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First Electrochemical Monitoring of Bensulide in Aqueous Media via DPV and SWV Using a Glassy Carbon Electrode

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ABSTRACT

This study aims to determine the voltammetric determination of Bensulide (BNS), a pesticide that has critical importance to be monitored due to its toxicological and environmental effects. BNS is classified as an organophosphorus herbicide used primarily in agricultural production, attracting attention due to its residue levels in both human and ecological health contexts. No study was found in the literature on the electrochemical determination of BNS. In this context, this study presents an original research analysis of BNS voltammetrically for the first time. The proposed analytical method was carried out by applying differential pulse voltammetry (DPV) and square wave voltammetry (SWV) techniques on the surface of a bare glassy carbon electrode (GCE). Voltammetric measurements were applied in pH 8.00 phosphate buffer (PBS) medium. In the voltammograms obtained with both techniques, it was observed that BNS gave a well-defined oxidation peak and it was determined that the analytical signals were sensitive to concentration. As a result of standard calibration studies, a wide linearity was obtained in the range of 6.00×10^{-7} – 8.00×10^{-5} M with DPV technique and in the range of 2.00×10^{-6} – 8.00×10^{-5} M with SWV. R^2 values calculated from the calibration curves were found to be 0.995 for DPV and 0.993 for SWV, respectively. Detection and determination limits were calculated using the classical formulas LOD = 3.3σ/m and LOQ = 10σ/m. The LOD values were 1.99×10^{-7} M for DPV and 8.04×10^{-7} M for SWV, while the LOQ values were 6.02×10^{-7} M and 2.44×10^{-6} M, respectively.

Keywords: Electrochemistry, Differential pulse voltammetry, Square wave voltammetry, Glassy carbon electrodes, Bensulide

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Introduction

BNS is known as an organophosphorus compound that is highly preferred in agricultural applications as a selective pre-emergent herbicide [1]. The chemical name of BNS is O-(p-tert-butylphenyl)-O-methylphosphorodiamidothiate, the molecular formula is $C_{14}H_{24}NO_4PS$, and its chemical structure contains amidic and thiophosphate groups attached to a phosphorus atom [1]. The phosphorus atom, positioned at the center of the chemical structure of BNS, is characterized by a methoxy (-OCH_3) group, an amidic (–NH_2) structure, and a p-tert-butylphenyl ring connected by a thiophosphate bridge [1]. BNS is widely preferred, especially in vegetable farming, and to protect lawn areas from weeds [2]. On the other hand, when the behavior of BNS in the soil ecosystem is examined, it is known that it exhibits a medium level of persistence in the soil and is slowly degraded by microbial activities [1]. Additionally, in cases of unconscious or excessive use, it mixes with groundwater, causing contamination. Therefore, the dosage and timing of use are crucial in terms of agricultural environmental management. The intake of BNS into the human body through food consumption is a critical issue that should be carefully evaluated in terms of potential health risks. Although BNS is classified as a selective herbicide with low acute toxicity, organophosphorus compounds have adverse effects on human health due to their common structural features [3]. Organophosphates damage nervous system functions by inhibiting the enzyme acetylcholinesterase [4]. BNS also shows this effect on the central and peripheral nervous system in high doses or cases of chronic exposure. Considering the potential effects of herbicides such as BNS, which are widely used in agriculture and turf areas, on human health and the environment, it is essential to monitor these compounds in food, soil, and water resources [5]. In particular, BNS residues, which can enter the human system through foodstuffs, pose a risk of causing toxic, genotoxic, and neurological effects in the long term. Therefore, it is imperative to develop and implement sensitive and reliable detection techniques. It is a basic requirement for the protection of human health that BNS is below the maximum residue limits (MRL) [6]. Therefore, residue determinations made with sensitive analysis techniques both ensure consumer safety and provide the opportunity to control agricultural practices. Sensitive detection of BNS is not only limited to food safety but also crucial for environmental sustainability [7]. BNS accumulated in soil and surface water can cause toxic effects on ecosystem components. Therefore, it is vital to be able to detect BNS at low concentrations within the scope of environmental monitoring studies. Electrochemical detection techniques, particularly voltammetric methods, offer significant advantages in pesticide and herbicide detection. With these techniques, it is possible to reach very low detection limits and to determine trace amounts of residues in environmental and food samples. With its high sensitivity and

selectivity, it facilitates the easy distinction between pesticides and other compounds in complex matrices. For instance, DPV [8], SWV [9], and anodic stripping voltammetry [10] allow accurate and reproducible determination of targets even at very low concentrations. In addition, electrochemical techniques require less equipment compared to classical chromatographic techniques, sample preparation is simpler, and analysis time is considerably shortened. Thanks to these advantages, on-site analysis can be performed using portable sensor platforms, thereby providing continuous and easier traceability of pesticide residues in the field, water sources, or food chain. Another critical point is the use of conductive and inert electrodes in electrochemical analysis. GC electrodes are frequently preferred in analytical applications due to their high chemical stability, expansive potential working windows, low background currents, and smooth surface morphologies [11].

