Nanohybrid sensor based on carboxyl functionalized graphene dispersed palygorskite for voltammetric determination of niclosamide

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Abstract

The stably dispersible palygorskite (Pal) nanorods onto carboxyl functionalized graphene (Gr-COOH) nanosheets were successfully employed for the facile fabrication of a novel nanohybrid sensing platform and the voltammetric determination of niclosamide (NA) in tablet and paddy water samples. A water-processable Gr-COOH can improve the dispersion and stability in water of Pal. The structure and properties of the formed Pal-Gr-COOH nanohybrid were characterized. The electrochemical behavior of NA, analytical conditions and the sensing performance of the prepared sensor were investigated. The sensor displayed the enhanced synergistically electrocatalytic ability toward NA in a linear range from 0.02 - 0.99 μM with low limit of detection (4.6 nM), good sensitivity, high reproducibility and satisfactory practicability, suggesting Pal-Gr-COOH nanohybrid will provide a promising platform for sensing application and facile preparation and potential application of other nanoclay mineral nanocomposites.

Keywords Palygorskite, Nanoclay mineral, Carboxyl functionalized graphene, Niclosamide, Nanohybrid

1. Introduction

Niclosamide (2,5-dichloro-4-nitrosalicylanilide, NA), an effective antihelmintic medicine, is widely used in public health, veterinary and aquaculture (Swan, 1999; Datta et al., 2000; Abreu et al., 2002). The World Health Organization has put it as the only commercially available molluscicide for the large-scale use in schistosomiasis control programs (World Health Organization, 1993). However, nowadays, many researchers found it has high toxicity to some aquatic snails and organisms (Andrews et al., 1982; Oliveira-Filho et al., 2000). Thus, it is a great challenge to develop a sensitive and selective method for the NA determination. At present, electrochemical methods have received the increasing attention owing to their many excellent advantages such as rapid, easy operation, high sensitivity, online testing and low-cost. Electrochemical sensor based electroanalytical methods have widely used for various fields such as environmental protection, pharmaceutical monitoring, and so on (Janssen et al., 2002; Karimi-Maleh et al., 2016, 2017). There were also some reports for the NA determination by electroanalytical methods (Sridevi et al., 1991; Alemu et al., 2002, 2003). However, electrochemical sensors based on nanomaterials modified electrodes were mainstream methods of the NA determination in recent years (Ghalkhani et al., 2010; Dede et al., 2014; Yao et al., 2014). Ghalkhani et al fabricated carbon nanoparticle/chitosan nanocomposite modified electrode for detecting NA, and it showed high stability, good reproducibility and excellent electro-catalytic activity toward NA (Ghalkhani et al., 2010). Mehretie et al also investigated poly

(3,4-ethylenedioxythiophene) modified glassy carbon electrode (GCE) towards the electrochemical determination of NA with excellent performance (Mehretie et al., 2012). In our previous work, we also built electrochemical sensors based on carbon nanomaterials modified GCE with wonderful properties (Yao et al., 2014). Nevertheless, these nanomaterials were more expensive. Thus, low-cost nanomaterials with excellent properties were necessary to seek for producing novel electrochemical sensors for detecting NA with high effective, cheap and good sensitivity.

Nanoclay minerals are good candidate as modified electrodes for applications in sensors and biosensors due to their exclusive features, such as abundance, high specific surface areas, inexpensive availability, environmental stability, high adsorptive and ion exchange properties (Mousty, 2004; Bergaya et al., 2013). Palygorskite (Pal), a type of hydrated magnesium aluminum silicate rich in the content of natural nanoclay minerals, which has fibrous crystal structure and chemical structural formula $\text{Si}_8\text{O}_{20}(\text{Mg}, \text{Al}, \text{Fe})_5(\text{OH})_2(\text{OH}_2)_4 \cdot 4\text{H}_2\text{O}$ (Haden et al., 1967; Drits et al., 1971; Gonzalez et al., 1989). Nowadays, Pal has been widely used in foodstuff, environmental protection and medicine owing to its cheap, large specific surface area, strong absorptive capacity and so on, but there were few reports on Pal and its nanocomposites as modified electrodes for applications in sensors and biosensors relatively to other nanoclay minerals like montmorillonite, kaolinite and halloysite (Zen et al., 2004). Therefore, it is well select to fabricate novel sensors for the

electrochemical determination of analytes based on Pal nanocomposites combined with merits of other nanomaterials.

