Application of nitrogen-doped reduced graphene oxide-persimmon tannin nanocomposites for electrochemical detection of Cd (II) in water resources

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Research Article

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Abstract

A ultrasensitive electrochemical biosensor was successfully constructed for the detection of Cd (II) based on nitrogen-doped reduced graphene oxide-persimmon tannin (PT-N-RGO) and gold nanoparticles (Au NPs) modified screen printing electrode (SPE). The structure and morphology of the prepared PT-N-RGO nanocomposite were characterized by ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (TEM). And each step in the sensor preparation was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). Au NPs as a material of modified electrode not only increased the effective surface area of the electrode, but also promoted the electron transfer during the redox reaction. The high specific surface area of PT-N-RGO composites further increased the electron transfer rate, thus improving the conductivity of the electrode and providing more binding sites for the subsequent capture of Cd (II). Under the optimized experimental conditions, the sensor exhibited good linearity in the range of 3.0~30 µg/L Cd (II) with a minimum detection limit of 0.46 µg/L. The sensor showed good selectivity, stability and reproducibility for the determination of Cd (II). The sensor was applied to the analysis of real water samples and the results were verified by inductively coupled plasma mass spectrometry (ICP-MS), and satisfactory results were obtained, providing a new idea for the effective determination of trace heavy metals in the environment.

1. Introduction

Heavy metals have been broadly used in lots of domains as the speed growth of industrial and agricultural horticulture, for instances: metallurgy, electroplating, alloy, cells, and phosphate fertilizer in agriculture (Judy et al. 2021). Cd (II) was one of the most problematic heavy metals, which was ranked seventh among the 275 most dangerous substances (Agency for Toxic Substances and Disease Registry of the USA) (Maity et al. 2019; Piyanuch et al. 2021). Cd (II) enters the human body through the digestive or respiratory tract and then binds to proteins containing hydroxyl, amino, sulfhydryl, and sulfhydryl groups, thereby inhibiting various enzyme systems and causing various diseases of the immune and nervous systems (Cao et al. 2021; Godt et al. 2019; Reyes et al. 2019; Ahmadijokani et al. 2021), including cardiovascular disease, Alzheimer's disease, renal insufficiency, leukemia, disorders of calcium metabolism, Parkinson's disease, and cancer (Jiang et al. 2020; Chen et al. 2019), long-term exposure to cadmium can cause heart, lung, bone, and kidneys causing irreversible damage and the threat to human health is enormous (Ding et al. 2021; Farhad et al. 2021). Cadmium pollutes the environment by absorbing rice and other plants, which easily absorb it from water and the environment. Cd (II) pollution in plants can adversely affect plant development and growth, resulting in a decline in crop yield and quality. Over accumulation of Cd (II) restrains the plant growth and nutrient absorption by interfering with the homeostasis of zinc, iron, and calcium and binding to sulfhydryl-containing amino acids in plants. In view of the universality and toxicity of cadmium, the US Environmental Protection Agency (EPA) sets a maximum permissible concentration of Cd (II) in drinking water at 5 µg/L (Chen et al. 2020).
Conventional ways of Cd (II) detection concentrate upon colorimetry (Ding et al. 2019), atomic absorption/fluorescence spectrometry (Brij et al. 2019; Rahmat et al. 2020), graphite furnace atomic absorption spectrometry (Luciane et al. 2019), fluorescence spectroscopy (Yulieth et al. 2022) and inductively coupled plasma mass spectrometry (ICP-MS) (Rahmat et al. 2020; Jaromir et al. 2019). Due to the simplicity of real sample handling and proximity to the field, these traditional Cd (II) testing methods have high sensitivity and specificity as well as a wide detection range, but they also have drawbacks such as being expensive, cumbersome, time-consuming, bulky and non-portable (Wang et al. 2020; Xu et al. 2020; Masoumeh et al. 2022; Mohammad et al. 2021). By contrast, electrochemical technologies have the advantages of fast analytical output, low cost, lightweight and reliability and sensors built so them have proven to be effective for rapid detection of Cd (II), without the need to operate complex and cumbersome preprocessing processes (Zohreh et al. 2020; Smart et al. 2020; Maizatul et al. 2020; Wu et al. 2019; Hassan et al. 2022; Chen et al. 2019; Wang et al. 2019). Hassan et al. developed an electrochemical sensor based on bimetallic mercury-Bi composite for single and simultaneous voltammetric sensing of Pb (II), Cd (II) and Zn (II) (Khalid et al. 2020).

Lately, Screen printed electrodes (SPEs) are easy to use, portability, inexpensive, mass production and easily miniaturized compared to traditional bulky batteries. SPEs have been widely used in the devise of single use biosensors (Smart et al. 2020). Besides, SPEs is an significant field in electroanalysis for need small sample bulks and reagents. Nowadays, SPEs can manufacture three-electrode systems on a low-cost flexible substrate, for instance: chancery paper, ceramics and plastic membrane, so it has attracted a lot of people's interest. Moreover, by the modification of the nanomaterials, the applicability of these SPEs can then be heightened.

