

POLY(3,4-ETHYLENEDIOXYTHIOPHENE METHANOL)/ASCORBATE OXIDASE/NAFION-SINGLE-WALLED CARBON NANOTUBES BIOSENSOR FOR VOLTAMMETRIC DETECTION OF VITAMIN C*

Yang-ping Wen^{a,b}, Dong Li^{a,b}, Yao Lu^{b,c}, Hao-hua He^{a**}, Jing-kun Xu^{b**},
Xue-min Duan^c and Ming Liu^{a,b}

^a Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education, and Key Laboratory of Physiology, Ecology and Cultivation of Double Cropping Rice, Ministry of Agriculture, Jiangxi Agricultural University, Nanchang 330045, China

^b Jiangxi Key Laboratory of Organic Chemistry, Jiangxi Science and Technology Normal University, Nanchang 330013, China

^c School of Pharmacy, Jiangxi Science & Technology Normal University, Nanchang 330013, China

Abstract Vitamin C (VC) content in commercial juices was voltammetrically determined using a highly selective and sensitive poly(3,4-ethylenedioxythiophene methanol)/ascorbate oxidase/Nafion-single-walled carbon nanotubes (PEDOTM/AO/Nafion-SWCNT) biosensor. The biocompatible PEDOTM matrix was prepared facily by the one-step electrochemical deposition technique in lithium perchlorate aqueous solutions. AO was dip-coated on the surface of the biocompatible PEDOTM matrix. The mixture of Nafion-SWCNT was dip-cast onto the surface of AO layer when it was obtained by blending Nafion solution and SWCNT dispersion together in a volume ratio of 1:1. The prepared PEDOTM/AO/Nafion-SWCNT biosensor was used for the voltammetric determination of VC, which exhibited the good linear range (4.0×10^{-5} – 3×10^{-3} mol/L), low detection limit (13 μ mol/L), pronounced sensitivity ($1.4072 \text{ mA (mmol/L)}^{-1} \text{ cm}^{-2}$), high bioaffinity (low apparent Michaelis-Menten constant), good stability (good repeatability), high specificity (good anti-interference ability) coupled with the good reliability and feasibility (the determination of VC in commercial juices). Meanwhile, the good aqueous solubility and the low onset oxidation potential of EDOTM will be more beneficial to the application in biosensor field compared to 3,4-ethylenedioxythiophene. Moreover, the good biocompatibility of PEDOTM matrix and high selectivity of Nafion-SWCNT films also provide a promising platform for the development of biosensing devices.

Keywords: Biosensor; Vitamin C; Poly(3,4-ethylenedioxythiophene methanol); Nafion; Single-walled carbon nanotubes.

INTRODUCTION

Recent trends in biosensors have been extensively reported in the development of new biomaterials and the improvement of biosensing techniques, as well as the application of biosensors in clinical diagnostics, the analysis of pharmaceuticals, environmental monitoring, the fermentation and food industries and a number of

*This work was financially supported by NSFC (Nos. 50963002, 51073074), Key Projects in the National Science & Technology Pillar Program in the Eleventh Five-year Plan Period (Nos. 2006BAD02A04, 2006BAD01A01), Jiangxi Provincial Department of Science and Technology (No. 2006BAD01A01-2-5), Jiangxi Provincial Department of Education (Nos. GJJ11590, GJJ10678), Natural Science Foundation of Jiangxi Province (No. 2010GZH0041), Key Laboratory of Photochemical Conversion and Optoelectronic Materials, TIPC, CAS, and Jiangxi Provincial Innovation Fund of Postgraduates (No. YC10A063).

** Corresponding author: Hao-hua He (贺浩华), E-mail: hhhua64@163.com

Jing-kun Xu (徐景坤), E-mail: xujingkun@tsinghua.org.cn

Received August 1, 2011; Revised October 17, 2011; Accepted October 19, 2011

doi: 10.1007/s10118-012-1140-4

similar fields^[1, 2]. Intrinsically conducting polymers (ICPs) as excellent biomaterials for the immobilization matrix of biologically-active species and act as mediator for the rapid electron transfer have attracted much attention in the fabrication of efficient biosensors^[1-4]. Poly(3,4-ethylenedioxythiophene) (PEDOT), as one of the most stable ICPs available today, is a promising candidate for the electrode modified material owing to inherent high electrical conductivity, high environmental stability, ease of preparation, controllable surface properties and good biocompatibility with organism, tissue, cell and biologically-active macromolecules^[5-8]. Moreover, it has been proven to be promising as the immobilization matrix of biologically-active species and direct the rapid electron transfer. There have been a number of reports describing the use of PEDOT as enzyme carriers^[9-13]. Although PEDOT has so many advantages, the poor water solubility (approximately 10 mmol/L) of 3,4-ethylenedioxythiophene (EDOT) is an important drawback, with this limitation, the improvement and modification of PEDOT should be performed to overcome these drawbacks. The poor aqueous solubility of EDOT can be improved by addition of an appropriate pendant side group onto the backbone^[5] or surfactants^[14, 15]. Reynolds's research group first synthesized EDOT with hydroxymethylate functional group 2,3-dihydrothieno[3,4-b]-1,4-dioxyn-2-yl methanol (EDOTM, usually named hydroxymethylated-3,4-ethylenedioxythiophene or 3,4-ethylenedioxythiophene methanol)^[5]. The electrochemical polymerization and properties of poly(3,4-ethylenedioxythiophene methanol) (PEDOTM) also were studied in comparison with PEDOT. EDOTM exhibited higher solubility in water, lower onset oxidation potential of monomer^[16-18], and the resulting conducting PEDOTM film displayed better biocompatibility^[16, 17], higher conductivity (700 S cm^{-1})^[19], better electrochemical, electrooptical and spectroelectrochemical properties^[16-21]. Moreover, the conducting PEDOTM film has also been applied in neural probes^[16] and polymer photovoltaic devices^[19].

