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Development of electrochemical method for the determination of olaquindox using multi-walled carbon nanotubes modified glassy carbon electrode

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ABSTRACT

A simple and highly sensitive method for the electrochemical determination of olaquindox (OLA) was developed, which was carried out on the multi-walled carbon nanotubes (MWCNT) modified glassy carbon electrode (MWCNT/GCE) using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results showed that MWCNT remarkably enhanced the reduction of OLA, which improved the cathodic peak current of OLA significantly. Under the optimum condition, the linear range for the calibration curve was 0.3–18.0 μg mL^{-1} with a detection limit of 0.26 μg mL^{-1}. The MWCNT/GCE showed a well reproducibility and the relative standard deviation (R.S.D.) was 3.5% ($n=9$). And a great anti-interference ability of the MWCNT/GCE was also observed. Finally, the MWCNT/GCE was satisfactorily employed to analyze some synthetic and real water samples.

1. Introduction

Olaquindox (OLA; 2-(*N*-2-hydroxyethylcarbamonyl)-3-methyl-quinoxaline-N^1,N^4-dioxide) being a well known growth promoting and antibacterial agent was widely used to promote growth, to improve feed efficiency, and to control swine dysentery and bacterial enteritis in the swine industry during the last century. However, its severe phototoxicity, mutagenicity, genotoxicity and carcinogenicity have been demonstrated by large numbers of studies [1–11]. Due to its toxicities and food safety concerns, the Commission of the European Community forbad the usage of OLA as animal growth promoter in 1998 [12]. But OLA is still broadly used as feed additive for swine, poultry and aquatic products in some regions of the world, which has caused wide public concern. During the feeding process and metabolism of animals, OLA will be accumulated in water and soil, which will have potential negative effects on the ecological environment. Therefore, the development of a simple, sensitive, rapid, and reliable method for the on-line monitoring of OLA is of great importance.

Various analytical methods for OLA monitoring have been reported in the literature such as liquid chromatography, liquid chromatography–tandem mass spectrometry, liquid chromatography–electrospray-mass spectrometry [13–18], spectrophotometry [19–21] and single-sweep oscillopolarography [22]. The chromatographic method is a common method to test OLA, which has high accuracy and recovery, well reproducibility, and reliable ability of qualitative analysis, but the main problems encountered in using such methods are either the need for derivatization or the need for time-consuming extraction procedures and high cost. There were also some reports on the determination of OLA by spectrophotometry in the last century, but the background interference and low sensitivity restrict its application especially in the trace analysis of OLA. The electrochemical method has great potential for environmental monitoring because of its inherent advantages such as fast response speed, sensitivity, ease of miniaturization, low cost, simplicity, timesaving and in vivo real-time determination [23]. Especially single-sweep polarography and pulse voltammetry (DPV), have high sensitivity and excellent selectivity, make it possible to decrease the analysis time as compared to the time exhaustive chromatographic methods. The literature reported that the mercury film electrode was used in the determination of OLA by single-sweep oscillopolarography [22], and the detection limit was low (0.0026 μg mL^{-1}), but the mercury is so poisonous that can pose a great threat to the environment and human health. Therefore, building an environment-friendly electrochemical analysis method of OLA is urgently needed.

Carbon nanotubes (CNTs), ever since their discovery, have attracted extensive attention due to their unique physicochemical and electrical properties. Because of their ability to promote charge transfer reactions, CNTs are considered to be extremely superior electrode material and have been widely utilized for the modification of electrodes [24–27].

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However, literatures on the electrochemical techniques for OLA determination are limited. As far as we know, studies on the application of the multi-walled carbon nanotubes (MWCNT) modified glassy carbon electrode (MWCNT/GCE) for OLA determination has not been reported. The proposed method has advantages such as no time-consuming sample preparation step prior to OLA assay, high sensitivity, rapid response, good reproducibility, and low detection limit compared to other reported methods. In this paper, the electrochemical behavior of OLA on the MWCNT/GCE was studied. The conditions of OLA determination by difference pulse voltammetry (DPV) were optimized, the interference experiment was carried out, and some synthetic and actual water samples were determined by the proposed method. An attempt was made to build an electrochemical analysis method of OLA.

