Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Report:

An anti-passivation ink for the preparation of electrodes for use in electrochemical immunoassays*#

Qi-qi ZHENG[1,2], Yuan-chao LU[1,2], Zun-zhong YE[1,2], Jian-feng PING[1,2], Jian WU[†‡1,2], Yi-bin YING[1,2,3]

[1]*College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China*

[2]*Key Laboratory of on Site Processing Equipment for Agricultural Products, Ministry of Agriculture, Hangzhou 310058, China*

[3]*Faculty of Agricultural and Food Science, Zhejiang A&F University, Hangzhou 311300, China*

[†]E-mail: wujian69@zju.edu.cn

Received Oct. 11, 2017; Revision accepted Dec. 4, 2017; Crosschecked Aug. 31, 2018

Abstract: *p*-Nitrophenylphosphate (PNPP) is usually employed as the substrate for enzyme-linked immunosorbent assays. *p*-Nitrophenol (PNP), the product of PNPP, with the catalyst alkaline phosphatase (ALP), will passivate an electrode, which limits applications in electrochemical analysis. A novel anti-passivation ink used in the preparation of a graphene/ionic liquid/chitosan composited (rGO/IL/Chi) electrode is proposed to solve the problem. The anti-passivation electrode was fabricated by directly writing the graphene-ionic liquid-chitosan composite on a single-side conductive gold strip. A glassy carbon electrode, a screen-printed electrode, and a graphene-chitosan composite-modified screen-printed electrode were investigated for comparison. Scanning electron microscopy was used to characterize the surface structure of the four different electrodes and cyclic voltammetry was carried out to compare their performance. The results showed that the rGO/IL/Chi electrode had the best performance according to its low peak potential and large peak current. Amperometric responses of the different electrodes to PNP proved that only the rGO/IL/Chi electrode was capable of anti-passivation. The detection of cardiac troponin I was used as a test example for electrochemical immunoassay. Differential pulse voltammetry was performed to detect cardiac troponin I and obtain a calibration curve. The limit of detection was 0.05 ng/ml.

Key words: Electrochemical immunoassay; Electrode ink; Anti-passivation; Ionic liquid; *p*-Nitrophenol
https://doi.org/10.1631/jzus.B1700510 **CLC number:** O652.1

1 Introduction

Electrochemical detection is a simple, fast, sensitive, and cost-effective method suitable for on-site applications (Bansod et al., 2017). The sensitive element of the testing system is the working electrode. Various types of electrodes are used in research, such as glassy carbon electrodes (GCEs) (Dinesh et al., 2017), precious metal electrodes (Tang and Wu, 2014; Yu et al., 2014), carbon paste electrodes (Idris et al., 2017), and screen-printed electrodes (SPEs) (González-Sánchez et al., 2016; Li S et al., 2017). To improve electrochemical performance, the electrode is usually modified with other materials such as nanoparticles (Budnikov and Shirokova, 2013) and polymers (Sajid et al., 2016). For instance, Ma et al. (2012) used a graphene-modified glass carbon electrode to detect dopamine selectively in the presence of epinephrine, uric acid, and ascorbic acid by measuring the reduction peak current. Molazemhosseini et al. (2017) fabricated a gold film electrode modified with CuO nanoparticles for the detection of glucose. Cinti et al. (2017) modified an SPE with a nanocomposite formed by carbon black and Prussian blue nanoparticles to

‡ Corresponding author
* Project supported by the National Natural Science Foundation of China (No. 31571918)
# Electronic supplementary materials: The online version of this article (https://doi.org/10.1631/jzus.B1700510) contains supplementary materials, which are available to authorized users
ORCID: Qi-qi ZHENG, https://orcid.org/0000-0003-1639-0739; Jian WU, https://orcid.org/0000-0003-4518-7027

detect ethanol in beer. In recent years, flexible electrodes have become popular, such as a paper-based electrode (Aneesh and Berchmans, 2017) and electrode ink (Secor et al., 2015). Ghosale et al. (2017) fabricated a low-cost paper electrode by directly writing with silver nanoparticle-based ink for detection of hydrogen peroxide in wastewater. Yang et al. (2015) fabricated serpentine gold electrodes by means of thermal evaporation for use as multi-parametric epidermal sensor systems (Yang et al., 2015).

Our research group has also made great efforts to improve the performance of electrodes. Graphene, also known as single atomic layer graphite, was used to fabricate a free-standing paper electrode by vacuum filtration for use as an impedimetric immunosensor (Wang et al., 2013) because of its high conductivity, fast electron transfer rate, large specific surface, and good biocompatibility (Kuila et al., 2011). We also fabricated a screen-printed graphene electrode for simultaneous determination of ascorbic acid, dopamine, and uric acid (Ping et al., 2012b). However, the manufacturing process requires the use of an organic solvent such as acetone cyclohexanone which is harmful to health and environment. Chitosan is a kind of thickener and coating agent in numerous fields, such as medicine, food, and chemical industry because of its biocompatibility, safety, and biodegradability. It can be used as binder to modify the surface of electrode (Nesakumar et al., 2016). In our previous studies, chitosan was used not only as the binder for producing nano-silver/acetylcholinesterase electrode ink, but also as the insulating compound to control the area of the electrode. It was the first time that chitosan had been used as the insulating compound (Zheng et al., 2016).

