

Poly(Acid Chrome Blue K) Modified Glassy Carbon Electrode for the Determination of Vanillin

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Received: 6 June 2012 / Accepted: 13 June 2012 / Published: 1 July 2012

Poly(acid chrome blue K) modified glassy carbon electrode (GCE) was electropolymerized by cyclic voltammetric sweep in the potential range from -0.2 to 0.9 V. The characteristic of Poly(acid chrome blue K) film was studied by different methods such as electrochemical impedance spectroscopy (EIS) and cyclic voltammetry. This modified electrode showed excellent electrocatalytic response to vanillin with the increase of the electrochemical responses. Under the optimal conditions a good linear voltammetric response could be obtained over the range of $1.0 \times 10^{-7} \sim 7.0 \times 10^{-5}$ mol dm⁻³ and the detection limit was got as 3.2×10^{-8} mol dm⁻³ (3σ). The proposed method was successfully applied to biscuits and dark-chocolate samples with satisfactory results.

Keywords: Electropolymerization, acid chrome blue K, glassy carbon electrode, determination, vanillin

1. INTRODUCTION

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of nature vanilla, which is one of the world's flavor extracts obtained primarily from *Vanillia planifolia*. Over 12,000 t of vanillin are produced per year, but less than 1% of this is natural vanillin from Vanilla; the remainder is synthesised much more cheaply via chemical or biochemical processes [1]. Consumers prefer natural ones than synthetic flavours even though the latter is available at very low prices. Owing to its flavour and aroma properties, vanilla has been widespread used in desserts, baked goods, confectionery, beverages, pharmaceuticals, foods and perfumery. Due to its leading to lower rates of

cardiac disease mortality, vanillin is also used as inhibition of the oxidation of human low density lipoproteins [2], and used as an antisickling effect in sickle cell anaemia sufferers [3].

Several methods have been reported for the determination of vanillin, such as UV spectrophotometry [4], GC-MS [5,6], high-performance liquid chromatography (HPLC)[3,7,8] and capillary electrophoresis (CE) [9,10]. These methods may be costly and involve time-consuming sample pretreatment processes. Recently, novel approaches for vanillin detection using electrochemical sensor have been reported, which are environmental friendly and using extremely low sample consumption as well [11-16]. Due to the simplicity, fast response, high sensitivity and online detection ability, electrochemical sensors have potential advantages to determine vanillin.

Since the discovery of the formation of polymer semiconductor films by the electrochemical oxidation of pyrrole [17] and aniline [18], there has been a growing interest in studying electrogenerated polymers. Recently, due to the polymer can be *in situ* formed on the electrode surface with controllable thickness, high conductivity and good stability, polymer modified electrodes prepared by electropolymerization have been receiving extensive interest in the fields of chemical sensors and biosensors [19-23].

To our knowledge, voltammetric determination of vanillin using a Poly(acid chrome blue K) modified glassy carbon electrode (GCE) has not been reported yet. By electropolymerization of acid chrome blue K on the GCE surface, a layer of conducting film was formed, which exhibited improved electrochemical performance. The electrocatalytic behaviors of Poly(acid chrome blue K)/GCE to the oxidation of vanillin were carefully investigated. Finally, this modified electrode was used for the analysis of vanillin in biscuits and dark-chocolate samples.

2. EXPERIMENTS

2.1. Reagents and apparatus

Acid chromeblue K and vanillin were purchased from Aladdin-Reagent Company (Shanghai, China). Vanillin was dissolved in redistilled water to form a standard solution of $1.0 \times 10^{-3} \text{ mol dm}^{-3}$ and stored at 4 °C. All reagents were of analytical-grade and used without any further purification. Phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of 0.2 mol dm^{-3} KCl and 0.2 mol dm^{-3} $\text{KH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$, and then adjusting the pH with 0.2 mol dm^{-3} H_3PO_4 or 0.2 mol dm^{-3} NaOH. Aqueous solutions were prepared with redistilled water.

