Sensitive Amperometric Detection of L-Glutamic Acid in Agricultural and Biological Samples Using the Biocompatible, High-effective and Integrated Biocompatible Poly(3,4-ethylenedioxythiophene) Nanocomposite Bioelectrode

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Received: 15 October 2015 / Accepted: 11 November 2015 / Published: 1 December 2015

An efficient glutamate oxidase (GluOx) amperometric biosensor for sensitively detecting L-glutamic acid (Glu) in agricultural and biological samples using an integrated biocompatible poly(3,4-ethylenedioxythiophene) (PEDOT) nanocomposite bioelectrode was facilely fabricated by the one-step electrochemical deposition technique in ionic liquid-in-water microemulsion containing multi-walled carbon nanotubes, GluOx, 3,4-ethylenedioxythiophene, 1-ethyl-3-methylimidazolium ethyl sulfate, sodium N-lauroylsarcosinate and LiClO₄. Cyclic voltammetry and electrochemical impedance spectroscopy indicated that the as-prepared PEDOT nanocomposite bioelectrode exhibited better electron-transfer ability than individual component, and GluOx were successfully immobilized into this bioelectrode. The sensing parameters such as working potentials, pH values, and temperature and performance like sensitivity, response time, limit of detection (LOD), stability, specificity and applicability of the as-fabricated bioelectrode were assessed. This biosensor demonstrated good bioelectrocatalytic response towards Glu in a linear range from 0.6 μM to 2 mM with a pronounced sensitivity of 10.12 μA mM⁻¹ cm⁻², short response time within 5-10 s, low LOD of 0.27 μM, high stability, good selectivity and satisfactory practicality. All these indicate that the proposed GluOx biosensor will provide a promising platform for determining Glu in agricultural and biological samples.

Keywords: Biosensor, Poly(3,4-ethylenedioxythiophene), L-Glutamate oxidase, L-Glutamic acid, Multi-walled carbon nanotube, Ionic liquid-in-water microemulsion

1. INTRODUCTION

L-glutamic acid (Glu), one of non-essential amino acids and proteinogenic amino acids, has various functions in the human body. It is very important for learning, memory, brain function, muscle
growth and maintenance, Glu as a neurotransmitter plays an important role in the pathology of neurological and psychiatric disorders. Besides, Glu is also a nutrient substance and flavor enhancer in the human diet and growth-enhancer of plant growth, its sources include all protein-rich plant foods (rice, wheat), dairy products, meats, poultry, even fish, eggs, and kombu. Thus the determination of Glu is very indispensable in both food and medical samples, especially in the case of in vivo analysis [1-3]. In addition, Glu is closely related to many aminotransferase such as glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.2, EC 2.6.1.2, L-aspartic acid + 2-oxoglutaric acid $\rightarrow_{\text{GOT}}$ oxaloacetic acid + Glu and glutamate-pyruvate transaminase (GPT, EC 2.6.1.2, L-alanine + 2-oxoglutaric acid $\rightarrow_{\text{GPT}}$ pyruvic acid + Glu), which can reversible catalyze the transfer of an amino group from amino acid to a-ketoglutarate and produce new amino acid (including Glu) in amino acid metabolism. The rapid and precise determination of Glu can indirectly detect enzymatic bioactivities such as GOD and GPT and/or other amino acids content (L-aspartic acid, L-alanine) [4-10], which are also very important in both animal and plant biochemistry and clinical medicine.

Glu have been routinely assayed by chromatographic and potentiometric titration, however, these methods are time-consuming and quite complicated. In comparison with well-established, lab-based methods, amperometric biosensors are more promising analytical tool due to superior sensitivity, simple fabrication, convenient use, easy miniaturization and low cost. L-glutamate oxidase (GluOx, EC 1.4.3.11) biosensor is one of Glu biosensors. GluOx belongs to the family of oxidoreductases, this can catalyze the oxidation of Glu (Glu + O2 + H2O $\rightarrow_{\text{GluOx}}$ 2-oxoglutarate + NH3 + H2O2)[4-10], which is very important to how to develop a multifunctional GluOx-based biosensor for the determination of the enzymatic bioactivities and amino acids content in animal and plant biochemistry, clinical medicine, crops and food. A variety of GluOx biosensors were fabricated using different matrix materials and analytical methods for the determination of Glu (Table 1).

