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Carbon Screen-Printed Electrode Coated with Poly (Toluidine blue) as an Electrochemical Sensor for the Detection of Tyramine †

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Abstract: In the present work the surface modification of a carbon screen-printed electrode by electrochemical polymerization of toluidine blue (TB) for determination of tyramine is described. The electrochemical polymerization of the electrode with TB was done by cyclic voltammetry at a scan rate of 50 mV/s and a potential sweep between –0.7 V and 1.0 V in the presence of 0.5 mM TB in an electrolyte solution. At each cycle, the polymer film started to deposit on the carbon screen-printed electrode which was repeated 20 times. For parameter optimization the electrochemical behavior of the modified electrode was analyzed by amperometric methods such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A phosphate buffer solution (PBS) was used as an electrolyte for all the amperometric experiments. The electrochemically modified poly-TB-coated carbon-screen-printed electrode showed an oxidation peak potential of tyramine at 0.67 V. The unmodified carbon-screen-printed electrode showed the tyramine oxidation peak potential at 0.9 V. Based on the voltammetric results, it was found that the poly-TB-modified carbon-screen-printed electrode showed higher sensitivity (1.78 μA nM^{-1} cm^{-2}) than a bare carbon-screen-printed electrode toward tyramine detection. Tyramine in 0.1 M PBS (pH 7.4) was analyzed by cyclic voltammetry from the potential of –0.7 to 1.0 V at a scan rate of 50 mV/s. The poly-TB-modified carbon-screen-printed electrode exhibited a linear response between catalytic peak current and tyramine concentration from 0.02 μM to 270.5 μM with a lower detection limit of 0.007 μM (S/N = 3).

Keywords: tyramine; poly (toluidine blue); sensor; polymerization; oxidation potential

1. Introduction

Tyramine (1-hydroxy-4-ethylaminobenzene) is a bioamine which are organic nitrogenous compound that may naturally form in food by bacterial decarboxylation. Tyramine is relatively present in the byproduct of microbial activity, fermented foods, dairy products, beverages, fish, and meat etc., [1–4]. High concentration of tyramine-contaminated foods consumption may cause negative impact on the human health such as, diarrhea, hypotension, migraine, cardiac failure, and low blood pressure etc., [5–8]. Tyramine belongs to the group of biogenic amines, including histamine, putrescine, and cadaverine. Considering the undesirable physiological effect of tyramine, it is important to develop highly sensitive, selective, and low cost methods for tyramine determination in food and beverages. The European food safety authority encourages researchers and provide finding for toxic bioamine analysis from fermented foods [9,10]. Currently numerous methods are available for tyramine analysis such as high-performance liquid chromatography [11],

mass spectrometry analysis [12], capillary electrophoresis [13], and quantitative PCR [14]. Although these methods provide lower detection limits and selectivity, they are highly required qualified methods, consuming more time, with difficult pretreatment steps before the experiments, and expensive instrumentation. Thus, alternative tyramine detections are highly desirable. Compared with the traditional analytical methods, electrochemical sensors development for bioamine detection have gained increasing attention because of their selectivity, sensitivity, low cast, user friendly instruments handling, on spot sample analysis and short analysis time [15]. Recently various electro active materials are used for electrochemical sensors development such as conducting polymers, nano materials, nano particles, nanotubes, metal complexes, and graphene etc., [16–18]. Among all, conducting polymers, modified electrochemical sensor has become a hot topic and opens a new domain in the area of analytical science.

The present work focuses on the design and development of conducting polymer-coated electrochemical screen-printed sensor for detection of tyramine. Poly (toluidine blue) is a well-known conducting polymer that acts as an electron transfer mediator over the electrode surface. The electrochemical performance of the developed poly (toluidine blue) modified screen-printed electrode was used for tyramine detection and investigated by voltammetric techniques.

2. Materials and Methods

2.1. Reagents

Screen printed carbon electrode was purchased from Metrohm Drop Sens (Spain). Tyramine, phosphate buffer saline (PBS), and toluidine blue were purchased from Sigma Aldrich (Sigma-Aldrich Handels Gmbh, Vienna, Austria). All other reagents and solvents were obtained commercially and used as-received unless otherwise specified.

2.2. Equipment

Cyclic voltammetry and differential pulse voltammetry were performed by Gamry instruments reference 600+ (potentiostat/Galvanostat ZR) Echem Analyst. The carbon screen-printed electrode (DRP-110) contains a single electrochemical cell, and each cell contains the carbon paste (CP) as the working electrode (WE), a silver/silver chloride as the reference electrode (RE), and a carbon paste as the counter electrode (CE). Electrical connections are made by silver and strips general dimensions: 3.4 × 1.0 × 0.05 cm. pH of the electrolyte solutions were measured by pH meter (VWR).