In this study, we used both graphene and chitosan for the fabrication of electrode ink. *n*-Octylpyridinium hexafluorophosphate (*n*-OPFP), a kind of ionic liquid comprising organic cations and anions (Ping et al., 2010; Tang et al., 2014), was also included in the electrode ink. We found that *n*-OPFP was superior in resisting fouling of the electrode when *p*-nitrophenol (PNP) was present in the solution. PNP is the product of an enzyme-linked immunosorbent assay (ELISA) (Saita et al., 2017) when *p*-nitrophenylphosphate (PNPP) is used as substrate and alkaline phosphatase (ALP) is used as catalyst (Preechaworapun et al., 2008). The fouling of the electrode by PNP may limit the application of immunosensors. Hence, we developed an anti-passivation electrode ink to overcome the fouling. The ink also has advantages of high performance and safety, and is pro-environment owing to the use of graphene and chitosan. We chose cardiac troponin I (cTnI) as a test example for electrochemical immunoassay using our anti-passivation electrode. The concentration of cTnI in serum is usually employed in the diagnosis and risk stratification of acute myocardial infarction (Li SJ et al., 2017).

2 Materials and methods

2.1 Reagents

Chitosan (>400 mPa·s), PNP, PNPP, gelatin, and diethanolamine were purchased from Aladdin (Shanghai, China). ALP from bovine intestinal mucosa, casein from bovine milk, Tween 20 and silver powder (5–8 μm) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Bovine serum albumin (BSA) and sterile Tris-buffer solutions (pH 8.5 and pH 7.5) were purchased from Sangon Biotech (Shanghai, China). Graphene was obtained from XFNano Co., Ltd. (Nanjing, China). *n*-OPFP was obtained from Shanghai Chengjie Co., Ltd., China. Potassium ferrocyanide, potassium chloride, magnesium chloride hexahydrate, and sodium hydroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Human cTnI monoclonal antibody used as capture antibodies; human cTnI and ALP-labeled cTnI monoclonal antibodies were obtained from Kitgen Biotech Co., Ltd. (Hangzhou, China). Reagents were of analytical grade and were used without further purification. All aqueous solutions were prepared using ultrapure water (Millipore, 18.2 MΩ/cm).

2.2 Apparatus

Images of the electrodes were taken with a scanning electron microscope (SEM; SU8010, Hitachi, Japan). Cyclic voltammetry, amperometric method, and differential pulse voltammetry (DPV) were performed using a CHI760 electrochemical workstation (CH Instruments, Austin, TX, USA). When the GCE was employed as the working electrode, an Ag/AgCl electrode and a platinum wire were used as the reference and auxiliary electrodes, respectively. The SPE was fabricated on a Z-C3050A screen printer (Zhengting Screen Printing Machine Co., Ltd., China) using commercial graphite ink, silver ink, and insulating

ink obtained from Acheson Co., Ltd. (USA). Spectrophotometry was performed using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). ELISA was carried out in 96-well plates (Jet Biofil, Guangzhou, China).

2.3 Fabrication of electrodes

Four kinds of electrodes were used in this work: a commercial GCE, a home-made SPE, a graphene/chitosan (rGO/Chi)-modified SPE, and a graphene/ionic liquid/chitosan composited (rGO/IL/Chi) electrode. The GCE was polished with aluminium oxide powder (0.05 μm). The SPE was fabricated according to the method of Ping et al. (2012a). In brief, the first layer was printed with silver ink on a polyvinyl chloride sheet as the reference electrode. The second layer was printed with graphite ink for use as the working electrode and counter electrode. The printed electrodes were heated in an oven at 120 °C for 30 min to evaporate the solvents. Insulating ink was printed on the film electrodes to form an insulating layer which solidified through exposure to ultraviolet radiation. The rGO/Chi-modified SPE was prepared by adding a drop of 2 μl rGO/Chi solution to the surface of the working SPE electrode. Chitosan (0.1 g) was dissolved in a 10-ml aqueous solution containing acetic acid (58 μl). Graphene was added to the solution of chitosan at a ratio of 1:60 (w/v). The rGO/IL/Chi-composited ink was made from the mixture of graphene, ionic liquid, and chitosan at a ratio of 1:19:60 (w/w/v). The rGO/IL/Chi-composited ink was used to fabricate a working electrode by directly writing on a single-side conductive gold strip. The reference electrode and counter electrode were written on the reverse side of the gold strip using home-made silver ink made from a mixture of silver powder and chitosan solution at a ratio of 2:1 (w/v). Then, the rGO/IL/Chi electrode was heated in an oven at 75 °C for 10 min to evaporate the moisture and melt the ionic liquid. The rGO/IL/Chi electrode was polished on a weighing paper to obtain a smooth surface. The solution of 3% (0.03 g/ml) chitosan was used as insulating ink to control the area of the electrodes.

