according to reference [17]. So this was the evidence that GNPs were modified on the electrode successfully.

Potassium ferricyanide was selected as a probe and the electrochemical impedance spectroscopy (EIS) was employed to evaluate the performance of the fabricated electrodes. The whole EIS has two parts (linear segment and semicircle part). The linear segment at lower frequencies shows a controlled diffusion process; the semicircle part at higher frequencies corresponds to the electron transfer limited process or the electron transfer resistance (R_{ct}). Fig. 3 shows the Nyquist plots of EIS using different

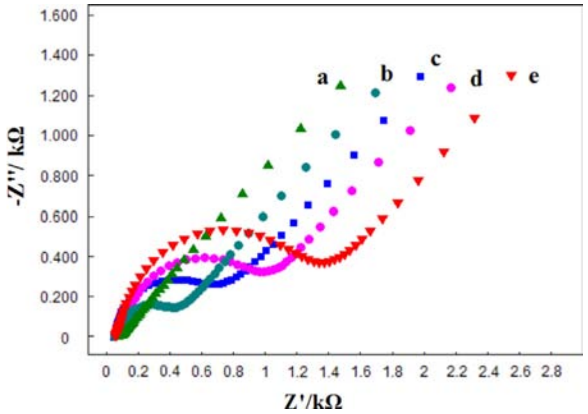

Fig. 3. Nyquist plots of EIS with different electrodes: (a) bare GCE; (b–d) one, two, three layers of GNPs/PAn-LB/GCE and (e)three layers of PAn-LB/GCE. Solution: 5×10^{-3} mol L^{-1} $Fe(CN)_6^{3-/4-}$ + 0.2 mol L^{-1} KCl.

electrodes. It is obviously seen that the R_{ct} of these electrodes were in a sequence of: bare GCE < GNPs/PAn-LB/GCE < PAn-LB/GCE (Fig. 3, curve a–e), which demonstrated GNPs was successfully modified on the GCE just as designed. This is because the layer of GNPs can promote electron transfer to further accelerate the diffusion of ferricyanide toward the electrode surface.

In the further research that an equivalent circuit was designed [18] and R_{ct} obtained was about 290 Ω, 516 Ω,and 679 Ω for 1 layer, 2 layers and 3 layers of GNPs/PAn-LB/GCE, respectively. It is clear that R_{ct} increased in linearity with the number of film layer, which suggests the same amounts of GNPs/PAn-LB film increased with increasing layers and the immobilizing process is uniform.

3.3. *The morphology of GNPs/PAn-LB film*

Fig. 4 shows SEM image of two layers of GNPs/PAn-LB on Si surface. It was obvious that GNPs was evenly distributed on the silicon surface, indicating successful immobilization of GNPs LB film on the substrate surface. Moreover, the size of GNPs did not change, which would benefit to make full use of the surface properties of GNPs in the analytical response. The density of GNPs observed in the Fig. 4 is relatively low. That is because that the concentration of nanoparticles in the subphase is low, and the electrostatic attachment of the GNPs to the PAn film is relatively weak, so the silicon surface cannot be fully coveraged.

3.4. *Electrochemical behavior of EP and UA at GNPs/PAn-LB/GCE*

The electrochemical behavior of EP and UA at GNPs/PAn-LB/GCE was investigated by cyclic voltammetry. Fig. 5 presents the cyclic voltammograms of EP and UA at different electrodes. As shown in Fig. 5, bare GCE exhibited poor electrochemical response to EP and UA (curve a) and PAn-LB/GCE (curve b) only showed small oxidation peaks of them. Whereas at GNPs/PAn-LB/GCE (curve c), both EP and UA showed well-defined oxidation peaks at about 0.25 V and 0.37 V respectively(curve c). The anodic peak currents of EP and UA were about 13-fold and 5-fold in comparison with that at bare GCE (curve a). It is evidence that GNPs/PAn-LB/GCE can significantly improve the response of EP and UA. This could be attributed to the excellent conducting capability and high surface-to-volume ratio of GNPs, which would increase the electrode surface area and make EP and UA easily to be accumulated and oxidized. Fig. 6 shows that the peak current and potential of EP or UA has no obviously change when they were exist singly compared to that of coexistence. The result demonstrated that GNPs/PAn-LB/GCE could be used for simultaneous determination of EP and UA.

