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A Novel Differential Pulse Polarographic Method for the Electroanalytical Characterization of Thiamethoxam

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Abstract: ***A novel Differential Pulse Polarographic (DPP) method has been developed and validated for the electroanalytical characterization of thiamethoxam, a widely used neonicotinoid insecticide. Given the environmental persistence and increasing detection of thiamethoxam residues in water, food, and biological matrices, this study aims to establish a sensitive, cost-effective, and rapid electroanalytical alternative to conventional chromatographic and immunoassay techniques. Electrochemical behavior was investigated using a dropping mercury electrode in Britton-Robinson buffer (pH 3.0–11.0), with optimal response at pH 7.0. The method was optimized with a scan rate of 6 mV/s and pulse amplitude of 100 mV, yielding a well-defined cathodic peak at –0.95 V. The reduction process was found to be pH-dependent, involving a two-step conversion of the nitroguanidine group. The proposed method showed excellent linearity in the 1.0–20.0 µg/mL range ($R^2 = 0.997$), with a limit of detection (LOD) and quantification (LOQ) of 0.035 and 0.116 µg/mL, respectively. The intra- and inter-day precision (%RSD <1.1%) and recovery rates (98.75–101.24%) confirmed the method's accuracy and reproducibility. Furthermore, the technique demonstrated robustness under slight variations in pH, reinforcing its suitability for routine monitoring of thiamethoxam residues in environmental and agricultural samples. This study is among the first to detail the electrochemical reduction mechanism of thiamethoxam using DPP, offering a validated and practical tool for trace-level quantification.***

Keywords: Differential Pulse Polarographic, thiamethoxam, Method Validation

1. Introduction

The widespread use of **neonicotinoid insecticides**, particularly **thiamethoxam**, in modern agriculture has raised significant environmental and toxicological concerns. Thiamethoxam is known for its systemic action, high efficacy, and relatively low mammalian toxicity. However, its frequent application and environmental persistence have necessitated the development of sensitive and specific methods for its detection in various matrices such as water, food, soil, and biological samples.

Several **analytical techniques** have been employed to determine thiamethoxam and other neonicotinoids in environmental and food samples. Among these, **enzyme-linked immunosorbent assay (ELISA)** has been utilized for its simplicity and suitability for high-throughput screening. Studies have reported the detection of thiamethoxam in stream and tap water, potato, cucumber, and apple samples using ELISA [1]. Similarly, ELISA has been applied for the simultaneous determination of imidacloprid and thiamethoxam in water [2], fruit juices [3], honey [4], and a wide range of food and environmental samples [5].

High-performance liquid chromatography (HPLC) remains a widely accepted and validated method due to its sensitivity and specificity. Numerous researchers have developed HPLC protocols to determine thiamethoxam residues in honeybees [6], drinking water [7], agricultural produce [8], soil [9], and milk [10]. Moreover, simultaneous detection of multiple neonicotinoids including thiamethoxam, imidacloprid, acetamiprid, and thiacloprid in complex matrices such as vegetables, fruits, and environmental water has also been reported [11–15].

While chromatographic and immunoassay-based methods have demonstrated accuracy and reliability, they often require sophisticated instruments, trained personnel, and extensive sample preparation. In contrast, **electroanalytical techniques**, particularly **Differential Pulse Polarography (DPP)**, offer cost-effective, rapid, and sensitive alternatives for the quantification of electroactive compounds. DPP provides enhanced resolution and lower detection limits, making it suitable for trace-level analysis in environmental and agricultural matrices.

Despite these advantages, limited literature is available on the **electrochemical behavior of thiamethoxam using DPP**. Hence, the present study focuses on the **electroanalytical characterization of thiamethoxam** using DPP, examining its reduction mechanism and optimum analytical conditions. Additionally, the method is **validated** according to **ICH Q2(R1)** guidelines to ensure accuracy, precision, linearity, and sensitivity for routine analysis applications.