Graphene (Gr), one of two-dimensional carbon nanomaterials, has given rise to great interest among physical, chemical and material fields since produced in 2004 (Novoselov et al., 2004). Gr possesses ideal geometric construction and unique optic, thermotic, electric and mechanic properties. Thus, potential applications and fundamental research of Gr have appeared the exponential growth in many fields such as supercapacitors, solar cells, nanometer electrode transistors, electrochemical sensors and composite materials (Stankovich et al., 2006; Geim et al., 2007; Pumera et al., 2010; Brownson et al., 2012; Zhao et al., 2016). In addition, Gr has highly-focused in sensing analysis due to its stable chemical structure, good conductivity, large specific surface area and excellent electrocatalytic properties. However, the stacking interactions between sheet layers and bad solution-processability of Gr limit its ranges of applications. So far, researchers tried to overcome Gr defects mainly through specific functional groups modified on its surface (Si et al., 2008; Ribeiro et al., 2011; Nie et al., 2012). Consequently, carboxyl group functionalized graphene (Gr-COOH), one of Gr derivatives with hydrophilic groups, was produced. It not only retained the excellent performance of Gr, but also enhanced its water dispersibility, which also made the excellent performance of Gr-COOH composite materials easy to prepare via strong interaction with other materials.

In this paper, a low-cost, simple, sensitive, and selective Pal-Gr-COOH modified GCE was fabricated for the voltammetric determination of NA in tablet and paddy water samples. The Pal-Gr-COOH nanohybrid was prepared by introducing Pal into Gr-COOH dispersion. The structure and properties of Pal-Gr-COOH were characterized, and the nanohybrid sensor based on Pal-Gr-COOH/GCE was fabricated and applied.

2. Experimental

2.1. Materials and reagents

Gr-COOH was purchased from Naijing Xianfeng Nanomaterials Technology co., Ltd. Pal and NA was purchased from Sigma-Aldrich. Lithium perchlorate trihydrate ($\text{LiClO}_4 \cdot 3\text{H}_2\text{O}$), D-(+)-Glucose, sodium chloride (NaCl), potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$), potassium hexacyanoferrate trihydrate ($\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$), disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) and sodium dihydrogen phosphate dehydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) were all obtained from the Sinopharm Chemical Reagent Co., Ltd. Nicotinic acid, nickel chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) and potassium nitrate were purchased from Beijing lark technology Co., Ltd. The paddy water was obtained from the rice base of Jiangxi agricultural university, and the pesticide tablets containing NA was purchased from local agro-chemical market. All chemicals were of analytical grade.

2.2. Apparatus

Electrochemical experiments were performed using CHI660B electrochemical

workstation (Shanghai Chenhua Instrument Company, China). The pH of phosphate buffer solution was measured by a portable pH meter PHB-5. Scanning electron microscope (SEM) images were obtained using JSM-6700F (JEOL, Japanese). Transmission electron microscope (TEM) images were conducted on TecnaiG220. Fourier transform infrared spectroscopy (FTIR) was recorded on Bruker Vertex 70 FT-IR spectrometer. The X-ray diffraction (XRD) patterns were acquired on X-ray power diffractometer using XRD-6100 (Shimadzu, Japanese). The UV-vis was conducted by Perkin Elmer Lambda 900 UV-vis NIR spectrometer.