2. Materials and methods

2.1. Apparatus and chemicals

Cyclic voltammetry (CV) and DPV were performed using a CHI 660D electrochemical workstation (Chenhua Instrument Company of Shanghai, China) coupled with a conventional three-electrode cell. The working electrode was the MWCNT/GCE (3 mm diameter), the auxiliary electrode platinum wire, and the reference electrode saturated calomel electrode (SCE). A S-3C Model pH meter (Shanghai Precision Scientific Instrument Co., China) was used for measuring the pH of solutions.

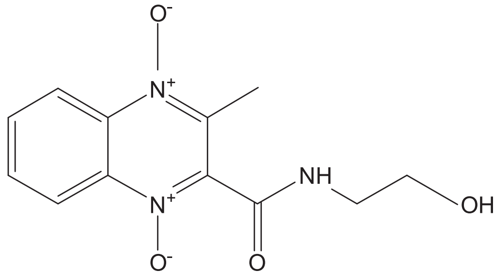
MWCNT (length 10–30 μm; specific surface area $>500\ m^2\ g^{-1}$; outer diameter <8 nm; 95% purity) was purchased from Chengdu Organic Chemicals Co., Ltd., Chinese Academy of Sciences. The analytical standard OLA ($C_{12}H_{13}N_3O_4$; MW 263.25; solubility at 25 °C: 5 mg mL^{-1} in water; CAS no. 23696-28-8; 98.4% purity) was purchased from China Institute of Veterinary Drug Control (Beijing, China), and its molecular structure is shown in Scheme 1.

The analytical reagent grade *N*,*N*-dimethylformamide (DMF) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade and purchased from Shenyang Chemical Company, China. Ultrapure water used throughout experiments was purified using a Sartorius Arium 611 system (Sartorius, Göttingen, Germany).

A 600.0 mg mL^{-1} stock standard solution of OLA was prepared by dissolving 609.8 mg OLA in ultrapure water and diluting to the mark in a 250 mL brown volumetric flask, and was kept in the dark below 4 °C. A 3.0 mg L^{-1} MWCNT–DMF dispersion was prepared by dissolving 3.2 mg MWCNT in 1 mL DMF by ultrasonication for 60 min.

2.2. Construction of the MWCNT/GCE

The GCE was polished with three grades of α-Al_2O_3 slurries (1.0, 0.3 and 0.05 μm) respectively, followed by sonicated in anhydrous ethanol and ultrapure water after each stage of polishing successively. A 23 μL drop of 3 mg L^{-1} MWCNT-DMF dispersion was coated on the surface of GCE, and then the solvent was evaporated under the infrared lamp to obtain the MWCNT/GCE.



Scheme 1. Molecular structure of OLA.

2.3. Procedure for OLA analysis

The electrochemical behavior of OLA was studied by CV; the determination of OLA was performed by DPV. All the experiments were carried out in a conventional electrochemical cell holding 5.00 mL 0.06 mol $L^{-1}H_2SO_4$ (pH 1.1) and certain amount of OLA at room temperature (25 ± 1 °C). Before OLA analysis, the experimental solution was deaerated by highly pure nitrogen for 10 min. The CV was recorded from −0.60 to 0.80 V at a scan rate of 0.1 V s^{-1}; the DPV was recorded from −0.60 to 0.80 V (with an amplitude of 50 mV and a pulse period of 0.5 s), and the cathodic peak current at −0.280 V was chosen for the quantitative analysis of OLA.

2.4. Water samples

The real water samples were collected from two different rearing ponds in Yuhong Borough Shenyang City (real Sample 1 and Sample 2), one aquatic product market in Huanggu Borough Shenyang City (real Sample 3), the Xinkai River in Shenyang City (real Sample 4) and the North Canal in Shenyang City (real Sample 5). Four synthetic water samples were prepared by dissolving a certain amount of OLA and 20-fold common existing substances with ultrapure water. Suitable amounts of 1.0 mol L^{-1} HCl were added to adjust the pH of real samples to be 2 for storage. All above samples were stored at 4 °C in low-density polyethylene (LDPE) bottles.