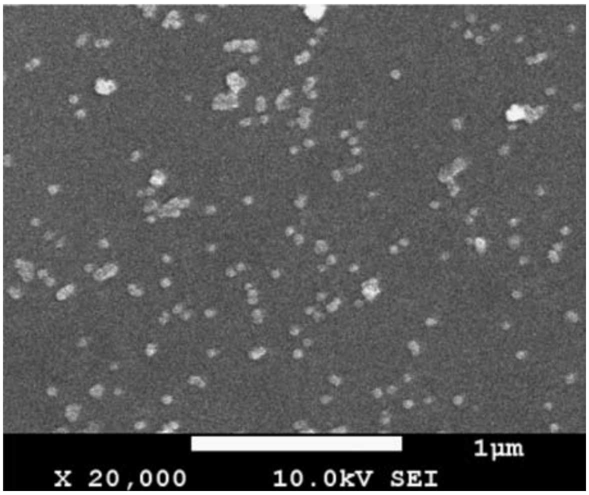

Fig. 4. SEM images of two layers of GNPs/PAn-LB deposited on a silicon wafer.

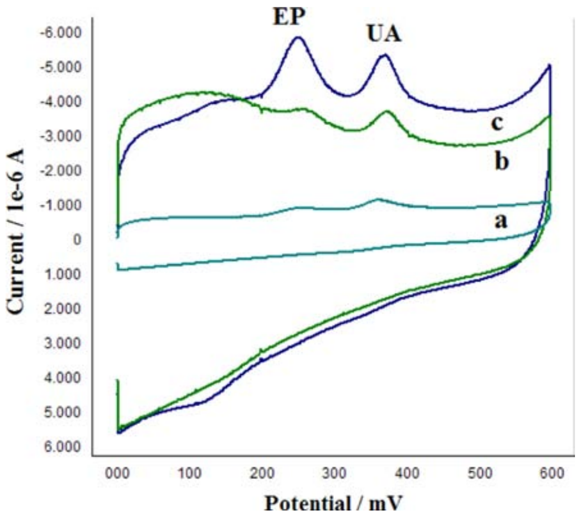

Fig. 5. Cyclic voltammograms of EP (4.0×10^{-6} mol L^{-1}) and UA (1.0×10^{-5} mol L^{-1})at different electrodes (a) bare GCE; (b) PAn-LB/GCE; (c) GNPs/PAn-LB/GCE. Blank solution: 0.2 mol L^{-1} pH 6.6 PBS. Scan rate: 100 mV s^{-1}.

To further elucidate the electrode reaction of EP and UA at GNPs/PAn-LB/GCE, the influence of scan rate (ν) and pH value of solution on i_p of EP or UA was studied by CV respectively. In the scan range of 10–500 mV s^{-1}, the peak current of EP or UA growed linearly with the increasing of scan rate, indicating electrode process both of EP and UA at GNPs/PAn-LB/GCE were controlled by adsorption. Based on Laviron's theory [19] of adsorption controlled irreversible anodic reaction, the Ep–ν relation of EP or UA can be described in the following equations: $Ep=0.323+0.0246\ \ln\nu$ (peak EP, $r=0.996$) and $Ep=0.420+0.0218\ \ln\nu$ (peak UA, $r=0.999$), and the number of electrons participating in the electrode reaction process can be calculated both to be 2.

Over the pH range of 5.0–8.0, the oxidation peak potential of both EP and UA shifted negatively with the increment of the solution pH. The relationship between the anodic peak potential and pH could be fitted into the regression equations: $Ep=0.684-0.064$ pH (peak EP, $r=0.998$) and $Ep=0.841-0.066$ pH (peak UA, $r=0.997$), respectively. The slope values of 64 mV/pH and 66 mV/pH (theoretical value of −59 mV) indicated equal number of electrons and protons involved in the redox process of EP and UA. This suggested that the redox of EP and UA at the GNPs/PAn-LB/GCE were both $2e^-/2H^+$ reaction.