Electrochemical methods have also been used for the fabrication of Glu biosensors either directly or in combination with other techniques because of their high sensitivity, excellent selectivity, simplicity, and rapidity. With much progress on reports in Glu biosensors field, the immobilization of GluOx on the interface of electrodes for the architecture of Glu biosensors has attracted many attention. But still there are a few issues such as high response time, high working potentials, lack of stability, and low reproducibility (see Table 1). The biocompatible matrices have opened a bright field towards the exploitation of efficient biosensors between the enzyme and the electrode.

Recent advances and trends in chemo/bio-sensors have been reported worldwide for exploring novel sensing materials and practical applications, which focus more on medicine, food, environment and biology, but less on agriculture. Nowadays, electrically conducting polymers (ECPs), carbon nanotubes (CNTs), ionic liquids (ILs) are widely used as sensing materials[18-26]. ECPs, particularly polyaniline (PANi), polypyrrole (PPy), poly(3,4-ethylenedioxythiophene) (PEDOT) and their derivatives prepared via electrosyntheses have been reported worldwide for the development of efficient chemo/bio-sensors in combination with other different materials, preparative methods, and analytical methods due to exceptional properties[18-20]. In particular, PEDOT-based biosensors as one of most excellent ECPs-based biosensors are a relatively new one. Moreover, PEDOT-based biosensors have displayed extraordinary advantages in different fields related to human health like the drug discovery, disease diagnosis, genetic mutations, forensics and food technology compared with
both PANi-based biosensors due to its hypotoxicity and PPy-based biosensors owing to its structural stability. [27-34].

ILs, consisting of positively and negatively charged ions at room temperature, as “green” solvents are opening up burgeoning new fields. ILs, the availability, application, and combination of a wide range of ECPs with new biosensing techniques, could cause a remarkable innovation in the design and construction of biosensing devices, especially the electrochemical ones. ILs-water is an excellent, low-cost and non-toxic system for the electrochemistry of ICPs by integrating advantages of the two. The preparation of high-performance and weak-toxic ECPs in ILs with alkyl sulfate anions microemulsion system is very beneficial to enhance the performance of biosensors like bioactivity, stability, and selectivity, especially the immobilization of biologically active species [17, 24-26, 31, 35-38].

CNTs are an excellent tubular nanostructural carbon-based material, which display large specific surface area, readily modifiable surface, strong electrocatalytic activity, high electrical conductivity, good biocompatibility, good adsorption capacity, excellent chemical stability, and so on. Exceptional properties of CNTs easy made they can foresee their promising applications in electroanalytical chemistry for CNT-based biosensors. The integration of different materials has contributed to the versatility, sensitivity, selectivity, and stability of biosensors. Polymeric-inorganic nanocomposite enzyme electrodes based on ECPs-CNTs have been widely employed for the architecture of efficient biosensors, including electrochemical ones. These nanocomposites can use as both the immobilization matrices/binders of enzymes and mediators of the electron transport. The capability of immobilization and stability of enzyme improve in the integrated ECPs-CNTs matrices as a result of the large surface area and good adsorption capacity of CNTs which, as a true biomaterial, effectively adsorb biomacromolecules (enzymes) contrasted with the corresponding CNTs-free biosensors, and the CNT-based biosensors can present the enhanced sensitivity and stability [21-23, 32, 39-41]. However, the immobilization of GluOx on the surface of ECPs-ILs-CNTs composite matrix for the fabrication of Glu biosensor has not been studied yet.

In our previous work, ascorbate oxidase (AO) and PEDOT were selected as the model enzyme and immobilization matrix of VC electrochemical biosensors, respectively. Carbon nanomaterials MWCNTs, biocompatible amino acid surfactant N-lauroylsarcosinate (SLS), and ILs with alkyl sulfate anions 1-ethyl-3-methylimidazolium ethyl sulfate ([Emim][EtSO4]) was used as synergetic reinforcer, respectively. A series of vitamin C electrochemical biosensors based on AO immobilized into PEDOT composite matrices were developed using different reinforcing materials and immobilization methods. The fabricated biosensors displayed superior sensing performance like low limit of detection (LOD), acceptable sensitivity, good bioaffinity towards matrices and satisfactory long-term stability by combining the merits of different materials. Finally, the fabricated biosensors were used for determining L-ascorbic acid in the commercial fruit juice and crops [31, 32, 38-40, 42, 43]. Moreover, the poly(thiophene-3-acetic acid) film electrosynthesized in ILs for the cross-linking immobilization of biologically active species such as AO and GluOx could enhance the biocompatibility, conductivity, bioaffinity, sensitivity and storage stability [17, 37].

