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Rapid and Simple Electrochemical Detection of Pterostilbene in Food Supplements Using a Carbon Paste Electrode

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ABSTRACT

Pterostilbene (PTS) is a naturally occurring polyphenol, known as the 3,5-dimethoxy derivative of resveratrol, that occurs in several plant sources such as Pterocarpus marsupium and blueberries, and is recognized for its wide-ranging pharmacological benefits. In this study, an electrochemical sensing platform employing a carbon paste electrode was developed for the sensing of PTS using square-wave voltammetry. Cyclic voltammetry experiments performed in 0.1 M H_2SO_4 revealed that PTS undergoes an irreversible, characterized by one well-defined anodic peak at approximately +0.89 V vs. Ag/AgCl. The oxidation behavior was found to be influenced by both the pH of the medium and the nature of the supporting electrolyte, indicating possible proton-coupled electron transfer mechanisms. Quantitative analysis relied on the most intense anodic peak, observed near +0.79 V under optimized experimental conditions. In an acidic environment, the calibration plot showed strong linear behavior across concentrations from 0.10 to 15.0 µg mL^{-1}, achieving a detection limit of 0.027 µg mL^{-1}. The proposed method demonstrated high sensitivity and selectivity, enabling accurate determination of PTS in commercial food supplement formulations, highlighting its potential for routine quality control in nutraceutical analysis.

Keywords: Carbon paste electrode, Detection limit, Food supplements, Pterostilbene, Square-wave voltammetry

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Introduction

Pterostilbene (PTS), a natural polyphenolic compound and 3,5-dimethoxy analogue of resveratrol, is found in various plants—particularly *Pterocarpus marsupium* and blueberries—and is valued for its broad therapeutic potential [1,2]. Compared to resveratrol, PTS exhibits greater lipophilicity due to the presence of two extra methoxy groups on its aromatic ring. This structural modification not only enhances its cellular uptake but also contributes to improved oral bioavailability and an extended half-life in the body. As a result, PTS demonstrates stronger and more sustained antioxidant activity, effectively scavenging free radicals, protecting cellular components from oxidative damage, and reducing lipid peroxidation. These properties make PTS highly valuable in the food and nutraceutical industries, where it is utilized not only as a natural antioxidant and preservative but also as a functional ingredient to promote health and prevent oxidative deterioration in food products [3-5]. PTS demonstrates diverse health-promoting effects, including antioxidant, anti-inflammatory, anticancer, antidiabetic, cardioprotective, neuroprotective, lipid-lowering, and anti-aging properties, acting through multiple mechanisms such as AMP-activated protein kinase (AMPK) activation and reactive oxygen species (ROS)-mediated apoptosis in cancer cells [6-8]. Owing to these benefits and its role in managing conditions like diabetes, hypertension, and neurodegenerative diseases, reliable and cost-effective methods for its qualitative and quantitative detection in dietary supplements, herbal formulations, and biological samples are essential for quality control and further research.

Chromatographic and spectrophotometric techniques were widely employed for determining and/or assessing the antioxidant capacity of PTS in beverages and food products, as well as in pharmaceutical preparations [9-10]. These analytical approaches were also applied in preclinical research [11] to investigate the bioavailability and tissue distribution of PTS in specific animal models [12], and to study its biochemical kinetic behavior [13]. In contrast, electrochemical analysis gained attention as a practical alternative for straightforward drug testing, offering benefits such as low equipment costs, rapid analysis, minimal sample preparation, and ease of use. Additionally, these methods were valued for their accuracy, good sensitivity, precision, and generally satisfactory selectivity in drug detection [14,15]. Polyphenolic compounds represent one of the most abundant and structurally diverse classes of phytochemical metabolites. The major subclasses include stilbenes, phenolic acids, and flavonoids. Among them, PTS belongs to the stilbene family [16]. Voltammetric techniques are widely employed for the electrochemical determination of polyphenolic compounds due to their capability to provide sensitive and reproducible responses. This approach has been successfully applied to

