Xác định dư lượng kháng sinh amoxicillin trong nước thải nuôi trồng thủy sản bằng phương pháp điện hóa sử dụng điện cực nano platin trên nền glassy cacbon

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TÓM TẮT

Platin nano hình hoa (Platinum nanoflowers) được kết tủa lên bề mặt điện cực glassy cacbon (GC) để hình thành điện cực mới (PtNFs/GCE). Đặc tính của điện cực PtNFs/GCE cho việc xác định Amoxicillin (AMX) bằng phương pháp Von - Ampe hòa tan anot sóng vuông (SWASV) đã được nghiên cứu. Sau khi tối ưu hóa các điều kiện thí nghiệm, cường độ dòng đỉnh hòa tan AMX cao nhất thu được trong dung dịch đệm photphat 0,2 M (pH = 7,5) với thời gian làm giàu 150 s, biên độ xung 60 mV và tốc độ quét là 0,25 V.s^{-1}. Giới hạn phát hiện và giới hạn định lượng của phương pháp phân tích được xác định lần lượt là 0,113 µM và 0,372 µM. Trong điều kiện thích hợp, phương pháp đạt được độ tái lặp cao: RSD = 9,65% (n=5). Ảnh hưởng của một số kháng sinh nhóm β-lactam và các chất gây nhiễu có thể có khác đã được nghiên cứu. Cuối cùng, điện cực PtNFs/GCE đã được áp dụng để xác định dư lượng kháng sinh trong nước thải nuôi trồng thủy sản với kết quả hoàn toàn phù hợp về mặt thống kê với phương pháp sắc ký lỏng hiệu năng cao (HPLC).

Từ khóa: *Glassy cacbon, platinum nanoflowers, kết tủa điện hóa, Von – Ampe hòa tan anot sóng vuông, Amoxicillin.*

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Determination of Amoxicillin antibiotic residues in aquaculture wastewater by the electrochemical method using platinum nanoflowers modified glassy carbon electrode

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ABSTRACT

The glassy carbon electrode electrodeposited platinum nanoflowers on the surface to form a new electrode (PtNFs/GCE). The behavior of PtNFs/GCE has been studied for Amoxicillin (AMX) determination by square wave anodic stripping voltammetry (SWASV). After optimizing the experimental and voltammetric conditions, the best stripping peak obtained current intensity in 0.2 M phosphate buffer solution (pH = 7.5) with 150 s accumulation time, 60 mV pulse amplitude, and 0.25 V.s^{-1} scan rate. The limit of detection and quantity was observed as 0.113 μM and 0.372 μM, respectively. Under suitable conditions, the method gained high reproducibility: RSD = 9.65% (n=5). Interference effects of some β-lactam antibiotics and other possible interferent were studied. Finally, the PtNFs/GCE was successfully applied to analyze Amoxicillin antibiotic residues in aquaculture wastewater, resulting in satisfactory statistical agreement with high-performance liquid chromatography (HPLC).

Keywords: Glassy carbon, platinum nanoflowers, electrodeposition, square wave anodic stripping voltammetry, Amoxicillin.

1. INTRODUCTION

Antibiotics are one of the most important drugs used in medicine because they can inhibit or kill bacteria that cause infectious diseases in humans and animals.[1,2] Amoxicillin (AMX) is the most used antibiotic from the β-lactam group to treat bacterial infections that affect humans and other animals. After its action mechanism, 60 - 70% of the drug is excreted and unaltered through urine and feces because of its low metabolism;[3,4] thus, Amoxicillin is rapidly released into the environment.[5] Because of its long persistence in the environment, the drug is considered a severe contaminant is leading to adverse effects on living organisms.

Accordingly, it is necessary to design a quantitative detection approach for AMX. Amoxicillin has been determined by spectrophotometry,[6] capillary electrophoresis,[7] and high-performance liquid chromatography.[8] However, they still have some disadvantages such as complicated procedures, time-consuming, expensive instrument requirement, and low detection sensitivity. Recently, the electrochemical technique has become a promising method used in medicine

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determination because of its high sensitivity and selectivity, fast measurement, low-cost instrument, and simple operation.