2. Materials and Method

2.1 Equipment

Polarographic studies were performed using a CL-362 Polarographic Analyzer (Elico Ltd., Hyderabad) with ELICO's Windows-based software, connected via RS-232C. The setup included a dropping mercury electrode (working), saturated calomel electrode (reference), and platinum wire (auxiliary). pH measurements were taken using an Elico pH meter. Absorbance of neonicotinoids in Britton-Robinson buffer was measured using a PerkinElmer Lambda 25 UV-Vis spectrophotometer.

2.2 Reagents

Analytical reagent grade chemicals were used to prepare all solutions in doubly distilled water. Reference standards used were thiamethoxam (99.9%) and Britton-Robinson (0.04 M), carbonate, and phosphate buffers were prepared using standard procedures and adjusted to required pH. These buffers were used to support electroanalytical studies across a wide pH range.

3. Result and Discussions

3.1 Scan Rate Effect (*v*):

The scan rate (mV/s) significantly influences peak resolution in voltammetric analysis. A balance is required between resolution and analysis time. Too high a scan rate reduces resolution, while too low increases analysis time. After optimizing pH, scan rate was varied from 3 to 12 mV/s with constant conditions and 2-minute nitrogen purging. The peak current (Ip) was plotted against scan rate, and the optimal ν was observed at 6 mV/s in BRB pH 7.0. Ip decreases slowly for the increasing υ from 6 to 12 mV/s. The increasing υ has shifted the Ep of **thiamethoxam** towards a more negative direction according to the equation; as shown in **Figure 3.20B**. Therefore, the optimum ν adopted for further studies was 6.0 mV/S (Figure.1)

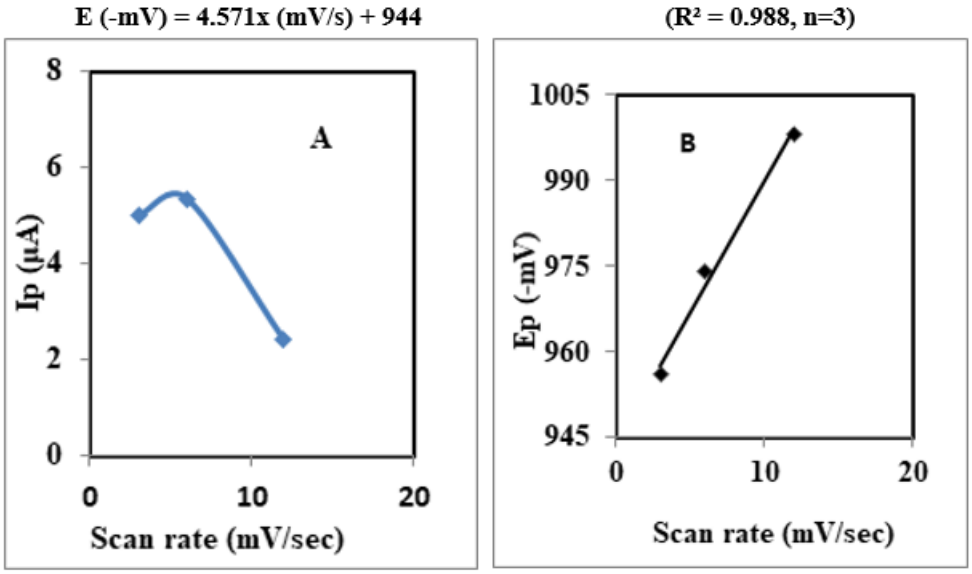

Figure 1: Effect of various υ on [A] Ip and [B] Ep of 10. µg ml^{-1} **thiamethoxam** peak in BRB pH 7.0.

3.2. Effect of pulse amplitude

The effect of the pulse amplitude on the Ip shows that the Ip increased and Ep displaced towards less negative direction when the pulse amplitude was increased from the range of 5.0 to 100mV as shown in **Figure 2A**. The result shows that a maximum value of Ip of insecticide were obtained at pulse amplitude of 100 mV is higher as compared to that obtained by unoptimized parameters. At the higher value of pulse amplitude, the Ip is slightly increased but peak broadening was observed, so 100 mV is chosen for optimum pulse amplitude.