Since carbon nanotube (CNT) was first discovered by Iijima^[22], CNT has received widespread attention recently in development of sensing and biosensing devices due to its high surface area, excellent electrical conductivity, significant mechanical strength and good chemical stability^[23-26]. Moreover, CNT composites with different substances as new subjects were also developed^[27-32] and widely employed for the fabrication of biosensing devices due to their high biocompatibility, selectivity and sensitivity^[25, 26, 33]. Nafion, as outer films of biosensing devices, can provide the biocompatible environment for biologically-active species, enhance the anti-interference ability of sensing and biosensing devices, prevent the leakage of the enzyme and improve the adhesion and binding force. Moreover, Nafion modified electrodes can be easily prepared by casting a small volume of the solution onto the surface of the electrode and allowing the solvent to evaporate. The resulting films possess a high adhesion to the surface of the modified electrode and a low swelling in aqueous media^[34, 35]. Therefore, Nafion has attractive properties for immobilizing enzymes at the surface of the electrode. In addition, Nafion-single-walled carbon nanotubes (Nafion-SWCNT) composite films could enhance the selectivity and sensitivity of the electrochemical sensor and the interfacial adhesion of the electrode^[36-39].

In our previous studies, the VC amperometric biosensors based on different PEDOT composite materials were investigated^[40-42]. The different parameters of the fabricated biosensor based on PEDOT/AO were optimized. In addition, the different immobilization methods were also employed for the fabrication of the VC electrochemical biosensor based on PEDOT/AO, the AO molecules physically adsorbed on the surface of PEDOT matrix easily peeled off from the surface of matrix. The co-electrodeposition technique is a good method for immobilization of the AO molecules in PEDOT matrix, but AO molecules due to their high molecular weight (140 kDa) sterically hindered the one-step electrochemical co-deposition with PEDOT, which resulted in the degradation of composite films from the surface of electrode. The covalent attachment and cross-linking must possess the various functional groups such as amino groups and carboxyl groups. Therefore, the entrapment method or in combination with other methods can solve the problem mentioned above.

In the present work, we studied the electrosynthesis and properties of PEDOTM film in LiClO_4 aqueous solutions. AO molecules were dropped onto the surface of the biocompatible PEDOTM matrix. The outer film of the biosensor was obtained by dip-coating method. The fabricated PEDOTM/AO/Nafion-SWCNT biosensor was used for the voltammetric detection of VC and performance analysis. In addition, the VC in commercial juices was successfully determined using the fabricated PEDOTM/AO/Nafion-SWCNT biosensor.