2.4 Electrochemical analysis of different electrodes

Cyclic voltammetry was carried out for the characterization of the four kinds of electrodes in a solution of 1 mmol/L potassium ferrocyanide and 0.1 mol/L potassium chloride. The potential range was −0.1 to 0.6 V at a scan rate of 0.05 V/s. Cyclic voltammetry was also performed to compare the responses of the four kinds of electrodes to 0.6 mmol/L PNP in Tris buffer solution (0.5 mol/L, pH 8.5) in the potential range of 0.2 to 1.1 V at a scan rate of 0.05 V/s. Amperometric method was carried out in 10 ml Tris buffer solution (0.5 mol/L, pH 8.5) under stirring at 1.04 V for SPE, 0.99 V for GCE, 0.90 V for rGO/Chi-modified SPE, and 0.84 V for the rGO/IL/Chi electrode. A drop of 10 μl PNP (0.6 mol/L) was added to the solution. The change of current was recorded. The microscopic structures of working electrodes of SPE, rGO/Chi-modified SPE, and rGO/IL/Chi electrodes were characterized by SEM at a magnification of ×3000.

Cyclic voltammetry was carried out to investigate the responses of the rGO/IL/Chi electrode to PNPP (3 mmol/L) and PNPP (3 mmol/L) with PNP (0.3 mmol/L) in Tris buffer solution (0.5 mol/L, pH 8.5) in the potential range of 0.2 to 1.1 V at a scan rate of 0.05 V/s. Amperometric method was carried out in 10 ml Tris buffer solution (0.5 mol/L, pH 8.5) including 0.5 mmol/L magnesium ions (Mg^{2+}) and 4.5 mmol/L PNPP under stirring at 0.84 V using the rGO/IL/Chi electrode. ALP (50, 100, and 200 U/L) was added to the solution. The change of current was recorded.

2.5 Immunoassay

The procedures for ELISA were as follows: the capture antibodies diluted with Tris buffer solution (0.05 mol/L, pH 7.5, 100 μl) were added to the 96-well plates for 12 h at 4 °C. Then, the blocking solution (200 μl), cTnI antigen (100 μl), and ALP-labeled antibody (100 μl) were sequentially added to the plates for 1 h at 37 °C. After each procedure, the plates were washed three times with Tris-Tween buffer solution (0.05% Tween 20, 0.05 mol/L Tris buffer, pH 7.5). Then, the solution of PNPP (4.5 mmol/L, 200 μl) was added to the plates for 15 min at 37 °C. NaOH solution (3 mol/L, 50 μl) was added to stop the reaction. The absorbance was measured by spectrophotometry at a wavelength of 405 nm. The concentrations of capture antibody and ALP-labeled antibody were optimized. The concentration gradient of the capture antibody was 3.3, 6.6, 13.2, and 26.4 μg/ml. The concentration gradient of ALP-labeled antibody

was 5, 10, 15, and 20 μg/ml. Solutions of BSA, casein, and gelatin (0.01 and 0.02 g/ml) were used to optimize the blocking solution. The concentration gradient of cTnI used to make the calibration curve was 0.05, 1, 10, 20, and 30 ng/ml.

DPV was carried out from 0.5 to 1.0 V with 4 mV incremental potential, 50 mV amplitude, 0.05 s pulse width, 0.0167 sample width, and 0.2 s pulse period for the detection of cTnI. The rGO/IL/Chi electrode was inserted in the ELISA plate containing 250 μl reactant solutions. The concentration gradient of cTnI was the same as that used in spectrophotometry. Each data point used to obtain a calibration curve was calculated from three parallel tests.

3 Results and discussion

3.1 Analysis of the performance of electrodes

Results from the characterization of the four kinds of electrodes in the solution of potassium ferrocyanide are shown in Fig. 1. There were two redox peaks in each curve, which could be attributed to the redox of ferric ions. The upward peak is an anodic peak, reflecting the oxidation process from ferrous ion to ferric ion. Correspondingly, the downward peak is a cathodic peak, reflecting the reduction process from ferric ion to ferrous ion. The peak potential difference of the rGO/IL/Chi electrode was 60 mV, which was smaller than that of the other three electrodes. The peak current of the rGO/IL/Chi electrode was the largest among the four kinds of electrodes. This indicates that the rGO/IL/Chi electrode performed better than the other electrodes. The surface textures of the working electrodes are shown in Fig. 2. There were many holes among the graphite flakes of the working electrode of SPE (Fig. 2a), leading to a non-uniform structure. In Fig. 2b, irregular particles can be seen on the graphite flakes, which may be the chitosan. The performance of the rGO/Chi-modified SPE was better than that of the SPE because of its smaller peak potential difference and larger peak current (Fig. 1), which was due to the use of graphene. The smooth surface of the rGO/IL/Chi electrode (Fig. 2c) may have been attributable to the ionic liquid and the polishing procedure. Ionic liquid has advantages of high ionic conductivity and a wide electrochemical window. Polishing can wipe impurities from the surface of working electrodes. A homogeneous surface texture leads to superior performance.

