Analytical Methods

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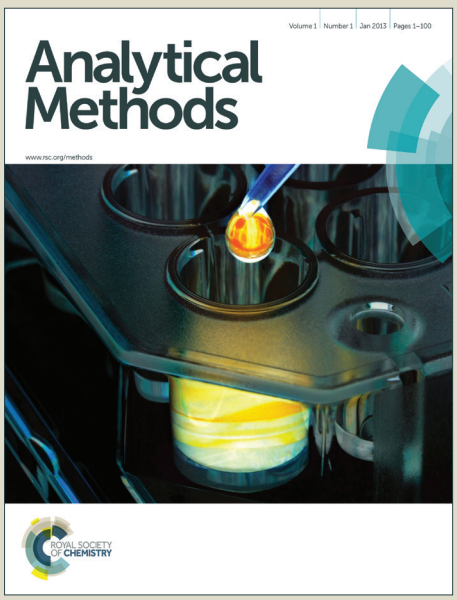

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Sensitive determination of natamycin based on a new voltammetric sensor: single-walled carbon nanotubes composite poly(L-serine) film modified electrode

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Abstract:

A novel voltammetric sensor, based on single-walled carbon nanotubes (SWNTs) composite poly(L-serine) film modified electrode, was fabricated by simple dipping-drying and electrodeposited methods and used for high-sensitive determination of natamycin. At this sensor, the electrochemical response of natamycin was improved greatly due to the synergistic effect of SWNTs and poly(L-serine). A new voltammetric method for determination of natamycin was proposed with detection linear range of 6×10^{-8} mol L^{-1} ~ 6×10^{-6} mol L^{-1} and detection limit of 4×10^{-8} mol L^{-1}. Furthermore, the electrode reaction of natamycin was investigated in detail and some dynamics parameters were calculated for the first time. Using the present method, the residual quantity of natamycin in available claret and beverage samples was evaluated with good satisfactory.

Introduction

Natamycin, known as pimaricin, is a polyene macrolide antimycotic (Fig. 1) produced by *Streptomyces species*.[1] It is a strong antifungal agent and can inhibit the growth of both yeasts and molds. Due to its low toxicity for mammalian cells, natamycin is suitable for surface treatment of food products to increase their shelf time. Therefore, natamycin is widely used as a fungicidal food preservative in cheese, sausage, fruits and beverages.[2] Furthermore, natamycin is the only available antifungal medication approved by the U.S. Food and Drug Administration, which was used to treat several human clinical fungal infections, such as candidiasis and trichomoniasis [3] due to its inhibiting effect on fungus.

However, if consumed in excess, natamycin can damage health by neutralizing antibodies to certain infections.[4] According to the 95/2/EC directive (Annex III Part C), natamycin may be used for the surface treatment of semi-hard and semi-soft cheeses and dry, cured sausages at a maximum level of 1 mg dm^{-2} in the outer 5 mm of surface.[5] Based on the application of natamycin in food preparations and various fungal infections, it is necessary to explore sensitive and reliable analytical method for natamycin. Up to now, some analytical techniques have been established for determination of natamycin, such as microbiological assay,[6] elemental analysis,[7] molecular absorption spectrophotometry, [8] chromatographic analysis with UV [9] and liquid chromatography coupled to mass spectrometry (LC/MS).[10-11] But complicated preconcentration, multisolvent extraction technique or a relatively high detection limit are coupled with these techniques, due to the complexity of real samples and low concentration of analyte.

Recently, electrochemical methods have attracted more and more attentions due to the advantages of fast response, easy operation, time-saving, low-cost instrumentation, high sensitivity and excellent selectivity. More importantly, the electrochemical technique also help for identifying the redox characters of drug compounds and provide some information about pharmacological action. To our knowledge, only one paper was reported for determination of natamycin using electrochemical method so far.[12] C. Aki developed a simple voltammetric method based on carbon paste electrode for the determination of natamycin, with a narrow linear range of 2×10^{-6}–8×10^{-5} mol L^{-1} and a poor detection limit of 1.5×10^{-6} mol L^{-1} yet. Therefore, to develop a sensitive, better selective and reliable electroanalytical method for natamycin is still interesting and significant.

Carbon Nanotubes (CNTs, called bucky tube) have aroused widespread concern since their appearance in 1990. Due to their high electrical conductivity, large specific surface area, good electrical characteristics and mechanical properties, CNTs have been widely employed in electroanalytical chemistry, especially in fabrication of voltammetric sensors and biosensors.[13] Poly(L-serine) is also used in fabrication of electrochemical sensor, owing to its versatility and easy preparation.[14] The composite

materials combining CNTs and polymer have increasing attention due to the synergistic contribution of two or more function components and many potential application. In the present approach, a sensitive and novel electrochemical sensor, based on single-walled carbon nanotubes (SWNTs) composite poly(L-serine) film modified glassy carbon electrode (GCE), was fabricated and used for natamycin both in researching its redox characters and determining concentration. This proposed sensor displayed a significant voltammetric response for natamycin with high sensitivity and selectivity, as well as a wider detection linear range and lower detection limit. Besides, the electrochemical properties of natamycin were investigated systematically and the dynamic parameters of the electrode process were calculated for the first time. Finally, the new proposed electroanalytical method was applied to determine the residual quantity of natamycin in commercially available claret and beverage samples with satisfactory recovery.

