Development of a Method for the Voltammetric Determination of Ascorbic Acid on a Palladium Doped Carbonaceous Electrode

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Abstract

Ascorbic acid (AA) is a vital antioxidant with critical roles in human health, including immune system support and protection against oxidative stress. Accurate and sensitive determination of AA is essential in clinical diagnostics, pharmaceutical analysis, and food quality control. In this study, a novel voltammetric method was developed using a glassy carbon (GC) electrode modified with palladium-doped carbonaceous material for AA detection. A mixture of equal amounts of raw coal tar pitch (r-CTP) and glass fiber (GF) was pyrolyzed in a high-temperature furnace at 1000°C for 1 h, with a heating rate of 10°C/min under a nitrogen atmosphere. The resulting carbonized material was ground using a planetary ball mill (Retsch, PM100), and palladium nanoparticles (PdNPs) were then doped onto the material via the sol-gel method. The obtained material is denoted Pd/CTP-GF. Pd/CTP-GF was characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD). The glassy carbon (GC) electrode modified with Pd/CTP-GF was characterized by cyclic voltammetry (CV). A method was developed for the determination of ascorbic acid using differential pulse voltammetry (DPV) on the Pd/CTP-GF modified GC electrode. The detection limit (S/N=3) of the developed method was determined to be 5 nM. The intraday and interday precision and accuracy of the method were also evaluated. In addition, the interference of dopamine, uric acid, and certain cations on ascorbic acid was investigated. In real sample applications, the developed method achieved a recovery of over 95% for ascorbic acid in orange juice samples.

Keywords: Ascorbic acid; differential pulse voltammetry; palladium nanoparticle; carbonaceous electrode.

Paladyum Nanoparçacık Katkılı Karbonsu Elektrot Üzerinde Askorbik Asidin Voltametrik Tayini İçin Yöntem Geliştirilmesi

Öz

Askorbik asit (AA), bağışıklık sistemini destekleme ve oksidatif strese karşı koruma gibi insan sağlığında kritik rolleri olan hayati bir antioksidandır. AA'nın doğru ve hassas bir şekilde belirlenmesi, klinik tanı, farmasötik analiz ve gıda kalite kontrolü için önemlidir. Bu çalışmada, AA tespiti için palladyum katkılı karbonlu malzeme ile modifiye edilmiş camsı karbon (GC) elektrot kullanan yeni bir voltametrik yöntem geliştirilmiştir. Eşit miktarda ham kömür katranı (r-CTP) ve cam elyaf (GF) karışımı, azot atmosferi altında 1000°C'de 1 saat boyunca 10°C/dk ısıtma hızıyla yüksek sıcaklık fırınında piroliz edilmiştir. Elde edilen karbonize malzeme, gezegensel bilyeli öğütücüde (Retsch, PM100) öğütülmüş ve daha sonra üzerine paladyum nanoparçacıkları (PdNP'ler) sol-gel yöntemiyle doplanmıştır. Elde edilen malzeme Pd/CTP-GF

olarak adlandırıldı. Pd/CTP-GF, taramalı elektron mikroskobu (SEM) ve X-ışını kırınımı (XRD) yöntemleri ile karakterize edilmiştir. Pd/CTP-GF ile modifiye edilmiş cam karbon (GC) elektrotu
ise dönüşümlü voltametri (CV) ile karakterize edilmiştir. Askorbik asitin tayini için Pd/CTP-GF modifiye edilmiş GC elektrotu üzerinde diferansiyel puls voltametrisi (DPV) kullanılarak bir yöntem geliştirilmiştir. Geliştirilen yöntemin tespit limiti (S/N=3) 5 nM olarak belirlendi. Geliştirilen yöntemin gün içi ve günler arası doğruluk ve kesinliği değerlendirilmiştir. Ayrıca, dopamin, ürik asit ve bazı katyonların askorbik asit üzerindeki interferans etkileri araştırılmıştır. Geliştirilen yöntemin gerçek numune uygulamasında, portakal suyu örneklerinde askorbik asit için %95'in üzerinde bir geri kazanım değeri elde edilmiştir.

Anahtar Kelimeler: Askorbik asit; diferansiyel puls voltametrisi; paladyum nanoparçacık; karbonsu elektrot.