Electrochemical measurements were performed on a CHI 660D Electrochemical Workstation (Shanghai CH Instruments, China). A conventional three-electrode system was used throughout the experiments. The working electrode was a bare, a pretreated or Poly(acid chrome blue K) modified GCE (2.0mm in diameter); the auxiliary electrode was a platinum wire and a saturated calomel electrode (SCE) as the reference. All potentials in this paper refer to this reference electrode. PBS were carried out with a PHS-3 pH-meter (Shanghai Leici Instrument Plant, China) at room temperature.

All experiments were carried out at room temperature.

2.2. Preparation of polymer modified GCE

Prior to preparation of the poly(acid chrome blue K)/GCE, the GCE (with a diameter of 2 mm) was prepared by polishing with 0.05 μm alumina slurry on microcloth pads to a mirror-like finish and rinsed with redistilled water. Subsequently, it was ultrasonicated thoroughly with HNO_3 (1:1, v/v), ethanol (1:1, v/v), and doubly distilled water. After pretreating, the GCE was cycled in 0.5 mol dm^{-3} H_2SO_4 with the potential range from -0.5 to $+1.5$ V at a scan rate of 100 mV s^{-1} until the reproducible background was obtained. Then, the poly(acid chrome blue K) film was electrochemically deposited in 0.2 mol dm^{-3} PBS (pH 7.0) containing 0.5 mmol dm^{-3} acid chrome blue K with the cycling potential from -1 to $+2$ V at scan rate of 100 mV s^{-1} for 25 cyclic times. After the electropolymerization, the modified electrode was rinsed thoroughly with redistilled water for further application.

3. RESULTS AND DISCUSSION

3.1. Electropolymerization of poly(acid chrome blue K) on GCE

The continuous cyclic voltammograms for the electrochemical polymerization of acid chrome blue K over the range of -1.0 to 2.0 V at 100 mV s^{-1} by 25 cycles was shown in fig. 1. In process of the electrochemical polymerization, it is clear that an anodic peak at 0.66 V corresponding to the oxidation of acid chrome blue K descended gradually with cyclic time increasing and trended to be stable after 10 scans. The gradually descended of the anodic peaks in the cyclic voltammogram indicated the gradually conversion of acid chrome blue K into its polymeric product. The polymeric product formed on the surface of GCE was a thin blue colored film.