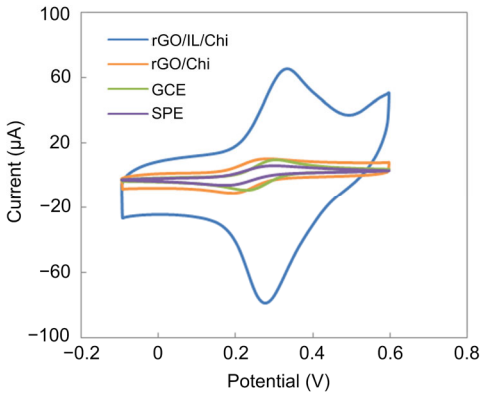

Fig. 1 Cyclic voltammogram of characterization of different electrodes (different color lines) in a solution of 1 mmol/L potassium ferrocyanide and 0.1 mol/L potassium chloride with a potential range of −0.1 to 0.6 V at a scan rate of 0.05 V/s

Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article

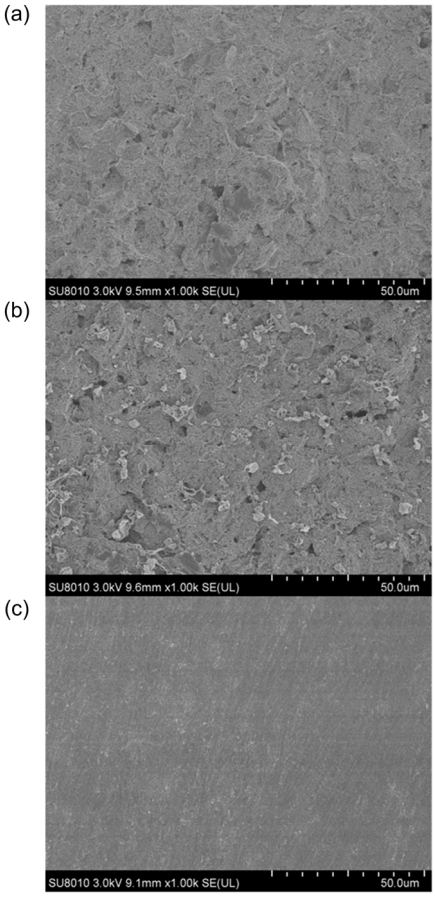

Fig. 2 SEM images of the surfaces of working electrodes

(a) SPE; (b) rGO/Chi-modified SPE; (c) rGO/IL/Chi electrode

The responses of the electrodes to PNP with cyclic voltammetry are shown in Fig. 3. The anodic peak potential was 0.84 V for the rGO/IL/Chi electrode, 0.90 V for the rGO/Chi-modified SPE, 0.99 V for the GCE, and 1.01 V for the SPE. Comparing the curves for the rGO/Chi-modified SPE and SPE, the performance of the electrodes was improved through the modification of graphene. The response of the rGO/Chi-modified SPE to PNP was also higher than that of GCE. The rGO/IL/Chi electrode had the lowest peak potential and the largest peak current, which means that it had higher sensitivity and better anti-interference ability than the other three types of electrodes. Amperometric responses of the electrodes to PNP are shown in Fig. 4. The currents of all four curves in Fig. 4 went up when PNP was added to the solution. However, only the curve in Fig. 4d kept the current stable. This indicates that PNP will passivate electrodes and the rGO/IL/Chi electrode has capability for anti-passivation.

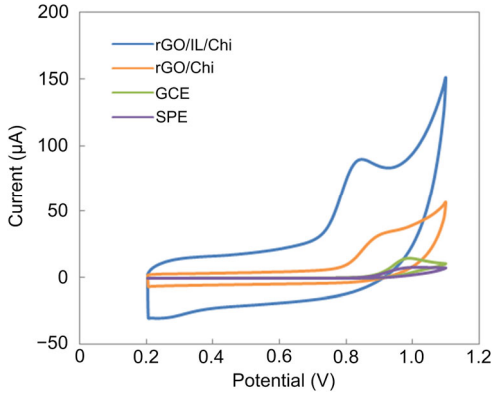

Fig. 3 Cyclic voltammogram of the responses of different electrodes (different color lines) to PNP (0.6 mmol/L) in Tris buffer solution (0.5 mol/L, pH 8.5) with a potential range of 0.2 to 1.1 V at a scan rate of 0.05 V/s

Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article

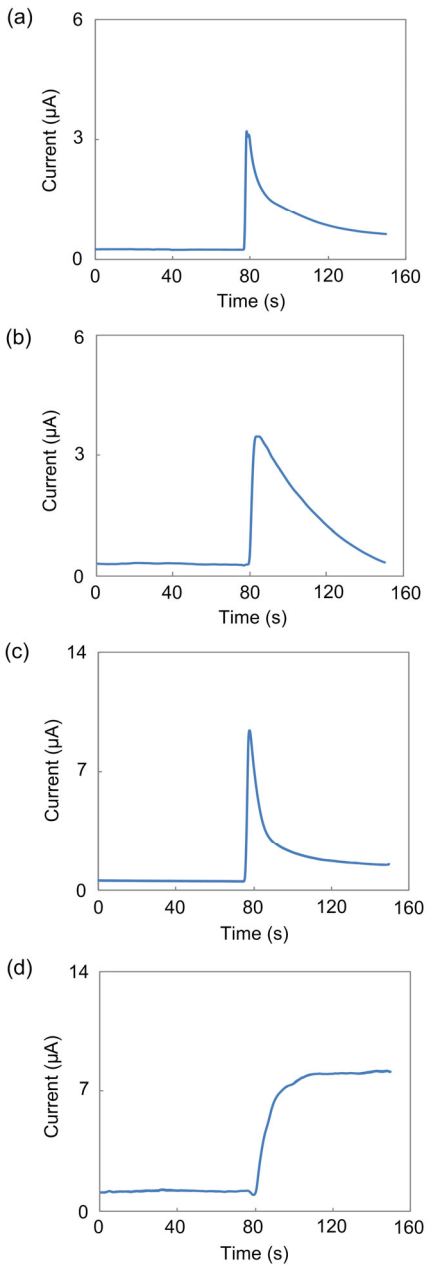

Fig. 4 Amperometric responses of different electrodes to PNP (0.6 mmol/L) in Tris buffer solution (0.5 mol/L, pH 8.5)

