Determination of triflumizole by differential pulse polarography in formulation, soil and natural water samples

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Abstract

A novel electroanalytical procedure is proposed for the determination of the triflumizole in formulation, soil and natural water samples using differential pulse polarography (DPP). Triflumizole exhibited a single well-defined cathodic peak over the pH range of 1.0–9.0 in Britton–Robinson (B–R) buffers. The peak potentials were shifted to more negative values upon increasing the pH and a plot of reduction potentials ($E_p$) versus pH exhibited two linear segments with a break at pH 4.0 which corresponded to the $pK_a \pm 1$ value of triflumizole. The peak appeared as a maximum at pH 2.0 ($−810$ mV versus saturated calomel electrode (SCE)) was well resolved and suitable to be investigated for analytical use. The current–concentration plot obtained using this peak was rectilinear over the range from 2.0 to 91.0/9262 mol L$^{-1}$ with a correlation coefficient of 0.993. The limit of detection (LOD) and limit of quantification (LOQ) were obtained as 0.72 and 2.39/9262 mol L$^{-1}$, respectively. The proposed method was successfully applied to the determination of triflumizole in spiked soil and lake water. The mean recoveries of the pesticide were 102.1 and 103.6% with a relative standard deviation of 1.01 and 2.68% in soil and lake water, respectively. The method was extended to the determination of triflumizole in agrochemical fungicide formulation Trifmine® and results were in agreement with that obtained by high-performance liquid chromatographic analysis (HPLC). The influence of some diverse ions and some other pesticides was also investigated.

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1. Introduction

Although, the use of pesticides provides unquestionable benefits in providing a plentiful, low-cost supply of high-quality fruits and vegetables, their incorrect application may leave harmful residues, which involve possible health risk [1]. Pesticides are widely utilized at various stages of cultivation and during post harvest storage to protect fruit and vegetables against a range of pests and fungi or to provide quality preservation. The risk of pesticide residues depends on their ability to cause adverse health effects and the potential human exposure to their residues in the diet.

Triflumizole, ($E$)-4-chloro-$\alpha$, $\alpha$, $\alpha$-trifluoro-$N$-(1-imidazol-1-yl-2-propoxy-ethylidene)-o-toluidine (IUPAC), is a novel systemic fungicide discovered and developed by Nippon Soda Co. Ltd., Japan [2], whose general structure is:

The pest control product (pesticide) triflumizole is a fungicide for the control of powdery mildew and scab on apples, grapes, pears, sour cherries, and sweet cherries. Ecological studies indicate triflumizole is practically non-toxic to honeybees and birds. It is categorized as being moderately toxic to highly toxic to fish. Since this is a minor use registration and since greenhouse applications have been traditionally treated as indoor uses, the hazards to non-target species and endangered species are considered to

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be minimal [3]. The hazard rankings are based on LD50 values (oral, dermal, and inhalation) for all mammals that are reasonable analogs for humans, including rats, mice, rabbits, monkeys, dogs, cats, gerbils, and guinea pigs. The LD50 is the lethal dose (in milligrams of substance per kilogram of body weight) that kills 50% of the test animals in a standard assay. Triflumizole is a toxic class III pesticide and acute oral LD50, for male and female rats are 750 and 695 mg kg\(^{-1}\), respectively [3].

The most analytical procedures for the determination of various pesticides are the chromatographic ones [4–6], but electroanalytical techniques have also been used for the determination and study of several pesticides in different matrices like water, soils, plants, and food [7–9]. Only a few methods for analyzing triflumizole compound in environmental samples can be found in the literature. They are mainly based on high-performance liquid chromatography (HPLC). Miyake et al. developed an analytical method for the determination of fungicidally active metabolites of triadimefon and triflumizole in beer using liquid chromatography–tandem mass spectrometry (LC–MS) [10]. A procedure for HPLC determination of triflumizole and its metabolite in crops was also examined [11], the detection limit was 0.01–0.02 ppm and the recoveries from spiked crops (0.5 ppm) were 73–99% for triflumizole and 74–94% for metabolite. The electroanalytical or polarographic technique presents some advantages in relation to traditional methods. When compared to chromatography, the polarographic procedures have several advantages such as their low cost and possibility of analysis without the need of extraction or pre-treatments, as well as the short time required for analysis [12–14]. On the other hand, HPLC can be used with relatively minor modifications for a large number of analytes. In polarography, it is often necessary to find different optimum conditions for each analyte and each matrix. The dissolving of mercury electrode at +0.4 V prevents reaching more positive potentials. Hence with mercury electrode only the more easily oxidized species can be followed. The sensitivity of differential pulse polarographic methods of analysis, enabling determination of electroactive species in up to about \(-10^{-7}\) mol L\(^{-1}\) solutions was superior or at least comparable to most other contemporary techniques.