In this paper, the authors used the electrochemical technique to investigate the electrochemical behavior of Amoxicillin which is also the first use of the electrochemical method in Vietnam. This research reported the application of platinum nanoflowers modified glassy carbon electrodes (PtNFs/GCE) to detect AMX. It has numerous advantages in electrochemical analysis such as stability, easy fabrication, high conductivity, low background current, and reproducibility.[9,10] By using the electrodes, we obtained good electrochemical signals of AMX.

2. MATERIALS AND METHODS

2.1. Reagents and Apparatus

Reagents: Hexachloroplatinic (IV) acid hexahydrate ($H_2PtCl_6.6H_2O$) used for fabrication of PtNFs/GCE was of pure grade (KGaA, 64271 Darmstadt, Germany). The electrolyte solution comprises 1.0 mM H_2PtCl_6, 0.1 M H_2SO_4 solution, and a $[Fe(CN)_6]^{3-}$ used redox system in 0.2 M phosphate buffer solution pH = 7 for the study of electrochemical properties. Amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S\cdot3H_2O$, AMX) was purchased from Merck (Germany). AMX was prepared daily from a 1 mM stock solution. Phosphate buffer solution (PBS) is the supporting electrolyte of electrochemical measurements.

Apparatus: Square wave anodic stripping voltammetry (SWASV) using PtNFs/GCE were performed on a system Autolab Electrochemical CPA-HH5 (Hanoi, Vietnam), with a three-electrode configuration (GCE and PtNFs/GCE as a working electrode, Ag/AgCl reference electrode, and platinum wire as an auxiliary electrode).

2.2. Preparation of modified electrode

The glassy carbon (GC, d = 3.0 mm) electrode was polished with a water slurry of 0.2 μm Al_2O_3, rinsed with ethanol and water, and then dried. The electrodeposition of platinum nanoparticles on the bare glassy carbon electrode was carried out in an H_2SO_4 0.1 M solution containing 1.0 mM H_2PtCl_6 at a constant potential of -0.2 V and deposition time of 150 s. Following that, the Pt/GCE was gently cleaned with distilled water before use.

2.3. Electrochemical measurements

The formation of PtNFs/GCE and their properties were investigated by the cyclic voltammetric method (CV). Measurements performed in 5 mM $[Fe(CN)_6]^{3-}$ from -0.3 V to 0.7 V at a scan rate of 0.1 V.s^{-1} were used to predict electrochemical features of electrodes and compare their electrochemically active surface areas. The analytical solution containing AMX, 0.2 M phosphate buffer (PBS, pH = 7.5) was put into a cell with the three electrodes. It was determined by square wave voltammetry (SWV) with (0.3, 1.1 V) of the potential range.

All experiments described in this section were performed at room temperature (25 ± 1 °C).

3. RESULTS AND DISCUSSION

3.1. Morphological characterization of the GCE, PtNFs/GCE by SEM

The surface morphology of the bare GCE, PtNFs/GCE are investigated by SEM with different scales. The SEM image shows that the GCE surface has a smooth and homogeneous morphology (Fig. 1a). However, the surface of the electrode's appearance changed dramatically after PtNFs/GCE (Fig. 1b). According to the SEM images (Fig. 1b), the GCE surface is densely developed with Pt nanoflowers. After electrodeposition, many Pt nanoflowers are produced on the GCE surface; the surface of PtNFs/GCE was rougher with irregular cubic nanoflowers shape whose size varies in the range (50 − 400 nm). Electrochemical methods also characterize the PtNFs/GCE.
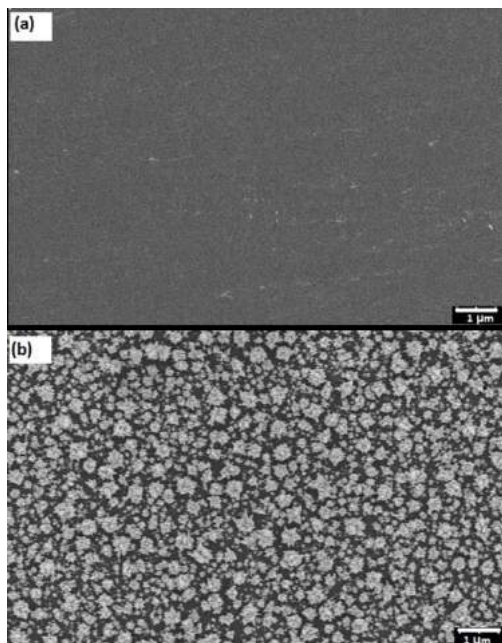
Figure 1. SEM images of GCE (a), PtNFs/GCE(b)