Inspired by preceding studies, a biocompatible integrated polymeric-inorganic nanocomposite matrix based on PEDOT-EtSO4-SL-MWCNTs was employed as an immobilization carrier of the
fabricated GluOx biosensors for highly efficient amperometric determination of Glu in agricultural and biological samples.

2. EXPERIMENTAL

2.1 Chemicals

GluOx (12.2 U mg\(^{-1}\), EC 1.1.3.4, from streptomycetes), sodium dihydrogen phosphate dihydrate (Na\(\text{H}_2\text{PO}_4\cdot2\text{H}_2\text{O}\)), disodium hydrogen phosphate dodecahydrate (Na\(_2\)HPO\(_4\)·12H\(_2\)O) and lithium perchlorate trihydrate (LiClO\(_4\)·3H\(_2\)O) were procured from Sinopharm Chemical Reagent Co., Ltd. MWCNT (0.10 wt%) was obtained from Nanjing XFNANO Materials Tech co., Ltd (Nanjing, China). EDOT, Glu, sodium N-lauroylsarcosinate (SLS) were bought from J&K Scientific Ltd. [Emim][EtSO\(_4\)] was purchased from Tokyo chemical industry Co., Ltd. 0.1 M phosphate buffer solutions (PBS; pH 7.5) were prepared with 0.1 M Na\(_2\)HPO\(_4\)·12H\(_2\)O and 0.1 M NaH\(_2\)PO\(_4\)·2H\(_2\)O. All chemicals were analytical grade and employed without further purification. The double-distilled deionized water was employed throughout this work.

2.2 Apparatus

All electrochemical tests were carried out on a CHI660E electrochemical workstation (Shanghai Chenhua Instrument Company, China) with a three-electrode system. This electrode system consisted of a glassy carbon electrode (GCE, diameter of 3 mm), platinum wire and saturated calomel electrode (SCE), which was used as a working electrode, reference electrode and auxiliary electrode, respectively. The pH values in PBS were measured using a PHB-5 portable pH meter (Hangzhou Qiwei Instrument, China). The addition of samples was performed by a micropipettor. Electrochemical impedance spectroscopy (EIS) was carried out in 0.1 M PBS (pH 7.5) containing 0.1 M LiClO\(_4\), and impedance spectra were recorded in the frequency range from 100 kHz to 1 Hz.

2.3 Fabrication of PEDOT-EtSO\(_4\)-SL-MWCNTs-GluOx/GCE bioelectrode

Prior to modification, the GCE was carefully polished with chamois leather containing 0.05 μm alumina slurry, and was ultrasonically cleaned with deionized distilled water, absolute ethanol, and deionized distilled water each for 5 min, respectively. The counter electrode was carefully polished with abrasive paper (1500 mesh), cleaned successively with water and acetone, then dried in air before each experiment. The PEDOT-EtSO\(_4\)-SL-MWCNTs-GluOx/GCE bioelectrode was prepared by one-step potentiostatical polymerization in 0.1 M PBS (pH 7.5) containing 0.02 M EDOT, 0.1 U mL\(^{-1}\) GluOx, 1 mg mL\(^{-1}\) MWCNTs, 0.1 M [Emim][EtSO\(_4\)], 0.02 M SLS, and 0.02 M LiClO\(_4\) at a polymerization potential of 1.1 V vs. SCE for 60 s on GCE surface. The thickness of PEDOT-EtSO\(_4\)-SL-MWCNTs-GluOx film was controlled by the total charge passing through the cell, which was read directly from current-time curves by computer. The bioelectrode was washed repeatedly with 0.1 M PBS (pH 7.5) to remove any loosely bound GluOx, EDOT, ILs, MWCNTs and others from the surface.
of electrode, and then placed in 0.1 M PBS (pH 7.5) with a refrigerated temperature of 4°C when not in use. The schematics of as-fabricated PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE were presented in Scheme 1.

**Scheme 1.** The fabrication of the integrated GO biosensor based on the biocompatible PEDOT-EtSO₄-SL-MWCNTs nanocomposite matrix.