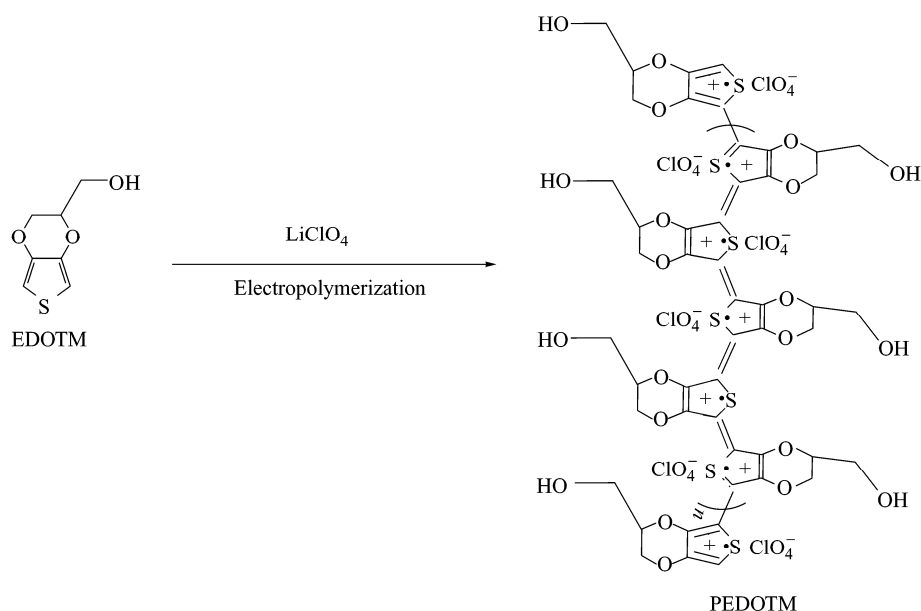
EXPERIMENTAL

Materials

AO (from Cucurbita sp, 250 U g⁻¹) was purchased from Sigma. 5% Nafion solution was obtained from DuPont Co., Ltd. SWCNT was purchased from Chengdu Institute of Organic Chemistry (out diameter 1–2 nm, length 5–30 μm). Lithium perchlorate trihydrate (LiClO₄·3H₂O), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O), and sodium dihydrogen phosphate dihydrate (NaH₂PO₄·2H₂O) were purchased from Sinopharm chemical reagent Co., Ltd. 50 mmol/L phosphate-buffered solution (PBS, pH 6.5) was prepared from 50 mmol/L NaH₂PO₄·2H₂O and 50 mmol/L Na₂HPO₄·12H₂O. VC was purchased from Bio Basic Inc. All the used reagents were analytical grade, and used as received without further purification. All solutions were prepared using deionized distilled water as the solvents. EDOTM was synthesized in our laboratory.

Electrochemical Measurements

All electrochemical experiments were performed in a three-electrode cell. A glassy carbon electrode (GCE) with a diameter of 3 mm served as working electrode, and a platinum wire with a diameter of 0.5 mm was used as counter electrode. The reference electrode was a saturated calomel electrode (SCE). They were placed 5 mm apart during the electrochemical measurements. GCE was polished with alumina (Al₂O₃, 0.05 μm), rinsed with deionized distilled water and cleaned ultrasonically, and then dried in air before each experiment. All solutions (except for the solution containing VC) were deoxygenated by bubbling with dry argon for 10 min prior to each experiment. All experiments (except for the solution containing VC) were performed at 25°C under slight argon overpressure. The cyclic voltammograms (CVs) of monomer and polymer were obtained at the potential scan rate of 100 mV s⁻¹. Electrochemical impedance spectroscopy results were recorded with an Autolab Frequency Response Analyzer system at a potential of 0.3 V, E_{ac} = 10 mV, and the frequency range in 10 kHz to 0.1 Hz. To prepare the high-quality PEDOTM film, low polymerization potential (1.0 V versus SCE) was selected to prevent overoxidation and degradation of PEDOTM matrix, as well as to avoid the performance deterioration of the biosensor. In combination with our previous reports, the low working potential of the biosensor was also selected to avoid the interference of reducing agents in real samples^[14, 40].



Scheme 1 The chemical structures of EDOTM and PEDOTM, and the electrochemical preparation of PEDOTM matrix in LiClO₄ aqueous solution

The Electrochemical Preparation of PEDOTM Films

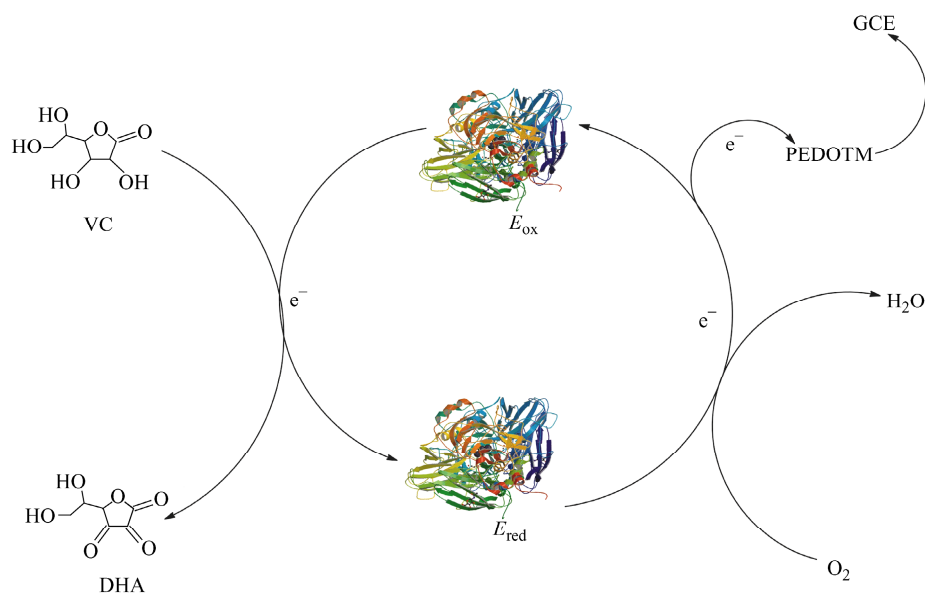
The PEDOTM film was prepared by one-step potentiostatical polymerization in 20 mmol/L EDOTM aqueous solution containing 0.1 mmol/L LiClO_4 at a constant potential of 1.0 V versus SCE at 25°C for 60 s on the surface of bare GCE (Scheme 1). The obtained PEDOTM modified GCE electrode was washed repeatedly with double-distilled deionized water to remove the electrolyte and monomer, then the properties of PEDOTM modified GCE electrode were studied by different electrochemical methods.