Nanomaterials are especially well fitted to immobilizing sensitive aptamer molecules, sensing and amplifying signals on account of big specific surface area, powerful surface reactivity, high electron transfer capacity and ideal adsorption ability (Akbar et al. 2020). Among them, reduced graphene oxide (RGO) has the advantages of good electrical conductivity, biocompatibility, large specific surface area, easy to functionalization and recombination (Wang et al. 2019), so it is broadly used in the field of electrochemistry to build a working electrode sensing interface. RGO is rich in oxygen-containing functional groups, which will further raise the electron transfer rate and gathering of Cd (II) on the electrode surface. As can be seen from formerly researches, the bring nitrogen atoms into reduced graphene oxide (N-RGO) can effectively raise its electronic properties and conductivity, thereby remarkably enhancing its functionality (Wei et al. 2019; Dai et al. 2018). Wen et al. prepared N-RGO-MnO₂ nanomaterials to detect Hg (II), with a minimum detection limit of 0.0414 nM (Wen et al. 2018). Hence, by adding nitrogen atoms to graphene, it promises to bring about better electrochemical properties. Li et al. (Li et al. 2018) reported a novel electrochemical sensor based on N-doped carbon quantum dot-GO (NCQDs-GO) and simultaneous determination of Cd (II) and lead Pb (II) by anodic stripping voltammetry. The conclusions indicate that limits of detection of Cd (II) and Pb (II) were 7.45 μg/L and 1.17 μg/L, independently.
Persimmon tannin (PT) is a substance extracted from green persimmons and is mainly composed of catechin (C), gallocatechin (GC), gallocatechin gallate (GCG) and catechin gallate (EG) (Qian et al. 2020). The ability of persimmon tannins to form stable chelates with many metal ions is due to the adjacent phenolic hydroxyl structure of the molecule which gives it a strong ability to bind metal ions (Wang et al. 2019). Meng et al. reported a persimmon tannin-immobilized gelatin/PVA nano-fiber band for extraction of U(VI) from simulated seawater, which was fabricated by electrostatic spinning and covalent cross-linking reaction (Meng et al. 2019). Complex polyphenol hydroxyl structures on persimmon tannins enable modified screen-printed electrodes to provide more binding sites for subsequent capture of Cd (II). Therefore, if the electrode can be modified with nanomaterials, an electrochemical biosensor with good biocompatibility, high specificity and sensitivity, and low cost detection of heavy metal Cd (II), which will provide new ideas for the quantitative detection of Cd (II) in the environment.

In this study, we developed a high-performance electrochemical biosensor for the detection of trace Cd (II) by using persimmon tannins modified nitrogen referenced reduced graphene oxide nanocomposite (PT-N-RGO). AuNP$_S$ was deposited on the SPE surface at a constant potential to accelerate the electron transfer during the redox reaction. PT-N-RGO is a conductive material with high specific surface area, which is dropped onto the Au NPs/SPE surface by electrostatic adsorption, thus providing more binding sites for subsequent capture of Cd (II) and improving the conductivity and affinity of the electrochemical sensor. PT-N-RGO specifically recognizes and combines with Cd (II) to form stable chelates that are deposited on the electrode surface and subsequently oxidizes Cd$^0$ to Cd (II) at a specific potential, resulting in a stripping peak current signal. Therefore, Cd (II) can be easily detected by measuring the peak current change of Cd (II) in Au NP$_S$/PT-N-RGO/SPE. Compared with the currently used methods for Cd (II) detection, the electrochemical biosensor prepared in this project is easier to realize the needs of miniaturization, portability and low cost of detection instruments. In addition, it allows us to detect very low concentrations of Cd (II) in water resources, such as tap water and lake water, with recoveries in the range of 91.95-108.55%.

2. Experimental section

2.1 Regents and instruments

Graphene oxide (GO) was purchased from Nano Pioneer Company Nanjing, China. HAuCl$_4$ were obtained from Xiqiao Technology Co., Ltd (Guangdong, China). CdCl$_2$ and were purchased from Tianjin Kermel Chemicals (China). Cd (II) standard solutions were purchased from Tong Rome Technology Co., Ltd (Beijing). Samples of dry persimmon tannin powder (the feed material extracted from astringent persimmon) were amicable donated by Huikun Company of Agricultural Products (Guangxi, China). All other chemicals and reagents used in these experiments were of analytical and used without further purification. All solutions were prepared with ultra-pure water (18.25MQ·cm) from a Millipore system.

All electrochemical experiments were performed on a CHI660E electrochemical workstation at room temperature (Shanghai Chen hua Instrument Co., Ltd., China) at room temperature (RT). All
electrochemical experiments are conducted based on a conventional screen-printed electrode electrochemical system (SPE, Nanjing Yun you Biotech Co., Ltd., China) in which a carbon paste electrode (ϕ=3 mm, the electrode area is 0.07 cm) is used as the working electrode, another carbon paste electrode is used as the counter electrode and an inert Ag/AgCl electrode is used as a reference electrode.

Scanning electron microscopy (SEM) was implemented with a Quanta 200 field environmental scanning electron microscope (FEI COMPANY USA). Transmission electron microscopy (TEM) was performed on a JEM-2100F electron microscope under an accelerating voltage of 100 kV (JEM-2100F, Japan). Fourier transform infrared spectra (FT-IR) were recorded in the range of 4000-400 cm\(^{-1}\) (FT-IR Bruker Tensor 27, Germany). Ultraviolet visible spectroscopy was performed in the wavelength range of 200-800 nm (UV-vis, UH5300, HITACHI, Japan). The X-ray diffraction (XRD) pattern and digital micro-graph image of adsorbents after adsorption were recorded by using Rigaku L-094 X-ray diffractometer and KEYENCE VHX-1000 micro-scope. XPS data were performed on a PHI Quantera II X-ray photoelectron spectrometer (Japan) with 300w Al Kα radiation.

