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# The Micro Network of Polyacrylonitrile (PAN)-polyaniline (Pani)-graphene (GRA) Hybrid Nanocomposites for Effective Electrochemical Detection of Glucose and Improved Stability

*Zupeng Yan*[1], *Hao Zheng*[1,*], *Jianfang Chen*[1,2], *Ying Ye*[3]

[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, State Oceanic Administration, Hangzhou 310012, People's Republic of China
[3] Ocean College, Zhejiang University, Zhoushan 316021, People's Republic of China
[*]E-mail: zhenghao@zju.edu.cn

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A glucose biosensor was developed by immobilizing glucose oxidase (GOD) into the micro network of polyacrylonitrile (PAN)-polyaniline (Pani)-graphene (GRA) hybrid nanocomposite fabricated through phase inversion process. PAN with molecular weight of $M_w$ around $2.93\times10^4$ was synthesized by single rare-earth catalyst-$Y(OAr)_3$ and GRA with few-layers was prepared by electrochemical expansion of graphite in propylene carbonate electrolyte, respectively. The morphologies of nanocomposites and the fabricated process of biosensor were performed by scanning electron microscopy (SEM). The cyclic voltammetry (CV) was employed to evaluate electrochemical performance of the as-prepared biosensor. The apparent activation energy (Ea) of enzyme-catalyzed reaction based on Arrhenius equation was estimated to be 16.21 kJ $mol^{-1}$. The constructed glucose biosensor exhibited a short response time within 5 s, and a superior storage stability of preserving 96.28% of the original response over a period of 2 weeks and 91.95% of that even after a month. The linear range, sensitivity, detection limit, anti-interference, and practical application were also investigated. The micro network of PAN-Pani-GRA hybrid nanocomposite provides a hopeful candidate for construction of biosensors.

**Keywords:** Polyacrylonitrile; Polyaniline; Graphene; Glucose biosensor.

## 1. INTRODUCTION

As the central biological compound of the photosynthesis and respiration processes, glucose is essential to successful growth and reproduction of both autotroph and heterotroph[1], especially in ocean. Being clearly aware of the glucose concentration can make it convenient to measure

heterotrophic potential of the marine ecosystem[2]. Moreover, the real time detection of glucose may provide specific evidence for the respiration of the marine planktons. Meanwhile, the quantification of glucose in other realms, such as waste water, food products as well as the blood and urine of the diabetics, are also of great significance[3–5]. Consequently, the development of fast, sensitive, and precise way for the determination and monitoring of glucose concentration in different environment is greatly significant nowadays[6–8]. The glucose enzyme biosensor can be considered to be a prosperously developing analysis method since the concept firstly was proposed by Clark and Lyons in 1962[9].

PAN is a porous polymeric material with the strong polar group (-CN) and high specific surface area, which can provide enough matrix to absorb enzyme strongly, making it a promising material to improve the longtime stability of biosensor. Good operational stability and the longtime stability are premise of accurate measurement. Zheng[10] firstly reported a PAN-based glucose biosensor with a linear range of 0-5 mM and an apparent activation energy (Ea) of 35.9 kJ $mol^{-1}$, but significant decrease of current response after three weeks, and a response time as long as 20 s. The enzyme desorbed easily from the membrane after three weeks due to the poor biocompatibility of the PAN membrane. Therefore, researches about the combination of PAN and Pani, a representative conducting polymer with good environmental stability and superior biocompatibility and other excellent features[11–18] were studied. A glucose enzyme biosensor based on PAN-Pani nanocomposite exhibited a linear range of 2 μM-12 mM, and the response signal remained unchanged in 100 days with a response time of around 30 s[19]. The nanocomposite referred above was also applied to a polyphenol biosensor, indicating that the proposed biosensor had no loss of activity after six months[20]. The merits of PAN and Pani were successfully combined, making the PAN-Pani nanocomposite more appropriate for longtime enzyme immobilization. Nevertheless, when it comes to practical detection of biosensor, fast response should be taken in to account as an important electrical characteristic as well as the most urgent challenge for the PAN-Pani composite.

GRA, a $sp^2$ hybridization bonded carbon, two-dimensional sheet composed of honeycomb crystal-lattice[21], has attracted enormous number of researchers from different fields specifically in electrochemical sensing due to its superior performance, such as extreme mechanical strength, exceptionally high electronic and thermal conductivities, pH sensitivity, and impermeability to gases[22–28]. The amperometric response of GRA-GOD was linearly proportional to the concentration of glucose in the range from 0.1 mM to 27 mM with a fast response time of within 5 s but a relatively low sensitivity of 1.85 μA $mM^{-1}cm^{-2}$[29]. Efforts have thus been directed at combining GRA with other materials, such as Pani[30–33], to improve the sensitivity of GRA-based biosensor, because of the synergistic effect between GRA and Pani[34,35]. Feng constructed a biosensor by immobilizing GOD into the Pani-GRA nanostructure through a simple electrochemical polymerization process[34], the sensor showed a response time of within 3 s and a relatively high sensitivity of 22.1 μA $mM^{-1}cm^{-2}$. A direct electron transferring GOD biosensor based on Pani-GRA-AuNPs was also investigated[12]. These GRA-based biosensors responded quickly to glucose because of the good electron transfer kinetics of GRA[23]. What's more, those GRA-Pani-based biosensors showed enhanced performance, such as longtime stability and large linear range, in glucose detection due to the strong electronic interactions and the synergistic effect between the matrix of GRA, Pani, and other applied

nanomaterials.

