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In situ electrochemical synthesis of graphene-poly(arginine) composite for p-nitrophenol monitoring

Yiwei Li a,b, Yaohong Ma a,b, Eric Lichtfouse e,f, Jin Song a,b, Rui Gong g, Jinheng Zhang a,b, Shuo Wang a,b, Leilei Xiao a,c,d,*,1

a Biology Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250103, PR China
b Shandong Provincial Key Laboratory of Biosensors, Jinan 250103, PR China
c Key Laboratory of Coastal Biology and Biological Resources Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, PR China
d CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, PR China
e Aix-Marseille Univ, CNRS, IRD, INRA, Coll France, CEREGE, Avenue Louis Philibert, Aix en Provence 13100, France
f State Key Laboratory of Multiphase Flow in Power Engineering, Xi’an Jiaotong University, Xi’an, Shaanxi 710049, PR China
g National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing 100101, PR China

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ABSTRACT

Para-Nitrophenol (p-nitrophenol) is a common industrial pollutant occurring widely in water bodies, yet actual monitoring methods are limited. Herein we proposed a fully electrochemically in situ synthesized graphene-polyarginine composite functionalized screen printed electrode, as a novel p-nitrophenol sensing platform. The electrode was characterized by morphologic, spectrometric and electrochemical techniques. p-Nitrophenol in both pure aqueous solution and real water samples was tested. Results show a detection limit as low as the nanomolar level, and display a linear response and high selectivity in the range of 0.5–1250 μM. Molecular simulation reveals a detailed synergy between graphene and poly-arginine. The preferable orientation of nitrophenol molecules on the graphene interface in the presence of poly-arginine induces H- and ionic binding. This sensor is an ideal prototype for p-nitrophenol quantification in real waters.

1. Introduction

Nitrophenols, such as p-nitrophenol, is vastly used in pharmaceuticals, pesticides, dyes, explosives and petroleum industries (Wang et al., 2016; Kumar et al., 2017). In air, water and soil, p-nitrophenol is found with high chemical stability and reluctant biodegradability, which make its removal challengeable (Han et al., 2019). It is highly toxic and can cause irreversible damages to human liver and kidneys. Therefore, p-nitrophenol has been classified priority pollutant among top 114 organic pollutants by the US Environmental Protection Agency, with allowed limit of 0.43 μM (60 ppb) in drinking water (Menazea and Mostafa, 2020; Chakraborty et al., 2021).

Although these methods present some advantages, they are limited by the need for man-sized instruments, expenses, experienced operators and slowness. Alternatively, electrochemical methods feature outstanding performances such as high-sensitivity, fast response, low-cost, easy operation and instrumental miniaturization (Karaman et al., 2021a, 2021b; Karimi-Maleh et al., 2021a, 2021b; Balasubramanian et al., 2019; Li et al., 2012). Electrochemical detectors of nitrophenols have been recently designed using various active materials as well as electro-analytical techniques, such as semiconductive metal composites (Zhang et al., 2018; Wang et al., 2019a, 2019b; Su et al., 2021), metal nanoparticles (Madhu et al., 2014; Chao et al., 2017), polymers (Zhu et al., 2020) and carbon nanoparticles (Karaman et al., 2021a, 2021b; Karimi-Maleh et al., 2017, 2018).
In particular, carbon nanomaterials were successfully applied to the detection of both small compounds and bio-macromolecules (Luo et al., 2020; Shen et al., 2019; Sengupta et al., 2020). However, optimizing all specifications such as high sensitivity, low detection limit, wide linear response, easy handling and low cost is challenging, as depicted in a comparison of actual methods (Table 1).

Among carbon materials, graphene has unique properties including high electrical conductance, huge surface area and absorbability, excellent electro-catalytic activity and versatile surface chemistry, which makes graphene an ideal electrochemical sensing material for \( p \)-nitrophenol (Cinti and Arduini, 2017; Shari et al., 2019). Wiench et al. (2017) demonstrated that the hydrothermally reduced graphene oxide interface is an excellent \( p \)-nitrophenol electro-catalyst, and extraordinary sensing stability within pH 4.0–5.0 was achieved. Nevertheless, interface of raw graphene is not optimal for detection, thus requiring architecture engineering and design of graphene composites. Mono-dispersions prevent graphene from the loss of electrochemical activity caused by graphene sheet restacking, which limits the contact of the analyte with graphene active sites (Shang et al., 2008; Chen et al., 2012; Cheng et al., 2017). As a consequence, scientists have designed composites by grafting functional materials such as metal nanoparticles, metal compounds, polymers, carbons and peptides (Yang et al., 2008; Cuenya, 2013; Su et al., 2021). Manganese cobalt oxide was introduced into graphene oxide (GO) as a dispersant to acquire better dispersed GO micro-architecture for \( p \)-nitrophenol analysis. Results showed that the metal oxide-GO composite exhibited much better

