SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
http://dergipark.gov.tr/akademik-gida

Akademik Gıda 18(1) (2020) 1-11, DOI: 10.24323/akademik-gida.729976

Research Paper / Araştırma Makalesi

Low Cost, Sensitive and Selective Chronoamperometric Determination of Vanillin at a Disposable Poly(Eriochrome Black T)/Pencil Graphite Electrode

Serkan Karakaya ✉

Department of Chemistry, Faculty of Science and Arts, Çanakkale Onsekiz Mart University, 17020 Çanakkale, Turkey

Received (Geliş Tarihi): 15.11.2019, Accepted (Kabul Tarihi): 23.03.2020

✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): skarakaya@comu.edu.tr (S. Karakaya)*
☎ +90 286 218 00 18 / 1955 📠 +90 286 218 05 33

ABSTRACT

In this study, low cost, sensitive and selective chronoamperometric determination of vanillin (VNL) is firstly achieved by using a disposable poly(Eriochrome Black T) modified pencil graphite electrode (poly(EBT)/PGE). Electro-oxidation behavior of 1.0 mM VNL was investigated at both poly(EBT)/PGE and PGE by the cyclic voltammetry (CV) method. CV measurements showed that oxidation current of VNL at poly (EBT)/PGE was much higher than the bare one. Furthermore, the pH effect on electro-oxidation current of VNL was tested at different pH values (2.0-8.0) of Britton Robinson Buffer solution (BRBS) for poly(EBT)/PGE and the highest current was obtained at pH 7.0 BRBS. Significant analytical parameters such as linear response range (LRR), limit of detection (LOD), and sensitivity were found as 0.050-10.0 μM, 0.013 μM and 5355 μA mM^{-1} cm^{-2}, respectively. The fabricated sensor was tested on a VNL-containing sample (VNL content: 3.0%) and the result (3.04% ±0.01) obtained from the applicability study showed that the proposed sensor enables the determination of VNL with high accuracy and precision.

Keywords: Vanillin, Pencil graphite electrode, Chronoamperometry, Cyclic voltammetry, Electrochemical sensor

Tek Kullanımlık Poli(Eriokrom Siyahı T) Modifiye Kalem Grafit Elektrotta Vanilinin Düşük Maliyetli, Seçici ve Duyarlı Kronoamperometrik Tayini

ÖZ

Bu çalışmada, vanilinin (VNL) düşük maliyetli, duyarlı ve seçici kronoamperometrik tayini tek kullanımlık bir poli(Eriokrom Siyahı T) modifiye kalem grafit elektrodun (poly(EBT)/PGE) kullanılması ile ilk defa gerçekleştirilmiştir. 1.0 mM VNL'nin elektro-yükseltgenme davranışı, hem PGE'de hem de poly(EBT)/PGE'de döngüsel voltammetri (CV) metodu ile incelenmiştir. CV ölçümleri poly(EBT)/PGE'deki yükseltgenme akımının yalın elektroda göre daha yüksek olduğunu göstermiştir. Ayrıca, poly(EBT)/PGE'de VNL'nin yükseltgenme akımına pH'nın etkisi, farklı pH değerlerindeki (2.0-8.0) Britton Robinson tampon çözeltilerinde (BRBS) test edilmiş ve en yüksek akımın pH 7.0 BRBS'de alındığı gözlenmiştir. Doğrusal yanıt aralığı (LRR), belirtme alt sınırı (LOD) ve duyarlılık gibi önemli analitiksel parametreler sırasıyla 0.050-10.0 μM, 0.013 μM ve 5355 μA mM^{-1} cm^{-2} olarak bulunmuştur. Tasarlanan sensör, VNL içeren bir örnekte (VNL içeriği %3.0) başarıyla test edilmiş ve uygulanabilirlik çalışmasından elde edilen sonuç (%3.04 ±0.01), önerilen sensörün, VNL'nin yüksek doğrulukta ve kesinlikte tayinine imkân sağladığını göstermiştir.

Anahtar Kelimeler: Vanilin, Kalem grafit elektrot, Kronoamperometri, Döngüsel voltammetri, Elektrokimyasal sensör

---

INTRODUCTION

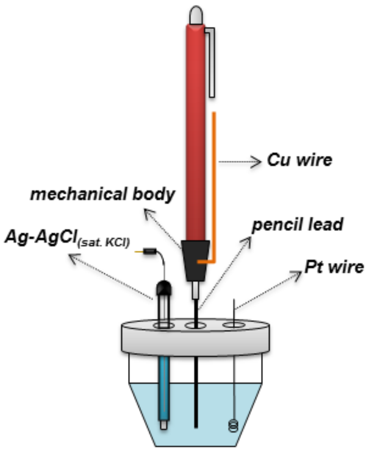
A food flavor, vanillin (VNL) known as 4-hydroxy-3-methoxybenzaldehyde is extensively utilized for many purposes, such as drugs, food products and cosmetics [1, 2]. The source of vanilla is the bean or shell of a tropical VNL orchid [3]. VNL can be obtained either by extraction from natural vanilla or artificially synthesis in laboratory. Natural VNL is generally preferred by many consumers instead of artificial one. Below 100 ppm for VNL capsules or natural VNL extracts or 250 ppm for synthetic vanillin are used in food products by food companies. Intaking of higher doses of VNL to human body causes serious metabolic disorders such kidney injury, vomiting, nausea and headaches [2]. For all these mentioned reasons, rapid, reliable, accurate and sensitive detection of low VNL concentrations in samples is always a critical issue for the protection of human health and food security. For this purpose, many traditional methods have been utilized for the determination of VNL such as mass spectrometry - gas chromatography coupled solid-phase microextraction technique [4, 5], electrophoresis [6, 7], high performance liquid chromatography (HPLC) [8, 9], etc. Although these techniques have been extensively used in VNL determination, they have significant restrictions such as needing high-cost equipment, including complicated procedures, inconvenient and time-sink sample preparation steps, high consumption of organic solvent and the need for well-experienced specialists. Among these procedures, electrochemical methods have aroused specific interest in sensing applications because of their considerable and useful advantages like fast response, simplicity low cost, better sensitivity and ease of operation [10-16]. In addition, the selection of electrode material is highly significant in construction of electrochemical sensors, because the type of electrode can affect the sensitivity, cost, stability and selectivity of an electrochemical sensor [17-20]. Glassy carbon electrode (GCE) [1, 21-24] and carbon paste electrode (CPE) [25-27] have been extensively used in fabrication of electrochemical VNL sensors. However, GCE and CPE require hard and time-consuming surface polishing and cleaning steps. Among these carbon-originated electrodes, PGEs have numerous advantages such as disposable, low cost, commercially availability, ease of modification, high mechanical rigidity and electrochemical reactivity and also, PGE offers a simpler and faster renewable surface than the other solid electrodes. Therefore, it has been reported that PGE provides beneficial and reproducible results due to their single-use properties [17, 19, 20, 28]. To get better sensitivity and response, bare electrode surfaces have been generally modified with functional materials including metal nanoparticles [29, 30], multiwall carbon nanotubes (MWCNTs) [31, 32], quantum dots [33-35] and conducting polymer films [36, 37]. In recent years, polymer film modified electrodes have aroused grate notice with their good repeatability and stability properties [38]. An azo naphthol dye, EBT is a metachromic indicator [39] and it is commonly used for coloring materials such as wool, silk and nylon [40]. EBT have been also used to modify on surfaces of various kinds of electrode to improve the response and sensitivity towards many analytes due to the fact that $–SO_3^-$ groups and electron-rich oxygen atoms of EBT provide electrochemically active surface area and accelerate interaction and electron transfer between analytes and the electrode [41-44].