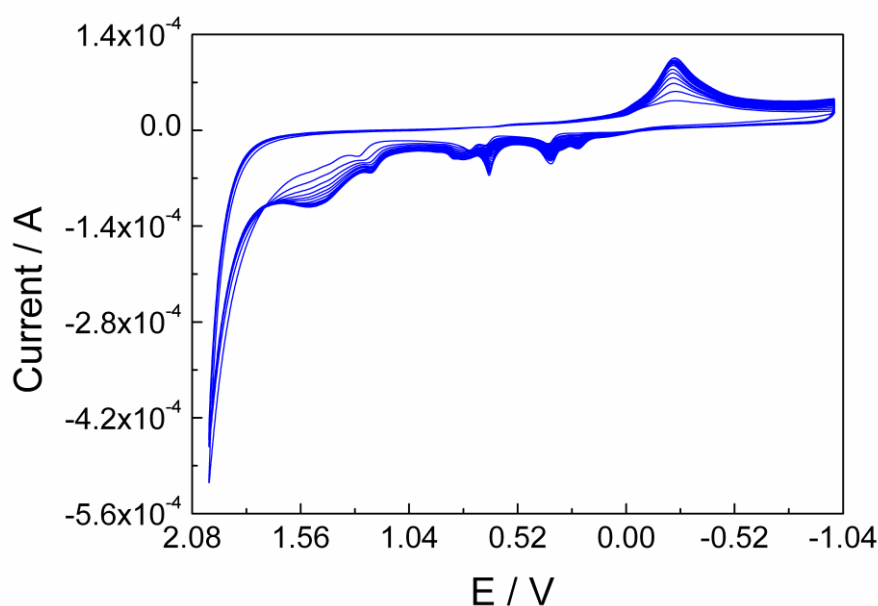


Figure 1. Cyclic voltammograms of polymerization of acid chrome blue K on GCE

3.2. Electrochemical characteristics of the modified electrode

The electrochemically effective surface areas (A) of poly(acid chrome blue K) /GCE were investigated by chronocoulometry using $1.0 \times 10^{-4} \text{ mol dm}^{-3} \text{ K}_3[\text{Fe}(\text{CN})_6]$ based on Anson equation: [24]

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

where c is substrate concentration, D is the diffusion coefficient, n is electron transfer number, Q_{dl} is double layer charge which could be eliminated by background subtraction, Q_{ads} is Faradic charge. Other symbols have their usual meanings. Based on the slopes of the curves of Q versus $t^{1/2}$, the geometric surface area of poly(acid chrome blue K)/GCE was calculated to be 0.34 cm^2 .

The poly(acid chrome blue K)/GCE was characterized by electrochemical impedance spectroscopy (EIS) (shown in Fig. 2). From Fig. 2, It can be seen that the redox process of the $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ ions showed an electron transfer resistance of about 900Ω at a bare GCE. When a bare GCE was electropolymerized with acid chrome blue K for 25 cyclic times, it showed a higher interfacial electron transfer resistance ($R_{ct} = 3500 \Omega$), indicating that the poly(acid chrome blue K) resisted electron transfer of the electrochemical probe. The result of AC impedance also supported that the poly(acid chrome blue K) film had been assembled onto the bare GCE surface.

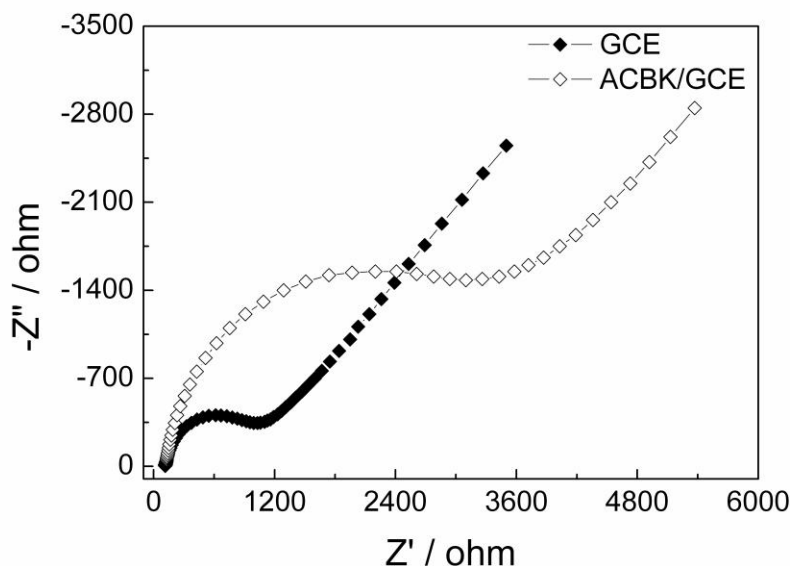


Figure 2. Nyquist plots in impedance measurements of a bare GCE and the poly-ACBK film modified GCE with 25 electropolymerization cyclic times.