From **Figure 2B** it was observed that the Ip is linearly proportional to pulse amplitude according to the following equation:

Ip(µA) = 0.024x(mV) - 0.00 (R^2=0.998)

From this study, the optimum conditions for electroanalytical determination of selected neonicotinoids by DPP technique are shown in **Table 3.2.** Using these optimized parameters, the Ip and Ep of 10.0 µgml^{-1} were found to be, 2.503 µA and -0.98V for thiamethoxam.

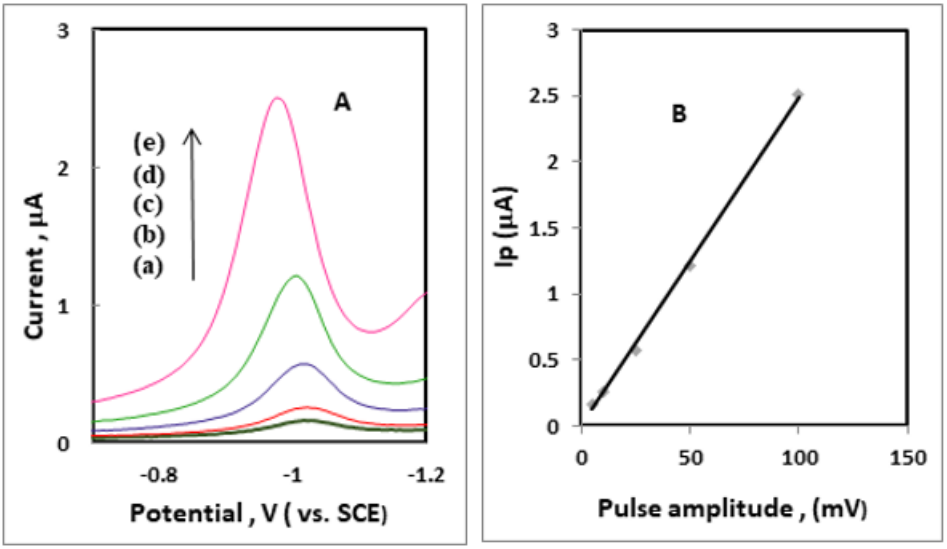

Figure 2: Effect of pulse amplitude on A) DPP polarograms and B) Ip of 10.0 µg $ml^{-1}$of thiamethoxam insecticide at a) 5.0 mV b) 10.0 mV c) 25.0 mV d) 50.0 mV and e) 100.0mV, pH 7.0, current range 10µA, scan rate 6mV/sec, drop time 1sec,

3.4 DPP behavior of thiamethoxam at different pH

The DPP study of 10.0 µg/ml thiamethoxam in BRB buffer (pH 3.0–11.0) revealed pH-dependent electrochemical reduction starting at pH ≥ 3.0, with peak potential shifting due to proton involvement [19]. Peak current and area increased with pH, peaking at pH 11.0, despite hydrogen evolution at pH 5.0–6.0. In alkaline media (pH > 8.0), alkaline hydrolysis of the nitroguanidine group enhanced the response [17]. pH 7.0 was chosen as optimal for stability. Thiamethoxam showed a two-step reduction (–0.5 V to –1.8 V vs. Ag/AgCl), consistent with NO_2 to NH_2 conversion via hydroxylamine and amine intermediates [20], as shown in Figure 3.6 (a) and (b).(figure 3)

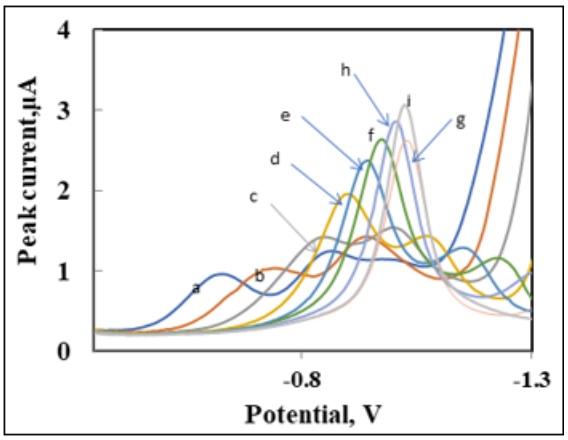

Figure 3: Differential pulse polarographic behavior of 10.0 µg ml^{-1}thiamethoxam insecticide at different BRB pH values; (a) 3.0(b) 4.0 (c) 5.0 (d) 6.0 (e) 7.0 (f) 8.0 (g) 9.0, (h) 10.0 and (i) 11.0.