According to the literature research, bare PGE was used without any modification for differential pulse voltammetric determination of VNL [10]. In this study, EBT and PGE have been firstly used together for low cost, disposable, sensitive and selective chronoamperometric determination of VNL and better analytical performances were obtained with proposed method compared with published study [10]. In this work, EBT was electro polymerized on PGE surface by cyclic voltammetry (CV) method and proposed electrode was successfully applied on real samples.

MATERIALS AND METHODS

Chemicals and Apparatus

Vanillin, 99% was supplied from Alfa Aesar. $NaH_2PO_4.2H_2O$, $Na_2HPO_4.2H_2O$, CH_3COOH, H_3PO_4, H_3BO_3, NaOH, KCl, $CaCl_2.2H_2O$, $MgCl_2$, $Al(NO_3)_3$, Na_2SO_4, $NaNO_3$, $Cu(NO_3)_2$, $ZnCl_2$, D-(+)-glucose and D-(+)-sucrose, $K_4[Fe(CN)_6].3H_2O$ and $K_3[Fe(CN)_6]$ were supplied from Sigma Aldrich or Merck. A Q7B Elga Option system for water purification was used to obtain ultrapure water for the preparation of buffer solutions. The pH of buffer solutions was ordered with a pH meter, HI-1332 Hanna. An electrochemical Compactstat Interface Analyzer (Ivium Technologies, Eindhoven, Netherlands) was used to perform the electrochemical measurements. Triple electrode system containing Ag-$AgCl_{(sat.\ KCl)}$ (as reference electrode), Pt wire (as counter electrode) and Tombow 2B pencil polymer lead (0.50 mm) (as working electrode) were used to perform all electrochemical measurements. A Rotring mechanical pencil (Germany) that bought from a book store was used to hold the pencil lead. A copper wire was brazed on metallic body of mechanical pencil for the connection of electricity. A schematic representation for the triple electrode system including reference electrode, counter electrode and working electrode, was given in Scheme 1.



Scheme 1. A schematic representation for the triple electrode system

Preparation of Poly(EBT)/PGE

Firstly, the effect of monomer concentration, anodic potential and number of cycle at electrodeposition step were optimized by examining the cyclic voltammetric response of 1.0 mM VNL in pH 7.0 PBS including 100 mM KCl. Then, poly(EBT)/PGE was prepared by recording CVs of Ar saturated 0.50 mM EBT in 0.10 M NaOH under optimized conditions (Potential range: -0.40 V - +0.90 V; scan rate: 50 mV/s and number of cycle: 15).

Electrochemical Applications

In all electrochemical applications, a total of 1.0 cm length (0.159 cm^2 of geometric area) of pencil lead was used for all electrochemical studies. Electrochemical impedance measurements of poly(EBT)/PGE and PGE were realized in 10.0 mM solution of $Fe(CN)_6^{3-/4-}$ including 100 mM KCl at a +0.18 V constant potential with a frequency range of 0.10–100000 Hz (sinusoidal signal: 0.005 V). CV behaviors of the poly(EBT)/PGE and PGE were investigated towards 1.0 mM VNL prepared in 0.10 M PBS pH 7.0 including 100 mM KCl. The effects of pH and scan rate on oxidation current of VNL were examined towards 1.0 mM VNL by CV method. Chronoamperometric responses of both poly(EBT)/PGE and PGE were investigated in presence of increased concentrations of VNL in 0.10 M BRBS pH 7.0 at an applied potential of +0.60 V (Interval time: 0.20 s).

Real Sample Study

The practical usability of the VNL sensor was tested on vanillin powder that supplied from a local market by combination of chronoamperometry and standard addition methods. For the determination of VNL content, 5.0 g (1 pocket) of powder was dissolved in 100 mL of pure water. After obtaining a stable background current, prepared solution was diluted with a ratio of $1/10^4$ with pH 7.0 BRBS in electrochemical cell. Then, equal additions (1.0 μM) from standard VNL solution (500 μM) were performed. Calibration curves from the obtained chronoamperomograms were drawn and the percentage of VNL content in vanillin powder was calculated.

RESULTS AND DISCUSSION

Electrochemical Polymerization of EBT on PGE

Figure 1 displays the voltammograms of electro-polymerization step (Potential range: -0.40 V - +0.90 V; scan rate: 50 mV/s and number of cycles: 15). During the electropolymerization process, two anodic peaks (I and II) at +0.07 V and +0.51 V, which correspond to oxidation of EBT gradually decreased with increase of cycle. A cathodic peak (III) observed at approximate −0.30 V, which corresponds to reduction of EBT with continuous scans and also this peak decreased continuously. After the cycles completed, both cathodic and anodic peaks were completely vanished, which points the formation of the polymer film on the PGE and became less conductive and more compact with the scan cycles, exhibiting a self-regulating feature. A mechanism for redox processes of EBT can be clarified as in Scheme 2.

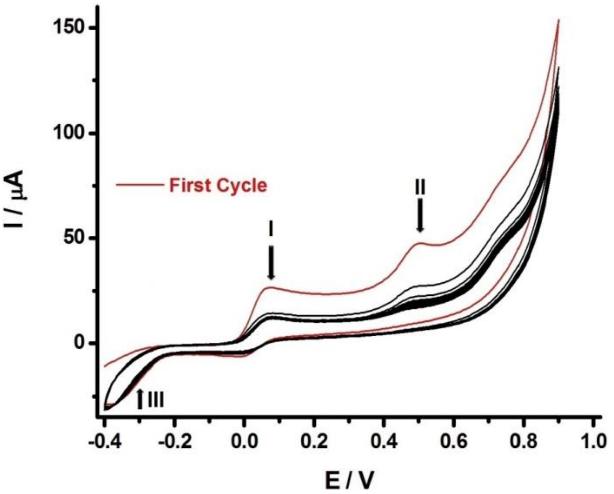
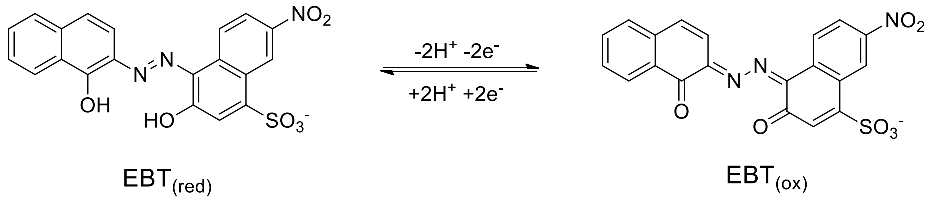


Figure 1. Cyclic voltammograms of Ar saturated 0.50 mM EBT in 0.10 M NaOH recorded in the potentials between -0.40 V and +0.90 V for 15 cycles (Scan rate: 100 mV/s).



Scheme 2. A proposed mechanism for EBT occurred during the electrodeposition process [43, 45].