various stilbene derivatives, such as resveratrol [17,18], polydatin [19], and piceatannol [20], demonstrating its reliability in characterizing and quantifying structurally related compounds like PTS. To date, only two electrochemical strategies have been reported for the quantification of PTS. In one work, its electrochemical characteristics and quantification were explored by monitoring the oxidation peak of PTS on a boron-doped diamond (BDD) electrode in the existence of a cationic surfactant, with the procedure validated through direct testing of commercial dietary supplements [21]. Another investigation employed a screen-printed diamond electrode (SPDE) and utilized Britton–Robinson buffer at pH 9.0 for the analysis of PTS in dietary supplement samples [22].

Carbon paste electrodes (CPEs) were versatile electrochemical sensors prepared by mixing conductive carbon powders, such as graphite, with a suitable binder like mineral oil or organic paste to form a malleable composite packed into an electrode holder with a metallic contact. They were valued for their low cost, ease of preparation, renewability of the sensing surface, and adaptability, as modifiers including nanoparticles, enzymes, or ion-selective agents could be incorporated directly into the paste to enhance selectivity and sensitivity. CPEs exhibited a broad potential window, low background current, and compatibility with various voltammetric and amperometric techniques, making them suitable for detecting pharmaceuticals, antioxidants, heavy metals, and other environmental or biological analytes. However, their performance could vary depending on the paste composition and preparation method, requiring careful optimization for consistent analytical results [23-25].

Given the limited number of voltammetric investigations on PTS, this study aims to develop and evaluate an electrochemical method based on an unmodified CPE as a simple, economical, and reliable sensing platform. The research focuses on optimizing experimental parameters to achieve a sensitive and well-defined oxidation response of PTS using square-wave voltammetry (SWV). Furthermore, the applicability and analytical performance of the proposed method are demonstrated through the determination of PTS in commercially available dietary supplement formulations, highlighting its potential for practical analytical applications.

Materials and Methods

Analytical-grade reagents were used throughout the study. Graphite powder and mineral oil were purchased from Sigma-Aldrich, while acids, potassium salts, and potassium ferricyanide ($K_3[Fe(CN)_6]$) were obtained from Merck. A 1.0 mg mL^{-1} standard solution of PTS was prepared in ethanol, stored at +4 °C, and protected from light when not in use. Supporting electrolytes and buffer solutions were prepared with ultrapure water (Millipore Milli-Q). Detailed preparation procedures were provided in the Supplementary Information.

Electrochemical measurements were performed using an Autolab PGSTAT 101 system equipped with NOVA software. A three-electrode configuration was employed, consisting of a carbon paste electrode (CPE) as the working electrode, an Ag/AgCl (3 M NaCl) reference electrode, and a platinum wire counter electrode. The CPE was prepared by mixing graphite powder and paraffin oil (70:30, w/w) and packing the mixture into a Teflon tube (3.0 mm i.d.). Further fabrication details were available in the Supplementary Information.

Cyclic voltammetry (CV) was initially used to investigate the electrochemical behavior of PTS at the CPE. Subsequently, square-wave voltammetry (SWV) was applied for quantitative determination, optimizing parameters such as supporting electrolyte, pulse amplitude, and frequency to enhance sensitivity. Measurements were conducted in 0.1 M H_2SO_4 within a potential range of 0 to +1.0 V, using optimized SWV settings (6 mV step potential, 40 mV pulse amplitude, and 75 Hz frequency).

The developed method was applied to commercial dietary supplements labeled to contain 100 mg PTS per capsule. The capsule contents were homogenized, dissolved in ethanol, and sonicated prior to dilution with 0.1 M H_2SO_4. Quantification was carried out using the standard addition method (1.0–10 µg mL^{-1}), and all measurements were performed in triplicate. Full sample preparation steps are detailed in the Supplementary Information.

