Chapter - 4
INTRODUCTION

Organophosphates are organic esters of phosphoric acid, thiophosphoric acid and other phosphoric acids. The organophosphorus compounds, first developed by Gerhard Scharder in Germany, are employed over the world for their broad insecticidal spectrum and short persistence in the environment (1,2). After the banning of organochlorinate pesticides due to their high persistence in the environment, organophosphorus pesticides (OPs), which have higher degradation rates, are currently the most employed pesticides in agriculture against several types of pests (3). Malathion also known as Carbophos or Mercaptothion is an important and widely used contact insecticide and acaricide for the control of aphids, red spider mites, leafhoppers and thrips on a wide range of vegetable and other crops. It is also used to control insect vectors like mosquitoes (4). In addition to its use for mosquito control, it has registered residential uses for insects on lawns, gardens and ornamental trees, shrubs and plants (5). Phorate also known as Thimet or 3911 is a widely used systemic insecticide against various sap feeding insects and active mites (6). The growth of rice was also reported to be stimulated by the application of phorate (7). Dimethoate also known as Rogor or Dimor, is another widely used systemic insecticide and acaricide. It is mainly used against fruit fly and olive fly (8,9). It is also highly toxic to mosquitoes (10).

Organophosphorus pesticides are characterized by their higher toxicity, which inhibits the enzyme acetyl-cholinesterase (an important neurotransmitter), and for remaining in food products due to their indiscriminate application by farmers (3, 11-12). They are rapidly absorbed by practically all routes including the gastrointestinal tract, skin, mucous membranes, and lungs (4). Essentially all OPs are nerve poisons that cause severe toxicity to the infected organism. The OPs cause the inhibition in activity of both red blood cell and plasma cholinesterase (13). Cholinesterase inhibition in humans can overstimulate the nervous system and result in nausea, dizziness, confusion and at very high exposures (e.g., accidents, major spills), respiratory paralysis and death (5). They are a common means of self-harm in the developing world (14). It has been reported that there were marked changes in
the pesticides responsible for the majority of deaths in Anuradhapura hospital, Sri Lanka, due to pesticide poisoning during the 1990s. At the beginning of the decade, OP insecticides predominated, with both the class I OPs and the class II OPs causing many deaths. Currently the majority of deaths are due to class II OPs, in particular the dimethyl OPs fenthion, dimethoate and paraquat (15). Malathion has been reported in soft drinks. The average concentration of malathion reported in soft drink samples was 0.17 ppb, which is 1.7 times the Bureau of International Standards limit for the individual pesticide in soft drinks (4). Malathion has low toxicity to birds and mammals and is not expected to pose a hazard to them (16). It degrades rapidly in the environment, especially in moist soils. But there are some environmental concerns with malathion. It is highly toxic to insects and to aquatic organisms, including fish (5). The pesticide tolerance set by the Food and Drug Administration ranges from 1 - 8 μg ml⁻¹ for all these pesticides (17).

Due to wide applicability and high toxicity, numerous methods like titrimetric (18), voltammetric (19), potentiometric (20), polarographic (21), flow injection amperometric (22), flow injection analysis (23-24), mass spectrophotometric (25), gas chromatography (26), GC-FPD (27-30), GLC-NPD (31), SPME-GC (32-35), SPE-GC, SDME-GC (36), matrix solid-phase dispersion and gas chromatography (37), membranec extraction (38), GC-MS (39-42), HPLC (43), TLC (44), liquid chromatography (45-46), LC-MS (47-48), matrix solid phase dispersion biosensors (49), capacitive field-effect sensor (50), crosslinked sol-gel SPME fiber (51), through oven transfer adsorption desorption (TOTAD) interface (52), electron ionization LC-MS interface (53) and stir bar sorptive extraction (54) have been reported in the literature for the determination of organophosphates. A few spectrophotometric methods (55-61) have also been reported. Most spectrophotometric procedures involve the determination of organophosphorus pesticide by total phosphorus measurement, based on the development of blue color when a heteropoly acid formed by the union of phosphate and molybdate is treated with a reducing agent (58-59). Some of these methods suffer from poor sensitivity, instability of color or involve extraction whereas others suffer from interference from arsenic and copper, blank absorption or longer time required for color
development. To overcome some of these drawbacks a rapid and sensitive method has been proposed for the determination of organophosphorus pesticides.

The present method is based on the oxidation of the organophosphorus pesticides with slight excess of NBS to its respective oxidised forms. The unconsumed NBS is then estimated with the decrease in the color intensity of rhodamine B. The method has been successfully applied for the determination of organophosphorus pesticide residues in various vegetable samples.

EXPERIMENTAL

Apparatus

A Toshniwal TVSP 25 spectrophotometer with 1 cm matched quartz cells was used for spectral measurement. A Systronics type 331- pH meter was employed for pH measurements.