To our knowledge, methodology for the determination of triflumizole using electrochemical or differential pulse polarographic (DPP) techniques has not yet been described in the literature. Besides, the aim of this study is to examine the polarographic behavior of triflumizole, find out optimum analysis conditions and apply the method for the determination of triflumizole in agrochemical fungicide formulation Trasmine®, spiked soil and lake water samples.

2. Experimental

2.1. Instrumentation

A PAR (Princeton Applied Research Company, USA) model 174A polarographic analyzer system, equipped with a PAR mercury drop timer, was used. A Kallouk electrolytic cell with reference-saturated calomel electrode (SCE), separated by liquid junction, was used in a three-electrode configuration. The counter electrode was platinum wire. The natural drop time of the mercury electrode was 3.2 s (2.04 mg s\(^{-1}\)). The polarograms were recorded with a Linseis LY 1600 X–Y recorder (Linseis, Selb, Germany). pH values were measured with a Hanna HI 8521 (Hanna Instruments, Singapore) pH meter. The HPLC system (Agilent 1100 HPLC system, Agilent Technologies, Palo Alto, CA, USA) consisted of a quaternary pump, a Rheodyne injector equipped with a 20 μL sample loop. Zorbax Eclipse XDB C\(_{18}\) (150 mm × 4.6 mm; i.d. 5 μm) column, and a model of L-7455 diode array and multiple wavelength UV–visible detector controlled by Agilent Chem Station software.

2.2. Chemicals and reagents

Triflumizole was provided by Nippon Soda Co. Ltd. with a purity of 99.9%. Stock solutions of triflumizole (1.0 × 10\(^{-3}\) mol L\(^{-1}\)) were daily prepared in 50% ethanol solution and kept in the dark in a refrigerator. Supporting electrolytes namely Britton–Robinson buffer (B–R buffer, 0.04 M, pH 3.0–9.0) and HCl (pH 1.0–2.0) were prepared in doubly distilled water. Working solutions were prepared by dilution of the stock solution with selected supporting electrolyte to give the solution containing triflumizole in the concentration range of 2.0 × 10\(^{-6}\)–9.1 × 10\(^{-5}\) mol L\(^{-1}\). The agrochemical fungicide formulation Trasmine® (30% triflumizole by mass) was obtained from Nippon Soda Co. Ltd. Tokyo. Salts used for supporting electrolyte, solvents and other reagents were of analytical reagent grade (Merck, Darmstadt, Germany). All solutions were protected from light and used within 3–6 h to avoid possible decomposition.

The mercury (pro-analysis) was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO\(_3\) and water columns, in the form of fine droplets. The collected mercury was dried between sheets of filter paper. Before use, a differential pulse polarogram of this mercury was recorded in order to confirm the absence of impurities. Britton–Robinson (B–R) buffer solution was prepared in such a way that 2.3 mL glacial acetic acid, 2.7 mL phosphoric acid and 2.4720 g boric acid dissolved by dilution with water to 1.0 L. Fifty millilitres portions of this solution were taken, and the desired pH was adjusted between 2.0 and 10.0 by addition of appropriate amount of 2.0 mol L\(^{-1}\) NaOH.