1. Introduction

Ascorbic acid (AA) is an antioxidant found in the human body [1]. AA acts as a reducing agent that protects the human body from the side effects of oxidative processes and plays a significant role in various biological activities. These include vascular protection, cellular development, bone-cartilage repair, collagen synthesis, and the healing of burns and wounds [2]. Additionally, ascorbic acid has demonstrated therapeutic effects in the treatment of life-threatening clinical conditions such as cancer [3], Alzheimer's disease [4], lymphoblastic leukemia [5] and tumors [6].

Chromatographic and spectrometric methods used for the determination of AA are expensive, complex, and involve challenging steps. Due to difficulties in sample preparation, the high cost of traditional methods, and other similar factors, there is a growing need for high-efficiency and cost-effective alternative methods for real-time AA detection [7] Electrochemical techniques, owing to their simplicity, lower cost, and ability to operate with minimal materials, have garnered significant interest among researchers. Electrochemical methods for the determination of ascorbic acid (Vitamin C) are widely employed due to their sensitivity, simplicity, and rapid response times [8, 9]. These methods often include potentiometric, voltammetric, and amperometric techniques, which are further enhanced through various sensor modifications to improve performance.

Electrochemical sensing provides numerous benefits, such as quick analysis, reduced sample volume, lower costs, and the removal of pre-concentration procedures. In this context, techniques such as pulse voltammetry (DPV and CV), and chronoamperometry have enabled the development of portable, user-friendly electrodes [10]. Electrochemical methods for AA detection are favored for their low cost, high sensitivity, ease of use, and quick response times. However, many sensing materials have limitations, such as low surface areas, poor chemical design, and difficult modifiability. Thus, designing well-structured, porous materials is key to improving performance. PdNPs-loaded carbon-derivatived electrodes offer selective and sensitive electrochemical detection of ascorbic acid [11]. Electrodes modified with PdNPs, such as Pd/CNFs, PdNPs/GR/CS, and PdNPs-GO, exhibit superior electrochemical activity toward AA and achieve lower oxidation potentials compared to bare electrodes [12].

Designing and modifying electrodes for AA electrochemical oxidation is way to improving reproducibility.. PdNPs-loaded carbon nanofiber-modified electrodes are used for AA determination in literature 13].
In a study [14], an electrochemical sensor for AA was designed utilizing a GC modified with PdNPs supported on graphene oxide (PdNPs-GO). The electrochemical behavior of AA oxidation was evaluated using cyclic voltammetry (CV) and amperometric techniques. Compared to a bare GC or PdNPs-modified electrode, the anodic peak potential for AA on the PdNPs-GO modified electrode shifted to a more negative value of 0.006 V. This shift, along with a notable separation of the anodic peak potentials of 0.172 V between AA and dopamine (DA), was attributed to the synergistic effect of GO and PdNPs. In a study, a highly sensitive and selective electrochemical sensor for ascorbic acid detection was developed, featuring a rapid response, broad linearity, and successful application in Vitamin C tablet analysis. The sensor demonstrated exceptional performance for AA detection [14].

In this study, an equal mixture of raw coal tar pitch (r-CTP) and glass fiber (GF) was pyrolyzed in a high-temperature furnace at 1000°C for 1 hour under a nitrogen atmosphere, with a heating rate of 10°C/min [15]. The resulting carbonized material was ground using a Spex-type mill (Retsch, PM100), followed by the synthesis of palladium nanoparticles (PdNPs) on its surface [7]. The final material was designated as Pd/CTP-GF. The Pd/CTP-GF was characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD). The glassy carbon (GC) electrode modified with Pd/CTP-GF was characterized by cyclic voltammetry (CV). A method for AA determination in the concentration range of 0.01–1.0 µM was developed using the Pd/CTP-GF-modified GC electrode surface. This method was successfully applied for the determination of Vitamin C in commercial orange juice samples.

2. Materials and Methods

2.1. Experimental

The chemicals used in this study were obtained from local suppliers of Merck, Sigma-Aldrich, and Fluka. The water used for solution preparation was of ultra-pure water (UPW) quality. Electrochemical measurements were conducted at room temperature using an Ivium-CompactStat (Netherlands). The glassy carbon electrode (MF-2012, Basi, USA, with a diameter of 3 mm) was used as a working electrode. A three-electrode system was employed for voltammetric measurements, with the working electrode being a glassy carbon electrode modified with pyrolyzed materials, the reference electrode being Ag/AgCl (saturated), and the auxiliary electrode a platinum wire.