2.3. Fabrication of Pal-Gr-COOH/GCE

Prior to the modification, the GCE was carefully polished with a leather containing 0.05 μm alumina slurry, and then ultrasonically cleaned in deionized water, absolute ethanol and deionized water each for 5 min, respectively. For preparing 1mg/ml Gr-COOH dispersion, 10 mg Gr-COOH was dissolved in 10 ml deionized water, and ultrasonic two hours. Then, Pal was added into 1 mg/ml Gr-COOH dispersion until the concentration of Pal in Gr-COOH dispersion was up to 10 mg/ml, and Pal-Gr-COOH was attained after the homogeneous dispersion. The Pal-Gr-COOH/GCE was fabricated by drop-coating 5 μl of Pal-Gr-COOH dispersion on the surface of GCE, and dried in infrared lamp. The preparative procedure of Pal-Gr-COOH/GCE is shown in Scheme 1.

2.4. Electrochemical measurements

The electrochemical measurement was carried out in a three-electrode cell with a modified electrode as the working electrode, a Pt wire as the counter electrode and a saturated electrode (SCE) as reference electrode, in 5 ml 0.1 M phosphate buffered solution at pH = 7.0. Before the measurement, electrolyte solutions were purged with nitrogen for 5 min. The electrochemical response was measured by differential pulse anodic stripping voltammetry (DPASV).

2.5. Samples preparation

After being centrifuged and filtrated, paddy water was adjusted pH into 7.0 and stored for following use. For preparing 0.1 mM pesticide sample solution, the tablet was ground into a powder and then dissolved in purified DMF.

3. Results and discussion

3.1. Characterization of Pal-Gr-COOH

A white Pal suspension (10 mL mg^{-1}) (Scheme 2a) with visible large particulates was obtained with the ultrasound for 2 h in water, and this suspension was prone to aggregate and some white precipitate was observed on the bottom of the bottle. On the contrary, a yellow-brown Gr-COOH (Scheme 2b) homogeneous dispersion (concentration 1 mL mg^{-1}) was prepared by ultrasonic agitation in water for 2h. More interestingly, the Pal was added into Gr-COOH aqueous dispersion, a stable dark-brown Pal-Gr-COOH dispersion (Scheme 2c) was obtained by sufficient ultrasound, and Pal showed an improved dispersion and stability in water, which were mainly ascribed to carboxy groups of Gr nanosheets.

3.1.1. UV-vis

UV-vis spectroscopy is a useful tool for monitoring any possible change, the strong absorption peak at 230 nm of Gr-COOH in Fig. 1Ab is attributed to the characteristic absorption of Gr, originating from the π - π^* transitions of aromatic C = C bond, and a weak shoulder at 307 nm attributed to n- π^* transitions of C = O bonds, corresponding to n- π^* transitions of C = O bond, which maybe closely related to the degree of reduction of Gr (Deng et al., 2013). The Pal has a weak shoulder at 257 nm in Fig. 1Aa, probably because of the mutual interference of polyatomic species (Mg, Al, Fe, Mn, etc (Zhou et al., 2013)). When Pal was added into Gr-COOH dispersion, Pal-Gr-COOH appeared a weak shoulder at 246 nm in Fig. 1Ac, while absorption

peak of Gr-COOH shifted or disappeared, which were attributed to the interaction between two nanomaterials.