2.3. Poly (Toluidine blue) Coated Screen-Printed Electrode Preparation

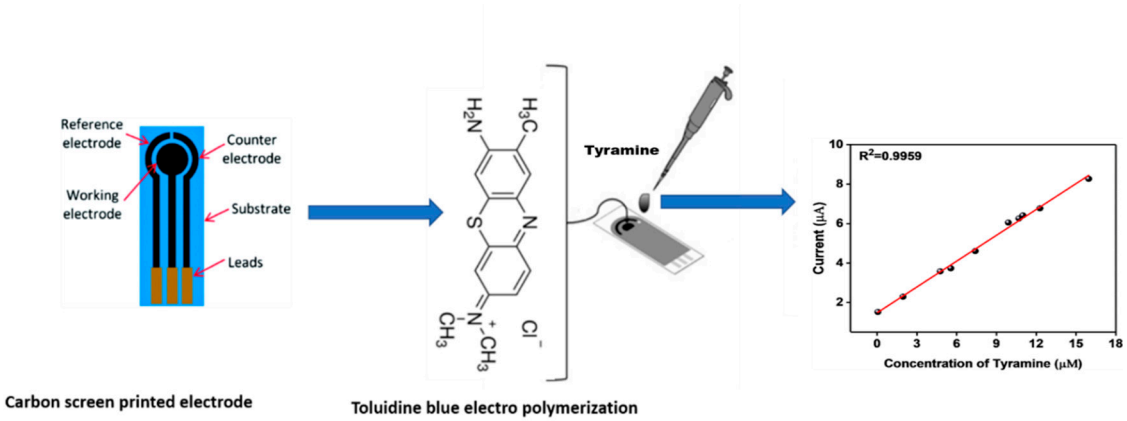
The screen-printed carbon electrode was fixed with the Greamy 600+ electrochemical instrument. Silver leads provide the electrical connection between sensor and the potentiostat, thereby avoiding use of cables and making the device user friendly. Toluidine blue (5 mM) was prepared with PBS (0.1 M. pH 7.4). Toluidine blue containing electrolyte solution (100 μL) was drop casted on top of the three electrode system. Cyclic voltammetry was used to run 20 cycles from –0.7 V to 1.0 V at a scan rate of 50 mV/s. Polymerization slowly increased from 1st cycle to 20th cycle. After the polymerization, the poly-TB modified screen-printed carbon electrode was removed from the electrical connection and washed with double distilled water.

3. Results and Discussion

3.1. Electrochemical Characterization of Poly (Toluidine blue) Screen Printed Carbon Electrode

Electrochemical coating and sensor development are clearly demonstrated in Scheme 1. Three printed electrode chips were coated with poly (toluidine blue) polymer film and used for tyramine analysis. To assess the electrochemical performance, the Poly-TB-modified screen-printed carbon electrode was fabricated and compared with an unmodified screen-printed electrode by cyclic

voltammetry in presence of tyramine (82 μM) and PBS (0.1 M, pH 7.4) at the scan rate of 50 mV/s. Figure 1 shows the cyclic voltammetry results of a bare and a modified electrode. Based on the results the poly-TB-modified screen-printed carbon electrode showed the tyramine oxidation peak at 0.67 V and the bare screen-printed carbon electrode showed the tyramine oxidation peak at 0.9 V with significant lower current response. This current variation and potential shift represent that the poly-TB-modified screen-printed carbon electrode has a higher conductivity, catalytic activity, larger active surface area and sensitivity. Furthermore, the modified electrode showed the oxidation peak at 0.19 V and the reduction peak at –0.06 V which represents the poly (toluidine blue) polymerization [19,20].



Scheme 1. Electrochemical analysis of tyramine using a poly-TB-carbon screen printed electrode.

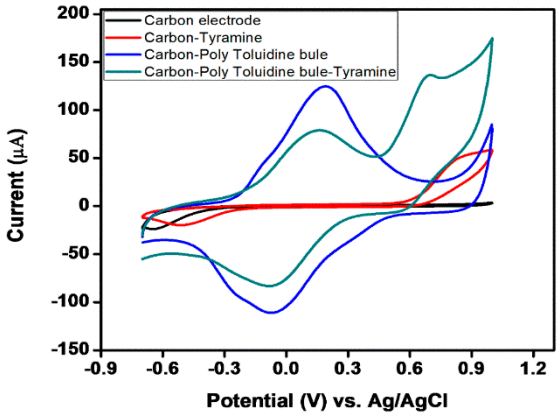


Figure 1. Cyclic voltammetry (CVs) obtained at (black) carbon screen-printed electrode, (red) containing tyramine, (blue) PTB-carbon screen-printed modified, (green) containing tyramine 82 μM in 0.1 M PBS pH 7.4 and scan a rate of 50 mV/s.