The Fabrication of PEDOTM/AO/Nafion-SWCNT Bioelectrode

5 μL of 250 U AO was dip-coated on the surface of the biocompatible PEDOTM modified GCE electrode using a Finnipette. After PEDOTM/AO electrode was dried in air, the mixture of Nafion-SWCNT were prepared by blending 0.5% Nafion and 0.835% SWCNT together and stirring thoroughly in a volume ratio of 1:1, and then 5 μL of the mixture was dip-cast onto the surface of AO layer and dried in air. Finally, the prepared PEDOTM/AO/Nafion-SWCNT composite modified GCE electrode was stored in 50 mmol/L PBS at 4°C when not in use. The oxidation peak current (i) of the prepared biosensor for the detection of VC was carried out using voltammetric method in PBS at 25°C.

Working Mechanism of PEDOTM/AO/Nafion-SWCNT Biosensor

The working mechanism of PEDOTM/AO/Nafion-SWCNT biosensor for the bioelectrocatalytic oxidation of VC in the presence of the AO molecules can be summarized as Scheme 2.



Scheme 2 The structures of VC and DHA, and the working mechanism of the fabricated biosensor for the detection of VC

The as-fabricated biosensor could catalyze the bioelectrocatalytic oxidation of VC to L-dehydroascorbic acid (DHA) and water with the oxygen present in solutions, and then the PEDOTM matrix acted as an electron-transfer mediator. SWCNT could promote the electron transfer between the AO molecules and the matrix interface, but also enhance bioelectrocatalytic oxidation of VC during electrochemical detection.

Determination of VC in Commercial Juices

All commercial juices containing VC were purchased from a local supermarket, VC sample solutions were prepared from different kinds of commercial juices containing VC and 50 mmol/L PBS (pH 6.5). Finally, the as-fabricated PEDOTM/AO/Nafion-SWCNT biosensor was used to detect all kinds of VC sample solutions. The results obtained by the fabricated biosensor were compared to that given by manufacturers.

Characterization

All electrochemical experiments were performed with a potentiostat-galvanostat (Model 263A, EG&G Princeton Applied Research). The addition of solutions to the cell was performed with the Finnpiette (Labsystems, Helsinki, Finland). The pH value was measured with a Delta 320 pH meter (Mettler-Toledo Instrument, Shanghai, China). The temperature was controlled with an HHS type thermostat (Shanghai, China). The EIS were performed with an Autolab Frequency Response Analyzer System (AUT30. FRA2-Autolab, Eco Chemie, BV, The Netherlands).

RESULTS AND DISCUSSION

Electropolymerization of EDOTM

The anodic polarization curve and cyclic voltammetry of EDOTM in LiClO_4 aqueous solutions are presented in Fig. 1 inset. 20 mmol/L EDOTM was added in LiClO_4 aqueous solution, after 5 min of ultrasound treatment, a clear and transparent EDOTM aqueous solution was obtained, suggesting that EDOTM had better aqueous solubility in comparison with EDOT. The polarization curve revealed that the onset oxidation potential (E_{ox}) of EDOTM was about 0.92 V, which was lower than E_{ox} of EDOT^[14, 15]. The lower value of E_{ox} was very beneficial to the preparation of the high-quality PEDOTM film. In addition, the higher polymerization potential could result in the decomposition of water ($E_{\text{ox}} = 1.23$ V) in the electrolyte, overoxidation and degradation of polymer film, which would influence the sensitivity, stability and reproducibility of the sensing and biosensing devices. Therefore, 1.0 V versus SCE was selected as the polymerization potential of EDOTM in subsequent experiments.

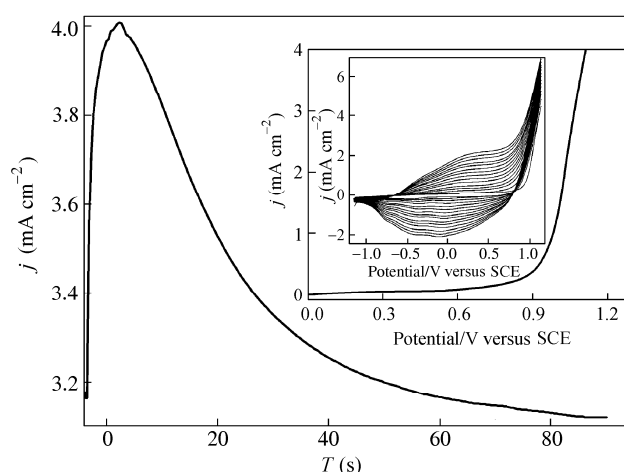


Fig. 1 Chronoamperogram of EDOTM at the applied potential of 1.0 V versus SCE at 25°C for 90 s
Inset: anodic polarization curves and CVs of the PEDOTM films at potential scan rates 100 mV s⁻¹

