Electrochemical Simultaneous Analysis of Fenitrothion and Its Metabolites

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INTRODUCTION

Gas-liquid chromatography is usually used for analysis of residues of the organophosphorus insecticide fenitrothion (I) [O, O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate] in foods and environmental samples. Since natural products sometimes interfere the detection of peaks given by a pesticide in chromatograms, a cleanup procedure is usually necessary before analysis.

Electrochemical methods have been successfully used as a basis for analysis of pesticides possessing a moiety capable of oxidation or reduction. In many cases they are less liable to interference, and when co-extractives are electrochemically inactive or undergo a redox reaction at a different potential, laborious cleanup steps can be omitted.

(I) and its major metabolites, O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphate (II) and 3-methyl-4-nitrophenol (III), possess the nitro group in the 4-position of the phenyl ring, which is susceptible to electrochemical reduction. We developed residue analytical methods for (I), (II) and (III) by employing differential pulse polarography and cyclic voltammetry using a glassy carbon electrode. We also examined a possibility of distinguishing (I), (II) and (III) by using the differences in hydrolysis rates and reduction potentials among (I), (II) and (III), with an aim at establishing a “simultaneous” analytical method.

MATERIALS AND METHODS

(I), (II) and (III) were synthesized at the laboratory of Sumitomo Chemical Co., Ltd. and purified by thin-layer chromatography (silica gel 60 F254, E. Merck), as previously reported. A supporting electrolyte, tetraethylammonium perchlorate (TEAP) was purchased from Tokyo Kasei and used without further purifications. Britton-Robinson (BR) buffer solutions (0.04 M) at desired pH were prepared by mixing stock solutions A (3.92 g of 85% phosphoric acid, 2.40 g of 96% acetic acid and 2.47 g of boric acid dissolved in 1.0 l of distilled water) and B (0.2 M aqueous sodium hydroxide solution) at different ratios.

Electrochemical analyses were carried out with a Yanaco P-1100 voltammetric analyzer in conjunction with a three-electrode cell system possessing a glassy carbon working electrode (Yanaco GC-P2, 7.1 mm²) and a platinum counter electrode. The Ag/Ag⁺ (0.01 M AgNO₃ acetonitrile solution containing 0.1 M TEAP) electrode and a saturated calomel electrode (SCE, Yanaco MR-P2-05-1) were used as references of potential in acetonitrile and BR buffer, respectively. Current-potential curves in cyclic voltammetry and differential pulse polarography were recorded with a GRAPHTEC A4/X-Y recorder (Model WX2400).

The acetonitrile solution containing 0.1 M TEAP or BR buffer (6.0 ml) was transferred to an electrochemical cell and aspirated with N₂ gas (>99.99%) for 10–45 min. A 1–10 µl aliquot of (I), (II) or (III) (1–2 × 10⁻⁴ M in acetonitrile) was added, and the solution was further aspirated with N₂ gas for 45 sec. Electrochemical measurements were conducted under a nitrogen atmosphere.

Hydrolysis rates of (I) and (II) were determined in 0.05 M NaOH aqueous solution at 25°C. Current-potential curves from −0.4 to −1.4 volt (vs. SCE) in the differential pulse polarographic mode were periodically recorded for up to 60 min. The hydrolysis rate constants of (I) (k₁) or (II) (k₂) were estimated by plotting log (ipt/ipt') against (t−t'). Expressions ipt and ipt' are the currents (µA) at peak potentials due to (I) or (II), which are recorded at time t and t', respectively. In this experiment, t' was 2–3 min after (I) or (II) was added to 0.05 M NaOH. Differential pulse polarograms of the mixtures of (I), (II) and (III) were measured in a similar manner.

