Accepted Manuscript

Title: Biosensor based on polyaniline-polyacrylonitrile-graphene hybrid assemblies for the determination of phenolic compounds in water samples

Authors: Hao Zheng, Zupeng Yan, Minghui Wang, Jianfang Chen, Xinzheng Zhang

PII: S0304-3894(19)30657-0
DOI: https://doi.org/10.1016/j.jhazmat.2019.05.107
Reference: HAZMAT 20714

To appear in: *Journal of Hazardous Materials*

Received date: 11 January 2019
Revised date: 17 May 2019
Accepted date: 30 May 2019

Please cite this article as: Zheng H, Yan Z, Wang M, Chen J, Zhang X, Biosensor based on polyaniline-polyacrylonitrile-graphene hybrid assemblies for the determination of phenolic compounds in water samples, *Journal of Hazardous Materials* (2019), https://doi.org/10.1016/j.jhazmat.2019.05.107

Biosensor based on polyaniline-polyacrylonitrile-graphene hybrid assemblies for the determination of phenolic compounds in water samples

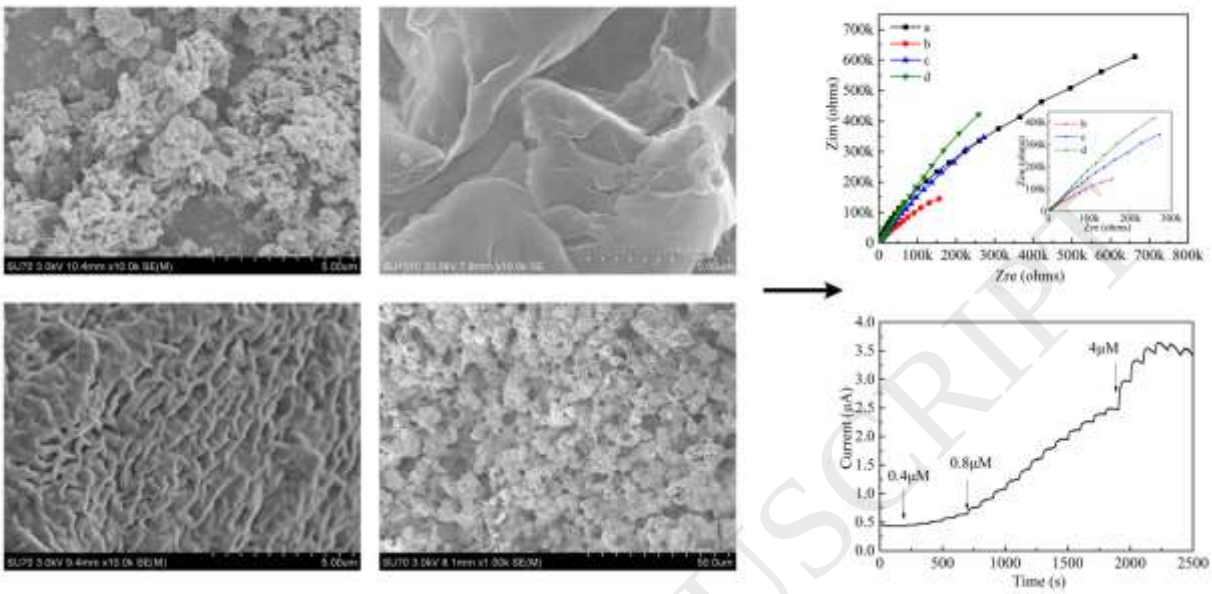
Hao Zheng[1,*], Zupeng Yan[1], Minghui Wang[1], Jianfang Chen[1,2], Xinzheng Zhang[1]

[1]*Institute of Marine Chemistry and Environment, Ocean College, Zhejiang University, Zhoushan 316021, People's Republic of China*

[2]*Key Laboratory of Marine Ecosystems and Biogeochemistry, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, People's Republic of China*

[*]*Corresponding author: Hao Zheng E-mail: zhenghao@zju.edu.cn Telephone: +86-580-2092260 Fax: +86-580-2092260*

Graphical abstract



Research Highlights

- A novel polyphenol biosensor based on hybrid assemblies of polyaniline-polyacrylonitrile-graphene has been fabricated.
- The excellent smooth steady-state currents were obtained to avoid errors caused by the current drifting and fluctuation.
- The proposed biosensor exhibited high sensitivity and selectivity for determination of phenolic compounds in water samples.

Abstract: Phenolic compounds are major environmental pollutants due to their toxic and hazardous nature on human health. A fast, sensitive and stable sensor for determination of phenolic compounds in the environmental water remains challenging. Herein, a biosensor platform with stable response current was fabricated by

entrapment of polyphenol oxidase (PPO) into hybrid assemblies of the conducting polyaniline (PAni)-porous polyacrylonitrile (PAN)-nanostructured graphene (GRA) and phase inversion process. The porous structure of PAN provided a favorable microenvironment for easily binding to PAni and GRA to obtain hybrid assemblies for effective immobilization of enzyme and increased synergistic effect. The morphologies and the electrochemical behaviors of the as-prepared biosensor were investigated using scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV), respectively. The proposed biosensor showed excellent sensitivity (6.46 μA μM^{-1} cm^{-2}) and fast response time (~5 s) with low detection limit (2.65×10^{-7} M) under the optimal pH value and applied potential. The biosensor was highly selective towards p-cresol that almost no signal was detected from common interferents. The biosensor was used for determination of phenolic compounds in water samples with satisfactory results compared with that of UPLC, demonstrating its great potential as a biosensor for the rapid determination of phenolic pollutants.

Keywords: Polyaniline; Nanocomposite; Polyphenol oxidase; Biosensor; Sensitivity

1. Introduction

Phenolic compounds are a class of refractory organic matters due to unique chemical structures that consists of an aromatic ring with one or more hydroxyl functional groups [1]. As is well known, it is harmful to ecosystem, natural

environment and human health even when present at a low concentration [2,3]. Phenolic compounds pose severe risks to human health with irreversible toxic effects on living organism. Its accumulation in phytoplankton and zooplankton has profound consequences to marine food webs and biogeochemical cycles [4,5]. Therefore, the determination of these phenolic compounds in environmental matrices and biological metabolites is of great significance. The various methods such as HPLC, GC-MS and spectrometry have been widely employed in the determination of phenolic compounds [6–10]. However, both chromatography and spectrometry have many weaknesses including the laborious cleaning work, tedious separation steps, expensive equipment and complex sample pretreatment. These disadvantages limit their practical applications, especially in long-term, real-time monitoring of phenolic compounds. Electrochemical technique with low cost and rapid analysis is regarded as a promising method [11,12]. Many reports have focused on the construction of polyphenol biosensor for evaluating phenolic compounds [15,16]. However, high sensitivity and stable response currents of biosensor are still major problems[13,14]. In order to overcome these drawbacks, it is crucial to have suitable support material and microenvironment for efficient entrapment of enzyme. Various functional materials have been developed, like carbon materials including graphene (GRA) [15] and carbon nanotubes (CNTs) [16], conductive polymers such as polyaniline (PAni) [17,18], polypyrrole [19,20] and polythiophene [21], other materials involving polyacrylonitrile (PAN) [22], polyvinyl chloride [23], bismuth oxide [24], and polydopamine [25]. GRA is a two-dimensional structure material that consists of