The cyclic voltammetry of EDOTM demonstrated that the current densities increased with the increasing cycle number of potential scans. It implied that the conducting PEDOTM film was formed, and gradually grew on the surface of GCE. Moreover, this phenomenon was very similar to our previous reports on the electropolymerization of EDOT^[14, 15]. In addition, the thickness of PEDOTM film was also investigated. The chronoamperogram of EDOTM in Fig. 1 indicated that the current densities initially experienced a sharp increase and then gradually decreased. Finally a plateau was reached after 60 s, which was similar to the chronoamperograms of EDOT^[14, 15]. Two or more stages were clearly observed during the one-step potentiostatical polymerization of EDOTM. The change in current densities in the initial stage was attributed to the nucleation process, while a current plateau region was owed to layer-by-layer growth mode, which demonstrated the electrochemical feasibility of the ultrathin polymer film, and the ultrathin polymer film played an important role in modern biosensing devices^[14]. The thickness of PEDOTM films was controlled by the total

charge passing through the cell, which was read directly from the current–time curve by computer. The very thin or thick polymeric films influenced the properties of the conducting polymer film and even the performance of biosensing devices^[14, 15, 40–42]. Therefore, a deposition time of 60 s was found to be optimal for the high-quality and ultrathin PEDOTM film, and so was selected for subsequent experiments.

Impedance Plots of PEDOTM Matrix

The electrochemical properties of the PEDOTM matrix in PBS are shown in Fig. 2. The CVs did not show any clear oxidation or reduction peaks, but merely a rectangle-like shape, indicating good capacitive characteristics of PEDOTM film, which was very beneficial to the application in the capacitors and supercapacitors. Moreover, the low-frequency region in Nyquist plots was related to the capacitance of the polymer film (corresponding to the low-frequency region of Bode plots). The Z_{im} of the impedance sharply increased and tended to a vertical line at the low frequency region, also indicating good capacitance behavior. The low ionic resistance suggested that ion transport in PEDOTM films was much faster.

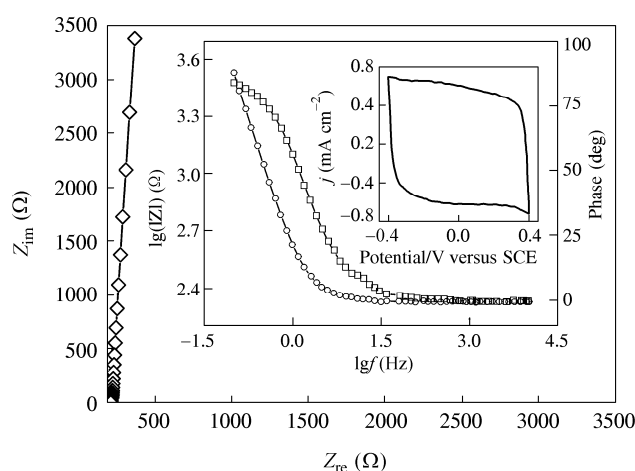


Fig. 2 Nyquist plots of the PEDOTM electrode in PBS
Inset: bode plots and CVs of the PEDOTM electrode in PBS

Bioelectrocatalytic Oxidation of VC

The peak current densities of the prepared PEDOTM/AO/Nafion-SWCNT biosensor for the bioelectrocatalytic oxidation of VC are shown in Fig. 3. It could be seen that the bioelectrocatalytic oxidation of VC began to appear from approximately -0.05 V and reached the maximum value at 0.13 V, which was different from the CVs of the biosensor in PBS without VC. This fact also suggested that the prepared biosensor could catalyze the bioelectrocatalytic oxidation of VC, indicating that the PEDOTM/AO/Nafion-SWCNT biosensor possessed a good bioelectrocatalytic activity for the oxidation of VC. In addition, the oxidation peak potential of VC was gradually shifted to a less-positive potential when the concentrations of VC were higher than 3×10^{-3} mol/L, which might be related to the dissolved oxygen consumption in surrounding enzymes.

Detection of VC

The linear relationship between the peak current densities and the concentrations of VC is shown in Fig. 3 inset. Obviously, the values of peak current densities linearly increased with the increasing concentrations of VC, revealing that the unknown concentrations of VC could be detected successfully by the fabricated PEDOTM/AO/Nafion-SWCNT biosensor. The linear range of the biosensor response to the concentrations of VC from 4.0×10^{-5} mol/L to 3×10^{-3} mol/L ($R^2 = 0.998$) could allow to determine the content of VC in crops, which lies within the narrow limits of 2×10^{-3} – 2.5×10^{-2} mol/L^[43]. Moreover, the survey indicated that the content of VC in most commercial juices was in the range of 0.05 – 0.25 mg/mL, which was feasible for detecting by using the PEDOTM/AO/Nafion-SWCNT biosensor. In addition, this method had a high sensitivity of 1.4072 mA (mmol/L) $^{-1}$ cm $^{-2}$ (from the slope of the linear part in Fig. 3 inset) and a low detection limit of 13 μ mol/L ($S/N = 3$).