3. Results and discussion

3.1. Electrochemical behavior of OLA

The CVs of OLA on the bare GCE and the MWCNT/GCE in 0.06 mol $L^{-1}H_2SO_4$ (pH 1.1) are illustrated in Fig. 1. As can be seen, no visible signal was found on the bare GCE (Curve a) but two sharp and well-resolved reduction peaks at –0.225 and –0.406 V (Curve c; P1, P2) were found on the MWCNT/GCE,

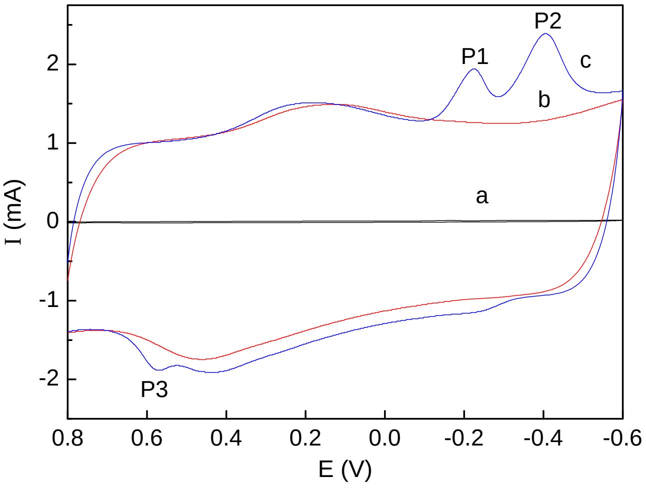


Fig. 1. CVs of OLA in 0.06 mol L^{-1} pH 1.1H_2SO_4 solution (C_{OLA}: 5.9 μg mL^{-1}; a: OLA on the bare GCE; b: blank on the MWCNT/GCE and c: OLA on the MWCNT/GCE). Scan rate 0.1 V s^{-1}, quiet time 2 s.

suggesting that the MWCNT has a strong enhancement ability for the electric signal of OLA. At low scan rates, the reoxidation peaks, which are relative to P1 and P2, were not found. But at high scan rates, the corresponding reoxidation peak appeared which was extremely weak and broad, indicating the irreversible electrode process.

Useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of OLA at different scan rates from 0.05 to 0.2 V s^{-1} was studied and shown in Fig. 2. There is a good linear relationship between cathodic peak current and scan rate. Take P2 for instance, the equation for this line is $I_{P2}=0.0105\,\nu-2.075\times10^{-4}$; $r=1$. This indicates that the electrode process was controlled by adsorption rather than diffusion. A plot of logarithm of reduction peak current (P2) versus logarithm of scan rate gave a straight line with a slope of 1.2615 (Fig. 3A) close to the theoretical value of 1.0, which is expected for an ideal reaction for the adsorption-controlled electrode process [28].

As for an adsorption-controlled and irreversible electrode process, the relationship between peak potential and the scan rate follows Laviron's equation [29]:

$$E_{Pc}=E^{o\prime}+\frac{RT}{\alpha nF}\ln\frac{RTk_s}{\alpha nF}-\frac{RT}{\alpha nF}\ln\nu \qquad (1)$$

where E_{Pc} is the cathodic peak potential (V), $E^{\circ\prime}$ is the formal potential (V), α is the electron-transfer coefficient, k_s is the standard rate constant of the surface reaction (s^{-1}), ν is the scan rate (V s^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is temperature (K) and F is Faraday constant (96500 C mol^{-1}). The electron-transfer coefficient (α) can be calculated from Laviron's equation. For OLA, the electron number n is equal to 2 [22,30] in each reaction step. Fig. 3B showed the plots of E_P versus ln ν, the equations of the straight line were

$$E_{P1}=-0.3160-0.0482\ln\nu;\ r_1=-0.992 \qquad (2)$$

$$E_{P2}=-0.5490-0.0724\ln\nu;\ r_2=-0.989 \qquad (3)$$

The values of α for P1 and P2 can be obtained from the slope respectively: $\alpha_1=0.266$, $\alpha_2=0.177$. The value of k_s can be determined from the intercept if the value of $E^{\circ\prime}$ is known. The value of $E^{\circ\prime}$ in Eq. (1) can be obtained from the intercept of the E_p versus υ curve by extrapolating to the vertical axis at $\upsilon=0$. In our system, for P1, $E^{\circ\prime}$ was obtained to be −0.156 V, k_s was calculated to be 0.750 s^{-1}; for P2, $E^{\circ\prime}$ was −0.308 and k_s was 0.495 s^{-1}.