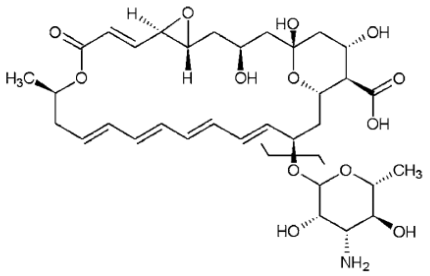


Fig. 1 Chemical structure of natamycin.

Experimental

Instruments and reagents

All electrochemical experiments were carried out using a RST3000 electrochemical workstation (Zhengzhou Shiruisi Instrument Co. Ltd., China.). A three-electrode system was employed, consisting of GCE (3 mm diameter) or modified GCE working electrode, Ag/AgCl reference electrode（the internal solution was saturated KCl solution） and platinum wire counter electrode, respectively. The type of pH meter was pHS-3C (from Shanghai Techcomp Jingke Scientific Instruments Co.,Ltd). The centrifuge used for processing actual sample and purifying SWNTs was provided by Hukang Centrifuge Co., Ltd (TG16-WS, Hunan, China).

All reagents were of analytical grade and were used as received. SWNTs (95% purity, Beijing Nachen S&T Ltd, China) were purified and carboxylated according to the

method described in reference.[15] N,N-dimethylformamide (DMF) was provided by Tianjin Yong Da Chemical Reagent Development Center (Tianjin, China). L-serine was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Natamycin (98%) was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). The stock solution of natamycin (1×10^{-3} mol L^{-1}) was prepared with methanol and stored under 4°C darkly. Double distilled water was used for all preparation. All electrochemical experiments were carried out at room temperature.

Fabrication of SWNTs composite poly (L-serine) modified glassy carbon electrodes

Before modification, the GCE was polished to a mirror finish using finer emery-paper and 0.5 μm alumina slurry respectively. Functionalized SWNTs (2.0 mg) were dissolved in DMF (2 mL) solution and sonicated in an ultrasonic bath for 3 h to obtain an uniformly black suspension (1.0 mg mL^{-1}). Firstly, a poly(L-serine) modified GCE (noted as poly(L-serine)/GCE) was obtained in 0.1mol L^{-1} phosphate buffer solution (pH 5.0) containing 2.0×10^{-3} mol L^{-1} L-serine by cyclic voltammetric sweeps in potential window between −1.1V and 2.5 V. After 5 cycles, the surface of poly(L-serine)/GCE was washed with deionized water to remove the physically adsorbed L-serine. The cyclic voltammograms of polymeric process was shown in Fig. 2. An oxidation peak was observed near 1.70 V in the anodic scan and a reduction peak was appearance near -0.47 V in the reverse scan during the polymerization of L-serine. The redox peak currents were increased with successive cyclic scans, indicating that an electro-conductive polymer film was formed on the GCE surface. The SWNTs/poly(L-serine) modified GCE (named as SWNTs/poly(L-serine)/GCE) was established by dropping the suspension of SWNTs/DMF (8μL) on the poly(L-serine)/GCE surface using a micro-injector, and then dried naturally. For comparison, a SWNTs modified GCE (named as SWNTs/GCE) was established by depositing above suspension (8 μL) on the fresh GCE surface. Prior to use, each modified electrode was pretreated in 0.05 mol L^{-1} H_2SO_4 solution by cyclic scans between potential of 0.60 V and 1.20 V for 10 cycles to activate surface.

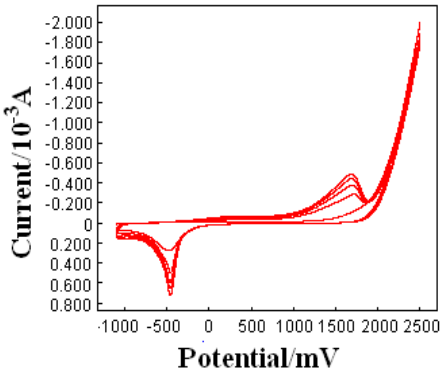


Fig. 2 Cyclic voltammograms of L-serine (2×10^{-3} mol L^{-1}) during electro-polymerization on GCE. Supporting electrolyte: pH 5.0 phosphate buffer; Scan rate: 0.1 V s^{-1}

Analytical procedure

Unless otherwise statement, 0.05 mol L^{-1} H_2SO_4 solution (pH 1.0) was used as blank supporting electrolyte. An accumulation step was carried out firstly under open-circuit while stirring the solution for 180 s in a 10 mL voltammetric cell. After 10 s rest, the positive-going cyclic voltammetry (CV) or linear sweep voltammetry (LSV) scan was then performed and corresponding voltammogram was recorded. After each measurement, the modified electrode was transferred to blank solution by stirring for 20 s to obtain a renew electrode surface.