2.2. Electrode Modification

The glassy carbon (GC) electrode was first treated with a 100 nm and then a 50 nm diameter alumina suspension (Baikowski, USA) and subsequently cleaned by sonication in an ultrasonic bath (Bandelin, Germany) for 5 minutes each, using deionized water and a mixture of acetone/isopropyl alcohol. A homogeneous mixture of raw coal tar pitch (r-CTP) and glass fiber (GF) was placed in a high-temperature furnace (Protherm, USA/Turkey) at 1000 °C at (rate of head=10 °C/min) under N2, followed by pyrolysis at this temperature for 1 h. The resulting carbonized product was designated as CTP-GF. This material was then ground into micro- and nano-powder by milling in a Spex-type grinder (Retsch, PM100, Germany) at 400 rpm for 6 hours.

Five grams of CTP-GF were mixed with 100 mL of ultra-pure water (UPW) to form a homogeneous suspension. To this suspension, 525 mg of polyvinylpyrrolidone (PVP, MW = 38,000), 300 mg of L-ascorbic acid, 1500 mg of potassium bromide (KBr), and 200 mg of palladium chloride ($PdCl_2$) were added in 55 mL of water [16]. The solution was heated to 114 °C, and the reaction was allowed to proceed under homogeneous stirring for 3 hours. Subsequently, the solution was cooled to room temperature. The resulting solution was centrifuged at 5000 rpm for 10 minutes. To ensure the complete removal of the reactants, a washing procedure was performed, wherein the material was washed three times with anhydrous ethanol and UPW, followed by centrifugation. The obtained Pd/CTP-GF was dried in a vacuum oven at 55 °C for 6 hours. Two hundred fifty milligrams of Pd/CTP-GF were weighed and mixed in 1.0 mL of acetonitrile to create a homogeneous suspension. From this solution, 10 µL was pipetted and dropped onto the surface of the GC electrode, and the electrode surface was dried using an infrared lamp (75 W).

3. Results and Discusion

3.1. Characterization studies

In order to assess the particle size, homogeneity, and elemental composition of the prepared electrode materials, scanning electron microscopy (SEM) image (Figure 1) and energy-dispersive X-ray spectroscopy (EDX) measurement (Figure 2) were conducted.
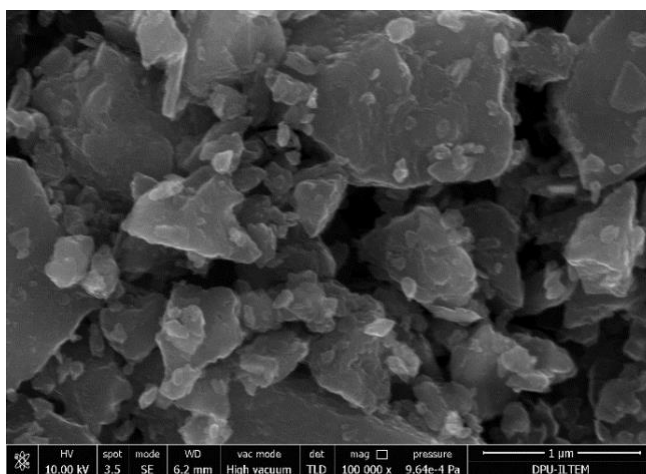

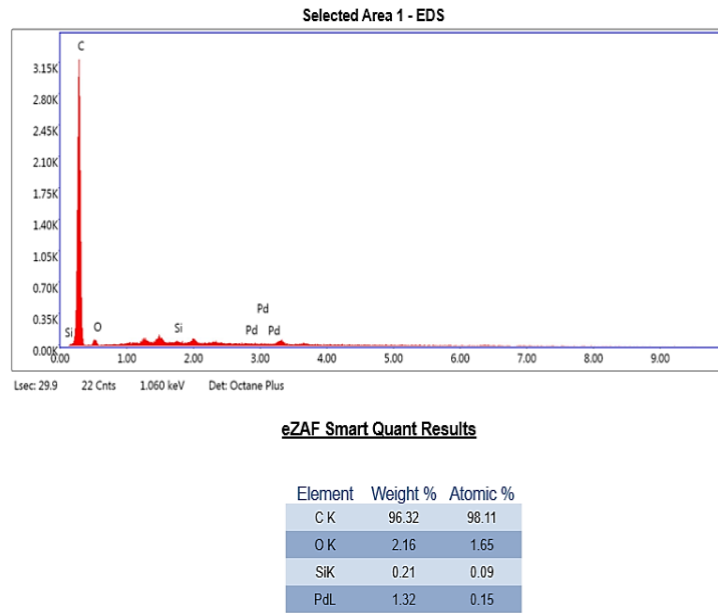**Figure 1.** SEM image of Pd/CTP*-GF.