Firstly, initial form of EBT ($EBT_{(red)}$) oxidized to benzoquinone di-imine form ($EBT_{(ox)}$) (observed as peaks I and II) and then the di-imine structure ($EBT_{(ox)}$) was reduced to initial form ($EBT_{(red)}$) (observed as peak III) on the surface of PGE. The electrodeposition behavior of EBT on PGE was found to be similar with some reports [43, 45]. As a result, EBT shows two-step one electron redox process in CVs (mentioned in Scheme 1), which is in good agreement with the mechanism reported by Yao et al. [43].

Investigation of Voltammetric Response of VNL at Poly(EBT)/PGE

In order to find the most suitable conditions for modification of EBT on PGE, firstly, modified electrodes were prepared in different concentrations of EBT (0.25, 0.50, 0.75 and 1.0 mM) and the responses of these modified electrodes were tested in presence of 1.0 mM VNL in pH 7.0 PBS. Obtained current vs. monomer concentration graphs showed that the highest oxidation current was obtained for the modified electrode that prepared with 0.50 mM EBT and this concentration was chosen as the optimum value (Figure 2A). Secondly, the effect of anodic potential range during the electropolymerization step was optimized. For this purpose, modified electrodes were prepared in 0.50 mM EBT solution including 0.10 M NaOH between increased anodic potential ranges (-0.40 V → 0.80, 0.90, 1.0, 1.2, 1.4, 1.6, 1.8 and +2.0 V) and these obtained electrodes were in presence of 1.0 mM VNL in pH 7.0 PBS. Obtained current vs. anodic potential graphs supported that the highest response was observed with the potential range of -0.40 V → +0.90 V at electropolymerization step (Figure 2B). In final step of optimizations, modified electrodes were prepared with increased cycles (0, 5, 10, 15, 20, 25 and 30) under optimized conditions. Current vs. number of cycle graph proved that the best response was obtained with 15 cycles (Figure 2C).

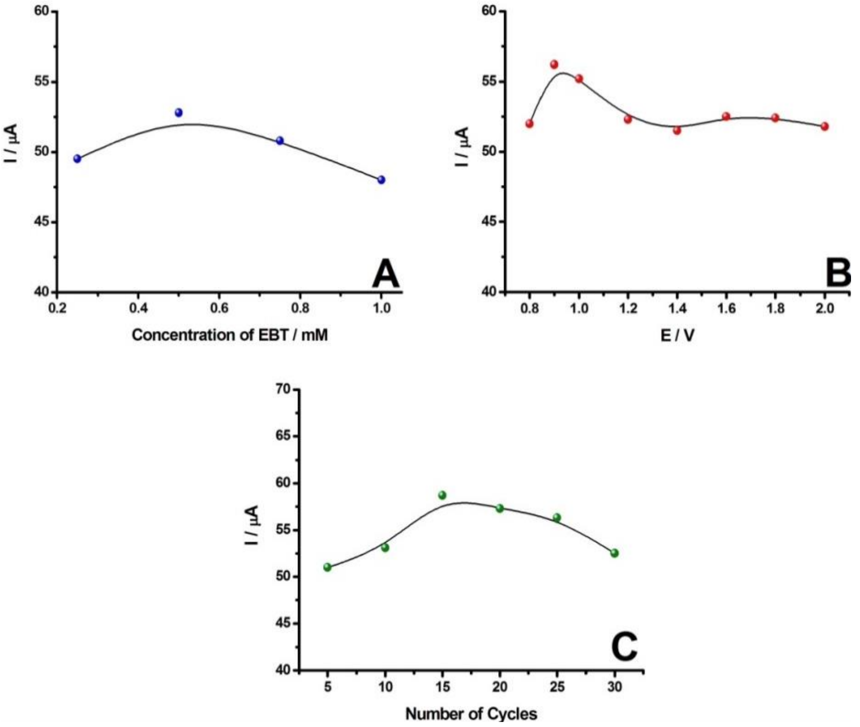


Figure 2. A) Current vs. EBT concentration (0.25, 0.50, 0.75 and 1.0 mM), B) current vs. anodic potential (0.80, 0.90, 1.0, 1.2, 1.4, 1.6, 1.8 and +2.0 V) and C) current vs. number of cycles graph (0, 5, 10, 15, 20, 25 and 30) towards 1.0 mM VNL in PBS 7.0 including 100 mM KCl.

After the determination for the most suitable condition for preparation of the modified electrode, the cyclic voltammetric behavior of 1.0 mM VNL was evaluated at both PGE and poly(EBT)/PGE in pH 7.0 PBS between the potentials of 0 − +1.0 V (Figure 3). As a result of the CV measurements, it is clear that no significant change was seen at oxidation potential of VNL and oxidation peak for bare and modified electrodes was observed at +0.65 V and +0.60 V, respectively. Furthermore, oxidation current of VNL was found to be much higher at poly(EBT)/PGE compared with bare PGE. Consequently, it is concluded that poly(EBT) facilitates the electron transfer between vanillin and PGE and the remarkable enhancement in oxidation current was obtained. A mechanism for oxidation process of VNL has been previously explained for GCE [21], CPE [27] and screen-printed carbon electrode (SPCE) [46]. The same redox behavior can be taken in consider for poly(EBT)/PGE. In this mechanism, phenolic hydroxyl and methoxy groups oxidized to carbonyl groups after losing equal numbers of protons ($2H^+$) and electrons ($2e^-$) (Scheme 3).

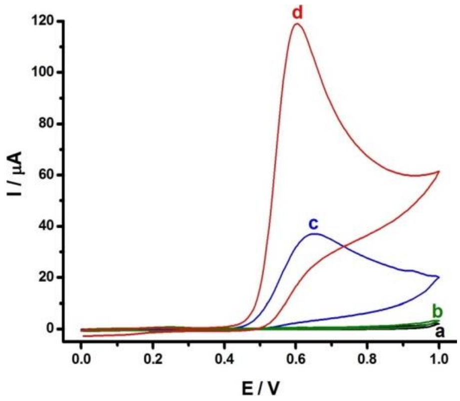
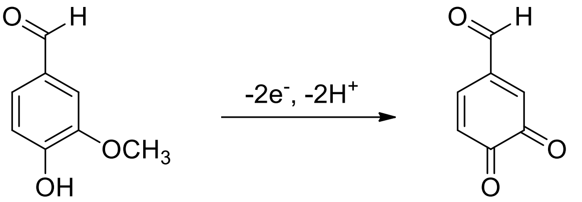


Figure 3. CV responses of PGE (a and c) and poly(EBT)/PGE (b and d) in presence of 0 mM (a and b) and 1.0 mM (c and d) VNL in 0.10 M PBS pH 7.0 including 100 mM KCl (Potential range: 0 V - +1.0 V; scan rate: 50 mV/s).



Scheme 3. Electrochemical oxidation mechanism for VNL [21, 27, 46].

pH value of supporting media is a significant parameter that effects oxidation potential and oxidation current of polyphenolic compounds. To examine the most suitable condition for the pH of supporting electrolyte, CVs of 1.0 mM VNL were recorded in BRBS between the pH values of 2.0 - 8.0 for poly(EBT)/PGE (Figure 4A). The recorded voltammograms indicated that oxidation peak potential shifts to cathodic direction with increment of pH and also, a significant enhancement in peak current occurred at pH 7.0 (Figure 4B). Because of the highest current obtained at 7.0, this pH value was chosen as the optimum condition for further studies. The linear relationship between potential vs. pH was also given in Figure 4C. The obtained slope (0.053) was found to be near to Nernstian slope (0.059) and indicated that equal numbers of protons and electrons act in electro-oxidation reaction of VNL on the electrode surface.

