Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi
Afyon Kocatepe University Journal of Science and Engineering

AKÜ FEMÜBİD **21** (2021) 011201 (84-96) AKU J. Sci. Eng. **21** (2021) 011201 (84-96)
DOI: 10.35414/akufemubid.819216

Araştırma Makalesi / Research Article

Voltammetric Determination of Vanillin in Commercial Food Products Using Electrochemically Fabricated Graphene Oxide Modified Electrode

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Geliş Tarihi: 01.11.2020 Kabul Tarihi: 01.02.2021

Abstract

Determination of vanillin, which is used increasingly common in the food, beverage, and drug industry, is important for human health. Therefore; it is essential to determine the amount of vanillin using a fast, simple and reliable method. In this study; an electrode modified with graphene oxide prepared in one-step, in a one-pot, with an environmentally friendly method, was prepared and used for the electrochemical determination of vanillin (VAN), as flavoring. The electrochemically fabricated graphene oxide electrode (EGO/GE) was prepared by electrochemically producing the graphene oxide structure on the graphite electrode surface. The characterization of the prepared EGO/GE was performed using techniques such as field emission scanning electron microscopy and atomic force microscopy and it was concluded that the proposed EGO/GE material was successfully prepared. While the investigation of the electrochemical behavior of the VAN was performed by the cyclic voltammetry, the differential pulse voltammetry method was used for the voltammetric determination. It was determined that EGO/GE, is used in the electrochemical determination of VAN, exhibits high electrocatalytic performance, a wide linear range (0.1-1000 μM), and a low detection limit (0.05 μM) compared to unmodified GE. The selectivity of the prepared sensor was studied in the presence of ions and organic substances. The sensor (EGO/GE) was used for VAN detection in ice-cream, coffee, and cookie samples and high recovery values were obtained. As a result of the stability and reproducibility studies carried out for the sensor, it has been observed that it is quite stable and suitable for reproduction.

Keywords
Vanillin;
Graphene Oxide;
Voltammetry;
Choronoamperometry;
Flavoring

Elektrokimyasal Olarak Üretilmiş Grafen Oksit Modifiye Elektrot Kullanılarak Ticari Gıda Ürünlerinde Vanilinin Voltametrik Tayini

Öz

Gıda, içecek ve ilaç sanayisinde kullanımı giderek yaygınlaşan vanilinin belirlenebilmesi insan sağlığı açısından önem arz etmektedir. Bu nedenle; hızlı, basit ve güvenilir bir yöntemle vanilin miktarının belirlenmesi esastır. Bu çalışmada; tek basamakta, tek kapta, çevre dostu bir yöntem ile hazırlanan grafen oksit ile modifiye edilmiş elektrot hazırlanmış ve bir aroma verici olan vanilinin (VAN) elektrokimyasal olarak tespiti için kullanılmıştır. Elektrokimyasal olarak üretilen grafen oksit elektrot (EGO/GE), grafen oksit yapısının grafit elektrot yüzeyinde elektrokimyasal olarak üretilmesi ile hazırlanmıştır. Hazırlanan EGO/GE'nun karakterizasyonu, alan emisyon taramalı elektron mikroskobisi ve atomik kuvvet mikroskobisi gibi teknikler kullanılarak gerçekleştirilmiş ve önerilen EGO/GE materyalin başarılı bir şekilde hazırlandığı sonucuna varılmıştır. VAN'in elektrokimyasal davranışının incelenmesi dönüşümlü voltametri ile gerçekleştirilirken, voltametrik tayini için diferansiyel puls voltametri yöntemi kullanılmıştır. VAN'in elektrokimyasal belirlenmesinde kullanılan, EGO/GE'un, modifiye edilmemiş GE'a kıyasla yüksek elektrokatalitik performans, geniş doğrusal aralık (0,1-1000 μM) ve düşük algılama limiti (0,05 μM) sergilediği belirlenmiştir. Hazırlanan sensörün seçiciliği, iyonlar ve organik maddeler varlığında incelenmiştir. Sensör (EGO/GE), dondurma, kahve ve kurabiye örneklerinde VAN tespiti için kullanılmış ve yüksek geri kazanım değerleri elde edilmiştir. Sensör için gerçekleştirilen stabilite ve tekrar üretilebilirlik çalışmaları sonucunda, oldukça kararlı ve tekrar üretime uygun olduğu gözlemlenmiştir.

Anahtar kelimeler
Vanilin;
Grafen Oksit;
Voltametri;
Kronoamperometri;
Aroma Verici

1. Introduction

Food safety is a scientific discipline that deals with the processing and storage of food by preventing foodborne diseases. As it is known, food safety has become an increasingly important issue for many countries due to its public health and economic dimension (Kılınç vd. 2017, Karakaya 2020). Vanilla is one of the most important flavoring used in the food industry. Vanilla plant is known since 1816. In addition, vanillin (VAN) is the main aromatic compound in natural vanilla and pure vanillin is chemically obtained from the extraction of vanilla seed with alcohol. The systematic name of vanillin is 4-hydroxy-3-methoxy benzaldehyde ($C_8H_8O_3$) (Dignum *et al*. 2001, Rao and Ravishankar 2000, Ranadive 1994). Vanillin biosynthesis is more demanded as it is more economical to obtain by extraction methods. It constitutes an important part of the flavorings market in the world. Vanillin is a unique and extremely valuable food additive as flavoring. This compound is commonly used to enhance the smell and flavor of commercial foods such as pudding, ice cream, confectionery, custard, cookies, chocolate, beverages and pastry products (Sinha *et al*. 2008). However, taking too much vanillin can trigger allergic reactions and it causes headaches, nausea and vomiting, and it could affect liver and kidney functions when large amounts of this flavor enhancer is ingested from the viewpoint of food safety and addiction prevention. For this reason, determining the amount of vanillin in foods is important for food safety (Saint Denis *et al*. 1996, Shang etal. 2014).