2.3. Procedures

2.3.1. Polarographic procedure

Ten millilitres of supporting electrolyte solution of Britton–Robinson (B–R) buffer or HCl was put into the polarographic cell and de-oxygenated with high-purity nitrogen (99.999%) for about 5 min. The background polarograms were obtained by scanning the potential from 0.0 V to about −1200 to −2000 mV (versus SCE) depending on the pH of the solution. The polarographic response was obtained using a pulse repetition time of 1.0 s, with an amplitude of 50 mV and a scan rate of 5.0 mV s\(^{-1}\) at an ambient temperature of 25 ± 3 °C. The analytical curves for the determination of triflumizole were obtained.
by standard addition of the pesticide and evaluation of the peak currents. Triflumizole hydrolyses readily at high and low pH and hydrolysis rate is pH dependent. The registrant-calculated half-lives were 8–9 days (pH 5), 64.6 days (pH 7), and 3.9 days (pH 9) [3]. The stability of the triflumizole solutions was also investigated by applying polarographic control at neutral pH and no hydrolysis occurred during the study (the recovery of the triflumizole was about 98.8%). Stock solutions of triflumizole were prepared in 50% ethanol solution at neutral pH and kept in the dark in a refrigerator, in order to avoid hydrolytic process. The optimum conditions for the analytical determination of the investigated compound by DPP were found to be: pH 2.0, peak potential −810 mV, scan rate of 5.0 mV s⁻¹, pulse amplitude of 50 mV with pulse duration of 50 ms at an ambient temperature of 25 ± 3 °C.

2.3.2. Formulation assay procedure

A suitable amount of pesticide formulation Trifmine® (30% triflumizole m/m) equivalent to 1.0 × 10⁻⁴ mol L⁻¹ triflumizole was accurately measured and transferred into a 100.0 mL of calibrated flask and completed to the mark with 50% ethanol solution, and sonicated 15 min. In the DPP experiments, 1.0 mL of an aliquot of this clear supernatant liquor was added to 9.0 mL of the pH 2.0 solution in the electrochemical cell (previously de-aerated for 5 min with humidified, 99.999% ultra-pure nitrogen.) and measured under calibration conditions. The triflumizole in pesticide formulation was analyzed by using the maximum polarographic peak current obtained at about −760 mV (versus SCE), using the standard addition method.

The results obtained were compared by HPLC. The procedure involves the use of acetonitrile/H₂O (80%-20%, v/v) as the mobile phase and 150 mm Zorbax Eclipse XDB C₁₈ (5 μm particle size) column. Triflumizole was determined by HPLC using diode array and multiple wavelength UV–visible detectors at 234 nm. The results obtained were compared statistically with HPLC using Student’s t-test and variance ratio F-test.

2.3.3. Soil and lake water analysis procedure

Two grams of soil (ground and dried) was weighed and spiked with a stock triflumizole solution at concentration level of 2.0 × 10⁻⁴ mol L⁻¹ in 20.0 mL of 50% ethanol solution. The same procedure was also followed in parallel for pesticide-free soil sample. After homogenizing the samples for 10 min, they were placed in centrifuge tubes, shaken for 2 h and centrifuged for 10 min at 3000 rpm. From the supernatant, 1.0 mL of pesticide-free aliquots were collected, transferred to the polarographic cell containing 9.0 mL pH 2.0 solution. Polarograms were recorded using the differential pulse polarographic (DPP) mode and the values of current (Iₚ) versus the corresponding concentrations of triflumizole in soil extracts were plotted to get the calibration graph. The determination of the pesticide was performed from peak current appeared at about −750 mV (versus SCE), using the multiple standard additions.

10.0 mL of lake water samples (obtained from Göksu Lake, Ankara/Turkey) were taken from the sample without any pre-separation or pre-concentration. The samples were spiked with a stock triflumizole solution at concentration level of 20.0 μmol L⁻¹ in 20.0 mL 50% ethanol solution. After the completion the above procedure for soil, 1.0 mL aliquots then added to the electrochemical cell containing 9.0 mL pH 2.0 supporting electrolyte solutions. The determination of the pesticide in lake water was carried out by DPP from the peak current appeared at −760 mV (versus SCE), under the experimental conditions described above, using multiple standard additions.