### 2.4 Amperometric tests

The amperometric tests of Glu were performed by using a working potential of 0.3 V vs. SCE for 30 s. The 0.1 M air-saturated PBS (pH 7.5) was selected as supporting electrolyte. Each test was repeated three times at the same cells with a stirring rate of 400 rpm. Each data point is an average value of data collected three times.

### 2.5 Preparation of practical samples

The chicken blood was drawn from the key laboratory of College of Animal Science and Technology in Jiangxi Agricultural University. The soil and ripened tomato were obtained from the experimental field in Jiangxi Agricultural University. Eggs was purchased from the local supermarket and made egg soup according to a traditional Chinese way. 10g the soil and ripened tomato were homogenized respectively with 100 ml double-distilled deionized water for 10 min, then homogenates were filtered to remove residues. The chicken blood and egg soup were homogenized respectively for 10 min, and then homogenates were filtered to remove residues. 0.1 M Na₂HPO₄·12H₂O and 0.1 M NaH₂PO₄·2H₂O were added respectively into all obtained filtrates mentioned above, and then the prepared sample solutions (pH 7.5) was obtained. The as-obtained biosensor based on GluOx was employed for detecting all freshly prepared samples, and then all these solutions were added different content of Glu using the standard addition method, respectively.
3. RESULTS AND DISCUSSION

3.1 Voltammetric behaviors of PEDOT-EtSO$_4$-SL-MWCNTs-GO/GCE

![Graph showing voltammetric behaviors](image)

Figure 1. CVs of the fabricated GluOx biosensors in PBS with 1 mM Glu (a) and without Glu (b) at a scan rate of 100 mV s$^{-1}$.

Figure 1 presented cyclic voltammograms (CVs) of PEDOT-EtSO$_4$-SL-MWCNTs-GluOx/GCE in potential ranges from -0.4 to 0.4 V. A couple of broad redox waves were observed in Figure 1a, this was not the same as CVs of PEDOT-EtSO$_4$-SL-MWCNTs-GluOx/GCE in PBS without Glu (no obvious redox waves, Figure 1b). This fact suggested that the fabricated PEDOT-EtSO$_4$-SL-MWCNTs-GluOx/GCE could catalyze the bioelectrochemical oxidation of Glu. This preliminarily indicated that GluOx molecules might be incorporated into PEDOT-EtSO$_4$-SL-MWCNTs matrix, and PEDOT-EtSO$_4$-SL-MWCNTs-GluOx/GCE might possess a good catalytic ability for the bioelectrochemical oxidation of Glu.

3.2 EIS of PEDOT-EtSO$_4$-SL-MWCNTs-GluOx/GCE

Figure 2 shows the Nyquist plots. The GCE is a small semicircle (Figure 2a) while PEDOT-EtSO$_4$-SL (Figure 2b), PEDOT-EtSO$_4$-SL-MWCNTs (Figure 2c) and PEDOT-EtSO$_4$-SL-MWCNTs-GluOx (Figure 2d) films are almost straight lines, this is a typical shape of the impedance spectra of PEDOT in water sytem [36], indicating a very low electron-transfer resistance, this is as a result of on high conductivity PEDOT. The imaginary part of impedance at low frequencies was almost perpendicular to real part, which indicated a good capacitance behavior. The real impedance at low frequencies, where the capacitive behavior dominates, is an indication of the combined resistance of the electrolyte and the films including both electronic and ionic contributions [36]. The values in real impedance at 0.1 Hz were 1310 Ω, 890 Ω and 1960 Ω for PEDOT-EtSO$_4$-SL, PEDOT-EtSO$_4$-SL-MWCNTs and PEDOT-EtSO$_4$-SL-MWCNTs-GluOx films, respectively. It could be found that the resistance of PEDOT-EtSO$_4$-SL-MWCNTs was lower than that of PEDOT-EtSO$_4$-SL, indicating the incorporation of MWCNTs into PEDOT-EtSO$_4$-SL matrix led to a faster electron transport in the bulk-
films and charge transfer in the parallel PEDOT-EtSO₄-SL-MWCNTs films/solution interface compared to that in the originally PEDOT/solution interface. This suggested that the presence of MWCNTs makes the composites have more active sites for faradic reactions and a larger redox capacitance than pure PEDOT films. Meanwhile, the macromolecular structure of GluOx hindered the electron-transfer and ion-transport, and the immobilization of GluOx into composite films provided much lower overall conductivity contrasted with the other two films. The above results also confirmed the successful immobilization of GluOx.