Different analytical techniques are used in vanillin detection and most of these are based on chromatography and spectroscopy. However, the search for new methods that can replace these methods, which have some disadvantages such as expensive instrumentation, complex sample preparation procedures and long analysis times, is increasing day by day (Arvas *et al*. 2020, Dokur *et al*. 2020, Gorduk *et al*. 2020, Koçak 2019). High performance thin layer chromatography (Meyyanathan *et al*. 2013, Sujalmi *et al*. 2005), gas chromatography (Li *et al*. 2009), spectrophotometric (Sanchez *et al*. 1990), electrochemical methods (Chethana *et al*. 2012, Liu *et al*. 2015, Luo and Liu 2012) have been used for determination of VAN. Among these methods, electrochemical methods can be an alternative to common methods used for the determination of vanillin and have advantages such as simple instrumentation, high sensitivity, low cost, short analysis time and easy use (Özcan 2019).

Electrodes have a very important place in electrochemical determinations. In particular, the modification of bare electrode with various agents has been studied extensively in recent years (Arvas *et al*. 2019, Gorduk 2020, Tahtaisleyen *et al*. 2020b). The modified electrode represents a modern approach to the electrochemical system. It is known that modification of unmodified electrode surfaces with different materials brings additional advantages, such as increased selectivity, high sensitivity and a decrease in the potential where the electrochemical reaction occurs (Koyun *et al*. 2018, Koyun and Sahin 2018a, Koyun and Sahin 2018b). The main purpose of the electrode modification is to obtain new surfaces of completely different properties on the surface of the working electrode, this enables the determination of electroactive species (Koyun *et al*. 2017, Koyun *et al*. 2019, Koyun *et al*. 2017). Graphene oxide is an oxidized form of graphene, laced with oxygen-containing groups. Recently, graphene oxide have been used to prepare modified electrodes due to its excellent conductivity and convenience to functionalization (Ersozoglu *et al*. 2020, Tahtaisleyen *et al*. 2020a). Thanks to its superior graphene structure in terms of conductivity and surface area, load transfer, redox reaction and mechanical strength, graphene oxide has been extensively studied on electrode designs that exhibit superior mechanical, thermal and electrical properties (Gürsu *et al*. 2017, Gürsu *et al*. 2018). In addition, graphene is hydrophobic and tend to form irreversible agglomerates or even restack to form graphite through strong π–π stacking or *van der Waals* interaction. Although the conductivity of graphene oxide is not as high as graphene, it is also regarded as a suitable candidate for sensing analysis due to its nano-scale effect, surface properties, and strong affinity with biological molecules. Besides, graphene oxide preparation methods using electrochemical methods have gained importance. Graphene oxide electrode synthesis in a single step by electrochemical method is simpler, less costly, environmentally friendly and stable than existing chemical methods. We have prepared graphene oxide modified graphite electrode, which is produced electrochemically with a simple and very low cost method.

In this study, the sensor was prepared using chronoamperometry method at one-step, and environmentally friendly and used in the determination of vanillin, as flavoring. Electrochemical behavior of VAN on EGO/GE was investigated by cyclic voltammetry and differential pulse voltammetry methods. The dependence of peak current and potential on pH, scanning speed, electrodeposition time and other experimental variables were investigated. The performance of the prepared sensor was examined in real samples such as cookie, coffee, and ice-cream. With this proposed method, a low detection limit and a wide linear range are obtained for VAN electrochemical analysis. Thus, a different perspective has been given to the literature for the determination of vanillin.

2. Material and Method

2.1 Chemicals, Apparatus and Instruments

All chemicals were purchased from Sigma-Aldrich and are analytically reagent grade pure. Distilled water was used in all experiments. K_2HPO_4 and KH_2PO_4 salts were used to prepare phosphate buffer solutions prepared and used at different pH values. All standard VAN solutions were prepared in phosphate buffer solution.

All of the electrochemical measurements were carried out with a three electrode system using the pencil graphite electrode (GE) (Tombow HB, 0.5 mm) as the working electrode, the Ag/AgCl electrode as the reference electrode, and the Pt wire as the counter electrode.

All electrochemical measurements were carried out with Autolab (PGSTAT128N) Potentiostat/Galvanostat. High-resolution AFM images were recorded using Shimadzu Scanning Prope Microscope SPM-9600. Morphological images of the electrode surface were recorded with the Philips-FEI XL30 ESEM-FEG brand SEM device.

2.2 Fabrication of EGO/GE

The fabrication of the EGO/GE to be utilized in the electrochemical detection of the vanillin was performed in one-step. For this purpose, based on previous studies (Ersozoglu *et al*. 2020, Gürsu *et al*. 2017, Gürsu *et al*. 2018, Tahtaisleyen *et al*. 2020a), graphene oxide formation was performed on the surface of the graphite electrode platform in 5.0 M H_2SO_4 solution, at +1.9 V constant potential and chronoamperometry method, and it was used for the first time for VAN determination. To investigate the effect of the applied potential time, a constant potential of +1.9 V was applied for periods of 15, 30, 60, 90, and 120 seconds. The performances of the prepared electrodes were compared using both CV and DPV methods in the solution containing 1000 µM VAN and it was observed that the highest oxidation response belongs to the electrodeposition time of 60 seconds. Therefore, the optimized electrodeposition time was chosen as 60 seconds. The electrode obtained after this procedure was coded as EGO/GE and washed by double-dipping in distilled water and dried at room temperature in a desiccator. The representative picture showing the preparation and application stages of the sensor is as seen in Figure 1.