RESULTS AND DISCUSSION

1. Reduction of Nitro Group

The cyclic voltammograms of (I) and (II) in...
Acetonitrile at a scan rate of 50 mV/sec showed that cathodic peak potentials ($E_p$) were between -1.57 and -1.58 volt (vs. Ag/Ag⁺) and that the anodic peak potentials were between -1.46 and -1.48 volt (vs. Ag/Ag⁺), respectively (Fig. 1). Cathodic half-wave potentials ($E_{p/2}$) of (I) and (II), where half of the cathodic peak current was detected, were graphically determined between -1.51 and -1.52 volt (vs. Ag/Ag⁺) from Fig. 1. Since the difference between $E_p$ and $E_{p/2}$ is 65-70 mV, the observed electrochemical reaction of (I) or (II) is considered one-electron quasi-reversible reduction. In contrast, (III) showed a cathodic peak only at -1.45 volt (vs. Ag/Ag⁺), and its $|E_p - E_{p/2}|$ was 140-160 mV (Fig. 1). For an irreversible electrochemical reaction, the following relationships can be cited,$^5$

$$|E_p - E_{p/2}| = \frac{47.7}{n} \text{ (at 25°C)} \quad (1)$$

$$\ln(i_p) = \frac{nF(E_p - E_o)/RT + A}{R} \quad (2)$$

where "n" is the number of electrons in the molecule participating in an electrochemical reaction and "a" is a transfer coefficient. $F$, $R$, $A$ and $T$ are the Faraday constant, gas constant, the constant determined from the sweep rate and temperature in $K$, respectively. $E_o$ is the initial potential where no current due to a chemical is observed. Current-potential curves at scan rates of 20, 50, 100 and 200 mV/sec were measured to estimate the "zn" value. The results are listed in Table 1. "zn" calculated from Eq. (1) was 0.92 ± 0.02. The least-square approximation method was applied to the $i_p$ vs. $(E_p - E_o)$ by using Eq. (2), and the following relationship was obtained, with $r$ as the correlation coefficient.

$$\ln(i_p) = -14.11(E_p - E_o) - 18.1 \quad (r^2 = 0.97)$$

From the value of a slope in the above equation, "zn" was calculated 0.36 at 25°C. Since "a" is considered 0.3-0.5,$^6$ the cathodic peak of (III) seems to arise from a one-electron reduction of (III). By analogy with the electrochemical properties of nitrobenzene,$^7$ $^8$ these results show that (I), (II) and (III) undergo a one-electron reduction of a nitro group in acetonitrile.

By utilizing the one-electron reduction of a nitro group in acetonitrile, the electrochemical analyses of (I), (II) and (III) were carried out in differential pulse polarography. The scan rate and pulse potential were 5 mV/sec and 50 mV, respectively. (I), (II) and (III) showed cathodic peaks at -1.52, -1.53 and -1.40 volt (vs.

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**Table 1** The potential and current at the cathodic peak of (III) at various scan rates in acetonitrile.

| Scan rate (mV/sec) | $E_p$ a) | $i_p$ b) | $|E_p - E_{p/2}|$ c) | $E_p - E_o$ c) |
|-------------------|---------|---------|----------------|----------------|
| 20                | -1.460  | 9.48    | 140            | -460           |
| 50                | -1.485  | 14.40   | 155            | -485           |
| 100               | -1.510  | 19.45   | 150            | -510           |
| 200               | -1.540  | 26.00   | 160            | -540           |

a) volt vs. Ag/Ag⁺.
b) in µA.
c) in mV.
$E_o$: -1.0 volt vs. Ag/Ag⁺.
Concentration of (III): 1.08 x 10⁻⁴ M.
Ag/Ag⁺), respectively. There was good linearity between the concentration of any one of the three chemicals and the peak current in a concentration range of 0.5–300 ppm, but the cathodic peak potentials of (I) and (II) were too close to be separated. Therefore, the pH dependence of $E_p$ was examined by using BR buffers, as shown in Fig. 2. Slight breaks were observed in the $E_p$ vs. pH plots at pH 5 and 8. It seems that a hydroxylamine intermediate formed by electrochemical reduction of the nitro group is protonated at these pH values.⁷ The $E_p$ of (III) is significantly different from those of (I) and (II) in a pH range of 5–10 (100–200 mV). However, since (I) and (II) still showed a similar $E_p$ over the pH range tested, the reliable amount of (I) or (II) cannot be determined separately by electrochemical analysis when these two chemicals generate together. In the meantime, the $i_p$ ($\mu$A) vs. concentration $C$ (ppm) plot was linear for all three compounds (Fig. 3). The detection limit was 30–50 ppb depending on the product. As our study showed, differential pulse polarography can be used to analyze (I), (II) and (III) quantitatively, with a high sensitivity over a wide range of concentrations, if they are present singly or separated before analysis by column chromatography as previously reported.⁷