3.5. Analytical applications and method validation

3.5.1. Accumulation conditions

In consideration of detection sensitivity and the fact of EP and UA adsorbed on the GNPs/PAn-LB/GCE surface, square wave voltammograms (SWV) coupled with accumulation step was employed for researching the analytical method. For this system containing 5.0×10^{-6} mol L^{-1} EP and UA, the accumulation potential (E_{acc}) was no more effect on the response of peak currents, so the accumulation was carried out under open circuit. The peak currents were increased with increasing of t_{acc} up to 60 s and then kept stable. So we chose 60 s as the t_{acc} in following study.

3.5.2. Calibration curve, detection limit, reproducibility and stability

The three layers of GNPs/PAn-LB/GCE showed excellent ability for the simultaneous determination of EP and UA in mixed solution. In the optimized working conditions, Fig. 7A shows the square wave voltammograms for the different concentrations of EP in the presence of a fixed concentration of 1×10^{-5} mol L^{-1} UA. It was clearly seen that the peak current of UA decreased with the increase of EP concentration, meaning that "competitive adsorption" existed between them. Although there is a competitive adsorption at electrode, the linear relation between peak currents and EP concentrations was observed in the range of 4×10^{-7} to 1×10^{-5} mol L^{-1} with detection limit of 8×10^{-8} mol L^{-1} ($i_{pa}=3.973+3.004\times10^{6}c_{EP}$, $r=0.998$). Similarly, when 4×10^{-6} mol L^{-1} EP coexisted with various concentrations of UA, the same trend could be observed (Fig. 7B). The peak currents of UA were linear with its concentration in the range of 4×10^{-7} to 6×10^{-5} mol L^{-1} ($i_{pa}=0.292+0.293\times10^{6}c_{UA}$, $r=0.999$), in spite of existing 4×10^{-6} mol L^{-1} EP. The detection limit for UA was 2×10^{-7} mol L^{-1}. These results indicate that the competitive adsorption equilibrium can be attained at the suitable concentrations of EP and UA. Therefore, the EP and UA could be estimated from a mixture at the concentration range studied by this method. The comparison of the proposed electrode with other modified electrodes for EP and UA detection was listed in Table 1. From Table 1, it can be seen that this method has the comparable sensitivity and detection limit for determination.

To estimate the reproducibility of the proposed electrode, relative standard deviations (RSD) of 10 times determination of 5×10^{-6} mol L^{-1} EP and UA were 1.97% and 4.62%, respectively. To assess the consistency of GNPs/PAn-LB/GCE, cyclic voltammetric scanning was recorded on one day and a week later and compared under the same condition. The peak currents of EP and UA both changed less than 4.8%, which indicated that GNPs/PAn-LB/GCE had good stability.

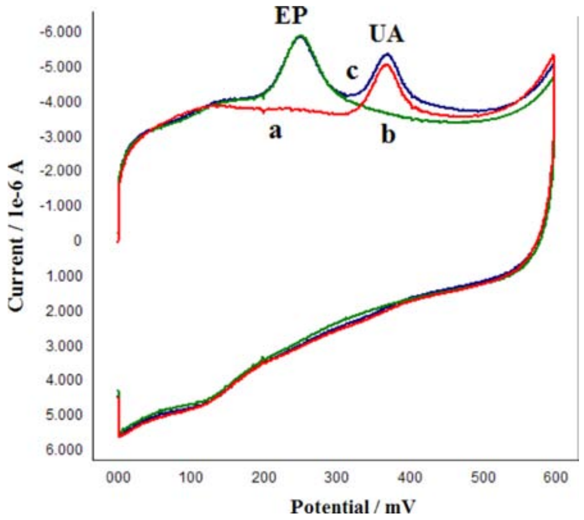

Fig. 6. Cyclic voltammograms of (a) EP (4.0×10^{-6} mol L^{-1}), (b) UA (1.0×10^{-5} mol L^{-1}) and (c) EP (4.0×10^{-6} mol L^{-1})+UA (1.0×10^{-5} mol L^{-1}) at GNPs/PAn-LB/GCE in 0.2 mol L^{-1} pH 6.6 PBS. Scan rate: 100 mV s^{-1}.