Sample preparation

A kind of commercially available claret and one orange juice were employed as real samples for validation and application studies. Prior to analysis, the claret and orange juice were filtered through a 0.50 mm PTFE membrane filter firstly, and then appropriate amount of samples were directly injected into the supporting electrolyte for determination of natamycin.

Results and discussion

Electrochemical characteristic of modified electrode

For explaining the function of different modifying layer, the electrochemical probe, $K_3[Fe(CN)_6]$, was employed to investigate their response. Fig.3 displayed the cyclic voltammograms of bare GCE (a), poly(L-serine)/GCE (b) SWNTs/GCE (c) and SWNTs/poly(L-serine)/GCE (d) in 1.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ containing 0.1mol L^{-1} KCl. At the bare GCE (curve a), a well-defined CV, characteristic of diffusion-limited and nearly reversible electron transfer redox process, was observed with i_{pa} = 11.95 μA, i_{pc} = 11.81 μA and peak-to-peak separation (ΔE_p) of 72mV. When L-serine was electrodeposited on the electrode surface (curve b), the charging current increased greatly and the reversibility of $Fe(CN)_6^{3-}$ decreased slightly

(i_{pa} = 9.40 μA, i_{pc} = 10.89 μA). This might be due to the combination between amino (from L-serine) and carboxyl (from electrode), which made the electrode surface negatively charged and thus some electrostatic repulsion existed with $Fe(CN)_6^{3-}$. However, at SWNTs/GCE, the redox peak currents apparently increased to i_{pa} = 38.32 μA and i_{pc} = 35.50μA with larger charging current (curve c). In comparison, the maximum redox peak currents (i_{pa} = 41.16 μA, i_{pc} =38.22μA) was obtained when SWNTs was immobilized on the poly(L-serine)/GCE (curve d). The obtained results demonstrated that different modified films were successfully immobilized on the GCE surface as designed step by step.

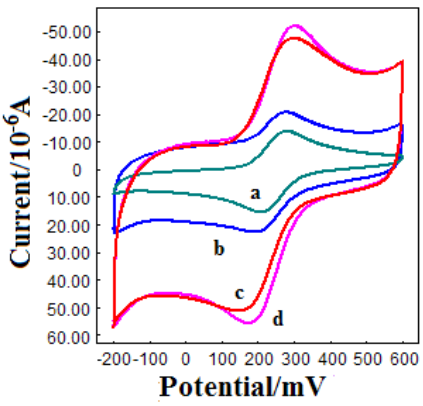


Fig. 3 Cyclic voltammograms of $K_3[Fe(CN)_6]$ (1×10^{-3} mol L^{-1}) in 0.1mol L^{-1} KCl solution at different electrodes: (a) bare GCE (b) poly(L-serine)/GCE (c) SWNTs/GCE (d) SWNTs/poly(L-serine)/GCE. Scan rate: 0.05 V s^{-1}

Electrochemical impedance spectroscopy (EIS) is another technique for characterizing the property of surface-modified electrode. The electron transfer resistance (Ret) at the electrode surface is derived from the semicircle domain of Nyquist plot and can be used to describe the interface property of sensor. Fig.4 presented the typical Nyquist plots obtained at bare GCE (curve a), poly(L-serine)/GCE (curve b) and SWNTs/poly(L-serine)/GCE (curve c) respectively in 5×10^{-3} mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution containing 0.2 mol L^{-1} KCl. At the bare GCE, there was a very small semicircle (Ret = 40.12 Ω) at the high frequency region relating to the interfacial electron transfer resistance. In contrast, the value of Ret decreased to 24.93 Ω after the GCE modified with L-serine, indicating that poly(L-serine) acted as a good electron relay between the electrochemical probe and sensor. When SWNTs was immobilized on the poly(L-serine)/GCE, the resistance value of Ret decreased to 18.70 Ω, meaning that the electrode process was controlled by diffusion in all frequency regions (1.0MHz to

0.01Hz) almost. These data demonstrated the high electrochemical activity and conductibility of CNTs.

