

Square Wave Voltammetric Determination of Indole-3-acetic Acid Based on the Enhancement Effect of Anionic Surfactant at the Carbon Paste Electrode

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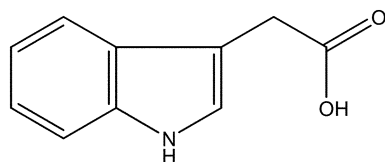
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Sodium dodecyl sulfate (SDS), an anionic surfactant, can strongly adsorb at the surface of a carbon paste electrode (CPE) via the hydrophobic interaction. In pH 3.0 Na₂HPO₄-citric acid buffer (McIlvaine buffer) and in the presence of SDS, the cationic indole-3-acetic acid (IAA, pK_a = 4.75) was highly accumulated at the CPE surface through the electrostatic interaction between the negative-charged head group of SDS and cationic IAA, compared with that in the absence of SDS. Hence, the oxidation peak current of IAA increases greatly and the oxidation peak potential shifts towards more negative direction. The experimental parameters, such as pH, varieties of surfactants, concentration of SDS, and scan rate were optimized for IAA determination. The oxidation peak current is proportional to the concentration of IAA over the range from 5 × 10⁻⁸ mol/L to 2 × 10⁻⁶ mol/L. The detection limit is 2 × 10⁻⁸ mol/L after 3 min of accumulation. This new voltammetric method was successfully used to detect IAA in some plant leaves.

Key Words : Indole-3-acetic acid, Surfactant effects, Carbon paste electrode, Voltammetric determination

Introduction

Auxins were discovered early in the twentieth century as plant-regulating substances. Indole-3-acetic acid (IAA) with the following molecule structure is a naturally occurring



auxin with broad physiological effects, such as regulating division, elongation and differentiation of cells. Recently, determination of IAA attracts increasing interest, and a large variety of methods including high performance liquid chromatography (HPLC),¹⁻³ gas chromatography (GC),⁴⁻⁶ capillary electrophoresis^{7,8} as well as immunoassays such as enzyme-linked immunosorbent assay (ELISA) and radio immunoassay (RIA),^{9,10} have been reported. Capillary electrophoresis and chromatographic methods including HPLC and GC have excellent selectivity and high sensitivity, but they require very expensive instruments and complicated sample treating. Although the detection limit and determining accuracy of immunoassay such as ELISA and RIA are very attracting, the analytical process is very complex and time-consuming. Therefore, it is very necessary and important to develop a fast, sensitive and simple analytical technique for the determination of IAA. Compared with the above-mentioned analytical methods, electrochemical method has some advantages such as rapid response, high sensitivity, extreme simplicity and low-cost requirements. To date, some

electrochemical techniques have also been developed for the determination of IAA. For instance, carbon fiber ultramicro-electrode¹¹ and OV-17 modified carbon paste electrode¹² were used to detect IAA. However, the determining sensitivity of these electrochemical methods is not enough high since the detection limit is about 10⁻⁷ mol/L.

In this paper, a voltammetric method for the determination of IAA based on the surfactant effects is reported. Surfactant, with a long hydrophobic chain and a hydrophilic head group, can alter the properties of electrode/solution interface and finally influence the electrochemical process of electroactive species.¹³⁻¹⁶ In pH 3.0 McIlvaine buffer, IAA exists in the cationic species. Due to the electrostatic interaction between cationic IAA and the negative-charged head group of SDS, IAA was induced to preconcentrate at the CPE surface, and consequently its electrode surface concentration improves sharply. Finally, the oxidation peak current of IAA increases remarkably and the oxidation peak potential shifts towards more negative potential in contrast to that in the absence of SDS. A sensitive and simple voltammetric method was developed for the determination of IAA. Compared with other published electrochemical methods, this new-proposed method possesses very high sensitivity and the detection limit is as low as 2 × 10⁻⁸ mol/L.

Experimental Section

Reagents. IAA (BoAo Biotechnology Corporation in Shanghai, China) was dissolved into ethanol to prepare 1 × 10⁻³ mol/L standard stock solutions. All types of long-chain surfactants (Shanghai Reagent Corporation, China) were made into 1 × 10⁻² mol/L aqueous solutions. Spectroscopic graphite powder and paraffin oil were purchased from Shanghai Reagent Corporation, China. Other chemicals used

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were of analytical reagents. All the chemicals were used without further purification.