2.2 Synthesis of PT-N-RGO

GO (10 mg) was dispersed in 10 mL water for 2h by ultrasound, then 0.3g urea was added to the above suspension. After stirring continuously for 30 min, the mixture was transferred to a Teflon-lined stainless steel autoclave and heated at 180°C for 12 h. The resulting N-RGO was centrifuged and washed three times with water and ethanol.

20mL ultra-pure water was added to 100mg of persimmon tannin material, and then it was magnetically stirred and dispersed on a magnetic stirrer for 8 hours to obtain persimmon tannin solution. 10mL of N-RGO solution was added with the same volume ratio of persimmon tannin solution, which was magnetically stirred on a magnetic stirrer for 4 hours at room temperature and washed with water 3 times to obtain PT-N-RGO solution. Seal with plastic wrap and store in the refrigerator until ready to use.

2.3 Fabrication of Cd (II) electrochemical biosensor

Firstly, the screen-printed electrode (SPE) was immersed in a 0.5 mol L\(^{-1}\) H\(_2\)SO\(_4\) solution and activated by the CV scanning method for 20 cycles at a scanning speed of 100 mV s\(^{-1}\) in voltage range of -0.2 V to 1.0 V. Secondly, the activated SPE was electrodeposited in 0.01% HAuCl\(_4\) solution at a potential of -0.5 V for 120 s to obtain Au NPs/SPE. Thirdly, 5.0 µL 1.0 mg mL\(^{-1}\) of PT-N-RGO suspension was added to Au NPs/SPE and incubated for 30 min to obtain Au NPs/PT-N-RGO/SPE. After the incubation was completed, the sensing interface was washed with PBS solution and dry the electrode surface. Finally, 6.0 µL of 0.5% BSA solution was added dropwise to the surface of the modified electrode to block nonspecific adsorption and rinsed with PBS. Thus, the Cd (II) electrochemical biosensor was fabricated and stored in a refrigerator at 4°C before use. After each step, ultra pure water was used to clean the electrodes and dried them.

2.4 Electrochemical detection of Cd (II) in standard solution
The electrochemical behavior was studied by differential pulse anodic solvation voltammetry (DPASV) for the detection of Cd (II). First, the prepared electrode (Au NP₅/PT-N-RGO/SPE) was placed in an acetic acid-sodium acetate buffer solution (pH=5) containing Cd (II) solution. And the deposited Cd (II) was enriched on the SPE surface for 180 s by applying a deposition potential of -1.3 V. The deposited Cd (II) was reduced to Cd⁰ at a negative potential under stirring conditions. After 10s of equilibrium, The reduced Cd⁰ could be oxidized to Cd (II) and dissolved during the negative potential scan, generating a peak stripping current signal. DPASV method measurements of Cd (II) stripping were performed in the potential range of -1.6V to 0.2V with increments of 4 mV, amplitude of 50 mV, sample width of 0.0167 s, and interval of 0.5 s. Finally, before the next cycle, Cd (II) was completely removed from the surface of the SPE by a cleaning step at 0.6 V for 60 s. All experiments were carried out in room temperature air.

2.5 Detection of Cd (II) in real samples

To investigate the practical value of Au NP₅/PT-N-RGO/SPE sensor, tap water and lake water were used as actual samples, and the recovery of Cd (II) in actual samples was detected by standard addition method. Two different types of water samples, including tap water and lake water, were collected from the local area of Guilin city (Guangxi Zhuang Autonomous Region, China). First, the collected tap water and lake water were filtered to avoid solid particles. Then, cellulose membrane with a pore size of 0.22µm and syringe were filtered three times, and the filtered solution was added with 0.1 M nitric acid solution to adjust its pH value to 5.0, and Cd (II) standard solutions of 5, 10, and 25µg/L were added to different actual water samples, respectively, and then diluted with deionized water to a final volume of 50 mL. Finally, different types of environmental water samples, such as tap water, lake water and standard solution with different concentrations of Cd (II), were quantified under the optimized experimental parameters. In addition, we also tested Cd (II) in the prepared water samples using inductively coupled plasma mass spectrometry (ICP-MS). The tests were repeated three times for each water sample and the average values were calculated.

3. Results and Discussion

3.1 Characterization of PT-N-RGO nanocomposites

The surface morphology of the PT-N-RGO composite material was characterised via SEM. Seen from Fig. 1a, the surface of N-RGO has a lot of pore structure and silk-like fold structure, which proves that N-RGO has been successfully prepared. After persimmon tannin doping in N-RGO, SEM images (Fig. 1b) showed different morphology from precursors, which became more compact.

The TEM technique was employed to observe the morphologies of the involved materials. Fig. 1c shows that there are many 5nm nanoparticles and porous structures distributed on the surface of N-RGO, and the wrinkled silk wavy morphology and highly defective structure of N-RGO can also be observed. The TEM image (Fig. 1d) shows that the surface of PT-N-RGO nanocomposites has a typical laminar flow structure with loose wrinkles. N-RGO became loose after reacting with persimmon tannin, most of its
pores were closed and many pore structures on the surface disappeared, showing different morphology indicating that PT-N-RGO nanocomposites was successfully prepared.