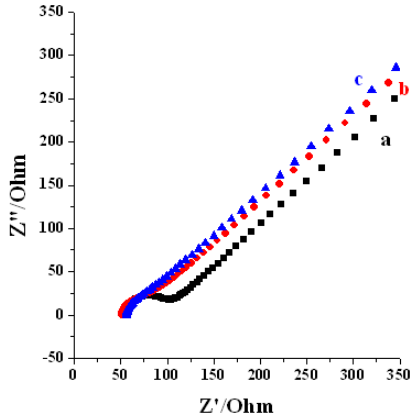


Fig. 4 Nyquist plots of $[Fe(CN)_6]^{3-/4-}$ (5×10^{-3} mol L^{-1}) in 0.2 mol L^{-1} KCl at different electrodes: (a) GCE, (b) poly(L-serine)/GCE, (c) SWNTs/poly(L-serine)/GCE. Frequency range: 1.0MHz-0.01Hz.

Electrochemical behavior of natamycin

Fig. 5 displayed the cyclic voltammograms of natamycin (5×10^{-6} mol L^{-1}) at bare GCE (curve a), poly(L-serine)/GCE (curve c), SWNTs/GCE (curve d) and SWNTs/poly(L-serine)/GCE (curve e) in 0.05mol L^{-1} H_2SO_4, respectively. A background voltammogram at SWNTs/poly(L-serine)/GCE was presented as curve b in Fig 5. The natamycin showed two oxidation peaks (P1 and P2) at each of these sensors. At bare GCE, the electrochemical response of natamycin was very weak. Only two small bulges were appearance in the anodic branch. However, the two oxidation peak currents of natamycin increased obviously at the poly(L-serine)/GCE, which could be attributed to poly(L-serine) membrane providing more active adsorption sites for natamycin. In the case of SWNTs/GCE, it was observed that the electrochemical response of natamycin dramatically increased, which could be reasonably ascribed to the large specific surface area and excellent conductive activity of SWNTs. Compared with those electrodes above, the SWNTs/poly(L-serine)/GCE exhibited the best electrochemical response to natamycin. The anodic peak current for P1 increased strikingly up to 23.58 μA with a well-defined peak shape at 0.925V and P2 was located at 1.1V. These results might be attributed to the synergistic effect of poly(L-serine) and SWNTs. Poly(L-serine) contained a lot of $-NH_2$ with intermolecular hydrogen bonding with natamycin, improving the adsorption of natamycin on the electrode surface. Carbon nanotubes, possessing a large specific surface area and superior conductivity, enlarged the specific surface area of electrode and accelerated the electron transfer between the electrode

and analyte. The synergy of both improved significantly the electrochemical response of natamycin. So, we were choosing the SWNTs/poly(L-serine)/GCE as the voltammetric sensor for natamycin in following study.

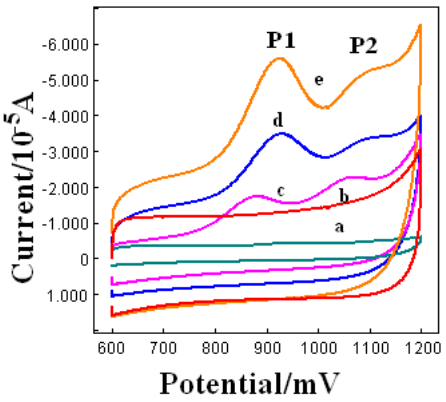


Fig. 5 Cyclic voltammograms of natamycin (5×10^{-6} mol L^{-1}) at bare GCE (a), poly(L-serine)/GCE (c), SWNTs/GCE (d), SWNTs/poly(L-serine)/GCE (e) and SWNTs/poly(L-serine)/GCE in blank solution (b) in 0.05mol L^{-1} H_2SO_4 (pH=1.0). Scan rate: 0.05 V s^{-1} .

To further assess the electrochemical properties of natamycin, we controlled the scan potential window within different range. Firstly, the potential window was limited within 0.6 -1.0V for 2 cyclic scans, a sensitive oxidation peak (P1) was observed in the first cycle and no any redox peak was observed in the following scan (Fig. 6 curve a). Secondly, when the potential was set between 1.0V and 1.20V, a small oxidation peak (P2) was obtained at E_{p2}=1.1 V in the first cycle and no any redox peak was observed in the following scan (Fig. 6 curve b). Finally, two consecutive cyclic scans were performed with potential window between 0.60V and 1.20V in the above solution, both of the oxidation peaks (P1 and P2) were observed in the first cycle and disappeared in the following scan (Fig. 6 curve c). This phenomenon indicated that the electrode surface may be passivated by the nonconducting oxidation products of natamycin, and P1 and P2 were from two different effective groups of natamycin.

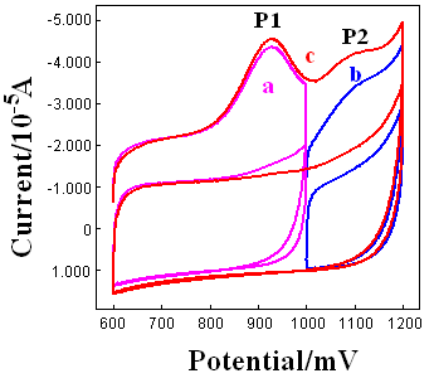


Fig. 6 Cyclic voltammograms of natamycin (5×10^{-6} mol L^{-1}) in 0.05mol L^{-1} H_2SO_4 (pH 1.0) with different potential window: (a) 0.6 V~1.0 V; (b) 1.0 V~1.2 V; (c) 0.6 V~1.2 V. Other conditions were same as Fig. 5.