3.5.3. Interference studies

The major interfering substance for the electrochemical analysis of EP and UA was from AA. It was found that AA (up to 50 fold) did not affect the detection of EP and UA. Other molecules such as glucose, glutamic acid, xanthine and adenine have no obvious effect on the signal of EP and UA.

3.5.4. Determination of EP and UA in sample

In order to assess the applicability of the proposed method in physiological sample and its background interference, human blood serum was selected as sample. There was no distinct signal of EP or UA observed in health human blood serum sample. For evaluating the veracity, some EP and UA standard solution was added in blood serum before analysis. And the recovery was determined by standard addition method. The results of determination were listed in Table 2. The mean recovery of EP and UA were 97.40% and 99.75%, and the R.S.D. was calculated to be 2.73% and 4.85%, respectively.

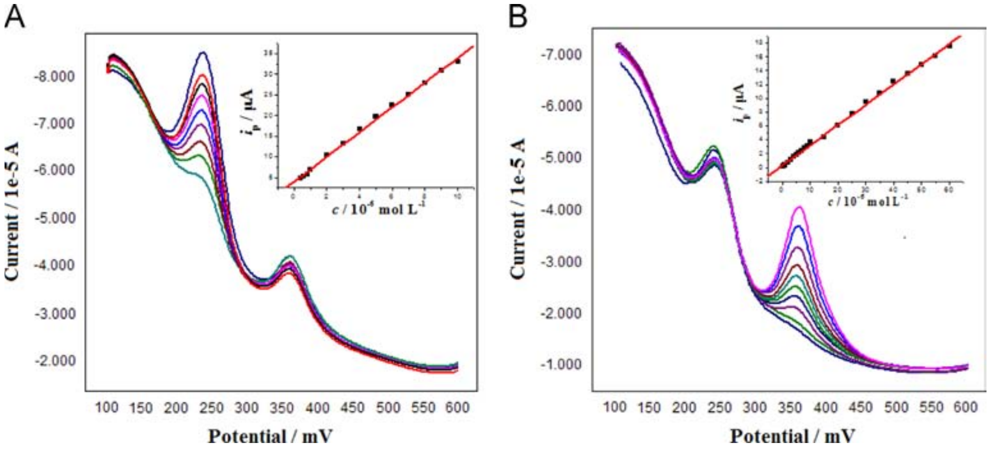

Fig. 7. Square wave voltammetric study of bases at GNPs/PAn-LB/GCE in 0.2 mol L^{-1} PBS (pH 6.6) with coexistence of another base. (A) EP concentration from 4.0×10^{-7} to 1.0×10^{-5} mol L^{-1} with 1.0×10^{-5} mol L^{-1} UA and (B) UA concentration from 4.0×10^{-7} to 6.0×10^{-5} mol L^{-1} with 4.0×10^{-6} mol L^{-1} EP.

Table 1
Comparison of different modified electrodes for EP and UA determination.

Electrode	Linear range ($mol\ L^{-1}$)		Detection limit ($mol\ L^{-1}$)		Ref.
	EP	UA	EP	UA	
DPD-Gold electrode	7.0×10^{-6}–5.0×10^{-4}	1.0×10^{-5}–7.5×10^{-4}	5.0×10^{-7}	9.0×10^{-6}	[20]
Activated/GCE	1.0×10^{-7}–5.0×10^{-4}	2.0×10^{-7}–1.0×10^{-5}	8.9×10^{-8}	1.6×10^{-7}	[21]
DOHM/CPE	1.0×10^{-5}–1.0×10^{-4}	1.0×10^{-4}–6.1×10^{-4}	8.0×10^{-7}	9.5×10^{-6}	[22]
Poly(caffeic acid)/GCE	2.0×10^{-6}–8.0×10^{-5}	5.0×10^{-6}–3.0×10^{-4}	2.0×10^{-7}	6.0×10^{-7}	[23]
PXSP/GCE	1.0×10^{-7}–5.6×10^{-4}	1.0×10^{-6}–3.9×10^{-4}	8.0×10^{-8}	1.0×10^{-7}	[24]
GNPs/PAn-LB/GCE	4.0×10^{-7}–1.0×10^{-5}	4.0×10^{-7}–6.0×10^{-5}	8.0×10^{-8}	2.0×10^{-7}	This work

Table 2
Determination result of EP and UA in human blood serum.