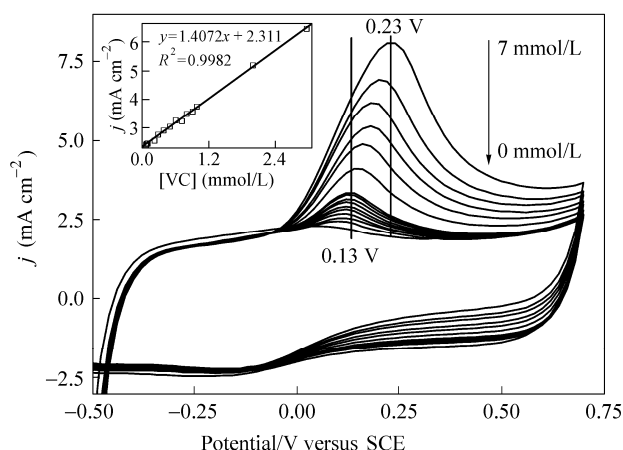


Fig. 3 The CVs of the PEDOTM/AO/Nafion-SWCNT biosensor in 0.5 mol/L PBS containing different concentrations of VC

Inset: the linear relationship between the oxidation peak current and the concentrations of VC

Reaction Kinetics of the Prepared Biosensor

The kinetic parameters of the PEDOTM/AO/Nafion-SWCNT biosensor were obtained by Lineweaver-Burk graphs in Fig. 4. The graphs were plotted by studying the relationship between peak current densities and the concentrations of VC. Kinetic parameters were calculated according to the Lineweaver-Burk equation as follows:

$$1/I = 1/I_{\max} + K_m/I_{\max}C$$

K_m (the apparent Michaelis-Menten constant) and $I_{\max}$ (the apparent maximum peak current densities) were obtained by Lineweaver-Burk plot in Fig. 4 inset. After linear regression, an equation of form $y = Ax + B$ was obtained. The inverse of B is $I_{\max}$, as at that point, x is zero; whereas K_m is calculated as $A(B)^{-1}$, as at that point, y is zero. The values of K_m and $I_{\max}$ were calculated as 4.82 mmol/L and 1.582 mA cm⁻². Michaelis-Menten constant is a measure of enzymatic affinity for its substrate and corresponds to the substrate concentration at $1/2 V_{\max}$. It is inversely proportional to the enzymatic affinity for its substrate. Hence, the lower value of K_m indicated that the prepared biosensor had good bioaffinity.

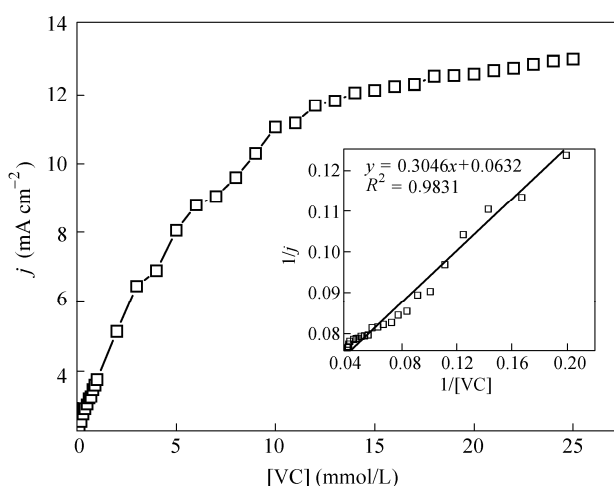


Fig. 4 Lineweaver-Burk plots of the PEDOTM/AO/Nafion-SWCNT biosensor under optimal parameters conditions

Operational Stability of the Prepared Biosensor

The operational stability of the prepared PEDOTM/AO/Nafion-SWCNT biosensor in terms of repetitive use in PBS containing 0.5 mmol/L VC is presented in Fig. 5. The biosensor was repetitively used for the determination of VC. The relative standard deviation was 0.31% for 30 successive assays, showing a fairly good repeatability of the PEDOTM/AO/Nafion-SWCNT biosensor.

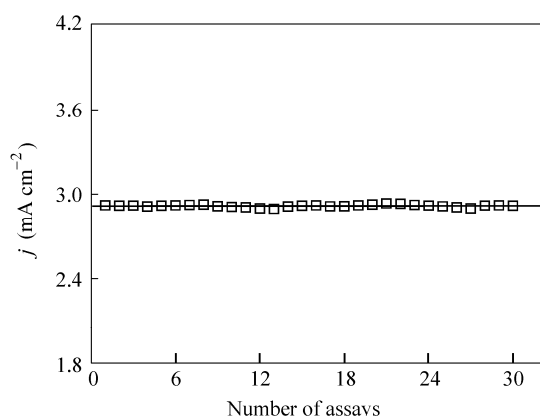


Fig. 5 The operational stability of the PEDOTM/AO/Nafion-SWCNT biosensor

Specificity of the Prepared Biosensor

Table 1 shows the specificity of the prepared PEDOTM/AO/Nafion-SWCNT biosensor. The different interferents with 0.5 mmol/L and 1 mmol/L were used for specificity tests of the biosensor in PBS containing 0.5 mmol/L VC. The peak current densities of the prepared biosensor for the detection of VC were accepted as 100%. The results indicated that these substances did not cause significant interference in peak current densities of the biosensor. This was because the AO molecules provided good specificity for the determination of VC. Moreover, Nafion and SWCNT enhanced the anti-interference ability of the outer films. Hence, the PEDOTM/AO/Nafion-SWCNT biosensor exhibited good specificity.