Reagents

All reagents used were of analytical reagent grade and double distilled water was used throughout the experiment.

Stock solution of organophosphorus pesticides (malathion, phorate, dimethoate) [NORTHERN MINERALS LTD., INDIA]: Stock solution of organophosphorus pesticides were prepared by dissolving 100 mg of pesticides (technical forms and formulations) in minimum amount of glacial acetic acid and then diluting to 100 ml with distilled water. Working standards were prepared by appropriate dilution.

\textit{N- bromosuccinimide (NBS) [LOBA CHEMIE]}: An aqueous solution of 0.01\% (w/v). Freshly prepared NBS solution was used.

\textit{Rhodamine B [BDH]}: An aqueous solution of 0.02\% (w/v)
**Hydrochloric acid**: 4 M aqueous solution

**Acetic acid [M:RCK]**: Glacial acetic acid was used

**Recommended Procedure**

An aliquot of sample solution containing 2.7 - 27 μg of malathion, 1.4 - 14 μg of phorate and 0.7 - 7.0 μg of dimethoate were transferred into a series of 25 ml calibrated flasks, to which 1.5 ml of NBS, 1 ml of glacial acetic acid and 0.5 ml of hydrochloric acid were added successively; the solution was kept aside with occasional shaking for about 10 min at ~30°C and then 0.7 ml of rhodamine-B solution was added and mixed thoroughly. The absorbance was measured at 550 nm against a reagent blank. A blank without pesticide (dye and NBS) and dye (devoid of pesticide and NBS) were prepared in similar manner and its absorbance was measured against distilled water. The decrease in absorbance corresponding to consumed oxidant, which reflects the pesticide concentration was obtained by subtracting the decrease in absorbance of the test solution (dye minus test) from that of the blank solution (dye minus blank). Calibration graph was prepared by plotting the decrease in absorbance of dye against the amount of the pesticide.

**RESULTS AND DISCUSSION**

**Spectral characteristics**

The maximum absorbance of rhodamine-B was found to be at 550 nm against distilled water. The reagent blank (dye and NBS) showed negligible absorbance at this wavelength (Fig. 1).

**Adherence to Beer’s law, molar absorptivity and Sandell’s sensitivity**

Beer’s law was obeyed in the concentration range 0.108 - 1.08, 0.05 - 0.56 and 0.028 - 0.28 μg ml⁻¹ for malathion, phorate and dimethoate respectively (Fig. 2). The curve was found to be linear with different slopes for malathion, phorate and dimethoate and has a good correlation coefficient in all the cases. The molar absorptivities and Sandell’s sensitivity of malathion, phorate and dimethoate are given in Table 1.
Effect of reagent concentration

In the first stage of oxidation of the organophosphorous pesticide, the use of 1.0-1.5 ml of NBS was found necessary. In the second stage 0.7 ml of rhodamine-B was found optimal (Fig. 3). It was found that acidic medium was necessary for better results. Since nitric acid and sulphuric acid are oxidizing in nature, glacial acetic acid and hydrochloric acid were tried. It was found that best results were obtained with 1ml of glacial acetic and 0.5 ml of 4 M hydrochloric acid.

Effect of time and temperature

It was observed that all the samples required 10 min for oxidation. After addition of rhodamine-B, only 2 min was required for bleaching. The most suitable temperature range was found to be 25°C - 30°C. Above and below this temperature absorbance was affected (Fig. 4).

Reproducibility

The reproducibility of the method was checked by seven replicate analyses for malathion, phorate and dimethoate. The standard deviation and relative standard deviation data are summarized in Table 1.

Effect of foreign species

To assess the validity of the proposed method, known amounts of various common foreign species and other pesticides associated with organophosphorus pesticides were added to the sample and analyzed by the proposed method. Phenol was the most expected interferent.

COLOUR REACTION

Rhodamine-B gives maximum absorbance at 550 nm. The following reaction occurs (Scheme 1):

- Oxidation of organophosphorus pesticides by NBS in acidic medium to its respective oxidised forms.
- The unconsumed NBS then bleaches the colour of rhodamine-B dye and the absorbance is measured at 550 nm
APPLICATION

In order to check the validity as well as to compare the proposed method with the reported method (60), various samples of vegetables were collected from different fields where organophosphorus pesticide was sprayed as insecticide. The surface residues in these samples were then analyzed by the proposed as well as conventional method and the results are shown in Table 3.

To check the recovery of organophosphorus pesticide, various vegetable samples free from organophosphorus pesticide were taken and treated with known amounts of pesticide and kept for 24 h. The samples were then washed with ethanol and the washings were collected in a boiling tube. Aliquots of these washings were used for the determination of organophosphorus pesticides by the proposed method. The recoveries range from 95-96% and the results are summarized in Table 4.