Figure 2. EDX spectrum of Pd/CTP-GF.

The SEM images of Pd/CTP-GF indicate that the particle size is below 2 µm and that nanoparticles are also present. The EDX spectrum shows peaks corresponding to Al, Si, C, and O from the glass fiber, as well as C and O from the CTP, along with reduced Pd on the surface. EDX analysis at the selected surface point reveals that the majority of the material is composed of C, while it is evident that Pd is effectively present on the surface.

X-ray diffraction (XRD) measurements (Figure 3) indicate that the material consists of carbon (in the range of 20-30°) and other components[17], with elements such as Pd, Si, and Al exhibiting distinct peaks in a poly crystalline structure [15].
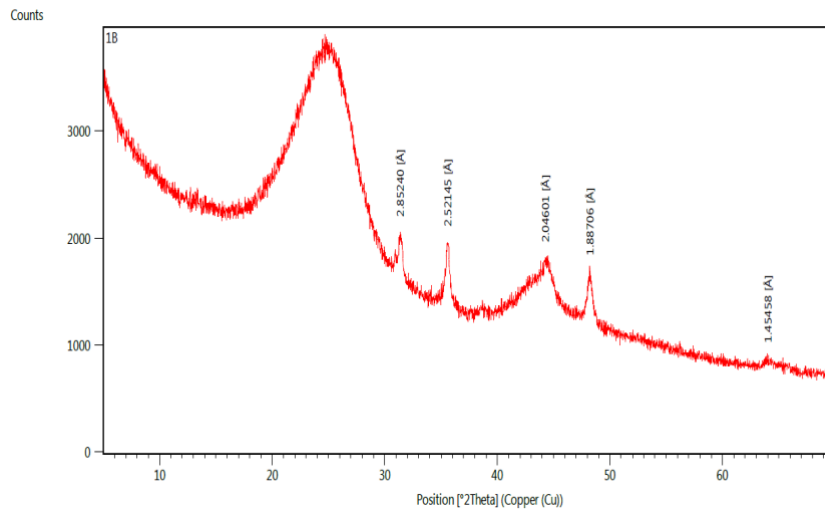

Figure 3. XRD pattern of Pd/CTP-GF.

In order to compare with the palladium-containing electrode, CTP obtained through the pyrolysis of r-CTP and CTP-GF were modified onto GC surface. CV measurements were conducted at a scan rate of 100 mV/s for 0.05 mM ascorbic acid (AA) in a 0.1 M phosphate buffer at pH 7.2, utilizing the CTP, CTF-GF, and Pd/CTP-GF modified GC electrodes (Figure 4).
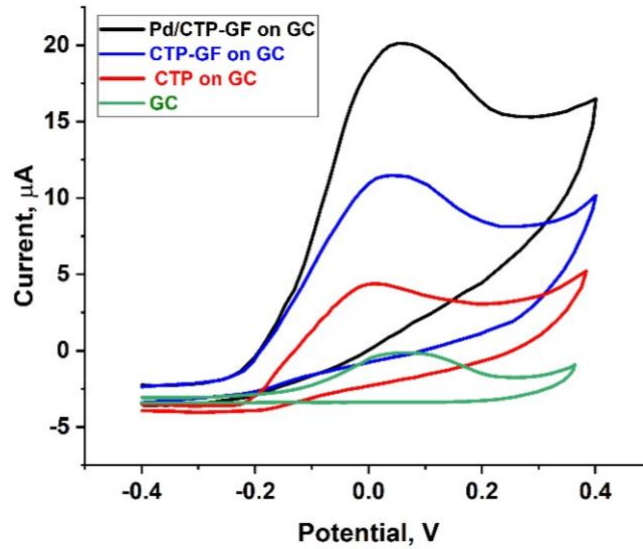
Figure 4. CVs of 0.05 mM AA in 0.1 M PBS on bare GC, CTP, CTP-GF, and Pd/CTP-GF on GC