3.1.2. FTIR

FTIR spectra of Pal (a), Gr-COOH (b) and Pal-Gr-COOH (c) were presented in Fig. 1B. The presence of peaks at 1026 and 472 cm^{-1} (Si-O-Si asymmetric stretching vibrations), 3617, 3547 and 3406 cm^{-1} (different hydroxyl stretching vibration adsorptions) were characteristic spectra of Pal (Fig. 1Ba), which was consistent with the previous report (Li et al., 2010). The peaks at about 1047, 1384, 1624, 1722, 2926 and 3418 cm^{-1} were feature peaks of Gr-COOH (Fig. 1Bb). Stretching vibrations of COOH and C-O-C in Gr-COOH produced peaks at 1722, 3418 and 1047 cm^{-1} , and other peaks at 1384, 1624 and 2926 cm^{-1} were assigned to OH bending vibration, carbon atoms sp^2 hybridization and the vibration of $-\text{CH}_2$, respectively (Deng et al., 2011; Bora et al., 2012; Han et al., 2014). As we could see from Fig. 1Bc, peaks location of Pal-Gr-COOH were similar with that of Pal, meanwhile, it also had feature peaks of Gr-COOH. Moreover, peaks of Pal-Gr-COOH hybrids became weak, even disappear, implying Pal interacted with Gr-COOH.

3.1.3. XRD

Diffraction reflections at $2\theta = 8.3^\circ$, 13.8° , 19.7° and 27.7° (Fig. 1Ca) were corresponding to (110), (200), (040), (400) crystal planes of Pal, respectively, suggesting that Pal had a typical tetrahedral structure (Xu et al., 2009), and the special

structure was good for the manufacture of sensing devices. After mixed Pal with Gr-COOH, the nanohybrids had diffraction reflections of both Pal and Gr-COOH, indicating that the crystal structure of Pal was not destroyed by Gr-COOH (Fig. 1Cb-c).

3.1.4. SEM

SEM images of Gr-COOH displayed the typical shape of Gr-COOH (Fig. 2A-B), the thin paper-like surface topography had some cracks and crimples, and the high surface roughness of Gr-COOH surface was clearly observed. SEM images of Pal showed short rod-like nanostructure of Pal (Fig. 2C-D), and they stacked together like rice straw. Pal was adhered onto the surface of Gr-COOH nanosheets in comparison with Fig. 2C-D. They were got together tightly with each other and formed nanorod network (Fig. 2E-F). Thus, Pal-Gr-COOH nanohybrids had larger surface structure comparing with Gr-COOH and Pal, which was beneficial to form larger electroactive area at electrode surface and in favor of the electrochemical determination of analytes.

3.1.5 TEM

TEM images of Gr-COOH showed that the Gr-COOH was wrinkle-sheet nanostructures, and even a single or double layered Gr-COOH was observed (Fig. 3A-B). TEM images of Pal displayed rod-like nanostructures with common diameter, and the diameter was approximately 25 nm (Fig. 3C-D). When Pal was introduced into Gr-COOH, Pal nanorods covered onto the surface of Gr-COOH nanosheets, and

its intensive degree and diameter have increased (Fig. 3E-F). According to above surface features, it demonstrated that Pal-Gr-COOH was well mingled together, and Gr-COOH has no effect on the crystal form of Pal. In addition, SEM results were in accordance with that of TEM, they also revealed the reason of the improved dispersion and stability in water of Pal.

3.1.6. EIS

Nyquist plots of GCE, Pal/GCE, Gr-COOH/GCE and Pal-Gr-COOH/GCE (Fig. 4a-d) were measured in the presence of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. The EIS of the bare GCE displayed a small semicircle at high frequencies (Fig. 4a), indicating that the charge transfer resistance at GCE was very low. After modifying with Pal, a large semicircle was emerged (Fig. 4b) due to its non-conductivity. Subsequently, when the GCE was modified with Gr-COOH, the size of the semicircle of Gr-COOH/GCE was larger than that of Pal/GCE (Fig. 4c), which was mainly ascribed to the electrostatic repulsion between negatively charged functional group of Gr and negative charge of the redox probe $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (Devi et al., 2015; Punckt et al., 2016). Finally, after Pal-Gr-COOH nanohybrid was drop-coated onto the GCE (Fig. 4d), the size of the semicircle of Pal-Gr-COOH was between that of both Gr-COOH/GCE and Pal/GCE, this is most likely to be related to the introduction of negative charges of Gr-COOH and bad conductivity of Pal, and also implying Pal and Gr-COOH has been mixed absolutely from another aspect.