2.3 Preparation of Real Samples

In this study, real sample studies were made with coffee and ice-cream samples bought from a local shop in Turkey and utilized with no pretreatment. For examination, 2.0 mL of the coffee was filtered and diluted to 10.0 mL with PBS pH 7.0. The ice-cream sample was melt mixed at room temperature. Then, 2.0 mL of the melted ice-cream sample was diluted to 10 mL with phosphate buffer solution (pH 7.0). The cookie sample was sourced from a local bakery. It was ground to a powder in a mortar with the cookie sample. The ground sample was mixed thoroughly to make a homogeneous mixture and 0.5 g of the mixed sample was taken and placed in a beaker. Then, 10 mL of ethanol solvent was added to the beaker containing the sample and it was mechanically mixed for 30 minutes. Then, the sample mixture was taken into a centrifuge tube and centrifuged at 3000 rpm for 15 min. The vanillin determination was carried out for the sample taken from the clear filtrate part of the tube, whose centrifugation was completed. Before the analysis, the cookie, coffee, and ice-cream samples were freshly prepared right. It was mixed in the ultrasonic bath for 10 minutes, and then analysis was carried out.

2.4 Experimental Procedures

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods were used to determine vanillin by an electrochemical method. In order to determine optimum conditions, measurements performed in 0.1 M pH 7.0 PBS containing 1000 µM VAN were recorded. Throughout the experimental studies, a length of 1.0 cm each time was immersed in the EGO/GE analyte solution to keep the surface area of the working electrode constant. DPV measurements were performed in the potential range 0.0 to 1.0 V at scan rate 50 mV/s in pH 7 PBS. Freshly prepared electrodes were used for each measurement.

3. Results and Discussion

3.1 Preparation and Characterization Studies of EGO/GE

The electrochemically fabricated graphene oxide electrodes were prepared using the chronoamperometric method on the surface of the pencil graphite electrode, which is an inexpensive and disposable graphitic material for optimization. Evidence of graphene oxide formation on the graphite electrode surface has been presented in detail in our previous studies (Ersozoglu *et al*. 2020, Gürsu *et al*. 2017, Gürsu *et al*. 2018). Optimizing the developed electrode material for electrochemical vanillin determination is the first in this sense in the literature. When graphene oxide modified electrodes prepared under a constant potential of +1.9 V in 5.0 M sulfuric acid solution and at different deposition times (15, 30, 60, 90, and 120 seconds) are used for the determination of vanillin, the higher oxidation signal was found to belong to the electrode that was electrodeposited for 60 seconds (Figure 2a). Each measurement was repeated three times (n=3). The shifts in the potential value occurring with each oxidation time applied confirm that the time is effective in the modification process. When a potential of +1.9 V is applied for blank solution and 0, 15, 30, and 60 seconds, the increase in peak current is directly proportional to time, while the change in current is inversely proportional when the deposition time is 90 and 120 seconds (Figure 2b). The response of the incoming signal at 0.56 V of the oxidation of vanillin gradually decreased when more than 60 seconds (for 90 and 120 seconds). However, all prepared electrodes exhibited high peak current and capacity for vanillin sensing in comparison to the Bare/GE. The electrode prepared in 60 seconds deposition time has superior performance for vanillin sensing among the prepared all modified electrodes due to the altered oxygen-containing functional groups via the deposition time of electrodes (Ersozoglu *et al*. 2020).

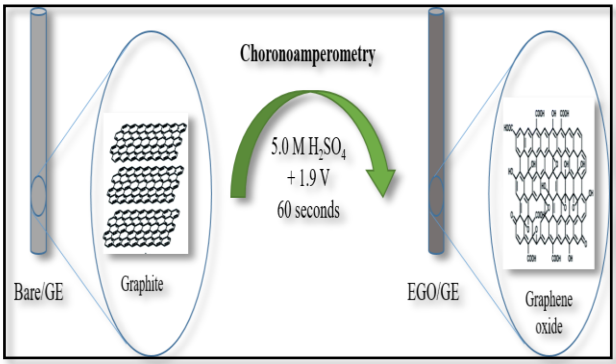

Figure 1. Schematic representation of the preparation of the sensor.

Field Emission Scanning Electron Microscopy (FESEM) and Atomic Force Microscopy (AFM) methods were used in the characterization process of the sensor optimized for vanillin determination. Advanced characterization studies of the electrode are presented in our previous study (Tahtaisleyen *et al*. 2020a). The Field Emission Scanning Electron Microscopy (FESEM) characterization technique provides information on surface morphologies such as shapes and sizes of carbon nanostructures.

Figure 3 shows FESEM images of EGO/GE at different magnification scales. The images shows that different sizes of graphene oxide are obtained by the chronoamperometric method. As a result of the oxidation process, the flat graphite structure of the Bare/GE was disintegrated into powder of small sizes as indicated with an arrow: submicron and nano. Mild electrochemical oxidation was strong enough to partially break the *van der Waals* interaction and intercalation occurred (Ersozoglu *et al*. 2020, Tahtaisleyen *et al*. 2020a). However, it does not allow the graphene oxide flakes to exfoliate. Due to its strong π→π interaction, the GO sheets tend to restacked and agglomerated as shown in the images. Based on this information, it was morphologically confirmed that graphene oxide structures were formed on the graphite electrode surface by electrochemical method.