Apparatus. All the electrochemical measurements were performed with a CHI 660A Electrochemical Workstation (CH Instrument, USA). The active surface area of the carbon paste electrode (CPE) is 0.071 cm^2 . A three-electrode system, including CPE working electrode, a platinum wire counter electrode and a saturated calomel reference electrode (SCE), was employed.

Preparation of the CPE. The carbon paste electrode was prepared by mixing 50 mg graphite powder and 20 μl . paraffin oil in a small mortar, and this mixture was then homogenized. After that, the paste was pressed manually into the cavity of the electrode body, and the surface was smoothed against clean paper. Unless otherwise stated, the paste was carefully removed prior to pressing a new portion into the electrode after every measurement.

Determination of IAA. The carbon paste electrode was activated in pH 3.0 Na_2HPO_4 -citric acid buffer (Mellvaine buffer) using successive cyclic sweep from 0.0 V to 1.1 V until the cyclic voltammograms was stable. After that, a known concentration of IAA and $4 \times 10^{-4} \text{ mol/L}$ SDS were added into, and the square wave voltammograms were recorded after 3 min open-circuit accumulation. The oxidation peak current was measured at 0.66 V.

Results and Discussion

Electrochemical behaviors of IAA. Representative cyclic voltammograms of IAA in pH 3.0 Na_2HPO_4 -citric acid buffer at the carbon paste electrode (CPE) are presented in Figure 1. In the initial anodic sweep from -0.10 to 1.10 V, two oxidation peaks, I_a and II_a , were observed for $5 \times 10^{-5} \text{ mol/L}$ IAA. The peak potential (E_p) for peak I_a is 0.77 V; for peak II_a , $E_p = 0.90 \text{ V}$. On the reversal sweep from 1.10 to -0.10 V, a reduction peak III_c appeared. However, another oxidation peak III_a , apart from the two oxidation peaks I_a and II_a , was observed on the second anodic sweep, which formed

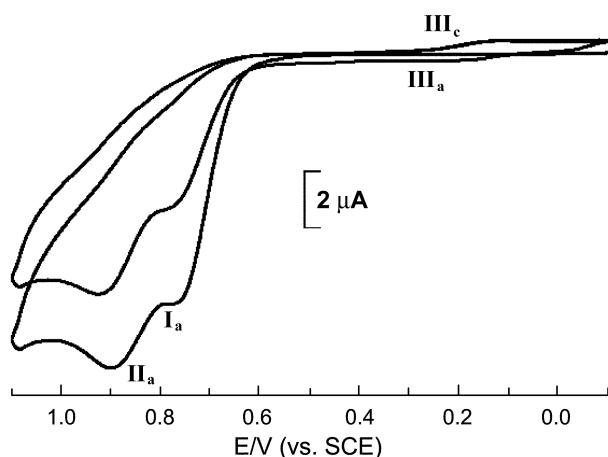


Figure 1. Cyclic voltammograms of $5 \times 10^{-5} \text{ mol/L}$ IAA in pH 3.0 Mellvaine buffer (0.1 mol/L) at carbon paste electrode. Scan rate is 100 mV/s.

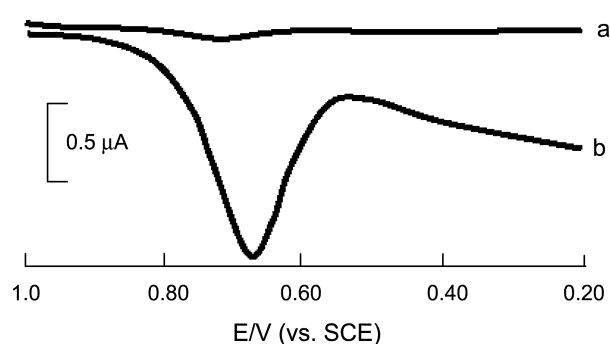


Figure 2. SW voltammograms of $5 \times 10^{-7} \text{ mol/L}$ IAA in pH 3.0 Mellvaine buffer. Curve a: in the absence of SDS, and curve b: in the presence of $4 \times 10^{-4} \text{ mol/L}$ SDS. SW amplitude is 50 mV, SW frequency is 15 Hz and SW step is 4 mV, accumulation time: 3 min.

a reversible couple with III_c . The E_p for peak III_c is 0.13 V, for peak III_a , $E_p = 0.19 \text{ V}$. If the initial anodic sweep is reversed before 0.90 V (*i.e.* before peak II_a), the redox couple $\text{III}_c/\text{III}_a$ will disappear, indicating that the $\text{III}_c/\text{III}_a$ redox couple results from the electrode reaction of the oxidation product of peak II_a .

