Differential Pulse Polarographic Method for the Determination of Thiophanate-Methyl in its Fungicide Formulation (Topsin M) and Agricultural Produces

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Abstract: The voltammetric behaviour of the thiophanate-methyl in its fungicide formulation (Topsin M) and agricultural produces has been studied in the presence of surfactant, Triton-X-100 in acetonitrile medium at dropping mercury electrode (DME) by differential pulse polarography (DPP). The proposed DPP method based on the investigation that the electrochemical response of thiophanate-methyl facilitated in presence of Triton X-100 (0.1%V/V) at the DME electrode surface in term of enhanced peak current as well as sharpening of diffused controlled peak at 140 mV (vs SCE.). The method has successfully been applied to both commercial fungicide formulation and agricultural produces. Recoveries of the active ingredient from formulation analysis and fortified agricultural produces samples (wheat, rice and apple fruit) were good, ranging from 88.8-101 % with maximum RSD value of 0.98 %.

Keywords: Thiophanate-methyl, differential pulse polarography; Dropping mercury electrode (DME), formulation analysis.

1. Introduction

Thiophanate-methyl [1,2-bis (3-methylcarbonyl-2-thioureido) benzene (Figure 1)] is a systemic fungicide based on a bis-thiourea derivative. It exhibits a rather broad antifungal spectrum and is very effective for the control of Cercospora leaf spot, powdery mildew, Sclerotinia rot, Botrytis mold of various crops as well as apple and pear scab and sheath blight of rice [1],[2]. Though non-phytotoxic, it has a long residual effect due to its stability under the sunlight [3]. In view of the wide use of this fungicide in agriculture, there is a need of a convenient, reliable and sensitive method for its analysis not only at the formulations (for the purpose of quality control) but at residual level (for monitoring residues in different environmental samples in order to ensure safe and judicious use of pesticides) as well.

Various methods mostly HPLC and spectrophotometric [4]-[6] used for the analysis of thiophanate-methyl. Commonly employed method [5] for its determination involves its reaction with an excess of copper(II)-triethanolamine-alkali(alkali-amine-copper) reagent. The insoluble product is removed by filtration and the unconsumed copper(II) in the filterate is reacted with pyridine-thiocyanate reagent. The coloured product is extracted in chloroform and absorbance of the extract measured at 435nm. In another method, the sample is reacted with copper(II) and absorbance of the product formed in colloidal suspension, measured at 390 nm (a shoulder between 370 and 400nm in the spectrum of the colloidal suspension). The first method besides being indirect (based on the measurement of excess reagent) is tedious and time-consuming; it involves steps like filtration and solvent extraction, which if not performed with utmost care could result in the loss of material and thus vitiate the results. With regard to the second method, it may be mentioned that the methods based on the absorption of colloidal suspensions are always feared to be of doubtful accuracy because a large error generally arises from the difficulty of preparing and maintaining all standards and unknown suspensions in a uniform and reproducible degree of dispersion.

Polarographic technique has emerged as a reliable and sensitive tool for trace analysis of pesticides [8]-[15]. Though oxidation behaviour for some thioureas has been investigated voltammetrically [10],[17] but thiophanate-methyl has not been studied much. In a significant effort in this direction , we have been able to work out a remarkably sensitive differential pulse polarographic method for the determination of thiophanate-methyl by exploiting electrochemical oxidation properties of thiourea moiety of this compound at DME in presence of surfactant, Triton-X-100 in acetonitrile.
2. Experimental

2.1 Reagents and standards

Acetonitrile (Merck) was kept over phosphorus pentoxide (5 g L⁻¹) and distilled twice. Tetraethylammonium perchlorate (TEAP) was prepared by the reported method [17].

Its standard solution was prepared by dissolving 2.296 g of pure compound in one litre of acetonitrile. Triton-X-100 polarographic cell containing acetonitrile (0.1% in acetonitrile) were taken in each of solution at room temperature (25±1°C). Cyclohexane (Merck) and ethylacetate (Merck) were used as eluting solvent. Silica gel (60-120 mesh, Sisco Research) was heated at 500 °C for 4 h and stored at 120 °C. The analytical standards of thiophanate-methyl, EPA (Environmental protection agency), was used. The commercial formulation of thiophanate methyl (Topsin M, 75 %) was procured from agrochemical retail shop of the local market.

