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Vanillin Determination in Food Products with Pd Nanoparticles Modified Poly(Methylene Blue) Film Electrode

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Abstract

Here, metal nanoparticles modified conductive polymer film electrode was fabricated via electrochemical technique. Methylene blue was electrochemically polymerized on the bare glassy carbon electrode surface. Then palladium nanoparticles were modified on the polymer surface by consecutive potential cycles. Resulting composite electrode was characterized with scanning electron microscopy and electrochemical impedance spectroscopy. Palladium nanoparticles modified poly(methylene blue) film glassy carbon electrode was used for sensitive and selective determination of vanillin with two linear ranges between 0.02 - 1 µM and 2 - 50 µM and a limit of detection as 0.01 µM. Proposed electrode accurately determine vanillin content in commercial biscuit and cake samples.

Keywords: Vanillin, Pd Nanoparticles, Methylene Blue, Modified Electrode, Voltammetry.

1. Introduction

4-hydroxy-3-methoxybenzaldehyde, known as vanillin ($C_8H_8O_3$) is a phenolic compound that is found naturally in vanilla seeds [1]. Vanillin (VAN) is the most commonly ingredient that responsible from flavoring in beverages and foods, used in perfumery and cosmetic industry, and also in pharmaceutical aids [2]. VAN extract from natural seeds is expensive that synthetic form was used in many industries. The excess use of VAN led to various diseases such as headache and kidney problems [3]. Therefore, it is significant to determine its amount both for health issues and control the quality of foods.

VAN can be determined with capillary electrophoresis [4], liquid chromatography-quadrupole linear ion trap mass spectrometry [5] and chemiluminometry [6]. In spite of the fact that these techniques require time consuming sample preparation step and operational expertise. Besides they constructed expensive equipment and consume lots of reagents. Electrochemical techniques overcome these mentioned disadvantages of other technique with its simplicity, fast response, and low costed equipment. Sensitivity of the electrochemical techniques are mainly affected by the electrode type that many researchers used modified electrodes to enhance the poor response of conventional electrodes towards analytes [7]. For this purpose; conductive polymers [8] and metal nanoparticles [9] were widely used.

Methylene blue (MB), (3,7-bis(dimethylamino)phenothiazine), is a polycyclic structure that contains sulphur and nitrogen atoms. It is the member of phenothiazine family and known as a common organic dye which is water soluble [10]. MB is also used for electrode modification due to its polymerization ability, strong adherence to the surface that led a stable modifier and high conductivity that promote electron transfer [11]. Polymerized MB film electrodes were used for determination of wide range of organic/inorganic molecules such as dopamine [12], ascorbic acid [13], sulphide [14].

Metal nanoparticles attracted significant interests for various fields of research area due to their large surface area, ability to promote electron transfer rate and good catalytic activity that reduce over-potential [15]. Pd is one of the most studied metal particles, among all kind of nanoparticles. Pd nanoparticles were used in determination of food additives, antidepressants, detection of hydrogen peroxide, and catalysis of methanol and ethanol oxidation in fuel cells, promotion of methane oxidation [16, 17].

Here, palladium nanoparticles decorated conductive methylene blue polymer was prepared by two-step for highly sensitive determination of VAN. In first step, MB monomer was electrochemically polymerized on the glassy carbon electrode surface and then Pd nanoparticles were modified on the polymer film via electrochemical deposition technique by cyclic voltammetry in the second step. Resulting composite surface enhanced the electrocatalytic activity of bare electrode towards VAN that has high stability and sensitivity. Moreover, proposed electrode provides accurate determination of VAN in different food products.

2. Materials and Methods
2.1. Reagents and Apparatus

$PdCl_2$, H_2SO_4 and HCl were supplied from Merck. All reagents used without further purification. Milli-pore ultra-pure water was used for dilution of the solutions. pH of PBS solution was adjusted with NaOH.