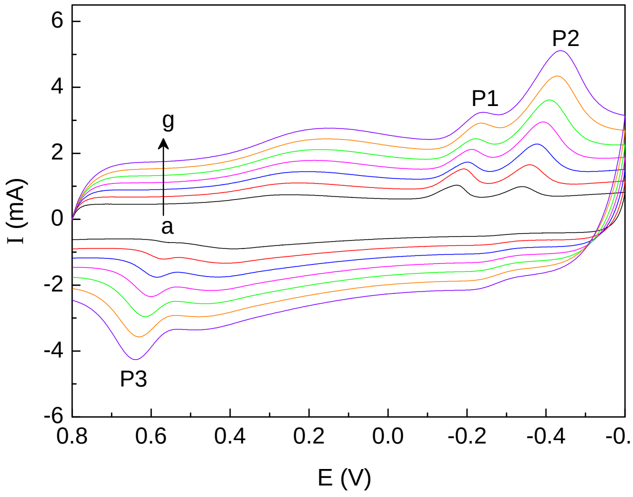


Fig. 2. CVs of OLA at different scan rates. C_{OLA}: 5.9 μg mL^{-1}; the scan rate from (a) to (g) respectively (V s^{-1}): 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, 0.2. Other conditions as in Fig. 1.

3.2. Mechanism of OLA electro-reduction

In 0.06 mol L^{-1}H_2SO_4 (pH 1.1) OLA existed as a cation due to the protonation of the amino [31]. Usually two electrons are involved in each step of OLA reduction on the electrode [22,30]. The two reduction peaks in the cyclic voltammogram (Fig. 1; P1, P2) indicated that OLA was reduced in two steps. The coordination group N→O on the quinoxaline ring and the carbonyl in the side chain are probably the groups reduced, and it is a two-electron/two-proton process (Scheme 2).

3.3. Effect of supporting electrolytes and pH

The following determination of OLA and its condition optimization were performed by DPV, and the cathodic peak chosen for the quantitative analysis is relative to P2 in Fig. 1.

Table 1 showed the experimental results of the determination of OLA in several different electrolytes (H_2SO_4, HNO_3, H_3PO_4, HCl, citric acid–sodium citrate, CH_3CO_2H–CH_3CO_2Na and Na_2HPO_4–NaH_2PO_4). Only in the strongly acidic electrolytes could the electric signal of OLA be observed. Among the four acids, 0.06 mol L^{-1}H_2SO_4 (pH 1.1) showed the most sensitive and stable current response for OLA. Besides it was found that with the pH of 0.06 mol L^{-1}H_2SO_4 gradually increased and the peak current decreased till OLA electric signal disappeared. So the original 0.06 mol L^{-1}H_2SO_4 (pH 1.1) was chosen as a supporting electrolyte in this experiment.