The optimized conditions used for analysis of three neonicotinoids was current range10µA, data acquisition slow, scan rate 6mV/sec, drop time 1sec, scan type forward, scan range start -200 mV end -1700mv, pulse amplitude 100mV, BRB pH 7.0 and cc compensation is 0%.

3.5 Electroanalytical study of thiamethoxam

The differential pulse polarograms of thiamethoxam recorded at pH 7.0 using Britton-Robinson buffer (BRB) as the supporting electrolyte is shown in Figure 3.27. The polarograms exhibits a well-defined cathodic peak around –0.95 V vs. SCE, which corresponds to the electrochemical reduction of thiamethoxam. As the concentration of thiamethoxam increases from curves (a) to (k), the peak current (Ip) increases accordingly, demonstrating a concentration-dependent response. However, upon further addition of the standard solution, the current reaches a maximum and begins to level off. This plateauing behavior indicates that the electrochemical process becomes limited by the amount of analyte available at the electrode surface, suggesting diffusion-controlled kinetics or adsorption saturation [21]. Such saturation behavior is characteristic of systems where the electrode surface or diffusion layer can no longer accommodate additional analyte molecules efficiently. This observation confirms that the process is governed by analyte availability, as expected for electroanalytical techniques under mass-transport limitations.

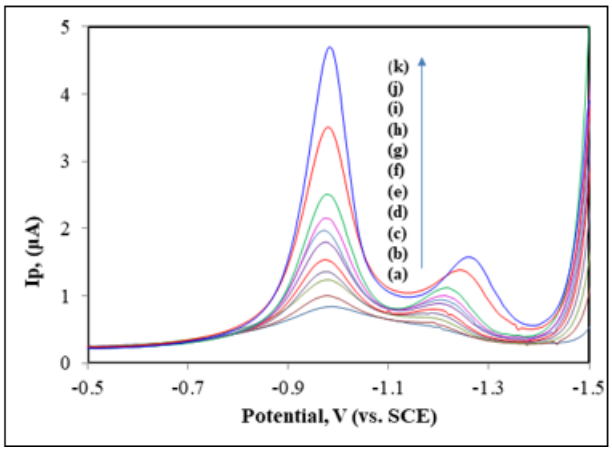

Figure 3.27: Differential pulse polarograms of increasing concentration of thiamethoxam insecticide at pH 7.0 in BRB buffer solution as a supporting electrolyte

3.6. DPP proposed reduction mechanism of thiamethoxam

The proposed reduction mechanism of imidacloprid and thiamethoxam are as shown in **Figure 3.6 (a) and (b).** The general reduction of NO_2 group leads to formation of NH_2 group. The pesticides studied showed two peaks clearly indicating that the process of reduction of NO_2 to NH_2 was not conceited one but took place in two distinct steps. In the first step only partial reduction has taken place .Thus it can be concluded that the NO_2 group gets reduced to NHOH group in the first step and subsequently NHOH group get converted in to NH_2 group.