In this study, the oxidation behavior of BNS on the GC electrode was investigated and its analytical determination was carried out by means of two different voltammetric techniques, DPV and SWV.

Experimental

Chemicals and Reagents

The chemicals preferred in electrochemical experiments and measurements were of analytical purity and were used without any additional purification process. BNS, all solutions and other supporting electrolytes were supplied by Sigma-Aldrich (USA). Ultrapure water (Millipore system) with a resistivity of 18.2 MΩ cm was used in the preparation of all solutions. Commercial human serum was used in real sample studies. Serum was purchased from Sigma-Aldrich. The spike technique was used in sample preparation.

Instrumentation

Voltammetric measurements were performed using an Autolab PGSTAT204N model potentiostat/galvanostat (Metrohm Autolab, Netherlands) and NOVA software. The moving average module was applied to all techniques except CV to ensure good readability of Ip values. A classic three-electrode system was used in all experiments: a glassy carbon electrode (GCE, 3 mm diameter) as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl (3 M KCl) electrode as the reference electrode.

Method

Electrode Preparation

Before each voltammetric measurement, the GC electrode surface was polished with 0.05 µm alumina suspension in a polishing kit and then thoroughly washed with distilled water. The electrode was cleaned in an ultrasonic bath for 5 min in a 1:1 (v/v) mixture of ethanol and distilled water to remove possible contaminants from its surface.

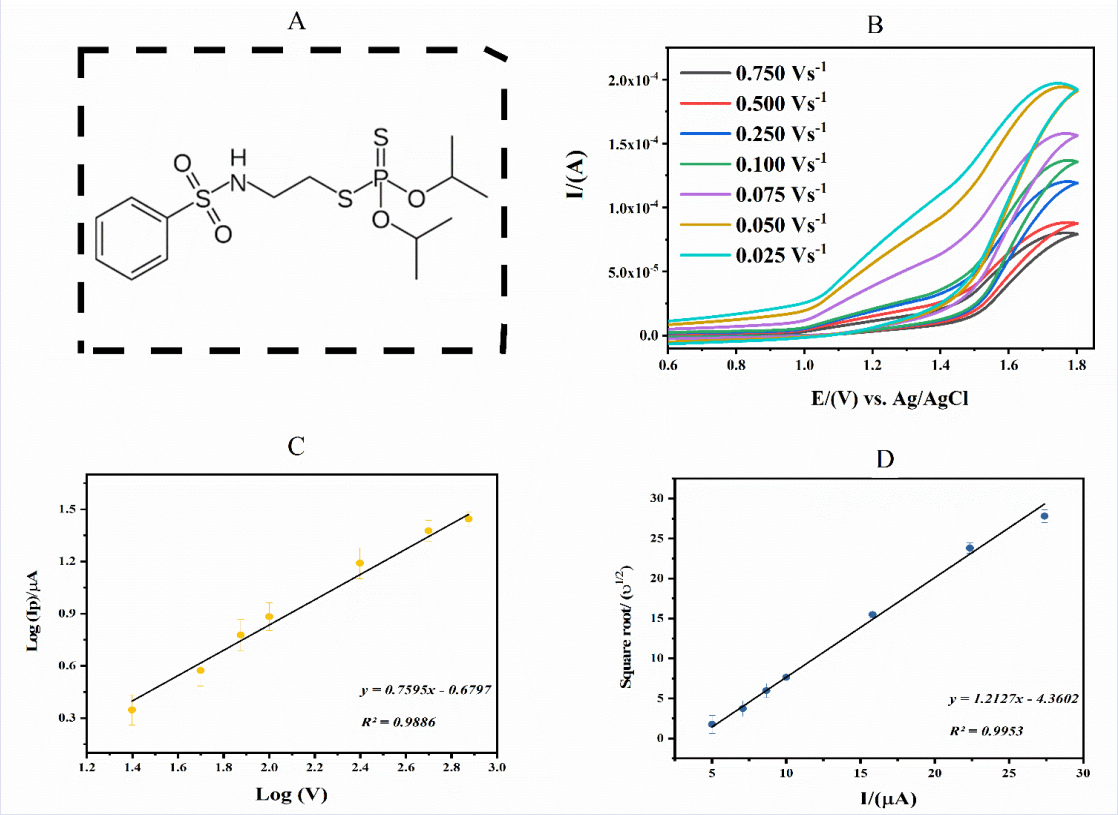

Figure 1. A) Chemical structure of BNS B) CVs performed at different scan rates and pH 8.00 PBS media in presence of 4.00×10^{-4} M BNS (0.025, 0.050, 0.075, 0.100, 0.250, 0.500, and 0.750 V s^{-1}) graph of C) $Log(Ip) - logv$ (R^2=0.9886) D) graph of $Ip - v^{½}$ (R^2=0.9953)