As shown in Figure 4, the highest peak current for the oxidation of ascorbic acid (AA) was obtained at 13.254 µA on the Pd/CTP-GF modified glassy carbon (GC) electrode. The peak currents for AA were measured at 8.546 µA on the CTP-GF modified GC surface, 3.246 µA on the CTP modified GC surface, and 2.09 µA on the bare GC surface. In order to enable the voltammetric determination of ascorbic acid (AA), an investigation of peak current as a function of scan rate was conducted during the oxidation scan to evaluate whether the electron transfer process is diffusion-controlled. Cyclic voltammetry measurements of 0.05 M AA were performed on the Pd/CTP-GF modified glassy carbon (GC) electrode across a range of scan rates: 25, 40, 60, 150, and 250 mV/s (Figure 5).
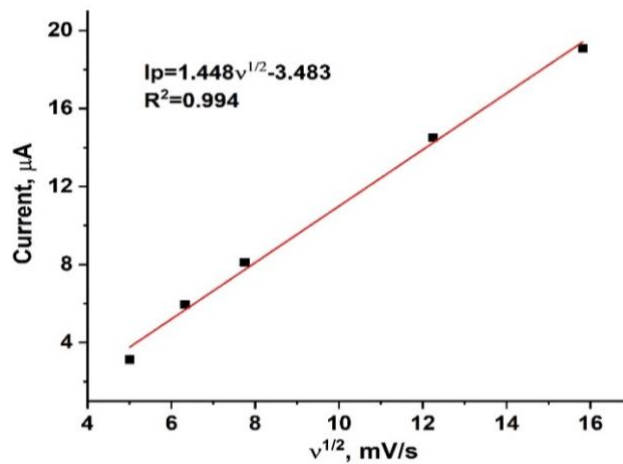

Figure 5. Ip vs. $\nu^{1/2}$ plot for 0.05 mM AA on the Pd/CTP-GF modified GC electrode at various scan rates.

Figure 2 shows a linear plot of Ip and the $\nu^{1/2}$. This suggests that the Randles-Sevcik equation is applicable, implying that the voltammetric studies are diffusion-controlled [18].

3.2. Development of an Analytical Method for AA

DPVs of ascorbic acid (AA) at concentrations of 0.01 -1.0 µM in 100 mM PBS on the surface of the Pd/CTP-GF modified GC are presented in Figure 6. The calibration curve for AA is shown in Figure 7.
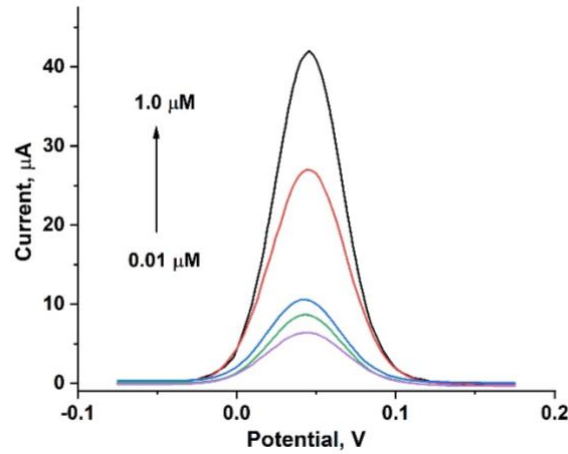
Figure 6. Differential pulse voltammogram of ascorbic acid (AA) in the concentration range of 0.01 to 1.0 µM.
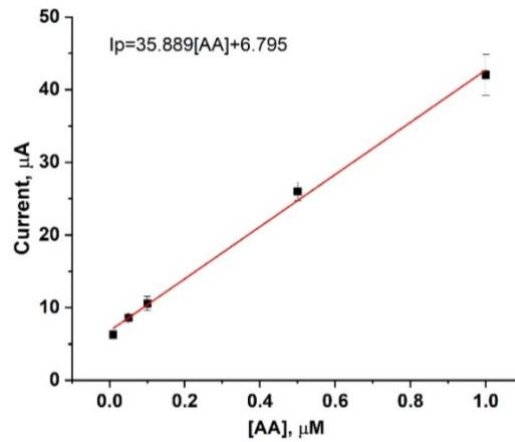

Figure 7. Calibration curve of peak current versus concentration obtained using the DPV technique on the surface of the Pd/CTP-GF modified glassy carbon (GC) electrode in the concentration range of 0.01 to 1.0 µM.

The analytical parameters obtained from the differential pulse voltammetry (DPV) measurements and calibration curve of the developed method are presented in Table 1. As shown in the table, the limit of detection (LOD) for the developed method was found to be 0.005 µM (5 nM).

Table 1. Analytical results of the developed AA determination method on the surface of the Pd/CTP-GF modified glassy carbon (GC) electrode.