As previously reported,¹⁷ the electrochemical behaviors of IAA are closely related to its concentration. At low concentration of IAA (such as lower than $5 \times 10^{-6} \text{ mol/L}$), the oxidation peak II_a vanishes and just an oxidation peak I_a is observed. There is no exception in this work. Moreover, the oxidation peak I_a is more sensitive. Therefore, the oxidation peak I_a was chosen as the analytical signal for the determination of IAA in the following studies.

The oxidation peak current of $5 \times 10^{-7} \text{ mol/L}$ IAA at the carbon paste electrode in the absence and presence of SDS were compared by square wave voltammetry (SWV). In the absence of SDS, the oxidation peak current of IAA, which at about 0.72 V, is very low (Fig. 2a). However, the oxidation peak current increases significantly in the presence of SDS, meanwhile, the oxidation peak potential shifts negatively to 0.66 V (Fig. 2b). In conclusion, SDS can significantly improve the determining sensitivity of IAA and make the electron transfer of IAA more easily.

Effect of pH. The pH value of buffer solution heavily affects the existing form of IAA and further influences its oxidation peak current. The oxidation peak current of $1 \times 10^{-6} \text{ mol/L}$ IAA in the presence of $4 \times 10^{-4} \text{ mol/L}$ SDS over the pH range from 2.0 to 8.0 were examined using linear sweep voltammetry (LSV). As can be seen from Figure 3, the oxidation peak current of IAA increased gradually as pH decreasing from 8.0 to 3.0, and then kept stable when pH shifting from 3.0 to 2.0. When pH is higher than 4.75, IAA ($\text{p}K_a = 4.75$) exists in anionic form. According to the principle of electrostatic interaction, IAA will be repulsed by SDS, and can not reach the CPE surface. This makes the electron transfer between CPE and IAA more difficult. Therefore, the peak current of IAA decreases in the presence of SDS as pH value is higher than 4.75. On the contrary, IAA exists in cationic form when pH is lower than 4.75, and is attracted by the SDS. Consequently, the peak current

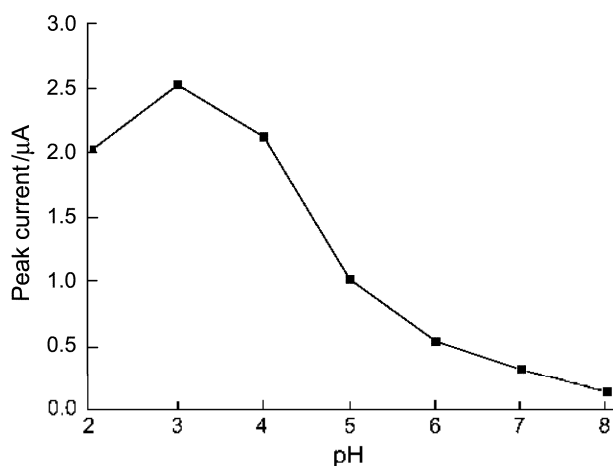


Figure 3. The relationship between the oxidation peak current of 1×10^{-6} mol/L IAA and pH in the presence of 4×10^{-1} mol/L SDS. Other conditions are the same as in Fig. 2.

increases due to the electrostatic interaction. In this work, pH 3.0 Na_2HPO_4 -citric acid buffer (Mellvaine buffer) is chosen as determining medium.

Surfactant effects. The oxidation peak current of 1×10^{-6} mol/L IAA in the presence of various long-chain surfactants, such as anionic sodium dodecyl benzene sulfonate (SDBS), sodium dodecyl sulfate (SDS); cationic cetyltrimethylammonium bromide (CTAB), stearyltrimethylammonium bromide (STAB), cetylpyridine bromide (CPB); and nonionic Triton X-100, were examined, and the results shown in Table 1. In the presence of cationic and nonionic surfactants, the oxidation peak currents of IAA decrease and the peak potential shifts positively in comparison with that in the absence of surfactant. Cationic surfactants, which also adsorb at the CPE surface, will reject cationic IAA. Hence, the electrochemical oxidation of IAA becomes harder in the presence of cationic surfactants. Nonionic surfactant also can adsorb at the CPE surface and block the mass transportation as well as the electron transfer of IAA. Undoubtedly, the oxidation peak current of IAA shows decline in the presence of nonionic surfactants, too. However, in the presence of anionic surfactants, such as SDS and SDBS, the peak current increases greatly accompanied by peak potential shifting to more negative direction, which caused