carbon atoms placed in a honeycomb crystal lattice bonded by sp^2 bond [26]. The unique fascinating properties of graphene such as fast electron transportation, excellent electrical conductivity and good mechanical strength make it a potential matrix for electrochemical biosensor[27,28]. However, graphene sheets tend to form graphite by agglomerating and restacking owing to π-π bonds and the van der Waals forces interaction between layers [29]. So far, versatile methods have been developed to disperse graphene sheets with polymer such as conducting PAni. PAni have been considered to be chemical stability polymer with good biocompatibility and controllable electrochemical properties [30–33], so the nanocomposites of GRA and PAni have successfully been employed by our research group for glucose biosensor [34]. Unfortunately, the nanocomposite is not suitable for other biosensors such as polyphenol biosensor due to the mechanism of enzyme catalytic reaction. PAN was employed as a redox mediator for redox benzoquinones to enhance stability of the response currents due to the electron-withdrawing of PAN with the nitrile group[35]. Furthermore, PAN is a porous polymeric material with satisfactory high surface area and excellent adsorption ability. The size of micropore is a little larger than that of an enzyme particle, so that enzymes could be adsorb into a favorable microenvironment provided by the PAN [36–38]. Zheng et al [39] firstly immobilized glucose oxidase into PAN micropore and obtained a fast response, good operational stability and reproducibility glucose biosensor. Moreover, it may also provide suitable microenvironment for preparing composites with PAni, which expands its usage [40]. The PAni-PAN-GRA hybrid assemblies could combine the excellent electrocatalytic

characteristics of PAni and GRA, porosity and good redox mediator of PAN, and exhibit better performance than those of the single pure component. Moreover, the strong interactions between PAni, PAN and GRA matrix could improve the electron transfer rate and the stability of response current. This is particularly important for investigating the polyphenol oxidase on catalytic reaction of phenolic compounds.

Based on our previous work [34,39,41], a polyphenol biosensor was fabricated by entrapment of PPO into hybrid assemblies of the conducting polyaniline-porous polyacrylonitrile-nanostructured graphene by an environmental friendly, reproducible and controllable method, then followed by a chemical crosslinking step with glutaraldehyde. The nanocomposite provided a stable platform for reduction catalytic reaction, which was suitable for polyphenol biosensor. The response currents of the biosensors based on different matrix materials have also been investigated.

2. Experimental

2.1 Materials

Polyphenol oxidase (PPO, BC grade) was purchased from Worthington (USA) with an activity of 1800 U mg^{-1}. P-cresol, aniline, ascorbic acid, Na_2HPO_4, KH_2PO_4, NaCl, $CaCl_2$, $MgSO_4$, KNO_3, $FeCl_3$, $ZnSO_4$, $CoCl_2$, $FeCl_2$ and $CuSO_4$ were obtained from Sinopharm Chemical Reagent Co., Ltd. All other reagents and chemicals were used as received without pretreatments. Milli-Q water was used throughout all test.

Polyaniline (PAni) was synthesized by a facile mechanochemical polymerization of aniline monomers with ammonium persulfate (APS) as an initiator under

solvent-free condition according to our previous report [41]. Polymerization of acrylonitrile was carried out using single component rare-earth catalyst-$Y(OAr)_3$ mentioned in our previous paper to obtain polyacrylonitrile (PAN) with molecular weight (M_w) of 2.93×10^4 [39]. Graphene (GRA) was prepared from exfoliation of graphite to form few-layer graphene sheets by electrochemical method in propylene carbonate electrolyte according to the literature [37].

2.2 Instrumental

All electrochemical studies were performed on PARSTAT P4000 electrochemical workstation (AMETEK, USA) with a conventional three-electrode system including reference electrode (saturated calomel electrode, SCE), working electrode (modified Pt disk electrode, diameter of 2 mm) and counter electrode (bare Pt disk electrode, diameter of 2 mm). The surface morphologies of the electrodes were observed by scanning electron spectroscopy (SEM) (SU70, Hitachi, JAPAN). The ACQUITY UPLC H-CLASS System equipped with a C18 column (Waters, BEH, 1.7μm, 2.1× 50 mm) and a PDA eλ Detector (Waters Corporation, USA) was utilized to detect p-cresol in water samples as a standard method.

2.3 Preparation of PAni-PAN-GRA modified electrodes

The bare platinum electrode was successively polished with 1.5 μm, 0.5 μm and 50 nm Al_2O_3, respectively, then washed using Milli-Q water. The electrode was treated by ultrasonic cleaning in ethanol and Milli-Q water sequentially.

The PAni-PAN-GRA solution was fabricated by hybrid assembling method. The

details were as follow: 3.4 mg PAN was added into 0.2 mL N, N-Dimethylformamide (DMF) then placed overnight to allow the PAN to be fully dissolved. The 2.2 mg PAni and 1.1 mg GRA were added into the PAN solution and treated with ultrasonic oscillation for 1 h. The PAni-PAN-GRA modified electrode was obtained by coating 0.5 μL PAni-PAN-GRA solution onto the polished electrode through phase inversion process [32].

2.4 Fabrication of the polyphenol biosensor

PPO was dissolved in 0.02 M phosphate buffer at pH 6.0 to obtain concentration of 4 mg/mL, followed by the addition of 0.5wt% acidic chitosan (CS) solution at a 1:3 (v/v) ratio. The PPO was immobilized onto the surface of PAni-PAN-GRA modified electrode by coating 5 μL of the prepared PPO solution and phase inversion process. The modified electrode was finally hanged in a saturated glutaraldehyde vapor vessel for 2 h at room temperature, in order to promote the cross-linking of the enzyme molecules. After exhaustively washed with Milli-Q water, the modified electrode was dried and stored at room temperature.

2.5 Electrochemical measurements

All amperometric measurements were carried out at an applied potential of -0.05 V (vs. SCE) in 0.02 M phosphate buffer (pH 6.0) without specific description, requiring the transient background to decrease to a steady-state value. A magnetic stirring was employed to achieve convective mass transfer during amperometric measurements.

The cyclic voltammetric experiments were carried out in 0.02 M phosphate buffer (pH 6.0) with potentials ranging from -0.4 to 0.4 V (vs. SCE) at a scan rate of 50 mV/s. Electrochemical impedance spectroscopy (EIS) were recorded in 0.02M phosphate buffer (pH 6.0) containing 1mM $Fe(CN)_6^{4-/3-}$ from 100 kHz to 1 Hz. Amperometric currents-time responses were monitored at an applied potential of -0.05 V versus saturated calomel electrode (SCE).