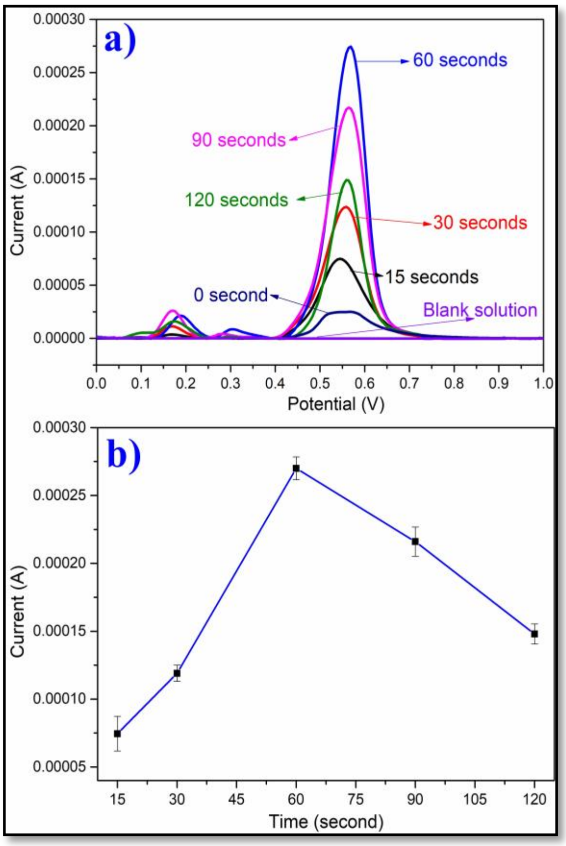

Figure 2. a) Differential pulse voltammograms and b) current-time relationship graph of EGO/GEs with different electrodeposition time in PBS pH 7.0 containing 1000 µM VAN at scan rate of 50 mV/s and the potential range of 0.0 V to 1.0 V.

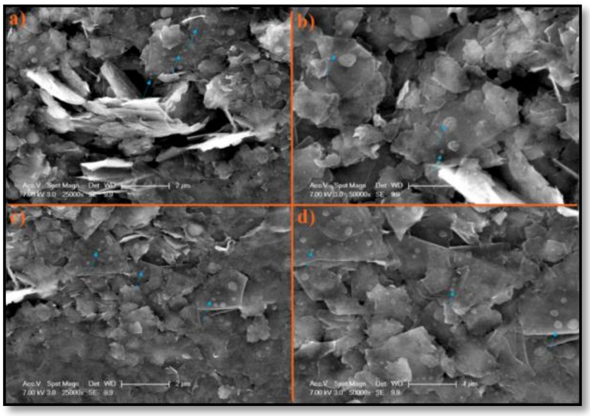

Figure 3. FESEM micrographs of a) EGO/GE with 25000 magnifications, b) EGO/GE with 50000 magnifications, c) EGO/GE with 25000 magnifications, and d) EGO/GE with 50000 magnifications (from different areas).

Figure 4 shows the atomic force microscopy (AFM) amplitude two and three dimensional images of unmodified and electrochemically fabricated graphene oxide modified electrode surfaces. The surface roughness of electrodes were clearly seen in images. The change of topography of the surface is distinctly seen after oxidation process. When the average roughness value (Ra) of the electrodes from AFM images were examined, it were found as 60.69 and 62.81 for Bare/GE and EGO/GE, respectively. The square root of the surface height distribution of the smoothness (Rq) is defined as the root mean square (RMS). RMS roughness (Rq) defines the final version of optical surfaces. Rq were found as 76.89 and 77.43 for Bare/GE and EGO/GE, respectively. The roughness of EGO/GE was higher than that of Bare/GE due to the intercalation and doping of groups containing oxygen into graphitic structure to produce graphene layers. When the functional groups containing oxygen were oxidized and reduced at the electrode surface, some of the graphene flakes fell off the surface of the graphitic electrode (Ersozoglu *et al*. 2020). This causes a rough texture on the electrode surface subjected to the deposition process. Thus, it is possible to say that the AFM images shows a similar trend with FESEM images.

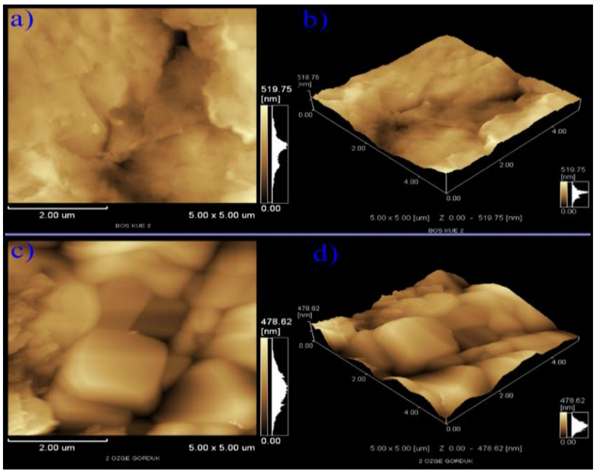

Figure 4. AFM images of a) Bare/GE (2-dimensional), b) Bare/GE (3-dimensional), c) EGO/GE (2-dimensional), and b) EGO/GE (3-dimensional).

3.2 Investigation Voltammetric Behavior of Vanillin at the EGO/GE

As is known, vanillin is an electroactive compound. Therefore, cyclic and differential pulse voltammograms were recorded on the surfaces of different electrodes in order to investigate the redox behavior and also evaluate the ability of the modifier agent to improve electrode performance for VAN determination. Both bare PGE and EGO/GE have demonstrated oxidation responses that confirm the electroactive nature of VAN. Figure 5 displays the cyclic voltammograms obtained for 1000 µM VAN at the bare PGE and EGO/GE in PBS (0.1 M) pH 7.0 with a scanning rate of 50 mV/s. The VAN shows the oxidation current with a good response. The oxidation peak potentials were seen at 0.62 V and 0.70 V at a bare pencil graphite electrode. However, in the same condition, EGO/GE displayed significant improvement in the current signal, and the potential of oxidation was found at 0.60 V. It was observed that the oxidation signal of the vanillin shifts in the anodic direction when the working electrode is EGO/GE. When the differential pulse voltammograms seen in Figure 6 are examined, it is possible to say that the oxidation signal for vanillin is slightly split at 0.55 and 0.57 V in the voltammogram recorded with Bare/GE.