Table 1. The oxidation peak current and potential of 1×10^{-6} mol/L IAA in the presence of different 4×10^{-1} mol/L surfactants

Surfactants	Oxidation peak current (μA)	Oxidation peak potential (V)
No surfactant	0.26	0.72
Anionic surfactants	SDBS	2.48
	SDS	2.52
Cationic surfactants	CTAB	0.18
	STAB	0.16
	CPB	0.20
Nonionic surfactant	Triton X-100	0.18

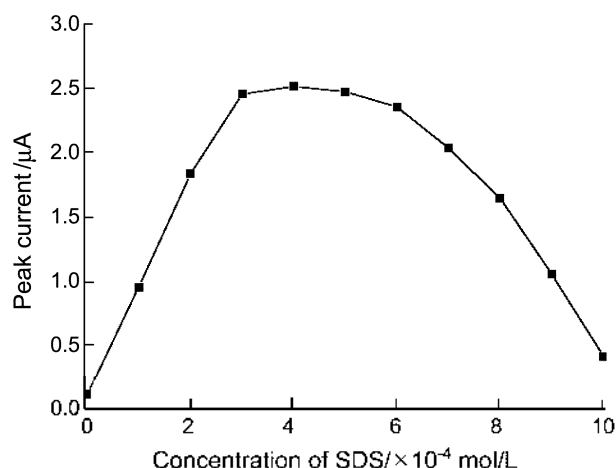


Figure 4. Plots of the oxidation peak current of IAA versus concentration of SDS. Other conditions are the same as in Fig. 2.

by the fact that the anionic surfactants induce cationic IAA to accumulate at the CPE surface *via* electrostatic interaction. No obvious difference on the peak current and peak potential was observed for IAA in the presence of SDS and SDBS. In this work, the SDS is used to improve the sensitivity of determination of IAA.

Although SDS can improve the oxidation peak current of IAA, the peak current enhancement is closely related to the concentration of SDS. The relationships between the oxidation peak current of IAA and the concentration of SDS are illustrated in Figure 4. As gradual improving the concentration of SDS, the oxidation peak current firstly increases gradually, and then changes slightly. However, the oxidation peak current of IAA decreases conversely when SDS concentration is higher than 5×10^{-1} mol/L. This is may be caused by the fact that the interaction of SDS with IAA in solution phase drastically influences the oxidation signal by activity change of IAA. In this paper, the concentration of SDS is chosen as 4×10^{-1} mol/L.

Influence of scan rate. The relationships between the oxidation peak current of IAA and scan rate were investigated using linear sweep voltammetry (LSV). It is found that the peak current is linearly with the scan rate from 25 mV/s to 300 mV/s, suggesting that the oxidation of IAA in the presence of SDS is adsorption-control.

Effects of accumulation potential and time. The oxidation peak current of IAA in the presence of 4×10^{-1} mol/L SDS was measured at different accumulation potential from -0.5 V to 0.5 V. No significant change on the oxidation peak current was observed after 3-min accumulation at different potential, in contrast to that under open-circuit condition. The results reveal that accumulation potential has no obvious influence on the oxidation peak current of IAA. Therefore, the accumulation of IAA was performed under the open-circuit condition.

Figure 5 shows the influence of the accumulation time on the oxidation peak current of IAA. The peak current increases linearly with prolonging accumulation time within the first 3 min. When the time exceeds 3 min, the oxidation

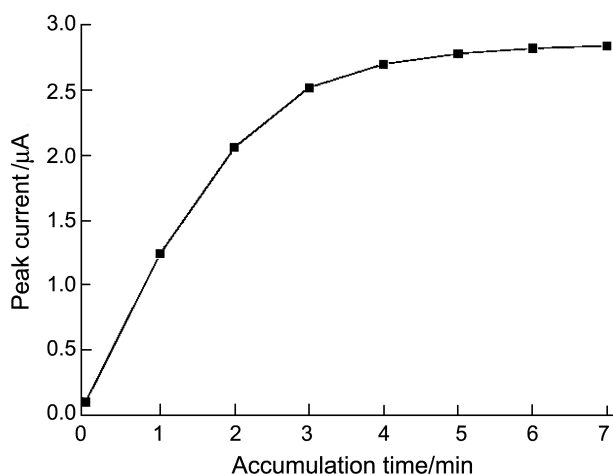


Figure 5. Influence of the accumulation time on the oxidation peak current of 1×10^{-6} mol/L IAA. Other conditions are the same as in Fig. 2.

peak current increases slightly and finally almost keeps unchangeable. This is may be due to the fact that the adsorption of IAA at the CPE surface tends to saturation. In this work, accumulation time is chosen as 3 min.