Results and Discussion

Electrochemical Behavior of PTS

To elucidate the redox behavior of PTS on the CPE, two successive CV measurements of 25 µg mL^{-1} PTS were performed in a 0.1 M H_2SO_4 solution (as subsequently shown, this medium is appropriate for analytical applications). The CV that excluded PTS was also illustrated in the graphs for comparison purposes. Figure 1A was illustrated that in the initial cycle, PTS exhibited one oxidation peak at around +0.89 V relative to Ag/AgCl. It was concluded that the PTS electrode reactions on the CPE were completely irreversible since there was no reduction peak in the reversed scan. Through the recording of consecutive CVs, a reduction in the anodic peak current was observed, potentially due to the deactivation of the electrode surface, possibly from fouling or the adsorption of oxidation products of PTS specifically on the CPE surfaces. The experiment using CV in a 0.1 M H_2SO_4 solution to determine how the scan rate affected PTS oxidation at the CPE. The oxidation peak potentials showed a small movement towards higher positive values as the scan rate was increased.

A linear correlation between the oxidation peak currents and the scan rate (v) was observed within the 100-600 mV s-1 range (n=6). It was articulated using the equations presented below;

I_p (µA) = 0.009 v (mV s^{-1}) + 0.696, r = 0.993 (1)

Also, a linear correlation between the oxidation peak currents and the square root of the scan rate ($v^{1/2}$) was observed within the 100-600 mV s^{-1} range (n=6). It could be articulated using the equations presented below;

I_p (µA) = 0.325 $v^{1/2}$ (mV s^{-1}) - 1.913, r = 0.996 (2)

A linear relationship was identified among the logarithm of peak current (log I_p) and the logarithm of the scanning rate (log v) for both peaks, expressed in the following equations:

log I_p (µA) = 0.826 log v (mV s^{-1}) - 0.149, r = 0.994 (3)

The obtained slope (~0.83) falls between the theoretical limits of 1.0 and 0.5, which correspond to adsorption- and diffusion-governed processes. Based on the earlier equations, it was inferred that the electrooxidation of PTS on the CPE was likely influenced by a combination of both mechanisms. Similar findings were documented in the voltammetric analysis of several compounds using different carbon-based electrodes [27,28].

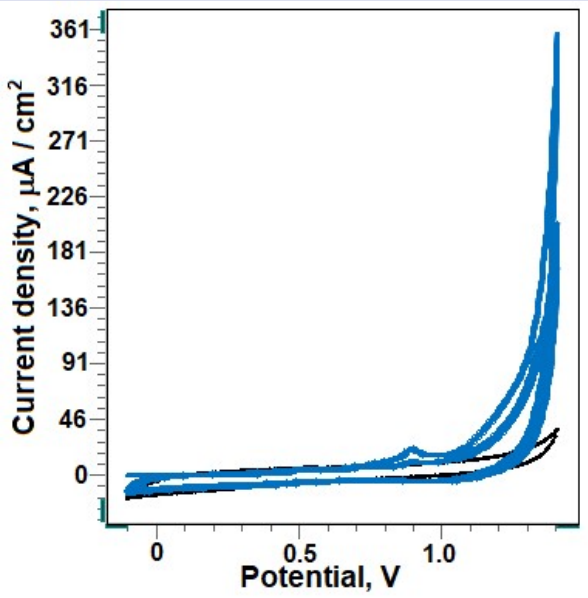


Figure 1. Cyclic voltammograms were recorded two times at 100 mV s^{-1} for 25 µg mL^{-1} PTS at CPE surface in 0.1 M H_2SO_4. The background current was depicted using black lines.