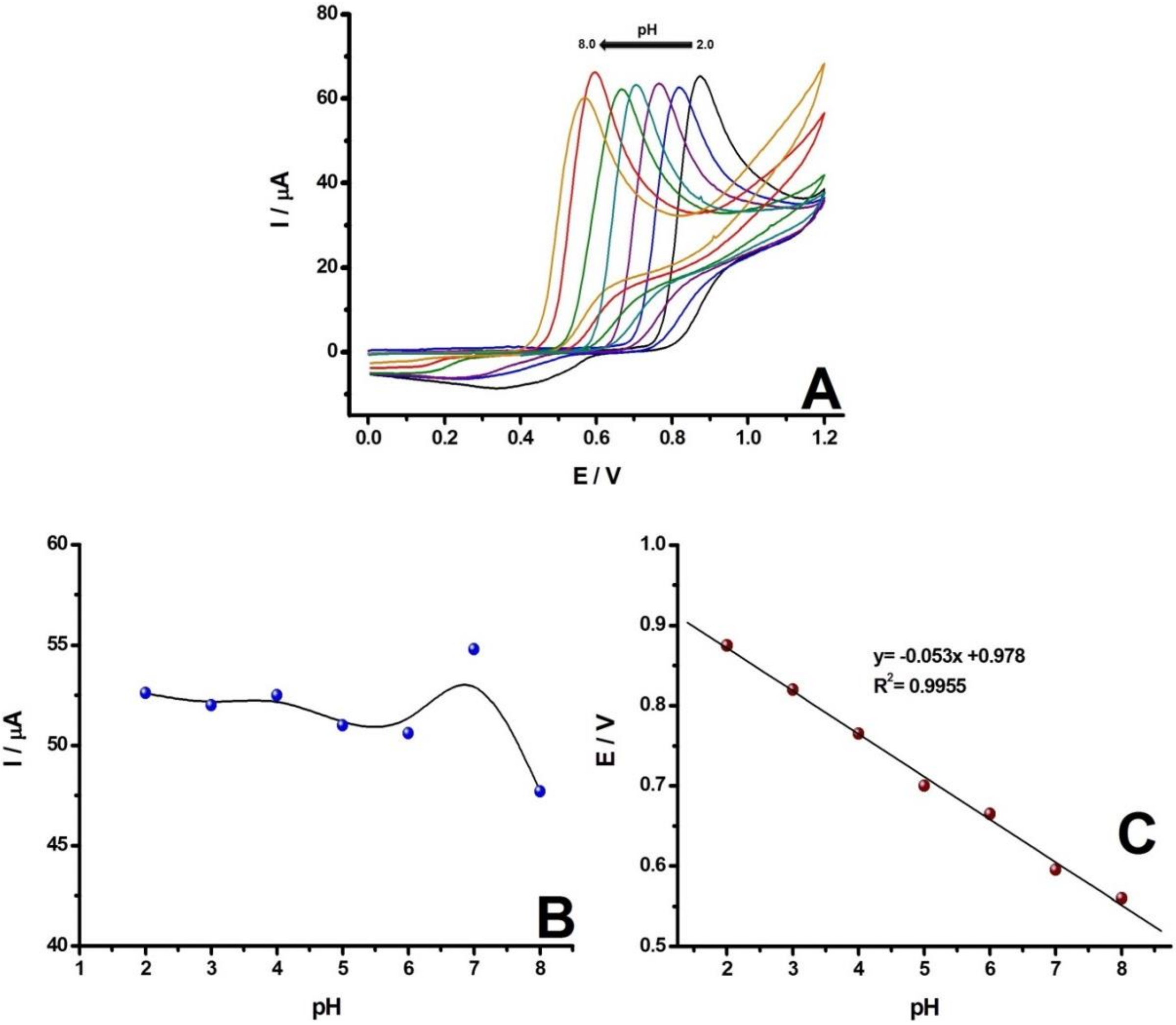


Figure 4. A) CV responses of 1.0 mM VNL solution in BRBS at increased pH values (2.0-8.0) including 100 mM KCl at poly(EBT)/PGE (Potential range: 0 V - +1.2 V; scan rate: 50 mV/s), B) current vs. pH graphs obtained from the voltammograms and C) the graph for linear relationship between pH and potential (E(V)= -0.053(pH) +0.978 and R^2= 0.9955).

The effect of scan rate on electro-oxidation of VNL was also studied (Figure 5A). It is clear from the Figure 5B that oxidation current increases linearly between 10-800 mV/s. The equation was found as I(µA) = 3.2804 $v^{1/2}$ +9.9759 with correlation coefficient of 0.9968 from I vs. $v^{1/2}$ plots the (Figure 5B). Also the high linear relationship between the I and $v^{1/2}$ indicates that electrode reaction occurs by diffusion controlled process.

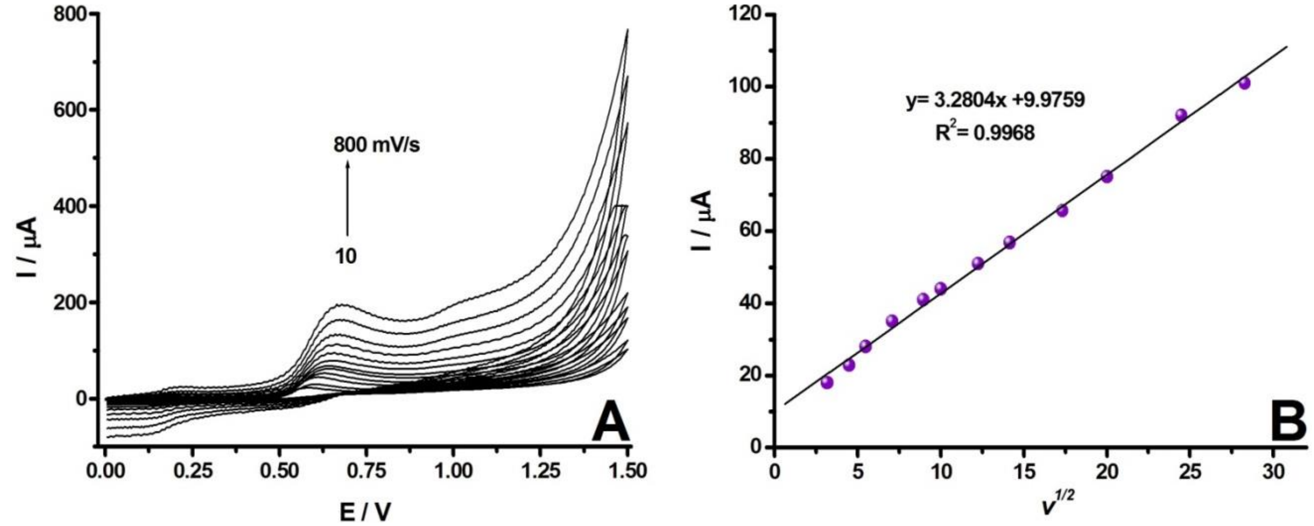


Figure 5. A) CV responses of 1.0 mM VNL solution in pH 7.0 BRBS at increased scan rates (10-800 mV/s) at poly(EBT)/PGE (Potential range: 0 V - +1.5 V) and B) the calibration curve obtained from I vs. $v^{1/2}$ plots (I(µA) = 3.2804 $v^{1/2}$ +9.9759 and R^2= 0.9968).