An Autolab electrochemical system (Metrohm) equipped with a NOVA software was used for voltammetric studies. All measurements were carried out in a three-electrode system consisting of; a platinum wire as a counter electrode, Ag/AgCl/KCl as a reference electrode, and glassy carbon electrode (GCE) as a working electrode. The electrodes were characterized with scanning electron microscope (SEM; JEOL JSM-7500F).

2.2. Preparation of Commercial Food Samples

Commercial biscuit and cakes were obtained from a local market. Each food samples were ground in a mortar until homogenized particles were obtained. 0.5 g of this resulting powder of each biscuit and cake was mixed with 10.0 mL ethanol. The mixture was mechanically shaken for 90 min and then centrifugated for 20 min at 4000 rpm. Finally, above of this solution tube was used for VAN determination.

3. Results and Discussion
3.1. Preparation and Characterization of Electrode

Fig.1a shows the polymerization of MB monomer on bare GCE surface in alkaline media. Polymerization potential window was selected as wide as possible to see the all oxidation and reduction peaks. Potential cycles were applied between -0.7 to +1.2 V in PBS (pH 10) containing 2 mM MB monomer. Cationic radical species were formed at higher potentials (1.1 V) which were necessary for polymerization. In addition, an anodic peak (−0.13 V) and cathodic peak (-0.35 V) were observed in the first cycle. By the formation of polymeric film on the electrode surface, the anodic peak was decrease while cathodic peak was slightly shifted to negative values. With the consecutive cycles, another redox pair was observed. These new pair of peaks reduced at −0.10 V, while oxidized at more positive potentials with successive cycles. The successful polymerization was occurred at GCE surface by electropolymerization technique which is also confirmed with scanning electron microscopy and electrochemical impedance spectroscopy techniques. In addition, Pd nanoparticles were deposited on the PMB/GCE surface by cyclic voltammetry that was presented in Fig.1b. PMB/GCE was immersed in the 1 mM Pd^{2+} containing H_2SO_4 solution and ten repetitive cycles were applied between + 0.30 V and -0.75 V. Both anodic and cathodic peaks were increased with the increase in the potential cycles that depicts the formation of Pd nanoparticles on the polymer film surface.

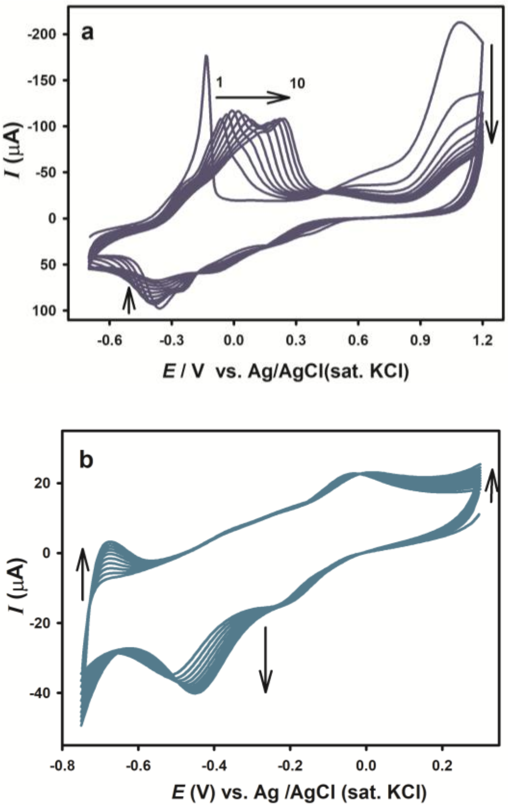

Figure 1. a) Polymerization of 2 mM MB on GCE in pH 10 PBS with 0.1 V s^{-1} scan rate. **b)** CVs of palladium nanoparticles deposition on PMB/GCE.