3. Results and discussion

3.1. Polarographic behavior

The Britton–Robinson buffer (0.04 mol L⁻¹) was chosen as a supporting electrolyte because of its wide pH range applicability. The polarographic behavior of triflumizole exhibited a single well-defined differential pulse (DP) peak over the whole pH range (1.0–9.0) in B–R buffer solution (Fig. 1). As shown in Fig. 2, the peak potentials were strongly pH-dependent in that they shifted to more negative values with increasing pH and showed two linear segments with their slopes of 34.0 and 84.0 mV within the pH 1.0–4.0 and 4.0–9.0, respectively. The linear segments can be expressed by the following regression equations:

\[ E_p(\text{mV}) = \begin{cases} -34.0p\text{H} - 760 & (\text{pH} 1.0–4.0), \quad r = 0.973 \\ -84.0p\text{H} - 554 & (\text{pH} 4.0–9.0), \quad r = 0.988 \end{cases} \]

![Fig. 1. DPP polarograms of 20.0 μmolL⁻¹ triflumizole at some selected pH’s.](Image)
The intersection point observed at pH 4.0 (Fig. 2), which corresponds to the $pK_a \pm 1$ value of triflumizole could be attributed to the acid-dissociation constant ($pK_a$) of triflumizole. The reported $pK_a$ of the triflumizole was 3.70 [15].

The pH dependence shows a reduction process involving addition of H$^+$ to the oxidized species. According to the structure of the pesticide molecule, the peak may correspond to the reduction of azomethine group via the well known 2e$^-$/2H$^+$ reduction mechanism of azomethine compounds [16]. This is common behavior for azomethine ($\overset{\text{C-N}}{\text{C-N}}$) containing organic compounds [16], and the peak probably corresponds to the reduction of the azomethine ($\overset{\text{C-N}}{\text{C-N}}$) group of triflumizole molecule. The mechanistic study was not pursued.

Fig. 3 shows the dependence of peak current of triflumizole on the pH of B–R buffer solution within the pH range of 1.0–9.0. The maximum sensitivity and response peak current was found at pH 2.0 comparing with other pH’s. The sensitivity of the peak currents in more acidic solution decreases slightly due to the background discharge at lower pH’s. As the pH approaches to neutral or moderately basic region, reduction peak decreases because of the controlling of the overall rate by protonation kinetics [17].

The effect of the other electrolytes on the DPP peak of triflumizole were also investigated by using the different unbuffered media, e.g., H$_2$SO$_4$, H$_3$PO$_4$, HClO$_4$, and HCl at pH 2.0. Therefore, we found that the peak current at pH 2.0 (HCl) was optimum, not only because of its highest sensitivity but also well resolved characteristics and suitability for analytical use.

3.2. Analytical methodology in supporting electrolyte

The optimum conditions for the analytical determination of the of triflumizole compound by DPP were found to be pH 2.0 at a reduction potential of $-810$ and 50 mV pulse amplitude, 5 mV s$^{-1}$ sweep rate, 1 s drop time, at 25 ± 3 °C. The consecutive additions of triflumizole to the pH 2.0 prepared with HCl in double-distilled water resulted in calibration curve displayed in Fig. 4. The peak currents obtained from the polarograms were linearly related to the pesticide concentration between 2.0 and 91.0 μmol L$^{-1}$, with the analytical equation given by:

$$I_p = 0.0112C - 0.061, \quad r = 0.993 \ (n = 10).$$

where $C$ is the concentration in μmol L$^{-1}$ and $I_p$ is the peak current in μA. The limit of detection (LOD) and limit of quantification (LOQ) were obtained as 0.72 and 2.39 μmol L$^{-1}$, respectively, according to the relation $k \times $S.D./$b$ (where $k = 3$ for LOD and $k = 10$ for LOQ, S.D. the standard deviation of the blank, and $b$ is the slope of the calibration curve). The straight line has a slope of 0.0112 μA/μmol L$^{-1}$, an intercept of 0.061 μA and a correlation coefficient of 0.993. The high sensitivity of differential pulse polarography is accompanied by very good repeatability. The precision from five repeated measurements of electrochemical signal of 20.0 μmol L$^{-1}$ triflumizole solution for supporting electrolyte was 5.87% (Table 1). These values confirmed the sensitivity of the proposed method for the determination of triflumizole.