3. Results and discussion

3.1 Effect of matrix materials on response currents

Amperometric measurements were recorded for characterizing the performance of the biosensors based on different matrix materials. As shown in Fig. 1, the currents drifting appeared in the response currents of the GRA/CS-PPO (curve a) and PAni-GRA/CS-PPO electrodes (curve b). In contrast, more stable response currents were observed from the PAN-GRA/CS-PPO (curve c) and PAni-PAN-GRA/CS-PPO (curve d) electrodes. These results implied that PAN might played a significant role in obtaining more reliable response current due to the electron-withdrawing nature of nitrile groups. The functional nitrile groups of PAN used as a redox mediator in electron transfer shuttle, which was suitable for biosensors especially based on reductase reaction [42]. The PAN component in nanocomposite has enhanced the current stability of biosensor and avoided the current drifting. In addition, comparing curve c with curve d, the response current of the curve d (PAni-PAN-GRA/CS-PPO) was obviously greater than that of the curve c (PAN-GRA/CS-PPO), which might be

assigned to the excellent conductivity and biocompatibility of PAni and The synergistic effect of PAni-PAN-GRA hybrid assemblies. Therefore, the PAni-PAN-GRA nanocomposite was chosen as the biosensor matrix for the immobilization of PPO.

(The preferred position for Fig. 1)

3.2 Morphology characterization of the biosensor

The surface morphologies of (A) PAni, (B) GRA, (C) PAN, (D) PAni-PAN-GRA and (E) PAni-PAN-GRA/CS-PPO were characterized by scanning electron microscopic (SEM). As shown in Fig. 2A, PAni through a facile mechanochemical method based on various aniline-ammonium persulfate molar ratios displayed a rod-like structure and a length of about 300 nm with an average diameter of 200 nm [41]. The small size of PAni was beneficial for dispersing the graphene sheets and provide large surface area. GRA (Fig. 2B) from electrochemical process exhibited a curved, flake-like and few-layers structure composed of merely carbon atoms and could be reasoned out according to the Raman spectra [43], which endowed GRA excellent conductivity. The meshed uniform structure of PAN was observed from Fig. 2C, it can be seen that network contained high pore volume. When PAni, GRA and PAN were assembled together (Fig. 2D), the nanocomposite film was mostly occupied by GRA which became much rougher and shaggier, and the microporous structure of PAN encouraged PAni to fully disperse around the film. This specific appearance in Fig. 2D could be explained by the concentrations of these three components, as well

as the specific surface area for each of them were taken into account. As seen in Fig. 2E, a large amount of PPO was uniformly embedded into the PAni-PAN-GRA nanocomposite.

(The preferred position for Fig. 2)

3.3 Electrochemical characterization of the biosensors

Fig. 3 showed the cyclic voltammograms (CVs) of various modified electrodes in 0.02 M phosphate buffer (pH 6.0) with potential from -0.4 to 0.4 V (vs. SCE). As shown in Fig. 3A, no obvious difference was observed for PAni-PAN-GRA between curve a in the absence of p-cresol and curve b in the presence of 4 μM p-cresol without the participation of PPO and the same results were found in PAni-PAN-GRA/CS (curve c and curve d), indicating that PAni-PAN-GRA and PAni-PAN-GRA/CS based electrode had no catalytic activity toward the reaction of p-cresol. The increased oxidation current can be clearly seen from Fig. 3B in the presence of p-cresol (curve b) compared with that of the absence of p-cresol (curve a) due to the direct electrochemistry of PPO, which suggested that the PPO-catalyzed reaction of p-cresol occurred on the biosensor. It could be clearly confirmed that the response of the biosensor to p-cresol resulted from the catalytic activity of enzyme immobilized in the nanocomposite. The catalytic role of PPO might be considered to be important in the reaction process of modified electrode. Therefore, the PAni-PAN-GRA/CS-PPO electrode could be employed to determine the p-cresol concentration.

(The preferred position for Fig. 3)

Electrochemical impedance spectroscopy (EIS) was used to evaluate and analyze the interface properties of the surface-modified electrodes. Fig. 4 listed the EIS of different modified electrodes including of PAni-PAN, PAni-PAN-GRA, PAni-PAN-GRA/CS and PAni-PAN-GRA/CS-PPO, displaying apparent differences of the electrochemical impedance spectra. The electron transfer resistance Rct of PAni-PAN-GRA modified electrode (curve b) was much smaller than that of PAni-PAN modified electrode (curve a), which might be assigned to excellent conductivity of GRA, improving the electron transfer of the electrochemical probe. However, when CS was incorporated onto the modified electrode, the value of Rct increased slightly (curve c), which indicated that CS was of nonconductive property and hindered the electron transfer. After the modified electrode was further assembled with PPO, the value of Rct was found to be increase in small increments for the same reason as CS (curve d), implying the successful immobilization of PPO.

(The preferred position for Fig. 4)

3.4 Optimal conditions of the biosensors

The operational conditions such as solution pH and potential have a significant influence on the electrochemical behavior of the biosensor. The effects of various applied potential on the biosensor behavior were studied over a range of potential from -0.3 V to 0.05 V (vs. SCE). As shown in Fig. 5A, maximum value of response current was observed at -0.05 V and the response current to p-cresol decreased when

applied potential varied in more negative potential. The result was in agreement with the previously reported work [18,44], indicating that the decrease of the response current at negative potentials was attributed to the decreased enzymatic activity resulting from the oxygen depletion in the vicinity of the PPO immobilized at the electrode surface. Therefore, the applied potential at -0.05 V was selected in the following experiments.

The dependence of the biosensor response on the phosphate buffer pH was also investigated in the pH range of 3.5 to 7.8. It can be found from Fig. 5B, the electrochemical response current was quite stable at pH values ranging from 5.5 to 7.0 and the maximum response current was obtained at pH 6.0. The similar results have been already reported [22]. On the other hand, the current response decreased at lower or higher pH values due to the changing activity of PPO as a function of phosphate buffer pH [45]. It was well known that highly acidic or alkaline solution would damage the bioactivity of immobilized enzymes. Therefore, the optimum pH with the high performance of the biosensor was chosen at pH 6.0 in the subsequent experiments.