The following equation applies: [25]

$$R_{ct} = \left(\frac{RT}{n^2 F^2 A k^0} \right) \left(\frac{1}{C_0^{(1-\alpha)} C_R^\alpha} \right) \quad (2)$$

With an electron transfer coefficient (α) of 0.5, the geometric surface area of poly(acid chrome blue K)/GCE is 0.34 cm^2 , the standard heterogeneous electron transfer coefficient (k^0) of bare GCE and poly-ACBK/GCE are $2.35 \times 10^{-6} \text{ cm s}^{-1}$ and $2.26 \times 10^{-7} \text{ cm s}^{-1}$, respectively. Evidently, k^0 ($2.35 \times 10^{-6} \text{ cm s}^{-1}$) of bare GCE is larger than k^0 ($2.26 \times 10^{-7} \text{ cm s}^{-1}$) of poly(acid chrome blue K)/GCE, proving that the poly(acid chrome blue K) film resisted electron transfer of the electrochemical probe. The electrode coverage (θ) is a key factor which can be used to estimate the surface state of the electrode, and the charge-transfer resistance is also related to it. The surface coverage (θ) of poly(acid chrome blue K)/GCE can be evaluated from the EIS according to the equation: [26, 27]

$$\theta = 1 - \frac{R_{ct}^{Bare}}{R_{ct}^{Poly-ACBK}} \quad (3)$$

where R_{ct}^{Bare} denotes the charge transfer resistance of the bare GCE, $R_{ct}^{Poly-ACBK}$ is the charge transfer resistance of the poly(acid chrome blue K)/GCE. The surface coverage (θ) of electrode was estimated to be 72.9%.

3.2. Electrochemical behaviors of Vanillin on the Poly(acid chrome blue K)/GCE

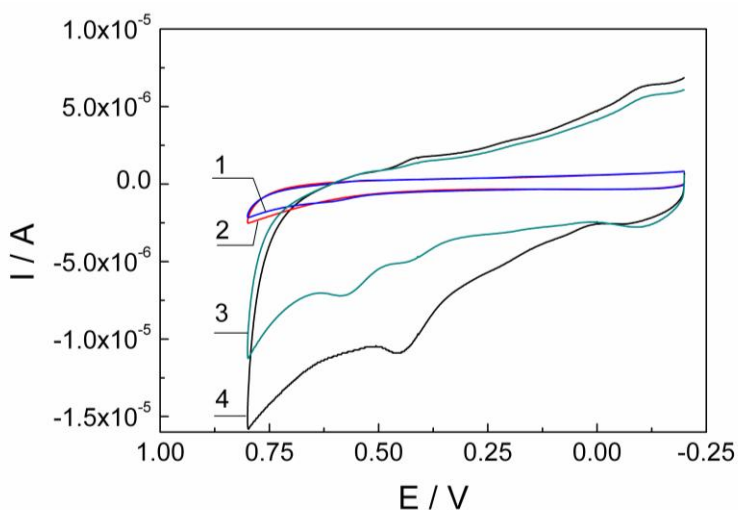


Figure 3. voltammograms of bare GCE (1, 2) and Poly(acid chrome blue K)/GCE (3, 4) in solution without (1, 3) or with $5.0 \times 10^{-6} \text{ mol L}^{-1}$ vanillin (2, 4)

The voltammograms of vanillin at bare GCE and Poly(acid chrome blue K)/GCE in PBS (pH 6.4) were shown in Fig. 3. It showed that an oxidation peak was observed in the reverse scan. At a bare GC electrode, oxidation peak at 0.606 V was observed in the reverse scan. After electropolymerization, the anodic peak potential shifted positively to 0.562 V. The oxidation peak current value (I_p) of vanillin at Poly(acid chrome blue K) /GCE was $2.213 \times 10^{-6} \text{ A}$, which is 12.2 times larger than the one at the bare GCE. The data obtained clearly show that the combination of Poly(acid

chrome blue K) improve the characteristics of vanillin oxidation. This suggested the Poly(acid chrome blue K)/GCE could considerably catalyze the electrooxidation of vanillin.