To further explore the electrochemical performance of different modified

electrodes, CVs of GCE (a), Pal/GCE (b), Gr-COOH/GCE (c) and Pal-Gr-COOH/GCE (d) were carried out in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. All modified electrodes demonstrated a pair of reduction-oxidation peaks, but all peak currents were smaller than that of the GCE (Fig. 4 inset). Moreover, peak currents of Gr-COOH/GCE, Pal/GCE, and Pal-Gr-COOH/GCE were increasing in turn, which were attributed to their high electron transfer resistance. These results of CVs were in accordance with that of EIS. In addition, the peak current of Gr-COOH/GCE was smaller than bare GCE, this was completely different from Gr-COOH/GCE, (In general, the peak current of Gr-COOH/GCE was higher than bare GCE), the decreased current also revealed that increase in the negative charges at the electrode surface and enhances electrostatic repulsion between the Gr-COOH and the redox probe (Devi et al., 2015; Punckt et al., 2016).

3.2. Electrochemical behaviors of NA

CVs of 50 μM NA at GCE (a), Pal/GCE (b), Gr-COOH/GCE (c) and Pal-Gr-COOH/GCE (d) in 0.1 M phosphate buffered solution (pH 7.0) were presented in Fig. 5A. All modified electrodes exhibited an irreversible reduction peak and a pair of redox peaks, which was consistent with previous reports (Yao et al., 2014). The peak current of Pal/GCE was higher than that of bare GCE, which might be assigned to its large specific area and good electrocatalytic activity (Fig. 5Aa). Nevertheless, the peak current of Gr-COOH/GCE was enhanced after GCE was modified with Gr-COOH, Interestingly, the peak current of Gr-COOH/GCE was larger than that of

Pal/GCE and GCE (Fig. 5Ac), which was obviously different from Fig. 4 inset. The main reason is that Gr-COOH showed excellent electrocatalytic ability for the redox of NA, and the analyte NA is no charge. In addition, quantum chemical calculations in our studies revealed that carboxyl group of function materials could easily interact with in analytes containing amide via the hydrogen bond (Zhang et al., 2013; Yao et al., 2014). Besides, the peak current of Pal-Gr-COOH/GCE was largest than that of three modified electrodes, which might be the synergistic effect between Pal and Gr-COOH (Fig. 5Ad).

To more clearly demonstrate the difference of the electrochemical behaviors of NA at different modified electrodes, DPASV of NA at GCE (a), Pal/GCE (b), Gr-COOH/GCE (c) and Pal-Gr-COOH/GCE (d) were performed in 0.1 M phosphate buffered solution (pH 7.0) containing 20 μ M NA. It could be seen from Fig. 5B that the electrochemical behaviors of NA at all modified electrodes by DPASV were more obviously. The GCE has a weak oxidation peak at around -0.13 V (Fig. 5Ba). The oxidation peak of NA was enlarged when the GCE was modified with Pal (Fig. 5Bb). Subsequently, the height of the oxidation peak at Gr-COOH/GCE was about two times higher than that of Pal/GCE (Fig. 5Bc). In comparison with other three modified electrodes, the Pal-Gr-COOH/GCE exhibited the highest peak height and the sharpest peak shape (Fig. 5Bd). All the above results were consistent with CVs of all modified electrode.

3.3. Optimization of experimental conditions

3.3.1. Effect of mass ratios both Gr-COOH and Pal

The different mass ratio between Gr-COOH and Pal on the electrode surface has an effect on the DPASV current response toward NA. Here, the Pal-Gr-COOH composite with different mass ratio was investigated in 0.1 M phosphate buffered solution (pH 7.0) containing 20 μ M NA. It could be seen in Fig. 6 that the current response of NA at Pal-Gr-COOH/GCE decreased with the increase of the ratio of Gr-COOH towards Pal from 0.1 to 0.9, which was closely related to Pal content with large specific area onto Gr-COOH sheet and the water dispersibility of Gr-COOH sheet (A large amount of Gr-COOH easily agglomerated and precipitated). Therefore, the optimum ratio of Pal-Gr-COOH was chosen as 0.1.