The effect of electrolyte and pH

The type of supporting electrolyte played a key role in the voltammetric response of analyte. To obtain the best

response of natamycin, some common supporting electrolytes were estimated, such as 0.1M phosphate buffer (pH=2.0), 0.1M Britton-Robinson (pH=2.0), 0.1M acetate buffer, 0.1M sulfuric acid and muriatic acid solutions spiked with 5×10^{-6} mol L^{-1} natamycin. The results showed that higher peak current and better peak shape were obtained in sulfuric acid solution. Therefore, sulfuric acid solution was adopted. To further elucidate the electrode reaction properties of natamycin, the effect of different H_2SO_4 concentration from 0.01 to 0.2 mol/L was optimized and the results were displayed in Fig. 7. When solution pH (the value of pH was calculated by the full dissociation of H_2SO_4) changed from 0.4 to 1.70, the peak potential of P1 and P2 was nearly unaffected, staying at about 0.925V and 1.1V (floating value ≤10 mV), respectively. This result suggested that there was no proton included in the P1 and P2 anodic process. Meanwhile, as was shown in Fig 1 of Supplementary, the oxidation peak current firstly increased, and then decreased with the increase of solution pH value. The maximum oxidation peak current was obtained at pH 1.0 (0.05mol L^{-1} H_2SO_4). Therefore, 0.05mol L^{-1} H_2SO_4 was selected for further experiments.

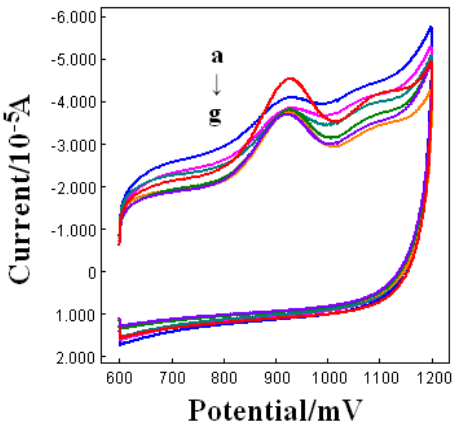


Fig. 7 The superimposed voltammogram of natamycin (5 $\times10^{-6}$ mol L^{-1}) at different concentration of H_2SO_4 (from curve a to g): 0.01, 0.02, 0.03, 0.05, 0.10, 0.15, 0.20mol L^{-1} the corresponding pH: 0.4, 0.52, 0.7, 1.0, 1.22, 1.4, 1.70. Other conditions were same as Fig. 5.

Characteration of natamycin at different scan rates

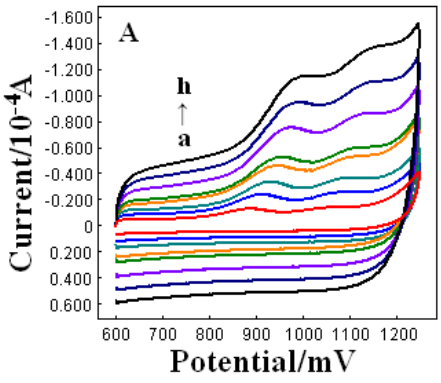
The reaction characters of natamycin at SWNTs/poly(L-serine)/GCE were further investigated by the influence of potential scan rate (v) on the peak current (i_p) and peak potential (E_p). Fig. 8A showed the superimposed cyclic voltammograms of natamycin (5× 10^{-6} mol L^{-1}) in 0.05 mol L^{-1} H_2SO_4 (pH=1.0) with different scan rates varying from 0.02V s^{-1} to 0.2V s^{-1}. The peak potential of P1 and P2 shifted positively with scan rate increasing. For P1, there was a good linear relationship

between log i_{p1} and log v obeying following equation (shown in Fig. 8B): log i_{p1} (μA) = 0.824 log v (V/s) +2.221 (R=0.999). The slope value of 0.824 suggested that the anodic peak P1 was governed by both adsorption and diffusion. Meanwhile, a linear relationship between the peak potential (E_{p1}) and ln v was also obtained with regression equation of E_{p1} (V) = 0.0497 ln v (V s^{-1}) +1.0710 (R=0.997). Based on Laviron's theory for an irreversible electrode process,[16] the E_p-v relation can be described by following equation (1):

$$E_p(V)=E^{0'}-\frac{RT}{\alpha nF}\ln v\frac{RTk_s}{\alpha nF}+\frac{RT}{\alpha nF}\ln v \quad (1)$$