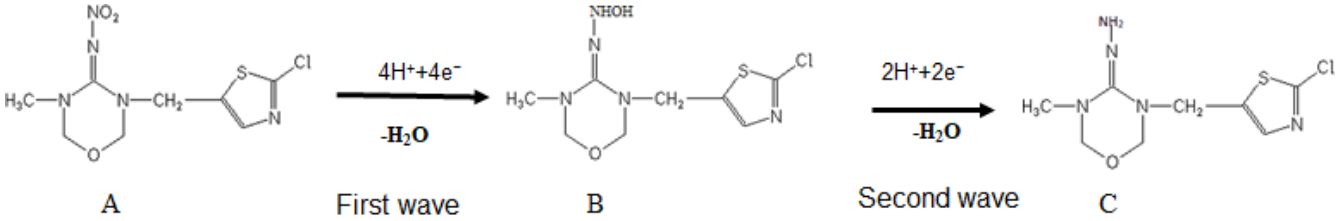

Figure 4: The proposed reduction mechanism of thiamethoxam

A = (4*E*)-3-[(2-chloro-1, 3-thiazol-5-yl) methyl]-5-methyl-*N*-nitro-1, 3,5-oxadiazinan- 4-imine
B = (4*E*)-3-[(2-chloro-1, 3-thiazol-5-yl) methyl]-5-methyl-1, 3,5-oxadiazinan-4-one Hydroxyhydrazone
C = (4*E*)-3-[(2-chloro-1, 3-thiazol-5-yl) methyl]-5-methyl-1, 3, 5-oxadiazinan-4-one hydrazone

4. Method Validation

4.1 Linearity of the Method

To ensure the reliability and applicability of the developed electroanalytical method for the determination of thiamethoxam, method validation was conducted in accordance with standard analytical guidelines. Linearity was assessed by plotting the peak current (Ip) obtained from differential pulse polarography against a series of increasing concentrations of thiamethoxam. The analysis was performed in Britton-Robinson buffer (BRB) solution at pH 7.0 as the supporting electrolyte. The concentrations tested were 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 16.0, and 20.0 µg ml^{-1}, corresponding to the labeled polarograms (a) through (k), respectively (Figure 3.28). A strong linear relationship was observed between the peak current and analyte concentration over this range. The regression equation was found to be **Ip = 187.7C + 0.428**, where *Ip* is the peak current in µA and *C* is the concentration in µg ml^{-1}. The method demonstrated excellent linearity, with a correlation coefficient (**R²**) of **0.997**, indicating a highly reliable and reproducible response within the studied concentration range. (figure 5)

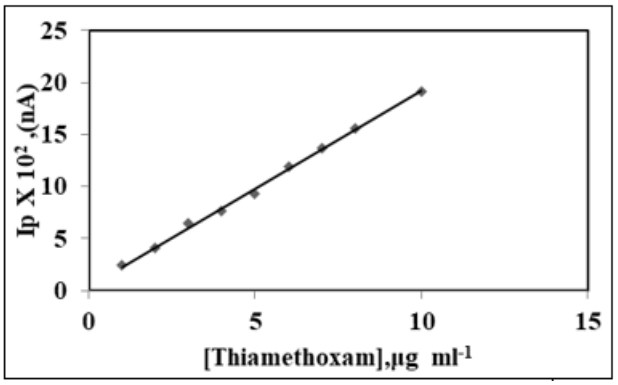

Figure 5: Linear plot of Ip versus concentration of thiamethoxam in BRB at pH 7.0.

4.2. Precision

The precision of the proposed DPP method was evaluated at 3.0 µg ml^{-1} and 6.0 µg ml^{-1} concentrations of thiamethoxam. Intra-day %RSD values were 0.98% and 0.59%, respectively, indicating good repeatability. Inter-day studies over three days showed %RSD values ranging from 0.38% to 1.09%, confirming the method's excellent reproducibility and reliability for quantitative analysis.(table 1)

Table 1: Ip (µA) obtained for intra-day and inter-day precision studies of thiamethoxam by proposed DPP procedure (n=5)

[Thiamethoxam] µg ml^{-1}	Intra-day Ip±SD(%RSD)	Inter-day measurement Ip±SD(%RSD)		
		Day 1	Day 2	Day 3
3.0	0.6 ± 0.005 (0.98%)	0.6 ± 0.005 (0.98%)	0.59 ± 0.005 (0.87%)	0.58± .0022 (0.38%)
6.0	1.17 ± 0.006 (0.59%)	1.17 ± 0.006 (0.59%)	1.15 ± 0.014 (0.512%)	1.18± 0.012 (1.09%)