3.3. Interference study

The influence of other commonly used electro-inactive pesticides; penconazole and prochloraz, and electroactive pesticide; thifen sulfuron methyl on the determination of 10.0 μmol L$^{-1}$ triflumizole has been evaluated. The degree of recoveries in...
the presence of equal amounts of penconazole, prochloraz, and thifen sulfuron methyl were 96.0 ± 1.2, 96.8 ± 2.4, and 90.3 ± 1.7%, respectively (n = 3). The relatively large interference effect for thifen sulfuron methyl could be attributed to its cathodic reduction peaks at potentials −880 and −950 mV, very close to the triflumizole peak and overlapped to some extent.

The influence of some cationic and anionic species, which are commonly found in soil and irrigation water, on the polarographic determination of triflumizole was investigated. The interference studies were performed using the various interfering ions, most of them are electro-active, e.g., Ni²⁺, Cu²⁺, Pb²⁺, Zn²⁺, Cr³⁺, Co²⁺, and Cd²⁺ and the others inactive, e.g., Mg²⁺, Ca²⁺, SO₄²⁻, NO₃⁻, F⁻, Cl⁻, and I⁻. The interfering ions were taken at the same concentrations and five times the amount of triflumizole. The degree of interference effects were treated as the ratio of the peak currents (by percentage) in the presence of the interfering ions to that in their absence (Table 2).

Under the optimum polarographic conditions for the determination 20.0 μmol L⁻¹ triflumizole, Cu²⁺ and Pb²⁺ appeared at less negative potentials, Ni²⁺ and Zn²⁺ at more negative potentials compared to the peak potential of triflumizole. Thus, the polarographic peaks of these ions did not overlap the pesticide peak. The recovery of 20.0 μmol L⁻¹ triflumizole was between 93 and 102% even five-fold excess of the mentioned ions. Co²⁺ did not seriously effect the triflumizole peak, since there was no peak response for this cation at the studied pH and concentrations.

Serious effects were observed in the presence of relatively higher concentrations of Cr³⁺ and Cd²⁺ since their peaks gave a shoulder near the triflumizole peak. The influence of interference effect in the presence of relatively high concentration Cd²⁺ could not be evaluated since the triflumizole peak was completely distorted. Mg²⁺, Ca²⁺, SO₄²⁻, and NO₃⁻ are polarographically inactive species and therefore had no serious effect on the polarographic peak of triflumizole. However, in the presence of relatively high concentrations of halide ions (F⁻ and I⁻) the peak current of the pesticide decreased markedly. Consequently, the degree of recoveries of triflumizole in the presence of five-fold F⁻ and I⁻ were 77 and 50%, respectively. This could be attributed to the saturation of the electro-active side of the pesticide molecule or oxidation of the I⁻ to the molecular iodine. The chloride ion had no significant effect on the triflumizole peak.

### 3.4. Determination of triflumizole in agrochemical product, soil and lake water

Validation of the proposed pulse polarographic method for the assay of triflumizole in agricultural dosages, soil and lake water were carried out via estimation of the range of linearity, the limit of detection (LOD), the limit of quantification (LOQ), repeatability, and selectivity. The accuracy of the developed method was checked by calculating the recovery of the known amount of triflumizole in agricultural pesticide formulation Trifmine® or spiked triflumizole to soil and lake water, and analyzed via the optimized differential pulse polarographic procedure.