3.2. Formation and characterization of PtNFs/GCE

The PtNFs modified the GCE in 0.1 M H_2SO_4 containing 1.0 mM H_2PtCl_6 solution by a cyclic voltammetry sweep in the range between -0.3 and 1.4 V. Fig. 2 shows the cyclic voltammetric responses of a PtNFs/GCE and bare GCE in 0.2 M phosphate buffer solution (PBS), pH, 7.0 containing 5 mM $K_3Fe(CN)_6$ at a scan rate of 0.1 V.s^{-1}.
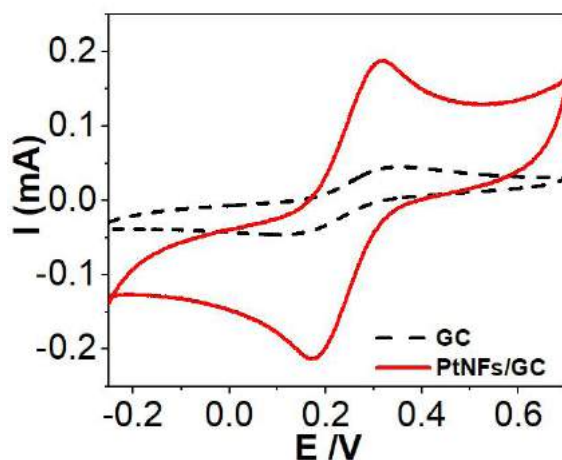

Figure 2. Cyclic voltammograms obtained at a bare GCE and PtNFs/GCE in 0.2 M phosphate buffer solution (PBS), pH, 7.0 containing 5 mM $K_3Fe(CN)_6$ at a scan rate of 0.1 V.s^{-1}.

It could be found that the electrochemical peaks of the bare GCE in the PBS are low. Their electrochemical peaks could also be observed at the PtNFs/GCE, with the intensity increasing significantly. The SEM images that well-dispersed nanoscale Pt particles were deposited on the GCE.

We determined the electrochemically active surface areas (AEAS) of GCE and the PtNFs/GCE with cyclic voltammetry method based on Randles – Sevcik equation[11]:

$$I_p = 2.69 \times 10^5 \times n^{3/2} \times A_{EAS} \times D^{1/2} \times v^{1/2} \times C$$

According to the above equation, the A_{EAS} of the PtNFs/GCE fabricated with the electrodeposition time 150 s under stirring 50 rpm was calculated as 0.184 cm^2 larger than the bare GCE (0.044 cm^2).

The results clearly explain that the modified electrode with platinum nanoflowers is beneficial for improved sensitivity in analysis thanks to the larger active surface area. Thus, the intensity of the stripping peak is increased by almost 4 times that of the bare GCE; AMX could be detected sensitively at the PtNFs/GCE. Therefore, the PtNFs/GC electrode allows for improved selectivity and sensitivity.

3.3. Optimization of experimental parameters

3.3.1. Effect of solution pH

For the development of the analytical method, the influence of the pH of the supporting electrolyte (hydrogen ion concentration) on the oxidation reaction of AMX (20 μM) was simultaneously evaluated by the SWV technique using the PtNFs/GC electrode.
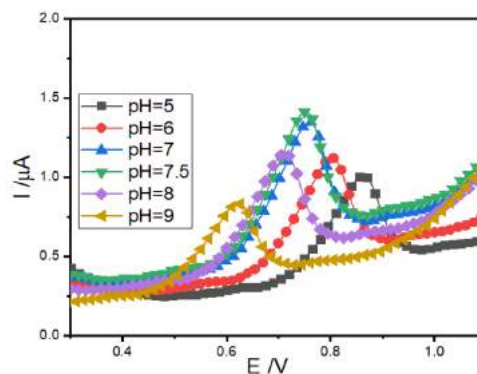