where E^0 is the formal potential, α is the charge transfer coefficient; n is the number of electron transfer; k_s is the standard heterogeneous electron rate constant, R , F and T have their usual meaning. From the slope of E_{p1} against ln v straight line, the value of n can be calculated as 1.03 by assuming α to be 0.5, meaning that one electron transfer was involved in the anodic process of P1. Based on the above mentioned equation (1), the value of α was 0.51 and the value of k_s was calculated to be 0.59 s^{-1}. In calculation, the formal standard potential (E^0 = 0.895V) was obtained through another linear relation of E_p-v by extrapolating v =0. Similarly, based on the good linear relationship between E_{p2} and ln v with regression equation: E_{p2} (V)=0.0426 ln v (V s^{-1}) +1.21872 (R=0.997), one electron transfer was involved in the anodic process of P2, α and k_s was also estimated to be 0.52 and 0.63 s^{-1}, respectively.

From the discussion above, the electrode reaction of natamycin was via two well-separated one-electron steps with no proton taking part in. It was difficult to conjecture the reaction mechanism at the sensor surface. It might be that two kind of cation radicals were generated by oxidizing the natamycin, which passivated the sensor surface easily.



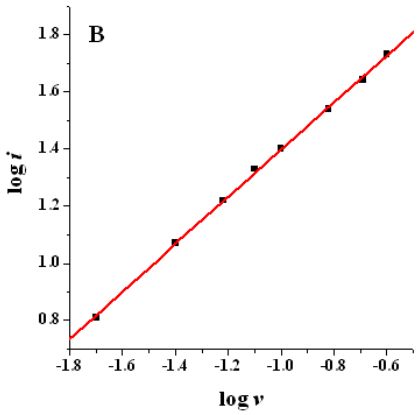


Fig. 8 (A) A superimposed voltammogram of natamycin (5×10^{-6} mol L^{-1}) in 0.05mol L^{-1} H_2SO_4 with different scan rates (from a to h): 0.02, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25 V/s. (B) The relationship of logarithm of i_{p1} vs. the logarithm of scan rate (log *v*).

Chronocoulometric study

For an adsorption along with diffusion driven electrode process, it was necessary to calculate its saturating adsorption capacity (Γ*) and diffusion coefficient (D) at the sensor surface. In chronocoulometric experiment, the SWNTs/poly(L-serine)/GCE was immersed in a natamycin solution (5×10^{-6} mol L^{-1}) for several minutes firstly to achieve saturated absorption, and then a potential step from 0.60 V–1.0 V was performed. The Q~t curve was recorded to calculate the saturated absorption capacity (Fig. 9A, curve b). For comparison, Q~t curve was recorded in blank H_2SO_4 solution too (Fig. 9A, curve a). Corresponding linear relation of Q~ $t^{1/2}$ plots were also performed and shown in Fig. 9B. The linear equations of Q~$t^{1/2}$ was Q (10^{-4} C) = 0.1512 $t^{1/2}$ ($s^{1/2}$) +0.9256 (R = 0.998) and Q (10^{-4}C) = 0.4688 $t^{1/2}$ ($s^{1/2}$) + 2.9446 (R=0.997) for curve a' and curve b', respectively. The slopes and intercepts of two straight lines were different, indicating that the oxidation process of natamycin for P1 was controlled by adsorption accompanying the diffusion process. According to the formula given by Anson: [17]

$$Q = \frac{2nFAC(Dt)^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads} \quad (2)$$

Here, Q_{dl} is double-layer charge, Q_{ads} is the Faradaic charge due to the oxidation of adsorbed natamycin and it is the intercept difference from curve a' and curve b'. Using Laviron' s theory of Q_{ads} = nFAΓ*, the Γ* value of 2.95×10^{-8} mol/cm^2 was obtained. Meanwhile, the D was calculated to be 1.47×10^{-6} cm^2/s from the slope of curve b'.

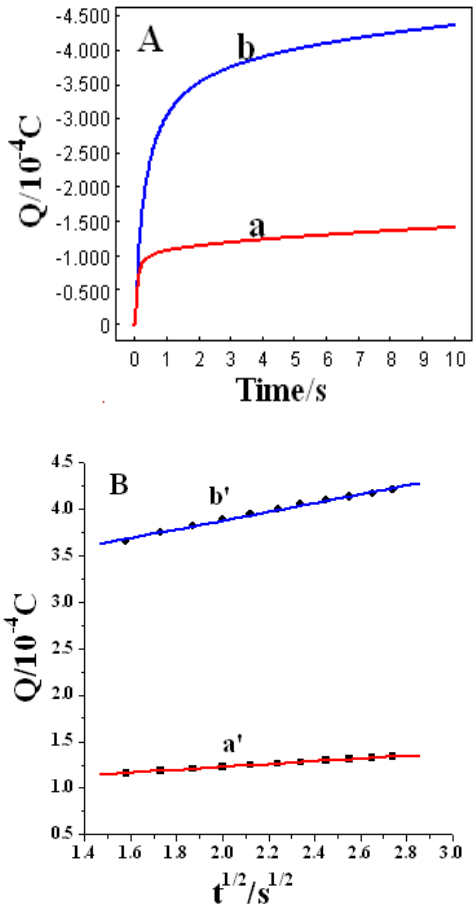


Fig. 9 (A) Chronocoulometric response curves of absence (curve a) and presence of 5×10^{-6} mol L^{-1} natamycin (curve b). (B) The corresponding Q ~ $t^{1/2}$ plots.