Calibration graph of IAA. The relationship between the oxidation peak current and the concentration of IAA was examined by using SWV, and the results shown in Figure 6. SW amplitude is 50 mV, SW frequency is 15 Hz and SW step is 4 mV. The oxidation peak current is proportional to the concentration of IAA over the range from 5×10^{-8} mol/L to 2×10^{-6} mol/L and obeys the following equation: $i_p = -0.017 + 2.55 \times 10^6 C$ ($r = 0.998$, C in mol/L). The detection

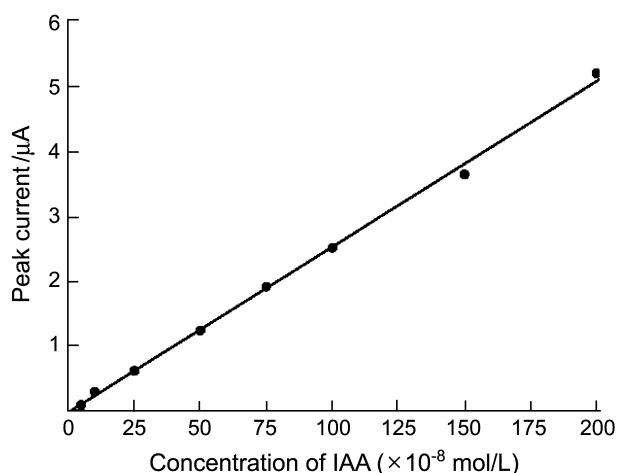


Figure 6. The calibration curve of IAA from 5×10^{-8} to 2×10^{-6} mol/L. Other conditions are the same as in Fig. 2.

limit was obtained by gradual decreasing the concentration of IAA and it was found that this method can detect IAA as low as 2×10^{-8} mol/L. The relative standard deviation (RSD) of eight measurements is 4.7% for 1×10^{-6} mol/L IAA.

Interference of other species. To evaluate the potential effect of foreign species on the determination of IAA at 1×10^{-6} mol/L level, a systematic study was carried out under the above-optimized conditions. The peak currents of IAA in the absence and presence of foreign species were measured by SWV, respectively, and the error was consequently obtained. The results are listed in Table 2. It is found that 5×10^{-5} mol/L K^+ , Na^+ , Ca^{2+} , Mg^{2+} , NH_4^+ , Zn^{2+} , Al^{3+} , Fe^{2+} , Fe^{3+} , Cu^{2+} , Cl^- , I^- , NO_3^- , SO_4^{2-} , Br^- , CO_3^{2-} , ClO_3^- , 2.5×10^{-5} mol/L Cd^{2+} , Pb^{2+} , Hg^{2+} as well as 1×10^{-5} mol/L 1-naphthylacetic acid (NAA), phenol, 2-nitrophenol, 2-aminophenol, and humic acid, almost do not interfere with the oxidation signal of IAA (error $< 4\%$).

Determination of IAA in leaf samples. It is clear that this new voltammetric method can be used to determine IAA quantitatively in the ideal laboratory samples. In order to testify its practical application, this voltammetric method was used to detect IAA in the gladiola and phoenix tree leaves. A 100 g sample of the leaves was treated with liquid nitrogen and then pulverized. The compound was extracted with ethyl acetate and separated by an extraction method reported in reference 12. The voltammetric responses of IAA in the plant leaves at the CPE in the presence of SDS are illustrated in Figure 7. Curve a shows the SW voltammograms of IAA in a plant leaf samples, and curve b depicts the SW voltammograms of IAA in a plant leaf samples after addition of a certain concentration of IAA standard solution.