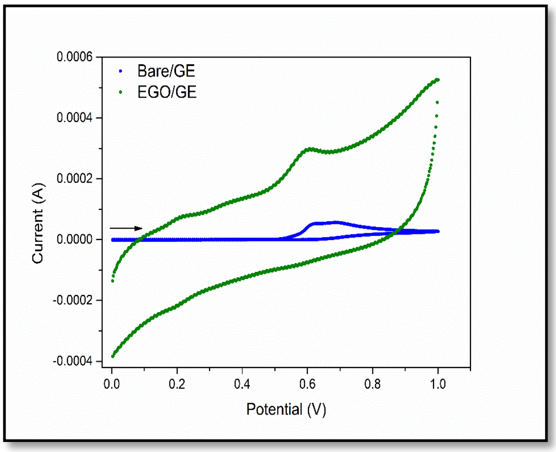

Figure 5. Cyclic voltammograms of bare/GE and EGO/GE in PBS pH 7.0 containing 1000 µM VAN, at potential range of 0.0-1.0 V, and scan rate of 50 mV/s.

According to this result, which appears as a single signal at 0.56 V according to the voltammogram recorded with EGO/GE under the same conditions, we can interpret that the electrochemical reaction occurs faster and easier. When Figure 6 is examined, it is seen that the current value of the sharp peak representing the oxidation of Vanillin on EGO/GE is approximately 13 times higher compared to the peak current value obtained with Bare/GE. Here, the noticeable enhancement of peak current demonstrates the electrocatalytic action of EGO/GE towards the detection of VAN (Koçak 2019). As a result, EGO/GE showed the maximum intensity of the electrochemical signal, demonstrating that electrochemically fabricated graphene oxide exhibited higher electroconductivity, which could be resulted from the synergic impact and good properties of modifiers, namely, high surface area, great electrical conductivity and much more sites for electroactive species.

3.3 Influence of pH on the detection of VAN at EGO/GE

The pH of the PBS has a considerable influence on the electrochemical response of VAN at the EGO/GE by influencing peak potential and peak current. The influence of the PBS pH value on determining VAN at EGO/GE was meticulously assessed in a wider pH interval of 4.0–8.0. Figure 7 shows differential pulse voltammograms recorded for 1000 µM VAN at EGO/GE. The oxidation peak potential moves to a more negative potential by higher pH. The peak potential versus pH plot showed that the peak potential is linearly dependent on the pH value in the interval of 4.0–8.0 with a slope of 41.8 mV/pH (R^2=0.994) as demonstrated in Figure 8a.

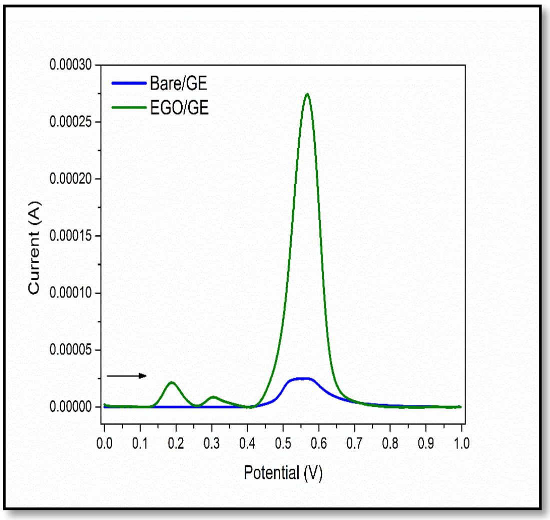

Figure 6. Differential pulse voltammograms of bare/GE and EGO/GE in PBS pH 7.0 containing 1000 µM VAN, at potential range of 0.0-1.0 V, and scan rate of 50 mV/s.

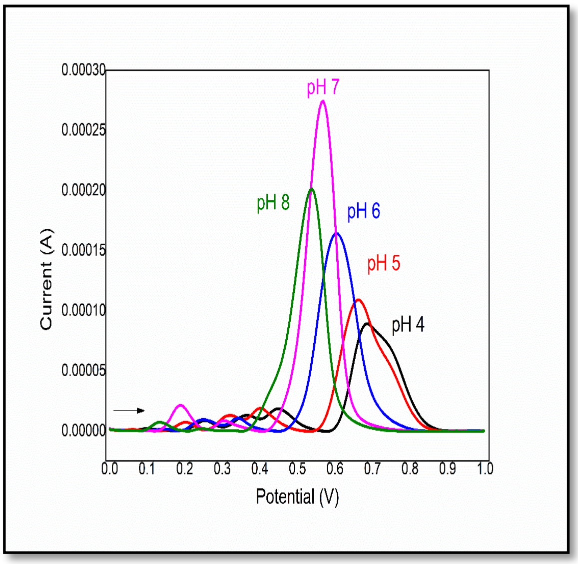

Figure 7. Differential pulse voltammograms of EGO/GE in the 4.0, 5.0, 6.0, 7.0, and 8.0 pH values PBS containing 1000 µM VAN.