(The preferred position for Fig. 5)

3.5 Performance of the biosensor

The current response of the PAni-PAN-GRA/CS-PPO modified electrode was investigated on successive addition of p-cresol with concentrations from 0.4 μM to 4.0 μM to the stirring phosphate buffer under the optimized experimental conditions

(Fig. 6A). As can be seen, the modified electrode indicated fast response achieving 95% of the steady state currents within 5 s due to excellent conductivity and best electron transfer of hybrid assemblies nanocomposite [34]. Furthermore, the smooth response currents were observed at every step from Fig. 6A attributed to the PAN used as electron transfer shuttle in a redox mediator and favorable microenvironment provided for adsorbing enzyme, avoiding the currents drifting. The smooth currents are superior to that of some already reported phenol biosensors [45–47]. As shown in Fig. 6B, the increased response current was no obvious with increasing p-cresol in the lower region of concentration while the current response attained a saturation level in the relatively high concentration region. More information can be obtained from Fig. 6C that the response currents for p-cresol determination exhibited high sensitivity of 6.46 μA μM^{-1} cm^{-2}, and a linearly range from 2×10^{-6} M to 1.16×10^{-5} M (R^2=0.9963) with detection limit of 2.65×10^{-7} M (S/N= 3). The sensitivity was much higher than that of PAni-PPO biosensor [45], PAni-PAN biosensor [22] and PAni-SWCNFs biosensor [48], but not as good as PAni-ionic liquid-PPO biosensor [18]. Apart from the anthropogenic PBS solution, the detection limits were also calculated in the other two environmental aqueous matrices acquired around our campus, 5.13×10^{-7} M (S/N= 3) in the lake and 3.65×10^{-7} M (S/N= 3), respectively, a little higher than that of PBS solution, but still acceptable. A further comparison of different electrochemical biosensors for the determination of p-cresol was summarized in table S1. An apparent Michaelis-Menten constant was estimated to be 2.53 μM from Fig. 6D.

(The preferred position for Fig. 6)

3.6 Stability and anti-interference ability of the biosensor

The operational stability of the biosensor was evaluated by monitoring the response currents in the presence of 4 μM p-cresol. The relative standard deviation (RSD) of the biosensor was found to be 3.9% for ten measurements respectively. In addition, the biosensor reproducibility was also examined from the response for 4 μM p-cresol at four independently prepared electrodes, which indicated the RSD of 5.4% for the determination of p-cresol concentration. This good stability and reproducibility might be assigned to favorable microenvironment provided by hybrid assemblies nanocomposite for effective immobilization of PPO.

The anti-interference ability was vital to the practical application of the biosensor. Herein, we chose Na^+, K^+, Ca^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}, NO_3^- and Cl^- as inorganic interference representatives, and glucose, aniline, and ascorbic acid as the organic ones to evaluate the property of the fabricated biosensor. The addition of up to ten times concentration higher than p-cresol of all these inorganic ions showed no considerable influence in 0.02M PBS (pH 6.0) solution, so did the organic interference in the same concentration of p-cresol. The excellent anti-interference ability could be contributed to excellent physical isolation of GRA and synergistic effect of PAni-PAN-GRA hybrid assemblies as well as the specific catalysis of polyphenol oxidase.

3.7 Determination of phenolic compound in water samples

To demonstrate the possible application of the proposed biosensor, the water

samples were estimated by standard addition method as reported previously [24,49]. No special sample pretreatment was performed except filtration and pH adjustment before the water samples obtained from the Qiantang River and East China Sea were analyzed. The experiment results were presented in Table S2. By adding standard p-cresol to the water sample over the concentration range from 4.0 μM to 9.0 μM, the recovery of the water samples was found to be from 102% to 106.7%, in general, indicating that the concentrations found were higher than those added. It was implied that both the Qiantang River and East China Sea contained refractory toxic phenolic compounds but very low level of the content, indicating relatively good quality of water. This is attributed to the effective environmental protection of Hangzhou city and the globalization.

Apart from those two kinds of water samples, we acquired another four samples around Zhoushan City, one from polluted adjacent sea, three from fresh water areas risking of p-cresol pollution. All but seawater sample were detected by both biosensor and UPLC method. Briefly, 2.5 ml of each water samples was added into a 25 ml 0.02 M PBS (pH 6.0) solution to acquire the signal change of the fabricated biosensor, then the concentration of p-cresol was calculated by the linear fitting curve of Section 3.5 and compared with the results from UPLC method (Table S3). The relative low difference between the biosensor detection and UPLC method indicated the as-prepared biosensor was qualified for real water sample detection.

4. Conclusions

We have developed the biosensor based on a nanocomposite through PAni, PAN

and GRA hybrid assemblies for the determination of phenolic pollutants. The PAni-PAN-GRA nanocomposite based biosensor demonstrated excellent performance, especially, a high sensitivity and stable response current. The synergistic effect of PAni-PAN-GRA hybrid assemblies not only improves the electron transfer ability of the system, but also exhibits the signal amplification of the nanocomposites and anti-interference ability. The proposed biosensor has been successfully applied for detecting p-cresol in real samples with satisfactory results compared with that of UPLC. Moreover, its advantage is also that there is no complex sample pretreatment in the analysis process. The hybrid assembly nanocomposites are promising to be highly useful for other biosensors based on redox reaction in the future. Further development of the new composition method may reveal more exciting microenvironment of the hybrid assembly nanocomposites for commercial and practical sensor platforms.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (U1709201, 91128212). It was also supported by the Global Climate Changes and Air-sea Interaction Program (GASI-02-PAC-ST-Wwin) and the National Key Research and Development Program of China (Grant No. 2016YFC0304905).

References:

[1] K. Min, C. Freeman, H. Kang, S.-U. Choi, K. Min, C. Freeman, H. Kang, S.-U. Choi, The Regulation by Phenolic Compounds of Soil Organic Matter Dynamics under a Changing Environment, Biomed Res. Int. 2015 (2015) 1–11. doi:10.1155/2015/825098.

[2] F.R. Caetano, E.A. Carneiro, D. Agustini, L.C.S. Figueiredo-Filho, C.E. Banks, M.F. Bergamini, L.H. Marcolino-Junior, Combination of electrochemical biosensor and textile threads: A microfluidic device for phenol determination in tap water, Biosens. Bioelectron. 99 (2018) 382–388. doi:10.1016/j.bios.2017.07.070.

[3] C.C. Mayorga-Martinez, M. Cadevall, M. Guix, J. Ros, A. Merkoçi, Bismuth nanoparticles for phenolic compounds biosensing application, Biosens. Bioelectron. 40 (2013) 57–62. doi:10.1016/j.bios.2012.06.010.

[4] P. Jin, T. Wang, N. Liu, S. Dupont, J. Beardall, P.W. Boyd, U. Riebesell, K. Gao, Ocean acidification increases the accumulation of toxic phenolic compounds across trophic levels, Nat. Commun. 6 (2015) 1–6. doi:10.1038/ncomms9714.

[5] Q. Lu, H. Hu, Y. Wu, S. Chen, D. Yuan, R. Yuan, An electrogenerated chemiluminescence sensor based on gold nanoparticles@C60 hybrid for the determination of phenolic compounds, Biosens. Bioelectron. 60 (2014) 325–331. doi:10.1016/j.bios.2014.04.044.

[6] C. Wang, Y. Zuo, Ultrasound-assisted hydrolysis and gas chromatography-mass spectrometric determination of phenolic compounds in cranberry products, Food Chem. 128 (2011) 562–568. doi:10.1016/j.foodchem.2011.03.066.

[7] A. Zafra-Gómez, B. Luzón-Toro, I. Jiménez-Diaz, O. Ballesteros, A. Navalón, Quantification of phenolic antioxidants in rat cerebrospinal fluid by GC-MS after oral administration of compounds, J. Pharm. Biomed. Anal. 53 (2010) 103–108. doi:10.1016/j.jpba.2010.03.003.

