

Graphene Ink Modified Glassy Carbon Electrode as electrochemical sensor for Galantamine Determination

Haobin Shi¹, Yuhong Zheng² Mengyao Wu¹ and Li Fu^{1,*}

¹ College of Materials and Environmental Engineering, Hangzhou Dianzi University, Hangzhou, 310018, P.R. China

² Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing Botanical Garden, Mem. Sun Yat-Sen, Nanjing 210014, P.R. China.

*E-mail: fuli@hdu.edu.cn

Received: 15 September 2018 / Accepted: 6 November 2018 / Published: 5 January 2019

In this study, commercial graphene ink has been used as a cost-effective modifier for glassy carbon electrode (GCE) surface modification. The excellent conductivity and defects triggered electrocatalytic property of the graphene ink showed a superior performance in electrochemical sensing. Galantamine, an alkaloid isolated from *Lycoris*, has been selected as an analyte for evaluating the graphene ink-modified GCE. A clear signal enhancement with reduction of overpotential was observed using a GCE after a simple graphene ink surface modification process. A linear detection range from 20 μM to 180 μM of galantamine was recorded on the proposed electrode with a low limit of detection of 4.1 μM .

Keywords: Graphene ink; Galantamine; Surface modification; Electrochemical sensor; Glassy carbon electrode

1. INTRODUCTION

Galantamine is a reversible anti-cholinesterase drug extracted from *Lycoris sativus*. This drug can easily enter brain tissue through the blood-brain barrier and has a strong effect on the central nervous system [1]. Galantamine can improve the conduction between nerve and muscle. Compared with physostigmine, neostigmine and piazimine, galantamine has a wide therapeutic range, low toxicity and weak muscarinic action [2, 3]. Galantamine is mainly used in the treatment of myasthenia gravis, poliomyelitis and sequelae but also for children with cerebral palsy, polyneuritis, spinal radiculitis, and nervous system diseases [4]. Cholinergic deficiency plays an important role in the pathogenesis of Alzheimer's disease. The cholinergic system is essential for maintaining short-term memory and attention [5, 6]. The dysfunction of the cholinergic system is associated with certain neuropsychiatric

symptoms and behaviours. Cholinesterase inhibitors increase the amount of acetylcholine by inhibiting the degradation of acetylcholine. New evidence suggests that long-term use of cholinesterase inhibitors not only improves cognitive and behavioural disorders, but may also affect neuronal function and survival. It can increase the level of acetylcholine in the brain and delay the process of brain cell dysfunction. Galantamine can significantly improve cognitive function and maintain daily living ability in mild and moderate Alzheimer's patients. Therefore, detection of galantamine level is highly important for the pharmaceutical industrial and clinical fields. Several analytical techniques have been developed for galantamine determination, such as liquid chromatographic–tandem mass spectrometric method [7], spectrofluorimetry [8, 9], micellar electrokinetic chromatography [10] and LC-MS/MS [11]. These established methods exhibited good performance for galantamine determination with a low detection limit. However, the complicated sample preparation process and expensive instrumentation limit their field applications. In contrast, the development of electrochemical analysis techniques with quick response and low cost provides an alternative approach for electroactive substance detection [12-15]. However, the electrochemical sensing performance is mainly based on the electrochemical reaction between the electrode and target analyte. To date, commercial electrodes, such as the glassy carbon electrode (GCE) and the screen-printed electrode, showed insufficient performance in trace substance detection. Electrode surface modification is considered to be a simple technique for enhancing the electrode for analytical applications [13, 16, 17]. Nanocarbon materials can be considered to be an excellent family for electrode surface modification due to their high conductivity, excellent stability and electrochemical inertness [18-20]. Among these materials, graphene is a type of carbon material with single-layer, two-dimensional hexagonal lattice structure. Graphene has the related excellent properties of graphite and carbon nanotubes. Due to the large specific surface area, high electrical conductivity, excellent mechanical, thermal properties, and biocompatibility, graphene exhibits many excellent properties and has been widely used in the preparation of chemically modified electrodes, successfully realizing the electrochemical sensitive detection of electroactive substances [18, 21-24].