The electrochemical active surface area of the CPE was determined using CV in a 0.1 M KCl solution containing 1.0 mM $K_3[Fe(CN)_6]$. Measurements were performed over a scan rate range of 10–300 mV s^{-1}, and the recorded CVs were shown in the supplementary information (Figure S1). The electroactive surface area was calculated from the slope of the peak current (I_p) versus the square root of the scan rate ($v^{1/2}$) plot, based on the Randles–Ševčík equation:

$I_p = (2.69 \times 10^5)\ n^{3/2}\ A\ D^{1/2}\ C\ v^{1/2}$ (4)

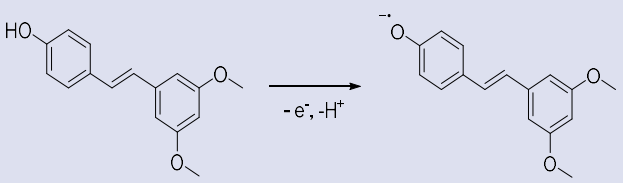
where I_p is the anodic peak current (A), n is the number of electrons transferred (n = 1), A is the electroactive surface area (cm^2), D is the diffusion coefficient (7.6×10^{-6} cm^2/s), C is the concentration of the redox probe (M), and v is the scan rate (V/s). The calculated electroactive surface area of the CPE was 0.11 cm^2.

Influence of Supporting Electrolyte Type and pH

By manipulating the pH and utilizing a range of supporting electrolytes, SWV investigated how pH affected the potentials and currents of PTS at the anodic peak. The pH influence of only this anodic peak potentials for the 15 µg mL^{-1} PTS compound was evaluated, as well as the results were shown in Figure 2A-B inside the pH range of 2.0-10.0 in 0.04 M BR buffer via potentials range from 0 to +1.0 V. The figure showed that PTS had one well-defined anodic peak on the working pH range. The data indicated that the peak potentials of PTS decreased with increasing pH, suggesting the participation of protons in the reaction of PTS at the CPE. Such behavior was in good agreement with previous electrochemical studies reported for PTS using BDD and SPD electrodes [21–22], where similar linear dependencies between oxidation potential and pH were observed. It was observed that the peak potentials exhibited a linear behavior in the pH range of 2.0 to 10.0, and were represented by the following equations:

E_{PA} (V) = -0.054 pH + 0.795 r=0.993 (5)

The PTS oxidation at the CPE was a pH-dependent process, as shown by the slopes of these equations. The slope of 0.054 V/pH, being proximate to the theoretical value of 0.059 V, denoted that an equivalent number of electrons and protons participated in the electrode reaction. The observed oxidative peak corresponds to the oxidation of the hydroxyl group in the PTS molecule, involving a one-electron/one-proton transfer to generate a free radical intermediate [17]. The proposed reaction pathway for this step was illustrated in Scheme 1.



Scheme 1. The mechanism of the redox reaction involving PTS at the CPE.

SW voltammograms of identical amounts of PTS were recorded in various electrolytes and were shown in Figure 2B. Using 0.1 M H_2SO_4, ABS (pH 4.7), and PBS at pH 2.5 and 7.4, anodic peak potentials of 0.78 V, 0.68 V, 0.76 and 0.49 V, respectively, were acquired. The respective anodic peak currents were 3.04 µA, 0.91 µA, 1.89 µA, and 0.71 µA.

Among these, the strongest signals at both anodic peaks were observed in 0.1 M H_2SO_4. Consequently, further analytical investigations were carried out using this medium.