In addition, Sabatani and coworkers proposed that the change of $R_{ct}$ is related to the apparent electrode coverage ($\theta$), which is as follows:

$$\theta = 1 - \frac{R_{ct}^b}{R_{ct}^{TP}}$$  \hspace{1cm} (1)

where $R_{ct}^b$ is the $R_{ct}$ measured at a bare electrode, and $R_{ct}^{TP}$ is the $R_{ct}$ measured under same conditions at the thiophenol (TP)-covered electrode, respectively [44]. It can be proved that whether TP was immobilized in electrode from this equation. Similarly, the $\theta$ of GluOx in biocompatible PEDOT nanocomposite electrode ($\theta^1$) was given as follows:

$$\theta^1 = 1 - \frac{R_{ct}^{PEDOT-EtSO_4-SL-MWCNTs}}{R_{ct}^{PEDOT-EtSO_4-SL-MWCNTs-GluOx}}$$  \hspace{1cm} (2)

where $R_{ct}^{PEDOT-EtSO_4-SL-MWCNTs}$ and $R_{ct}^{PEDOT-EtSO_4-SL-MWCNTs-GluOx}$ are $R_{ct}$ of the PEDOT-EtSO₄-SL-MWCNTs/GCE and PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE, respectively. The value of $\theta^1$ is about 54.59.8%, which further confirmed that GluOx molecules were successfully immobilized in the biocompatible PEDOT-EtSO₄-SL nanocomposite matrix.

![Figure 2](image_url)

**Figure 2.** EIS of different electrodes in PBS (pH 7.5) containing 0.1 M LiClO₄: (a) GCE; (b) PEDOT-EtSO₄-SL/GCE; (c) PEDOT-EtSO₄-SL-MWCNTs/GCE; (d) PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE.

### 3.3 Effect of working potential

The effect of working potentials on current responses of PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE towards Glu was shown in Figure 3. Response currents increased rapidly with the increase of applied potentials from 0 to 0.3 V, and then the response currents began to level off, thus
0.3 V was selected for subsequent measurements, which is also lower than that in the most of previously reported literature based on GluOx electrode (Table 1). 

![Graph showing the effect of potential on current response of fabricated GluOx biosensors.]  

**Figure 3.** Effect of working potentials on current responses of fabricated GluOx biosensors at 25°C.

### 3.4 Effect of pH

The bioactivity of the as-fabricated bioelectrode is strongly depended on pH values of the buffer system. A plot of amperometric responses towards samples containing 0.1 mM Glu as a function of pH exhibited a sharp maximum at pH 7.5 (Figure 4), this is in accordance with the previously reported literature on most of enzyme electrode based on GluOx (Table 1). So the pH value of 7.5 was used to be optimal for Glu detection in following experiments.

![Graph showing the effect of pH on current response of fabricated GluOx biosensors.]  

**Figure 4.** Effect of pH on current responses of fabricated GluOx biosensors at 25°C.

### 3.5 Effect of temperature

The biological activity of enzyme strongly depends on the temperature. Very high or low temperature can inactivate the enzyme. The effect of temperature between 10 and 50 °C on the relative activity of the as-fabricated bioelectrode was studied by monitoring response currents of freshly prepared enzyme electrodes (Figure 5). The relative biological activity of the bioelectrode was not
monitored at higher temperatures (above 50 °C) to prevent vaporization. The currents gradually increased as the temperature raised and reached a maximal value between 25 and 35 °C, which was in agreement with most of previous reports in literature (Table 1). The biosensor lost its bioactivity at temperatures above 35 °C and eventually underwent irreversible denaturation. For avoiding gradual loss in bioactivity of enzyme and preventing irreversible denaturation of GluOx at higher temperatures, 25 °C was selected as the standard operating temperature for all following experiments.

Figure 5. Effect of temperature on current responses of the fabricated GluOx biosensors in PBS (pH 7.5) containing 1 mM Glu at 25°C.

3.6 Detection of Glu

Figure 6. Current-time plots of the PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE in PBS with various Glu concentrations at a potential of 0.3 V vs SCE. Inset: I vs [Glu] at 30 s.