3.3. Optimal of experimental conditions

The effect of solution pH on the electrochemical response of 5.0×10^{-6} mol L⁻¹ vanillin at Poly(acid chrome blue K)/GCE was investigated with PBS solution in the pH range from 2.0 to 8.0 by cyclic voltammetry. As can be seen in Fig. 4, the maximum value of oxidation peak current appeared at pH 3.0, so this value was selected throughout the experiments. Fig. 4(B) showed that the oxidation (E_{pa}) potentials shifted negatively with increasing pH, and the regression equations can be expressed as $E_{pa} \text{ (V)} = -0.0516 \text{ pH} + 0.8831$ ($R = 0.9993$). The slope of -51.6 mV pH^{-1} demonstrated that the numbers of electron and proton transferred in the electrochemical reaction of vanillin were equal.

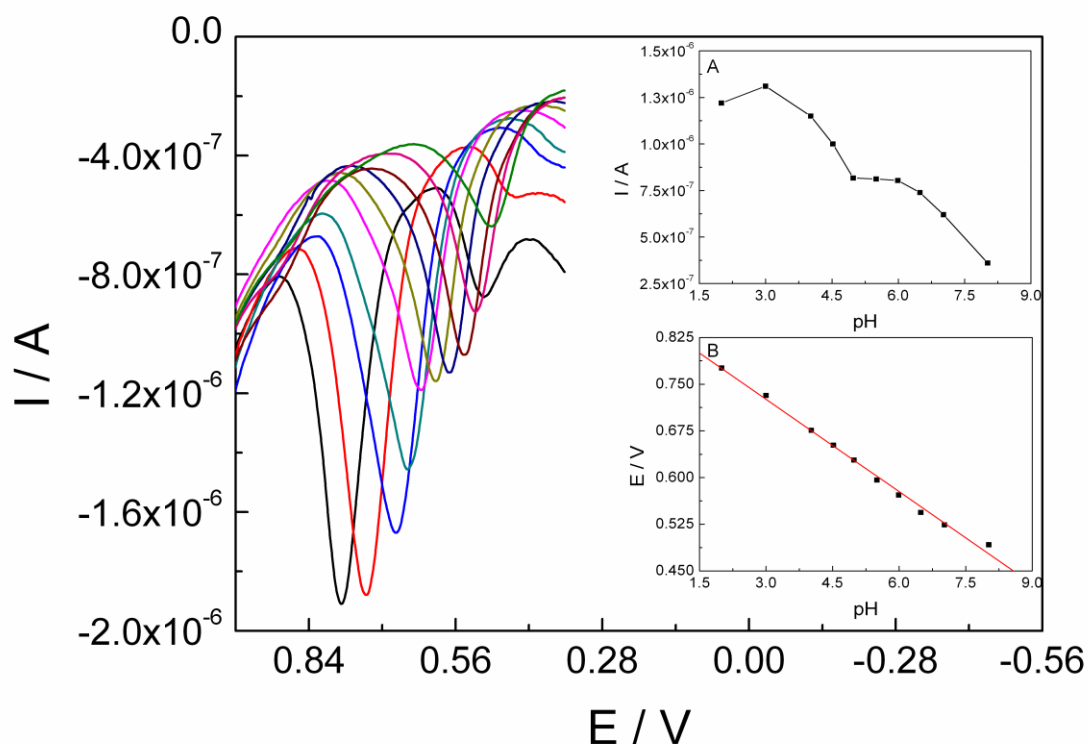


Figure 4 Influence of pH on the oxidation peak current and potential of 5.0×10^{-6} mol L⁻¹ vanillin at Poly(acid chrome blue K)/GCE in the sweep rate of 100 mV s^{-1} .