4.3. Accuracy

The accuracy of the DPP method was evaluated using standard concentrations of thiamethoxam (3.0 and 6.0 µg ml^{-1}). Intra-day Ip values were 0.60 ± 0.005 µA (0.98% RSD) for 3.0 µg ml^{-1} and 1.17 ± 0.006 µA (0.59% RSD) for 6.0 µg ml^{-1}. Inter-day results over three days showed consistent Ip values with %RSD ranging from 0.38% to 1.09%. For 3.0 µg ml^{-1}, Ip values were 0.60, 0.59, and 0.58 µA across days. For 6.0 µg ml^{-1}, values ranged between 1.15 and 1.18 µA. The low variability confirms high measurement accuracy. Thus, the method provides reliable and accurate quantification of thiamethoxam.(table 3)

Table 3: Mean values for recovery of thiamethoxam standard solution (n = 3)

No of experiments	Amount added (µg ml^{-1})	Ip (µA)	Amount found (µg ml^{-1})	Recovery %	%Recovery±*SD* (RSD)
1	3.0	0.60 0.59 0.61	2.98 2.93 3.03	99.46 97.68 101.24	99.46±1.78 (1.79%)
2	6.0	1.17 1.16 1.15	6.03 5.97 5.92	100.5 99.64 98.75	99.64±0.89 (0.89%)
3	10.0	1.92 1.89 1.90	10.0 9.88 9.93	100.0 98.82 99.35	99.39±0.58 (0.59%)

4.4. Determination of Limit of Quantification (LOQ)

The limit of quantification (LOQ) represents the lowest concentration at which an analyte can be quantitatively measured with acceptable precision and accuracy [22, 23]. According to ICH Q2 (R1) guidelines, the limit of detection (LOD) and LOQ were calculated using the formulas

$$\mathbf{LOD = 3.3 \cdot \sigma / S}$$
$$\mathbf{LOQ = 10 \cdot \sigma / S}$$

Where σ is the standard deviation of the response and S is the slope of the calibration curve. Using the regression equation **Ip = 187.7C + 0.428**, the LOD and LOQ were found to be **0.035µg ml^{-1} and 0.116 µg ml^{-1}**, respectively. These values demonstrate that the proposed method offers sufficient sensitivity to detect and quantify thiamethoxam at low concentrations, making it suitable for routine analytical use.

4.5. Robustness

The robustness of the method was tested by varying the pH slightly from the optimized value (pH 7.0) to 6.8 and 7.2. The recovery percentages for a 6.0 µg/mL sample were 99.22% at pH 6.8, 99.55% at pH 7.0, and 101.05% at pH 7.2. The corresponding relative standard deviations (% RSD) were 1.24%, 0.92%, and 0.58%, respectively. These results indicate that the method is robust with minimal variation in recovery and precision across the tested pH values (table 4)

Table 4: Robustness of the method at slight variation from the optimized pH parameters

Amount added (µg ml^{-1})	pH= 6.8		pH= 7.0		pH= 7.2	
	Amount found (µg ml^{-1})	% Recovery	Amount found (µg ml^{-1})	% Recovery	Amount found (µg ml^{-1})	% Recovery
6.0	5.98	99.67	6.03	100.50	6.1	101.67
6.0	6.01	100.17	5.97	99.50	6.06	101.00
6.0	5.87	97.83	5.92	98.67	6.03	100.50
Mean	5.95	99.22	5.97	99.55	6.06	101.05
SD	0.0737	1.2322	0.055	0.9163	0.0351	0.5875
% RSD	1.24%	1.24%	0.92%	0.92%	0.58%	0.58%

5. Conclusion

In conclusion, a novel Differential Pulse Polarographic (DPP) method was successfully developed for the determination of thiamethoxam. The insecticide exhibited a well-defined reduction peak at –0.95 V in Britton-Robinson buffer at pH 7.0. The method proved to be highly sensitive and allowed detection of trace levels of thiamethoxam, with a wide linear range and a low detection limit suitable for environmental monitoring. It showed good precision and accuracy, as confirmed by low relative standard deviation values and satisfactory recovery results. Moreover, common excipients and matrix components did not interfere with the analysis. This technique offers simplicity, cost-effectiveness, and minimal sample preparation, making it ideal for routine analysis of thiamethoxam in agricultural and environmental samples.

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