The current responses of the as-fabricated bioelectrode were obtained by the successive addition of various Glu concentrations into PBS. Figure 6 presents the current-time plots of the as-obtained bioelectrode in PBS containing various Glu concentrations (0.6 µM – 2 mM) at a potential of 0.3 V (Figure 6 inset, R² = 0.997). The sensitivity was obtained by the slope of the initial linear part of
the calibration in inset of Figure 6, and its value was 9.99 μA M⁻¹ cm⁻², and the LOD was 0.27 μM. Obviously, response currents increased with increasing concentrations of Glu showed a short response time (5–10 s, depending on the concentrations of Glu), indicating a fast electron transfer process. In comparison with the sensing performance of other GluOx-based biosensors, the sensing performance of this biosensor was listed in Table 1. It could be seen that the proposed biosensor displayed a wider linear range, faster response time, lower LOD and satisfactory sensitivity from the data presented in Table 1, this is due to the synergistic effect among PEDOT, SLS, [Emim][EtSO₄], and MWCNTs.

Table 1. The sensing parameters and performance of various Glu biosensors based on GluOx in recent years

<table>
<thead>
<tr>
<th>Immobilization matrices</th>
<th>Immobilization methods</th>
<th>Working potentials</th>
<th>pH</th>
<th>T (°C)</th>
<th>Response times (s)</th>
<th>LOD (μM)</th>
<th>Linear ranges (nA/μM/cm²)</th>
<th>Sensitivity (nA/μM/cm²)</th>
<th>Storage life (days)</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>GluOx/cMWCNT/AuNP/CHIT</td>
<td>Covalent linking</td>
<td>ND</td>
<td>7.5</td>
<td>35</td>
<td>2</td>
<td>1.6</td>
<td>5–500</td>
<td>55</td>
<td>120</td>
<td>9</td>
</tr>
<tr>
<td>PtNP/NAE</td>
<td>Entrapment</td>
<td>0.6 V vs Ag/AgCl</td>
<td>7.4</td>
<td>22</td>
<td>&lt; 5</td>
<td>0.594</td>
<td>&lt;800</td>
<td>10.76</td>
<td>14</td>
<td>11</td>
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<tr>
<td>CeO₂/TiO₂/GmOx/Chit/α-FD/Pt</td>
<td>Entrapment</td>
<td>0.6 V vs Ag/AgCl</td>
<td>7.4</td>
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<td>7.5</td>
<td>ND</td>
<td>&lt; 3</td>
<td>0.0001</td>
<td>0.02–400</td>
<td>ND</td>
<td>60</td>
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<tr>
<td>Pt/Ppy/MWCNT TTFT-CNQ</td>
<td>Electrodoposition</td>
<td>ND</td>
<td>7.4</td>
<td>ND</td>
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<td>0.3</td>
<td>140</td>
<td>0.384</td>
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<td>25</td>
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<td>0.68</td>
<td>58-1271</td>
<td>ND</td>
<td>60</td>
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<tr>
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<td>ND</td>
<td>0.5-8000</td>
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<tr>
<td>PTAA</td>
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<td>0.3 V vs SCE</td>
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<td>25</td>
<td>&lt; 5</td>
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<td>0.6-3000</td>
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<td>Electrodeposition</td>
<td>0.3 V vs SCE</td>
<td>7.5</td>
<td>25</td>
<td>5–10</td>
<td>0.27</td>
<td>0.6-2000</td>
<td>9.99</td>
<td>120</td>
<td>This work</td>
</tr>
</tbody>
</table>

3.7 Dynamics of GluOx biosensors

The bioaffinity of the bioelectrode was described by Lineweaver-Burk plots in Figure 7 inset. Its parameters were obtained by the equation as follows:

\[
\frac{1}{I} = \frac{K_{\text{mapp}}}{I_{\text{max}}} \frac{1}{[\text{Glu}]} + \frac{1}{I_{\text{max}}}
\]