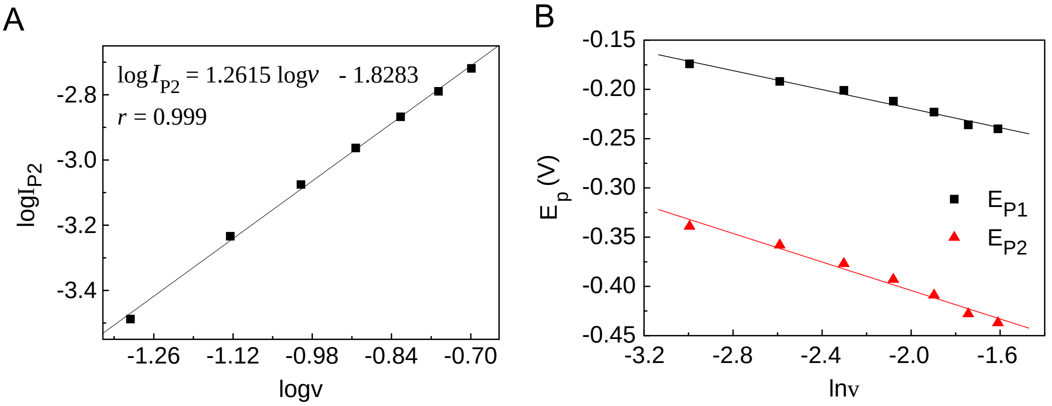
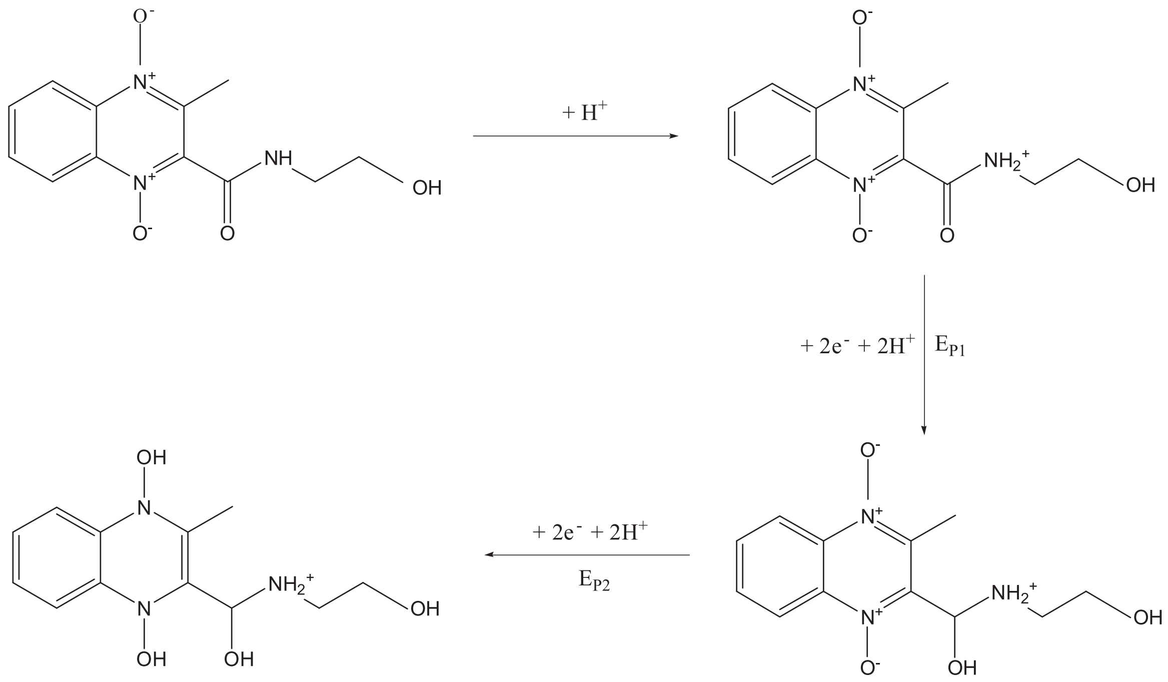


Fig. 3. Dependence of the peak current on the scan rate (A) and dependence of the peak potential on the scan rate (B).



Scheme 2. Proposed mechanism of electro-reduction of OLA on the MWCNT/GCE.

Table 1
Effect of supporting electrolytes on the peak current of OLA on the MWCNT/GCE (C_{OLA}: 5.9 μg mL^{-1}, in 0.06 mol L^{-1} pH 1.1H_2SO_4 solution). Amplitude 50 mV, pulse period 0.5 s, quiet time 2 s.

Supporting electrolytes (0.06 mol L^{-1})	pH	I_p ($\times 10^{-4}$ A)
H_2SO_4	1.1	1.109
HNO_3	1.2	1.058
H_3PO_4	1.1	0.926
HCl	1.2	0.760
Citric acid–sodium citrate	3.0	–[a]
CH_3CO_2H–CH_3CO_2Na	5.0	–
Na_2HPO_4–NaH_2PO_4	7.0	–

[a] No OLA signal.