In this article, we have attempted to modify the GCE by using commercial graphene ink. The modified GCE showed a superior electroanalytical performance towards galantamine oxidation. The proposed electrochemical sensor could achieve linear detection of galantamine between 20 to 180 μM with a detection limit of 4.1 μM . The results indicate the graphene ink could be simply used as an electrode modifier for enhancing the electroanalytical performance of commercial electrode.

2. EXPERIMENTAL

Graphene ink was purchased from Tanfeng Tech. Inc. (China) and diluted to 1 mg/mL using water as solvent. Galantamine and other common reagents were purchased from Aladdin (China) as analytical grade. The 0.1 M standard phosphate buffer solutions (PBS) was prepared by mixing K_2HPO_4 and NaH_2PO_4 water solution.

The UV-vis spectrum of the sample was recorded in a UV-vis spectrometer (Agilent Technologies, model 8453) from 200 to 700 nm. The morphology of the graphene ink modified electrode was characterized using a scanning electron microscope (SEM, S-4700, Hitachi High Technologies

Corporation, Japan). The Raman spectrum of the sample was recorded using a Raman Microprobe (Renishaw RM1000) with 514 nm laser light from 1000 to 1800 cm^{-1} . The FTIR spectrum of the sample was recorded using a Thermo Nicolet iS50 FTIR from 500 to 4000 cm^{-1} . All electrochemical characterizations and determinations were recorded in a CHI760e electrochemical workstation. A three-electrode system was used for all experiments. A GCE, Pt electrode and Ag/AgCl(3 M KCl) were used as working electrode, counter electrode and reference electrode, respectively. Cyclic voltammetry and differential pulse voltammetry were applied for galantamine determination.

For GCE surface modification, a specific amount of graphene ink (1 mg/mL) was drop casted on the GCE surface and dried naturally. The modified electrode was denoted as GCE-GP.

3. RESULTS AND DISCUSSION

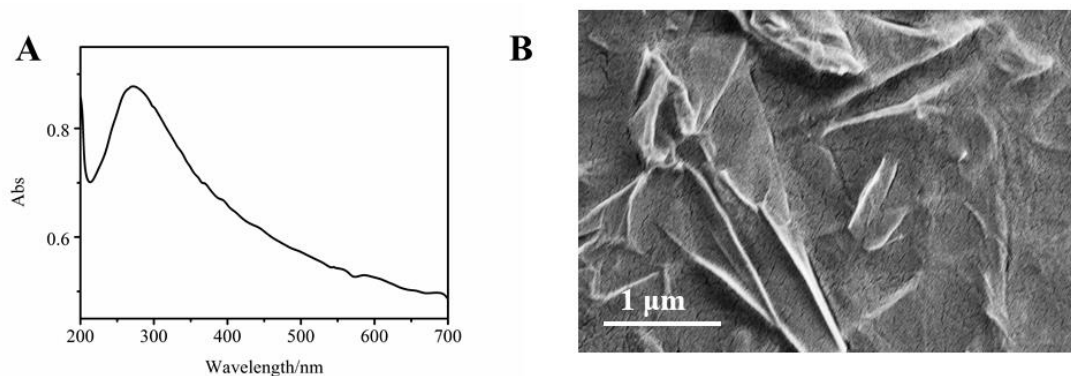


Figure 1. (A) UV-vis spectrum and (B) SEM image of graphene ink.