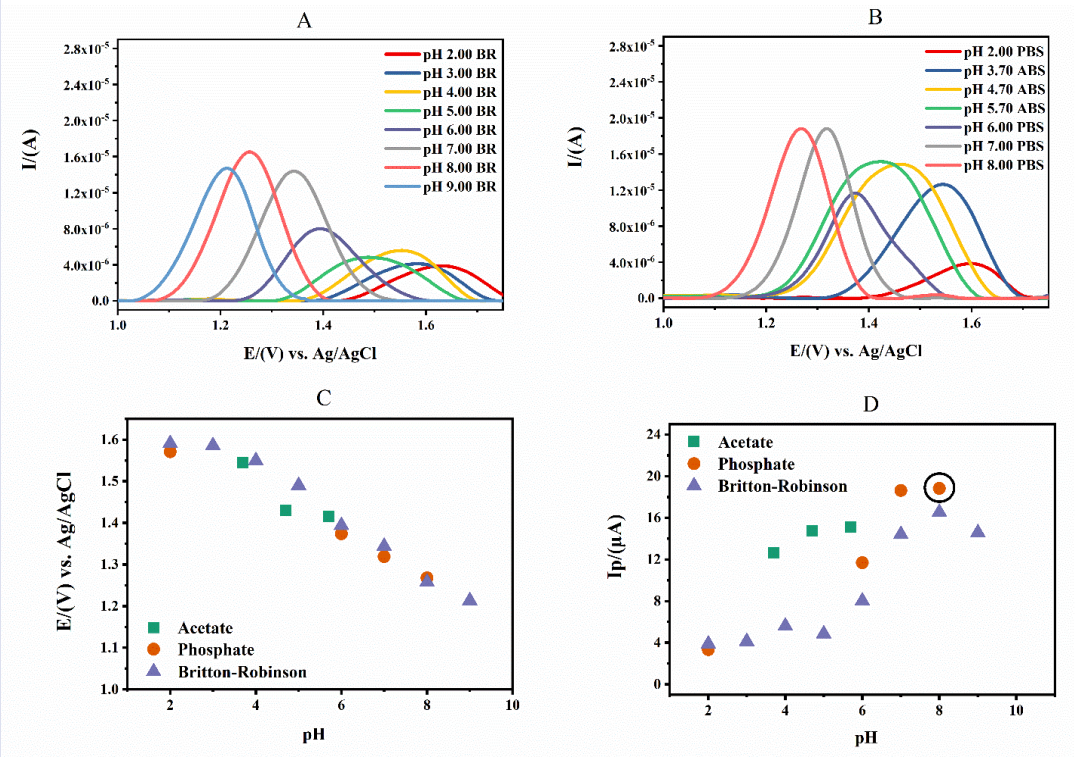

Figure 2. DP voltammograms of 1.00×10^{-4} M BNS on GC electrode surface A) in BR buffer solutions between pH 2.00 and 9.00; B) AB and PB solutions between pH 2.00 and 8.00; C) The graph of Ep-pH; D) The graph of Ip-pH.