3.4. Effect of scan rate

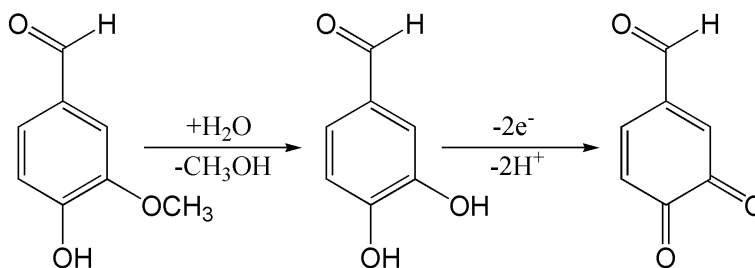
The electrochemical behaviors of vanillin at different scan rates were investigated on the surface of the Poly(acid chrome blue K)/GCE by cyclic voltammetry. A linear relationship ($I_p = 7.8 \times 10^{-9} v + 3.4 \times 10^{-7}$, $n=17$, $r=0.9978$) was obtained between the peak current and the scan rate in the

range of 0.01–0.9 V s⁻¹, which revealed that the oxidation of vanillin was an adsorption-controlled process.

The peak potential shifted to more positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate can be expressed as E_p (V) = 0.0135 ln v (V/s) + 0.6724 ($n=19$, $r=9965$). As for an irreversible electrode process, according to Laviron [28], E_p is defined by the following equation:

$$E_p = E^{\theta'} + \frac{RT}{\alpha n F} \ln v \quad (4)$$

where E_p is the peak potential (V vs. SCE), $E^{\theta'}$ is the formal potential (V vs. SCE), R is the universal gas constant (8.314 J K⁻¹ mol⁻¹), T is the temperature (K), α (alpha) is the charge transfer coefficient for the oxidation step, n is the number of electrons involved in the rate determining step, and F is the Faraday constant (96,485 C mol⁻¹). According to Bard and Faulkner,²⁴ for a totally irreversible wave, E_p is a function of scan rate, shifting (for an oxidation) in a positive direction by an amount $1.15RT/\alpha F$ for each ten fold increase in v . So, from this we get the value of α to be 0.85. The number of electron (n) transferred in the electro-oxidation of Vanillin was calculated to be 2.2 (approximately equal to 2). So the proposed mechanism on the Poly(acid chrome blue K)/GCE may be expressed with the following equation, which involved two electrons and two protons oxidation process (Scheme. 1).



Scheme 1. Electrochemical mechanism of vanillin.

Since the electrode process was adsorption-controlled. The accumulation time for vanillin adsorbing on the Poly (acid chrome blue K)/GCE was also investigated. It was found that the peak current reached its maximum at 70 s with accumulation potential at 0.7 V. So 70 s of accumulate time and 0.7 V of accumulation potential were used in the following procedure.

3.5. Calibration curve

Differential-pulse voltammograms obtained with increasing amounts of vanillin showed that the peak current increased linearly with increasing concentration, as shown in Fig. 5. Using the optimum conditions described above, linear calibration curves were obtained for vanillin in the range

of 1.0×10^{-7} – 7.0×10^{-5} mol dm⁻³. The linear equation were I_p (A) = $3.93 \times 10^{-2} c$ (mol dm⁻³) + 7.05×10^{-8} ($r = 0.9972$). The detection limit was estimated to be 3.2×10^{-8} mol dm⁻³ (3σ).