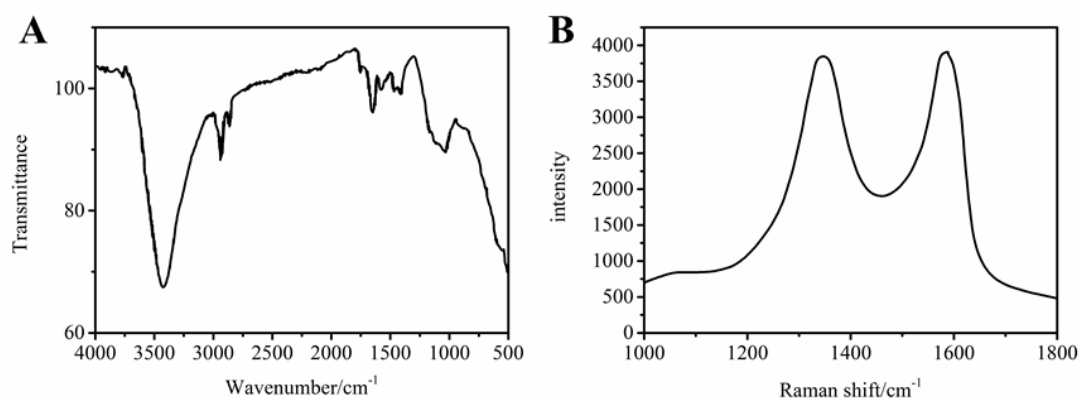


Figure 2. (A) FTIR and (B) Raman spectrum of graphene ink.

Figure 1A shows the UV-vis spectrum of graphene ink; a well-defined characteristic peak can be noted at 266 nm, corresponding to the excitation of the π -plasmon of the graphitic bond [25, 26], thereby suggesting the graphene flakes in the ink exist mainly in reduced form, rather than in oxidized form. Figure 1B shows the SEM image of the graphene ink. It can be seen that the graphene flakes are in a smooth sheet-like structure, rather than re-stacked thick clusters, suggesting the graphene ink could provide a larger electro-active surface area during the electrochemical reaction.

The chemical status of the graphene ink was further characterized using FTIR and Raman spectroscopy. Figure 2A shows the FTIR spectrum of the graphene ink. It can be seen that the spectrum of graphene ink shows peaks at 1724, 1582, 1409 and 1023 cm^{-1} , corresponding to the C=O stretching of COOH groups, C=C vibrations, C–OH vibrations and C–O vibrations, respectively [27-29]. These peaks suggest a certain number of oxygen-containing functional groups that are still attached on the graphene surface, which could affect its dispersibility. Compared with the literature [30-32], the intensity of these peaks was much lower than of graphene oxide, further indicating these graphene flakes retained most of their conductivity property. Figure 2B shows the Raman spectrum of the graphene ink. As shown in the figure, the spectrum exhibited two characteristic bands approximately located at 1568 and 1342 cm^{-1} , which can be ascribed to the first-order scattering of E_{2g} phonons by sp^2 carbon atoms and breathing mode or κ -point phonons of A_{1g} symmetry, respectively [33-35]. The intensity ratio between these two bands can be used for evaluating the quality of the graphene. In this case, the ratio is approximately 1, suggesting the graphene in ink contains many defects. For electrochemical applications, the defects on the graphene surface can trigger significant activity of electrocatalysis [36-39].