The morphologies of bare GCE, PMB/GCE and Pd/PMB/GCE were recorded by scanning electron microscopy (SEM). A smooth and uniform surface was observed for bare GCE (Fig. 2a). It can be directly observed that, bud like shapes formed on the bare surface after the electropolymerization MB (Fig.2b). The buds on the surface was growth with the Pd nanoparticles deposition and EDX analysis confirms the existence of Pd content (%13 weight gain) on the Pd/PMB/GCE surface (Fig.2c). Physical and interfacial properties of the bare and modified electrodes were characterized with electrochemical impedance spectroscopy (EIS). Spectra was performed in 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.1 M KCl solution. Frequencies between 5 to 75000 Hz were applied at GCE, PMB/GCE, Pd/PMB/GCE and comparative results were represented in Fig. 2d. The impedance circuit contains the solution resistance (Rs), , constant phase element (CPE or Q), and charge transfer resistance (Rct). In Nyquist diagrams, a straight line with a slope of nearly 45◦ is related to the mass transport process and semicircle diameter at high frequencies relates to the resistance characteristics of the electrode/electrolyte interface. A large semicircle related to the highest the charge transfer resistance was obtained for bare GCE (380 Ω, curve *i*). An obvious decrease in resistance observed for curve *ii* (95 Ω) indicates the formation of conductive PMB on the bare GCE surface that could accelerate the electron transfer rate. After the Pd nanoparticles deposition, the resistance of the electron transfer was significantly reduced (25 Ω, curve *iii*). Overall results depict the formation of both Pd nanoparticles and PMB film on the bare GCE surface.

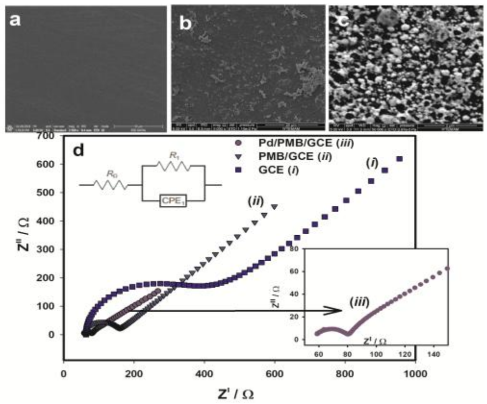

Figure 2. SEM images of **a)** Bare GCE, **b)** PMB/GCE, **c)** Pd/PMB/GCE and **d)** EIS spectra of electrodes. Inset contains related circuit and zoomed plot of Pd/PMB/GCE.

3.2. Voltammetric Behavior of VAN

The electrochemical behaviour of VAN was investigated at different electrodes using cyclic voltammetry to understand the effect of modifiers. Fig. 3 depicts the CVs of GCE, PMB/GCE, and Pd/PMB/GCE at 0.1 mM VAN containing pH 6 buffer solution. The quasi-reversible redox behavior observed for VAN at all electrodes. Bare GCE has very poor signal (3 µA) indicating slow electrode kinetics. After the polymerization of MB on GCE surface, peak current significantly enhanced (12.3 µA). VAN oxidation was observed at 0.80 V at both PMB/GCE and Pd/PMB/GCE. Another small oxidation peak was observed at 0.83 V in the reverse scan that can be attributed to the re-oxidation of adsorbed VAN compounds on the electrode surface. Morover, the highest peak current was obtained at Pd/PMB/GCE (39.3 µA) that can be attributed to the synergistic effect of metal nanoparticles and conductive polymer. The improved peak characteristics in terms of peak current was obtained for proposed Pd/PMB/GCE which is 13 times higher than bare GCE and 3 times higher than PMB/GCE.