3.3.1. Effect of buffer solutions

The concentration of all buffer solutions was 0.1 M. and pH value was adjusted to 7.0 by NaOH and HCl. NA presented a low peak height and ugly peak shape in CH_3COONa , LiClO_4 and KCl buffer solutions (Fig. 7). On the contrary, a pretty good oxidation peak was appeared in NH_4Cl and Britton-Robinson (B-R) buffer solutions. But there was a better peak shape and higher height in phosphate buffered solution. Hence, phosphate buffered solution was chosen as buffer solution in subsequent experiments.

3.3.2. Effect of scan rates

Obviously, a pair of reversible redox peak with well peak shape and a poor reduction peak were observed from CVs with scan rates from 20 to 500 mV s^{-1} (Fig.

8), which was related to the electrochemical reaction mechanism of NA (Yao et al., 2014). Moreover, the logarithm of oxidation peak currents ($\log I_{pa}$) and reduction peak currents ($\log I_{pc}$) show a better linear relationship with the logarithm of scan rates ($\log \nu$), and the regression equation was $\log I_{pa} (\mu A) = 0.788 \log \nu - 0.831$ ($R^2 = 0.9968$) and $\log I_{pc} (\mu A) = 0.745 \log \nu - 0.886$ ($R^2 = 0.9936$) (ν in $mV s^{-1}$, Fig. 8 inset), we could infer that the reaction of Pal-Gr-COOH/GCE was controlled by adsorption process according to slopes of two linear equations range from 0.5 to 1 (Uslu et al., 2008).

3.3.3. Effect of pH

NA is usually unstable in strong acid medium (Mehretie et al., 2012), while it is easily hydrolyzed in strong alkaline media (Al- Hadiya et al., 2005), thus the effect of pH values on the NA determination at Pal-Gr-COOH/GCE over the range of pH 4.0 – 8.0 was explored by DPASV. The peak currents of NA at Pal-Gr-COOH/GCE increased gradually with the increasing pH value until it reached the maximum at pH 7.0, but it decreased rapidly when pH increased further (Fig. 9). Therefore, phosphate buffered solution with pH 7.0 was chosen for following study. In addition, E_p decreased with the increase of pH values, and the linear relationship is $E_p (V) = -0.0468 \text{ pH} + 0.190$ ($R^2 = 0.9988$), and the slope is 46 mV pH^{-1} , which was close to the theoretical value of 58.5 mV pH^{-1} . According to the Nernst equation, we could speculate that the numbers of electrons and protons were equivalent, implying that the electrochemical redox of NA was a two-electron and two-proton process in

combination with our previous reports (Yao et al., 2014).

3.4. Determination of NA

DPASV method was used to determine NA at different concentrations in 0.1 M phosphate buffered solution (pH 7.0). Peak currents at Pal-Gr-COOH/GCE were proportional to NA concentrations (Fig. 10). The modified electrode showed a great linear range from 0.02 – 0.99 μM , a limit of detection is 4.6 nM, and a linear equation was $I (\mu\text{A}) = 0.736 + 0.68 C (\mu\text{M})$ ($R^2 = 0.9986$) (Fig. 10 inset B). In comparison with previous reports (Alemu et al., 2002 and 2003; Ghalkhani et al., 2010; Mehretie et al., 2012; Yao et al., 2014; Wang et al., 2014; Dede et al., 2014), the prepared sensor has higher sensitivity and lower limit of detection (Table 1), which mainly ascribe to the large surface area and strong adsorption of both Pal and Gr-COOH, including high surface catalytic activity of Gr-COOH.

Under the same conditions, five modified electrodes were used to determine NA, respectively, and the relative standard deviation was 2.5%. Besides, every modified electrode was carried out thirty consecutive measurements, and the relative standard deviation was 1.9%. Thus, the modified electrode possessed good repeatability.