Although lots of works have been focused on glucose biosensors based on individual PAN, Pani, GRA and their composites, micro network of nanocomposites as a matrix to immobilize glucose oxidase has not been reported. Based on our previous work[10,34], we used a one-pot synthesis followed by phase inversion process to fabricate glucose biosensor, making it a promising future in practical application. The PAN-Pani-GRA nanocomposite turned out to be a perfect micro network for enzyme immobilization. The as-prepared electrochemical biosensor preserved the biological activity of GOD for quite a long time, shortened the response time to glucose, and performed well in sensitivity, selectivity, and reproducibility.

## 2. EXPERIMENTAL SECTION

### *2.1. Reagents and materials*

The BC grade glucose oxidase (GOD) from Aspergillus niger was obtained from Sigma. Acetonitrile was acquired from Merck KGaA. Triethylamine, Glycine, D-galactose, urea, L-phenylalanine, L-tyrosine, aniline, acrylonitrile, dimethyl formamide (DMF), and chitosan (CS, deacetylation ≥ 95%) were purchased from Aladdin. After dried with molecular sieve, acrylonitrile was distilled and stored over $CaH_2$ under argon for further use[10]. The rest of the chemicals and reagents were of analytical grade when acquired and prepared with Milli-Q water (≥ 18.2 MΩ $cm^{-1}$) if necessary.

### *2.2. Instrumentation*

All the electrochemical measurements adopted were performed by a PARSTAT 4000 electrochemical workstation (AMETEK, USA) with a conventional three-electrode system composed with a platinum (Pt) disk electrode (a diameter of 2 mm) as the counter electrode, the nanocomposite modified Pt disk electrode as the working electrode, and a saturated calomel electrode (SCE) as the reference electrode. A Hitachi SU-70 scanning electron spectroscopy (SEM) was utilized to acquired SEM images. An ACQUITY UPLC H-CLASS System with an Evaporative Light Scattering Detector (Waters, USA) was applied to detect glucose as a standard method.

### *2.3. Preparation of the nanocomposite*

Graphene (GRA) was acquired by electrochemical expansion of graphite in propylene carbonate electrolyte, a method reported previously[36]. Polymerization of acrylonitrile was carried out with the presence of single rare-earth catalyst-$Y(OAr)_3$ prepared according to the previous literature of our group[10], and the molecular weight of the obtained polyacrylonitrile (PAN) was around $2.93\times10^4$. Polyaniline (Pani) was synthesized by gradually adding ammonium peroxydisulfate (APS) into aniline monomer under an argon atmosphere in a mortar according to the previous works[37,38].

After the synthesis steps, 4.7 mg PAN was added into 0.5 mL DMF and dissolved overnight at room temperature to obtain fully dissolved PAN solution with a mass fraction of 1%. Then, 1.0 mg GRA and 2.0 mg Pani were added into 200 μL acquired PAN solution, followed by sonicating for about 1 hour to obtain a uniformly dispersed PAN-Pani-GRA solution.

### *2.4. Fabrication of the modified biosensor*

The bare Pt disk electrodes were pretreated by the following steps before fabrication. Briefly, Pt disk electrodes were polished with 1.5 μm, 0.5 μm, and 50 nm alumina slurries in sequence, followed by successive sonication treatments with Milli-Q water, ethanol, and Milli-Q water. Afterwards, the Pt disk electrodes were treated by CV method with a potential window from -0.2 to 1.6 V (vs. SCE) in 0.2 M sulfuric acid at a scan rate of 0.2 V/s till the stable statement of CV was acquired. The Pt electrodes were rinsed with Milli-Q water and dried in the air under room temperature eventually.

1.0 μL of the PAN-Pani-GRA solution was dropped on the surface of the Pt disk electrode and transformed into a thin membrane by a phase inversion process[34]. 8 mg $mL^{-1}$ GOD in 0.02 M phosphate buffer (pH 7.0) was mixed with 0.5 wt% CS/acetic acid solution at 1:1 volume ratio to obtain CS-GOD solution. After that, another phase inversion process was employed to fabricate PAN-Pani-GRA/CS-GOD modified biosensor by coating 5 μL acquired CS-GOD solution on the surface of the PAN-Pani-GRA membrane.