FT-IR spectroscopy is useful for characterizing functional groups of nanomaterials. Figure 1e depicts the FT-IR spectra of PT (curve a), N-RGO (curve b), and PT-N-RGO nanocomposites (curve c). In the spectrum of persimmon powder, the peaks at 3415 cm\(^{-1}\) and 3241 cm\(^{-1}\) represent phenolic O-H stretching vibration, the peaks at 2934 cm\(^{-1}\) represent C-H stretching ring of phenolic resin, and the peaks at 1625 cm\(^{-1}\) represent ketone C=O bending vibration. The peak at 1450 cm\(^{-1}\) represents C-C stretching vibration of the ring, the peak at 1224 cm\(^{-1}\) is C=C-O stretching vibration, the peak at 1068 cm\(^{-1}\) is C-O-C stretching vibration and bending vibration, and the peak at 614 cm\(^{-1}\) can be attributed to the deformation vibration of C-H bond in the phenol ring. In the N-RGO spectrum, 3441 cm\(^{-1}\) corresponds to the stretching vibration peak of O-H, 1633 cm\(^{-1}\) corresponds to the stretching vibration peak of C=C, 1437 cm\(^{-1}\) corresponds to the deformation vibration peak of O-H, 1106 cm\(^{-1}\) corresponds to the stretching vibration peak of C-N, 872 cm\(^{-1}\) corresponds to the N-H characteristic absorption peak. Therefore, it can be judged that N-RGO is successfully prepared. After cross linking, the O-H stretching vibration intensity decreased, indicating that the cross linking occurred through the condensation reaction of phenol hydroxyl group, forming C-O-C bond. Due to the stretching vibration of keto carbonyl C=O, the peak at 1625 cm\(^{-1}\) moves to 1607 cm\(^{-1}\), which indicates the \(\pi-\pi\) interaction between PT and N-RGO benzene ring. These results fully prove the successful synthesis of PT-N-RGO under the action of phenol condensation and \(\pi-\pi\) interaction.

The three substances of N-RGO, PT and PT-N-RGO were characterized by UV-visible spectrophotometer, as shown in Fig.1f. PT (Fig 1f. curve a) has a characteristic peak at 274 nm, N-RGO (Fig 1f. curve b) has a characteristic absorption peak at 266 nm and the characteristic peak of PT-N-RGO nanocomposites (Fig 1f. curve c) is a flat peak between 260 nm and 280 nm. This is because the characteristic peak of persimmon tannin is similar to that of nitrogen and impurity reduced GO. This shows that PT-N-RGO nanocomposites have been successfully prepared by combining various substances.

The main application of XPS is determination of the binding energy of electrons for qualitative analysis of surface elements. Simultaneously, according to the intensity or area of photo-electron spectra, the relative concentration of atoms also can be obtained. Figure. 2a is the total element map of PT-N-RGO. It is not difficult to find that the sample mainly contains three elements, C, N and O. As shown in Fig. 2b, The high resolution C1s peak is 284.8 eV centered and the tail has a high binding energy, indicating the presence of carbon atoms attached to N and O hetero atoms, there are two peaks located at 285 eV, 286.3 eV, which were assigned to C=C and C-O, respectively. In addition to the above two peaks, a new peak at 287.1 eV corresponds to C=O, which may be the result of reduction of O-C=O and C-O. In case of N1s, only one peak centered at 399.37 eV was corresponded to C=N groups. In Fig 2d, the high-resolution O1s spectrum of PT-N-RGO could be satisfactorily divided into three peaks, located at 531.18 eV (C=O), 532.31 eV (C=O) and 532.20 eV (0-C=O), respectively. These evidences indicated the successful preparation of the PT-N-RGO composites.
3.2 Analysis principle of Cd (II) electrochemical biosensor

A novel electrochemical biosensor for the detection of Cd (II) was constructed based on Au NPs/PT-N-RGO modified SPE and the schematic diagram is shown in Figure 3a. First, the naked SPE is activated with sulfuric acid, and Au NPs are deposited on the SPE surface at a constant potential. Then, the PT-N-RGO nanocomposites was added drop to the surface of Au NPs/SPE by electrostatic adsorption to construct a highly sensitive electrochemical biosensor (Au NPs/PT-N-RGO/SPE). Cd (II) solutions were prepared with an acetic acid-sodium acetate buffer of pH 5, stirred and deposited at a potential of -1.3 V for 180 s, followed by differential pulse voltammetry (DPASV) at a potential of -1.6 V to 0.2 V. During the DPASV measurement, firstly, Cd (II) in solution is deposited on the electrode surface, and the deposited Cd (II) is reduced to Cd$^0$ at negative potential, then the anodic stripping stage, where the reduced Cd$^0$ can be oxidized to Cd (II) and dissolved during the negative potential scan, generates the stripping peak current signal. The reaction mechanism of the redox reduction of Cd (II) on the surface of Au NPs/PT-N-RGO/SPE is as follows:

Accumulation:

$$(\text{Au NPs/PT-N-RGO/SPE}) + \text{Cd (II)} + 2e^- \rightarrow (\text{Cd}^0 .. \text{Au NPs/PT-N-RGO/SPE})$$

Stripping step:

$$(\text{Cd}^0 .. \text{Au NPs/PT-N-RGO/SPE}) \rightarrow (\text{Au NPs/PT-N-RGO/SPE}) + \text{Cd (II)} + 2e^-$$

In this experiment, due to its excellent conductivity and high specific surface area, PT-N-RGO not only further improves the electron transfer rate and enrichment of Cd (II) on the electrode surface, but also provides more functional sites for capturing Cd (II), thus avoiding the uneven deposition of Cd (II) on the SPE surface, which greatly improves the sensitivity of the electrochemical sensor and the accuracy of Cd (II) detection.