2.2 Instrumentation

The polarographic measurements were made with an ELICO (India) Pulse Polarograph (model CL-90) with polarcard recorder (Model LR-180) equipped with DME as working electrode, SCE as reference electrode and a coiled platinum wire as an auxiliary electrode. All the polarography experiments were done in an inert atmosphere achieved by purging the cell solution with pure nitrogen for 5 min. All the differential polarographic studies were performed under following optimized and set instrumental parameters for each of solution at room temperature (25±1°C).

Table 1: Optimized and set instrumental parameters for DPP studies

<table>
<thead>
<tr>
<th>ELICO Pulse Polarograph parameters</th>
<th>Polarocard scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial potential=+400mV Final</td>
<td>X-axis mV</td>
</tr>
<tr>
<td>Potential= -200 mV</td>
<td>Y-axis mV/cm</td>
</tr>
<tr>
<td>Pulse amplitude= 50mV Sensitivity</td>
<td>1 µA/V</td>
</tr>
<tr>
<td>Drop time= 1 s</td>
<td>mV/cm</td>
</tr>
</tbody>
</table>

2.3 Differential Pulse Polarographic (DPP) procedure

2.3.1 Preparation of calibration graphs for thiophanate-methyl

Aliquots (0.1-2.0 mL) of the stock solution (0.001 M in acetonitrile) of thiophanate-methyl were taken in each of polarographic cell containing acetonitrile. Triton-X-100 (1 mL, 0.1% in acetonitrile) were added to one of polarographic cell and final volume were made to 50 mL with TEAP (0.01 M in acetonitrile). Nitrogen gas was passed through each solution for 5 min and differential pulse polarogram was recorded with the above instrumental parameters (Table 1). Similarly in other polarographic cell, aliquots (0.1-2.0 mL) of the other stock solution (0.005 M in acetonitrile) of thiophanate-methyl were taken without adding Triton-X-100 and final volume were made to 50 mL. Finally solutions were processed in same way as described above. Typical differential pulse polarograms of thiophanate-methyl in absence and presence of Triton X -100 were recorded and shown in Figure 2 and 3.

Calibration graph was constructed by plotting peak current, Iₚ (µA) versus of thiophanate-methyl (µg) added to polarographic cell in presence of Triton –X-100 under above optimized experimental conditions (Figure 4). The electrochemical and calibration characteristics of obtained from differential pulse polarographic studies are presented in Table 2 and 3.

2.3.2 Commercial Formulation analysis

A single large sample (Topsin-M, containing 70% active ingredient) equivalent to 4.28 mg of thiophanate-methyl was weighed, shaken with acetonitrile and filtered. The residue (if any) was washed 2-3 times with acetonitrile. The filtrate and washings were diluted to a known volume (25 mL) with the same solvent. Suitable aliquots of the solution were taken and processed for analysis in similar fashion as described in above section. The results are given in Table 4.

2.3.3 Recovery analysis

The samples of wheat and rice (5 g) were mixed with various amounts of standard solution of thiophanate-methyl (in acetonitrile). The samples were mixed well and extracted with 4 installments of 3 mL acetonitrile. The combined extracts were diluted to 20 mL with acetonitrile. Each resulting solution was transferred into the polarographic cell, and processed similar to calibration graph.

In case of apple fruits, 20 g sample was taken in containers and sprayed with various amounts of thiophanate-methyl (in acetonitrile). The samples were mixed well and blended with acetonitrile and filtered through Buchner funnel. The solid residues were washed 4 times with sufficient amount of acetonitrile and combined extracts cleanup on silica gel column using cyclohexane-ethylacetate (1:1 V/V) mixture as eluting solvent. The elute was concentrated by evaporation on hot water bath and residues were dissolved in acetonitrile and processed for analysis as described above. Assay results are given in Table 5.

3. Results and Discussion

3.1 Basis of proposed DPP method

The method is based on the observations that thiophanate-methyl gives an anodic polarographic peak at 150 mV( Figure 2) due its...
oxidisable behaviour at DME, working electrode using tetraethylammonium perchlorate (TEAP) as supporting electrolyte in acetonitrile medium. It is, however, interesting to observe that when thiophanate-methyl is added incrementally in presence of 0.1% Triton-X-100 (surfactant) and reaction studied quantitatively at DME, diffusion-controlled peak shifted slightly towards negative potential and appeared at 140 mV (Figure 3) with

The remarkable sensitivity of the new peak at 140 mV coupled with the excellent linear relationship obtained between concentration of thiophanate-methyl added and current intensity of anodic peak provides a sensitive differential pulse polarographic method for the determination of thiophanate-methyl (Figure 4).