\( K_{\text{mapp}} \) (apparent Michaelis-Menten constant) and \( I_{\text{max}} \) (apparent maximum steady-state response current) values were calculated by the mentioned-above equation. An equation of the form \( y = Bx + A \) was given after linear regression. \( K_{\text{mapp}} \) can be calculated as \( B (A)^{-1} \), as at that point, \( y \) is zero, whereas the inverse of \( A \) is \( I_{\text{max}} \), as at that point, \( x \) is zero. \( K_{\text{mapp}} \) and \( I_{\text{max}} \) values were 2.33 mM and 25.2 μA cm⁻², respectively. These values were significantly lower than that reports in previous literature[45]. A measure of enzymatic affinity for substrates and corresponds to the concentration of substrate at 1/2 \( V_{\text{max}} \) is defined as Michaelis-Menten constant, which is inversely proportional to the enzymatic affinity for its substrates. Thus the lower value of \( K_{\text{mapp}} \) revealed the high bioaffinity of the fabricated biosensor to substrates, which might be attributable to the biocompatibility of SLS and the affinity of MWCNTs, even synergistic effect among PEDOT, SLS, [Emim][EtSO₄], and MWCNTs.
**Figure 7.** Line weaver-Burk plots of the PEDOT nanocomposite bioelectrode under optimal parameter conditions.

**Figure 8.** The determination of $E_a$ using the fabricated PEDOT nanocomposite bioelectrode.

In addition, the apparent activation energy ($E_a$) of this bioelectrode was presented by $\ln I$ vs. $1/T$ plots in Figure 8. The $\ln I$ vs. $1/T$ graph reveals the relationship between the inverse of the temperature and the Napierian logarithm of $I$, and the $E_a$ value was obtained by the equation as follows:

$$\ln k = \ln k_0 - \frac{E_a}{RT} \Rightarrow \ln I = \ln I_0 - \frac{E_a}{RT}$$

An equation of $y = A + Bx$ was obtained after linear regression, then the apparent activation energy was given from $E_a = BR$. The $E_a$ value was 17.12 kJ M$^{-1}$, which was much lower than that in previous reports (Table 1). The lower $E_a$ value indicated that the prepared bioelectrode had higher bioaffinity and bioactivity towards matrix, which was consistent with the lower $K_{mapp}$ value.
3.8 Stability of GluOx biosensor

Stability is a very important parameter towards a biosensor. The operational stability of the fabricated bioelectrode was tested in 0.1 M PBS (pH 7.5) containing 0.1 mM Glu with 40 successive measurements using the same GluOx bioelectrode (Figure 9A). At 40th experiments for the Glu determinations, the fabricated bioelectrode retained almost 100% of its original activity. Moreover, the coefficient of variation was 1.61 % for 40 successive assays, indicating a fairly good repeatability of the PEDOT-EtSO₄-SL/MWCNTs/GluOx bioelectrode. Besides, the coefficient of variation was 1.29 % for assays of ten bioelectrodes (Figure 9B), revealing a very good reproducibility of the PEDOT-EtSO₄-SL/MWCNTs/ GluOx bioelectrode.

![Figure 9A](image1)
![Figure 9B](image2)
![Figure 9C](image3)

**Figure 9.** The operational stability, reproducibility, and long-term storage stability of the GluOx biosensor based on PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE.

The long-term stability of the fabricated bioelectrode is studied in the same solution. The response currents of the bioelectrode decreased gradually and retained about 85% of its initial value after 120 days (Figure 9C). There are two reasons for this good long-term stability. Good biocompatible and weak-toxic PEDOT-EtSO₄-SL /MWCNTs matrix on the GCE surface provide a good microenvironment for maintaining the native activity of GluOx. On the other hand, the network of MWCNTs and the firmly embedding of GluOx onto biocompatible conducting matrix might prevent the leakage of GluOx from the matrix.
The influence of different substances in animal and plant as potential interferents was investigated under the optimum conditions in PBS containing 10 µM Glu or 100 µM Glu, respectively. The results showed that common anions and cations like 10-fold of Na⁺, K⁺, ClO₄⁻, Cl⁻, NO₃⁻, H₂PO₄⁻, HPO₄²⁻ and SO₄²⁻, organic acids, carbohydrates, vitamins hormone, other natural amino acids and others did not interfere in corresponding concentration. But dopamine and vitamin C appeared observable interference when the their concentration exceeded 100 µM. Thus it was reasonably concluded that this method was relatively selective for the determination of Glu.