The feasibility of the prepared Cd (II) electrochemical biosensor was tested using the DPASV method, and the results are shown in Figure 3b. In the buffer solution without the addition of cadmium ions, there was no peak current, while in the solution with different concentrations of Cd (II), a significant peak current could be observed with a clear peak at around -0.72 V. The peak current increases with the increase of Cd (II) concentration. The reason for this phenomenon may be that more and more Cd (II) accumulates on Au NPs/PT-N-RGO/SPE with time for redox reaction to achieve higher Cd (II) stripping peak response. The gradual positive shift in the stripping potential of Cd (II) may be due to the increase in the thickness of the Cd (II)-Au NPs/PT-N-RGO/SPE interface. This is a common phenomenon during the determination of heavy metals by DPASV. There is a significant difference between the current response of the buffer with and without the addition of cadmium ions, indicating that the sensor can be used for Cd (II) detection.

3.3 Electrochemical characterization of Cd (II) electrochemical biosensor
In order to study the performance of the prepared electrochemical sensors, the electrochemical behavior of the electrodes after each preparation was monitored by Cyclic Voltammetry (CV) method. Fig. 4a shows the CV graphs of the different electrodes at the various modification stages in the potential range of -0.8 V to 1.0 V at a scanning speed of 100 mVs\(^{-1}\) in PBS solution (0.2 M, pH=6.0) including 5.0 m M \(K_3Fe(CN)_6/K_4Fe(CN)_6\) and 0.1 M KCl. For the bare SPE (curve a), a pair of weak redox peaks were observed with a small peak current value of 4.83 \(\mu\)A, indicating a clean and debris-free electrode surface as well as a slow electron transfer rate, which can be used for subsequent tests. Au NPs have good electrical conductivity and promote electron transfer, so a well-defined pair of quasi-reversible redox peaks (Ipa = 18.10 \(\mu\)A, Ipc = -19.96 \(\mu\)A) were obtained after Au NPs deposition, and the redox peak current was significantly higher than that of bare SPE (curve b). It indicates that Au NPs improve the conductivity of the electrode substrate. Au NP\(_S\)/PT-N-RGO/SPE has the highest peak current (Ip: 51.92 \(\mu\)A) (curve c), which is due to the PT-N-RGO composite with high specific surface area further increasing the electron transfer rate, thus improving the conductivity and sensitivity of the electrode. Moreover, we estimate the effective area of the modified electrode by Randles-Sevcik formula. The relationship between peak current (Ip) and effective surface area (A) of the electrode follows the Randles-Sevcik equation (Alejandro et al. 2018):

\[
Ip = 2.69 \times 10^5 AD^{1/2} n^{3/2} \frac{1/2}{C},
\]

where Ip is the current peak, A is the valid surface acreage of the sensor (cm\(^2\)), D is the proliferation parameter in the medium [Fe (CN)\(_6\)]\(^{3/-4-}\) (6.70 \(\times\) 10\(^{-6}\) cm\(^2\) s\(^{-1}\)), n is the number of electrons involved ([Fe (CN)\(_6\)]\(^{3/-4-}\); n = 1), \(\nu\) is the scan rate (Vs\(^{-1}\)) and c is the redox medium concentration (mol cm\(^{-3}\)).

The effective areas of different modified electrodes calculated by the above formula are as follows: bare SPE (0.0043cm\(^2\)) < Au NP\(_S\)/SPE (0.0160cm\(^2\)) < Au NP\(_S\)/PT-N-RGO/SPE (0.0470cm\(^2\)). This result demonstrates that the modification of Au NP\(_S\) and PT-N-RGO nanocomposites significantly enhances the electrical conductivity and expands the surface area, thus facilitating the electron transfer in electrochemical reactions.

Electrochemical impedance spectroscopy (EIS) is another method to characterize the interface characteristics of different modified electrodes. It has the advantages of fast, low cost, high sensitivity and simple operation, and is widely used in electrochemical analysis. The half-circle diameter of the Nyquist plot reflects the interfacial resistive charge transfer (Rct). The EIS spectra of bare SPE (curve a), Au NP\(_S\)/SPE (curve b), and Au NP\(_S\)/PT-N-RGO/SPE (curve c) are shown in Figure 4b. The equivalent circuit corresponding to the EIS spectrum is inserted in Figure 4b, where Rs and Rct are the solution resistance and charge transfer resistance, Zw is the Warburg impedance, and Cp is the capacitance at the electrode surface/solution interface. As can be seen in Figure 4b, the bare SPE yields a larger diameter semicircle with a Ret value of 1222 \(\Omega\) (curve a), the diameter of the semicircle for Au NPs/ SPE is considerably shorter with a Ret value of 682.39 \(\Omega\) (curve b), because Au NPs can promote easier electron transfer and lower impedance during redox reactions. With the adsorption of PT-N-RGO, the Ret was further reduced to
329.60 Ω (curve c) due to the good electron conduction ability of PT-N-RGO as well as the high specific surface area. Therefore, the EIS results are consistent with the CV results, further demonstrating that the Cd (II) electrochemical biosensor has been successfully prepared.