Table 2: Electrochemical characteristics of thiophanate-methyl solution at DME during differential pulse polarographic (DPP) studies

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Differential pulse polarographic (DPP) studies at DME</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In Acetonitrile (In absence of Triton-X-100)</td>
</tr>
<tr>
<td>Peak Potential, ( E_p ) (mV)</td>
<td>150</td>
</tr>
<tr>
<td>Half wave Potential, ( E_{1/2} ) (mV)=( E_p ) (mV) - 50/2</td>
<td>125</td>
</tr>
</tbody>
</table>
The method has successfully been applied to both commercial fungicide formulation and agricultural produces

3.2 Effect of Solvent and supporting electrolyte

Determination by non-aqueous polarography is advantageous as thiophanate-methyl shows poor solubility in water but it also undergoes decomposition in an aqueous medium containing acids or bases (commonly employed in aqueous polarography in the form of acidic/basic buffer or acidic/alkaline electrolytes). Acetonitrile has been a solvent of choice in the present studies because of its convenient liquid range, ready availability, wide electrochemical range, moderate dielectric constant (facilitating the solubility of this compound) and low toxicity. Since the solvent and supporting electrolyte can significantly affect the redox properties and voltammetric responses, the choice of proper solvent-electrolyte system becomes all the more important [18],[19]. Among the various supporting electrolytes such as lithium chloride, sodium perchlorate, tetrabutylammonium iodide studied, TEAP has been found suitable supporting electrolyte in acetonitrile. Using this system, a linear base line having a plateau parallel to the potential axis was obtained over quite a wide range of potential. The polarographic behavior of thiophanate-methyl and formulated product based on it, in the presence of Triton-X-100 at DME yielded well defined, more sensitive diffusion-controlled peak at 140 mV against SCE electrode at.

3.3 Effect of surfactant - Triton-X-100

In the present study Triton-X-100 was selected because of its capability to suppress the undesirable peaks of impurities and to prevent adsorption of oxidation products which generally interfere in analysis. Secondly, addition of optimized levels of Triton-X-100 is also reported to sharpen and increase the height of the analyte peak with slight shift in peak potential [20]. Figure 5 shows the results obtained for the peak current for different %age of Triton-X-100 (V/V) added to the solution. It was possible to use high concentrations of Triton without problems of foam formation. For low percentages of Triton, adsorption effects occur and the reproducibility was not good; at high percentages greater adsorption of the surfactant itself on the electrode surface caused diminution of the peak current, but good reproducibility was obtained. Addition of 0.1% Triton to the solution was found to be most suitable and in these optimized conditions. Similar results were obtained in present study due to apparently facilitating the oxidation mechanism and producing an associated increase in the peak height.

3.4 Validation of Proposed Method

3.4.1 LOD and LOQ

According to International conference on Harmonization (ICH) guidelines, following expressions are applied to evaluate limit of detection (LOD) and limit of quantitation(LOQ): LOD =3 SD/b and LOQ = 10 SD/b where SD is standard deviation of intercept and b is slope of regression line. The calculated LOD and LOQ are found to be 0.1 X 10^{-6}g and 0.3 X10^{-6}g respectively as shown below in Table 3.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.1 X10^{-6}g</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.3 X10^{-6}g</td>
</tr>
<tr>
<td>Linear range, µg/mL</td>
<td>0.5-16</td>
</tr>
<tr>
<td>Slope</td>
<td>0.2043</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.019</td>
</tr>
<tr>
<td>Correlation coefficient , R²</td>
<td>0.9873</td>
</tr>
</tbody>
</table>

3.4.2 Specificity

Specificity is ability of any analytical method to measure analytical response in the presence of all possible and potential impurities. For such test, DPP voltammogram of standard solution of commercial formulation excipients (additives) were recorded. Such additives exhibited no electroanalytical response in applied controlled potential range. Response of analyte in presence of excipients was compared with standard pure thiophanate methyl and found to be stable.
3.4.3 Stability
In the this voltammetric study, standard of pure thiophanate methyl stock solution was kept in dark at 4°C for 20 days in refrigerator and were analyzed at every day (at different time). Reproducibility of peak current of stock solution of thiophanate methyl was observed for 10 days and after that peak current decreased significantly.

3.4.5 Application of the proposed method
Electroanalytical peak at 140 mV appeared in the voltammetric study has been exploited and made the basis of the proposed differential pulse polarographic method. The method has been successfully applied to the analysis of a formulation of thiophanate-methyl for its active ingredient content. The recoveries were the range 97.6-101% with RSD’s in the range 0.64-0.88% for formulation analysis (Table 4).