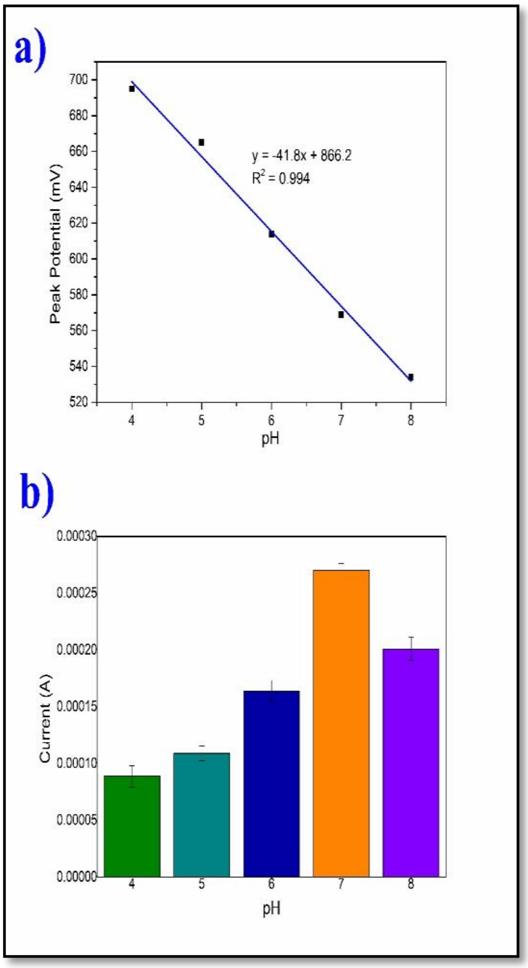

Figure 8. Graph showing the variation of a) the peak potential and b) the peak current responses of PBS containing 1000 µM VAN with pH value.

Figure 8b shows the change in current depending on the pH change. This finding suggests there exists an equal quantity of protons and electrons taking part in the redox process (Rosy 2015).

According to Bard and Faulkner (Bard and Faulkner, 2001), the transferred electron number in the VAN oxidation reaction was found as approximately 2 (Karakaya 2020, Koçak and Karabiberoğlu, 2018). Therefore, the reaction in Figure 9, for the electro-oxidation of VAN on the surface of EGO/GE can be suggested, where 2 electrons and 2 protons participate in the electrode reaction. This was in agreement with that stated in the literature (Karakaya 2020).

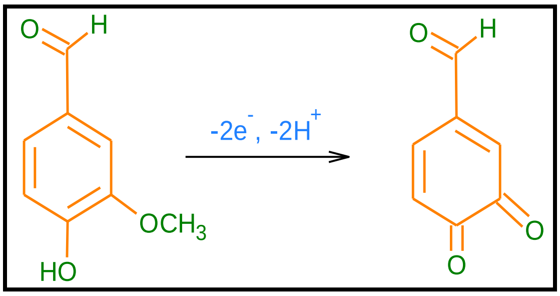

Figure 9. The possible oxidation mechanism of VAN at EGO/GE.

3.4 Influence of potential scan rate on response oxidation peak current of VAN

The influence of changing the employed scan rate for 1000 µM VAN in PBS pH 7.0 was examined by the CV technique at the EGO/GE as demonstrated in Figure 10.

The experiment findings acquired from the EGO/GE displayed a rise in the peak currents with higher applied scanning rate, and they were proportional to each other based on the Randles–Sevcik formula (Gorduk 2020). The finding suggested that there was a shift of the oxidation peak potentials towards the less negative side.

To validate the electrode process, the plot of the peak current versus square roots of scan rate was drawn, and the resulting plot was a straight line with satisfactory linearity in the interval from 25 to 500 mV/s as presented in Figure 11 with the correlation coefficient (R^2) of 0.9884. These results indicate that the electrode process is diffusion-controlled (Gorduk 2020, Koyun *et al*. 2019).

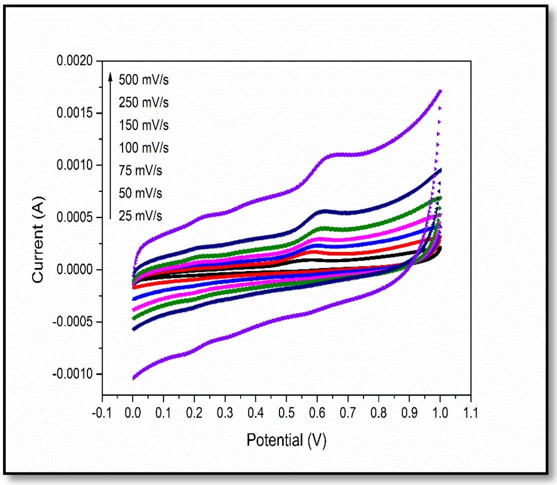

Figure 10. Cyclic voltammograms of the EGO/GE at scan rates of 25, 50, 75, 100*, 150*, 250, and 500 mV/s in PBS pH 7.0 containing 1000 µM VAN.

3.5 Analytical Determination of Vanillin

A set of VAN solutions was created at a falling concentration starting from 0.1 µM to compute the identification and determination limit of the created EGO/GE. The voltammograms acquired by differential pulse voltammetric method in the potential interval of 0.0 V to 1.0 V using the modified electrode in every solution are presented (Figure 12). By utilizing the voltammograms in Figure 12, a calibration plot that provides the current-concentration relationship shown in Figure 13 was obtained. As a consequence of the computations conducted from the calibration plot, the LOD value was found as 0.05 µM (S/N=3), and the LOQ value was found as 0.17 µM (S/N=10). The linear regression equation of EGO/GE is $I(A)= 0.2741C_{VAN}(M) + 2x10^{-6}$, $R^2= 0.9994$. The comparison of the analytical performances of some electrodes for vanillin determination in the literature is presented in Table 1. When the sensors mentioned in Table 1 are compared with EGO/GE; EGO/GE appears to be prepared fairly easily (one-step) and in a very short time (60 seconds), has a very low cost, offers a wide linear range and a relatively low detection limit. EGO/GE stands out because of not using a modifying agent prepared as a result of expensive and long efforts in the modification process of the electrode.