3.4 Optimization of experimental conditions

In order to obtain the maximum current response of the prepared electrochemical biosensor for Cd (II) detection, the experimental conditions of deposition time, deposition potential, buffer pH and PT-N-RGO dose were optimized using a single-factor variable experiments. In Figure 5, the response currents under different conditions were recorded using DPASV.

There is no doubt that effective deposition can increase the amount of target on the electrode surface, thus improving the sensitivity and reducing the detection limit. Therefore, the effect of deposition time on the peak Cd (II) stripping current was investigated within the deposition time of 80s to 210s. As shown in Fig 5a, the peak current gradually enhanced with increasing deposition time, which could be attributed to the increase in the amount of Cd (II) deposited on the electrode surface. However, with the saturation of the active sites on Au NP/S/PT-N-RGO/SPE, the peak current started to decrease when the deposition time exceeded 180 s. Therefore, we chose 180 s as the optimal deposition time.

Similarly, the effect of deposition potential was discussed between -1.5 to -1.1 V. The results are shown in Figure 5b, when the deposition potential is shifted from -1.5 V to -1.3 V, the electrochemical reduction of Cd (II) was promoted, the peak current of Cd (II) shows an increasing trend and reaches a maximum at -1.3 V. However, a further positive shift of the deposition potential from -1.3 V to -1.1 V leads to the occurrence of the hydrogen precipitation reaction, which hinders the deposition of Cd (II) on the electrode surface and leads to a decrease in the peak Cd (II) current. Therefore, we choose -1.3 V as the optimum deposition potential.

It is well known that the pH of a solution has a profound effect on the electrochemical behavior of Cd (II). Therefore, the effect of different pH values on the Cd (II) electrochemical biosensor was investigated to find its optimal pH, and we performed experiments on 0.2 M PBS buffer solutions with different pH values (3.0-7.0). Figure 5c shows the effect of the pH of PBS on the response current of the electrochemical biosensor. As shown in the figure, the response current increased from 4.49 µA to 9.24 µA when the pH of PBS was increased from 3.0 to 5.0. The response current decreased from 9.24 µA to 3.40 µA when the pH of PBS was increased from 5.0 to 7.0. This is due to the hydrolysis of metal ions with a further increase in pH and a sharp decrease in the peak cation current, it is clear that the peak response current (9.24 µA) was maximum at pH 5.0. Therefore, we chose pH=5.0 acetic acid-sodium acetate buffer for further experiments.

Figure 5d shows the effect of the dose of PT-N-RGO nanocomposite on the Cd (II) electrochemical biosensor. The current response of the sensor gradually increases as the dose of PT-N-RGO nanocomposite increases. Due to the good electron transfer ability of PT-N-RGO nanocomposite, the current response reached a maximum value of 13.80 µA when the dose of PT-N-RGO nanocomposite was
increased to 6.0 µL. As the dose of PT-N-RGO nanocomposite continued to increase, the current response of the sensor gradually decreased, so the optimum PT-N-RGO nanocomposite dose is 6.0 µL.

From the above study, the optimal experimental conditions for this electrochemical sensor experiment are as follows: (1) the optimal dose of PT-N-RGO is 6.0 µL; (2) the optimal pH value of PBS is 5.0; (3) the optimal deposition time 180 s; (4) the optimal deposition potential -1.3 V; Therefore, we choose these values as the best criteria for further study.

### 3.5 Analytical performance of Cd (II) electrochemical biosensor

In order to obtain the analytical performance of the prepared Cd (II) electrochemical sensor, the DPASV response of the Au NP$_S$/PT-N-RGO/SPE sensor to different concentrations of Cd (II) solution was investigated under optimal experimental conditions to evaluate the sensitivity of the sensor, and the results are shown in Fig. 6a shows the electrochemical DPASV response in the range of 3.0 µg/L-30.0 µg/L. The peak current signal of Cd (II) gradually increase with the increase in the Cd (II) concentration. This may be due to the increase in the amount of Cd (II) deposited on the electrode surface with increasing deposition time. The peak current ($I$) is linearly proportional to Cd (II) concentration from $3.0\mu g/L-30.0\mu g/L$, with the linear regression equation of $I=1.0019x-1.6748$ (where $x$ is the concentration of Cd (II) and $I$ is the response peak current of the sensor) with $R^2$ of 0.9969. (Figure. 6b).

The limit of detection (LOD) of the electrochemical biosensor is calculated to be 0.46µg/L at a signal-to-noise ratio of 3 using the formula $C_{LOD}=3S_b/b$ ($S_b$ is the standard deviation calculated by six repeated detection blank samples and $b$ is the slope of the standard curve). The detection limit (LOD) of the above formula is as low as 0.46µg/L, much lower than the 5.00 µg/L stipulated in the national drinking water sanitation standard. Therefore, the prepared sensor can realize the application of the electrochemical detection of Cd (II) in water resources. The sensitivity of the electrochemical biosensor is calculated to be 0.44 µA µM$^{-1}$ cm$^{-2}$ using the formula $K/A$ ($K$ is the absolute value of slope of standard curve, and $A$ is the effective surface area of the biosensor (cm$^2$)).