<table>
<thead>
<tr>
<th>Electrode architecture</th>
<th>Electrochemical technology</th>
<th>Linear range (μM)</th>
<th>Sensitivity (μA μM⁻¹ cm⁻²)</th>
<th>LOD (μM)</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>AgNWs-PANI/GCE</td>
<td>DPV</td>
<td>0.6–32</td>
<td>–</td>
<td>0.052</td>
<td>Chao et al., 2017</td>
</tr>
<tr>
<td>Cu-curcumin/GCE</td>
<td>DPV</td>
<td>0.1–1030</td>
<td>1.57</td>
<td>68.2</td>
<td>Dinesh and Saraswathi, 2017</td>
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<tr>
<td>FeO\textsubscript{0.5}/TiO\textsubscript{2}/nc/GCE</td>
<td>Amperometry</td>
<td>5–310</td>
<td>3.89</td>
<td>0.183</td>
<td>Wang et al., 2019a, 2019b</td>
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<td>AgPd@UGO-66- NH\textsubscript{2}/GCE</td>
<td>DPV</td>
<td>100–370</td>
<td>1259.1</td>
<td>32</td>
<td>Hira et al., 2019</td>
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<tr>
<td>HA-NP/GCE</td>
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<td>1–300</td>
<td>–</td>
<td>0.6</td>
<td>Yin et al., 2010</td>
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<tr>
<td>ZnCo\textsubscript{2}O\textsubscript{4}/GCE</td>
<td>DPV</td>
<td>1–4000</td>
<td>0.318</td>
<td>0.3</td>
<td>Zhang et al., 2018</td>
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<td>PANI-array/CNF/GCE</td>
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<td>0.01–0.1</td>
<td>1.09</td>
<td>0.0015</td>
<td>Zhu et al., 2020</td>
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<td>PEDOT-PSS/TTO</td>
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<td>0.24</td>
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<td>0.02</td>
<td>–</td>
<td>–</td>
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<td>GC/rGO-Ag</td>
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<td>12.65</td>
<td>0.0012</td>
<td>Ikhsan et al., 2016</td>
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<td>2.5</td>
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<td>Mn-Fe\textsubscript{3}O\textsubscript{4}/3D-G</td>
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<td>0.646</td>
<td>–</td>
<td>–</td>
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<td>AC/GCE</td>
<td>DPV</td>
<td>1–500</td>
<td>–</td>
<td>0.16</td>
<td>Madhu et al., 2014</td>
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<tr>
<td>P(Arg)/eG/SPE</td>
<td>SWV</td>
<td>0.5–1250</td>
<td>0.661</td>
<td>0.012</td>
<td>This work</td>
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<td>Amperometry (Ox)</td>
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<td>1.295</td>
<td>0.0024</td>
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<tr>
<td></td>
<td>Amperometry (Red)</td>
<td>5–200</td>
<td>0.793</td>
<td>0.0039</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1
Electrochemical biosensors for detection of \( p \)-nitrophenol.

Scheme 1. Schematic presentation of the fabrication of the electrodes.
electro-catalytic activity than the oxide and GO alone (Manjula and Chen, 2021). Moreover, modifying graphene morphology by preparing graphene sponges and graphene-carbon hybrids can improve electro-catalytic properties (Asadian et al., 2017; Mohamed et al., 2018; Zhu et al., 2018). Su et al. (2021) fabricated a 3D graphene framework by using urushiol as an inter-layer spacer, and the pores within the framework were adapted to accommodate metal oxide catalyst. Sensor prepared thereof presented a limit of detection (LOD) to p-nitrophenol as low as 19 nM. In one study (Jaiswal and Tiwari, 2021), graphene framework was prepared by zinc ion chelating with a GO suspension. The coordination product formed 3D multi-layered structure to fully expose electro-catalytically active sites. The framework was highly sensitive to p-nitrophenol with LOD about 96 nM. Moreover, it can be used for distinguishing p-nitrophenol from o-nitrophenol. But those systems are costly and poorly stable in real conditions. In fact, we discovered that graphene can be easily synthesized in situ from any carbonaceous precursor by simple electrochemical methods (Li et al., 2019).

In the present work, we present a novel electrochemical sensor based on electrochemically in situ synthesized graphene interface anchored with poly-arginine (Sch. 1). This is the first p-nitrophenol sensor based on fully electrochemical synthesized nano-interface. The sensor shows high sensitivity for p-nitrophenol, low detection limit as well as wide dynamic range. Ideal repeatability, stability, anti-interference ability and real-sample applications are also observed. This sensor is suitable for multiple in-the-field scenario uses with satisfactory performances, cost-effectiveness, scalability and user-friendliness. Finally, we hypothesized that poly-arginine grafting improved p-nitrophenol detection by optimizing graphene surface morphology and favoring H-bond and ionic interactions between arginine and nitrophenols.