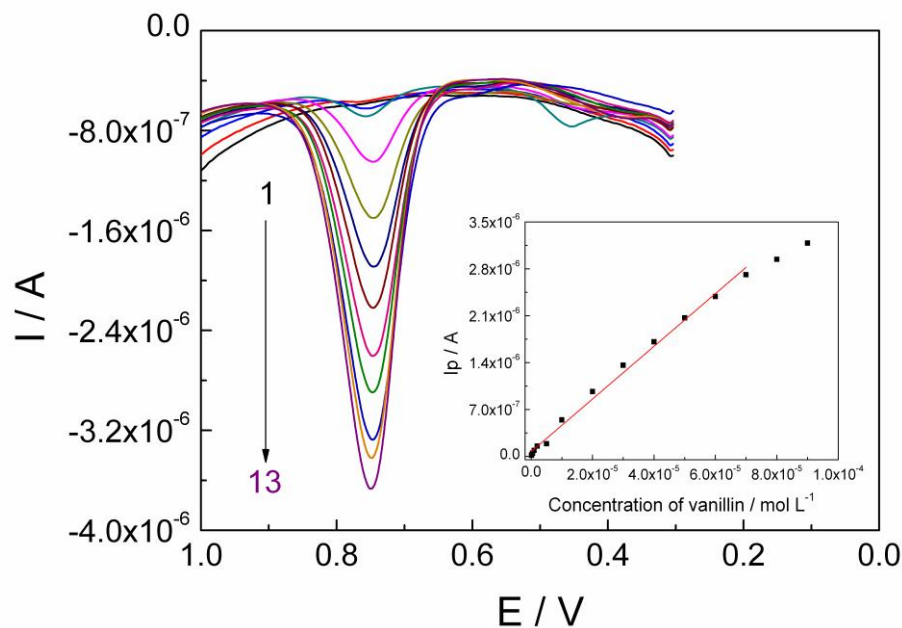


Figure 5. Ddifferential pulse voltammograms of Poly(acid chrome blue K)/GCE in different concentrations of vanillin solutions.

3.6. Reproducibility, stability and interferences

For investigating the fabrication reproducibility, a 1.0×10^{-5} mol dm⁻³ vanillin solution was measured by six modified electrodes prepared independently and the RSD of the peak current was 2.9 %, revealing excellent reproducibility. After the electrode was stored for 7 days at 4°C in humidity environment, it could retain 95.7 % of its original response, suggesting acceptable storage stability. In addition, 1000-fold concentration of glucose, lactose, sucrose, CaCl₂, AlCl₃; 300-fold concentration of NaCl, KNO₃, CuSO₄; 200-fold concentration of FeCl₂; 100-fold concentration of MgCl₂, citric acid; 60-fold concentration of Zn(Ac)₂, MnCl₂, aminoacetic acid; 20-fold concentration of FeCl₃; 10-fold concentration of V_c do not interfere with the reduction signal of 1.0×10^{-5} mol dm⁻³ vanillin (peak current change < 5%). These results indicated that Poly(acid chrome blue K)/GCE has an excellent selectivity for vanillin, and it might be applied to determine vanillin in real samples.

3.7. Analytical application

In order to test the practical application of the proposed method, the Poly(acid chrome blue K)/GCE was used to determine vanillin in commercial biscuits and dark-chocolate. The results obtained by the proposed and reference procedures (Spectrophotometric method⁴ for the assay of vanillin was adopted) were compared in Table 1 and were in good agreement.

Table 1. Determination of vanillin in real samples

Samples	reference method (mg g ⁻¹)	Proposed method (n=5)				
		Determination (mg g ⁻¹)	Added (mg g ⁻¹)	Founded (mg g ⁻¹)	RSD (%)	Recovery (%)
biscuits	1.58	1.62	1.20	2.81	2.15	99.2
dark-chocolate	1.13	1.17	1.20	2.35	3.02	98.3

4. CONCLUSIONS

Electrochemical polymerization of acid chrome blue K on the surface of GCE was performed and the properties of the resulted polymer films modified electrode were investigated. The polymer film on the electrode showed good electrocatalytic ability to the oxidation of vanillin and the electrochemical behaviors of vanillin on Poly(acid chrome blue K)/GCE was carefully investigated. Under the optimal conditions, a linear relationship of the oxidation peak current and the vanillin concentration was obtained within the range from 1.0×10^{-7} to 7.0×10^{-5} mol dm⁻³ by differential pulse voltammetry, and the method was further applied to determine vanillin in commercial biscuits and dark-chocolate with satisfactory results.

ACKNOWLEDGMENTS

This work was financially supported by the Program for Excellent Talents in Guangxi Higher Education Institutions.