Table 1. The specificity of the PEDOTM/AO/Nafion-SWCNT biosensor in different interferents

Interferents	Relative activity (%)	
	0.5 mmol/L interferents (0.5 mmol/L VC)	1 mmol/L interferents (0.5 mmol/L VC)
Oxalic acid	100.0	100.0
Malic acid	100.0	100.0
Inositol	100.0	100.0
Thiamine	100.0	100.0
Nicotinic acid	100.0	100.0
Glucose	100.0	100.0
Fructose	100.0	100.0
Sucrose	100.0	100.0
L-glutamic acid	100.0	100.0
L-aspartic acid	100.0	100.0
Ethanol	100.0	100.0

Determination of VC in Commercial Juices

The analytical results for the determination of VC in commercial juices are listed in Table 2. The values obtained by the fabricated biosensor were in agreement with that given by the manufacturers. This showed the reliability and feasibility of the present method. Therefore, the good results of the VC determination in commercial juices also indicated that the fabricated PEDOTM/AO/Nafion-SWCNT biosensor might be employed for the determination and analysis of VC content in agricultural crops.

Table 2. The determination of VC in commercial drinks using the as-fabricated PEDOTM/AO/Nafion-SWCNT biosensor

Commercial drinks	[VC] (mg/mL)	[VC] by voltammetric method (mg/mL) ^a	
		Mean $\pm$ SD	RSD (%)
Vitamin water	0.200	0.1945 $\pm$ 0.00084	0.43
Mizone	0.200	0.1924 $\pm$ 0.00146	0.76
Lemon juice	0.225	0.2187 $\pm$ 0.00198	0.91
Grape juice	0.100	0.09923 $\pm$ 0.00082	0.83

^a Average value of five replications; SD is standard deviation; RSD is relative standard deviation.

CONCLUSIONS

The VC content in commercial juices was voltammetrically determined using a highly selective and sensitive PEDOTM/AO/Nafion-SWCNT biosensor. The fabrication and properties of the biocompatible PEDOTM matrix were characterized. Good aqueous solubility, low onset oxidation potential and hydroxyl groups of EDOTM were very beneficial to application in biosensor field. Highly selective Nafion-SWCNT outer films were obtained by blending Nafion solutions and SWCNT dispersion together in a volume ratio of 1:1 and dried in air. The good biocompatibility of PEDOTM matrix and high selectivity of Nafion-SWCNT outer films also provide a promising platform for the development of biosensing devices. The prepared biosensor has good linear range, low detection limit, pronounced sensitivity, high bioaffinity and specificity and good stability, as well as good reliability and feasibility in application. It is, with all the evidence in mind, therefore reasonable to conclude that the biosensor is a potential candidate for the detection and analysis of VC in agricultural crops.

REFERENCES

- Gerard, M., Chaubey, A. and Malhotra, B.D., *Biotechnol. Bioeng.*, 2002, 17: 354
- Malhotra, B.D., Singhal, R., Chaubey, A., Sharma, S.K. and Kumar, A., *Curr. Appl. Phys.*, 2005, 5: 92
- Cosnier, S., *Anal. Bioanal. Chem.*, 2003, 377: 507
- Ahuja, T., Mir, I.A., Kumar, D. and Rajesh, *Biomaterials*, 2007, 28: 791
- Groenendaal, B.L., Jonas, F., Freitag, D., Pielartzik, H. and Reynolds, J.R., *Adv. Mater.*, 2000, 12: 481
- Groenendaal, L.B., Zotti, G., Aubert, P.H., Waybright, S.M. and Reynolds, J.R., *Adv. Mater.*, 2003, 15: 855
- Rozlosnik, N., *Anal. Bioanal. Chem.*, 2009, 395: 637
- Kirchmeyer, S., Elschner, A., Reuter, K., Lovenich, W. and Merker, U., "PEDOT as a conductive polymer: principles and applications", CRC Press, New York, 2010
- Park, J., Kim, H.K. and Son, Y.G., *Sensor. Actuat. B*, 2008, 133: 244
- Santhosh, P., Manesh, K.M., Uthayakumar, S., Komathi, S., Gopalan, A.I. and Lee, K.P., *Bioelectrochemistry*, 2009, 66: 7561
- Kros, A., Nolte R.J.M. and Sommerdijk, N.A.J.M., *J. Polym. Sci. Polym. Chem.*, 2002, 40: 738
- Nien, P.C., Tung, T.S. and Ho, K.C., *Electroanal.*, 2006, 18: 1408
- Richardson-Burns, S.M., Hendricks, J.L., Foster, B., Povlich, L.K., Kim, D.H. and Martin, D.C., *Biomaterials*, 2007, 28: 1539
- Wen, Y.P., Xu, J.K., He, H.H., Lu, B.Y., Li, Y.Z. and Dong, B., *J. Electroanal. Chem.*, 2009, 634: 49
- Zhang, S.S., Hou, J., Zhang, R., Xu, J.K., Nie, G.M. and Pu, S.Z., *Eur. Polym. J.*, 2006, 42: 149
- Xiao, Y., Cui, X., Hancock, J.M., Bouguettaya, M., Reynolds, J.R. and Martin, D.C., *Sens. Actuators, B-Chem.*, 2004, 99: 437
- Xiao, Y., Martin, D.C., Cui, X. and Shenai, M., *Appl. Biochem. Biotechnol.*, 2006, 128: 117
- Lima, A., Schottland, P., Sadki, S. and Chevrot, C., *Synthetic Met.*, 1998, 93: 33
- Kim, Y.S., Park, J.H., Lee, S.H. and Lee, Y., *Sol. Energy Mater. Sol. Cells*, 2009, 93: 1398
- Doherty, W.J., Wysocki, R.J., Armstrong, N.R. and Saavedra, S.S., *Macromolecules*, 2006, 39: 4418