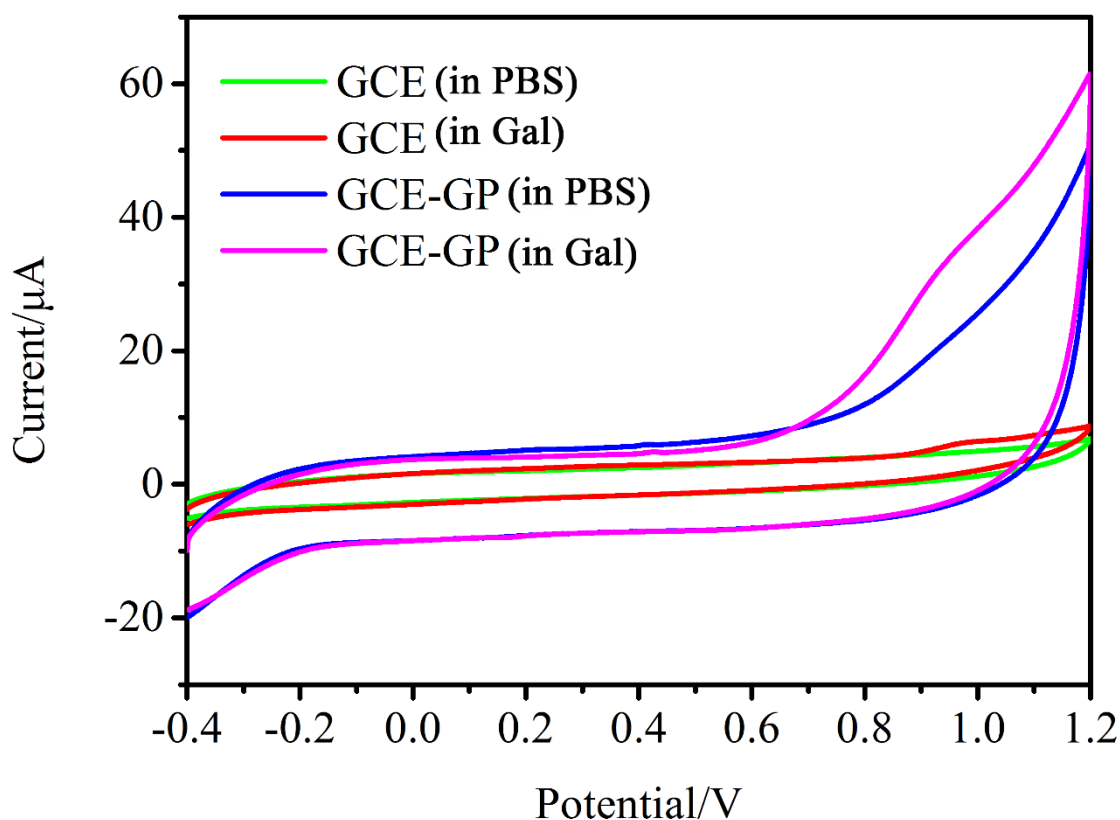


Figure 3. Cyclic voltammograms of bare GCE and GCE-GP in the absence and presence of 50 μM galantamine in pH 7.0 PBS. Scan rate=50 mV/s.

The electrochemical property of the graphene ink towards galantamine was tested and compared with bare GCE. As shown in Figure 3, both bare GCE and GCE-GP show no notable response from -0.4 to 1.2 V, suggesting the graphene ink itself has no clear redox reaction in the scan range. In contrast,

both electrodes show a clear oxidation peak when 50 μM galantamine was introduced into the electrolyte. More specifically, the bare GCE exhibits a small oxidation peak at 0.96 V, while the GCE-GP shows a clearer oxidation peak at 0.89 V. The peak current enhancement can be ascribed to the excellent conductivity and high specific surface area of the graphene ink, while the decreasing of the oxidation potential can be ascribed to the electrocatalysis triggered by the surface defects of the graphene flakes.