Electrochemical and Surface Characterizations of PGE and Poly(EBT)/PGE

Electrochemical impedance spectroscopy (EIS) was used to examine the conductivity properties of the carbon-based electrodes. EIS curves of the electrodes were recorded in 10.0 mM solution of Fe(CN)$_6^{3-/4-}$ including 100 mM KCl (Figure 6). As it can be clearly seen from the figure, charge transfer resistance (R_{ct}) at PGE (50 Ω) significantly increased (212 Ω) after polymer film (poly(EBT)) formed on the surface of PGE. The enhancement in R_{ct} was attributed to the electrostatic repulsion between negatively charged Fe(CN)$_6^{3-/4-}$ redox probe and negatively charged functional groups (–N=N– and –SO_3^-) of EBT. As a result, significant decrement observed in conductivity and electron transfer between poly(EBT)/PGE and redox probe. Although the decrease in conductivity was seen EIS curves, the enhancement in electrochemical response towards oxidation of VNL may be attributed to the possible interactions (H bonding and/or ion (–SO_3^- group of poly(EBT)) – dipole (-CHO group of VNL) interaction) which provide highly electrochemically active surface area and result acceleration in electron transfer between VNL and the poly(EBT)/PGE (Figure 3).

Surface characterizations of both electrodes were performed by recording their SEM micrographs and EDX spectrums (Figure 7). When the SEM images of the electrodes were considered, a flat and smooth surface that seen at PGE (Figure 7A) prominently changed after formation of layered polymer film (Figure 7B). In order to obtain more information about surface morphology, EDX analyzes of electrodes were also performed. When the EDX spectra are examined, the signals (90.5% C, 7.3% O, 2.1% Si, 0.1% Al) appearing on the lean electrode confirm that PGE is composed of graphite and clay minerals (Figure 7C) [12]. Apart from these signals, additional signals (1.6% N, 0.6% Na, 0.1% S correspond to –N=N– and –SO_3Na groups of EBT) and increment in C signal (90.5% to 97.8%) seen on the modified electrode indicate that formation of the polymer film certainly occurred on the PGE surface (Figure 7D). The relevant morphological findings indicate that EBT has been successfully modified on the PGE surface by electropolymerization

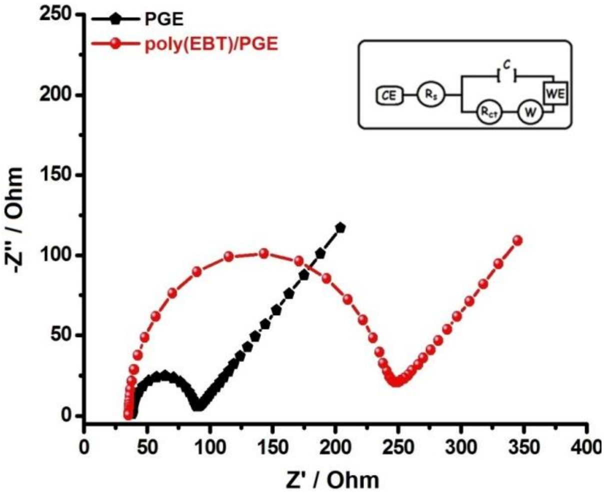


Figure 6. EIS curves of poly(EBT)/PGE (red) and PGE (black) that recorded in 10.0 mM solution of Fe(CN)$_6^{3-/4-}$ including 100 mM KCl (Frequency range: 0.10 – 100000 Hz, sinusoidal signal: 0.005 V and constant potential: +0.18 V) and inset: equivalent circuit model (CE as counter electrode, R_s as ohmic resistance, C: double layer capacitance, W: Warburg impedance, WE: working electrode).

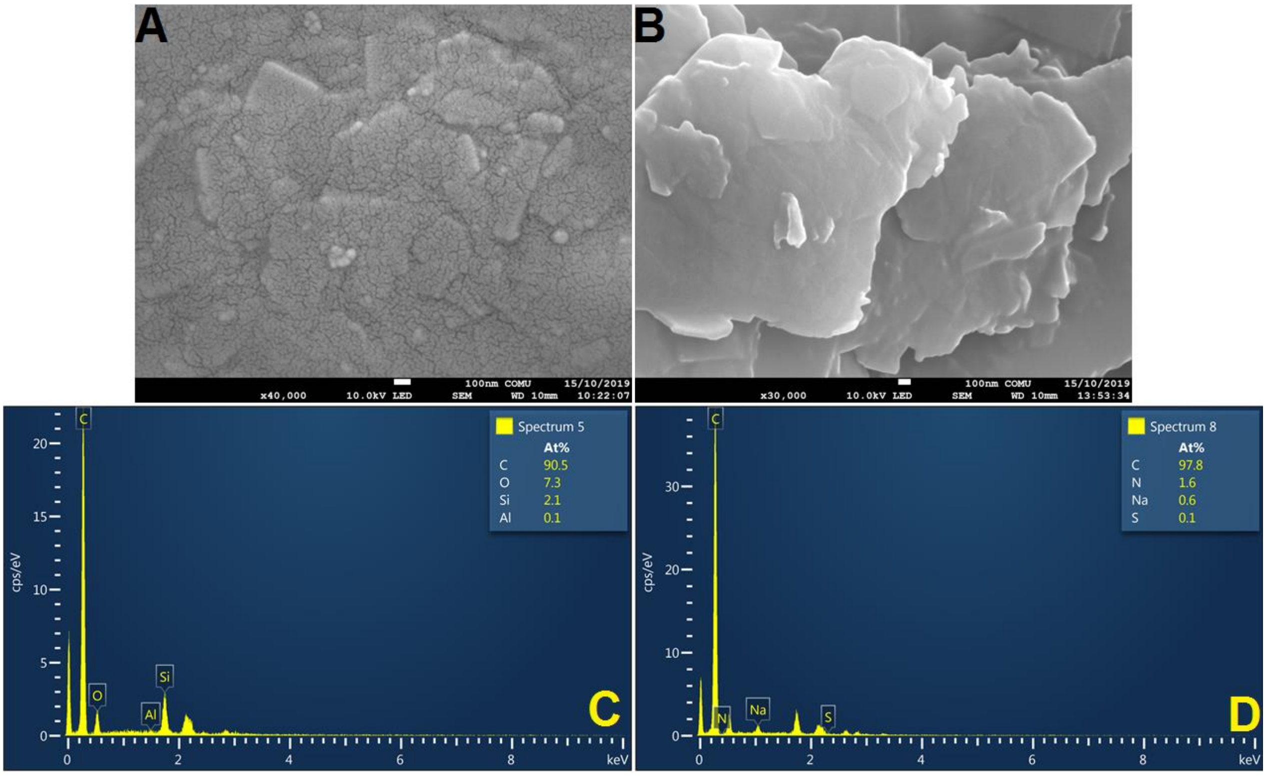


Figure 7. SEM micrographs of A) PGE and B) poly(EBT)/PGE and EDX spectrums of C) PGE and D) poly(EBT)/PGE.