Table 4: Assay of a commercial formulation of thiophanate-methyl containing 70% active ingredient

<table>
<thead>
<tr>
<th>Active ingredient taken (µg)</th>
<th>Mean peak Current obtained, ( I_p(µA) )</th>
<th>Amount found (µg)</th>
<th>Recovery* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.119</td>
<td>0.49</td>
<td>98.0± 0.78</td>
</tr>
<tr>
<td>1.0</td>
<td>0.183</td>
<td>0.98</td>
<td>98.0± 0.67</td>
</tr>
<tr>
<td>2.0</td>
<td>0.385</td>
<td>1.98</td>
<td>99.0± 0.64</td>
</tr>
<tr>
<td>4.0</td>
<td>0.844</td>
<td>4.04</td>
<td>101± 0.74</td>
</tr>
<tr>
<td>8.0</td>
<td>1.612</td>
<td>7.80</td>
<td>97.5± 0.70</td>
</tr>
<tr>
<td>16.0</td>
<td>3.188</td>
<td>15.70</td>
<td>98.12±0.88</td>
</tr>
</tbody>
</table>

*Values are mean of five determinations with relative standard deviations (±)

Recoveries of the fungicide from fortified grain samples (wheat and rice) and apple fruit were good, ranging from 88.8-98.4% with RSD’s in the range 0.69-0.98 % (Table 5).

Table 5: Recovery of thiophanate-methyl from fortified samples

<table>
<thead>
<tr>
<th>Active ingredient added (µg)</th>
<th>Wheat</th>
<th>Rice</th>
<th>Apple</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>94.2±0.69</td>
<td>96.6± 0.92</td>
<td>92.2± 0.98</td>
</tr>
<tr>
<td>3.0</td>
<td>97.8±0.94</td>
<td>97.0± 0.72</td>
<td>89.8± 0.87</td>
</tr>
<tr>
<td>6.0</td>
<td>98.4± 0.80</td>
<td>95.8± 0.78</td>
<td>90.8± 0.80</td>
</tr>
<tr>
<td>12.0</td>
<td>95.6± 0.79</td>
<td>98.2± 0.84</td>
<td>92.4± 0.96</td>
</tr>
</tbody>
</table>

*Values are mean of five determinations with relative standard deviations (±)

4. Conclusions
In our efforts to explore the electroanalytical aspects using voltammetry, we found differential pulse polargraphy as an emerging and remarkably sensitive technique for determination of thiophanate methyl. The electrochemical response of thiophanate-methyl facilitated in presence of Triton X -100 (0.1% V/V) at the DME electrode surface in term of enhanced peak current and sharpening of diffused controlled peak at 140 mV.

The simplicity and rapidity (due to non requirement of extraction apparatus for colored product, a common practice in colorimetric analysis) of the procedure: remarkable sensitivity of the technique and its use for both formulation and agricultural produces is significant advantages, which the method possesses over the colorimetric methods. That the method is indeed reproducible is evident from the good precision data (in terms of RSD’s) obtained in the analysis of the fungicides.

References


Author Profile

Presently Dr. Chetan Chauhan is working as an Associate Professor in the Department of Chemistry, College of Natural and Computational Science, Dilla University since Oct., 2011. Dr. Chauhan did his Ph.D (Chemistry) under the supervision of Prof. D.K. Sharma in 2006 from H.P. University, Shimla. He has served as an Assistant Professor in various reputed universities including Panjab University, Chandigarh; Punjabi University, Patiala and Shoolini University (Dec., 2006- Oct., 2011). His core area of research includes environmental and atmospheric chemistry specifically the pollution and monitoring of pesticides, heavy metals and surface ozone. He is also a recipient of NESA Junior Scientist Award of the Year -2006 (awarded by National Environmental Science Academy). During his academic tenure, Dr. Chauhan has guided two M.Sc students, three M.Phil students and one Ph.D student (for first two year). He has published 15 research paper in the journal of international and national repute including Journal AOAC International, Atmospheric Environment and Pesticide Research. He has presented 18 research paper in different international and national conferences. He is a life member of various science societies like Indian Society for Electroanalytical Chemistry (ISEAC), National Environmental Science Academy (NESA), Punjab Academy of Sciences, Indian Society of Analytical Scientists –Delhi Chapter. He is also the editorial member for the Journal of Applied Chemistry and Research (JACR).