3.5. Interference

The anti-interference ability of Pal-Gr-COOH/GCE was investigated in 0.1 M phosphate buffered solution (pH 7.0) containing 50 μM NA in the presence of some substances such as the same concentration for glucose, nicotinic acid, Na^+ , Ni^{2+} , K^+ .

As shown in Fig. 11, there are no obvious changes in the current responses for NA in the presence of different interferences due to the weak electrochemical activity or relatively far away from oxidation potentials, which did not cause significantly interference.

3.5. Samples analysis

To evaluate the feasibility and precision of Pal-Gr-COOH/GCE, the NA tablet and paddy water were conducted the practical sample analysis by standard addition method. Table 2 indicated that recoveries of NA tablet and paddy water samples were 96.0% - 102.5% and 96.2% - 102.0%, respectively, suggesting that electrochemical detection of NA using the fabricated composite modified electrode is feasible. The content of NA in a tablet was 490.2 mg, but the real content in a NA tablet sample was 500 mg, so the error between them was 1.96%. Moreover, each sample solution was submitted to three parallel detections, and the RSD for the different concentration levels was no more than 4.35%, suggesting that results obtained by the modified electrode were acceptable. To further confirm the accuracy of this electrochemical detection method in practical application, the same contents of NA were also detected using HPLC. The results obtained by HPLC and with the fabricated electrochemical sensor based on Pal-Gr-COOH/GCE were in good agreement, suggesting that the newly developed method is accurate and has promise for application. Thus, the modified electrode was an excellent sensing platform for the NA analysis of practical samples.

4. Conclusions

A novel nanohybrid film with advantages of the excellent performance of both Pal and Gr-COOH was successfully used to fabricate a cheap, simple, and sensitive sensor for electrochemically detecting NA. The nanohybrid was prepared by adding Pal into Gr-COOH dispersion. The structure and properties of Pal-Gr-COOH nanohybrid film were investigated by UV-vis, FT-IR, XRD, SEM and TEM. The Pal-Gr-COOH/GCE exhibited a linear detection range from 0.02 – 0.99 μM with low limit of detection (4.6 nM) under optimal conditions. The prepared sensor has high reproducibility and good sensing stability for NA determination. Moreover, the modified electrode was successfully applied to analyze the trace NA in real samples (NA tablets and paddy water) with satisfactory results. The work will provide a stably dispersible Pal nanohybrid with Gr-COOH for application in sensing NA, and enlarge the potential application in different fields of other nanoclay mineral nanocomposites with hydrophilic nanometer materials.

Conflict of interest

The authors declare that they have no conflict of interest.

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Scheme 1. The preparation process of Pal-Gr-COOH/GCE.

Scheme 2. The preparation of Pal (a), Gr-COOH (b) and Pal-Gr-COOH (c).

Figure. 1. UV-vis spectra (A), FT-IR spectra (B), XRD patterns (C) of modified electrodes materials Pal (a), Gr-COOH (b) and Pal-Gr-COOH (c).

Figure. 2. SEM images of Gr-COOH/GCE (A $\times$ 20000; B $\times$ 50000), Pal/GCE (C $\times$ 10000; D $\times$ 50000) and Pal-Gr-COOH/GCE (E $\times$ 10000; F $\times$ 50000).

Figure. 3. TEM images of modified electrodes materials Gr-COOH (A and B), Pal (C

and D) and Pal-Gr-COOH (E and F).

Figure. 4. Nyquist plots of different electrodes: GCE (a), Pal/GCE (b), Gr-COOH/GCE (c), and Pal-Gr-COOH/GCE (d) in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. Inset: CVs of different electrodes in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl. Scan rate: 50 mV s⁻¹.