2. Experimental section

2.1. Chemicals and reagents

Monopotassium phosphate (KH₂PO₄), potassium hydroxide (KOH), hydrochloric acid (HCl, 37%), potassium ferricyanide (K₃[Fe(CN)₆]), 99.5%, potassium ferricyanide trihydrate (K₃[Fe(CN)₆]·H₂O, 99.0%), phosphoric acid (H₃PO₄, 99.0%), graphite powder (C, 800 mesh, 99.95%), sodium chloride (NaCl, 98.8%), potassium chloride (KCl, 99.9%), calcium chloride (CaCl₂, 99.9%), potassium nitrate (KNO₃, 99.0%), sodium sulfate (Na₂SO₄, 99.0%), p-nitrophenol, o-nitrophenol (oNP, 98.0%), m-nitrophenol (mNP, 99.0%), p-aminophenol (pAP, 98.0%), catechol (99.0%), p-nitrobenzene (pNB, 99.0%), phenol (99.0%), hydroquinone (HQ, 99.5%), p-hydroxybenzaldehyde (pHBA, 98.0%), 2,3-dimethylphenol (DMP, 98.0%), bisphenol A (BPA, 99.0%) and sodium dodecylsulfonate (SDS, 98.0%) were purchased from Aladdin Biochemical Technology Co., Ltd., Shanghai, used at achievement without further purification. Other reagents are of AR grade. Ultrapure water (resistivity: ≥ 18 MΩ cm⁻¹) was used for all experiments.

2.2. Fabrication of screen printed electrode

The procedure to prepare the screen printed electrode (denoted as SPE hereafter) is presented in Scheme 1. The electrode comprises a working electrode, a counter electrode and a pseudo-reference electrode. The patterns distribute into 4 layers, i.e., the polyethylene terephthalate (PET) substrate, the silver conductive/reference layer, the carbon working/counter layer and the insulative layer. All the patterns are transferred to nylon films (200 mesh) for preparing stencils by the local supplier. Silver ink (NT-ST608, NanoTop Electronic Technology Ltd., PRC) was evenly spread onto the stencil and dragged the squeeze across the patterned area, leaving the ink transfer to the PET substrate. Curing the ink under 90 °C for 90 min to obtain the conductive areas as well as the pseudo-reference electrode. Carbon ink (C2030519P4, Gwent Electronic Materials Ltd., UK) and insulative coating (CR-18T-C, Asahi Chemical Research Laboratory CO., LTD., Japan) were sequentially applied with the corresponding stencils and identical procedure to that of the silver ink, to form the working electrode, counter electrode and the insulative areas, respectively. Cut the whole printed board to obtain single SPE electrodes with each has a 2.0 × 2.0 mm working electrode and counter electrode, and a 2.0 × 1.0 mm reference electrode.

2.3. Preparation of the electrodes cGO/SPE, eG/SPE and P(Arg)/eG/SPE

Preparation of chemically synthesized graphene oxide and the screen printed electrode modified thereof was modified from the literature (Marcano et al., 2010), and detailed in Supplementary Note 1, (denoted the electrode as cGO/SPE).

To prepare the electrochemically in situ synthesized graphene modified screen printed electrode (eG/SPE), bare screen printed electrode was immersed into 0.2 M H₃PO₄ solution contains 0.5 v/v% SDS, 1.8 V direct current potential was imposed to the working electrode vs. an external Ag/AgCl reference electrode for 200 s. Then the electrode was fully washed with water and processed by cyclic voltammetry scans (–2.0 to 0 V, 100 mV/s for 10 consecutive cycles) in 0.1 M KNO₃ solution. After rinsing with water, the electrode was designated eG/SPE. The electrochemically in situ synthesized graphene-poly(arginine) modified screen printed electrode (P(Arg)/eG/SPE) was fabricated by immersing eG/SPE into 0.1 M phosphate buffer solution (pH 7.0) contains 20 mM L-arginine, perform a 10 cycles cyclic voltammetry scans under 50 mV s⁻¹ between the potential range of –2.0–2.0 V.

2.4. Characterisations

Scanning electronic microscope (SEM, Hitachi, X-650), Raman spectroscopy (JY Labram HR 800, 632.8 nm laser), X-ray photoelectron spectroscopy (XPS, ESCALAB 250 using Al Kα radiation source), and Fourier transform infrared spectroscopy (FTIR, Nicolet 6700 with a DTGS detector and Omnic 8.0 software, Thermo Fisher Scientific, USA) were used to characterize surface state of the electrodes include morphology, chemical composition, and their changes. Unless stated otherwise, voltammetric, amperometric, and electrochemical impedance spectroscopy (EIS) experiments were performed with the build-in three-electrode system of the SPE. All electrochemical experiments were carried out with the electrochemical workstation (CH 760E, CH Instruments Inc., USA) equipped with a 5-mL homemade reactor under ambient condition.