- 21 Doherty, W.J., Wysocki, R.J., Armstrong, N.R. and Saavedra, S.S., *J. Phys. Chem. B*, 2006, 110: 4900
- 22 Iijima S., *Nature*, 1991, 354: 56
- 23 Yuan, K., Wang, X.F., Zhou, X.J., Ma, W.C., Zhu, Y.C., Liu, X.W. and Zhang, J.Y., *Chinese J. Polym. Sci.*, 2009, 27(3): 375
- 24 Tong, L.F., Ma, H.Y. and Fang, Z.P., *Chinese J. Polym. Sci.*, 2008, 26(3): 331
- 25 Wang, J., *Electroanal.*, 2005, 17: 7
- 26 Balasubramanian, K. and Burghard, M., *Anal. Bioanal. Chem.*, 2006, 385: 452
- 27 Thostenson, E.T., Ren, Z. and Chou, T.W., *Compos. Sci. Technol.*, 2001, 61: 1899
- 28 Harris, P.J.F., *Int. Mater. Rev.*, 2004, 49: 31
- 29 Wang, Z.K., Hu, Q.L. and Cai, L., *Chinese J. Polym. Sci.*, 2010, 28(6): 801
- 30 Zhao, X.F., Jiang, Q., Zhang, Y., Jiang, H.C., Sun, P.Q., Yi, C.J. and Zhao, Y., *Acta Polymerica Sinica (in Chinese)*, 2011, (4): 384
- 31 Guan, A.Z., Song, Y.H., Tan, Y.Q. and Zheng, Q., *Acta Polymerica Sinica (in Chinese)*, 2011, (2): 224
- 32 Wang, W.R., Liu, Y.T., Feng, Q.P., Xie, X.M. and Ye, X.Y., *Acta Polymerica Sinica (in Chinese)*, 2010, (2): 131
- 33 Jacobs, C.B., Peairs, M.J. and Venton, B.J., *Anal. Chim. Acta*, 2010, 662: 105
- 34 Peng, C., Yang, Y., Wang, L., Huang, M. and Shi, X.F., *Chinese J. Polym. Sci.*, 2009, 27(6): 755
- 35 Karyakin, A.A., Kotelnikova, E.A., Lukachova, L.V., Karyakina, E.E. and Wang, J., *Anal. Chem.*, 2002, 74: 1597
- 36 Landi, B.J., Raffaele, R.P., Heben, M.J., Alleman, J.L., VanDerveer, W. and Gennett, T., *Nano Lett.*, 2002, 2: 1329
- 37 Wang, H.S., Li, T.H., Jia, W.L. and Xu, H.Y., *Biosens. Bioelectron.*, 2006, 22: 664
- 38 Landi, B., Raffaele, R., Heben, M., Alleman, J., VanDerveer, W. and Gennett, T., *Mat. Sci. Eng. B-Solid*, 2005, 116: 359
- 39 Sun, D., Xie, X., Cai, Y., Zhang, H. and Wu, K., *Anal. Chim. Acta*, 2007, 581: 27
- 40 Liu, M., Wen, Y.P., Li, D., Yue, R.R., Xu, J.K. and He, H.H., *Sensor. Actuat. B*, 2011, 159: 277
- 41 Liu, M., Wen, Y.P., Xu, J.K., He, H.H., Li, D., Yue, R.R. and Liu, G.D., *Anal. Sci.*, 2011, 27: 477
- 42 Liu, M., Wen, Y.P., Li, D., He, H.H., Xu, J.K., Liu, C.C., Yue, R.R., Lu, B.Y. and Liu, G.D., *J. Appl. Polym. Sci.*, 2011, 122: 1142
- 43 Smirnoff, N., *Curr. Opin. Plant Biol.*, 2000, 3: 229