Table 1 shows the results of previous studies using different methods for the detection of Cd (II). Compared with the earlier reported methods, Au NP$_S$/PT-N-RGO/SPE has a satisfactory linear range and lower detection limits, even better than many methods. The good detection performance was attributed to the following: Au NP$_S$ was used as a material to modify the electrode and deposited on the SPE surface at a constant potential, which promoted the electron transfer between Cd (II) and the electrode. PT-N-RGO composites have the advantages of large specific surface area, strong surface reactivity, strong electron transfer ability and ideal adsorption capacity, thus providing more binding sites for subsequent capture of Cd (II) more binding sites to improve the conductivity and affinity of the electrochemical sensor for specific recognition of heavy metal ions. Therefore, Au NP$_S$/PT-N-RGO/SPE can be used as an excellent electrode material for electrochemical determination of heavy metal ions.

### 3.6 Specificity, stability, and reproducibility of Cd (II) electrochemical biosensor
In practical application, the types of metal ions in water samples are complex, the sensing performance of the electrochemical sensor may be affected by the potentially interfering ions. Therefore it is necessary to investigate the selectivity ability of the modified electrodes. We chose various possible coexisting ions.

The electrochemical sensor was immersed in 20.0 µg/L Cd (II) solution or one of the above-mentioned four interferents (200.0 µg/L) or a mixture (all the interferents mixing with Cd (II) with the substance concentrations of 20.0 µg/L). Testing the current response of the sample under optimal conditions using DPASV recordings, the results are shown in Figure 6c, the electrochemical sensor for detection Cd (II) has the biggest response current compared with detection interference substance. (Mn$^{2+}$, 3.63 µA; Pb$^{2+}$, 2.25 µA; Cu$^{2+}$, 3.53 µA; Zn$^{2+}$, 2.23 µA; Ca$^{2+}$, 2.87 µA; Mg$^{2+}$, 3.17 µA; Ag$^{+}$, 2.90 µA; Fe$^{3+}$, 2.94 µA; Cd$^{2+}$, 19.14 µA; mixture, 15.99 µA). The above results show that the sensor has good selectivity for Cd (II).

After preparation, the electrochemical sensor was stored in a wet refrigerator at 4°C. DPASV method was used to detect Cd (II) solution (15.0 µg/L) and record its peak current value at regular intervals (1, 3, 5, 7 and 10 days) and compared with those on the first day (Fig. 6d). The peak current of electrochemical sensor on that day of preparation was 13.16 µA and the current response was 100%. When the storage time is 3 days, the response current drops to 95.52% of the original current, and when the storage time is 10 days, the response current drops to 84.78% of the original current which shows that the biosensor had excellent stability.

To study the reproducibility of Au NPS/PT-N-RGO/SPE sensor under the same experimental conditions, five Cd (II) electrochemical biosensors were employed to investigate the reproducibility with Cd (II) (15.0 µg/L) using the DPASV method. All five electrodes displayed similar current responses (15.82, 15.65, 15.87, 15.54 and 15.30 µA) with a relative standard deviation (RSD) of 2.39%, indicating that the electrochemical sensor had good reproducibility.

### 3.7 Application of Au NPS/PT-N-RGO/SPE for Cd (II) Detection in Real Sample Analysis

To verify the practical performance of the prepared sensors, tap water and lake water were used as water samples for Cd (II) determination by the standard addition method. Before analysis, tap water and lake water were filtered through 0.22 µm membrane filters, and the treated water samples were diluted with nitric acid buffer solution and added with known Cd (II) standard solution. As shown in Table 2, the original samples prepared were analyzed without any added Cd (II) markers and low concentrations of cadmium were detected. When 5, 10 and 25 µg/L of Cd (II) standards were added to the sample, the recoveries of tap water and lake water were in the ranges of 91.95%-106.94% and 95.62%-108.55%, with relative standard deviations (RSD) were in the range of 2.52%-5.51 % and 1.52 %-7.02 %, respectively. This method is commensurate with the detection results of the inductively coupled plasma mass spectrometry (ICP-MS), indicating that the prepared electrochemical biosensor has good precision and accuracy for different water samples.
4. Conclusions

In this study, we report a screen-printed electrode modified with Au NPs and PT-N-RGO nanocomposites for the quantitative detection of Cd (II) by DPASV to detect the dissolved current value of Cd (II) at a specific potential, with the peak current increasing with the increase of Cd (II) concentration. Au NPs, PT-N-RGO and screen-printed the synergistic effect of Au NPs, PT-N-RGO and screen-printed electrode substrates improves the electrochemical detection sensitivity of Cd (II). The prepared sensors showed good selectivity and stability, with the current response maintained at 84.78 % after 10 days of storage in a wet refrigerator at 4 °C, as well as stable reproducibility with a RSD of 2.39 % for the current response of the same batch of five electrodes. The Cd (II) in the actual sample was determined using the prepared sensor with satisfactory recoveries of 91.95 % to 108.55 %. The electrochemical sensor created in this paper enriches the types of sensors for the detection of Cd (II) with low detection limits, high sensitivity and good specificity. We believe that this electrochemical biosensor will have a broad application prospect.

Declarations

Conflicts of interest

The authors declare that they have no conflict of interest or personal relationship that could have appeared to influence the work reported in this paper.