[8] M. Heinäaho, A.E. Hagerman, R. Julkunen-Tiitto, Effect of different organic farming methods on the phenolic composition of sea buckthorn berries, J. Agric. Food Chem. 57 (2009) 1940–1947. doi:10.1021/jf802797v.

[9] O.J. Lara-Guzmán, R. Álvarez-Quintero, E. Osorio, M. Naranjo-Cano, K. Muñoz-Durango, GC/MS method to quantify bioavailable phenolic compounds and antioxidant capacity determination of plasma after acute coffee consumption in human volunteers, Food Res. Int. 89 (2016) 219–226. doi:10.1016/j.foodres.2016.07.020.

[10] K. Pyrzynska, A. Sentkowska, Recent Developments in the HPLC Separation of Phenolic Food Compounds, Crit. Rev. Anal. Chem. 45 (2015) 41–51. doi:10.1080/10408347.2013.870027.

[11] J. Kudr, O. Zitka, M. Klimanek, R. Vrba, V. Adam, Microfluidic electrochemical devices for pollution analysis–A review, Sensors Actuators, B Chem. 246 (2017) 578–590. doi:10.1016/j.snb.2017.02.052.

[12] M. Arciuli, G. Palazzo, A. Gallone, A. Mallardi, Bioactive paper platform for colorimetric phenols detection, Sensors Actuators, B Chem. 186 (2013) 557–562. doi:10.1016/j.snb.2013.06.042.

[13] Z. Qiu, J. Shu, D. Tang, Bioresponsive Release System for Visual Fluorescence Detection of Carcinoembryonic Antigen from Mesoporous Silica Nanocontainers Mediated Optical Color on Quantum Dot-Enzyme-Impregnated Paper, Anal. Chem. 89 (2017) 5152–5160. doi:10.1021/acs.analchem.7b00989.

[14] Y. Lin, Q. Zhou, D. Tang, Dopamine-Loaded Liposomes for in-Situ Amplified

Photoelectrochemical Immunoassay of AFB 1 to Enhance Photocurrent of Mn^{2+} -Doped $Zn_3(OH)_2V_2O_7$ Nanobelts , Anal. Chem. 89 (2017) 11803–11810. doi:10.1021/acs.analchem.7b03451.

[15] H. Zhang, H. Zhang, A. Aldalbahi, X. Zuo, C. Fan, X. Mi, Fluorescent biosensors enabled by graphene and graphene oxide, Biosens. Bioelectron. 89 (2017) 96–106. doi:10.1016/j.bios.2016.07.030.

[16] M.S. Cosio, A. Pellicanò, B. Brunetti, C.A. Fuenmayor, A simple hydroxylated multi-walled carbon nanotubes modified glassy carbon electrode for rapid amperometric detection of bisphenol A, Sensors Actuators, B Chem. 246 (2017) 673–679. doi:10.1016/j.snb.2017.02.104.

[17] Y. Tan, X. Guo, J. Zhang, J. Kan, Amperometric catechol biosensor based on polyaniline-polyphenol oxidase, Biosens. Bioelectron. 25 (2010) 1681–1687. doi:10.1016/j.bios.2009.12.007.

[18] J. Zhang, J. Lei, Y. Liu, J. Zhao, H. Ju, Highly sensitive amperometric biosensors for phenols based on polyaniline-ionic liquid-carbon nanofiber composite, Biosens. Bioelectron. 24 (2009) 1858–1863. doi:10.1016/j.bios.2008.09.012.

[19] Y. Wang, X. Qing, Q. Zhou, Y. Zhang, Q. Liu, K. Liu, W. Wang, M. Li, Z. Lu, Y. Chen, D. Wang, The woven fiber organic electrochemical transistors based on polypyrrole nanowires/reduced graphene oxide composites for glucose sensing, Biosens. Bioelectron. 95 (2017) 138–145. doi:10.1016/j.bios.2017.04.018.

[20] J. Shu, Z. Qiu, D. Tang, Self-Referenced Smartphone Imaging for Visual Screening of H_2S Using CuxO-Polypyrrole Conductive Aerogel Doped with Graphene Oxide Framework, Anal. Chem. 90 (2018) 9691–9694. doi:10.1021/acs.analchem.8b03011.

[21] D. Voccia, M. Sosnowska, F. Bettazzi, G. Roscigno, E. Fratini, V. De Franciscis, G. Condorelli, R. Chitta, F. D'Souza, W. Kutner, I. Palchetti, Direct determination of small RNAs using a biotinylated polythiophene impedimetric genosensor, Biosens. Bioelectron. 87 (2017) 1012–1019. doi:10.1016/j.bios.2016.09.058.

[22] H. Xue, Z. Shen, A highly stable biosensor for phenols prepared by immobilizing polyphenol oxidase into polyaniline-polyacrylonitrile composite matrix, Talanta. 57 (2002) 289–295. doi:10.1016/S0039-9140(02)00028-0.

[23] S. Chawla, J. Narang, C.S. Pundir, An amperometric polyphenol biosensor based on polyvinyl chloride membrane, Anal. Methods. 2 (2010) 1106–1111. doi:10.1039/c0ay00165a.

[24] D. Shan, J. Zhang, H.G. Xue, Y.C. Zhang, S. Cosnier, S.N. Ding, Polycrystalline bismuth oxide films for development of amperometric biosensor for phenolic compounds, Biosens. Bioelectron. 24 (2009) 3671–3676. doi:10.1016/j.bios.2009.05.038.

[25] R. Ren, G. Cai, Z. Yu, Y. Zeng, D. Tang, Metal-Polydopamine Framework: An Innovative Signal-Generation Tag for Colorimetric Immunoassay, Anal. Chem. 90 (2018) 11099–11105. doi:10.1021/acs.analchem.8b03538.

[26] R. Stine, S.P. Mulvaney, J.T. Robinson, C.R. Tamanaha, P.E. Sheehan, Fabrication, optimization, and use of graphene field effect sensors., Anal. Chem. 85 (2013) 509–21. doi:10.1021/ac303190w.

[27] Q. Zhou, Y. Lin, K. Zhang, M. Li, D. Tang, Reduced graphene oxide/$BiFeO_3$ nanohybrids-based signal-on photoelectrochemical sensing system for prostate-specific antigen detection coupling with magnetic microfluidic device, Biosens. Bioelectron. 101 (2018) 146–152. doi:10.1016/j.bios.2017.10.027.

[28] D. Tang, J. Shu, K. Zhang, Y. Lin, Q. Zhou, Z. Yu, Reduced graphene oxide-functionalized FeOOH for signal-on photoelectrochemical sensing of prostate-specific antigen with bioresponsive controlled release system, Biosens. Bioelectron. 98 (2017) 15–21. doi:10.1016/j.bios.2017.06.033.

[29] A.T. Lawal, Synthesis and utilisation of graphene for fabrication of electrochemical sensors, Talanta. 131 (2015) 424–443. doi:10.1016/j.talanta.2014.07.019.