2.5. Molecular simulation

The calculation is based on a grid-based molecular docking method that employs CHARMM. Random ligand conformations are generated from the initial ligand structure through high temperature molecular dynamics, followed by random rotations. The Calculate Energy (DFT) protocol was used to calculate the energy or optimize the geometry of a set of small molecules using the density functional quantum mechanics method. Finally, the theoretical computational modeling were presented.

3. Results and discussion


We analyzed electrodes including the bare screen printed electrode (SPE), the electrochemically in situ synthesized graphene modified screen printed electrode (eG/SPE) and the electrochemically in situ synthesized graphene-poly(arginine) modified screen printed electrode (P(Arg)/eG/SPE). Cyclic voltammetry was applied to perform the in situ electrochemical polymerization of arginine in 0.1 M phosphate buffer solution (pH = 7.0) in presence of 2.0 mM L-arginine. (–2.0 to 2.0 V,
100 mV s\(^{-1}\)). Ten consecutive cycles of the electro-deposition process of P(Arg) onto newly prepared eG/SPE was shown in Fig. S1. Results show that the electrolytic current became weak cycle-wise with slower rates, approximating to a finally stable state. This indicates the accumulation of poly-arginine onto the eG/SPE surface with weak or none conductivity of acquired poly-arginine, according to previous studies (Behbahani et al., 2012; Ali et al., 2020).

The morphology of the three electrodes was analyzed by SEM (Fig. 1). The scattered graphite particles are clearly visible and show the surface state of the electrode (Fig. 1a). After the electrochemical processing, graphite particles are partially dissociated with some flat, edged plates (Fig. 1b). This morphology, generally observed in graphite contained electrodes under acoustic anodization conditions (Palanisamy et al., 2014; Niu et al., 2017; González-Sánchez et al., 2018), facilitates the in situ synthesis of a graphene oxide layer (Li et al., 2019).

FTIR results show that arginine has distinctive peaks at 1329 cm\(^{-1}\) and 1447 cm\(^{-1}\), corresponding to symmetric stretching of free carboxyls, whereas these peaks decrease in poly-arginine (Fig. 2a). Similarly, the peak of C—N and N—H stretching at 3348 cm\(^{-1}\) corresponding to free amino groups decreases in poly-arginine. The formation of amide bonds in polyarginine is supported by new peaks of C—N and N—H stretching at 1524 cm\(^{-1}\), and carbonyl stretching at 1644 cm\(^{-1}\). Overall, these data confirm arginine polymerization on the electrode surface (Zhang et al., 2019).

EDS mapping results show the differences of N and O contents in the raw electrode, graphene oxide electrode and polyarginine-grafted graphene oxide electrode (Fig. 1j). The oxygen increase supports the formation of graphene oxides during anodization. Then, the increase of nitrogen agrees with the formation and attachment of polyarginine on the surface (Fig. 2).

Fig. 1. Characterization of SPE, eG/SPE and P(Arg)/eG/SPE. Scanning electron microscopy of SPE (a), eG/SPE (b) and P(Arg)/eG/SPE (c). Energy dispersive spectroscopy of N (d, e, f) and O (g, h, i), and the corresponding column graph showing the atomic percentages (j) and weight percentages (k) on SPE (black), eG/SPE (blue) and P(Arg)/eG/SPE (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Raman spectra show two main peaks (Fig. 2b), D peak at 1323 cm$^{-1}$ and the G peak at 1580 cm$^{-1}$ representing in-plane vibrations of sp2 carbons and the intrinsic structural defects on graphene lattice, respectively (Ambrosi et al., 2014). The increase of the D/G ratio from 0.955 in the raw electrode to 1.062 in the anodized electrode is explained by the rising number of carbon defects. The 2D band at ~2700 cm$^{-1}$ and D$\text{G}^+$ band at ~2910 cm$^{-1}$ are suggesting the formation of a mono-layered graphenic structure, as observed previously (Li et al., 2019). The decrease of the D/G ratio from 1.062 in the anodized electrode to 0.998 in the poly-arginine grafted anodized electrode is explained by the growth of the poly-arginine layer (McColl et al., 2003).

