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A Novel Photoelectrochemical Sensor for Thiamphenicol Based on Porous Three-Dimensional Imprinted Film

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In this study, we designed a novel photoelectrochemical (PEC) sensor for thiamphenicol (TAP), which based on porous graphene (P-r-GO), MoS₂ nanoflower, dendritic Pt-Pd NPs (Pt-Pd NPs), amino multi-walled carbon nanotubes (NH₂-MWCNTs), molecularly imprinted polymer (MIP) and L-shape glassy carbon electrode (L-GCE). Firstly, MoS₂ and P-r-GO nanoflower composite was prepared by one-step hydrothermal method. Then, this composite suspension was coated on L-GCE surface to virtually form a porous interface. After that, the suspension of Pt-Pd NPs and NH₂-MWCNTs was dropped onto MoS₂-P-r-GO / L-GCE. Subsequently, TAP was imprinted on above modified electrode by cyclic voltammetry as o-phenylenediamine was monomer. Afterwards, ascorbic acid was selected as a photocurrent probe when TAP was removed from MIP film and adsorbed on sensing surface. The resulting PEC sensor possessed excellent response for TAP, and its linear range was $1.0 \times 10^{-9} \sim 3.5 \times 10^{-6}$ mol L⁻¹ with the detection limit of 5.0×10^{-10} mol L⁻¹. This sensor was used to determine TAP in real food samples with favorable results.

Keywords: Photoelectrochemical sensor; Thiamphenicol; Porous graphene; MoS₂; Molecularly imprinted polymer

1. INTRODUCTION

Thiamphenicol (TAP) belongs to amphenicols, and possesses the effective broad-spectrum antibiotics active against most of gram bacteria as chloramphenicol and florfenicol [1, 2]. Therefore, it is commonly employed to treat such bacteria for the food producing animals, and become a substitute for chloramphenicol due to its relative safety for human beings, but overdose intake also can cause a

series of the serious diseases [1]. Hence, many countries have set its maximum residue limit for food regulation system [1-3]. For this purpose, the chromatographic method and its derived method are usually used to detect its residue in food sample, such as high performance liquid chromatography (HPLC) [3, 4], gas chromatography (GC) [5], HPLC-mass spectrometry (HPLC-MS) [2, 6] and GC-MS [7], etc., but they often demand expensive equipment, tedious extraction and extract cleanup. Based on the disadvantages, it is necessary to design simple and economic analytical method to determine its residue for agricultural products.

For drug residue analysis, photoelectrochemical (PEC) sensor becomes a promising way due to its specific photocurrent signal, as well as lower cost and faster operation as traditional electrochemical sensor [8]. Generally, to enhance the sensitivity and/or selectivity, enzyme, antibody, nucleic acid and molecularly imprinted polymer (MIP) et al are usually employed as recognition receptors for this field [9]. For these receptors, MIP not only possesses the desired selectivity for target molecule as natural receptors, but also presents some advantages, such as easy and controllable design, and low cost, as well as reusable property. As a result, it becomes a popular receptor based on above inherent excellence in this field [10]. To construct this kind of sensor, the preparation of MIP on sensing surface is a critical step for PEC sensor, which involves polymerization method and electrode modification [11]. For the former, electrochemical polymerization is an easy, effective and controllable way, and the resulted film usually possesses excellent conductive and photoactive capability, which benefits the sensitivity and/or selectivity for the PEC sensor [10, 12]. For the latter, polymerized substrate should possess excellent photoactive capability, large surface area and fast mass transport. Based on these criterions, the three-dimensional nanostructure becomes a promising choice. For instance, TiO₂ nanotube array [13-15], porous Au [16], BiOI nanoflake arrays [17] and AgI nanoparticles (NPs)-BiOI nanoflake arrays [18] were used as the polymerized substrate in this field. At the same time, to easily capture illumination, sheet electrodes such as photoactive materials modified TiO₂ / Ti [13, 14], indium tin oxide (ITO) or fluorine-doped tin oxide glass (FTO) [17, 18] were commonly employed as a work electrode, but it resulted some disadvantages, such as it was difficult to accurately control its modified area, and it was tedious for fabrication procedure, etc. Therefore, to overcome these issues, the facile method for the preparation of photoactive electrode is still in need of exploration.