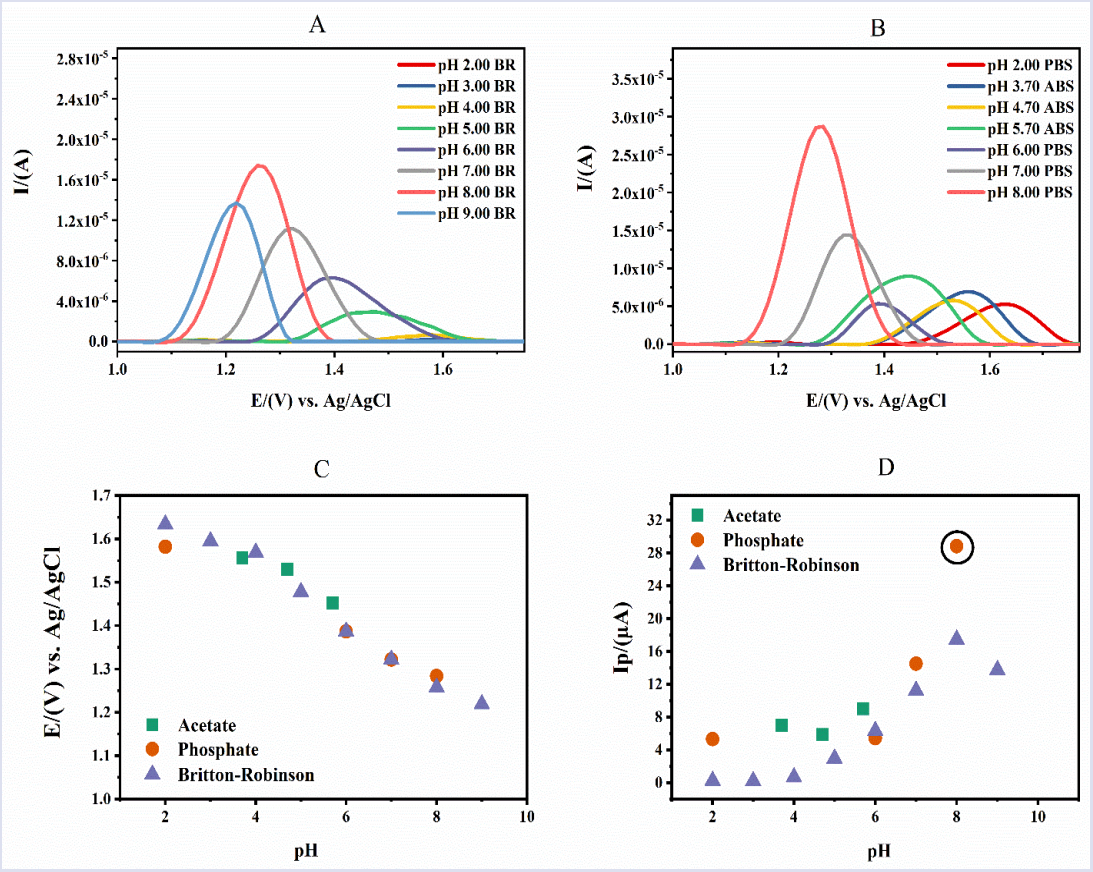

Figure 3. SW voltammograms of 1.00×10^{-4} M BNS on GC electrode surface A) in BR buffer solutions between pH 2.00 and 9.00; B) AB and PB solutions between pH 2.00 and 8.00; C) The graph of Ep-pH; D) The graph of Ip-pH.

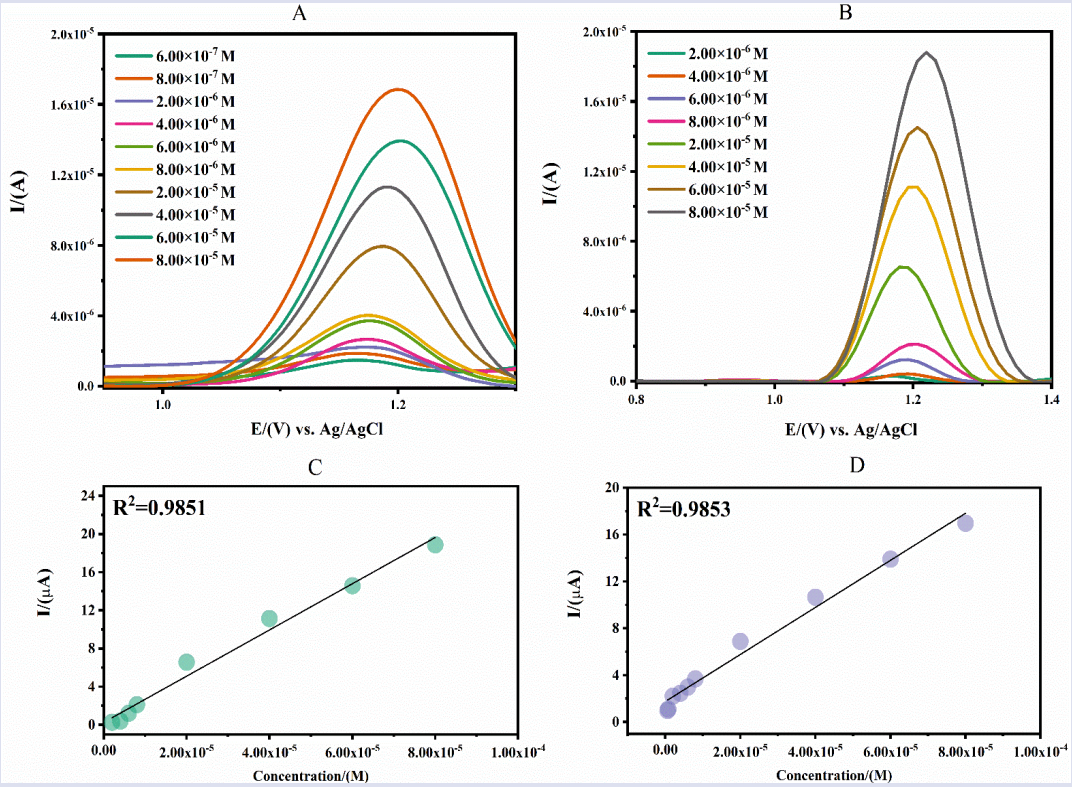

Figure 4. A) DP and B) SW voltammograms of GC electrode sensing performance in various BNS concentrations in pH 8.00 PBS solution (standard calibration curve), standard calibration plots for C) DPV and D) SWV measurement