Analytical Parameters	Results
Linear Concentration Range	0.01–1.0 µM (1.76 ng/mL–176.12 ng/mL)
Linear Equation, Ip (µA), [AA] (µM)	Ip=35.889[AA]+6.795
R^2	0.996
LOD, (S/N=3)	0.005 µM (0.88 ng/mL)

*Molar Mass of AA: 176.12 g/mol

3.3. The Accuracy And Precision

The accuracy and precision values of the method were determined by making intraday and interday measurements for five days. In Table 2, Due to RSD being below 5%, the precision of the method has been assessed as high. Below ± 5% RE, and below 5% RSD results show the precision of the method has been evaluated as high.. (**Table 2**).

Table 2. Precision and accuracy values of the developed method for intra-day and five consecutive days (n = 5 measurements).

Analysis	Added, µM	Found, µM	Precision (RSD), %	Relative Error, %
Inter-day	0.5 µM	0.497±0.012	2.41	-0.6
Intra-day*	0.5 µM	0.493 ±0.022	4.46	-1.4

3.4. Interference Study

In the developed method, the interference effects of potential species such as dopamine (DA), uric acid (UA), and nitrite (NO_2^-), which could affect the accurate determination of ascorbic acid (AA), were investigated. To evaluate this, the effects of 1 µM concentrations of these interferents on the voltammetric response of 1 µM AA were thoroughly examined. The results showed that the impact of 1 µM DA, UA, and NO_2^- on the peak current variation of AA was negligible, with changes remaining below 5%. The peak current variations for AA in differential pulse voltammetry (DPV) measurements are provided in Table 3. These findings indicate that these interferents have little to no significant effect on AA detection, thereby confirming the high selectivity and reliability of the developed method. This level of selectivity is essential for ensuring accurate AA determination, even in complex samples containing common biological and environmental interferents.

Table 3. Effect of 1 µM DA, UA, and NO2- on the DPV peak current of 1 µM AA.

Potential interfering species	Current variation, %
DA	-2.71
UA	1.11
NO_2^-	-0.35

3.5. Real Sample Analysis

The real sample tests were conducted to evaluate the developed method for the determination of ascorbic acid (AA) using a Pd/CTP-GF modified GC electrode, utilizing orange juice samples. The samples were diluted for analysis. Table 4 presents the concentrations of AA in 100 mL of orange juice. It was observed that the recovery values obtained by adding 10 mg of AA per 100 mL sample were quite satisfactory, ranging from 95% to 105%, indicating high recovery efficiency.

Table 4. Determination of AA* in real samples and recovery values.

Numune	Added, mg/100 mL	Found ± SD, mg/100 mL	Recovery, %
Orange juice	0	21.87±0.96	-
	10	32.11±1.12	102.4
Carbonated orange drink	0	10.05±0.27	-
	10	20.17±0.34	101.2

*Molar Mass of AA: 176.12 g/mol

4. Conclusion

A sensor has been developed for the determination of AA, a powerful antioxidant that plays a crucial role in human health. In this study, a Pd/CTP-GF on GC electrode was successfully fabricated and characterized using SEM, EDX, and CV. This electrode enabled the development of a highly sensitive voltammetric method for AA detection, with a broad working range of 0.01 µM to 1.0 µM and an impressive detection limit of 5 nM. Comparative analysis (Table 5) highlights the superior sensitivity of this method, which outperforms many existing DPV-based techniques by offering a detection limit in the nanomolar range. Additionally, the method demonstrated excellent recovery rates for AA in real samples, such as orange juice and carbonated orange drink, confirming its precision and practical applicability. These findings emphasize the potential of this method as a reliable tool for AA analysis in clinical, pharmaceutical, and food quality control applications.

Table 5. Comparison of some AA determination methods developed using the DPV technique in the literature.

Elektrode	Technique	Linear range (µM)	LOD	Reference
CQDs-rGO/GCE	DPV	70–1000 µM	3.33 µM	[14]
GCE/T-rGO	DPV	4.0-100.0 mM	0.61 µM	[19]
PdNPs-GO/GCE	DPV	20 µM to 2.28 mM	23 µM	[12]
Pd/CNF-CPE	DPV	0.05–4 mM	15 µM	[20]
PdNPs/CM/CPE	DPV	3.27–40.51	0.53 µM	[21]
CdO-SPCE	DPV	5-150 µM	53.5 nM	[22]
Pd/CTP-GF	DPV	0.01-1.0 µM	5 nM	This Work

6. References

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