## 3. RESULTS AND DISCUSSION

### *3.1. Morphology of the PAN-Pani-GRA/CS-GOD nanocomposite*

The images of mono or multiple nanomaterials obtained from scanning electron microscopic (SEM) were shown in Fig. 1. As could be seen in Fig. 1A, the GRA presented a curved and layered structure. Furthermore, few-layer GRA composed of merely carbon atoms could be reasoned out according to the Raman spectra, which was consistent with the results reported previous[36]. Consequently, the synthesized GRA could not only segregate the GOD from the electrode surface but also efficiently carry charges between the solution and the electrode surface. A microporous structure of PAN could be found in Fig. 1B, and provided an excellent immobilization matrix for other nanomaterials and enzyme, the porous matrix then could offer enough reactant like glucose and oxygen to the enzyme[39]. Fig. 1C showed an urchin-like structure of Pani, the size of them were smaller than the grid size of PAN, resulting in the PAN-Pani nanocomposite structure (Fig. 1D) with the disappearance of microporous PAN, while a perfect combination between PAN and Pani substituted. With the addition of GRA and the sufficient dispersion process, the structure of PAN-Pani-GRA nanocomposite was mostly occupied by GRA with the help of PAN to form a thin membrane, enhancing the stability of the biosensor by means of superior attachment of microporous PAN on the electrode. In the meantime, Pani dispersed all around the membrane, providing enough place for enzyme immobilization, and improving the stability and selectivity of biosensor as well. In the

meantime, the strong interactions between GRA and Pani could enhance the charge transfer and diffusion processes[34,40]. And this specific appearance in Fig. 1E could be illustrated when the concentrations of these three nanomaterials in DMF solution before phase inversion process (5 mg $mL^{-1}$ of GRA, 10 mg $mL^{-1}$ of Pani, and 23.5 mg $mL^{-1}$ of PAN), as well as the specific surface area for each of them were considered. The scene after the immobilization of GOD (Fig. 1F) showed a well-constructed micro three-dimensional network structure of PAN-Pani-GRA/CS-GOD, as the GOD embedded into the porous hybrid matrix with the help of the linker, CS, this could be a strong evidence of the synergistic effect within the micro network of the nanocomposite.

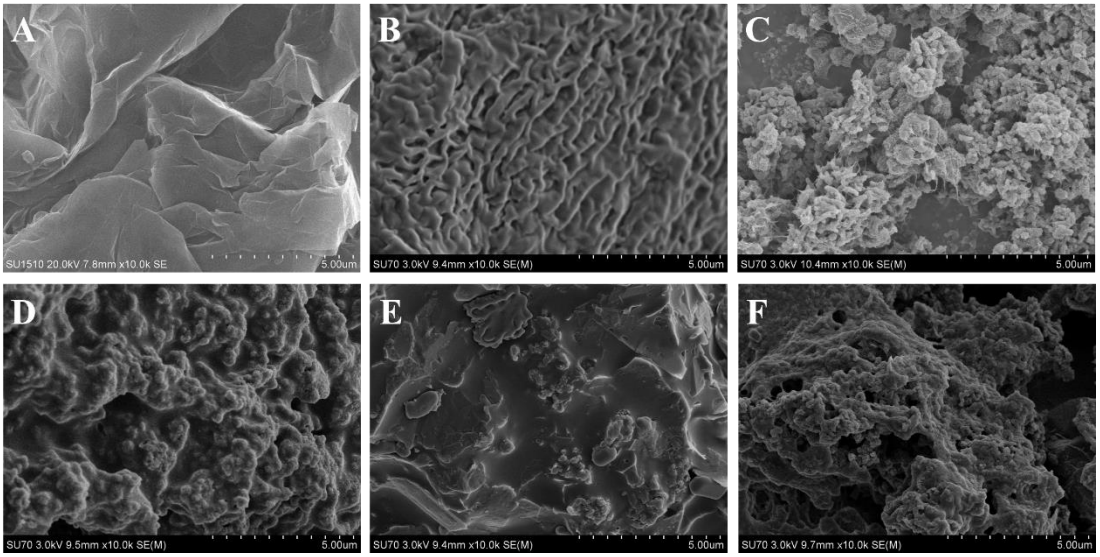


**Figure 1.** The SEM of nanomaterials: (A) GRA; (B) PAN; (C) Pani; (D) PAN-Pani; (E)PAN-Pani-GRA; (F) PAN-Pani-GRA/CS-GOD.

### *3.2. Electrochemical performances of the biosensor*

The cyclic voltammograms (CV) was utilized for evaluating the electrochemical performances of the PAN-Pani-GRA/CS-GOD biosensor. To examine and verify the electrocatalytic effect of all these mentioned materials, various electrochemical biosensors based on PAN-Pani, PAN-Pani-GRA, PAN-Pani-GRA/CS, and PAN-Pani-GRA/CS-GOD modified electrodes were constructed. As were shown in Fig. 2, the curves of PAN-Pani (curve a, b), PAN-Pani-GRA (curve c, d), and PAN-Pani-GRA/CS (curve e, f) modified electrodes in Fig. 2A showed no difference respectively in 0.02 M pH 6.5 buffer with or without the present of 0.598 mM glucose, which illustrated the no electrocatalytic effect of PAN, Pani, GRA and CS to glucose. Whereas, a significate increase of the oxidation current for PAN-Pani-GRA/CS-GOD modified electrode could be observed with the addition of glucose in Fig. 2B, which indicated that GOD was indispensable in the fabricate glucose biosensor. What's more, the enzyme electrocatalyzed (anodic) reaction could be illustrated by the follow two steps reported previously[3]:

$$Glucose + O_2 \xrightarrow{GOD} Gluconic\ acid + H_2O_2 \quad (1)$$

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^- \quad (2)$$

So the glucose detection could be realized by amperometric monitoring of the production of hydrogen peroxide[41].