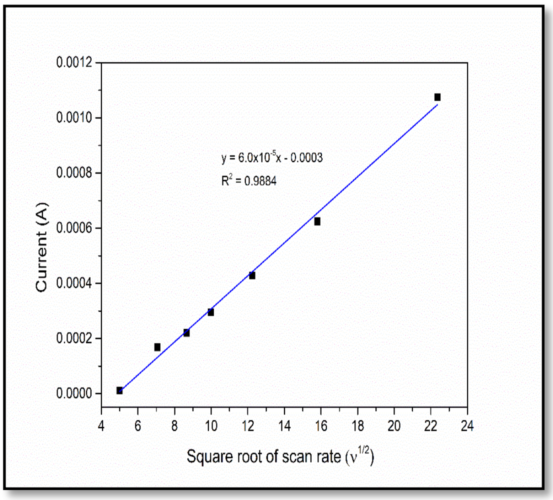

Figure 11. Graph showing the linear relationship between the square roots of the scan rate with oxidation peak current.

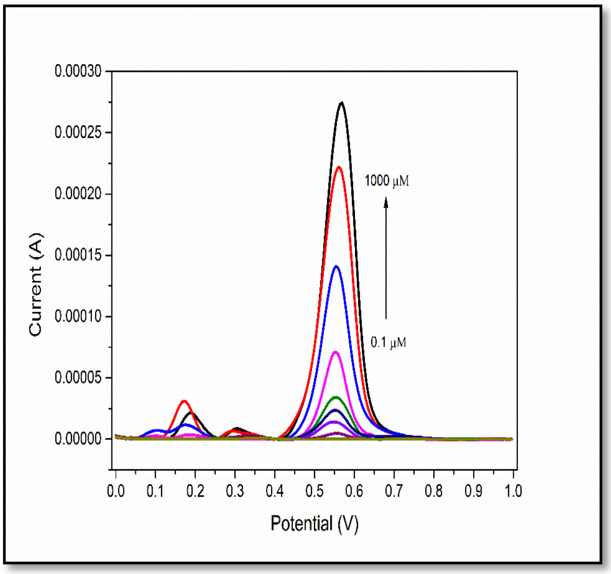

Figure 12. Differential pulse voltammograms of EGO/GE in different concentrations of VAN solutions.

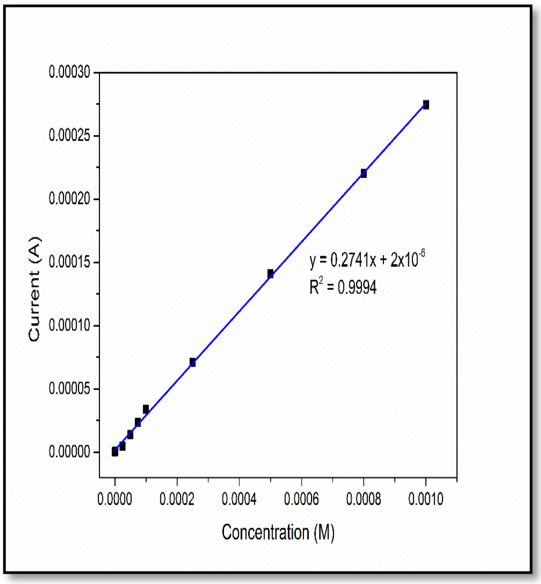

Figure 13. Calibration graph of current-concentration (1000, 800.0, 500.0, 250.0, 100.0, 75.0, 50.0, 10.0, 2.50, 1.0, and 0.1 µM concentrations of VAN in PBS pH 7.0).

Table 1. Comparison of some sensor platforms in the literature for VAN determination

Sensor	Electrochemical method	Linear range (µM)	Limit of detection (µM)	Reference
AuNP-PAH/GCE	Square wave voltammetry	0.9-15	0.055	(Silva *et al*. 2015)
AgNP/graphene/GCE	Square wave voltammetry	2.0-100	0.33	(Huang *et al*. 2014)
CoS nanorods/GCE	Differential pulse voltammetry	0.5-56	0.07	(Sivakumar *et al*. 2017)
C_3N_4/GCE	Differential pulse voltammetry	0.2-10	0.004	(Fu *et al*. 2020)
G-QD@Nafion/AuNPSPCE	Differential pulse voltammetry	0.66-33	0.32	(Durán *et al*. 2018)
Pd/PMB/GCE	Differential pulse voltammetry	0.02-1	0.01	(Koçak 2019)
Au-MWCNT/GCE	Differential pulse voltammetry	0.07-6.5	0.03	(Koçak and Karabiberoğlu 2018)
poly(EBT)/PGE	Amperometry	0.05-10	0.013	(Karakaya 2020)
EGO/GE	Differential pulse voltammetry	0.1-1000	0.05	This study

Abbreviations: AuNP-PAH; gold nanoparticles stabilized in poly(allylamine hydrochloride), AgNP; silver nanoplates, CoS; cobalt sulfide, C_3N_4; carbon nitride, G-QD; graphene quantum dot, AuNP; gold nanoparticle, Pd/PMB; Pd Nanoparticles/Poly (Methylene Blue), Au-MWCNT; gold nanoparticles modified multiwalled carbon nanotube, poly(EBT); Poly(Eriochrome Black T), GCE; glassy carbon electrode, SPCE; screen printed carbon electrode, PGE; pencil graphite electrode.