Here, porous graphene and MoS_2 (MoS_2 -P-r-GO) nanoflower composite was prepared by onestep hydrothermal method. Then, the suspension of MoS_2 -P-r-GO was modified on L shape glassy carbon electrode (L-GCE) surface to obtain MoS_2 -P-r-GO / L-GCE. After that, the suspension of NH₂-MWCNTs and Pt-Pd NPs was dropped onto MoS_2 -P-r-GO / L-GCE surface. Next, the TAP was imprinted in poly-o-phenylenediamine (o-PD) film by using cyclic voltammetry (CV) at above modified electrode surface. For this strategy, as far as we know, it was first time to construct such PEC sensor based on MIP for TAP, and the L-GCE made the illumination easily achieve on GCE surface. Additionally, the MoS_2 -P-r-GO nanoflower, NH₂-MWCNTs and dendritic Pt-Pd NPs formed a threedimensional imprinting film to enhance the sensitivity and selectivity. JEM-2100 transmission electron microscope (TEM).

2. EXPERIMENTAL

2.1. Apparatus and reagents

To construct a three-electrode system, we employed modified L shape glassy carbon (L-GCE) electrode as working electrode, a platinum foil as counter electrode and a saturated calomel electrode (SCE) as reference electrode. A 5 W laser resource (λ =405 nm) was used as a irradiation source with power switch, which was purchased from Beijing Changtuo Co. Ltd. The photocurrent test was carried on a CHI 660D electrochemistry workstation (Shanghai CH Instruments Co., China). Electrochemical impedance spectra (EIS) was performed with an Autolab PGSTAT302N electrochemistry workstation (Switzerland), and the frequency range was 0.1 Hz to 100 kHz. Morphological and structural

Amino multi-walled carbon nanotubes (NH₂-MWCNTs, amino ratio: ~0.45 wt %) was purchased from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). Graphene oxide (GO) was prepared by a modified Hummers method [19] and characterized as our previous report [20]. According to the report [21], porous GO (P-GO) was obtained by oxidizing and etching GO with KMnO₄ and HCl, but we lengthened oxidation time to 6 h in order to acquire bigger pore and better three-dimensional structure [22], and the details were shown in Supplementary Information. Dendritic Pt-Pd NPs were prepared respectively according to our previous report [20], and the details were shown in Supplementary Information. Hexadecylpyridinium chloride monohydrate (HDPC), H₂PtCl₆, Na₂PdCl₄, ascorbic acid (AA), thiamphenicol (TAP), chloramphenicol and florfenicol were purchased from Sigma (St. Louis, MO, USA). Unless stated otherwise, other reagents used were analytical grade. The porcine muscle, milk and honey samples were purchased from local supermarket. The phosphate buffer solution (PBS, pH= 7.0) was prepared with NaH₂PO₄ and Na₂HPO₄, and its concentration was 0.1 mol L⁻¹.

characterizations were performed with a Zeiss (German) scanning electron microscope (SEM) and

2.2. Synthesis of MoS₂-P-r-GO composite

 MoS_2 -P-r-GO nanoflower composite was prepared by a facile and one-step hydrothermal method. To prepare MoS_2 -P-r-GO nanoflower, 0.3 mmol (NH₄)₆Mo₇O₂·4H₂O and 9 mmol thiourea were added into 50 mL of 1.0 mg mL⁻¹ P-GO aqueous suspension, and made them completely dissolve. Then, the resultant mixture was transferred into a 100 mL Teflonlined stainless steel autoclave for hydrothermal reaction at 180 °C for 24 h. When the reaction was completed, the black precipitates were separated by centrifugation, and washed with H₂O and ethanol for some times to remove any possible ions, and followed by freeze-drying for 24 h.

2.3. Preparation of PEC sensor

Scheme 1A described the steps for the construction of PEC sensor. Firstly, 5 μ L of 2.5 mg mL⁻¹ MoS₂-P-r-GO suspension (in N,N-dimethylformamide (DMF)) was dropped on the L-GCE surface,

and made it dry at 80 °C. Then, 5 μ L of DMF suspension of Pt-Pd and NH₂-MWCNT consisting of 15 mg mL⁻¹ Pt-Pd NPs and 1 mg mL⁻¹ NH₂-MWCNT was coated on MoS₂-P-r-GO / L-GCE, and dried under same condition. In this way, Pt-Pt NPs-NH₂-MWCNTs / MoS₂-P-r-GO / L-GCE was achieved.