Acknowledgements

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References


Tables

Table 1 Comparative parameters of different detection methods for Cd (II)
<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Methods</th>
<th>Linear range (µg/L)</th>
<th>Detection limit (µg/L)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co₃O₄/SPE</td>
<td>DPASV</td>
<td>0-140</td>
<td>3.50</td>
<td>(Yogeeshwari et al. 2021)</td>
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<tr>
<td>CUiO-66/Bi/GCE</td>
<td>SWASV</td>
<td>10-50</td>
<td>1.16</td>
<td>(Ding et al. 2021)</td>
</tr>
<tr>
<td>Yb-BTC/GCE</td>
<td>DPASV</td>
<td>0-50</td>
<td>2.97</td>
<td>(Manh et al. 2021)</td>
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<tr>
<td>PtNF₅/GCE</td>
<td>DPASV</td>
<td>1-100</td>
<td>0.45</td>
<td>(Nguyen et al. 2019)</td>
</tr>
<tr>
<td>PEDOT/Schiff</td>
<td>SWASV</td>
<td>5-100</td>
<td>0.95</td>
<td>(Mohammed et al. 2021)</td>
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<td>MWCNTS-POCF/PIGE</td>
<td>SWASV</td>
<td>6.5-147.8</td>
<td>1.90</td>
<td>(Jayadevimanoranjitham et al. 2019)</td>
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<td>MoS₂-RGO/Nafion/GCE</td>
<td>SWASV</td>
<td>2.2-112.4</td>
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<tr>
<td>GC/ErGO</td>
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<td>(Jaime et al. 2021)</td>
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<td>Sb₂O₃/MWCNTs</td>
<td>ASV</td>
<td>80-150</td>
<td>1.93</td>
<td>(Tran et al. 2020)</td>
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<td>Au NPs/PT-N-RGO/SPE</td>
<td>DPASV</td>
<td>3-30</td>
<td>0.46</td>
<td>This work</td>
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DPASV method: differential pulse anodic stripping voltammetry; SWASV method: Square-wave anodic stripping voltammetry; ASV method: anodic stripping voltammetry; SPE, screen- printed electrodes; CUiO-66: carbonized UiO-66; Yb:ion; BTC: benzene-1,3,5-tricarboxylic (BTC), an organic ligand with selectivity for the target contaminants; PtNF₅: Platinum nanoparticles; PEDOT: Poly(3,4-ethylendioxithiophene), an electroactive polymer with high electrical conductivity and good environmental stability; MWCNTs: multiwalled carbon nanotubes; POCF: poly O-cresophthalein complexone; RGO: reduced graphene oxide; ErGO: electrochemicaly reduced graphene.

Table 2 Analysis of the Cd (II) test results in the water resources
<table>
<thead>
<tr>
<th>Added Cd (II) Concentration (µg/L)</th>
<th>Average Cd (II) Concentration Detected by ICP-MS (µg/L)</th>
<th>Average Cd (II) Concentration Detected by the sensor (µg/L)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
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Figures
Figure 1

SEM Characterization of (a) N-RGO, (b) PT-N-RGO. TEM images of (c) N-RGO (d) PT-N-RGO. (e) FT-IR Characterization of PT (curve a), N-RGO (curve b), PT-N-RGO (curve c). (f) UV-visible spectrophotometer of PT (curve a), N-RGO (curve b), PT-N-RGO (curve c).
Figure 2

XPS spectra of PT-N-RGO nanocomposites. (a) Full survey, (b) C 1s spectra, (c) N 1s spectra, (d) O 1s spectra.
Figure 3

(a) Scheme illustration of the fabrication procedure and detection principle of the Cd (II) electrochemical biosensor. (b) DPASV method curves of the electrochemical biosensor with 15, 20, 25 µg/L Cd (II) as experimental group and without Cd (II) as control group in acetate sodium acetate buffer pH=5 at a potential range from -1.6V to 0.2 V for feasibility analysis.
Figure 4

(a) CV characterization of bare SPE (curve a), Au NPs/SPE (curve b), Au NPs/PT-N-RGO/SPE (curve c) in PBS solution (0.2 M, pH 6.0) including 5.0 mM $\text{K}_3\text{Fe(CN)}_6/\text{K}_4\text{Fe(CN)}_6$ solution containing 0.1 M KC1. (b) EIS characterization of bare SPE (curve a), Au NPs/SPE (curve b), Au NP$_5$/PT-N-RGO/SPE (curve c) in PBS solution (0.2 M, pH 6.0) including 5.0 mM $\text{K}_3\text{Fe(CN)}_6/\text{K}_4\text{Fe(CN)}_6$ solution containing 0.1 M KC1.
Figure 5

(a) The influence of electrochemical deposition time of response current. (b) The influence of deposition potential of response current. (c) The influence of pH value of PBS of response current. (d) The influence of the dosage of PT-N-RGO nanohybrids of response current. The concentration of Cd (II) is 10.0 µg/L. All the data in the figure are the median values of three independent experimental analyses, and the relative standard deviation is represented by the error bars.
Figure 6

(a) DPASV method reaction of Au NPs/PT-N-RGO/SPE in acetic acid-sodium acetate buffer (pH 5.0) containing different concentrations of Cd (II). (b) Calibration curve between the stripping peak current and Cd (II) concentration. (c) Selectivity research of Au NPs/PT-N-RGO/SPE. (d) Histogram for stability investigation of the concentrations proposed Cd (II) electrochemical biosensor (Cd (II), 20.0 µg/L).

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