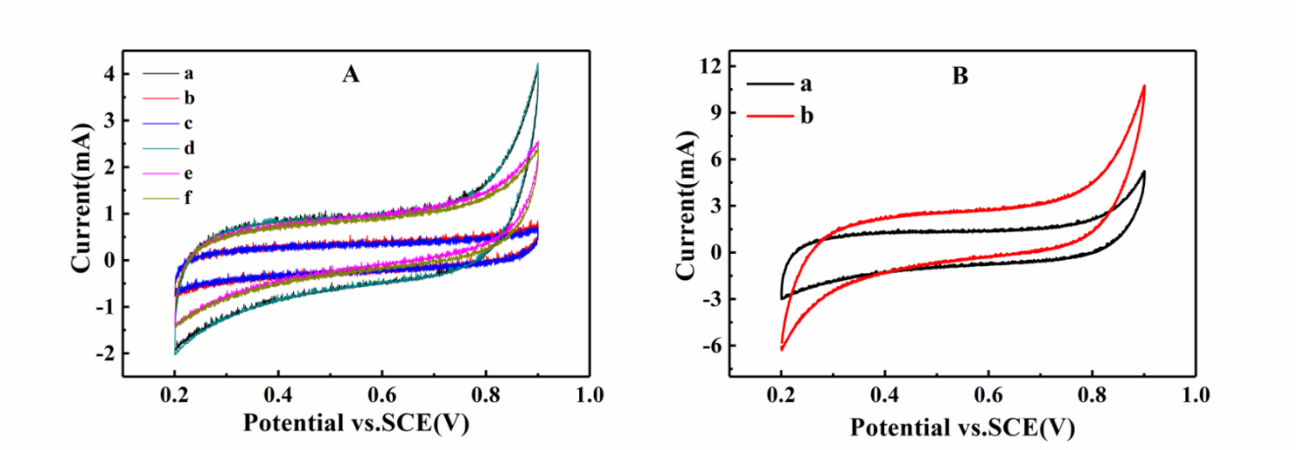


**Figure 2.** (A) CV of different electrodes in 0.02 M pH 6.5 phosphate buffer at a scan rate of 50mV/s : (a) PAN-Pani modified electrode in the absence of 0.598 mM glucose; (b) PAN-Pani modified electrode in the presence of 0.598 mM glucose; (c) PAN-Pani-GRA modified electrode in the absence of 0.598 mM glucose; (d) PAN-Pani-GRA modified electrode in the presence of 0.598 mM glucose; (e) PAN-Pani-GRA-CS modified electrode in the absence of 0.598 mM glucose; (f) PAN-Pani-GRA-CS modified electrode in the presence of 0.598 mM glucose; (B) CV of PAN-Pani-GRA/CS-GOD modified electrode without (b) or in (a) the presence of 0.598 mM glucose.

### *3.3. Optimization of the biosensor for glucose detection*

As for enzyme biosensor, applied potential, pH values, and temperature in the 0.02 M phosphate buffer for glucose detection were essential factors to be optimized.

As it could be obviously seen in Fig. 3A, the current response of the biosensor in three different pH value buffers for 0.598 mM glucose increased rapidly from 0.3 V to 0.65 V (vs. SCE), then reached a maximum value in 0.65 V (vs. SCE) followed by a decrease of current response from 0.65 V to 0.8 V (vs. SCE). Consequently, an applied potential of 0.65 V vs. SCE was chosen as an optimized choice in the following work. What's more, the noteworthy increase of the current response in pH 6.54 buffer compared with the other two buffers indicated that the optimized pH value was somewhere between pH 6.09 and pH 7.14, namely pH value of 6.54 could be approximately considered as the best pH value for glucose detection.

To verify the conclusion of the optimized pH value proposed above, the influence of different pH values of the adopted detection buffers on the amperometric response were investigated and shown in Fig. 3B. Three different concentrations of glucose (0.598 mM, 0.995 mM, and 1.784 mM) were chosen in the test. It could be seen that the oxidation current response to glucose gradually increased from pH 3.5 to pH 6.5, and reached maximums at pH 6.5, then decreased between pH 6.5 and pH 9.0. Notably, there was a sudden decrease of current response at pH 8.23 in the 1.784 mM glucose solution, which didn't happen in the other two lower concentrations (0.995 mM or 0.589 mM), and we

attributed this to the exceeding of the linear range of the biosensor at pH 8.23 in the 1.784 mM glucose solution. In conclusion, the optimized pH value of the phosphate buffer for the enzyme immobilization biosensor was pH 6.5, which was consistent with the free enzyme's good response in the pH 4~7 as well as the conclusion mentioned above, yet a little higher than the maximum activities of free enzyme in pH 5.5, which could be the consequence of enzyme immobilization and the cooperation of all these nanomaterials applied in the biosensor.