Results and Discussion

Scan Rate

Fig.1A shows the chemical structure of BNS. The thioether group in the structure of BNS is considered the most probable center in terms of electrochemical oxidation. Since thioethers generally tend to oxidize in the anodic region and mostly transform into sulfoxide (S=O) structures with the transfer of 1 electron and 1 proton [12]. This process is both kinetically fast and occurs through a single-step mechanism, thus yielding clear and distinct voltammetric peaks. On the other hand, the P=S bond in the organophosphate group of the structure can also be oxidized; however, such oxidations require a higher potential and generally occur through multi-step mechanisms that involve unstable intermediate products [13]. For this reason, it is seen as a less preferred center in electrochemical studies. The sulfamide group (–SO_2NH–) in the structure is a functional group with low electroactivity [14]. It is evaluated more with its properties, such as hydrolytic stability or solubility, rather than being electrochemically oxidized. For all these reasons, the 1-electron oxidation process of BNS observed on the GC electrode most likely corresponds to the oxidation of thioether sulfur. Fig. 1B shows the CV curves performed at scan rates of 0.025–0.750 V/s in pH 8.00 phosphate buffer medium. It is observed that Ip value increases as the scan rate increases. This situation suggests that an oxidation reaction occurring on the GC electrode surface is kinetically controlled. Additionally, the positive expansion of the CV curves indicates that the system may exhibit semi-reversible behavior. In Fig. 1C, the relationship between log (Ip) and log (v) is evaluated, and the slope obtained is determined to be approximately 0.7959. When this value is interpreted according to the diffusion control at around 0.5 [15] and the adsorption control at around 1.0 [15], it indicates that the oxidation of BNS occurs with a mixed control mechanism involving both diffusion and adsorption effects. In Fig.1D, the relationship between Ip and $v^{1/2}$ is investigated, and the existence of a linear fit (R^2 = 0.9953) is seen. This high correlation value confirms that the system primarily follows a diffusion-controlled electrochemical process. As a result, this figure reveals that BNS follows a diffusion-dominated, but single-electron oxidation mechanism on the GC electrode surface, as confirmed by scan rate analyses and an adsorptive effect.

pH Scanning

In Fig. 2, the voltammetric behavior of the BNS molecule on the bare GC electrode surface in different buffer solutions and pH ranges is examined in detail. In Fig. 2A, the DPV voltammograms obtained in the pH range of 2.00–9.00 in BR solution show that the peak potential (Ep) shifts to more negative values with increasing pH. Additionally, it is observed that the peak current (Ip) increases significantly in the pH range of 7.00–9.00, with pH 8.00 standing out as the optimum analysis condition. In Fig. 2B, a similar negative shift in Ep values is observed in AB and PB buffer environments, and especially the

highest Ip value obtained at pH 8.00 in phosphate buffer suggests that this buffer system is more suitable for the electrochemical determination of BNS. In the EP–pH graph in Fig. 2C, it is seen that EP decreases linearly depending on pH in all three buffer systems, indicating a proton-electron simultaneous transfer mechanism. In Fig.2D, the Ip–pH graph is evaluated, and especially the high peak current obtained at pH 8.00 in phosphate buffer supports that this medium is the most efficient condition for BNS determination in electrochemical terms. In general, the voltammetric behavior of BNS is sensitive to pH and buffer type; it is noteworthy that the optimum voltammetric response is obtained in phosphate buffer at pH 8.00.

The SWV results in Fig. 3 provide a detailed summary of the electrochemical behavior of the BNS molecule on the GC electrode in various buffer solutions and pH ranges. When these data are evaluated in conjunction with the DPV results in Fig. 2, they are essentially parallel. The SW voltammograms obtained in the pH range of 2.00–9.00 in the BR buffer medium are seen in Fig. 3A graph. A tendency to shift to more negative potentials is noticeable as the pH increases in the Ep values, which is parallel to the DP voltammograms shown in Fig. 2A graph. However, it is noteworthy that the oxidation signals of BNS are weakened and scattered significantly, especially in the pH range of 2.00–5.00 in the SWV technique. This shows that SWV gives more stable results in higher pH ranges in BR medium, but the electroanalytical performance of the system decreases with decreasing pH. In addition, Fig. 3B shows the pH effect in ABS and PBS buffer solutions. While the optimum Ip signal is obtained in DPV at pH 8.00 PBS, in SWV results, the highest peak current is obtained in PBS at pH 8.00. However, a higher peak current is obtained with the SWV technique in PBS pH 8.00 medium. This significant difference is due to the differences in the sensitivity of DPV and SWV techniques to surface interactions. While DPV generally provides more stability and sensitivity under low pH conditions, SWV can provide more distinct signals in diffusion-controlled processes and basic environments, as it operates at a higher frequency and modulation. This result confirms that BNS is more prone to oxidation under more basic conditions in PBS medium, and stronger signals are obtained with the SWV technique under these conditions. The pH trend in Fig. 3C summarizes how the peak potential changes with varying pH in three different buffer systems. Again, here, the Ep values systematically shift to negative potentials as pH increases. Although pH sensitivity is similar between BR and PBS buffers, it was observed that Ep values remained slightly more positive in the acetate buffer. This reflects the buffering capacity of the medium and the effect of the ionic structure in the solution. On the other hand, Fig. 3D illustrates the Ip–pH change, with the most striking point being the distinct peak current observed at pH 8.00 in phosphate buffer. This value represents the highest Ip signal among all buffer systems and pH ranges and is shown with a black circle. This finding demonstrates that BNS undergoes more effective oxidation in phosphate buffer and basic medium, as determined by the SWV technique, indicating that this condition is the most suitable environment for analytical determination. The fact that the optimum signal in DPV is obtained at lower pH proves that the electrochemical behavior of BNS can change significantly depending on the choice of technique.