Sample	**EP** ($n=3$)				**UA** ($n=3$)			
	Added/(10^{-6} mol L^{-1})	**Total Found** /(10^{-6} mol L^{-1})	**R.S.D** (%)	**Recovery** (%)	**Added**/(10^{-5} mol L^{-1})	**Total Found** /(10^{-5} mol L^{-1})	**R.S.D.**(%)	**Recovery** (%)
1	4.00	3.82	4.21	95.6	1.00	0.98	5.34	98.41
2	5.00	4.91	1.36	98.25	1.50	1.48	3.74	99.05
3	6.00	5.90	2.61	98.36	2.00	2.04	5.49	101.79

4. Conclusions

In summary, we adopted LB technique combined with Polyaniline to design a novel GNPs/PAn-LB GCE. Based on this technology, GNPs would maintain its original state and thereby full play its electric activity. In this research, EP and UA were employed to show the advantage of GNPs/PAn-LB/GCE and better sensitivity was obtained comparing with that of other voltammetric sensors. This work suggested that GNPs modified electrode by LB techniques might to be a very promising platform for analytical sensing.

Acknowledgments

The authors gratefully acknowledge the financial support of the National Natural Science Foundation of China (Grant no. 21275132).

References

[1] J. Wang, W.D. Zhang, J. Electroanal. Chem. 654 (2011) 79–84.
[2] T. Kajita, M. Oyama, J. Electroanal. Chem. 656 (2011) 264–268.
[3] L.P. Wang, W. Mao, D.D. Ni, J.W. Di, Y. Wu, Y.F. Tu, Electrochem. Commun. 10 (2008) 673–676.
[4] M.A. Raj, S.B. Revin, S.A. John, Colloids Surf. B 87 (2011) 353–360.
[5] Ch.Y. Li, Y. Ya, G.Q. Zhan, Colloids Surf. B 76 (2010) 340–345.
[6] M. Oyama, D. Nakashima, C.Y. Cummings, F. Marken, Electrochem. Commun. 13 (2011) 68–71.
[7] J.P. Bourgoin, Thin Solid Films 327–329 (1998) 515–519.
[8] F. Sbrana, M.T. Parodi, D. Ricci, Z.E. Di, Mat. Sci. Eng.: C 22 (2002) 187–191.
[9] M.D. Hanwell, S.Y. Heriot, T.H. Richardson, Colloids Surf. A 284–285 (2006) 379–383.
[10] Sh.W. Chen, Adv. Mater. 12 (2000) 186–189.
[11] R. Gangopadhyay, A. De, Chem. Mater. 12 (2000) 608–622.
[12] A. Maity, M. Biswas, J. Appl. Polym. Sci. 100 (2006) 819–824.
[13] S. Srivastava, N.A. Kotov, Acc. Chem. Res. 41 (2008) 1831–1841.
[14] P. Granholm, J. Paloheimo, H. Stubb, Synth. Met. 84 (1997) 783–784.
[15] S. Manigandan, A. Jain, S. Majumder, Sensor. Actuat. B: Chem. 133 (2008) 187–194.
[16] G. Tsutsui, S.J. Huang, H. Sakaue, J. Appl. Phys. 40 (2001) 346–349.
[17] G. Tanami, V. Gutkin, D. Mandler, Langmuir 26 (2010) 4239–4245.
[18] L.N. Zou, Y.M. Li, B.X. Ye, Microchim. Acta 173 (2011) 285–291.
[19] E. Laviron, J. Electroanal. Chem. 101 (1979) 19–28.
[20] M.M. Ardakani, H. Beitollahi, M.K. Amini, J. Electroanal. Chem. 651 (2011) 243–249.
[21] J.X. Qiao, H.Q. Luo, N.B. Li., Colloids Surf. B 62 (2008) 31–35.
[22] M.M. Ardakani, Z. Taleat, H. Beitollahi, Chin. Chem. Lett. 22 (2011) 705–708.
[23] W. Ren, H.Q. Luo, N.B. Li, Biosensors Bioelectron. 21 (2006) 1086–1092.
[24] A.A. Ensafi, M. Taei, T. Khayamian, Colloids Surf. B 79 (2010) 480–487.