3.2. Differential Pulse Voltammetry Studies

The electrochemical behavior of tyramine was analyzed by a poly-TB modified screen-printed carbon electrode. Figure 2a showed the continuous successive additions of tyramine from 0.02 μM to 16 μM with PBS (0.1 M, pH 7.4 V). The oxidation peak appeared at 0.67 V and the peak current was slowly increased based on the tyramine concentration. Figure 2b represents the analytical calibration plot of various concentrations of tyramine vs. the catalytic oxidation current. The anodic current (I_{pa}) was linearly increasing with elevation of the tyramine concentration and the correlation coefficient (R^2) was found to be 0.9959. The lower detection limit (LOD) was calculated as 0.007 μM according to the equation S/N = 3, and the sensitivity was 1.78 μA nM^{-1} cm^{-2}. It can be seen that the poly-TB-modified screen-printed carbon electrode is the most sensitive sensor for tyramine detection which offered satisfactory linear range and lower detection limits.

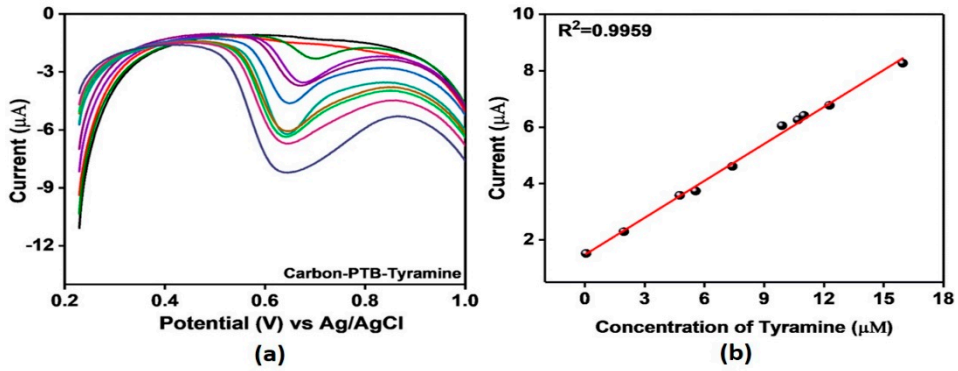


Figure 2. (**a**) Differential pulse voltammetry (DPV) response of PTB-Carbon screen printed electrode in presence of tyramine (0.02 to 16 μM) in 0.1 M PBS of pH 7.4 at a scan rate of 50 mV/s. (**b**) Calibration plot of the current response vs. the concentration of tyramine.

3.3. Long-Term Stability Studies

In order to know the lifetime of the poly-TB-modified screen-printed carbon sensor, measurements were done in a 5-day interval, in presence of tyramine (82 μM) with PBS (0.1 M, pH 7.4) at the scan rate of 50 mV/s. The analysis was done for almost 60 days and the results were recorded. Based on the results, the developed sensor retained 97% response at the end of 60 days (Figure 3). The operating long-term stability of Poly-TB-modified screen-printed carbon-modified electrode was investigated by injecting 82 μM of tyramine in the electrochemical cell every 30 min over extended period of 7 h (inserts). The recorded results do not show any significant changes during the time duration. These results confirmed that the poly-TB-modified screen-printed carbon sensor has both excellent storage capacity and operational stability as an amperometric tyramine sensor.

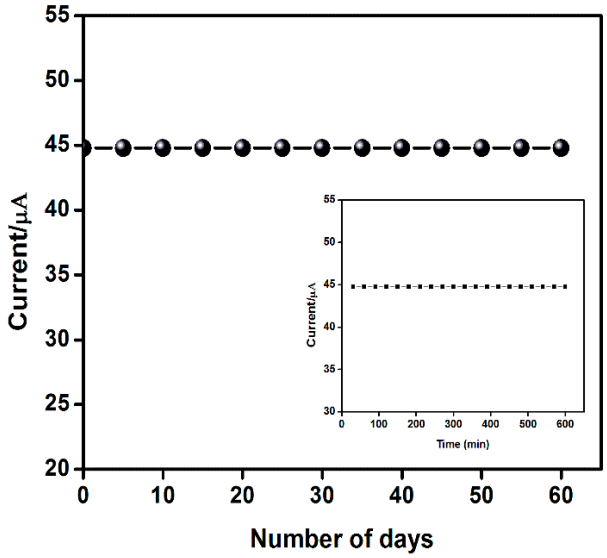


Figure 3. Long-term stability results of the poly-TB-modified screen-printed carbon sensor toward the oxidation current of tyramine (82 μM). Calibration plot of current variation of tyramine vs. different time intervals (Inserts).

4. Conclusions

In this work, a poly-TB modified screen-printed carbon electrode was successfully fabricated, characterized, and used for the first time for tyramine detection. The developed sensor showed good stability, higher sensitivity, lower detection limits, repeatability, long-term durability, and was

inexpensive. All these satisfactory results indicate that a poly (toluidine blue) polymer film has excellent properties as a electroactive material for sensor surface modification.

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