3.4. Effect of the amount of modifier

Fig. 4 showed the effect of the amount of MWCNT–DMF dispersion on the peak current. It was found that the peak current increased with the increasing amount of modifier, and when the amount exceeded 23 μL, the current decreased. Therefore, the modifier amount 23 μL was selected in the experiment.

The influence of the modifier amount on the peak current is related to the thickness of the film. Too thin film would decrease the adsorption amount of OLA on the electrode surface, resulting in the small peak current. On the contrary, too thick film would make the conductivity of electrode reduced and the film unstable as MWCNT could leave off the electrode surface. So the peak current also decreased.

3.5. Performance of the MWCNT/GCE for OLA determination

Under the optimum condition, the performance of the MWCNT/GCE for OLA determination using DPV was studied. DPVs obtained with increasing concentrations of OLA showed that the peak current increased linearly with increasing concentration as shown in Fig. 5. The linear equation was $I_P=0.0163C+0.00524$ ($r=0.996$) with the linear range of 0.3–18.0 μg mL^{-1}, and the detection limit was 0.26 μg mL^{-1} based on three times the background noise. The MWCNT/GCE showed a well reproducibility and the relative standard deviation (R.S.D.) was 3.5%, which was evaluated by nine repetitive measurements of 5.9 μg mL^{-1} OLA.

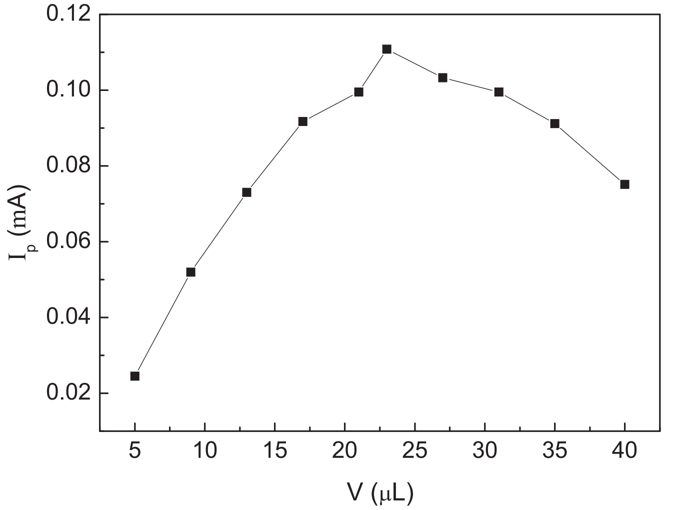


Fig. 4. Effect of the amount of modifier on the peak current of OLA on the MWCNT/GCE (C_{OLA}: 5.9 g mL^{-1}). Other conditions as in Table 1.

The comparison of the MWCNT/GCE with some other methods for the determination of OLA was listed in Table 2, which showed good stability and wide linear range of the proposed method. Compared to the mercury film electrode, the MWCNT/GCE is much more environment-friendly.

3.6. Interference studies

Under the optimum experimental conditions, the influence of various substances as potential interference component of fishery water on the determination of 5.9 μg mL^{-1} OLA was evaluated. The results (Table 3) showed that the peak current of OLA is not affected by all common existing substances under experimental

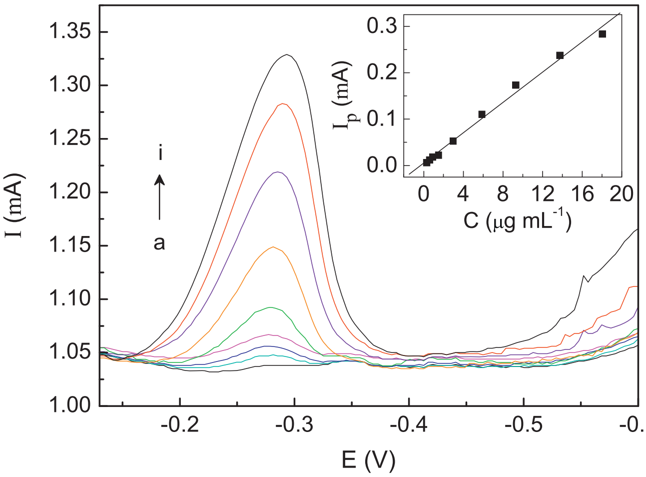


Fig. 5. DPVs of OLA at different concentrations; insert: the related calibration curve. The concentration of OLA from a to i respectively (μg mL^{-1}): 0.3, 0.6, 0.9, 1.5, 3.0, 5.9, 9.3, 13.7, 18.0. Other conditions as in Table 1.