#### 3.4.1. Agrochemical pesticide

Fig. 5 shows the differential pulse polarograms corresponding to the determination of triflumizole concentration in Trifmine®. As can be seen in Fig. 5, well-defined polarographic peaks allowed pesticide determination. To study the accuracy of the proposed method, and to check the possible interferences from common recipients, recovery studies were carried out. For these experiments, known amounts of the pure drug were added to the earlier analyzed formulation of Trifmine®. Each measurement was repeated four times. These data gave an average triflumizole content of 30.23 ± 1.59% for DPP, in close agreement with the 30.0% quoted by the manufacturer. The nominal content of the drug was calculated from the correspond-

### Table 2

Influence of interfering ions on the peak current of 20 μmol L⁻¹ triflumizole

<table>
<thead>
<tr>
<th>Concentrations of interfering ions (μmol L⁻¹)</th>
<th>Interfering ions and their influence on signal ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Ni²⁺, Cu²⁺, Pb²⁺, Zn²⁺, Cr³⁺, Co²⁺, Cd²⁺, Mg²⁺, Ca²⁺, SO₄²⁻, NO₃⁻, F⁻, Cl⁻, I⁻</td>
</tr>
<tr>
<td>100</td>
<td>Ni²⁺, Cu²⁺, Pb²⁺, Zn²⁺, Cr³⁺, Co²⁺, Cd²⁺, Mg²⁺, Ca²⁺, SO₄²⁻, NO₃⁻, F⁻, Cl⁻, I⁻</td>
</tr>
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* Overlap.
The proposed polarographic method applied for the analysis of Trifmine® needs no filtration of pesticide extract from undissolved recipients; just dilution of an aliquot from the supernatant layer with the pH 2.0 solution is required before each measurement. HPLC was also applied to the formulation Trifmine® to check the validity of the proposed method.

The results obtained were compared statistically with HPLC using Student’s t-test and variance ratio F-test (Table 3). Statistical analysis of the results by both methods using the Student’s t-test and variance ratio F-test, show no significant difference between the performance of the two methods regarding the accuracy and precision, respectively. The experimental values of t and F at 95% confidence level did not exceed the theoretical ones indicating the good agreement with the HPLC.

On the other hand, the main advantage of the proposed DPP method over the HPLC is that the DPP method offers high sensitivity, low limit of determination, easy operation and simple instrumentation.

3.4.2. Spiked soil and lake water

The present optimized procedures were also successfully applied to determination of triflumizole spiked to soil and lake water. The relationship between peak current ($I_p$) and concentration of triflumizole is rectilinear for both soil and lake water over the range cited in Table 1. Linear regression analysis of the data gave the following equations,

$$I_p = \begin{cases} 0.0114C + 0.049, & r = 0.997 \\ 0.0132C + 0.206, & r = 0.994 \end{cases}$$

for soil and lake water, respectively; where C is the concentration in μmol L$^{-1}$, and $I_p$ is the peak current in μA. The precision from five repeated measurements of electrochemical signal of 20.0 μmol L$^{-1}$ triflumizole for soil and lake water were 5.16 and 3.52%, respectively. These values confirmed the sensitivity of the proposed method for the determination of triflumizole in spiked samples.

The reliability of the proposed procedure for the determination of the triflumizole in agrochemical pesticide Trifmine®,
soil, and lake water was checked using spiked triflumizole. Fig. 6 shows the differential pulse polarograms corresponding to the determination of some triflumizole at selected concentrations in soil sample. The recoveries were estimated by measuring the peak heights of extracted spiked pesticide, soil or lake water samples and comparing them with the peak heights obtained after the standard additions of the same concentrations. The results are shown in Table 4. Recoveries calculated from pesticide formulation, soil and lake water samples are 102.8 ± 1.62, 102.1 ± 1.03, and 103.6 ± 2.78% with the relative standard deviations of 1.57, 1.01, and 2.68%, respectively. The results are satisfactorily accurate and precise.

4. Conclusion

The differential pulse polarographic method presented for the quantitative determination of triflumizole allows the accurate determination in pesticide formulation, soil and natural water sample and was found to be simple and highly sensitive. The main advantage of such a procedure is the possibility to determine the concentration of the active component directly from the pesticide formulation, soil or water without the need for any prior steps such as extraction, clean-up, or pre-concentration which are tedious, time consuming, and also polluting. Moreover, no sophisticated instrumentation is needed. The present method could possibly be applied for the determination of triflumizole in other environmental samples as well as for quality control laboratories.

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References