Figure 3. Effects of pH on the stripping peak current of 20 μM AMX on PtNFs/GCE

The pH was investigated in the range of 5.0 - 9.0 with 0.2 M phosphate buffer. The SW potential plots obtained in this investigation are shown in Fig 3. It can be seen that the oxidation peak current for AMX increased until it peaked at pH 7.5 when the pH increased. Therefore, we chose pH 7.5 as the optimal pH value for subsequent experiments.

3.3.2. Effect of accumulation time

There was a linear correlation between the AMX peak current (I_p) and accumulation time (t_{dep}) in the range of 60 - 240 s. The result (Fig. 4) shows that the peak current increased with an increase in accumulation time from 60 to 150 s, which can be attributed to the rapid adsorption of AMX on the surface of the electrode. However, the oxidation peak current was leveled off with a further increase in accumulation time beyond 150 s. It can be ascribed to the saturation of AMX on the surface of the electrode. Consequently, the accumulation time of 150 s was suitable.
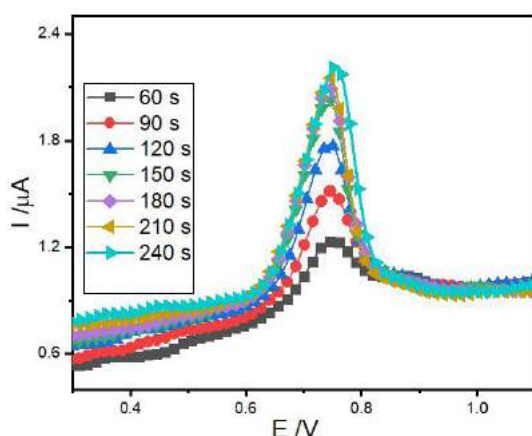

Figure 4. Effects of accumulation time on the stripping peak current of 20 µM AMX on PtNFs/GCE

3.3.3. Effect of scan rate

The Effect of scan rate on the AMX electrochemical responses was investigated by the SWASV technique, varying the potential scan rate from 0.05 to 0.5 V/s. The obtained results show that, as the scan rate increases, the oxidation peak current for AMX increases until it peaks at 0.25 V/s scan rate, the oxidation peak current for AMX declines slightly. Hence, recorded the best peak definition at when 0.25 V/s scan rate.

3.4. Reproducibility, linear range, and limit of detection

Reproducibility: Under the suitable conditions (0.2 M phosphate buffer (pH = 7.5); 20 µM AMX; accumulation time = 150 s: ΔE = 60 mV; v = 0.25 V/s; E_{range} = 0.3 V to 1.1 V, the SWASV with PtNFs/GCE gained high reproducibility of AMX stripping responses. The RSD value of reproducibility was calculated to be 9.65% indicating that the fabrication procedure was reliable (n = 5).

Linear range: The wide linearity range was good in the range of 0.5 − 60.0 µM, with a correlation coefficient of 0.996. The SWASVs and linear regression lines/equations for the AMX are shown in Fig. 5a and 5b, respectively.

Limit of detection (LOD): The limit of detection (LOD) was calculated as 3σ. The detection limits of 0.113 µM were estimated. With the low detection limit mentioned above, the SWASV using PtNFs/GCE can directly detect AMX in the local aquaculture wastewater without a concentration step before analysis.
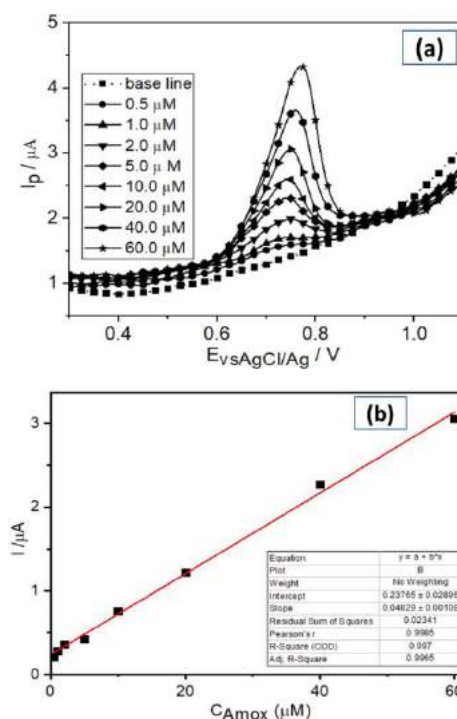