Chronoamperometric Determination of VNL at Poly(EBT)/PGE

The significant parameters of amperometric VNL sensor (such as LRR, LOD and sensitivity) were further investigated. For this aim, chronoamperomograms of increased concentrations of VNL were recorded at +0.60 V under optimized conditions for both electrodes (Figure 8A). The poly(EBT)/PGE showed a wider linear response in concentrations between 0.050 and 10.0 µM VNL compared with bare PGE (0.20 – 2.0 µM VNL). Calibration curves obtained from the chronoamperomograms were also given in Figure 8B. The equation for linear response was found as I(µA)= 0.851(µM) +0.036 with correlation coefficient of 0.9984 for poly(EBT)/PGE. LOD and LOQ values of proposed sensor were determined as 0.013 µM and 0.043 µM according to the 3(s/m) and 10(s/m), respectively (s: standard deviation of the currents for the ten times additions of the lowest concentration (0.05 µM) that gives a measurable signal and m: the slope obtained from the calibration curve). The sensitivity was found to be 5355 µA mM^{-1} cm^{-2} according to the ratio of slope/active surface area (1.0 cm of geometric area of PGE was measured as 0.159 cm^2).

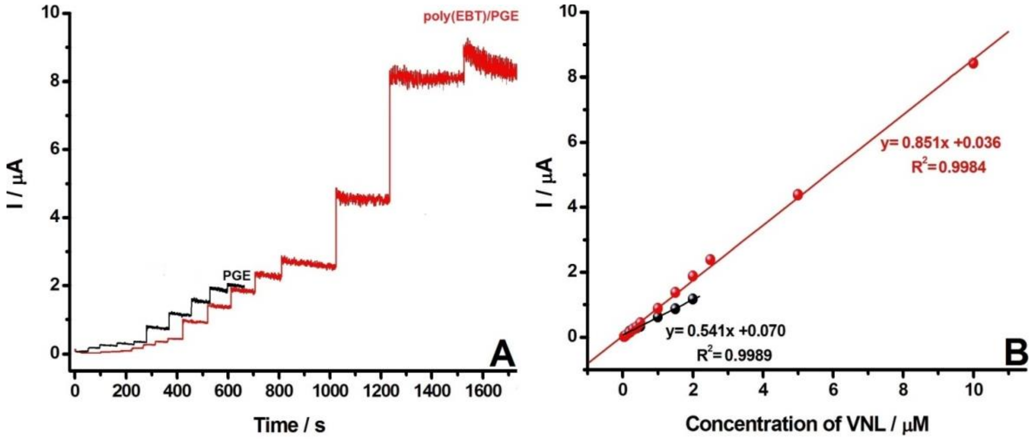


Figure 8. A) Chronoamperometric responses of poly(EBT)/PGE (red line) and PGE (black line) at increased concentrations (0.20–2.5 µM for PGE and 0.050–15.0 µM for poly(EBT)/PGE) of VNL in BRBS pH 7.0 (Applied potential: +0.60 V and interval time: 0.20s) and B) the calibration curve for PGE (black line, I(µA)= 0.541(µM) +0.070 and R^2= 0.9989) and poly(EBT)/PGE (red line, I(µA)= 0.851(µM) +0.036 and R^2= 0.9984) obtained from the chronoamperomograms.

For evaluation of the analytical performance of the poly(EBT)/PGE, the obtained parameters were compared with those in previously published works (Table 2). In these works, GCE [22, 24, 47, 48] and CPE [25-27] were intensively used for construction of VNL sensors. But as it was previously mentioned, the drawbacks such as hard and time-consuming cleaning/polishing steps limit their uses. On the other hand, PGE does not include these limitations and PGE has superior advantages compared with mentioned electrodes. PGEs also provide fast, renewable surfaces and high reproducibility. According to the literature research, pre-treated form of PGE was used in DPV determination of VNL without modification of any material [10]. In proposed study, VNL has been determined with polymer film (poly(EBT) modified PGE by chronoamperometric method for the first time. Also designed sensor shows wider LRR compared with published works [10, 22, 47] and has a lower LOD than many studies [10, 22, 24, 25, 47, 48]. After all, it is clear that poly(EBT)/PGE shows better and comparable analytical performance than the other published VNL sensors.

Table 1. Comparison of proposed sensor with the other published studies

The Type of Electrode	Method	LRR (µM)	LOD (µM)	Ref.
Graphene-GCE	DPV	0.60-48	0.056	[22]
CDA/Au–AgNPs/GCE	Amperometry	0.20-50	0.040	[24]
CuHCF thin film	CV	0.76-120	0.23	[48]
CoS nanorods/GCE	DPV	0.50-56	0.070	[47]
$CuFe_2O_4$/ionic liquid/CPE	SWV	0.10-700	0.070	[25]
CPE/CdO/SWCNTs/DPIB	SWV	0.03-1200	0.009	[26]
CPE/NiO-SWCNTs/$BPrPF_6$	SWV	0.01-350	0.007	[27]
p-PGE	DPV	0.50–10	0.16	[10]
poly(EBT)/PGE	*Amperometry*	*0.050–10.0*	*0.013*	*This work*

CDA: cellulose diacetate; CuHCF: copper hexacyanoferrate; DPIB: dipropylimidazolium bromide; $BPrPF_6$: 1-butylpyridinium hexafluorophosphate; p-PGE: pre-treated pencil graphite electrode.

Examination of the Selectivity at VNL Sensor

Selectivity of the poly(EBT)/PGE were investigated in presence of 1.0 µM VNL towards some potential interference species (100 folds of NO_3^-, SO_4^{2-}, CH_3COO^-, Cl^-, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Cu^{2+}, glucose and sucrose) and the recorded time vs. current graph was given in Figure 9. The recorded chronoamperomograms have proved that the interference effects of higher concentrations of these ionic and molecular species on electro-oxidation current of VNL were found to be insignificant. As a result, it can be said that the proposed method can be successfully used for selective determination of VNL in real samples, which include these interference species.

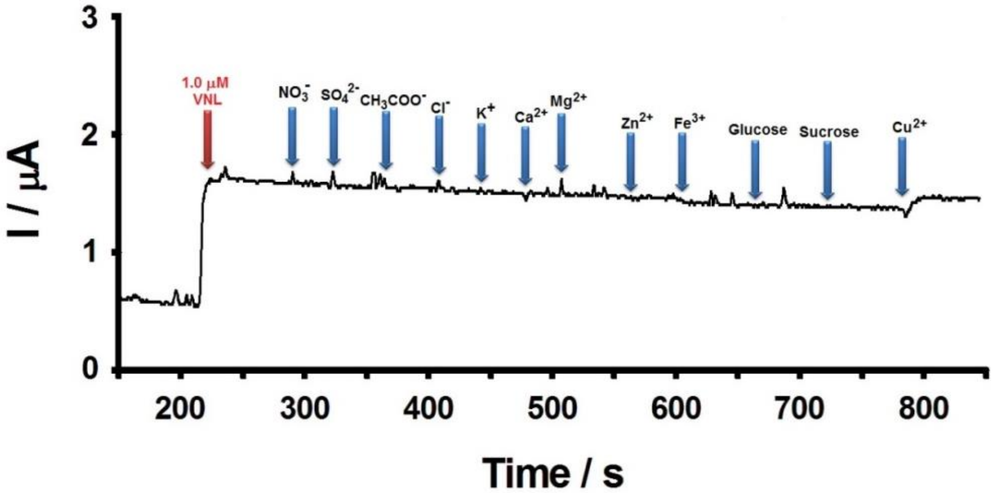


Figure 9. Chronoamperomerometric responses towards 1.0 µM VNL in presence of various ionic and molecular interferences (100 folds (100 µM) of NO_3^-, SO_4^{2-}, CH_3COO^-, Cl^-, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Cu^{2+}, glucose and sucrose) at poly(EBT)/PGE under optimized conditions.