Analytical Application and Method Validation

Accumulation conditions

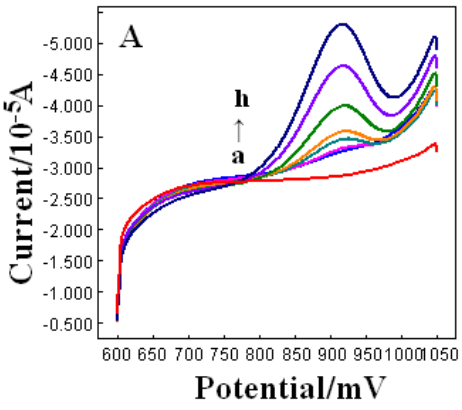
Accumulation step was usually a simple and effective way to enhance the determining sensitivity. The influence of accumulation time (t_{acc}) on response currents of 1.0×10^{-6} mol L^{-1} natamycin was estimated. The results showed that the peak currents increased greatly within t_{acc} of 180 s and then enhanced slowly. As well-known, a longer t_{acc} would get a lower detection limit, but lead to a narrow linear range. For comprehensive consideration, 180 s under open circuit was chosen here as accumulation condition for erecting an analytical method of natamycin.

Calibration curve, detection limit, reproducibility and stability

In this study, the current response of P1 was selected as the detection signal to establish calibration curve due to its better sensitivity. Fig. 10 displayed the response of different natamycin concentrations under optimized working conditions using linear sweep voltammetry (LSV). A good linear relationship could be established between the oxidation peak currents and natamycin concentrations in the range from 6×10^{-8} mol L^{-1} to 6×10^{-6} mol L^{-1}. The linear regression equation was expressed as i_p(μA)=3.5967C(μmol L^{-1}) +1.5976（R=0.997) and the detection limit was 4×10^{-8} mol L^{-1} based on the signal to noise of 3. Compared with the simple previous report about the determination of natamycin using carbon paste electrode [12], a wider linear range and lower detection limit

was obtained.

To estimate the reproducibility of proposed sensor, the relative standard deviation (RSD) of detected peak current for six successive measurements of natamycin (1.0×10^{-6} mol L^{-1}) was calculated to be 3.79%, which demonstrate the good reproducibility of proposed sensor. Similarly, five electrodes were modified in parallel under the same condition and the RSD obtained for the determination of a 1.0×10^{-6} mol L^{-1} natamycin was 4.35%. Furthermore, after a SWNTs/poly(L-serine)/GCE was stored under 4℃ for two weeks, the current response retained 95.1% of its initial response for a 1.0×10^{-6} mol L^{-1} natamycin solution. These experiments above indicated that the proposed sensor was long-term stability and good reproducibility for determination of natamycin.



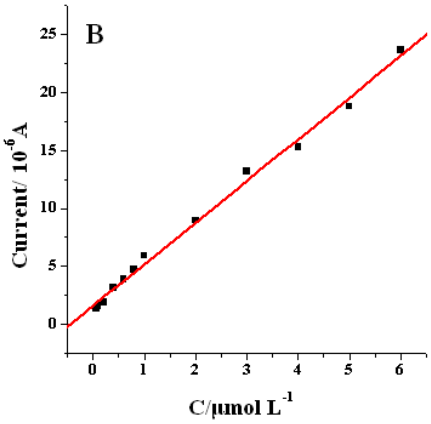


Fig. 10 (A) LCV curves of natamycin with different concentrations (from a to h): 0, 6×10^{-8}, 2×10^{-7}, 6×10^{-7}, 8×10^{-7}, 2×10^{-6}, 4×10^{-6}, 6×10^{-6} mol L^{-1}. (B) Calibration plot of peak current versus natamycin concentrations, the corresponding concentrations: 6×10^{-8}, 1×10^{-7}, 2×10^{-7}, 4×10^{-7}, 6×10^{-7}, 8×10^{-7}, 1×10^{-6}, 2×10^{-6}, 3×10^{-6}, 4×10^{-6} 5×10^{-6}, 6×10^{-6}mol L^{-1}. t_{acc}: 180 s. Scan rate: 0.05 V s^{-1} .