CONCLUSION

The method is very simple, rapid, sensitive and reproducible. The method does not suffer from instability of colors as bleaching of the dye is involved. The method has been successfully applied in the determination of pesticide residue in vegetable samples.
Scheme 1. Colour Reaction
Table 1. Optical characteristics and precision data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Malathion</th>
<th>Phorate</th>
<th>Dimethoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ nm</td>
<td>550</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>Beer's law/µg ml$^{-1}$</td>
<td>0.108-1.08</td>
<td>0.056-0.56</td>
<td>0.028-0.28</td>
</tr>
<tr>
<td>Molar absorptivity/1 mol$^{-1}$ cm$^{-1}$</td>
<td>2.37 x 10$^5$</td>
<td>1.35 x 10$^5$</td>
<td>6.58 x 10$^5$</td>
</tr>
<tr>
<td>Sandell's Sensitivity/µg cm$^2$</td>
<td>0.0014</td>
<td>0.0007</td>
<td>0.0003</td>
</tr>
<tr>
<td>Slope (b)*</td>
<td>0.7284</td>
<td>1.3546</td>
<td>2.8753</td>
</tr>
<tr>
<td>Intercept (a)*</td>
<td>-0.0038</td>
<td>-0.0029</td>
<td>-0.0016</td>
</tr>
<tr>
<td>Corr. coefficient(r)</td>
<td>0.9994</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>S.D.</td>
<td>± 0.0049</td>
<td>± 0.0034</td>
<td>± 0.0036</td>
</tr>
<tr>
<td>R.S.D</td>
<td>1.98%</td>
<td>1.33%</td>
<td>1.69%</td>
</tr>
</tbody>
</table>

*Regression equation $y = bx + a$, where $x$ is the concentration in µg ml$^{-1}$

Table 2. Effect of foreign species

Concentration of organophosphorus pesticide: 0.15 µg ml$^{-1}$

<table>
<thead>
<tr>
<th>Foreign Species</th>
<th>Tolerance limit* (µg ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr$^{2+}$</td>
<td>200</td>
</tr>
<tr>
<td>Se$^{2+}$</td>
<td>500</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>580</td>
</tr>
<tr>
<td>Aniline</td>
<td>250</td>
</tr>
<tr>
<td>Dithiocarbamate Pesticides</td>
<td>40</td>
</tr>
<tr>
<td>Organochlorine Pesticides</td>
<td>30</td>
</tr>
<tr>
<td>Carbamate Pesticides</td>
<td>15</td>
</tr>
<tr>
<td>Phenol</td>
<td>8</td>
</tr>
</tbody>
</table>

*Tolerance limit may vary by ± 2%
Table 3. Application of the method on unknown samples

<table>
<thead>
<tr>
<th>Vegetable sample*</th>
<th>Organophosphorus pesticide found (µg)</th>
<th>Present method</th>
<th>Reported method (60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauliflower</td>
<td>2.12</td>
<td>2.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.44</td>
<td>4.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.83</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>Cabbage</td>
<td>7.65</td>
<td>7.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.72</td>
<td>4.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.24</td>
<td>2.23</td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>1.46</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.64</td>
<td>2.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.85</td>
<td>3.82</td>
<td></td>
</tr>
</tbody>
</table>

*Amount of vegetable samples (from fields where organophosphorus pesticides were sprayed): 25 g

Table 4. Application of the method on pesticide free sample

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Malathion</th>
<th>Phorate</th>
<th>Dimethoate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amt. added (µg)</td>
<td>Amt. found (µg)</td>
<td>Rec. %</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>25</td>
<td>23.7</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>47.5</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>70.6</td>
<td>94.4</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.0</td>
<td>96.0</td>
</tr>
<tr>
<td>Cabbage</td>
<td>50</td>
<td>47.7</td>
<td>95.4</td>
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<td></td>
<td>75</td>
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<td></td>
<td>25</td>
<td>23.8</td>
<td>95.2</td>
</tr>
<tr>
<td>Spinach</td>
<td>50</td>
<td>47.5</td>
<td>95.4</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>71.4</td>
<td>95.2</td>
</tr>
</tbody>
</table>

*Amount of sample: 25 g
FIG 1. ABSORPTION SPECTRA OF RHODAMINE-B

FIG 2. CALIBRATION CURVE FOR MALATHION, PHORATE AND DIMETHOATE
FIG 3. EFFECT OF REAGENT CONCENTRATION

A: DYE (RHODAMINE-B)
B: DYE - NBS

FIG 4. EFFECT OF TEMPERATURE AND TIME ON OXIDATION OF ORGANOPHOSPHORUS PESTICIDES

CONCENTRATION OF MALATHION: 14 µg/25 ml
PHORATE: 7 µg/25 ml
DIME THOATE: 3.5 µg/25 ml
REFERENCES

14. Eddleston M., Quarterly Journal of Medicine, 2000, 93, 715.

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