Figure 5. SWVs of AMX samples ranging in concentration from 0.5 to 60.0 µM (a) and relationship between AMX concentrations with corresponding peak current (b)

3.5. Interference study

Some β-lactam antibiotics and another possible interference in the local aquaculture wastewater samples may be adsorbed onto the electrode surface and then influence the stripping response of AMX. We conducted experiments with the presence of variable concentrations of some β-lactam antibiotics and another possible interferent in a solution containing 20 μM AMX in 0.2 M phosphate buffer (pH 7.5). The results show that the maximum concentration of interfering species causes an error of less than ± 12,6% to determine AMX. The results showed that the concentration of ampicillin, penicillin G exceeded 50 times, and the concentration of K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{2+} exceeded 100 times did not affect the determination of the 20 μM AMX. The proposed sensor selectivity for AMX is based on the fact that AMX is the only phenolic penicillin and a medium-spectrum β-lactam antibiotic and that electro-oxidation occurs at the p-hydroxy substituent of AMX[13,14] that is absent from other members of the β-lactam group and also from all other possible inhibitors.

3.6. Analysis of real samples

The SWASV procedure using PtNFs/GCE with suitable conditions was applied to determine AMX in the local aquaculture wastewater at Binh Dinh province, Central Viet Nam.

Some organic compounds affecting the determination of AMX in aquaculture wastewater are removed by the extraction method. Then, all water samples were added to 0.2 M phosphate buffer (pH = 7.5) and determined optimal conditions were. The results in experimental values are shown in Table 1. We also compared the results with the same samples determined from high-performance liquid chromatography (HPLC). As shown in Table 1, the comparative results suggest that the concentrations of AMX were in good agreement with the results found in the HPLC measurement. The method reported in this work is also highly sensitive, reproducible, and accurate. Therefore, the PtNFs/GC electrode is potentially helpful for the analysis of AMX in real samples.

Table 1. Analytical results for the determination of AMX (n = 3) in real water samples

Sample	AMX (μM)			
	Sample location	SWASV	HPLC	Error (%)
Samples of wastewater from Tuy Phuoc shrimp farming	13°50'15.9"N 109°12'46.9"E	1.32 ±0.33	1.21±ND	9.09
Samples of wastewater from Tuy Phuoc shrimp farming	13°50'17.9"N 109°12'50.3"E	1.04±0.36	1.16 ±ND	10.34
Samples of wastewater from Phu My shrimp farming	14°21'51.4"N 109°07'18.3"E	0.76±0.42	0.88±ND	13.64
Samples of wastewater from Phu My shrimp farming	14°23'12.2"N 109°06'45.6"E	0.93±0.27	0.85±ND	9.41

SWASV: square wave anodic stripping voltammetry; HPLC: high-performance liquid chromatography. ND: Not determined.

4. CONCLUSIONS

In this study, a new electrochemical sensor for the determination of AMX antibiotic residues was fabricated simply by the electrochemical method. Traditional electrochemistry and microscopic analysis methods have been applied to characterize platinum nanoflowers electrodeposited on GCE. The electrochemical signals of AMX increased by using PtNFs/GCE. It helps produce the electrochemical Sensor for the determination of AMX at trace concentration.

A simple, sensitive, and inexpensive method for the determination of AMX is proposed. The PtNFs/GCE demonstrated better detection sensitivity and higher SWASV signals than the bare GCE, with a detection limit of about 0.113 µM.

Acknowledgements

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