[30] R. Devi, S. Relhan, C.S. Pundir, Construction of a chitosan/polyaniline/graphene oxide nanoparticles/ polypyrrole/Au electrode for amperometric determination of urinary/plasma oxalate, Sensors Actuators, B Chem. 186 (2013) 17–26. doi:10.1016/j.snb.2013.05.078.

[31] J. Lai, Y. Yi, P. Zhu, J. Shen, K. Wu, L. Zhang, J. Liu, Polyaniline-based glucose biosensor: A review, J. Electroanal. Chem. 782 (2016) 138–153. doi:10.1016/j.jelechem.2016.10.033.

[32] P. Marcasuzaa, S. Reynaud, F. Ehrenfeld, A. Khoukh, J. Desbrieres, Chitosan-graft-polyaniline-based hydrogels: Elaboration and properties, Biomacromolecules. 11 (2010) 1684–1691. doi:10.1021/bm100379z.

[33] R. Zeng, Z. Luo, L. Zhang, D. Tang, Platinum Nanozyme-Catalyzed Gas Generation for Pressure-Based Bioassay Using Polyaniline Nanowires-Functionalized Graphene Oxide Framework, Anal. Chem. 90 (2018) 12299–12306. doi:10.1021/acs.analchem.8b03889.

[34] X. Feng, H. Cheng, Y. Pan, H. Zheng, Development of glucose biosensors based on nanostructured graphene-conducting polyaniline composite, Biosens. Bioelectron. 70 (2015) 411–417. doi:10.1016/j.bios.2015.03.046.

[35] F. Xianjie, P. Yu, B. Morandi, Catalytic reversible alkene-nitrile interconversion through controllable transfer hydrocyanation, Science (80-.). 351 (2016) 832–836. doi:10.1126/science.aae0427.

[36] R. Nenkova, D. Ivanova, J. Vladimirova, T. Godjevargova, New amperometric glucose biosensor based on cross-linking of glucose oxidase on silica gel/multiwalled carbon nanotubes/polyacrylonitrile nanocomposite film, Sensors Actuators, B Chem. 148 (2010) 59–65. doi:10.1016/j.snb.2010.05.034.

[37] G. Bayramoğlu, M. Karakişla, B. Altintaş, A.U. Metin, M. Saçak, M.Y. Arica, Polyaniline grafted polyacylonitrile conductive composite fibers for reversible immobilization of enzymes: Stability and catalytic properties of invertase, Process Biochem. 44 (2009) 880–885. doi:10.1016/j.procbio.2009.04.011.

[38] G. Bayramoğlu, A.Ü. Metin, M.Y. Arica, Surface modification of polyacrylonitrile film by anchoring conductive polyaniline and determination of uricase adsorption capacity and activity, Appl. Surf. Sci. 256 (2010) 6710–6716. doi:10.1016/j.apsusc.2010.04.077.

[39] H. Zheng, H. Xue, Y. Zhang, Z. Shen, A glucose biosensor based on microporous polyacrylonitrile synthesized by single rare-earth catalyst, Biosens. Bioelectron. 17 (2002) 541–545. doi:10.1016/S0956-5663(02)00010-6.

[40] G. Zhai, Q. Fan, Y. Tang, Y. Zhang, D. Pan, Z. Qin, Conductive composite films composed of polyaniline thin layers on microporous polyacrylonitrile surfaces, Thin Solid Films. 519 (2010) 169–173. doi:10.1016/j.tsf.2010.07.088.

[41] H. Zheng, X. Feng, L. Zhou, Y. Ye, J. Chen, Intercalated polyaniline-kaolinite nanocomposite prepared via in situ mechanochemical synthesis, J. Appl. Polym. Sci. 133 (2016) 1–8. doi:10.1002/app.43551.

[42] D. Shan, C. Mousty, S. Cosnier, S. Mu, A New Polyphenol Oxidase Biosensor Mediated by

Azure B in Laponite Clay Matrix, Electroanalysis. 15 (2003) 1506–1512. doi:10.1002/elan.200302740.

[43] J. Wang, K.K. Manga, Q. Bao, K.P. Loh, High-yield synthesis of few-layer graphene flakes through electrochemical expansion of graphite in propylene carbonate electrolyte, J. Am. Chem. Soc. 133 (2011) 8888–8891. doi:10.1021/ja203725d.

[44] E. Han, D. Shan, H.G. Xue, S. Cosnier, Hybrid material based on chitosan and layered double hydroxides: Characterization and application to the design of amperometric phenol biosensor, Biomacromolecules. 8 (2007) 971–975. doi:10.1021/bm060897d.

[45] P. Wang, M. Liu, J. Kan, Amperometric phenol biosensor based on polyaniline, Sensors Actuators, B Chem. 140 (2009) 577–584. doi:10.1016/j.snb.2009.05.005.

[46] F.C. Vicentini, L.L.C. Garcia, L.C.S. Figueiredo-Filho, B.C. Janegitz, O. Fatibello-Filho, A biosensor based on gold nanoparticles, dihexadecylphosphate, and tyrosinase for the determination of catechol in natural water, Enzyme Microb. Technol. 84 (2016) 17–23. doi:10.1016/j.enzmictec.2015.12.004.

[47] Y. Qu, M. Ma, Z. Wang, G. Zhan, B. Li, X. Wang, H. Fang, H. Zhang, C. Li, Sensitive amperometric biosensor for phenolic compounds based on graphene-silk peptide/tyrosinase composite nanointerface, Biosens. Bioelectron. 44 (2013) 85–88. doi:10.1016/j.bios.2013.01.011.

[48] B. Wang, J. Zheng, Y. He, Q. Sheng, A sandwich-type phenolic biosensor based on tyrosinase embedding into single-wall carbon nanotubes and polyaniline nanocomposites, Sensors Actuators, B Chem. 186 (2013) 417–422. doi:10.1016/j.snb.2013.06.016.

[49] J. Kochana, A. Gala, A. Parczewski, J. Adamski, Titania sol-gel-derived tyrosinase-based amperometric biosensor for determination of phenolic compounds in water samples. Examination of interference effects, Anal. Bioanal. Chem. 391 (2008) 1275–1281. doi:10.1007/s00216-007-1798-6.

Figure captions

Fig. 1 Amperometric responses of bionsensors based on different matrix materials (a) GCE/GRA/CS-PPO, (b) Pt/PAni-GRA/CS-PPO, (c) Pt/PAN-GRA/CS-PPO, (d) Pt/PAni-PAN-GRA/CS-PPO.