Figure. 5. CVs of 50 μM NA at GCE (a), Pal/GCE (b), Gr-COOH/GCE (c) and Pal-Gr-COOH/GCE (d) in 0.1 M phosphate buffered solution (pH 7.0) (A). DPASVs of 20 μM NA at different electrodes: GCE (a), Pal/GCE (b), Gr-COOH/GCE (c) and Pal-Gr-COOH/GCE (d) in 0.1 M phosphate buffered solution (pH 7.0) (B).

Figure. 6. The effect of mass ratios of Gr-COOH to Pal on DPASV current response for NA at Pal-Gr-COOH/GCE.

Figure. 7. DPASV of NA in 0.1 M different buffer solutions (pH 7.0).

Figure. 8. CVs of 50 μM NA at Pal-Gr-COOH/GCE in 0.1 M phosphate buffered solution (pH 7.0) with different scan rates: 20, 50, 100, 150, 200, 300, 400 and 500 mV s^{-1} . Inset: the plot of $\log I_{\text{pa}}$ vs. $\log v$.

Figure.9. Effect of pH on peak currents and peak potentials.

Figure. 10. DPASV for NA detection at Pal-Gr-COOH/GCE. NA concentrations (from a to k): 0.02, 0.099, 0.199, 0.299, 0.399, 0.596, 0.794, 0.99, 1.96, 2.96, 4.76 μM . Inset: DPASV for 0.002 μM NA detection at Pal-Gr-COOH/GCE (A) and the calibration range from 0.02 to 0.99 μM (B).

Figure. 11. Influence of coexisting substances on the current response for NA by Pal-Gr-COOH/GCE in 0.1 M phosphate buffered solution (pH 7.0) containing 50 μM NA and several interferences (a, NA; b, NA + glucose; c, NA + nicotinic acid; d, NA + Na^+ ; e, NA + Ni^{2+} ; f, NA + K^+ ; g, NA + glucose + nicotinic acid + Na^+ + Ni^{2+} + K^+).

Table 1

The comparison of electrochemical sensors based on different materials previously used for the determination of NA

Electrodes	Buffer solutions	Linear ranges (μM)	LOD (nM)	Samples analysis	References
GCE	$\text{NH}_3\text{-NH}_4\text{OH}$ (pH 8.5)	0.05 – 1	20.5	NA tablet	Mehretie et al. (2012)
GCE	$\text{NH}_3\text{-NH}_4\text{OH}$ (pH 8.5)	1 – 100	80	NA tablet	Alemu et al. (2002)
Carbon Nanoparticle/ chitosan/GCE	PBS (pH 7.0)	0.01-2	7.7	NA tablet, Blood serum	Alemu et al. (2003)
PEDOT/GCE	PBS (pH 7.0)	0.075 – 7.5	10.9	NA tablet	Ghalkhani et al. (2010)
SWCNTs/GCE	PBS	0.023–19	15		
MWCNTs/GCE	(pH 5.0)	0.023–26.1	7.7	NA tablet	Yao et al. (2014)
ER-GO/GCE		0.020–23.3	6.6		
PEDOT:PSS–PVA–Gr-	PBS	0.17-42	50		Wang et al.

COOH/GCE	(pH 6.5)				(2014)
PEG	BR (pH 7.0)	0.05-10	15	NA tablet	Dede et al. (2014)
Pal-Gr-COOH/GCE	PBS	0.02–0.99	4.6	NA tablet	This work
				Paddy water	
	(pH 5.0)				

Table 2

The determination of NA in pharmaceutical tablets and paddy water

Sample		Added (μM)	Found (μM)	Recovery (%)	RSD (%)	Determined by HPLC
Tablet	1	0.05	0.048	96.0	4.35	0.051
	2	0.08	0.082	102.5	3.89	0.079
	3	0.15	0.151	100.7	3.67	0.149
Paddy water	1	0.05	0.051	102.0	4.17	0.049
	2	0.08	0.077	96.2	3.96	0.079
	3	0.15	0.148	98.7	3.52	0.151

Graphical abstract