(a) 1.01 V for SPE; (b) 0.99 V for GCE; (c) 0.90 V for rGO/Chi-modified SPE; (d) 0.84 V for rGO/IL/Chi electrode

3.2 Electrochemical immunoassay

PNPP is widely used as the substrate in ELISA. It is necessary to investigate the redox characteristics of PNPP. There was no redox peak in the curve of PNPP (3 mmol/L) in Fig. 5. The curve for PNPP (3 mmol/L) with PNP (0.3 mmol/L) showed an anodic peak at 0.84 V. The anodic peak could be attributed to PNP. This indicated that PNPP did not generate a redox reaction in this range of potential and did not influence the response of the electrode to PNP. Amperometric method was used to investigate the influence of the concentration of ALP in the enzymatic reaction, using PNPP as the substrate. The currents went up

when ALP was added in the solution (Fig. 6) because of the production of PNP. The final current increased with increasing ALP concentration. Thus, the concentration of ALP can be detected indirectly.

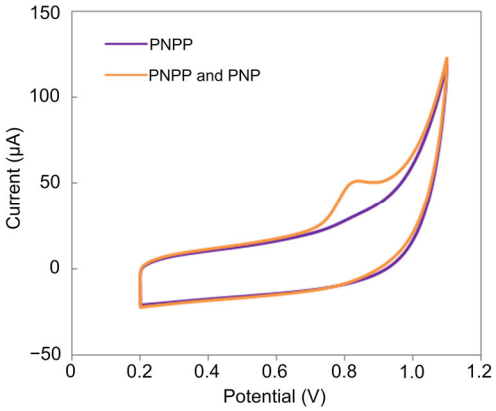

Fig. 5 Cyclic voltammogram of the response of the rGO/IL/Chi electrode to 3 mmol/L PNPP (purple line) and 3 mmol/L PNPP with 0.3 mmol/L PNP (orange line) in Tris buffer solution (0.5 mol/L, pH 8.5) with a potential range of 0.2 to 1.1 V at a scan rate of 0.05 V/s

Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article

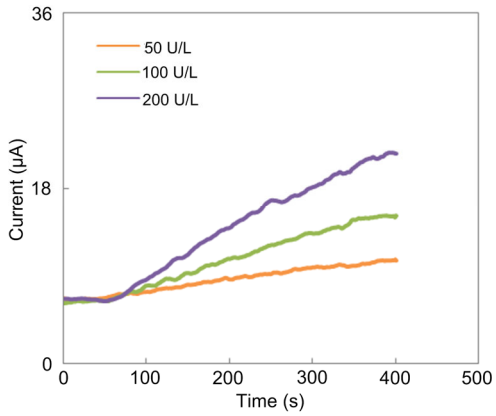

Fig. 6 Amperometric responses of the rGO/IL/Chi electrode to the product of PNPP (4.5 mmol/L) using ALP as a catalyst at 0.84 V

The concentration gradient of ALP is 50, 100, and 200 U/L (different color lines) (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

The immunoassay of cTnI is based on a sandwich structure. The blocking solution and the concentrations of capture antibody and ALP-conjugated antibody were optimized. As shown in Fig. S1, the absorbance was highest when BSA was used and lowest when gelatin was used as the blocking solution. However, high absorbance of background will decrease the signal-to-noise ratio when using BSA as the blocking solution. Hence, 0.01 g/ml casein solution was chosen because of its high signal-to-noise ratio. Absorbance was enhanced as the concentration of capture antibody increased (Fig. S2). The speed of the increase declined when the concentration exceeded 13.2 μg/ml. The signal-to-noise ratio was highest at a concentration of 13.2 μg/ml of capture antibody. So, 13.2 μg/ml of capture antibody was chosen to be used in the following experiments. Absorbance was also enhanced with increasing concentrations of ALP-conjugated antibody (Fig. S3). A concentration of 15 μg/ml was chosen because it had the highest signal-to-noise ratio.