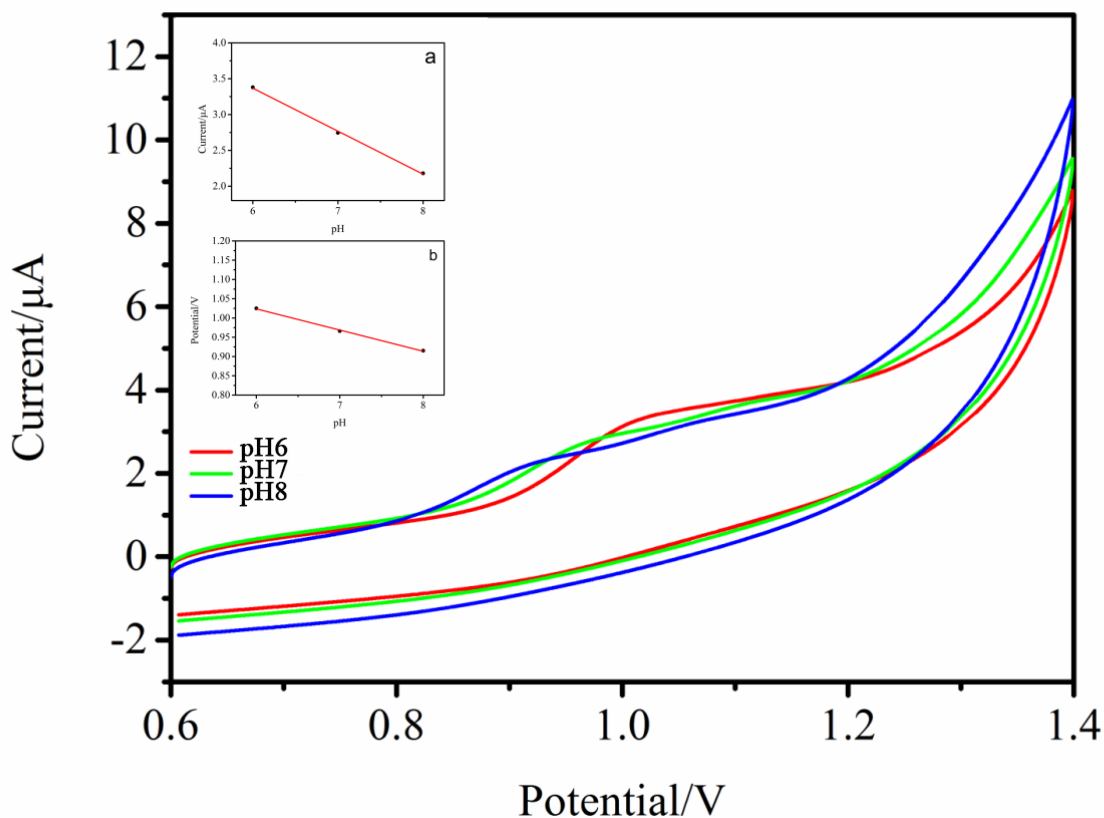


Figure 4. Cyclic voltammetry of GCE-GP responding to galantamine at different pH conditions in pH 7.0 PBS. Scan rate=50 mV/s. Inset: plots of pH value and oxidation peak currents; plots of pH value and oxidation potentials.

The electrochemical behaviour of the galantamine at the GCE-GP was studied in detail. Figure 4 shows the GCE-GP towards galantamine oxidation at different pH conditions. As shown in the figure, the current response of the galantamine at basic condition is slightly less sensitive than in the acidic condition. This phenomenon could be ascribed to the anionic reaction of the galantamine molecule [5]. Since the neutral environment is most common in biological conditions, a pH of 7 was selected for further analysis. The oxidation potential of the galantamine was negatively shifted along with the pH increase, suggesting the proton participated in the oxidation process. A linear regression equation can be obtained between galantamine oxidation potential and pH value: $E_{\text{pa}}(\text{V}) = -0.055\text{pH} + 1.35367$ ($R^2 = 0.9964$). The slope is close to the theoretical value, suggesting the galantamine oxidation at GCE-GP surface involves equal number of electrons and protons [40].

The effect of scan rate on galantamine oxidation has been investigated as well. Figure 5 shows the CV curves of GCE-GP towards galantamine oxidation from 20 mV/s to 200 mV/s. As shown in the inset of Figure 5, a linear relationship was observed for the logarithm of oxidation current vs. logarithm of scan rate. The equation can be expressed as: $\log I_{pa}(\mu A) = 0.79077 \log v (mV/s) + 1.04641$ ($R^2 = 0.99768$). Therefore, the electrochemical oxidation of galantamine at GCE-GP surface is a typical diffusion-controlled process.