Table 2
Comparison of the proposed method with the other OLA-determination methods.

Methods	Linear range (μg mL^{-1})	Detection limit	References
Liquid chromatography	0.002–0.1	40 μg kg^{-1} [a]	[13]
Liquid chromatography	2–20	2.0 mg kg^{-1}	[14]
Liquid chromatography–tandem mass spectrometry	1–20	40 μg kg^{-1} [b]	[17]
Liquid chromatography–electrospray-mass spectrometry	0.5–10	0.20 μg mL^{-1}	[18]
Spectrophotometry	5–30	–	[19]
Spectrophotometry	4–16	–	[20]
Spectrophotometry	1–16	–	[21]
Mercury film electrode	0.0105–5.265	0.0026 μg mL^{-1}	[22]
MWCNT modified GCE	0.3–18.0	0.26 μg mL^{-1}	Present work

[a] Based on two times the background noise.
[b] Based on 10 times the background noise.

Table 3
Influence of common substances on the determination of OLA (C_{OLA}: 5.9 μg mL^{-1}).

Influences	Tolerance limits ($C_{influences}/C_{OLA}$)
NO_3^-, PO_4^-, Cl^-	1200
K^+, Na^+	800
Mg^{2+}	650
NH_4^+	550
Ca^{2+}, tetracyclines	400

Table 4
Determination of OLA in water samples.

Samples	Added (μg mL^{-1})	Detected (μg mL^{-1})[a]	Recovery (%)
Synthetic Sample 1	0.6	0.55 ± 0.02	92
Synthetic Sample 2	0.9	0.97 ± 0.03	108
Synthetic Sample 3	1.5	1.64 ± 0.05	109
Synthetic Sample 4	3.0	2.90 ± 0.09	97
Real Sample 1	0	Not detected	–
	3.0	2.85 ± 0.10	95
Real Sample 2	0	Not detected	–
	6.0	6.28 ± 0.22	105
Real Sample 3	0	Not detected	–
	12.0	11.53 ± 0.40	96
Real Sample 4	0	Not detected	–
	3.0	3.19 ± 0.12	106
Real Sample 5	0	Not detected	–
	3.0	3.08 ± 0.10	103

[a] Average of seven replicate measurements (average ± standard deviation).

conditions, suggesting a great anti-interference ability of the MWCNT/GCE.

3.7. Application

To evaluate the practical application of the proposed method, the MWCNT/GCE was used to determine four synthetic water samples and five real water samples. The synthetic samples were prepared by dissolving a certain amount of OLA and 20-fold common existing interferences (Table 3) with ultrapure water. Real Sample 1 and Sample 2 were from rearing ponds, real Sample 3 was from the aquatic product market, and real Sample 4 and Sample 5 were from rivers. The data presented in Table 4 showed the satisfactory results.

4. Conclusion

In this paper, a rapid, simple and highly sensitive method for the electrochemical determination of OLA was developed, which was carried out on the MWCNT/GCE using DPV. The results showed that MWCNT remarkably enhanced the reduction of OLA, which improved the cathodic peak current of OLA significantly. Under the optimum condition, the calibration curve was $I_P = 0.0163C + 0.00524$ ($r = 0.996$) with the linear range of 0.3–18.0 μg mL^{-1} and the detection limit was 0.26 μg mL^{-1}. The MWCNT/GCE showed a well reproducibility with the relative standard deviation (R.S.D.) of 3.5% ($n = 9$) and a great anti-interference ability. Finally, the MWCNT/GCE was satisfactorily employed to analyze some synthetic and actual fishery water samples. The results obtained in this work suggest that the proposed electrochemical analysis method using the MWCNT/GCE has a potential application for the on-line monitoring of OLA.

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