The detection of cTnI was performed with both an electrochemical method and a spectrometric method. The comparison of the original curve with the self-corrected curve recoded in 30 ng/ml of cTnI by DPV is shown in Fig. 7. The original curve in Fig. 7a had a large background current. The self-correcting software designed by our research group was used to eliminate the background current, resulting in a flat baseline. The peak current in Fig. 7b was well defined after correction. Fig. 8 shows the relationship between the concentration of cTnI and the peak current recorded by DPV. The peak current increased with increasing concentrations of cTnI. The high response of the rGO/IL/Chi electrode to PNP means that there were many ALP-labeled antibodies in the solution (Fig. 6). The ALP-labeled antibodies were conjugated to the cTnI antigens. So, the concentration of cTnI was able to be detected indirectly through monitoring the peak current. The concentration gradient of cTnI was 0.05, 1, 10, 20, and 30 ng/ml. The calibration curve shown in Fig. 9b is linear (R^2= 0.996). The limit of detection of cTnI was 0.05 ng/ml. A spectrometric method was carried out for the comparison as shown in Fig. 9a. The concentration gradient of cTnI was the same as that used for the electrochemical method. The calibration curve was linear with the related coefficient of 0.996. The results shown in Fig. 9 indicated the feasibility of detection of cTnI by electrochemical immunoassay using the rGO/IL/Chi electrode.

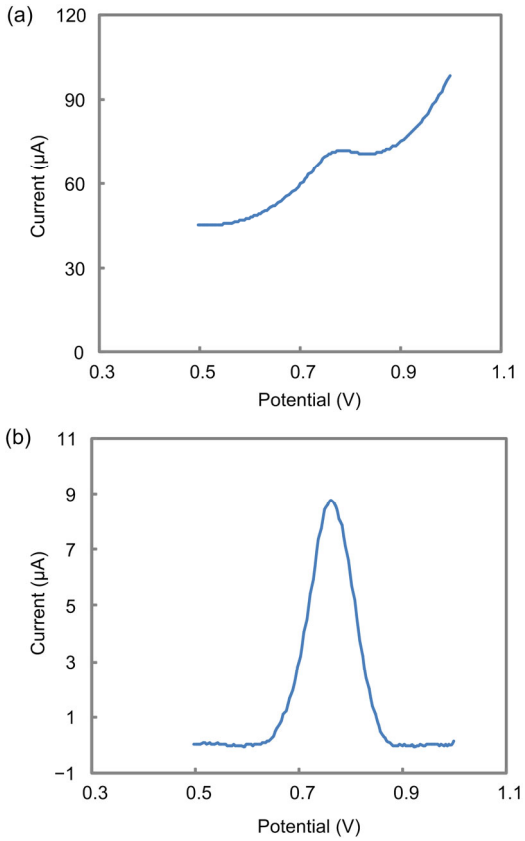

Fig. 7 DPV of the response of the rGO/IL/Chi electrode to 30 ng/ml cTnI in the potential range of 0.5 to 1.0 V with 4 mV incremental potential, 50 mV amplitude, 0.05 s pulse width, 0.0167 sample width, and 0.2 s pulse period
(a) Original curve; (b) Self-corrected curve

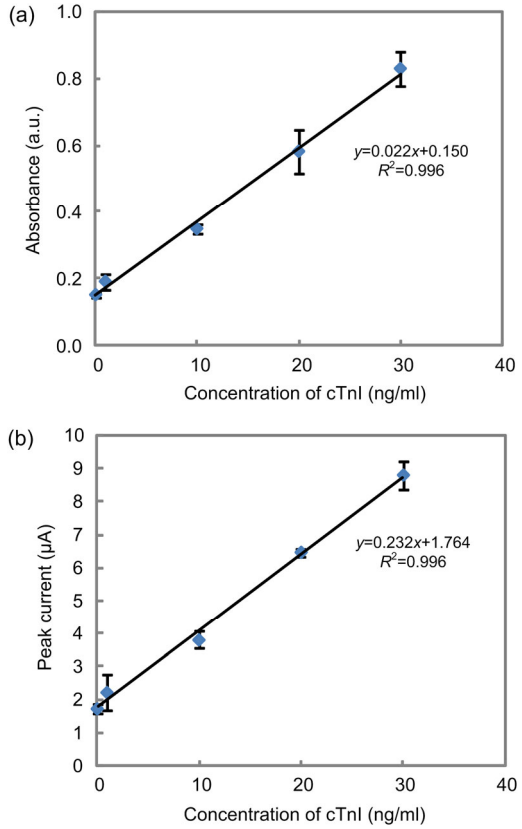

Fig. 9 Calibration curves of cTnI
(a) Spectrophotometry; (b) Differential pulse voltammetry. Each data point is expressed as mean±standard deviation from three parallel tests. a.u.: automatic unit

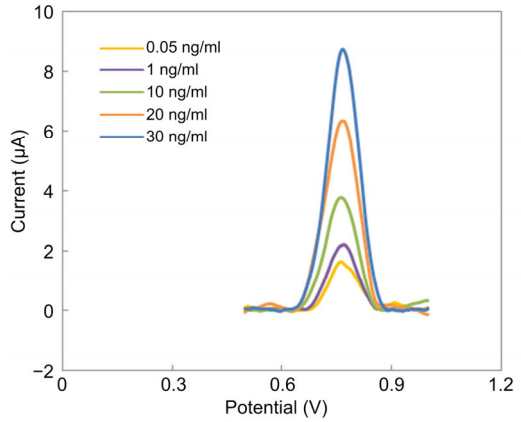

Fig. 8 DPV for the detection of cTnI using the rGO/IL/Chi electrode in the potential range of 0.5 to 1.0 V with 4 mV incremental potential, 50 mV amplitude, 0.05 s pulse width, 0.0167 sample width, and 0.2 s pulse period
The concentration gradient of cTnI is 0.05, 1, 10, 20, and 30 ng/ml (different color lines) (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