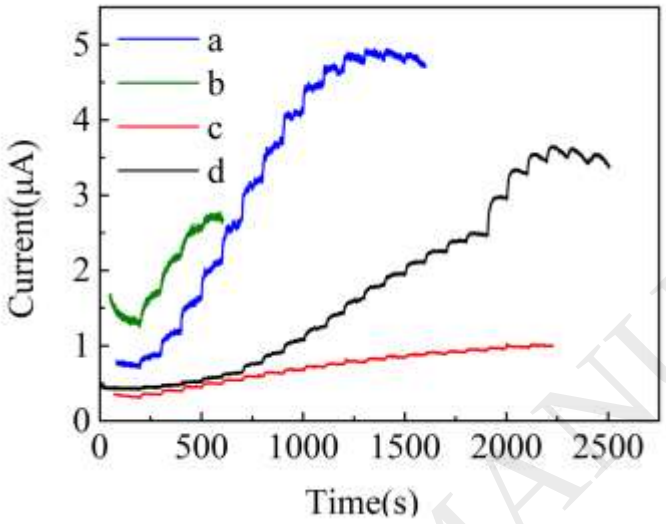


Fig. 2 SEM imagines of (A) PAni, (B) GRA, (C) PAN, (D) PAni-PAN, (E) PAni-PAN-GRA/CS-PPO.

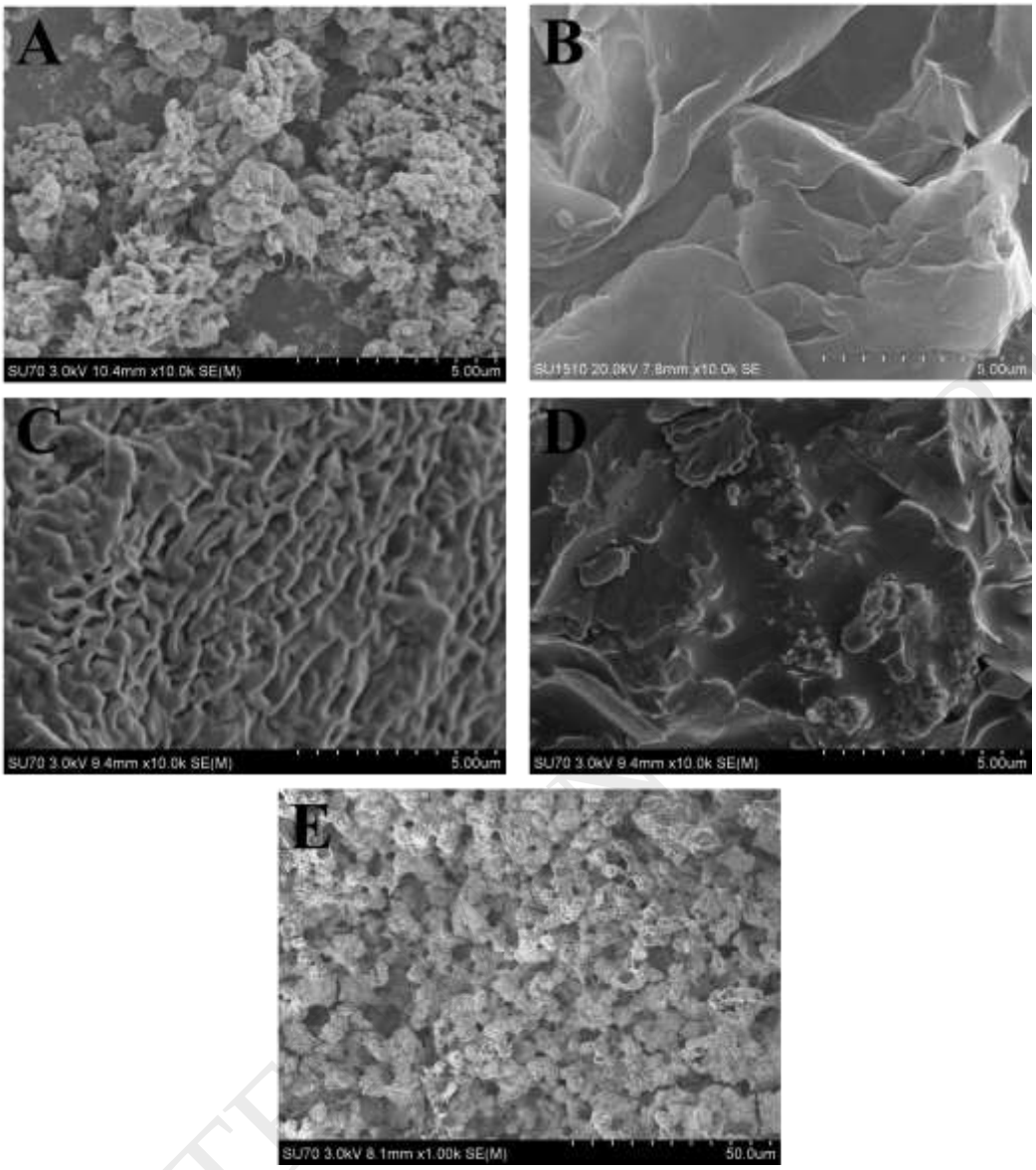


Fig. 3 (A) CVs in 0.02M pH 6.0 phosphate buffer at 50mV/s for (a) without p-cresol, and (b) with 4μM p-cresol of PAni-PAN-GRA modified electrode, (c) without p-cresol, and (d) with 4μM p-cresol of PAni-PAN-GRA/CS modified electrode. (B) CVs in 0.02M pH 6.0 phosphate buffer at 50mV/s for (a) without p-cresol, and (b) with 4μM p-cresol of PAni-PAN-GRA/CS-PPO modified electrode.