Besides, temperature was also a factor that couldn't be underestimate when it came to the current response of biosensor. As the temperature raised from 10 °C to 50 °C, the current response of biosensor to 0.598 mM glucose under the optimized conditions mentioned above persistently increased accompanied by a sustained decrease response time. Nevertheless, the noise largened when the temperature came to 35 °C and higher. Taking the feasibility into account, a temperature of 25 °C was selected. What's more, the apparent activation energy (Ea) of the enzyme biosensor could be estimated according to the Arrhenius equation (Fig. 3C). Ea is calculated to be 16.21 kJ mol$^{-1}$ from the slope of the ln $i$ vs. $T^{-1}$ relationship fitting line, which is smaller than that of the PAN (25.3 kJ mol$^{-1}$)[10] and PAN-Pani (23.9 kJ mol$^{-1}$)[19] based glucose biosensor. The smaller apparent activation energy may be ascribed to the fast electron transfer kinetics of GRA[23] and porous PAN in the micro network, indicating the superior electrocatalytic activity of the PAN-Pani-GRA/CS-GOD based biosensor.

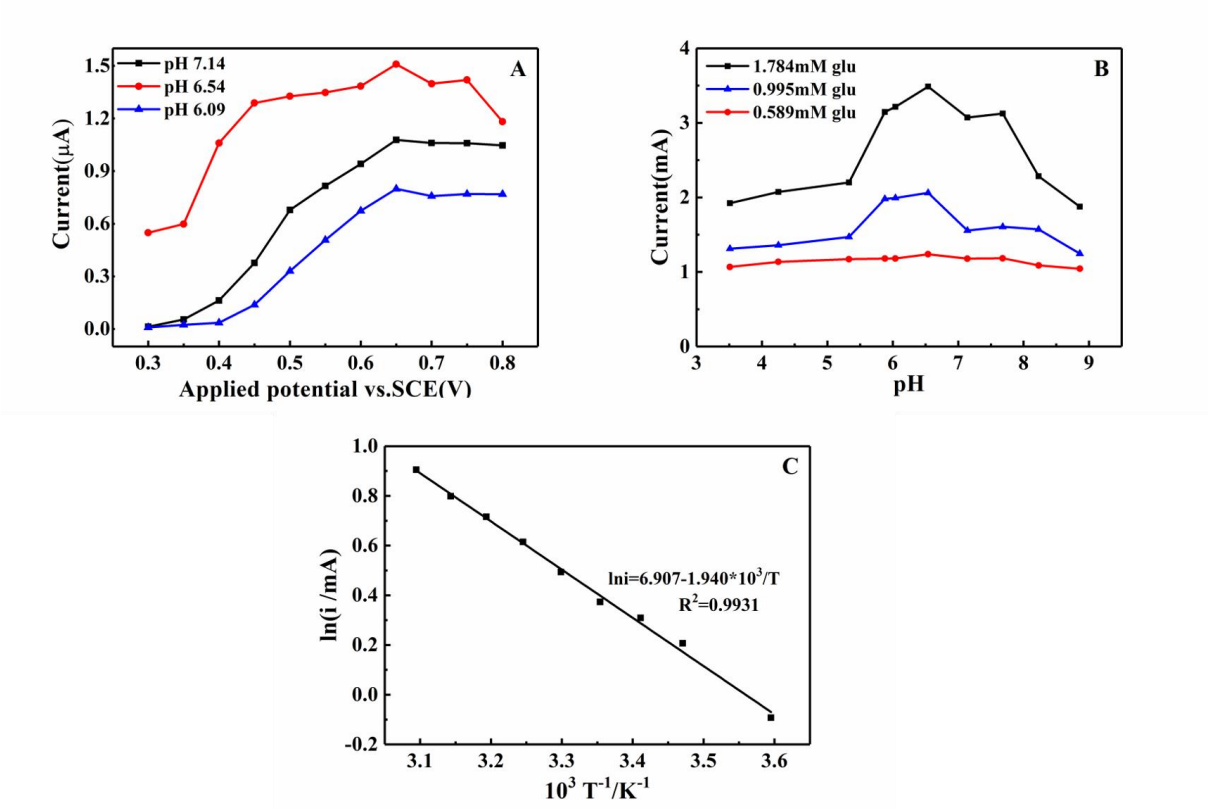


**Figure 3.** (A) Current response of PAN-Pani-GRA/CS-GOD biosensor to different applied potentials in three different pH values of 0.02 M phosphate buffer; (B) Current response of PAN-Pani-GRA/CS-GOD biosensor to different pH values varies from 3.51 to 8.89 with the addition of 0.598 mM, 0.995 mM, 1.784 mM glucose in 0.02 M phosphate buffer; (C) The ln $i$ vs. $T^{-1}$ relationship of PAN-Pani-GRA/CS-GOD biosensor.