3.6 Interference Studies

In electrochemical sensor studies, it is possible that other ions or compounds that may be in the same environment with analyte species can interfere. The prepared sensor is expected to respond selectively to the analyte among other interfering species. A differential pulse voltammetric study was conducted to investigate the selectivity of the EGO/GE according to the species interfering with VAN determination. The amperometric response of the EGO/GE sensor obtained by sequential addition of VAN and interfering species to 0.1 M PBS (pH 7.0) is presented in Figure 14. The ions (K^+, Fe^{3+}, Na^+, PO_4^{3-}, Cl^-, and NO_3^-) at a concentration 100 times higher than the concentration of VAN and ascorbic acid, glucose and phenylalanine, which were 10 times higher than the concentration of VAN, were added to the solution. No current response was observed for glucose and phenylalanine, while the oxidation signal for ascorbic acid was observed around -0.04 V. The potential difference between the oxidation signal of ascorbic acid and the oxidation signal of the VAN was not such as to cause interference. In addition, the variation created by these added species on the current response of the vanillin signal is less than 5%, indicating that the sensor is not affected by interference types. Based on these results, it was concluded that the EGO/GE has a good selectivity and sensitivity for determining VAN even in solution containing interfering species.

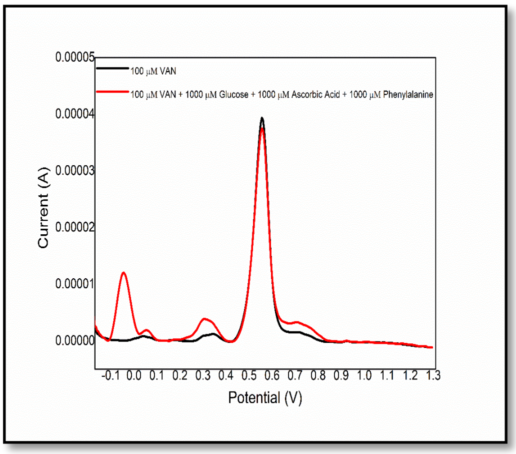

Figure 14. Differential pulse voltammograms recorded in the presence and absence of interference species (glucose (1000 µM), ascorbic acid (1000 µM), and phenylalanine (1000 µM)).

3.7 Stability and Reproducibility of EGO/GE

Intra-day and inter-day measurements were recorded to examine the reproducibility of the EGO/GE. To examine intra-day repeatability performance, DPVs of 10 µM VAN were conducted using ten different EGO/GEs prepared just prior to analysis. The percent relative standard deviation (RSD%) was found to be 2.24%. In order to investigate the repeatability performance inter-day, repeated measurements were made for ten days and the RSD% was found to be 2.93%. These results demonstrated that the EGO/GEs were replicable in their preparation. In addition, to examine the stability of the electrodes, DPV measurements were taken with electrodes held in a desiccator for 60 days. Hence, the difference between the starting and ending values was only 3.71%. In conclusion, these findings demonstrated that the EGO/GE was quite stable.

3.8 Practical Applications

The spike recovery method was used to examine the usability of the proposed sensor platform in real samples. Electrochemical measurements were performed with DPV method.

Table 2. Differential pulse voltammetric determination of VAN in the real samples with EGO/GE.

Sample	**Added concentration of vanillin (µM)**	**Found concentration of vanillin (µM)**	**Recovery (%)**	**Relative standard deviation (%)**
Coffee	0.0	31.50	-	-
	20.0	49.83	96.76	2.63
	40.0	70.13	98.08	2.40
Ice-Cream	0.0	11.94	-	-
	20.0	28.73	89.95	2.17
	40.0	49.72	95.73	3.06
Cookie	0.0	3.17	-	-
	10.0	14.11	102.94	3.49
	20.0	24.19	104.40	2.85

The presence of vanillin was investigated in the cookie, coffee and ice cream samples prepared as mentioned in the "2.3. Preparation of Real Samples" section. The 2.0 mL coffee was diluted with phosphate buffer solution (10 mL, pH 7.0). Then, 20 and 40 µM standard VAN solutions were introduced to the diluted sample solution. The 2.0 mL melted ice-cream was diluted with PBS (10 mL, pH 7.0). Then, 20 and 40 µM standard VAN solutions were introduced to the diluted sample solution. The 2.0 mL cookie filter was filled directly into the electrochemical cell. Then, 10 and 20 µM standard VAN solutions were introduced to the diluted sample solution. Table 2 presents the obtained findings. Every measurement was replicated three times, and the RSD% was computed. The recovery values were determined to be in the range of 89.95 to 104.40%. The findings showed that the EGO/GE could be applied to real sample analysis.

4. Conclusions

In summary, for the electrochemical determination of vanillin, a sensor that can perform graphene oxide formation on the graphite electrode surface with a very low cost, time-saving and environmentally friendly technique, in one step, in one-pot, was used. After this sensor was optimized for voltammetric determination of vanillin, it was characterized using different characterization techniques. The sensor exhibited a good response, a low detection limit, and a wide linear range for detecting vanillin. When the results of the studies performed in the use of EGO/GE for vanillin such as interference, stability, reproducibility, and applicability in commercial food products are examined, it is determined that it will be a good candidate for analytical applications. It is predicted that the prepared sensor can guide the electrochemical determination of different analytes that are important for human and the environment health.

Acknowledgement

Ozge Gorduk especially thanks Prof. Dr. Yucel Sahin for his support in this study.

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