Application on Real Sample

Applicability for proposed sensor was tested on vanillin powder, which was supplied from a local market. VNL determination at poly(EBT)/PGE was performed by using chronoamperometry combined with standard addition method and the result was given in Table 4. The result showed that poly(EBT)/PGE exhibits good applicability for determining VNL in real samples.

Table 4. The results for determination of VNL in vanillin powder (n=3).

Sample	Percentage (%) of Vanillin Written on Label	Found (%) with Proposed Method
Vanillin Powder	3.0	3.04±0.01

CONCLUSION

In present study, two useful and advantageous approaches were used for chronoamperometric determination of VNL for the first time: (i) PGE as a cheap and disposable electrode and (ii) EBT as redox mediator for enhancing the electrochemical response and sensitivity. Although many types of carbon originated electrodes and modification materials were used for sensitive electrochemical determination of VNL based on its electro-oxidation behavior, but as it mentioned before significant drawbacks limit their uses. In addition, the proposed sensor provides low cost, disposable, sensitive and selective chronoamperometric determination of VNL and comparable analytical performance was obtained among many published studies. The designed sensor also enables high selectivity towards the determination of VNL in presence of many potential interference species. The obtained results from real sample analysis proved that designed sensor can be successfully applied to daily used samples. It is expected that the proposed method may provide an alternative way for the low-cost, sensitive and selective determination of VNL in real samples in the future.

REFERENCES

[1] Sun, Y.J., Jiang, X.W., Jin H., Gui, R.J. (2019). Ketjen black/ferrocene dual-doped MOFs and aptamer-coupling gold nanoparticles used as a novel ratiometric electrochemical aptasensor for vanillin detection. *Analytica Chimica Acta*, 1083, 101-109.

[2] Sinha, A.K., Sharma, U.K., Sharma, N. (2008). A comprehensive review on vanilla flavor: extraction, isolation and quantification of vanillin and others constituents. *International Journal of Food Science Nutrition*, 59299-59326.

[3] Bettazzi, F., Palchetti, I., Sisalli, S., Mascini, M. (2006). A disposable electrochemical sensor for vanillin detection. *Analytica Chimica Acta*, 555, 134-138.

[4] Wang, Z.Y., Zeng, G.F., Wei, X.Q., Ding, B., Huang, C., Xu, B.J. (2016). Determination of vanillin and ethyl-vanillin in milk powder by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry. *Food Analytical Methods*, 9, 3360-3366.

[5] De Jager, L.S., Perfetti, G.A., Diachenko, G.W. (2008) Comparison of headspace-SPME-GC–MS and LC–MS for the detection and quantification of coumarin, vanillin, and ethyl vanillin in vanilla extract products. *Food Chemistry*, 107, 1701-1709.

[6] Shu, M., Man, Y.R., Ma, H., Luan, F., Liu, H.T., Gao, Y. (2016). Determination of vanillin in milk powder by capillary electrophoresis combined with dispersive liquid-liquid microextraction. *Food Analytical Methods*, 9, 1706-1712.

[7] Ohashi, M., Omae, H., Hashida, M., Sowa, Y., Imai, S. (2007). Determination of vanillin and related flavor compounds in cocoa drink by capillary electrophoresis. *Journal of Chromatography A*, 1138, 262-267.

[8] Pérez-Esteve, É., Lerma-García, M.J., Fuentes, A., Palomares, C., Barat, J.M. (2016). Control of undeclared flavoring of cocoa powders by the determination of vanillin and ethyl vanillin by HPLC. *Food Control*, 67, 171-176.

[9] Zhu, J.L., Chen, D.P., Ai, Y.H., Dang, X.P., Huang, J.L., Chen, H.X. (2017). A dummy molecularly imprinted monolith for selective solid-phase microextraction of vanillin and methyl vanillin prior to their determination by HPLC. *Microchimica Acta*, 184, 1161-1167.

[10] Dilgin, D.G. (2019). Voltammetric determination of vanillin using a pretreated pencil graphite electrode. *Akademik Gıda*, 17, 1-8.

[11] Dilgin, D.G. (2018) Determination of calcium dobesilate by differential pulse voltammetry at a disposable pencil graphite electrode. *Analytical Letters*, 51, 186-197.

[12] Teoman, İ., Karakaya, S., Dilgin, Y. (2019). Sensitive and rapid flow injection amperometric hydrazine sensor using a gold nanoparticle modified graphite pencil electrode. *Analytical Letters*, 52(13), 2041-2056.

[13] Ayaz, S., Dilgin, Y. (2017). Flow injection amperometric determination of hydrazine based on its electrocatalytic oxidation at pyrocatechol violet modified pencil graphite electrode. *Electrochimica Acta*, 258, 1086-1095.

[14] Dilgin, D.G., Ertek, B., Dilgin, Y. (2018). A low-cost, fast, disposable and sensitive biosensor study: flow injection analysis of glucose at poly-methylene blue-modified pencil graphite electrode. *Journal of the Iranian Chemical Society*, 15, 1355-1363.

[15] Karakaya, S., Dilgin, D.G. (2019). Low-cost determination of cetirizine by square wave voltammetry in a disposable electrode. *Monatshefte Fur Chemie*, 150, 1003-1010.

[16] Özcan, A., Gürbüz, M., Özcan, A.A. (2018). Preparation of a disposable and low-cost electrochemical sensor for propham detection based on over-oxidized poly (thiophene) modified pencil graphite electrode. *Talanta*, 187, 125-132.

[17] Wang, J., Kawde, A.N., Sahlin, E. (2000). Renewable pencil electrodes for highly sensitive stripping potentiometric measurements of DNA and RNA. *Analyst*, 125, 5-7.

[18] Aslışen, B., Koçak, Ç.C., Koçak, S. (2019). Electrochemical determination of sesamol in foods by square wave voltammetry at a boron-doped diamond electrode. *Analytical Letters*, 1-12.

[19] Jadon, N., Jain, R., Pandey, A. (2017). Electrochemical analysis of amlodipine in some pharmaceutical formulations and biological fluid using disposable pencil graphite electrode. *Journal of Electroanalytical Chemistry*, 788, 7-13.

[20] Abdul Aziz, M., Kawde, A.N. (2013). Gold nanoparticle-modified graphite pencil electrode for the high-sensitivity detection of hydrazine. *Talanta*, 115, 214-221.

[21] Silva, T.R., Brondani, D., Zapp, E., Vieira, I.C. (2015). Electrochemical sensor based on gold nanoparticles stabilized in poly(allylamine hydrochloride) for determination of vanillin. *Electroanalysis*, 27, 465-472.

[22] Peng, J.Y., Hou, C.T., Hu, X.Y. (2012). A graphene-based electrochemical sensor for sensitive detection of vanillin. *International Journal of Electrochemical Science*, 7, 1724-1733.

[23] Qianwen, M., Yaping, D., Li, L., Anqing, W., Dingding, D., Yijun, Z. (2019). Electrospun MoS_2 composite carbon nanofibers for determination of vanillin. *Journal of Electroanalytical Chemistry*, 833, 297-303.

[24] Zheng, D.Y., Hu, C.G., Gan, T.A., Dang, X.P., Hu, S.S. (2010). Preparation and application of a novel vanillin sensor based on biosynthesis of Au-Ag alloy nanoparticles. *Sensors and Actuators B-Chemical*, 148, 247-252.