Scheme 1. Diagram of steps for the PEC sensor (A) and detection device (B).

To prepare MIP, Pt-Pt NPs-NH₂-MWCNTs / MoS_2 -P-r-GO / L-GCE were immersed in an acetate buffer solution as its pH value was 5.2 and contained 0.2 mmol L⁻¹ TAP and 0.8 mmol L⁻¹ o-PD. Then, TAP was imprinted in ploy-o-PD film by using CV when the potential was changed from 0 to 800 mV, and the scan rate was kept 50 mV s⁻¹, and the scan segment was 10. When the polymerization was completed, the MIP modified electrode was dried at room temperature. After that, it was immersed in methanol/acetic acid (9/1, V/V) solution for 20 min in order to remove TAP. Thus, MIP / Pt-Pt NPs-NH₂-MWCNTs / MoS₂-P-r-GO / L-GCE was achieved. For comparisons, NIP sensor were prepared as MIP sensor except the absence of TAP, and the MIP / L-GCE, MIP / P-r-GO / L-GCE and MIP / NH₂-MWCNTs / MoS₂-P-r-GO / L-GCE were constructed by changing modified interface.

2.4. Electrochemical measurements

Firstly, the MIP sensor was immersed in a 10 mL PBS (pH= 7.0) containing a certain concentration of TAP, and gently stirred for 200 s. Afterward, it was rinsed with H₂O to remove some molecules by nonspecific adsorption. Then, it was assembled with platinum foil electrode and SCE to form a three-electrode system, which was immersed a new 5 mL PBS (pH= 7.0) containing 0.1 mol L⁻¹ AA in a quartz cell and linked with the electrochemical workstation (Scheme 1B). Next, nitrogen was used to purge oxygen in the electrolyte for 15 min. Finally, photocurrent was recorded under a constant potential of 0 V (versus SCE) as the 405 nm light irradiated on the MIP sensor surface.

3. RESULTS AND DISCUSSION

3.1. Morphological characterization

Firstly, TEM recorded the microstructure of MoS₂-P-r-GO composite (Fig.1A). It was found this composite showed flower shape, and the size of MoS_2 was about 500 nm ~ 1 μ m, which was similar to the previous report on hydrothermal preparation of MoS_2 as the same precursors [23]. To reveal its detailed architecture, its high-magnification TEM (HRTEM) image (Fig.1B) displayed two kinds of sheet structure, which indicated that the bigger sheet spacing represented MoS₂, and the smaller and crooked was P-r-GO, which possessed excellent hybrid architecture, meaning the MoS₂-Pr-GO composite could be facilely obtained by this method. For this, the formation of hybrid architecture was related to the excess amount of thiourea [23] and their sheet structure [24]. The thiourea can be adsorbed on P-r-GO surface, and made MoS₂ grow to form a flower shape, and made this structure stabilize on P-r-GO surface. At the same time, P-r-GO and MoS₂ were all sheet structure, which benefited MoS₂ to grow on P-r-GO surface due to the contact between the nanoflakes. Meanwhile, it was found that P-r-GO possessed porous structure, and the diameters of pore were about $10 \sim 20$ nm (Fig1.C). To further characterize MoS₂, its HRTEM image was recorded (Fig.1D), which indicated that its interplanar spacing was about 0.27 nm from the top-view image, proved it was (100) planes of hexagonal MoS₂, and a curled edge indicated that the layer-to-layer spacing of MoS₂ was about 0.63 nm, which was in accord with the previous report [23].



Figure 1. TEM images of MoS₂-P-r-GO (A), HRTEM image for P-r-GO (B) and MoS₂ (C), and TEM of dendritic Pt-Pd NP (E).

Additionally, the resulting Pt-Pd NPs presented shape with size of 80 ~ 90 nm, and its branches regularly arranged and formed a dendritic structure with the pore diameter of 1 nm (Fig.1E), which was in accord with our previous report [20]. For this, the HDPC play a role of structure directing agent for the growth of Pt-Pd NPs.