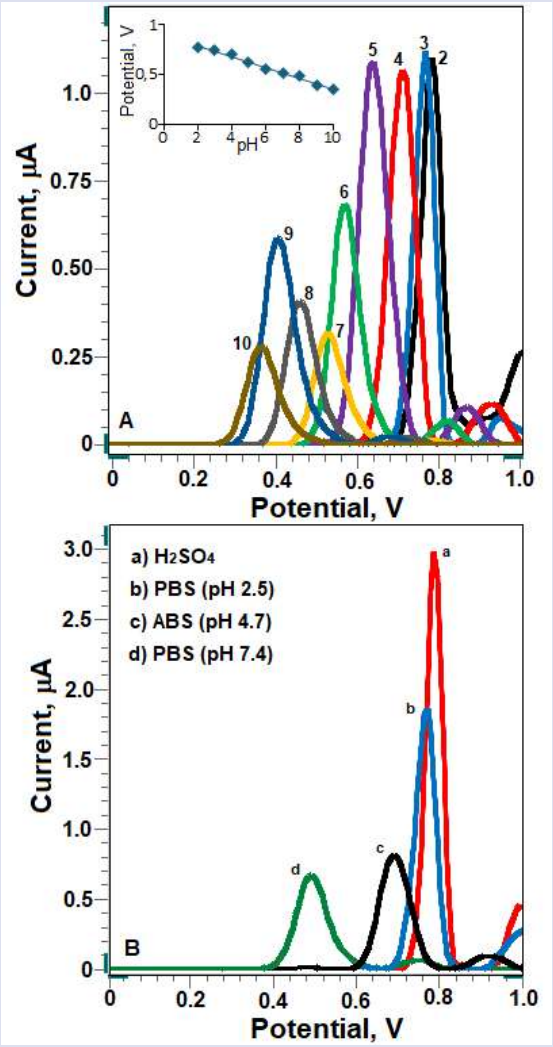


Figure 2. SW voltammograms for 15 µg mL^{-1} PTS were obtained on the CPE in BR buffer over pH 2.0–10.0 (A) and in different supporting electrolytes covering various pH ranges (B). SWV variables incorporate the subsequent: *f*, 50 Hz; ΔE_s, 6 mV; ΔE_{sw}, 30 mV. The graph (inset of A) depicts the correlation between the peak oxidation potentials of PTS and pH levels.

The Effect of SWV Variables

To obtain the highest, most distinct, and well-shaped peaks, the effects of key SWV parameters—frequency (*f*), scan increment (ΔE_s), and pulse amplitude (ΔE_{sw}) —were examined for 15 µg mL^{-1} PTS in 0.1 M H_2SO_4. The influence of the relevant variables was examined using a single-point optimization approach. However, certain other variables did not change when the analyzed variable did. Hence, PTS oxidation peak currents were tested to determine the overall effect of SWV factors. When the ΔE_s and ΔE_{sw} were stable at 6 and 30 mV, correspondingly, the *f* values were computed between 25 and 125 Hz. Raising any one of these factors resulted in an increase to the peak current height. Using *f* values greater than 75 Hz, however, causes their forms to become noticeably longer. This phenomenon has the potential to decrease analytical selectivity. Ultimately, it was determined that 75 Hz was the ideal *f* value. The influence of ΔE_{sw}, and other SWV potential factors (staying SWV variables: ΔE_s= 6 mV, *f* = 75 Hz), was deliberate by altering it from 30 to 80 mV. At this stage, an enhanced pulse amplitude was applied to promptly boost the PTS anodic peak current. However, subsequently 40 mV, the peak form grew. Consequently, 40 mV was chosen as the ideal ΔE_{sw} value. When the final device parameters, ΔE_{sw} & *f*, were maintained at 40 mV and 75 Hz, in that order, ΔE_s was examined at levels between 2 and 16 mV. Raising ΔE_s caused the SWV signals to increase in amplitude by as much as 6 mV. There was a consistent increase in oxidation signals after 6 mV, though. It is chosen as the optimal ΔEs value since the peak shapes subsequently 6 mV were expanded at the same time. Thus, 75 Hz *f*, 6 mV Hz ΔE_s, and 40 mV ΔE_{sw} were shown to yield the greatest and most stable anodic peak currents, which were then used for additional research.

Analytical Performance

The analytical efficiency was tested by analyzing the anodic peak current as a function of PTS concentrations after the chemical parameters and experimental variables had been optimized. Figure 3 presented the calibration curves for the oxidation peak of PTS, and Table 1 summarized the relevant analytical parameters.