[25] Khalilzadeh, M.A., Arab, Z. (2017). High sensitive nanostructure square wave voltammetric sensor for determination of vanillin in food samples. *Current Analytical Chemistry*, 13, 81-86.

[26] Cheraghi, S., Taher, M.A., Karimi-Maleh, H. (2017). Highly sensitive square wave voltammetric sensor employing CdO/SWCNTs and room temperature ionic liquid for analysis of vanillin and folic acid in food samples. *Journal of Food Composition and Analysis*, 62, 254-259.

[27] Gupta, V.K., Karimi-Maleh, H., Agarwal, S., Karimi, F., Bijad, M., Farsi, M., Shahisi, S.A. (2018). Fabrication of a food nano-platform sensor for determination of vanillin in food samples. *Sensors (Basel)*, 18, 2817.

[28] David, I.G., Popa, D.E., Buleandra, (2017). M. Pencil graphite electrodes: a versatile tool in electroanalysis. *Journal Analytical Methods in Chemistry*, 1905968.

[29] Khoshroo, A., Hosseinzadeh, L., Sobhani-Nasab, A., Rahimi-Nasrabadi, M., Ahmadi, F. (2019). Silver nanofibers/ionic liquid nanocomposite based electrochemical sensor for detection of clonazepam via electrochemically amplified detection. *Microchemical Journal*, 145, 1185-1190.

[30] Chen, H.Y., Yang, T., Liu, F.Q., Li, W.H. (2019). Electrodeposition of gold nanoparticles on Cu-based metal-organic framework for the electrochemical detection of nitrite. *Sensors and Actuators B-Chemical*, 286, 401-407.

[31] Duan, D.D., Yang, H., Ding, Y.P., Li, L., Ma, G.H. (2019). A three-dimensional conductive molecularly imprinted electrochemical sensor based on MOF derived porous carbon/carbon nanotubes composites and prussian blue nanocubes mediated amplification for chiral analysis of cysteine enantiomers. *Electrochimica Acta*, 302, 137-144.

[32] Mo, F., Xie, J., Wu, T., Liu, M., Zhang, Y., Yao, S. (2019). A sensitive electrochemical sensor for bisphenol A on the basis of the AuPd incorporated carboxylic multi-walled carbon nanotubes. *Food Chemistry*, 292, 253-259.

[33] Bhanjana, G., Chaudhary, G.R., Dilbaghi, N., Chauhan, M., Kim, K.H., Kumar, S. (2019). Novel electrochemical sensor for mononitrotoluenes using silver oxide quantum dots. *Electrochimica Acta*, 293, 283-289.

[34] Li, Y.X., Li, Z.P., Ye, W.X., Zhao, S., Yang, Q.C., Ma, S., Xiao, G., Liu, G., Wang, Y., Yue, Z. (2019). Gold nanorods and graphene oxide enhanced BSA-$AgInS_2$ quantum dot-based photoelectrochemical sensors for detection of dopamine. *Electrochimica Acta*, 295, 1006-1016.

[35] Abbas, M.W., Soomro, R.A., Kalwar, N.H., Zahoor, M., Avci, A., Pehlivan, E., Hallam, K.R., Willander, M. (2019). Carbon quantum dot coated Fe_3O_4 hybrid composites for sensitive electrochemical detection of uric acid. *Microchemical Journal*, 146, 517-524.

[36] Nemakal, M., Aralekallu, S., Mohammed, I., Pari, M., Reddy, K.R.V., Sannegowda, L.K. (2019). Nanomolar detection of 4-aminophenol using amperometric sensor based on a novel phthalocyanine. *Electrochimica Acta*, 318, 342-53.

[37] Zhao, L.Y., Li, J.G., Chen, X.L., Cheng, D., Zhang, J.Y., Yang, H.X. (2019). Highly sensitive electrochemical detection of hydrogen peroxide based on polyethyleneimine-Au nanoparticles-zinc protoporphyrin. *Journal of the Electrochemical Society*, 166, B631-B636.

[38] Gilbert, O., Swamy, B.E.K., Chandra, U., Sherigara, B.S. (2009). Electrocatalytic oxidation of dopamine and ascorbic acid at poly (eriochrome black-t) modified carbon paste electrode. *International Journal of Electrochemical Science*, 4, 582-591.

[39] Liu, X., Luo, L.Q., Ding, Y.P., Kang, Z.P., Ye, D.X. (2012). Simultaneous determination of L-cysteine and L-tyrosine using Au-nanoparticles/poly-eriochrome black T film modified glassy carbon electrode. *Bioelectrochemistry*, 86, 38-45.

[40] Kansal, S.K., Sood, S., Umar, A., Mehta, S.K. (2013). Photocatalytic degradation of eriochrome Black T dye using well-crystalline anatase TiO_2 nanoparticles. *Journal of Alloys and C*ompounds, 581, 392-397.

[41] Yao, H., Sun, Y.Y., Lin, X.H., Tang, Y.H., Liu, A.L., Guangwen, L., Wei, L., Zhans, S. (2007). Selective determination of epinephrine in the presence of ascorbic acid and uric acid by electrocatalytic oxidation at poly(eriochrome black T) film-modified glassy carbon electrode. *Analytical Sciences*, 23, 677-682.

[42] Liu, X., Luo, L., Ding, Y., Kang, Z., Ye, D. (2012). Simultaneous determination of L-cysteine and L-tyrosine using Au-nanoparticles/poly-eriochrome black T film modified glassy carbon electrode. *Bioelectrochemistry*, 86, 38-45.

[43] Yao, H., Sun, Y.Y., Lin, X.H., Tang, Y.H., Huang, L.Y. (2007). Electrochemical characterization of poly(eriochrome black T) modified glassy carbon electrode and its application to simultaneous determination of dopamine, ascorbic acid and uric acid. *Electrochimica Acta*, 52, 6165-6171.

[44] Wei, Y.L., Luo, L.Q., Ding, Y.P., Liu, X., Chu, Y.L. (2013). A glassy carbon electrode modified with poly(eriochrome black T) for sensitive determination of adenine and guanine. *Microchimica Acta*, 180, 887-893.

[45] Cittan, M., Celik, A. (2019). An electrochemical sensing platform for trace analysis of eriochrome black T using multi-walled carbon nanotube modified glassy carbon electrode by adsorptive stripping linear sweep voltammetry. *International Journal of Environmental Analytical Chemistry*, 1-13.

[46] Kutty, M., Settu, R., Chen, S.M., Chen, T.W., Tseng, T.W., Hatamleh, A.A., Yu, J., Ju, R., Huang, C.-C. (2019). An electrochemical detection of vanillin based on carbon black nanoparticles modified screen printed carbon electrode. *International Journal of Electrochemical Science*, 14, 5972-5983.

[47] Sivakumar, M., Sakthivel, M., Chen, S.M. (2017). Simple synthesis of cobalt sulfide nanorods for efficient electrocatalytic oxidation of vanillin in food samples. *Journal of Colloid and Interface Science*, 490, 719-726.

[48] Prabhu, P., Babu, R.S., Narayanan, S.S. (2019). Electrochemical determination of l-vanillin using copper hexacyanoferrate film modified gold nanoparticle graphite-wax composite electrode. *Journal of Materials Science: Materials in Electronics*, 30, 9955-9963.