Next, SEM images recorded the evolution for the preparation of sensor. It was clearly shown that MoS_2 -P-r-GO nanoflower formed a porous and three-dimensional interface (Fig.2A) on L-GCE. In succession, when the hybrid NH₂-MWCNTs-Pt-Pd NP suspension was dropped on above modified surface (Fig.2B), the NH₂-MWCNT NWs could firmly immobilized dendritic Pt-Pd NPs in their space due to the interaction between amino-group and Pt-Pd NPs (Fig.2B), which was in accord with our previous report [20]. Then, when the TAP was imprinted on above modified electrode, it was clearly observed the formation of MIP film (Fig.2C).



Figure 2. SEM images of MoS₂-P-r-GO / L-GCE (A), NH₂-MWCNTs-dendritic Pt-Pd NPs / MoS₂-Pr-GO / L-GCE (B) and MIP NH₂-MWCNTs-dendritic Pt-Pd NPs / MoS₂-P-r-GO / L-GCE (C).

3.2. Impedance characterization for the preparation of sensor

To evaluate electron transfer capability, the EIS of different modified electrodes was recorded. It was found that the EIS of bare L-GCE presented a small semicircle (Fig.3A(a)), and its resistance (Ret) was about 1500 Ω , but when MoS₂-P-r-GO composite was coated on L-GCE surface, its Ret decreased to about 500 Ω (Fig.3A(b)), which made the electron transfer rate enhance due to the role of P-r-GO, which was similar with CdS-r-GO modified electrode [25]. In succession, Pt-Pd NPs-NH₂-MWCNTs was coated on above surface, the EIS of the resulting electrode was almost a straight line (Fig.3A(c)), meaning the modified electrode provided excellent electron transfer interface. However, when TAP was imprinted on Pt-Pt NPs-NH₂-MWCNTs / MoS₂-P-r-GO / L-GCE, its Ret increased to about 7000 Ω (Fig.3B(a)), indicating that the MIP film blocked the electrochemical reaction of Fe(CN)₆^{3-/4-}. When TAP was removed from above MIP film electrode, its Ret decreased to about 2000 Ω (Fig.3B(b)), but when it captured TAP again, its Ret exhibited a large Ret (5200 Ω) (Fig.3B(c)), meaning TAP increased the resistance of the sensing surface. This change of modified electrode for MIP was in accord with previous report [26]. For this change, template molecule increased the resistance for polymer modified electrode, which indicated the imprinted sites was formed in sengsing surface.



Figure 3. (A) EIS of L-bare GCE (a), MoS_2 -P-r-GO / L-GCE (b) and NH_2 -MWCNTs-Pt-Pd NPs / MoS_2 -P-r-GO / L-GCE (c) in 1.0 mmol L⁻¹ K₃Fe(CN)₆+K₄Fe(CN)₆ containing 0.1 mol L⁻¹ KCl. (B) EIS of MIP / NH₂-MWCNTs-Pt-Pd NPs / MoS_2 -P-r-GO / L-GCE before removing TAP (a), removing TAP (b) and after rebinding TAP (c) in 1.0 mmol L⁻¹ K₃Fe(CN)₆ + K₄Fe(CN)₆ containing 0.1 mol L⁻¹ KCl.

3.3. Photocurrent characterization of different MIP electrodes

Firstly, photocurrent response was employed to evaluate the imprinted effect (Fig.4A). It was found that MIP sensor before the recognition for templates, it had lager photocurrent response for AA (Fig.4A(a)), but when it captured TAP, its photocurrent response became smaller (Fig.4A(a')), which indicated that TAP occupied in MIP film and blocked AA oxidation, which proved imprinted effect was obvious. However, when NIP sensor replaced (Fig.4A(b-b')) MIP sensor under the same conditions, its photocurrent response change was smaller, which indicated the nonspecifically adsorption of TAP on NIP sensing surface was weak. This change was in accord with the previous reports as AA was photocurrent probe [26].

Next, to decipher the role of the modified materials for MIP sensor, their photocurrent responses were compared (Fig.4B). The results indicated that MIP / L-GCE presented a smaller photocurrent response (Fig.4B(a-a')), the reason should be that polymerized o-PD had weak photoactivity to AA, but when the MIP was achieved on P-r-GO / L-GCE, its photocurrent response (Fig.4B(b-b')) was larger than that of MIP / L-GCE because P-r-GO possessed porous structure, which increased the imprinted surface area. When MoS₂-P-r-GO was used to modify L-GCE, the