2. Simultaneous Analysis

Based on the log $(i_p/t)/i_p$ vs. $(t-t')$ plots, the hydrolysis rates of (I) ($k_I$) and (II) ($k_{II}$) in 0.05 M NaOH were estimated $(2.59 \pm 0.29) \times 10^{-2}$ min⁻¹ ($r^2=0.97$) and $(2.98 \pm 0.31) \times 10^{-2}$ min⁻¹ ($r^2=0.99$), respectively, by the least-square approximation method. Since hydrolysis of both (I) and (II) obeys first-order reaction kinetics under the conditions tested,⁹ their half-lives of disappearance must be $114.1 \pm 5.5$ min and $9.67 \pm 0.68$ min, respectively.

When the concentration of NaOH was reduced to 0.005 M, hydrolysis hardly occurred within an hour. Furthermore, no significant change in peak potential was observed in the current-potential curves when differential pulse polarograms were measured in 0.05 M and 0.005 M NaOH. The $E_p$ values of (I), (II) and (III) were $-0.77$, $-0.77$ and $-1.10$ volt (vs. SCE), respectively. Based on these results, the $i_p$ ($\mu$A) vs. $C_0$ (ppm) calibration curves in 0.005 M NaOH were prepared for (I), (II) and (III) as shown below and used to calculate each of the concentrations at time 0 in 0.05 M NaOH.

\[
i_p(I)=0.131 C_0(I)+0.0272 \quad (3)
\]
\[
i_p(II)=0.0767 C_0(II)+0.0338 \quad (4)
\]
\[
i_p(III)=0.0452 C_0(III)-0.0256 \quad (5)
\]

The correlation coefficient was 0.99 for each chemical. The differential pulse polarogram of the mixtures of (I), (II) and (III) measured in 0.05 M NaOH showed two peaks at $-0.77$ and $-1.10$ volt (vs. SCE). Since (I) and (II) have the same peak potential, the observed current $(I_p)$ at $-0.77$ volt is the sum of those due to (I) and (II), as expressed in Eqs. (6) and (7).

\[
I_p=i_p(I)+i_p(II) \quad (6)
\]
It is the peak current measured in 0.05M NaOH at time t. Since $i_{pt}$ can be expressed by using the $i_{po}$ and the hydrolysis rate constants ($h_I$ and $h_{II}$), Eq. (7) can be rewritten as Eq. (8).

$$I_{pt} = i_{po}(I) + i_{po}(II)$$ (7)

$$I_{pt} = i_{po}(I) \times 10^{-T} + i_{po}(II) 10^{-T}$$ (8)

The estimation of initial concentrations $C_0$ (I) and $C_0$ (II) is thus possible by solving Eqs. (3), (4), (6) and (8) when $i_{po}$ and $i_{pt}$ are measured electrochemically. Separate calculation of $C_0$ (III) is also possible from the peak current at $-1.10$ volt in 0.005 m NaOH by using Eq. (5). In this way the concentrations of (I), (II) and (III) can be determined if the samples are analyzed by differential pulse polarography in 0.005 and 0.05 m NaOH. In order to validate this electrochemical method, the mixtures of (I), (II) and (III) at two different concentrations were analyzed for the three components simultaneously. The typical differential pulse polarograms are shown in Fig. 4. Measurement was repeated four times, and the results are expressed as the averaged values in Table 2. The experimental values of (I), (II) and (III) were in accord with the fortification levels within $\pm 10\%$ error.

The above results demonstrate the feasibility of using electrochemical procedures as an alternative basis for a "simultaneous" analytical procedure for fenitrothion and its two main metabolites.

**REFERENCES**


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要 約
フェニトロチオンとその代謝物の電気化学的同時分析
片木敏行, 三上信司, 松田 正
フェニトロチオンならびに, 環境中での主代謝物であるオキソ体, 3-メチル-4-ニトロフェノールについての同時分析法を検討するために, まずサイクリックボルタントメトリーによりニトロ基の還元について調べた。さらに, フェニトロチオンとオキソ体について加水分解反応速度の差を, 3-メチル-4-ニトロフェノールと他の 2 化合物についてはニトロ基の還元電位の差を利用し, 0.05 M 水酸化ナトリウム水溶液中, 微分パルスポータログラフ法による 3 化合物混合系での同時分析法を検討した。その結果, 約 10% の誤差範囲内で各化合物の濃度を求めることができた。