3.10 Practical application of GluOx biosensor

Table 2. The detection of Glu in real samples using GluOx amperometric biosensor compared with HPLC

<table>
<thead>
<tr>
<th>Samples</th>
<th>Added (µM)</th>
<th>Average found (µM)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>Average found (µM)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken blood</td>
<td>0</td>
<td>28.86 ± 0.154</td>
<td>0.53</td>
<td>-</td>
<td>27.12 ± 0.095</td>
<td>0.35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>49.36 ± 0.178</td>
<td>0.87</td>
<td>102.5</td>
<td>47.56 ± 0.143</td>
<td>0.7</td>
<td>102.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>137.83 ± 3.154</td>
<td>3.07</td>
<td>108.97</td>
<td>130.52 ± 1.58</td>
<td>1.21</td>
<td>103.4</td>
</tr>
<tr>
<td>Soil</td>
<td>0</td>
<td>21.264 ± 0.096</td>
<td>0.45</td>
<td>106.32</td>
<td>20.984 ± 0.053</td>
<td>0.25</td>
<td>104.92</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>98.319 ± 1.339</td>
<td>1.36</td>
<td>98.3</td>
<td>100.594 ± 1.265</td>
<td>1.26</td>
<td>100.59</td>
</tr>
<tr>
<td>Tomato</td>
<td>0</td>
<td>13.537 ± 0.126</td>
<td>0.93</td>
<td>-</td>
<td>13.247 ± 0.057</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>33.435 ± 0.196</td>
<td>0.58</td>
<td>99.49</td>
<td>33.254 ± 0.069</td>
<td>0.35</td>
<td>98.58</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>117.876 ± 1.56</td>
<td>1.48</td>
<td>105.34</td>
<td>115.468 ± 1.24</td>
<td>1.22</td>
<td>101.93</td>
</tr>
<tr>
<td>Eggs soup</td>
<td>0</td>
<td>54.9826 ± 0.316</td>
<td>0.58</td>
<td>-</td>
<td>53.699 ± 0.116</td>
<td>0.22</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>75.4635 ± 0.187</td>
<td>0.91</td>
<td>102.4</td>
<td>75.237 ± 0.186</td>
<td>0.92</td>
<td>101.27</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>158.6593 ± 2.96</td>
<td>1.87</td>
<td>103.68</td>
<td>157.9869 ± 1.08</td>
<td>1.05</td>
<td>103</td>
</tr>
</tbody>
</table>

To test the feasibility and applicability of the fabricated biosensor, the bioelectrode was used to detect the Glu concentration in agricultural and biological samples. Table 2 shows the content of Glu in agricultural and biological samples, which was obtained by the standard addition method using PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE. To test the accuracy of the proposed method, the Glu content was detected by HPLC. The results obtained by HPLC are in good agreement with that of the PEDOT-EtSO₄-SL-MWCNTs-GluOx/GCE, revealing that the proposed is suitable and feasible. In addition, known amounts of Glu were spiked in the samples, then analyzed according to the same procedure. The recoveries are in a range of 98.3% – 108.97.0%, indicating that the fabricated biosensor
could be used for determining Glu levels in real samples. In addition, from RSD, also indicating that high concentration of Glu will influence the accuracy of the fabricated biosensor.

4. CONCLUSIONS

An amperometric Glu biosensor was successfully constructed by the one-step electropolymerization technique in ionic liquid-in-water microemulsion containing multi-walled carbon nanotubes, GluOx, EDOT, [Emim][EtSO₄], SLS and LiClO₄, and CVs and EIS confirmed the immobilization of GluOx into the integrated biocompatible PEDOT-EtSO₄-SL-MWCNTs-GluOx matrix. The fabricated bioelectrode was used to determine the concentrations of Glu and showed satisfactory bioelectrochemical catalytic activity with a wide linear range from 0.6 μM to 2 mM, pronounced sensitivity of 10.12 μA mM⁻¹ cm⁻², short response time within 5-10 s, and its low LOD is 0.27 μM. In addition, the bioelectrode displayed good stability, specificity and applicability towards Glu sensing in real samples. The satisfactory results prove that the biocompatible PEDOT-EtSO₄-SL-MWCNTs matrix is a potentially promising immobilization platform for the architecture of enzyme-based sensor, and provide a good model for practical application in animal and plant biochemistry and clinical medicine.

ACKNOWLEDGEMENTS
The authors would like to acknowledge the financial support of this work by the NSFC (51302117), Jiangxi Provincial Department of Education (GJJ14289 & GJJ14301).

References

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