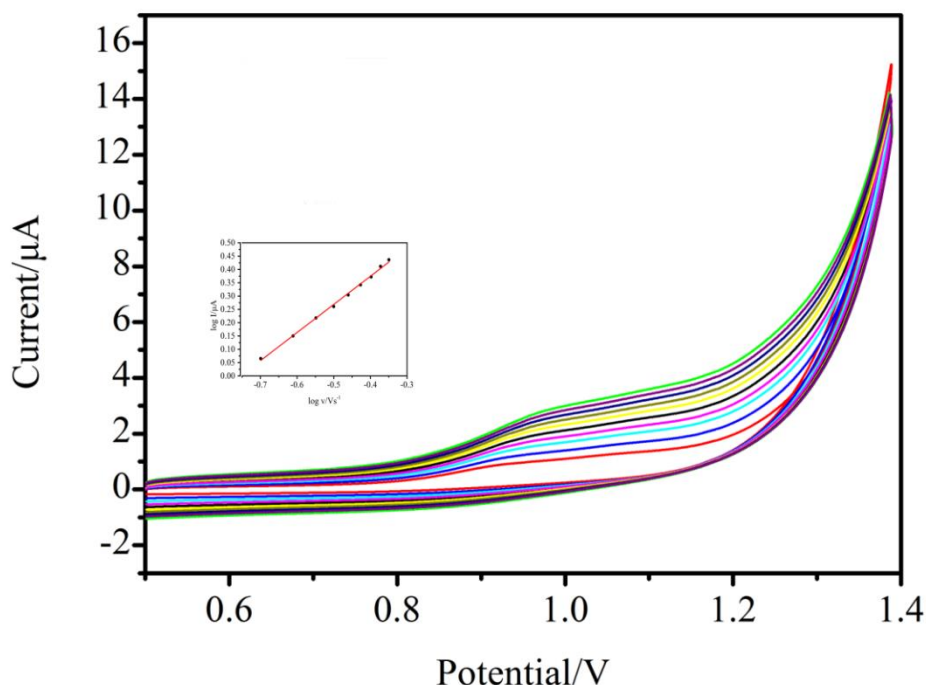


Figure 5. Cyclic voltammetry of GCE-GP responding to galantamine from scan rate of 20 mV/s to 220 mV/s in in pH 7.0 PBS. Inset: plots of logarithmic scan rate and oxidation peak currents; plots of logarithmic scan rate and oxidation potentials.

The Laviron equation can be used for calculating the irreversible electrode process:

$$E_{pa} = E^{\circ} + \frac{2.3RT}{(1-\alpha)nF} \log \frac{(1-\alpha)nF}{RTk_s} + \frac{2.3RT}{(1-\alpha)nF} \log v$$

where F , R , T , n , α , v , k_s and E° are the Faraday constant, gas constant, temperature, number of electron participated in reaction, transfer coefficient, scan rate, standard heterogeneous rate constant and formal potential, respectively. $(1-\alpha)$ can be calculated to be 0.116. According to the Bard and Faulkner proposed equation,

$$\alpha = \frac{47.7}{E_p - E_{p/2}} (mV)$$

where $E_{p/2}$ is the potential where the current is at half the peak value. α can be calculated to be 0.487, which is close to 0.5 in an irreversible electrode process. Based on the above results, n can be calculated to be 1.03. Therefore, one electron and one proton participated in the galantamine oxidation. The possible oxidation mechanism has been proposed in Figure 6.

The amount of modifier on the electrode surface could significantly influence the electrochemical signal. As shown in Figure 7, the oxidation current showed a clear enhancement when the amount of modifier increased from 2.5 μL to 4 μL . This enhancement can be ascribed to the increase of electroactive surface area during the oxidation process. However, a further increase of the amount of modifier could result in a decreasing current. It can be explained that a thicker modifier film was formed on the electrode surface, which prolonged the electron transfer pathway and resulted in a low current response. Therefore, 4 μL of graphene ink (1 mg/mL) has been used for GCE surface modification.

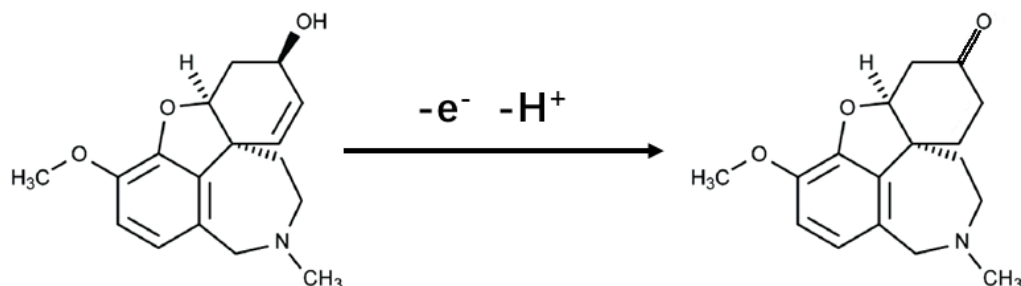


Figure 6. The proposed electrochemical galantamine oxidation.