XPS was applied to further identify the atomic changes of the electrodes. As is shown in Fig S2, C1s deconvolution spectrum of SPE shows peaks at 284.67 eV, 285.2 eV, 286.5 eV and 288.8 eV, attributed to sp2 carbons and the intrinsic structural defects on graphene lattice, respectively (Ambrosi et al., 2014).
carbons, sp3 carbons, C-O and C-O/COO group, respectively. The data show the composition of graphite and the polymer binder contained in the carbon ink (Fig S2a). For eG/SPE, the ratio of sp2 to sp3 increased, indicating the in situ production of graphene on the electrode surface (Fig S2b). For P(Arg)/eG/SPE, a new peak at 285.9 eV attributed to C–N linkage is observed, indicating the formation of P(Arg) (Fig S2c) (Ayiania et al., 2020). This peak was also found in the monomeric arginine. By comparison, the C-NH2 peak at 288.0 eV largely depleted in polyarginine, as previous report (Zhang et al., 2014). Overall, these data confirm electrochemical polymerization of arginine on the electrode.

To further understand the properties of these electrodes, electrochemical behaviors were first studied by cyclic voltammetry method. As shown in Fig. 3a, a characteristic reversible peak pair can be observed, showing their electro-catalytic activity towards ferro/ferricyanide ions. But manifestly, SPE (black) exhibited the worst electrochemical performance with lowest peak currents (Ipa = 43.0 μA), and a large peak potential difference (ΔEp) value, indicating a slow electrochemical kinetics. In comparison, eG/SPE markedly enhanced the performance. The Ipa enlarged to 329 μA and the ΔEp value reduced to ~135 mV. For P(Arg)/eG/SPE, Ipa further increased to 592.8 μA and the ΔEp value decreased to ~85 mV. These data directly displayed the highly electro-catalytic activity of P(Arg)/eG/SPE to the surface dependent electro-activity species. Further, Fig. 3b and c provided the results with P(Arg)/eG/SPE to detect ferro/ferricyanide ions at different scan rates. Within the range of 10–500 mV s⁻¹, good linear correlation was obtained between the peak currents and their corresponding square root of scan rates (Ipc = 7.1312c + 10.425, R² = 0.99; Ipa = -5.8902c - 13.391, R² = 0.99). This indicated the electrochemical reaction of the surface dependent species followed a diffusion controlled kinetics (Li et al., 2018), which is advantageous to sensor applications for the target of the same category.

For all three electrodes in the high frequency range analyzed by electrochemical impedance spectroscopy (Fig. 3d), a semi-circular region always appeared and became the predominate variable, indicating that the electron transfer resistance, Rct, is the main factor changed on
the electrodes. By further modeling and calculation, the equivalent circuits were acquired (Fig. 3d, inset). $R_{ct}$ drastically declined from 2136 Ω on SPE to 258.6 Ω on eG/SPE, showing the high conductance of the in situ synthesized graphene layer. The $R_{ct}$ value of P(Arg)/eG/SPE, however, increased weakly back to 343.5 Ω, revealing the weak conductivity of poly-arginine. This is consistent with the cyclic voltammetry result aforementioned. Actually, the in situ synthesized graphene layer predominantly contributed to the electro-catalytic activity and the surface conductivity. P(Arg) mainly enhanced the electrode interface to exert higher electro-catalytic activity (Fig. 4).

3.2. Electrochemical performances for p-nitrophenol detection

We compared the electro-catalytic performance of bare screen printed electrode (SPE), the electrochemically in situ synthesized graphene modified screen printed electrode (eG/SPE) and the electrochemically in situ synthesized graphene-poly(arginine) modified screen printed electrode (P(Arg)/eG/SPE), along with the counterpart, chemically synthesized graphene oxide modified screen printed electrode (cGO/SPE) in the presence of nitrophenol (Fig. 4). Peak I at – 1200 mV is the irreversible reduction of nitrophenol. The reversible peak II at – 200 mV and peak III at – 170 mV represent the reduction of p-hydroxyaminophenol and p-nitrosophenol (Renu et al., 2021). Peak IV at 750 mV is attributed to the oxidation of the phenolic moiety of nitrophenol. Results show that the polyarginine-grafted electrode displays the best performance with the highest currents, versus the oxidized electrode and the classical graphene oxide electrode, cGO/SPE. The raw electrode showed the weakest performance. The best potential for nitrophenol monitoring is at peak I.