In consideration of all factors above, the subsequent tests were conducted in 0.02 M pH 6.5 phosphate buffer at the temperature of 25 °C and the applied potential of 0.65 V (vs. SCE) if not mentioned.

### *3.4. Amperometric response of the modified biosensor to glucose*

After acquiring the optimized conditions, glucose detection performance of the as-prepared biosensor could then be evaluated. As such, a typical current response-time of the biosensor with constantly addition of glucose was shown in Fig. 4A. The amperometric response time was less than 5 s (reaching 95% of steady state current), which was consistent with the relatively small value of Ea.

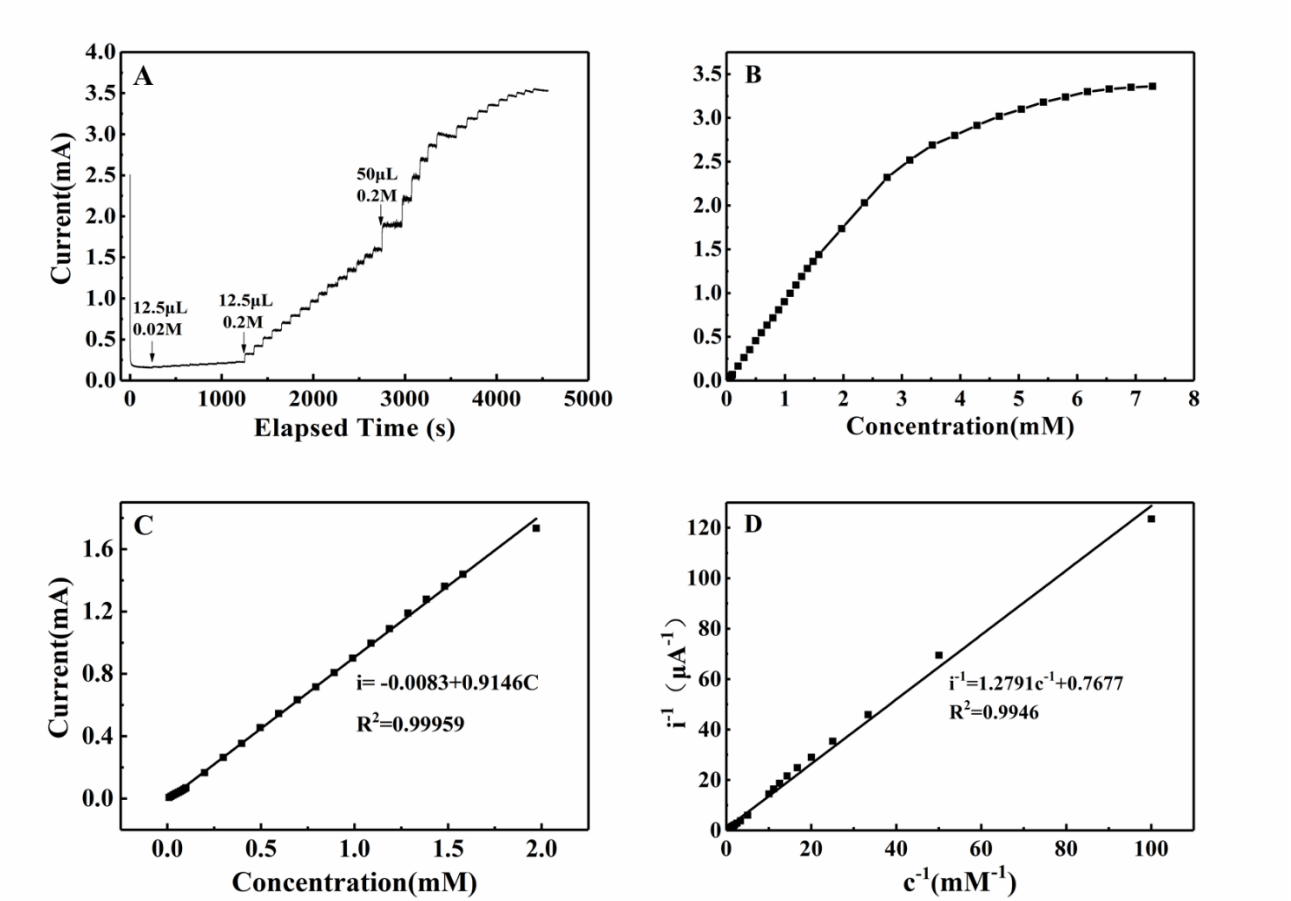


**Figure 4.** The amperometric responses of the PAN-Pani-GRA/CS-GOD modified biosensor to constantly addition of glucose at the optimized condition; (B) Steady state amperometric current-concentration curves; (C) Linear fitting curves; (D) The apparent Michaelis-Menten constant ($k_m$) evaluated by the Lineweaver-Burk equation.