4 Conclusions

We developed a novel anti-passivation electrode for electrochemical immunoassay by directly writing a graphene-ionic liquid-chitosan composite on a single-side conductive gold strip. The reference electrode was written on the other side of the gold strip using silver-chitosan ink. Three percent chitosan solution was used as an insulating compound to control the area of the electrode. No organic solvents were used in the fabrication. The electrode had superior performance in detecting PNP according to its low peak potential and large peak current, which could be attributed to the application of graphene and ionic liquid. Compared with the other three kinds of electrodes, only the rGO/IL/Chi electrode had anti-passivation capability when PNP was present in the solution. As a test example, cTnI was detected by electrochemical immunoassay. The limit of detection was 0.05 ng/ml.

In conclusion, the rGO/IL/Chi electrode has the advantages of anti-passivation, high response, and safety, and is also friendly to the environment and human health.

Compliance with ethics guidelines

Qi-qi ZHENG, Yuan-chao LU, Zun-zhong YE, Jian-feng PING, Jian WU, and Yi-bin YING declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Aneesh K, Berchmans S, 2017. Highly selective sensing of dopamine using carbon nanotube ink doped with anionic surfactant modified disposable paper electrode. *J Solid State Electrochem*, 21(5):1263-1271. https://doi.org/10.1007/s10008-016-3482-2

Bansod B, Kumar T, Thakur R, et al., 2017. A review on various electrochemical techniques for heavy metal ions detection with different sensing platforms. *Biosens Bioelectron*, 94:443-455. https://doi.org/10.1016/j.bios.2017.03.031

Budnikov HC, Shirokova VI, 2013. Term "Nano" in electroanalysis: a trendy prefix or a new stage of its development? *J Anal Chem*, 68(8):663-670. https://doi.org/10.1134/S1061934813080030

Cinti S, Basso M, Moscone D, et al., 2017. A paper-based nanomodified electrochemical biosensor for ethanol detection in beers. *Anal Chim Acta*, 960:123-130. https://doi.org/10.1016/j.aca.2017.01.010

Dinesh B, Saraswathi R, Kumar AS, 2017. Water based homogenous carbon ink modified electrode as an efficient sensor system for simultaneous detection of ascorbic acid, dopamine and uric acid. *Electrochim Acta*, 233:92-104. https://doi.org/10.1016/j.electacta.2017.02.139

Ghosale A, Shrivas K, Shankar R, et al., 2017. Low-cost paper electrode fabricated by direct writing with silver nanoparticle-based ink for detection of hydrogen peroxide in wastewater. *Anal Chem*, 89(1):776-782. https://doi.org/10.1021/acs.analchem.6b03512

González-Sánchez MI, Valero E, Compton RG, 2016. Iodine mediated electrochemical detection of thiols in plant extracts using platinum screen-printed electrodes. *Sens Actuators B Chem*, 236:1-7. https://doi.org/10.1016/j.snb.2016.05.152

Idris A, Saleh TA, Sanhoob MA, et al., 2017. Electrochemical detection of thiocyanate using phosphate-modified zeolite carbon paste electrodes. *J Taiwan Inst Chem Eng*, 72: 236-243. https://doi.org/10.1016/j.jtice.2017.01.015

Kuila T, Bose S, Khanra P, et al., 2011. Recent advances in graphene-based biosensors. *Biosens Bioelectron*, 26(12): 4637-4648. https://doi.org/10.1016/j.bios.2011.05.039

Li S, Zhang Q, Lu YL, et al., 2017. One step electrochemical deposition and reduction of graphene oxide on screen printed electrodes for impedance detection of glucose. *Sens Actuators B Chem*, 244:290-298. https://doi.org/10.1016/j.snb.2016.12.142

Li SJ, Zuo Y, Huang WF, 2017. Establishment of a reference interval for high-sensitivity cardiac troponin I in healthy adults from the Sichuan area. *Medicine*, 96(14):e6252. https://doi.org/10.1097/MD.0000000000006252

Ma XY, Chao MY, Wang ZX, et al., 2012. Electrochemical detection of dopamine in the presence of epinephrine, uric acid and ascorbic acid using a graphene-modified electrode. *Anal Methods*, 4(6):1687-1692. https://doi.org/10.1039/c2ay25040c

Molazemhosseini A, Magagnin L, Vena P, et al., 2017. Single-use nonenzymatic glucose biosensor based on CuO nanoparticles ink printed on thin film gold electrode by micro-plotter technology. *J Electroanal Chem*, 789:50-57. https://doi.org/10.1016/j.jelechem.2017.01.041

Nesakumar N, Sethuraman S, Krishnan UM, et al., 2016. Electrochemical acetylcholinesterase biosensor based on ZnO nanocuboids modified platinum electrode for the detection of carbosulfan in rice. *Biosens Bioelectron*, 77: 1070-1077. https://doi.org/10.1016/j.bios.2015.11.010

Ping JF, Wu J, Ying YB, 2010. Development of an ionic liquid modified screen-printed graphite electrode and its sensing in determination of dopamine. *Electrochem Commun*, 12(12):1738-1741. https://doi.org/10.1016/j.elecom.2010.10.010