$$Ep = -0.0551\,p + 1.6675\ (R2; -0{,}9917;\ pH: 2.00 - 9.00; BR\ media; CV;\ GCE) \quad (1)$$

$$Ep = -0.0679\,p + 1.8140\ (R2; -0{,}9923;\ pH: 2.00 - 9.00; BR\ media; DPV;\ GCE) \quad (2)$$

$$Ep = -0.0642\,p + 1.7845\ (R2; -0{,}9867;\ pH: 2.00 - 9.00; BR\ media; SWV;\ GCE) \quad (3)$$

The Ep–pH equations given above mathematically explain how the peak potential of the BNS molecule obtained with different electrochemical techniques, such as CV, DPV, and SWV, on the GC electrode in BR buffer medium changes depending on pH [16]. In all three equations, the Ep values shift to more negative potentials as pH increases, indicating that the electrochemical reaction occurs with proton participation and that the mechanism is pH-sensitive. Especially the slope values (CV: −0.0551, DPV: −0.0679, and SWV: −0.0642 V/pH), which are approximately parallel to the theoretical value of −59 mV/pH, indicate a redox mechanism in which the reaction occurs with equal numbers of proton and electron transfer [16]. When compared in terms of slope, the DPV technique exhibits the highest pH sensitivity (−0.0679 V/pH), while these values are slightly lower in the CV and SWV techniques. This difference may be due to the sensitivity of each method to the pulsed structure, time resolution and surface interactions. Moreover, the R^2 values being above 0.98 in all three equations support the high linear fit of the relationship between pH and Ep, as well as the reliability of the obtained data.

Analytical Performance

Fig. 4 shows the results obtained with DPV and SWV techniques for the determination of BNS at different concentrations on the GCE surface. Fig. 4A shows the DP voltammograms obtained with increasing BNS concentrations in a pH 8.00 phosphate buffer solution. It is observed that Ip increases regularly with concentration, which proves that a sensitive and reproducible oxidation reaction specific to BNS occurs on the electrode surface. Similarly, in the voltammograms obtained with SWV in Fig. 4B, the increase in Ip with the rise in concentration reveals that both techniques give clear and analytically strong signals suitable for the quantitative determination of BNS. In Fig. 4C, the current-concentration linear calibration graph obtained with the DPV technique is given, and the correlation coefficient (R^2 = 0.9853) supports a linear relationship with high accuracy. In Fig. 4D, the same evaluation can be made for the SWV technique, and a similarly high correlation coefficient (R^2 = 0.9851) is obtained.

Table 1. Regression data of the calibration lines for determination of BNS by DPV and SWV in standard solution pH 8.00 PBS.

	DPV	SWV
Linearity range (M)	6.00×10^{-7} – 8.00×10^{-5}	2.00×10^{-6} – 8.00×10^{-5}
Slope (µA/M)	2.01×10^{5}	2.42×10^{5}
Intercept (µA)	1.75×10^{1}	2.61×10^{-1}
Correlation coefficient	0.995	0.993
LOD (M)	1.99×10^{-7}	8.04×10^{-7}
LOQ (M)	6.02×10^{-7}	2.44×10^{-6}
Within-day of peak current (RSD %) [a]	0.67	0.51
Within-day of peak potential (RSD %) [a]	0.45	0.40
Between-day of peak current (RSD%)	0.82	0.79
Between-day of peak potential (RSD%)	0.48	0.53

[a] Each value is an average of five measurements.