The plot of the steady state amperometric current as a function of glucose concentration was shown in Fig. 4B, the linear range was from 10.0 μM to 1.97 mM ($R^2$ = 0.9992), wider than the biosensor fabricated based on Pani-GRA by our group previously[34], and a relatively high detection sensitivity was calculated from the linear portion as 29.11 μA $mM^{-1}cm^{-2}$, higher than some glucose biosensors reported previously[34,42] because of the porous surface of GRA-PAN and the

biocompatibility of Pani resulting in a large amount of GOD solidly immobilized in the micro network. Moreover, the apparent Michaelis-Menten constant ($k_m$) evaluated by the Lineweaver-Burk equation was 1.67 mM, lower than some already published biosensors[34,42]. And the detection limit was calculated to be 2.10 mM (S/N = 3). As for the plots with a glucose concentration higher than 5mM, they no longer exhibited as the linear pattern, suggested that the active sites of GOD are saturated and followed the zero-order reaction kinetics of enzyme. The parameters of the amperometric response indicated that the fabricated biosensor had an approving performance in glucose detection.

### *3.5. Anti-interference performance of the modified biosensor*

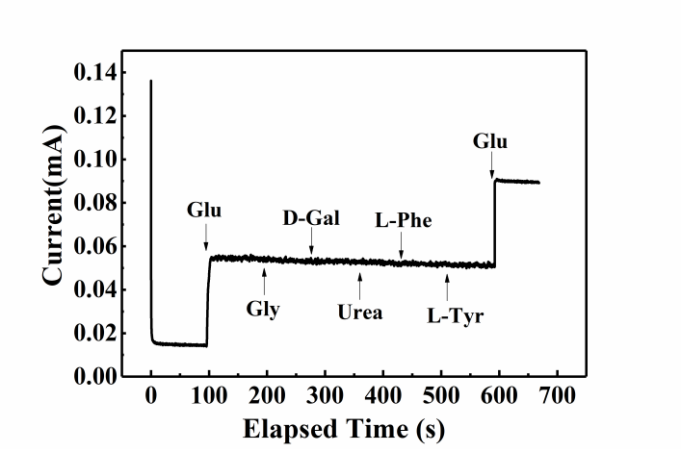


**Figure 5.** Anti-interference performance of the amperometic response of the biosensor to five interferences in the presence of glucose.

**Table 1.** Comparison of analytical performance of different glucose sensors

| Materials | Linear range (mM) | Sensitivity ($\mu AmM^{-1}cm^{-2}$) | $E_a$ (kJ $mol^{-1}$) | storage stability($i/i_0$) | reference |
|---|---|---|---|---|---|
| PAN-Pani | 0.002-12 | 67.1 | 23.9 | 100% after 100 days | [19] |
| Pani-GRA- AuNPs | 0.004-1.12 | - | - | 96% after 20 days | [12] |
| Pani-GRA- AuNPs | 0.2-11.2 | 20.32 | - | - | [42] |
| Pani-GRA | 0.01-1.48 | 22.1 | - | - | [34] |
| Pani-MWNT-PtNPs | 0.003-8.2 | 128 | - | 90% after 48 days | [16] |
| Pani-3D rGO-$SnO_2$ | 0.0055-27.66 | 0.00026 | - | 83% after 1 month | [43] |
| PAN-Pani-GRA | 0.01-1.97 | 29.11 | 16.21 | 91.95% after a month. | This work |

Abbreviations: PAN: polyacrylonitrile; Pani: polyaniline; GRA: Graphene; AuNPs: Au nanoparticles; MWNT: multi-wall carbon nanotube; PtNPs: Pt nanoparticles; 3D rGO: three-dimensional reduced graphene oxide.

For the sake of real sample detection, the selectivity of the glucose biosensor could not be overlooked. Glycine (Gly), D-galactose (D-Gal), Urea, L-phenylalanine (L-Phe), and L-tyrosine (L-

Tyr) were chosen as five representative interferences in both biological and non-biological circumstance. As was shown in Fig. 5, at the present of 0.199 mM glucose, nearly no response was found to glycine (0.199 mM), D-galactose (0.199 mM), Urea (0.199 mM), L-phenylalanine (0.099 mM), or L-tyrosine (4.916 μM), showing a quite superior selectivity of the PAN-Pani-GRA/CS-GOD modified biosensor.

### *3.6. Stability and reproductivity of the modified biosensor*

When it came to commercial application, the longtime storage stability, determined by the deactivation and desorption of the enzyme absorbed on the nanocomposite, was an important factor of the biosensor. In this work, the longtime stability experiment was carried out at 0.65 V (vs. SCE) in 0.02 M phosphate buffer (pH 6.5) containing 0.598 mM glucose at the temperature of 25 °C every 7 days for a month by the modified biosensor, which was kept under 4 °C in the 0.02 M pH 6.5 phosphate buffer when unused. The steady-state response versus storage time was shown in Fig. 6, the response only decreased to 96.28% in a fortnight, and 91.95% in a month, superior than some biosensors reported before[12,16]. The result indicated an excellent storage stability of the glucose biosensor due to the enzyme solidly embedded into the micro network of PAN-Pani-GRA matrix, making the enzyme difficult to desorb. Comparison of the analytical performance of the constructed glucose biosensor with other previous reported biosensors was summarized in Table. 1.