photocurrent change (Fig.4B(c-c')) was larger than that of P-r-GO / L-GCE, which should be ascribed to the outstanding photoactivity of MoS_2 for AA. When NH_2 -MWCNT was coated on MoS_2 -P-r-GO / L-GCE, the resulting sensor presented higher response because NH_2 -MWCNTs further increased the imprinted area (Fig.4B(d-d')). Meanwhile, when NH_2 -MWCNT space was loaded with Pd-Pt bimetallic NPs, the MIP electrode surface was further enhanced (Fig.4A(a-a')). This phenomenon was similar with MIP / ZnO-Au modifed electrode for pentachlorophenol [26]. For this strategy, the porous r-GO not only played a key role to immobilize MoS_2 and gave a choice for MoS_2 to form a porous and three-dimensional structure. At the same time, MoS_2 had excellent photoactivity response for AA and this structure provided three-dimensional frame to virtually space to load NH_2 -MWCNT and Pt-Pd NPs. Meanwhile, the NH_2 -MWCNT and Pt-Pd NPs further increased imprinted area and enhanced electron transfer ability. Importantly, the modified electrode presented porous and three-dimensional nanostructures, which benefited mass transfer and the growth of polymerization.



Figure 4. Photocurrent for different electrodes in PBS (pH= 7.0) containing 1.0 mol L⁻¹ AA. (A) Electrodes: MIP / NH₂-MWCNTs-Pt-Pd NPs / MoS₂-P-r-GO / L-GCE and NIP NH₂-MWCNTs-Pt-Pd NPs / MoS₂-P-r-GO / L-GCE before rebinding (a and b) and after rebinding in 1.75 × 10⁻⁶ mol L⁻¹ TAP (a' and b'). (B) Electrodes: MIP / L-GCE, MIP / MoS₂-P-r-GO / L-GCE, NH₂-MWCNTs / MoS₂-P-r-GO / L-GCE and MIP / NH₂-MWCNTs-Pt-Pd NPs / MoS₂-P-r-GO / L-GCE and MIP / NH₂-MWCNTs-Pt-Pd NPs / MoS₂-P-r-GO / L-GCE and MIP / NH₂-MWCNTs-Pt-Pd NPs / MoS₂-P-r-GO / L-GCE before rebinding in 1.75 × 10⁻⁶ mol L⁻¹ TAP (a' to d').

3.4. Optimization of experimental variables

3.4.1. Effect of MoS₂-P-r-GO composite

Firstly, the effect of MoS_2 -P-r-GO concentration for the photocurrent was considered as it concentration was 1.5 ~ 3.5 mg mL⁻¹ (Fig.S1A). It was found when its concentration increased from 1 mg mL⁻¹ to 2.5 mg mL⁻¹, the photocurrent response change gradually increased, and achieved a max value at 2.5 mg mL⁻¹, and then decreased with the concentration increasing. This reason was that its concentration increase caused electrode surface increasing, but it could be given a choice for the electrode resistance increasing. Thus, 5 µL of 2.5 mg L⁻¹ MoS₂-P-r-GO was chosen to modify L-GCE.

Then, P-GO concentration for the preparation of MoS_2 -P-r-GO composite was also discussed for the sensor response as its concentration was changed from 0.5 to 1.5 mg mL⁻¹ (Fig.S1B). It was found that the photocurrent response increased as its concentration increased 0.5 to 1.0 mg mL⁻¹, and then decreased gradually. This should be ascribed to the ratio variety of P-r-GO, and MoS₂ caused the change of conductive capacity and photoactive performance. For this, P-r-GO had excellent conductive capacity, but poor photoactive performance. However, the role of MoS₂ was contrary as CdS for hybrid CdS-r-GO modified electrode [25]. Therefore, 1.0 mg mL⁻¹ P-GO was selected to prepare MoS₂-P-r-GO and was balanced point for the photocurrent response for the MIP sensor.

3.4.2. Effect of Pt-Pd NPs concentration

To consider the Pt-Pd NPs for the preparation of MIP sensor, its concentration was varied from 5 mg mL⁻¹ to 15 mg mL⁻¹ as NH₂-MWCNTs concentration kept 1 mg mL⁻¹ (Fig.S1C). When its concentration increased 5 to 15 mg mL⁻¹, the photocurrent response increased. However, when it exceeded 15 mg mL⁻¹, the photocurrent response decreased. This was related to their amount ratio about the imprinted area and resistance of MIP film, when NH₂-MWCNT increased the film thickness also increased, leading to the increasing of resistance of this film according to our previous report [22]. Thus, we chose 10 mg mL⁻¹ dendritic Pt-Pd for the preparation of sensor.