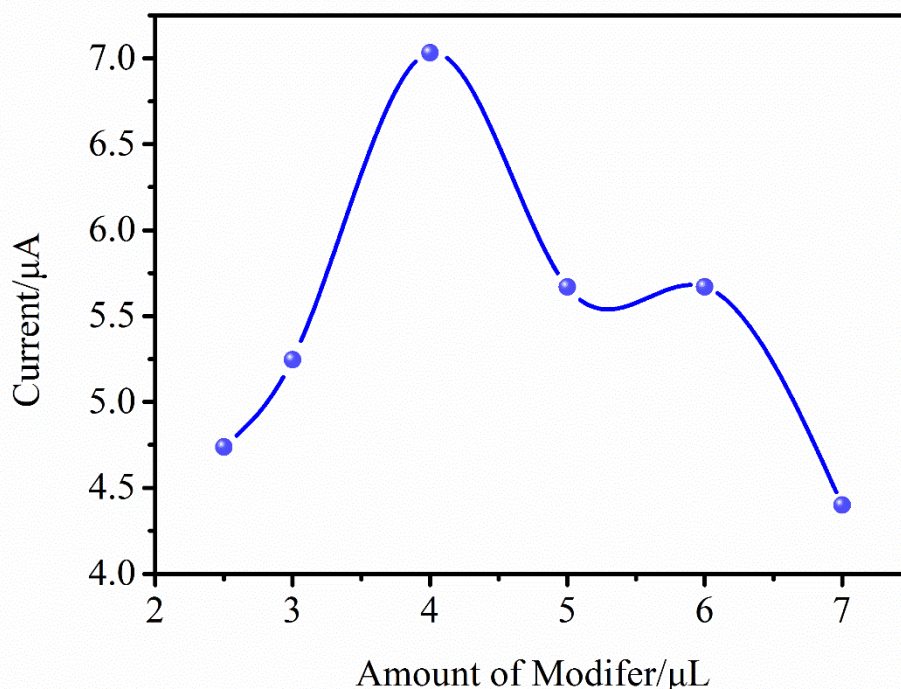


Figure 7. Influence of amount of modifier for electrochemical oxidation of galantamine. The electrochemical reaction was performed in pH 7.0 PBS with scan rate of 50 mV/s.

Differential pulse voltammetry (DPV) has been used as analytical technique to study the linear detection range and feasibility of GCE-GP due to its high sensitivity. Figure 8 shows the DPV curves of GCE-GP towards galantamine from 20 μM to 180 μM in 0.1 M PBS. The anodic peak responses of galantamine were proportional to its concentration. As shown in the inset of the Figure 8, the GCE-GP shows a linear detection range from 20 μM to 180 μM with a linear regression equation of $I=0.00743C(\mu\text{M})+1.54906$. The limit of detection was 4.1 μM based on a signal-to-noise ratio of 3.

Real sample test has been performed using a commercial galantamine hydrobromide injection with a dilution. Standard addition method has been adopted during the test. As shown in Table 1, the proposed GCE-GP can be successfully applied for galantamine detection in real drug.

Table 1. Electrochemical determination of galantamine content in a galantamine hydrobromide injection.

Sample No.	Detection (μM)	Addition (μM)	Detection (μM)	Recovery (%)	RSD (%)
1	47.4	20.0	66.4	98.5	3.1
2	76.6	20.0	95.8	99.2	2.6
3	98.7	20.0	119.9	101.0	3.4

Reproducibility and stability are important factors in electrochemical sensors. Four independent GCE-GPs were used to measure electrode reproducibility. As shown in Figure 9A, the four independent GCE-GPs exhibited highly similar detection results. A relative standard deviation (RSD) of 5.2% of was observed. The stability of the GCE-GP was tested by one electrode for six successive detections. As shown in Figure 9B, only very small degradation was noticed. The sixth detection retained more than 92% of its original performance. Therefore, the proposed GCE-GP showed excellent reproducibility and stability.