3.3. Effect of electro-deposition of poly-arginine, pH and scan rate on the electro-catalytic performance

We studied the effect of the electrochemically active surface area, pH and scan rate on the performance of the polyarginine-grafted graphene electrode. Areas were changed by varying cyclovoltametric deposition cycles. We observed little variation of current intensity from 10 to 30 cycles, whereas electrochemically active surface areas decreased above 10 cycles (Fig. 5a). Similar phenomena were observed in the previous reports (Zhang et al., 2012; Li et al., 2021). So 10 cycles were adopted for further experiments. The electrochemical behaviour of the electrode in response to nitrophenol at different scan rates is shown in Fig. 5b and linear correlations are observed at peak I, II, III and IV (Fig. 5c). Findings show that the nitrophenol transformation is controlled by diffusion in the 10–200 mV s$^{-1}$ range. The study of the effect of pH shows that the polyarginine electrode reached optimum current at pH 6 and pH is well

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**Fig. 6.** Square wave voltammetry responses of P(Arg)/eG/SPE to diverse concentrations of p-nitrophenol (a). Corresponding relation between peak current and concentration (b). Selectivity of P(Arg)/eG/SPE over the foremost possible interfering compounds (c).
correlated with potential according to $E_p = 52.229 \text{pH} + 545.65$ with $r^2$ of 0.99 (Fig. 5d). This finding indicates that the electro-reduction of nitrophenol is a proton-coupled process with equimolar transfer number of $\text{H}^+$ and $\text{e}^-$ in a wide acidity range (Zhu et al., 2020).

3.4. Voltammetric performances for sensitive $p$-nitrophenol detection

Square wave voltammetry was applied to examine P(Arg)/eG/SPE. Testing parameters are shown in Supplementary Note 3. Fig. 6a showed that P(Arg)/eG/SPE presented clear responses to $p$-nitrophenol concentration within range of 0–1250 μM. A good linear relationship was obtained between the $I_{pc}$ values and the concentration of $p$-nitrophenol with the range of 0.5–1250 μM (Fig. 6b). The regressive equation was $I_{pc} = 0.0342 C (\mu\text{M}) + 115.1$ ($R^2 = 0.99$). The sensitivity of the sensor was calculated to be 0.66 μA μM$^{-1}$ cm$^{-2}$ by the Eq. (1) (Chakraborty et al., 2021),

$$\text{Sensitivity} = \frac{k}{A}$$  \hspace{1cm} (1)

where, $k$ designates the slope of the above regressive equation, $A$ is the electrochemically active surface area calculated in Supplementary Note 4, with the calculated $A = 0.0514 \text{cm}^2$.

The limit of detection (LOD) was determined as 12 nM, calculated following the criterion of $S/N = 3$ (Su et al., 2021), using Eq. (2),

$$\text{LOD} = \frac{3N}{k}$$  \hspace{1cm} (2)

$N$ = standard deviation value of the response; $k$ = calibration curve slop.

Comparing with the previous results in Table 1, it can be seen the P(Arg)/eG/SPE fabricated in this research has excellent quantification performances as a $p$-nitrophenol electrochemical sensor.

Reproducibility of this sensor was evaluated by testing the square wave voltammetry responses with 5 P(Arg)/eG/SPE sheets prepared independently in 0.1 M phosphate buffer solution (pH = 7) containing 1.0 μM $p$-nitrophenol. The relative standard deviation (RSD) was found to be 5.2%. RDS was 1.2% for the repeatability analysis, which was tested by performing 5 independent measures in the same solution with each in triplet (Supplementary Fig S3a). For stability investigation, 3 newly prepared P(Arg)/eG/SPE sheets were stored under ambient condition and tested in the same solution every 24 h for 7 days. Over 90% signal retention rate was gotten within the 7-day experiment (Fig S3b and c).

In the real world, some possible interferents may cause detection distortion to the sensor (Su et al., 2021; Renu et al., 2021). We therefore chose some most likely interferent to investigate the sensor’s selectivity.

To investigate the selectivity of the sensor, we used 3 groups of possible interferents to test the sensor’s response in the presence of $p$-nitrophenol. The results are shown in Table 2.

Table 2
Recoveries of $p$-nitrophenol in real-world samples ($n = 3$).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Standard addition (μM)</th>
<th>Fund (μM)</th>
<th>Recovery</th>
<th>SD</th>
</tr>
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<tr>
<td>Industrial wastewater</td>
<td>5</td>
<td>5.02</td>
<td>100.4</td>
<td>1.7</td>
</tr>
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<td>Lab. wastewater</td>
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Fig. 7. Amperometric responses of P(Arg)/eG/SPE at −800 mV (a) and 800 mV (c) in presence of $p$-nitrophenol with different concentrations. The corresponding regressive relation between current and $p$-nitrophenol concentration sampled at 3rd second.
interferent to perform the anti-interference test (Fig. 6c). In the first group, common co-existing ions include Na\(^+\), K\(^+\), Ca\(^{2+}\), Cl\(^-\), NO\(_3^-\), SO\(_4^{2-}\) with almost no influence to the sensing signal of p-nitrophenol. In the 2nd group, homologs and isomers of p-nitrophenol are studied with signal recovery ratios ranging from 91.79% to 106.66%. The 3rd was group composed of phenols which are also homologous to p-nitrophenol. Slight signal deviation from 101.32% to 107.67% is detected. For all experiments, the signal recovery ratios are within range of 91.79–107.67%, with the most cases less than 5.0%. Among these, isomer o-nitophenol and phenol caused the most prominent interference due to high homology of the structure to p-nitrophenol, yet their influence is acceptable to real applications.