3.4.3 Effect of the mole ratio of TAP to o-PD and polymerization time

The mole ratio of TAP to o-PD was considered as the ratio changed from 1:2 to 1:6 (Fig.S1D). It was found the sensing photocurrent achieved a max response at the ratio of 1:4. For this, it was related to the variety for the number of available binding site in MIP film. The functional monomer played a key role to combine template, when its amount was too little to combine enough TAP. As a result, the number of available binding site was few. However, for this increasing trendy, which resulted the MIP film became thick, making many binding sites became ineffective because it was buried in MIP film. Meanwhile, this trendy also caused the increase of resistance of MIP according to our previous reports [20, 27]. Thus, the mole ratio was set at 1:4 to prepare MIP sensor.

Polymerization time was considered for the MIP sensor responses as it increased from 6 to 14 cycles (Fig.S1E). Result indicated that photocurrent change increased as polymerization time lengthened, but when the time exceed 10 cycles, the photocurrent gradually decreased. For this, it should be that the MIP film became thicker as the time increased, which leaded to the increase of resistance for this film. Therefore, we set 10 cycles as optimal polymerization time for this PEC sensor.

3.4.4. Effect of adsorption time

To discuss the effect of adsorption time for this PEC sensor, it ranged from 60 to 200 s (Fig.S1F) for the sensing response. When the sensor was used to capture TAP in a higher

concentration sample $(1.75 \times 10^{-6} \text{ mol L}^{-1})$, its sensing photocurrent increased with the adsorption time increasing, and achieved a balanced value as the time was about 120 s. When a lower TAP concentration solution (i.e. $5.0 \times 10^{-8} \text{ mol L}^{-1}$) was replaced by this test, the balanced adsorption time value lengthened to 200 s. Thus, we chose 200 s as adsorption time for this PEC sensor.

3.4.5. Effect of pH

The pH value of supporting electrolyte was a key parameter for PEC sensor. For this, it was discussed in the range of pH 6.0 to pH 8.0 (Fig.S1G). Result indicated when pH value was 7.0, the max sensing value was achieved. The reason for this should be that AA was easily to be oxidized in neutral environment.

3.5. The optimal response characteristic of PEC sensor

To evaluate the response characteristic of PEC sensor based on MIP, the photocurrent signals of sensor for TAP were recorded under the optimal experimental conditions. Fig.5A described the sensing photocurrent change increased as the TAP concentration increased, and presented linear responses as TAP concentration was $1.0 \times 10^{-9} \sim 3.5 \times 10^{-6}$ mol L⁻¹, and its regression equation was i_p (μ A) = 0.5159 + 0.4896 C (μ mol L⁻¹), r²= 0.9954) and its detection limit was 5.0×10^{-10} mol L⁻¹ (S/N= 3). This PEC sensor shown a reasonable linear range and a lower detection limit in comparison with these reports [3, 6, 7, 28, 29],the details were listed in Table S1.



Figure 5. (A) Photocurrents of the MIP / NH₂-MWCNTs-Pt-Pd NPs / MoS₂-P-r-GO / L-GCE in PBS (pH= 7.0) containing 1.0 mol L⁻¹ AA after binding different TAP concentrations in the range of $0 \sim 3.5 \times 10^{-6}$ mol L⁻¹. Inset is the calibration curve of TAP. Error bars represented standard deviation (n= 3). (B) Influence of similar compounds on the photocurrent of TAP in PBS (pH= 7.0) containing 0.1 mol L⁻¹ AA under optimal experimental conditions. Solution composition: (a) 1.75×10^{-6} mol L⁻¹ TAP, (b) a + 3.5×10^{-5} mol L⁻¹ florfenicol and (c) a + 3.5×10^{-5} mol L⁻¹ chloramphenicol. Error bars represented SD (n=3).

3.6. Selectivity

Florfenicol and chloramphenicol was employed as similar interferences to evaluate the selectivity of this PEC sensor for TAP. The results indicated that 20-fold of two compounds had almost no interference for the determination of 1.0×10^{-6} mol L⁻¹ TAP, but the NIP sensor had smaller response for these due to the little nonspecificity for these molecules, meaning MIP played a key role for the selectivity for this PEC sensor.