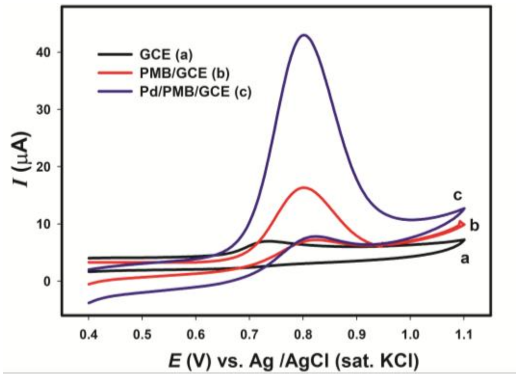

Figure 3. CVs of 0.1 mM VAN in pH 6 PBS with 0.05 V s-1 scan rate at a) GCE. b) PMB/GCE and c) Pd/PMB/GCE.

The effect of pH on the VAN behavior was studied by CV in 0. 1 M buffer solution with various pH values (from 2 to 8) (not shown here). A linear relationship between the peak potential and the pH was observed at Pd/PMB/GCE. The slope of related plot is close to the theoretical value of 59 mV/pH that suggested as electrochemical reaction of VAN occurred with two-proton/ two-electron transfer. In addition, the highest peak current was obtained at pH 6 was chosen as an optimum value for further studies. Moreover, to obtain more sensitive determination results, cycle number of polymerization, monomer and palladium concentration and Pd deposition cycle number were also optimized. Considering the overall results, parameters were optimized as follows; monomer concentration: 2 mM, cycle number of polymerization; 20, Pd concentration; 1 mM and cycle number of Pd deposition; 10. These optimized parameters were used for further studies.

In order to understand the surface reaction was controlled by diffusion or adsorption, VAN oxidation was investigated with various scan rates (10-250 mV s^{-1}). Based on these data, Ipa versus square root scan rate ($\upsilon^{1/2}$) graphs were plotted for bare GCE, PMB/GCE and Pd/PMB/GCE and it was presented in Fig. 4. VAN peak current was increased at all electrodes with increasing the scan rate. The slope value was getting higher with the electrode modification as expected. According to the results, it can be said that electrode reaction was all controlled by diffusion at each electrode.

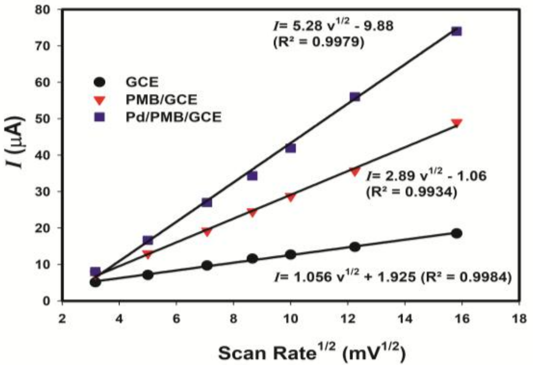

Figure 4. Dependence of VAN peak current on square root of scan rate at different electrodes.

Differential pulse voltammetry (DPV) was selected for determination of VAN by using Pd/PMB/GCE under the optimized experimental conditions (pulse amplitude: 50 mV, pulse time: 30 ms, scan rate: 10 mV s^{-1}). Fig. 5 exhibits the DP voltammograms of increasing concentrations of VAN at prepared electrode. Peak current of VAN was proportional with its concentration in the ranges of 20 nM - 1 µM and 2 µM - 50 µM with two segments. The related calibration graph was located in the inset of Fig.6. The linear regression equations were found as Ipa (µA) = 10.43 C_{VAN} (µM) + 1.83, (R^2

=0.9941), and Ipa (µA) = 0.260 C_{VAN} (µM)+ 12.35, (R^2 =0.9957). Calculated detection limit was 10 nM (S/N = 3). The proposed Pd/PMB/GCE has relatively lower detection limit value than those previously published literatures as in Table 1 [27-29].