Ping JF, Wang YX, Ying YB, et al., 2012a. Application of electrochemically reduced graphene oxide on screen-printed ion-selective electrode. *Anal Chem*, 84(7):3473-3479. https://doi.org/10.1021/ac203480z

Ping JF, Wu J, Wang YX, et al., 2012b. Simultaneous determination of ascorbic acid, dopamine and uric acid using high-performance screen-printed graphene electrode. *Biosens Bioelectron*, 34(1):70-76. https://doi.org/10.1016/j.bios.2012.01.016

Preechaworapun A, Dai Z, Xiang Y, et al., 2008. Investigation of the enzyme hydrolysis products of the substrates of alkaline phosphatase in electrochemical immunosensing. *Talanta*, 76(2):424-431. https://doi.org/10.1016/j.talanta.2008.03.025

Saita T, Yamamoto Y, Hosoya K, et al., 2017. An ultra-specific and sensitive sandwich ELISA for imatinib using two anti-imatinib antibodies. *Anal Chim Acta*, 969:72-78. https://doi.org/10.1016/j.aca.2017.03.034

Sajid M, Nazal MK, Mansha M, et al., 2016. Chemically modified electrodes for electrochemical detection of dopamine in the presence of uric acid and ascorbic acid: a review. *TrAC Trend Anal Chem*, 76:15-29. https://doi.org/10.1016/j.trac.2015.09.006

Secor EB, Ahn BY, Gao TZ, et al., 2015. Rapid and versatile photonic annealing of graphene inks for flexible printed electronics. *Adv Mater*, 27(42):6683-6688.

https://doi.org/10.1002/adma.201502866

Tang WZ, Wu J, 2014. Amperometric determination of organophosphorus pesticide by silver electrode using an acetylcholinesterase inhibition method. *Anal Methods*, 6(3):924-929. https://doi.org/10.1039/C3AY41932K

Tang WZ, Zhou JZ, Yang QQ, et al., 2014. Determination of methyl parathion by solid-phase extraction on an ionic liquid-carbon nanotube composite electrode. *Anal Methods*, 6(15):5886-5890. https://doi.org/10.1039/C4AY01062K

Wang YX, Ping JF, Ye ZZ, et al., 2013. Impedimetric immunosensor based on gold nanoparticles modified graphene paper for label-free detection of *Escherichia coli* O157:H7. *Biosens Bioelectron*, 49:492-498. https://doi.org/10.1016/j.bios.2013.05.061

Yang SX, Chen YC, Nicolini L, et al., 2015. "Cut-and-Paste" manufacture of multiparametric epidermal sensor systems. *Adv Mater*, 27(41):6423-6430. https://doi.org/10.1002/adma.201502386

Yu XW, Sheng KX, Shi GQ, 2014. A three-dimensional interpenetrating electrode of reduced graphene oxide for selective detection of dopamine. *Analyst*, 139(18):4525-4531. https://doi.org/10.1039/C4AN00604F

Zheng QQ, Yu YH, Fan K, et al., 2016. A nano-silver enzyme electrode for organophosphorus pesticide detection. *Anal Bioanal Chem*, 408(21):5819-5827. https://doi.org/10.1007/s00216-016-9694-6

List of electronic supplementary materials

Fig. S1 Absorbance of test solution with different kinds of blocking solutions

Fig. S2 Absorbance of test solution with different concentrations of capture antibody

Fig. S3 Absorbance of test solution with different concentrations of ALP-conjugated antibody

中文概要

题　目：一种用于电化学免疫分析的抗钝化的电极浆料

目　的：酶联免疫分析常用底物对硝基苯磷酸盐分解后产生对硝基苯酚（PNP），该物质对电极具有钝化作用，限制了该体系在电化学免疫分析领域的应用。因此，需要开发一种具有抗钝化作用的电极浆料。

创新点：石墨烯–子液体–壳聚糖（rGO/IL/Chi）电极浆料不仅具有抗钝化作用，且拥有良好的电化学性能，制备过程中无需使用任何有机溶剂，安全环保。

方　法：制备 rGO/IL/Chi 复合浆料，涂布于单面导电金箔上，75 °C，10 min，将其作为工作电极；背面涂布自制的银浆，作为参比电极和对电极；0.03 g/ml 壳聚糖溶液作为绝缘浆料。

结　论：本研究表明，在比较商品化的玻碳电极、自制丝网印刷电极、石墨烯修饰的丝网印刷电极和 rGO/IL/Chi 电极对 PNP 的循环伏安响应时，rGO/IL/Chi 电极具有最大的峰电流响应和最小的峰电位。同时，对四种电极进行性能表征时，rGO/IL/Chi 电极具有最小的峰电位差和最大的峰电流。这表明 rGO/IL/Chi 电极具有较好的电化学性能，且对 PNP 有较大的响应。比较四种电极对 PNP 的安培响应之后，发现只有 rGO/IL/Chi 电极具有抗钝化作用。可将该电极用于肌钙蛋白 I 的检测，其检测限为 0.05 ng/ml。

关键词：电化学免疫分析；电极浆料；抗钝化；离子液体；对硝基苯酚