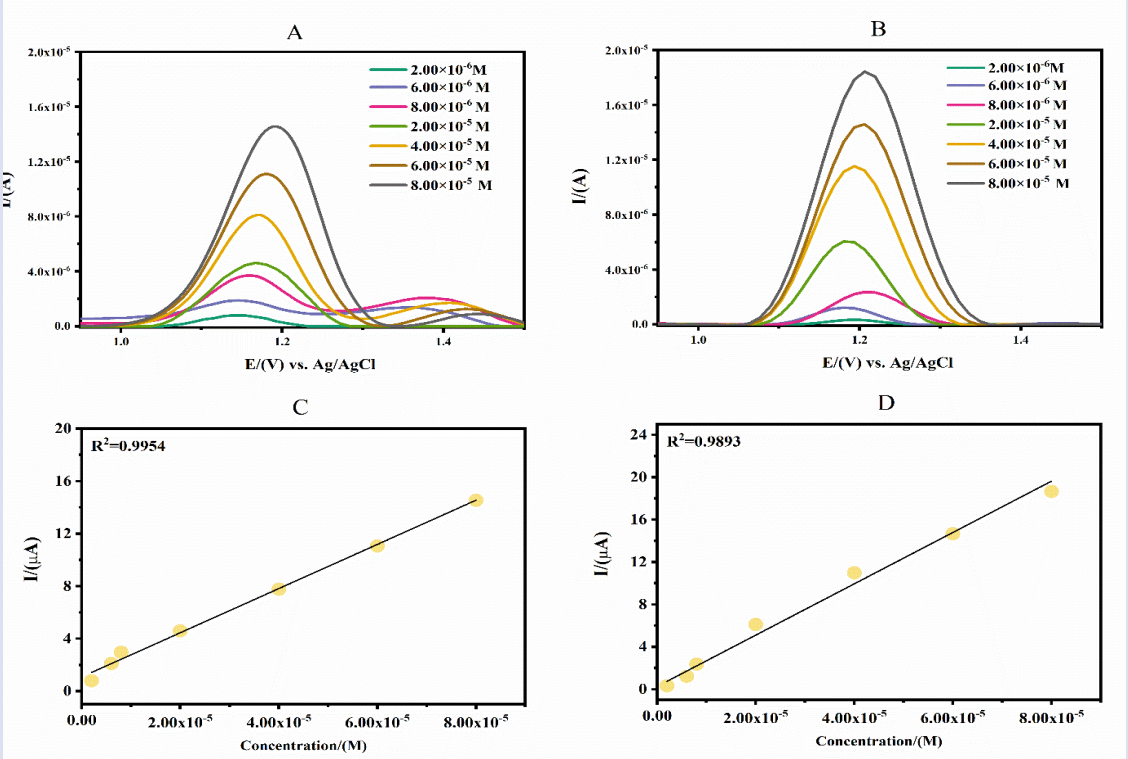

Figure 5. A) DP and B) SW voltammograms of GC electrode sensing performance in various BNS concentrations in commercial human serum (serum calibration curve), serum calibration plots for C) DPV and D) SWV measurement.

Table 2. Regression data of the calibration lines for the determination of BNS by DPV and SWV in commercialhuman serum.

	DPV	SWV
Linearity range (M)	2.00×10^{-6} – 8.00×10^{-5}	2.00×10^{-6} – 8.00×10^{-5}
Slope (µA/M)	1.68×10^{5}	2.36×10^{5}
Intercept (µA)	1.08×10^{1}	-4.96×10^{-1}
Correlation coefficient	0.997	0.995
LOD (M)	3.96×10^{-7}	9.17×10^{-7}
LOQ (M)	1.26×10^{-6}	2.78×10^{-6}
Within-day of peak current (RSD %) [a]	1.13	1.10
Within-day of peak potential (RSD %) [a]	0.44	0.56
Between-day of peak current (RSD%)	1.25	1.39
Between-day of peak potential (RSD%)	0.55	0.51

[a] Each value is an average of five measurements.

Table 3. Results obtained for BNS determination from the commercial human serum.

Values	DPV	SWV
Added (mg)	40.00	40.00
Found (mg)	40.00	40.20
Recovered (%)	100.12	101.30
Bias (%)	-0.38	-1.30
R.S.D. (%)	1.07	1.42

Table 4. Recent application of pesticides

Target	Type	Electrode	Modification	Electrochemical method	Real sample	Linearity range	LOD	Reference
Imidacloprid	insecticide	GCE	WS_2	Voltammetric	DI water, tap water, pond water, and collected soil samples	10-90 µM	0.28 µM	[18]
Imidacloprid	insecticide	GCE	rGO	Potentiometric	-	0.5 µM-1.0 mM	0.20 µM	[19]
Carbendazim	fungicides	GCE	NPG	Voltammetric	waste water	3.0–120 µM	0.24 µM	[20]