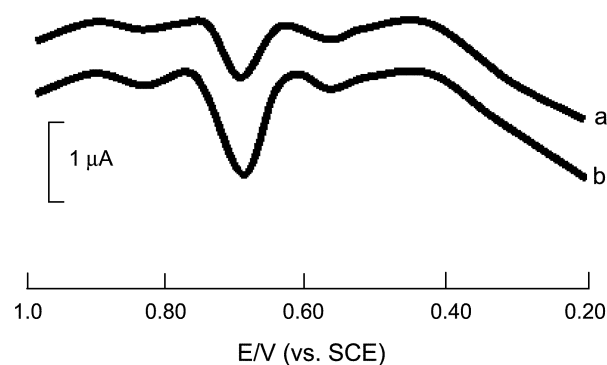


Figure 7. Illustration for the determination of IAA in leaf samples by this proposed method. Curve a: SW voltammograms of IAA in leaf samples, curve b: curve (a) + 5.00×10^{-7} mol/L IAA standard solution. SW amplitude is 50 mV, SW frequency is 15 Hz and SW step is 4 mV.

Table 2. Interferences of foreign species on the oxidation peak current of 1×10^{-6} mol/L IAA

Foreign species	Tolerance level (mol/L)*
K^+ , Na^+ , Ca^{2+} , Mg^{2+} , NH_4^+ , Zn^{2+} , Al^{3+} , Fe^{2+} , Fe^{3+} , Cu^{2+} , Cl^- , I^- , NO_3^- , SO_4^{2-} , Br^- , CO_3^{2-} , ClO_3^-	5×10^{-5}
Cd^{2+} , Pb^{2+} , Hg^{2+}	2.5×10^{-5}
1-Naphthylacetic acid (NAA), phenol, 2-nitrophenol, 2-aminophenol, humic acid	1×10^{-5}

*For 4% error.

Table 3. Determination of IAA in leaf samples using standard addition method. SW amplitude is 50 mV, SW frequency is 15 Hz and SW step is 4 mV. The concentration of SDS is 4×10^{-4} mol/L, accumulation time: 3 min

Samples*	Added (mol/L)	Expected found (mol/L)	Total found (mol/L)	Recovery (%)
Gladiola leaf	0		5.64×10^{-7}	
	4.00×10^{-7}	9.64×10^{-7}	9.79×10^{-7}	103.8
Gladiola leaf	0		5.76×10^{-7}	
	4.00×10^{-7}	9.76×10^{-7}	9.87×10^{-7}	102.8
Gladiola leaf	0		5.48×10^{-7}	
	4.00×10^{-7}	9.48×10^{-7}	9.61×10^{-7}	103.2
Phoenix tree leaf	0		4.32×10^{-7}	
	5.00×10^{-7}	9.32×10^{-7}	9.20×10^{-7}	97.6
Phoenix tree leaf	0		4.42×10^{-7}	
	5.00×10^{-7}	9.42×10^{-7}	9.38×10^{-7}	99.2
Phoenix tree leaf	0		4.18×10^{-7}	
	5.00×10^{-7}	9.18×10^{-7}	9.12×10^{-7}	98.8

*each leaf sample was measured three times.

It is apparent that the oxidation peak at 0.66 V attributes to IAA since this peak height increases as adding IAA standard solution. The IAA concentration was measured by standard addition method using the described voltammetric method as follows. A certain volume of leaf sample solution was added into a cell containing 10 mL pH 3.0 Na_2HPO_4 -citric acid buffer (McIlvaine buffer), and the peak currents of IAA were measured firstly by SWV after 3 min accumulation. Then a certain quantity standard solution of IAA was added and the peak currents were measured again. From the difference between the peak currents, the concentrations of IAA in leaf samples can be obtained. The results are listed in Table 3. The IAA content is about 10.23 μg per gram gladiola leaf, and for 1 g phoenix tree leaf is about 8.51 μg . The recovery was in the range from 97.6% to 103.8%. These results suggest that this method can be used in the clinical practice.

Conclusion

In this work, SDS adsorbs on the CPE surface individually with its hydrophobic C-H chains close to the surface, and its negative-charged head group directs towards the bulk solution. In pH 3.0 McIlvaine buffer, IAA ($\text{pK}_a = 4.75$) exists in cationic form and interacts with negative-charged head group of SDS via electrostatic interactions. IAA is subsequently induced to adsorb at the CPE surface. As a result, the electrode surface concentration of IAA enhances greatly, thus, the oxidation peak current increases remarkably and the oxidation potential shifts negatively. If pH is higher than 4.75, the oxidation peak current of IAA conversely decreases in the presence of anionic surfactants such as SDS and SDBS. However, the cationic surfactants such as CTAB and

STAB, will improve the oxidation peak current. In conclusion, based on the different existing form of IAA (cationic or anionic) in the buffer with various pH value, suitable ionic surfactants can be used to improve the sensitivity of determination of IAA using a carbon paste electrode.

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