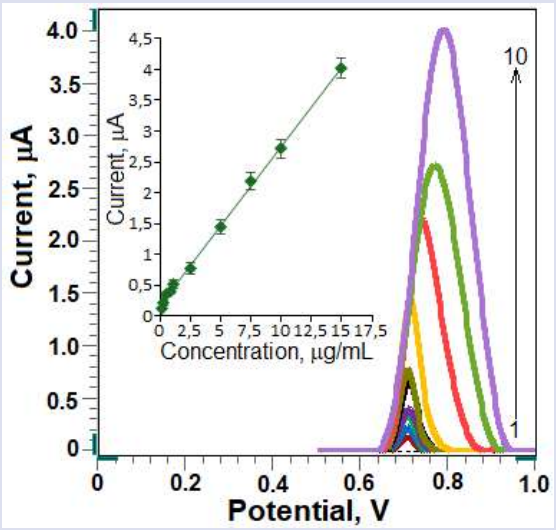


Figure 3. SW voltammograms for the PTS concentrations of 0.1, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 7.5, 10, and 15 µg mL^{-1} (1-10) for the oxidation peaks in H_2SO_4 solution. In the inset of Figure 3 you can see the PTS quantitation calibration curve. The variables that make up SWV are as follows: *f*, 75 Hz; ΔE_s, 6 mV; ΔE_{sw}, 40 mV.

Table 1. Analytical characteristics acquired for the oxidation peak of PTS utilizing square wave voltammetry on the CPE.

Analytical parameter	
E_p	+0.79
LWR	0.1-15 µg mL^{-1}
LRE	I_p (µA) = 0.2570 ± 0.008 C (µg mL^{-1}) + 0.181 ±0.006
r	0.998
LOD	0.02700 ± 0.0009 µg mL^{-1}
LOQ	0.09000 ± 0.0030 µg mL^{-1}
Intra-day repeatability (RSD%, n=10)	4.2
Inter-day repeatability (RSD%, n=3)	6.1

E_p=peak voltage; LWR=linear working range; LRE=linear regression equation; r=correlation coefficient; LOD=detection limit; LOQ, quantification limit

Based on what we are aware, no prior research has employed CPE for the detection of PTS. Compared to previous reports, our findings using a showed greater sensitivity, achieving a detection limit of 0.027 µg mL^{-1} (Table 2). Both previous studies employed commercial diamond-based electrodes, which were relatively costly. In contrast, the present study utilized a low-cost alternative, the CPE, and—similar to earlier work—successfully applied the method to PTS determination in food supplement products.

Table 2. Analyzing the established method in relation to other electrochemical investigations that have been published.

Electrode	Media	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	Sample	Reference
BDDE	HNO_3 with CTAB	0.005 – 1.0	0.0011	Dietary supplements	[21]
SPDE	BR buffer (pH 9)	0.003 – 0.233	0.0008	Dietary supplements	[22]
CPE	H_2SO_4	0.1 – 15.0	0.027	Dietary supplements	This study

BDDE; Boron-doped diamond electrode, SPDE; Screen–printed diamond electrode, CPE; carbon paste electrode, CTAB; cetyltrimethylammonium bromide, BR; Briton–Robinson.

Effect of Potential İnterfering Substances

By studying the impact of different interfering chemicals often found in dietary supplements under ideal experimental conditions, the selectivity of the suggested method was evaluated. This was done for 1 µg mL^{-1} PTS. The tolerance limit was defined as the maximum concentration of interfering substances that caused no more than a 5% deviation in the PTS anodic peak current. Common inorganic ions, including sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), and iron (Fe^{3+}), were tested at five-fold higher concentrations and were found to have no significant effect on the anodic peak current. Similarly, typical sugars such as fructose, lactose, glucose, and maltose did not interfere with the PTS signal, even at five-fold excess levels. In addition, common excipients found in dietary supplements, including starch, magnesium stearate, and cellulose, produced negligible changes in the anodic current under the same conditions. The effects of these compounds on the PTS oxidation response were summarized in Table 3.

Table 3. The impact of possible interferences on the present response of the oxidation signal of PTS.