When Fig. 4 and Table 1 are evaluated together, it is determined that DPV and SWV techniques exhibit good analytical performance in the determination of BNS. A wide linearity range is observed for both techniques. The linearity range for DPV is determined as 6.00×10^{-7}–8.00×10^{-5} M, and for SWV it is defined as 2.00×10^{-6}–8.00×10^{-5} M. The calibration curve slopes obtained are 2.01×10^{5} µA/M for DPV and 2.42×10^{5} µA/M for SWV, confirming that SWV technique gives slightly higher current response. LOD and LOQ values were calculated by means of the classically used formulas LOD = 3.3σ/m [17] and LOQ = 10σ/m [17], where σ is the standard deviation around the y-axis intercept of the calibration curve and m is the slope. According to these calculations, the LOD value obtained with the DPV method was 1.99×10^{-7} M and the LOQ value was 6.02×10^{-7} M, while these values were found to be 8.04×10^{-7} M and 2.44×10^{-6} M for the SWV method, respectively. These data reveal that the DPV method has lower detection and quantification limits, i.e., it is more sensitive in detecting BNS at lower concentrations. The fact that R^2 is above 0.99 in both techniques supports the high reliability of the linear calibration curves. In addition, the %RSD values calculated in terms of intra-day and inter-day peak current and potential repeatability are below 1% for both techniques, indicating that the system operates with high repeatability and stability. In conclusion, DPV and SWV techniques are reliable and sensitive in the voltammetric determination of BNS, and the DPV method stands out as a preferred method, especially for lower concentrations.

In addition, Fig. 5 and Table 2 include the DP (Fig. 5A) and SW (Fig. 5B) voltammograms obtained with different concentrations of the BNS molecule in the presence of commercial human serum, and the calibration plots obtained with the DPV (Fig. 5C) and SWV (Fig. 5D) technique, respectively. When the calculations given above are taken as reference, the LOD value obtained with the DPV method was found to be 3.96×10^{-7} M and the LOQ value was found to be $1.26\times10^{-}6$ M, while the LOD values obtained with the SWV method were found to be 9.17×10^{-7} M and 2.78×10^{-6} M, respectively. On the other hand, the %RSD values calculated for DPV and SWV, in terms of intra- and inter-day peak current and potential reproducibility, are slightly above 1% compared to those obtained from the standard solution. Higher %RSD values are observed due to the matrix effect of the biological environment. However, these values are within acceptable limits.

demonstrate the accuracy and precision of the method. Furthermore, the recovery percentages in Table 3 support the effectiveness of the preferred electrochemical techniques not only in standard solutions but also in complex biological matrices, providing results that are stable against matrix effects.

Recent Applications

In recent years, GC electrodes, preferred for the electrochemical detection of pesticides, have been modified with various nanomaterials such as tungsten sulfide (WS_2), reduced graphene oxide (rGO), and nanoporous gold (NPG), but it is noteworthy that LOD values obtained in some studies remain above 10^{-7} M. This is thought to be due to the materials applied to the GC electrode surface not accelerating electron transfer kinetics to the desired extent, not establishing sufficient affinity with the target pesticide, or the limited active surface area. LOD values in the µM range, reported particularly for molecules such as carbendazim and imidacloprid, indicate that while the modification improves the signal-to-noise ratio, analytical sensitivity is not achieved at the desired level.

Conclusion

In this study, BNS was determined voltammetrically in order to demonstrate the effectiveness of electrochemical approaches for monitoring pesticide residues and reducing environmental risks. In this analysis, which was performed for the first time in the literature, DPV and SWV techniques were applied via GC electrode, and each result obtained with these methods was evaluated in detail. It was determined that the thioether group in the chemical structure of BNS is an oxidizable center voltammetrically, and it was confirmed by the calculations that a single-electron-transfer oxidation mechanism occurs through this structure. As a result of pH scanning studies, it was determined that the highest oxidation signals were obtained at pH 8.00, and the optimum measurement medium was determined as PBS. Scan speed studies revealed that the peak current increased linearly with the square root of the scan speed, and a slope value of approximately 0.79 was obtained in the log-log plot, which proved that a mixed mechanism with diffusion control but adsorption contribution was involved in the electrochemical process. Moreover, as a result of standard calibration studies, wide and linear calibration curves were obtained in the range of 6.00×10^{-7} – 8.00×10^{-5} M with DPV technique and 2.00×10^{-6} – 8.00×10^{-5} M with SWV. The obtained R^2 values were 0.995 (DPV) and 0.993 (SWV), respectively, supporting that the methods can be applied with high sensitivity. In addition, LOD and LOQ values were calculated with the classical 3.3σ/m [17]and 10σ/m formulas [17] and were found to be 1.99×10^{-7} M (LOD) and 6.02×10^{-7} M (LOQ) for the DPV method and 8.04×10^{-7} M (LOD) and 2.44×10^{-6} M (LOQ) for the SWV method. These results revealed that DPV is more advantageous at low concentrations by offering lower detection limits, while SWV is preferable for high concentrations with its fast and strong signal response.

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Contributor roles taxonomy for author statement**.**

Selva Bilge: Conceptualization, Methodology, Formal Analysis, Resources, Writing - Original Draft, Supervision

Zeynep Kara: Investigation and Formal Analysis

Ethical Approval Statement

This study does not contain any human or animal subjects.

Conflicts of interest

There is no conflicts of interest in this work.

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