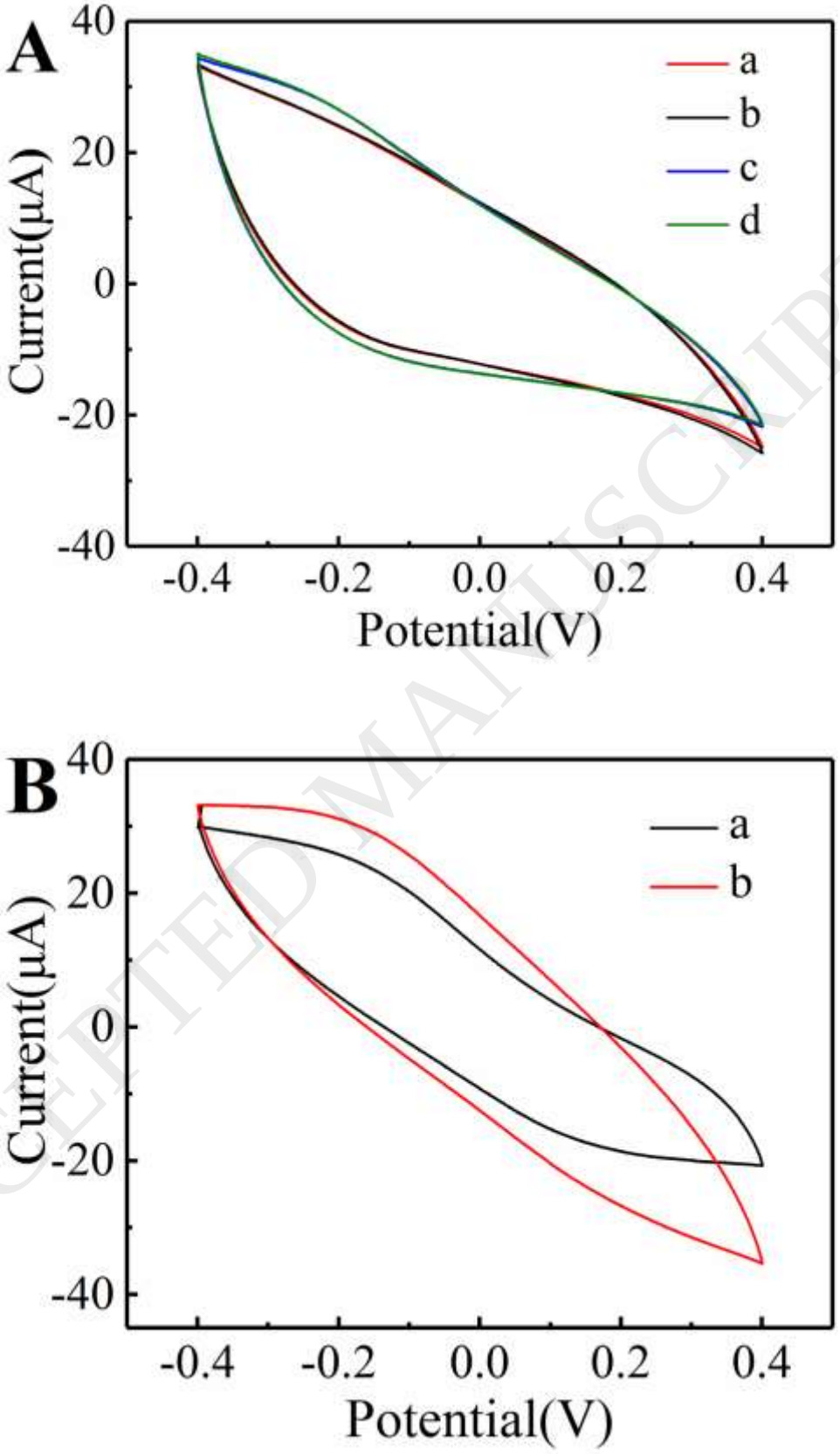


Fig. 4 EIS of different modified Pt disk electrodes in 0.02M pH 6.0 phosphate

buffer for (a) PAni-PAN, (b) PAni-PAN-GRA, (c) PAni-PAN-GRA/CS and (d) PAni-PAN-GRA/CS-PPO modified electrodes. The inset figure showed the EIS for (b) PAni-PAN-GRA, (c) PAni-PAN-GRA/CS and (d) PAni-PAN-GRA/CS-PPO.

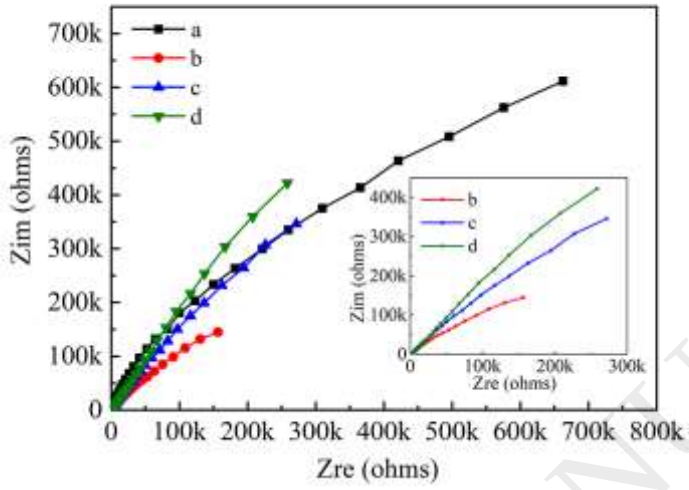


Fig. 5 Effects of (A) applied potential in 0.02M pH 6.0 phosphate buffer containing 4μM p-cresol and (B) pH values in different pH buffers containing 4μM p-cresol on the current responses of PAni-PAN-GRA/CS-PPO modified electrode.

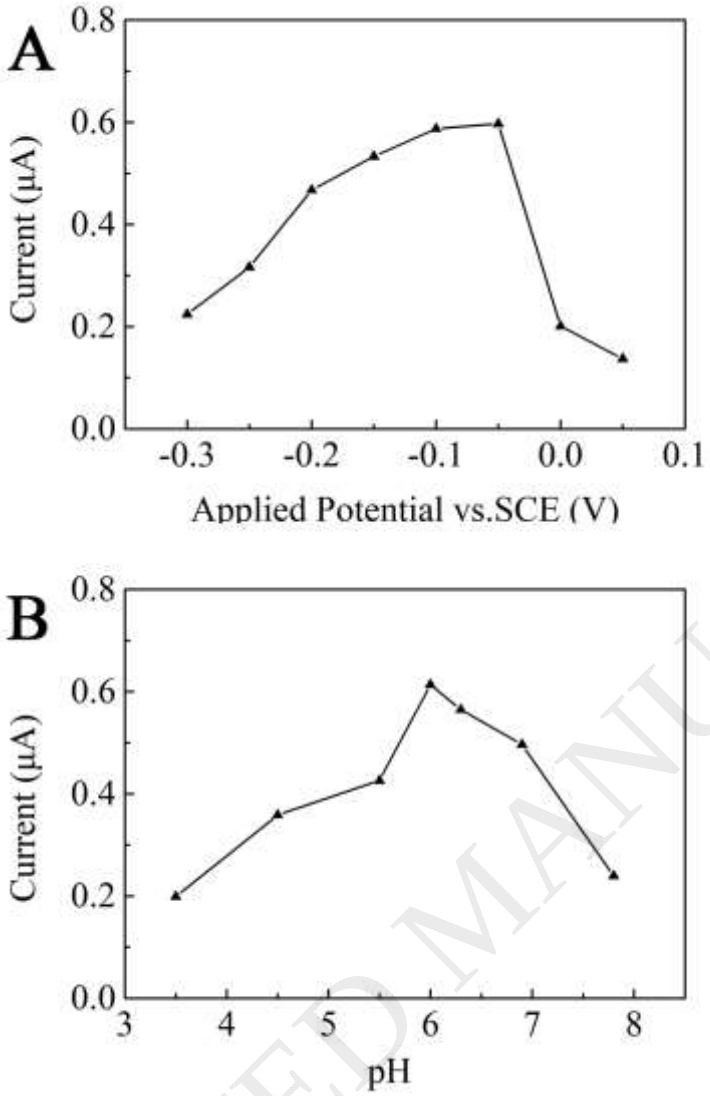


Fig. 6 (A) Amperometric responses to successive injection of p-cresol at an applied potential of -0.05V (vs. SCE) in 0.02M pH 6.0 phosphate buffer, (B) Current-Concentration curves, (C) calibration curves, and (D) calculation of apparent Michaelis–Menten constants according to the data in (B).