Interference study

In consideration of the analytical application of proposed method, the effect of various species that likely to be in food and beverage sample were evaluated in detail. We fixed the natamycin in 1.0×10^{-6} mol L^{-1}, and then spiked with various excess amount of the species under the same experimental conditions. The tolerance limit for a foreign species was taken as the largest amount yielding a relative error of $<\pm5\%$ for determination of natamycin. The results

were listed in Table 1 of Supplementary and it showed that 200-fold Ca^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, 50-fold glucose, sucrose, amylum, citrate acid, sodium citrate, oxalic acid, vitamin E, vitamin B_2 and edetate disodium caused negligible interference. Meanwhile, it was also observed that some common food preservatives such as 50-fold vitamin C, benzoic acid, sodium benzoate, sodium sorbate and methylparaben had no effects on the oxidation peak of natamycin. These data were clearly proved the reasonable selectivity of proposed method.

Residual quantity determination of natamycin in claret and beverage samples

Residual natamycin in commercially available claret and orange juice were detected to evaluate the practical applicability of proposed method. LSV technique and standard addition method were employed for determining the residual quantity and recoveries. The results were listed in Table 1. Regretfully, no anodic peak of natamycin was observed when appropriate amount of claret and orange juice was added to the electrolytic cell, which may be attributed to the low content or the degradation of natamycin in samples. This result coincided with the declaration by European Union (EU) that residues of natamycin were absent or in concentration below 5.0 μg L^{-1}. By adding a defined natamycin in the detected sample solution, recoveries were determined in the range of 96.2%~116.8%, which clearly indicated the applicability and reliability of proposed method.

Conclusion

In conclusion, a highly sensitive electrochemical sensor, based on single-walled carbon nanotubes (SWNTs) composite poly(L-serine) film modified glassy carbon electrode, was fabricated and used for investigating the electrochemical characters of natamycin and erecting a new electroanalytical method. The synergistic effect of poly(L-serine) and SWNTs could enhance the voltammetric response of natamycin remarkably. The electrode reaction of natamycin was investigated and the dynamics parameters of electrode process were calculated for the first time. Unfortunately, it was difficult to get the reaction mechanism here, which may be solved in the future study.

Table 1 Determination of natamycin in claret and orange juice

Sample	Original found [a] (μmol L^{-1})	Standard added (μmol L^{-1})	Total found [a] (μmol L^{-1})	RSD (%)	Recovery (%)
Claret	Not found	1.0	1.168	2.6	116.8
		2.0	2.886	3.8	96.2
Orange juice	Not found	1.0	1.099	1.3	109.9
		2.0	2.933	2.7	97.8

[a] average value of three replicate measurements.

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Notes and references

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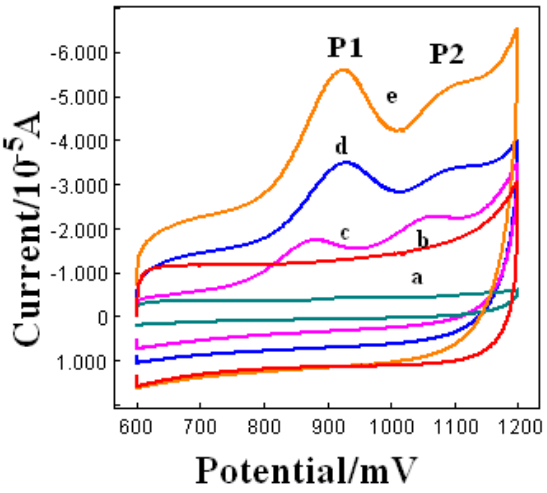
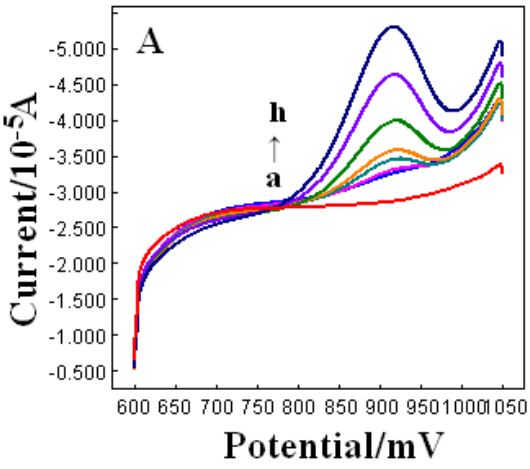
Graphical abstract

The response of the different electrodes.





bare GCE (a), ploy(L-serine)/GCE (c), SWNTs/GCE (d), SWNTs/ploy(L-serine)/GCE (e) with presence of natamycin and and blank voltammograms of SWNTs/ploy(L-serine)/GCE (b)

The modified electrode was applied in the determination of natamycin using LSV.

Figure: A simple sensor for high-sensitive determination of natamycin based on single-walled carbon nanotubes (SWNTs) composite poly(L-serine) film modified glassy carbon electrode. The proposed electrode improved the response of natamycin intensively. A simple and high-sensitive electroanalytical method of natamycin was also established using LSV and applied in commercially available claret and beverag analysis.