References

1. N. J. Walton, M. J. Mayer, and A. Narbad, *Phytochemistry*, 63 (2003) 505.
2. P. L. Teissedre, and A. L. Waterhouse, *J. Agric. Food Chem.*, 48 (2000) 3801.
3. D. Farthing, D. Sica, C. Abernathy, I. Fakhry, J.D. Roberts, D.J. Abraham, and P. Swerdlow, *J. Chromatogr. B*, 726 (1999) 303.
4. E. W. Ainscough, and A. M. Brodie, *J. Chem. Educ.*, 67 (1990) 1070.
5. L. S. D. Jager, G. A. Perfetti, and G. W. Diachenko, *Food Chem.*, 107 (2008) 1701.
6. T. Sostaric, M. C. Boyce, and E. E. Spickett, *J. Agric. Food Chem.*, 48 (2000) 5802.
7. V. S. Sobolev, *J. Agric. Food Chem.*, 49 (2001) 3725.
8. K. N. Waliszewski, V. T. Pardo, and S. L. Ovando, *Food Chem.*, 101 (2006) 1059.
9. M. C. Boyce, P. R. Haddad, and T. Sostaric, *Anal. Chim. Acta*, 485 (2003) 179.
10. M. Ohashi, H. Omae, M. Hashida, Y. Sowa, and S. Imai, *J. Chromatogr. A*, 1138 (2007) 262.
11. J. Peng, C. Hou, X. Hu, *Int. J. Electrochem. Sci.*, 7 (2012) 1722.
12. F. Bettazzi, I. Palchetti, S. Sisalli, and M. Mascini, *Anal. Chim. Acta*, 555 (2006) 134.
13. J. L. Hardcastle, C. J. Paterson, and R. G. Compton, *Electroanal.*, 13 (2001) 899.
14. D. J. Kong, S. F. Shen, H. Y. Yu, J. D. Wang, and N. S. Chen, *Chinese J. Inorg. Chem.*, 26 (2010) 817.
15. M. Luque, E. Luque-Pérez, A. Ríos, and M. Valcárcel, *Anal. Chim. Acta*, 410 (2000) 127.
16. D. Y. Zheng, C. G. Hu, T. Gan, X. P. Dang, and S. S. Hu, *Sens. Actuators, B*, 148 (2010) 247.

17. A. F. Diaz, K. K. Kanazawa, and G. P. Gardini, *J. Chem. Soc. Chem. Commun.*, 1979, 635.
18. A. F. Diaz, J. A. Logan, *J. Electroanal. Chem.*, 111 (1980) 111.
19. K. Lin, C. Yin, S. Chen, *Int. J. Electrochem. Sci.*, 6 (2011) 3951.
20. B. Wang, T. Kong, J. Zhao, C. Cui, R. Liu, Q. He, *Int. J. Electrochem. Sci.*, 7 (2012) 615.
21. R. Zhang, G. D. Jin, D. Chen, and X. Y. Hu, *Sens. Actuators, B*, 138 (2009) 174.
22. B. Wang, J. Zhao, J. Xiao, C. Cui, R. Liu, *Int. J. Electrochem. Sci.*, 7 (2012) 2781.
23. P. Kalimuthu, and S. A. John, *Electrochim. Acta*, 56 (2011) 2428.
24. F. C. Anson, *Anal. Chem.*, 36 (1964) 932.
25. A. J. Bard, and L. R. Faulkner, “*Electrochemical Methods Fundamentals and Application*”, 2004, 2nd ed., Wiley.
26. E. Sabatani, J. Cohen-Boulakia, M. Bruening, and I. Rubinstein, *Langmuir*, 9 (1993) 2974.
27. C. Henke, C. Steinem, A. Janshoff, G. Steffan, H. Luftmann, M. Sieber, and H. J. Galla, *Anal. Chem.*, 68 (1996) 3158.
28. E. Laviron, *J. Electroanal. Chem.*, 100 (1979) 263.