3.7. Stability and repeatability

To study stability and repeatability, this PEC sensor was used to carry out some tests. Firstly, the sensor was employed to successively detect 1.75×10^{-6} mol L⁻¹ TAP to obtain a variation coefficient (3.1 %, n= 5), and its sensing signal still remained up to 97 % of its initial value after 10 successive assays (RSD= 2.7 %). Then, this PEC sensor was stored at room temperature as it was not in use. After two weeks, its sensing signal kept 96.3 % (RSD= 1.9 %, n= 3) of its initial value for 1.75 $\times 10^{-6}$ mol L⁻¹ TAP, which meant the sensor presented good stability. For repeatability, five different electrodes were independently prepared under the same conditions, and resulting PEC sensors were used to detect 1.75×10^{-6} mol L⁻¹ TAP, respectively, and the obtained RSD was 5.8 %, meaning the method possessed the reliability of fabrication procedure.

3.8. Detection of real samples

To estimate the obtained PEC sensor for real sample, it was used to carry out recovery tests for TAP in milk, honey and porcine muscle, which were treated by according to our previous report [22], and the details were described in Supplementary Information. Results were presented in Table 1, which indicated the recoveries for TAP standards added were 90 % to 97 %. At the same time, according to previous report, we used HPLC method to further confirm TAP concentration in porcine muscle sample [3], the results (Table 2) was in accord with the resulting PEC sensor for TAP, meaning it possessed excellent performance for the determination of TAP in real sample.

Samples	Added (μ mol L ⁻¹)	Found (μ mol L ⁻¹)	Recovery (%)	RSD (%, n=5)
	0.00	0.00		
Mille	0.50	0.45	95	2.0
IVIIIK	1.00	0.92	92	1.9
	3.00	0.27	97	1.8
	0.00	0.00		
Honoy	0.05	0.0045	90	1.4
Honey	1.00	0.96	96	2.1
	2.00	1.90	95	2.0
	0.00	0.12		1.8
Porcine muscle	0.50	0.58	94	1.9
	1.00	1.08	90	2.1

Table 1. Recovery tests of TAP in different samples.

Samples	Added (μ mol L ⁻¹)	Found (µmol L ⁻¹)	Recovery (%)	RSD (%, n=5)
	0.00	0.10		7.4
Porcine muscle	0.50	0.56	93	7.3
	1.00	1.00	91	6.1

Table 2. HPLC method for porcine muscle samples

4. CONCLUSIONS

In this work, we designed a L-GCE, which gave PEC sensor to a facile choice to carry on the GCE surface. At the same time, we used one-step hydrothermal method to synthesize MoS_2 -P-r-GO composite, and MoS_2 nanoflower virtually array on P-r-GO surface, which gave choice for NH₂-MWCNTs-dendritic Pt-Pd NPs to fill into the porous space. Then, TAP was imprinted on this three-dimensional electrode by using CV. Thus, a novel PEC sensor for TAP was constructed, which possessed high sensitivity and selectivity for TAP. This work presented a choice for the determination of TAP in real sample.

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ELECTRONIC SUPPLEMENTARY INFORMATION

1. EXPERIMENTAL

1.1. Preparation of porous oxide graphene

100 mL of 0.5 mg mL⁻¹ GO suspension was mixed with 0.5 g KMnO₄ under magnetic stirring for 6 h. The above solution was merged with 30 mL HCl (36 %, wt %) and 30 mL H₂O₂ (30 %, wt %) for 3 h. After that, the products were separated by centrifugation, washed with water and dried in a vacuum oven at 60 °C.

1.2. Preparation of dendritic Pt-Pd NPs

According to our previous report [20], the dendritic Pt-Pd NPs was synthesized when HDPC as a template. Typically, 0.40 mL of 10 mmol L^{-1} Na₂PdCl₄, 0.40 mL of 10 mmol L^{-1} H₂PtCl₆ and 5 mL of 4.0 mg mL⁻¹ HDPC were mixed to form a homogeneous aqueous solution. Then, 0.60 mL of 0.10 mol L^{-1} AA was added into above solution, and kept undisturbed at 85 °C for 3 h. The obtained products were collected by centrifugation and washed with H₂O, followed by freeze-drying for 24 h.