Overall, results show good selectivity and anti-interference of the sensor. Moreover, real-world samples were used to perform the standard addition tests. As the results shown in Table 2, in all cases the error produced in the complex samples were less than 3%, which further demonstrated a worthy applicability. Collectively, P(Arg)/eG/SPE is an ideal sensor for portable p-nitrophenol detection with high sensitivity, wide quantification range and good reliability.

3.5. Amperometry for long-term p-nitrophenol monitoring

Many cases require long-term, consecutive quantifications of p-nitrophenol level other than the need for one-off detection. Considering this, the ability of the sensor to continual p-nitrophenol monitoring was investigated. The i-t curves recorded in 0.1 M phosphate buffer solution (pH = 7.0) in presence of p-nitrophenol with various concentrations (Fig. 7). Data in Fig. 7a and c were obtained using peak I and peak IV with the working potential of −800 mV and 800 mV, respectively. Regressive curves were obtained by sampling the current values at the 3rd second of each curve (Fig. 7b and d). The regressive equations gotten as follow: Ipc (μA) = 0.067 C (μM) + 8.7405 (R\(^2\) = 0.99) and Ipa (μA) = −0.041 C (μM) − 5.0597 (R\(^2\) = 0.99). The corresponding linear concentration ranges were 5–1250 μM and 5–200 μM. Limit of detections were 2.4 and 3.9 nM, and sensitivities were 1.30 and 0.79 μA μM\(^{-1}\) cm\(^{-2}\). It is noticeable that for measuring higher concentration gradient p-nitrophenol samples under 800 mV, the catalytic current Ipa no longer increased or even weakened (data not shown). Electro-oxidation of p-nitrophenol may lead to in situ self-polymerization, along with the irreversible adsorption of the oxidation product (Chen et al., 2008; Karabiberoglu et al., 2019). The great surface fouling could largely passivate the sensor. Therefore, the reduction mode seemed to be preferable for the long-term monitoring.

A long-term consecutive monitoring of varied concentration of p-nitrophenol in phosphate buffer solution base is shown in Fig. 8. As the concentration of p-nitrophenol changed, the reductive current intensity presented a swift response. Even when p-nitrophenol concentration dropped to lower level, the sensor responded quickly with a in-step signal, indicating that this electrode was fouling-free and adaptive for consecutive monitoring.

3.6. Molecular modeling

Theoretical computational modeling was performed to study the interactions of p-nitrophenol with the P(Arg)/eG/SPE. According to the potential binding energy (ΔE\(_{AB}\)) predictions, two models were considered. The first one illustrated the arrangement of a p-nitrophenol molecule freely onto a graphene sheet. It was modeled with an in-plane defect which is common in electro-catalytic analyses (Liu et al., 2018; Wang et al., 2019a, 2019b). As shown in Fig. 9a, p-nitrophenol nearly parallelly attached to the graphene plane with the closest inter-molecular distance of 3.51 Å (at the phenol moiety of p-nitrophenol), which is mainly induced by strongly non-specific adsorption. This is the reason why only limited performance gain harvested and the electrode fouling is severe on bare graphene interfaces.

In comparison, the existence of poly-arginine makes p-nitrophenol molecule re-orientate on graphene interface trough comprehensive effect from H-bonding, ionic attraction and hydrophobic force (Fig. 9b).
revealed by density function theory calculation result. More importantly, poly-arginine benefit to drawing -NO₂ moiety of p-nitrophenol towards the defect - the electrocatalytically active site on graphene plane. Consequently, P(Arg) assist preferable orientation of p-nitrophenol onto the sensing interface and present a synergy with in situ synthesized graphene to endow P(Arg)/eG/SPE with fast electrochemical kinetics. This verifies the vital importance of delicate molecular design of sensing interface to create optimized chemical microenvironment.

4. Conclusion

A fully electrochemically synthesized graphene screen printed electrode interface with poly-arginine anchored was successfully designed and applied for p-nitrophenol sensing. Using voltammetric technology, P (Arg)/eG/SPE fulfilled high sensitive p-nitrophenol detection with limit of detection as low as 12 nM. Moreover, a wide range quantification of 0.5–1250 μM was fitted. By adapting amperometric method, highly sensitive and long-term consecutive p-nitrophenol level monitoring can be realized, with the limit of detection of 2.4 nM and linear concentration range of 5–1250 μM. Specially, it is revealed by density function theory calculation that poly-arginine facilitates p-nitrophenol molecules to preferably orientate onto the graphene interface with fast electrochemical kinetics. Considering the very low cost of screen printed electrode fabrication, this sensor is an ideal prototype for the next generation versatile p-nitrophenol electrochemical sensing devices.