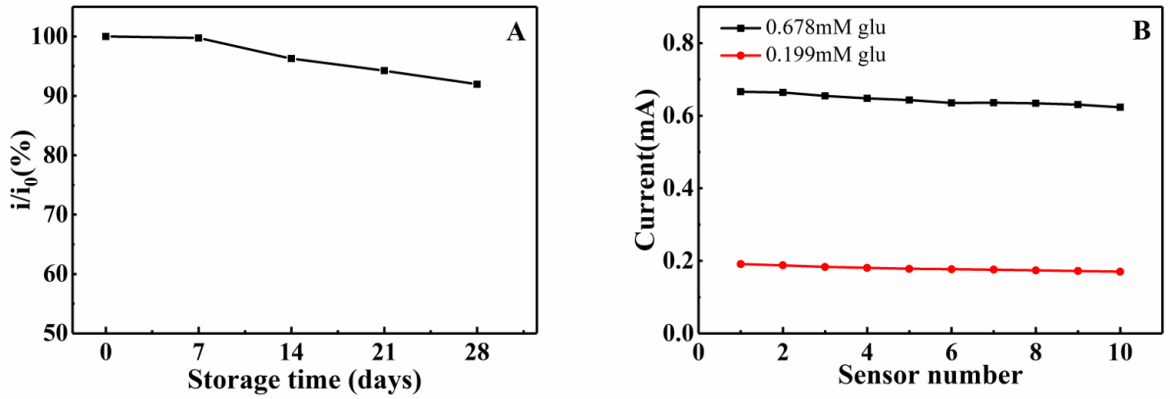


**Figure 6.** （A）Longtime stability of the glucose biosensor at 0.65 V (vs. SCE) in 0.02 M phosphate buffer (pH 6.5) containing 0.598 mM glucose at the temperature of 25 °C;（B）The reproductivity of the modified biosensor.

Apart from the storage stability, the reproductivity and the repeatability of the biosensor were also important factors for commercial utilization. The reproductivity of the modified biosensor was evaluated by the relative standard deviations (RSD) of four biosensors prepared independently at the glucose concentration of 0.598 mM (Table. 2), the RSD was 4.97%, which was a little high because the disperse status of GRA in DMF solution might be different in every biosensor preparation. Similarly, the repeatability of the biosensor was tested by 10 times detection in two different glucose

concentrations of 0.199 mM and 0.678 mM (Fig. 6B), the results were 3.16% and 2.13% respectively, indicating a fine repeatability of the biosensor.

**Table 2.** The repeatability of the biosensor

| Electrode number | Steady state current response (μA) |
|---|---|
| 1 | 0.5756 |
| 2 | 0.5423 |
| 3 | 0.5014 |
| 4 | 0.5290 |

*3.7 Real sample detection*

The metabolism process of *penicillium* was performed in order to verify the practical usage of the as-prepared biosensor. Briefly, a dip of penicillium was dispersed in a 0.1 M glucose solution by a stainless-steel wire after sterilization. Then, aqueous samples were collected and filtrated after 0 hour, 6 hours, and 9 hours, respectively. Two parallel experiments were simultaneously carried out to reduce errors. The constructed biosensor was utilized to detect the signal change of adding 12.5 μL aqueous sample into 25 mL of PBS solution )0.02 M, pH 6.5), and the glucose concentration was calculated by the linear fitting curves obtained in Section 3.4. As shown in Table 3, the results were in agreement with those measured by an UPLC standard method [44], and showed clearly that the prepared biosensor was capable and effective for real sample detection.

**Table 3.** Determination of glucose in *penicillium* samples by the proposed sensor and UPLC

| Sample Group | Collected Time (h) | Glucose concentrations (mM) | | Relative error (%) |
|---|---|---|---|---|
| | | UPLC method | Biosensor | |
| 1 | 0 | 99.14 | 99.68 | 0.54 |
| | 6 | 92.67 | 93.71 | 1.12 |
| | 9 | 89.49 | 87.70 | -0.20 |
| 2 | 0 | 99.71 | 104.03 | 4.33 |
| | 6 | 92.90 | 99.01 | 6.58 |
| | 9 | 89.24 | 95.00 | 6.46 |

The glucose concentrations from both methods had been converted based on the dilution ratios.

## 4. CONCLUSIONS

We have successfully developed an amperometric glucose biosensor based on PAN-Pani-GRA hybrid nanocomposite with micro network structure. The constructed biosensor exhibited a superior storage stability of preserving 91.95% of the original response in a month due to the micro network of

nanocomposite for the effective immobilization of enzyme. Moreover, a low Ea of 16.21 kJ $mol^{-1}$ leading to a relatively short response time within 5 s was detected owing to the fast electron transfer kinetics of GRA and the synergistic effect between PAN, Pani, and GRA. Meanwhile, the biosensor showed a wide linear range from 10.0 μM to 1.97 mM, excellent selectivity, good repeatability, and reproducibility. The biosensor could be applied for real sample detection like monitoring metabolism in aquatic environment, and the easy constructed micro network might be developed as a potential enzyme immobilizing platform in the near future.

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