1.3. The treament of samples

The milk samples were treated according to our report [22], which involved protein precipitation and extraction of TAP which involved protein precipitation and extraction of TAP for milk samples, and the similar treatment honey samples except the protein precipitation. Specifically, 10 mL milk sample and 1 mL of TAP standard solution with different concentrations was added in 7 mL water. Then, 2 mL of trichloroacetic acid (20 %, wt %) solution was added to sample for protein precipitation. The mixture was vortexed for 1 min, and then centrifuged at 4000 rpm for 10 min. The supernatant was collected and filtered through a 0.22 μ m membrane filter. After that, the TAP was extracted by using ethyl acetate (the amount is 10 mL for one time) as an extraction liquid for three times. Next, the organic extraction was collected and evaporated to dryness using a gentle stream of nitrogen gas. Afterwards, 10.0 mL PBS (0.10 mol L⁻¹ PBS) was added and vigorously vortexed for 10 s. Then, the resultant PBS containing TAP was transferred in a cell for the recovery determination. For honey samples, 1.00 g sample and 1 mL of TAP standard solution with different concentrations were added into 9.0 mL water. Then, the treatment was similar to that for milk sample except the protein

precipitation. For the porcine muscle samples, 1.00 g sample and 1 mL of TAP standard solution with different concentrations were added into 9.0 mL water. Then, the treatment was similar to that for milk sample.

1.4 HPLC method for the detection of TAP in porcine muscle samples [3]

Chromatography was performed on a Waters Alliance 515 LC System and a Waters multi λ 2475 fluorescence detector (Waters Corp., Milford, MA, USA). The separation was achieved on a LiChrospher C18 column (250 mm × 4.6 mm i.d., 5 µm; Merck KGaA). The column temperature was maintained at 30 °C. The injection volume was 200 µL manually with a 200 µL quantitative ring. The analysis was carried out using acetonitrile (A), 0.01 M sodium dihydrogen phosphate containing 0.005 M sodium dodecyl sulfate and 0.1 % triethylamine, adjusted to pH 4.8 with 85% phosphoric acid (B) (A/B, 35:65, v/v) as the mobile phase, at a flow rate of 1.0 mL min⁻¹. The fluorescence detector of HPLC was set at 224 nm for excitation wavelength and 290 nm for emission wavelength.

2. Figure



Fig. S1 Influence of different factors on the photocurrent change of 1.75×10^{-6} mol L⁻¹ TAP (A to F) in PBS (pH= 7.0) containing 0.1 mol L⁻¹AA. All error bars represent SD (n= 3). (A) P-r-GO-MoS₂ concentration; (B) P- GO concentration; (C) Dendritic Pt-Pd bimetallic NPs concentration; (D) ratio of template molecule to functional monomer; (E) electrochemical polymerization time; (F) adsorption time (a: 1.75×10^{-6} mol L⁻¹; b: 2.0×10^{-8} mol L⁻¹ TAP); (G) pH effect.

3. Table

Table S1 Com	parison of	different	methods	for T	AP o	determination
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Method	Linear range (mol L ⁻¹)	Detection limit (mol L^{-1})	References
RP-HPLC-FLD	$7.0 \times 10^{-9} \sim 1.4 \times 10^{-6}$	4.2×10^{-9}	[3]
UPLC-ESI-MS/MS	$2.8 \times 10^{-9} \sim 2.8 \times 10^{-7}$	1.4×10^{-9}	[6]
GC-NCI/MS	$0 \sim 1.4 \times 10^{-6}$	1.4×10^{-9}	[7]
LC-ESI-MS/MS	$1.2 \times 10^{-9} \sim 1.4 \times 10^{-7}$	3.4×10^{-9}	[28]
LC-MS	$8.4 \times 10^{-9} \sim 2.8 \times 10^{-7}$	2.8×10^{-9}	[29]
Photoelectrochemical Sensor	$1.0 \times 10^{-9} \sim 3.5 \times 10^{-6}$	5.0×10^{-10}	This work

Note:

RP-HPLC-FLD: Reversed-phase high-performance liquid chromatography with fluorescence detection. UPLC-ESI-MS/MS: Ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry.

GC-NCI/MS: Gas chromatography-negative chemical ionization mass spectrometry.

LC-ESI-MS/MS: Liquid chromatography-electrospray ionization-tandem mass spectrometry. LC-MS: Liquid chromatography-mass spectrometry.

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