Interference	Concentration ratios (PTS: interference)	The current change (%)
Sodium (Na^+)	1:5	< 4
Potassium (K^+)	1:5	< 4
Magnesium (Mg^{2+})	1:5	< 5
Calcium (Ca^{2+})	1:5	< 5
Iron (Fe^{3+})	1:5	< 5
Fructose	1:5	< 6
Lactose	1:5	< 6
Glucose	1:5	< 4
Maltose	1:5	< 6
Starch	1:5	< 3
Magnesium stearate	1:5	< 4
Cellulose	1:5	< 5

Evaluation of the Proposed Method's Applicability

To evaluate the feasibility of the methodology, the suggested electroanalytical method was used to identify PTS in samples of food additives using the standard addition technique. In the Materials and Methods, the procedures for sample preparation and measurement execution were detailed. Methods such as evaporation, filtering, or sample material extraction were not employed in the evaluation of the samples. Figure 4 displayed the sample's SW voltammograms together with the standards that were used to analyze it. In conjunction with the graphical analysis [I_p= 0.201 C (µg mL^{-1}) + 0.958 (r = 0.998)]. The results showed that there was a reasonable agreement between the label value of 100 mg per capsule and the actual contents of 95.3 mg per capsule (RSD of 4.1%) when the oxidation signal of the PTS was used for the evaluation. Considering the several dilutions of the material led to this conclusion. To evaluate the accuracy of the proposed method, a recovery test was performed by spiking the previously analyzed sample solution in the electrochemical cell with known amounts of PTS standard (final concentrations: 1.0, 2.5, and 5.0 µg mL^{-1}). The method was subsequently applied for quantification, yielding recovery values between 97.1% and 105.9% across three replicate measurements at each concentration. These results indicate minimal matrix interference in the dietary supplement formulation, as evidenced by the high recovery rates.

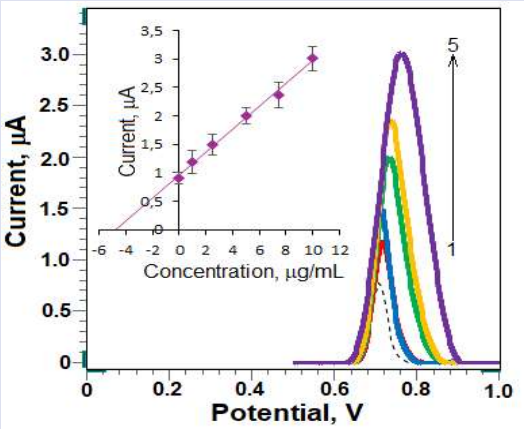


Figure 4. SW voltammograms by diluting the samples. Dashed lines illustrate the diluted sample (1-5) after standard additions of 1.0, 2.5, 5.0, 7.5, and 10 µg mL^{-1} PTS in H_2SO_4 solution on the surface of the CPE. The evaluation results of the standard addition technique are illustrated in the inset. Figure 3 illustrates further operational parameters.

Conclusions

The present study introduces a simple, cost-effective, and environmentally friendly electrochemical approach for the quantification of PTS using an unmodified CPE. Under optimized square-wave voltammetric conditions in 0.1 M H_2SO_4, PTS exhibited an irreversible oxidation peak at approximately +0.79 V (vs. Ag/AgCl). The developed method provided a linear response over the concentration range of 0.10–15.0 µg mL^{-1}, with a detection limit of 0.027 µg mL^{-1}, demonstrating excellent sensitivity for PTS determination. Compared with previously reported electrochemical studies employing BDD and SPD electrodes, the proposed CPE-based platform achieves comparable or even superior analytical performance while significantly reducing electrode cost and preparation complexity. Unlike diamond-based electrodes, which require specialized fabrication and conditioning, the unmodified CPE offers a renewable and easily prepared surface, making it a practical alternative for routine analysis. The developed method was successfully applied to the determination of PTS in commercially available dietary supplements, confirming its applicability to real samples with satisfactory accuracy. Overall, this work expands the current scope of voltammetric studies on PTS and highlights the potential of low-cost carbon-based electrodes as reliable analytical tools for quality control and routine monitoring in nutraceutical and pharmaceutical formulations.

Conflicts of interest

There are no conflicts of interest in this work.

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