CRediT authorship contribution statement

Y.L. and L.X. designed the research. Y.L. J.Z. and S.W. conceptualized and performed the experiments. Y. L. and Y. M. contributed reagents, materials and analysis tools. Y. L., Y. M., J.S., L.X. and E.L. analyzed the data. R. G. performed the molecular simulation calculation. Y. L., J.S., L.X. and E.L. wrote and revised the manuscript. All the authors discussed the results and commented on the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126718.

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Supplementary Material for

In situ electrochemical synthesis of Graphene-poly(arginine) composite for p-nitrophenol monitoring

Note 1: Preparation of chemically prepared graphene oxide (GO)-modified SPE (G/SPE)
Added 1 g of graphite flakes to a 1 L flask under magnetic stirring, 0.5 L concentrated H$_2$SO$_4$ / H$_3$PO$_4$ mixture with a volume ratio of 9:1 was then perfused. 6 g of KMnO$_4$ was added to the mixture after stirring for 15 min, keeping the temperature below 50 °C overnight. Then cooled the mixture to room temperature and poured into ice cooled 200 mL of deionized water containing 5 mL of 30% H$_2$O$_2$. The final mixture was centrifuged at 4,000 rpm for 1 h, and the supernatant was decanted. Washed the precipitate fraction several times by repeated centrifugation to remove excess acid and oxidant. The solid phase was then washed successively with 200 mL of water, 200 mL of 30% HCl, and 200 mL of ethanol by centrifugation at 4,000 rpm for 1 h. Finally, the precipitate was washed 3 times by centrifugation with 200 mL of deionized water to obtain pure graphite oxide. The purified GO was mixed with deionized water and sonicated for 4 h. Then diluted it to obtain 0.1 mg mL$^{-1}$ GO suspension. G/SPE was fabricated by casting 0.5 µL of the 0.1 mg mL$^{-1}$ GO suspension onto SPE working area, applying -1.5 V constant potential for 200 s in 0.1 M KCl solution, and washed with deionized water for use.

Note 2: Electrochemical impedance spectroscopy (EIS) experiments
EIS analyses were performed with the three-electrode system. The studied electrode was used as the working electrode, the counter electrode was a Pt disc electrode ($\phi$=2.0 mm) and an Ag/AgCl electrode as the reference. All the analyses were conducted in 0.1 M KCl solution containing 10 mM K$_3$[Fe(CN)$_6$] and K$_4$[Fe(CN)$_6$]. In the frequency range of $5 \times 10^{-3}$ - $1 \times 10^{5}$ Hz, a sinusoidal potential modulation of ±10 mV amplitude was superimposed on the formal potential measured in the ferro-/ferric-cyanide redox couple (0.24 V vs Ag/AgCl).

Note 3: Square-wave voltammetric (SWV) experiments
SWV measurements were performed within require potential ranges with the potential increment pulse of 4 mV·s$^{-1}$, amplitude of 25 mV and frequency of 15 Hz. The electrode system keeps being the build-in three electrode of the SPE electrode.

Note 4: Electrochemically active surface areas (EASA) calculation
The EASA of electrodes are calculated by using the Randles-Sevcik equation (Hrapovic, et al., 2004) using CV measurements in 0.1 M KCl contains 2 mM K$_3$[Fe(CN)$_6$] at the rate of 100 mV·s$^{-1}$,

$$I_p = 2.69\times10^{5}AD^{1/2}n^{3/2}\gamma^{1/2}C$$

where $A$ is the area of the electrode (cm$^2$), $D$ is the diffusion coefficient of Fe(CN)$_6^{3-}$ in solution (6.70 $\times$ 10$^{-6}$ cm$^2$·s$^{-1}$), $n$ designates the number of electrons participating in the redox reaction, $\gamma$ is the scan rate of the potential perturbation (V·s$^{-1}$), and $C$ is the concentration of the probe molecule in the bulk solution (mol·cm$^{-3}$).
**Fig. S1.** Cyclic voltammogram for electro-deposition of Arg on eG/SPE.

**Fig. S2** XPS C1s spectra of SPE (a), eG/SPE (b), P(Arg)/eG/SPE (c) and arginine (d).
Fig. S3 SWV graphs recorded from one single (a) and 5 different (b) P(Arg)/eG/SPEs in 0.1 M PBS (pH = 7.0) contains 0.5 mM pNP, and current changes with the corresponding current signal retention rate of P(Arg)/eG/SPEs made by different cycles of CV for P(Arg) deposition.

Supplementary reference