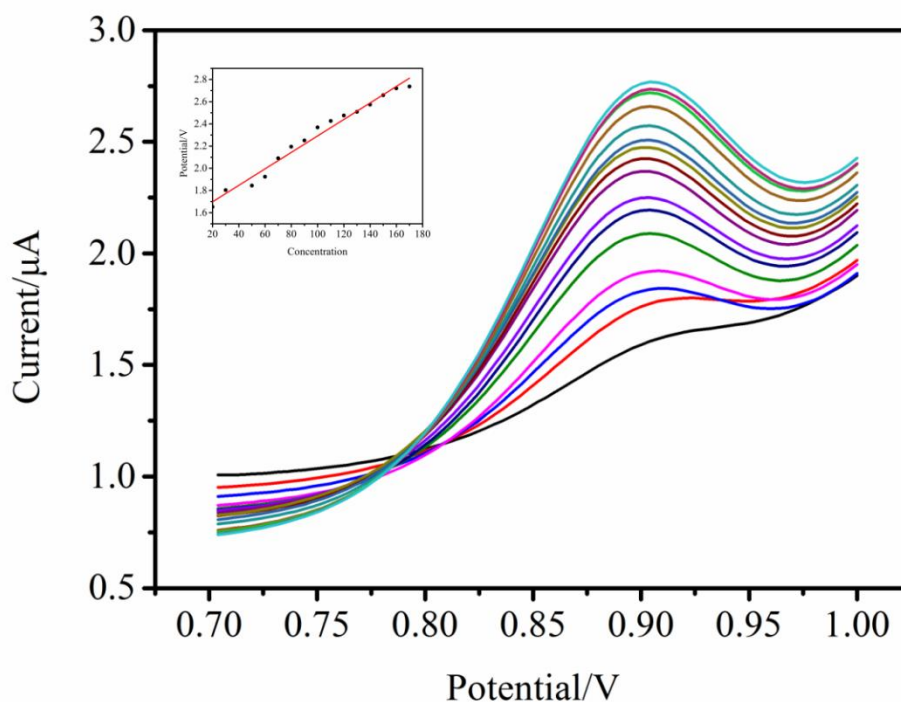


Figure 8. Differential pulse voltammograms of GCE-GP towards galantamine in concentration from 20 μM to 180 μM in 0.1 M PBS (pH 7). Inset: Plots of galantamine concentrations against peak currents.

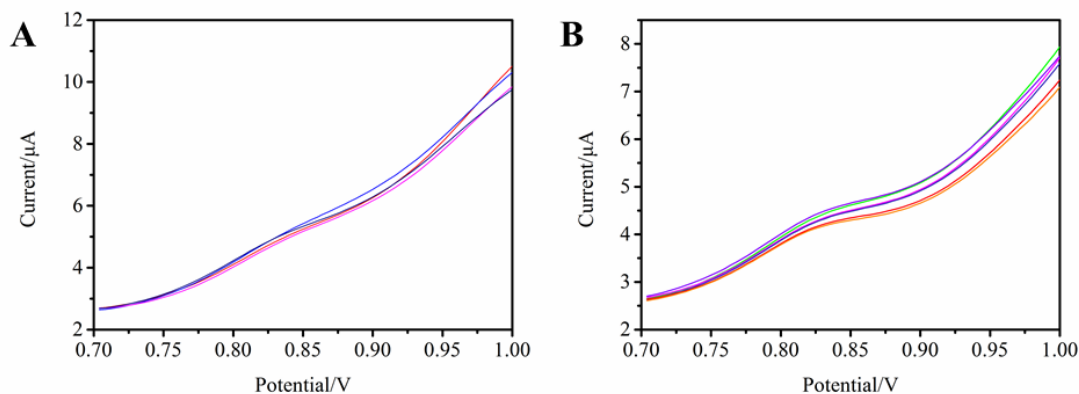


Figure 9. (A) Four individual GCE-GPs for galantamine detection. (B) A GCE-GP for six successive galantamine detections. All electrochemical measurements were performed in pH 7.0 PBS with scan rate of 50 mV/s.

4. CONCLUSIONS

Commercial ink can be used as a cost-effective electrode surface modifier for constructing an electrochemical sensor. The excellent conductivity of graphene can enhance the electrochemical signal, while its surface defects can trigger electrocatalytic reactions. In this report, a graphene ink modified GCE has been fabricated via a simple drop casting method and used for galantamine detection. A linear detection of galantamine was recorded at a concentration range from 20 μM to 180 μM with a low limit of detection of 4.1 μM.

ACKNOWLEDGMENTS

This work has been financially supported by Zhejiang Province Natural Science Foundation of China (LQ18E010001).

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