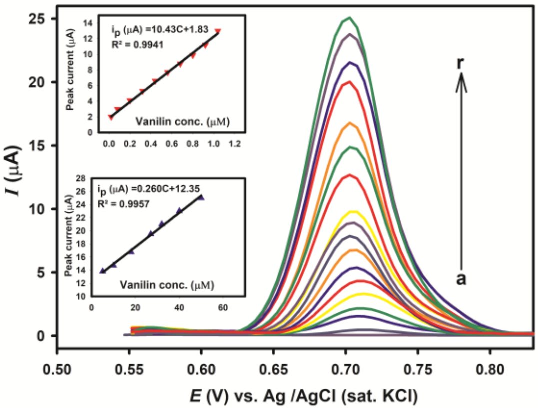

Figure 5. DPVs of Pd/PMB/GCE with increasing concentrations. (a: blank solution, b to r: 20 nM to 50 µM VAN). Insets are related calibration graphs.

Interference study gave information about selectivity of the proposed method. Herein, various interfering substances (benzoic acid, glucose, , sucrose, oxalic acid, caffeic acid, tartaric acid, citric acid) and some ions such as Zn^{2+} , Al^{3+} , Mg^{2+}, Na^{+} Fe^{3+}; Pb^{2+}, K^{+}, Cl^{-}, NO_3^{-}, SO_4^{2-} was studied. 1000 fold excess of each substance has no significant effect on 5 µM VAN (RSD ≤ %5).

One of the main aims of using composite electrode is to enhance the oxidation current and stability of the electrode surface towards the VAN. Thus, oxidation peak current of VAN has been recorded daily for two week with the same electrode. Pd/PMB/GCE kept at above the supporting electrolyte solution in a close system that prevents the entrance of oxygen. At the end of two weeks, the electrode maintain %97 of its original signal towards VAN. It can be said that highly stable electrode surface was achieved with this study. Moreover, 10 different fabricated Pd/PMB/GCE were employed for VAN detection and RSD was calculated as 3.1%. Repeatability studies of Pd/PMBGCE were utilized with measuring the VAN response of 50 repetitive measurements. Satisfactory results were obtained as 3.4% of RSD value.

Table 1. Comparison of literature results with the proposed method.

Method	Linear range	LOD	Ref.
Au-Pd/graphene/GCE	0.1-7.0 and 10.0-40.0 µM	0.020 µM	[18]
Au/MWCNT/GCE	0.02-6.5 and 7.0-75 µM	0.038 µM	[19]
Ag nanoplates graphene/GCE;	2-100 µM	0.33 µM	[20]
Pd/PMB/GCE	**0.02- 1 and 2-50 µM**	**0.01 µM**	**This work**

3.3. Real Sample Assay

The practical applicability of the proposed method was investigated with various food products at Pd/PMB/GCE via standard addition method. Commercial biscuit and cake samples pretreated according to the sample preparation method in Section 2.2.

Table 2. Vanilin determination in different foods (n=3).

Samples	Added µM	Found µM	Recovery %	RSD %
Cake	0	2.3	-	3.2
	4	6.1	96.8	2.7
	8	10.4	100.9	3.1
	12	14.5	101.4	2.4
Bisquite	0	2.5	-	2.3
	4	6.7	103.1	2.9
	8	10.3	98	3.2
	12	14.7	101.4	2.7

The obtained results are given in Table 2 and recovery values are found in the range of 96.8% and 103.1%. Vanillin content in biscuit and cake samples were found as 76.45 µg/g and 76.07 µg/g, respectively. These high recovery values reveal that the developed composite electrode has accurately determine VAN content in commercial cake and biscuit samples.

4. Conclusion

In this study, electrodeposition of Pd nanoparticles on electropolymerized methylene blue film was successfully fabricated on glassy carbon electrode. Based on the results, modification of Pd nanoparticles on PMB film significantly increases the activity of electrode towards vanillin. The common interferants have no influence in the oxidation signal of VAN. The proposed electrode has two linear segments (20 nM- 1 µM and 2 µM- 50 µM) and low detection limit of 10 nM by DPV. In conclusion, the proposed method is a promising alternative for accurate and sensitive determination of VAN in various food products.

Ethics

There are no ethical issues after the publication of this manuscript.

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