CHAPTER-6

Organophosphorus Pesticides

Esters of phosphoric acid

Acetylcholinesterase (AChE) Inhibitors

A CATALYTIC KINETIC SPECTROPHOTOMETRIC DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN VEGETABLE SAMPLES
Summary

A simple selective and sensitive catalytic kinetic spectrophotometric method for the determination of trace amount of organophosphorus pesticides has been proposed. The method is based on the catalytic effect of organophosphorus pesticides (malathion, dimethoate and phorate) on the oxidation of LCV (Leuco crystal violet) by potassium iodate in hydrochloric acid medium to give a violet colored dye. The dye shows maximum absorption at 592 nm. The fixed-time method was used for 15 minutes. The system obeys Beer’s law in the range of 0.02-0.2, 0.032-0.32, 0.03-0.3 µg mL⁻¹ respectively for malathion, dimethoate, and phorate. Important analytical parameters such as time, temperature, reagent concentration, acidity etc. have been optimized for complete color reaction. Sandell’s sensitivity and molar absorptivity for the system were found to be 0.0002, 0.0004, 0.0004 µg cm⁻², 1.2 × 10⁶, 5.21 × 10⁵, 6.3 × 10⁵ l mol⁻¹ cm⁻¹ respectively. The proposed method was satisfactorily applied to micro-level determination of organophosphorus pesticides in vegetable samples.

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INTRODUCTION

Organophosphorus pesticides are potentially hazardous substances widely used in agriculture due to their high insecticidal activity [1]. Organophosphates are organic
esters of phosphoric acid, thiophosphoric acid and other phosphoric acids [2]. These pesticides are toxic for mammals due to the inhibition of the acetyl cholinesterase (AChE), an enzyme necessary for the normal function of the central nervous system [3-4]. The pesticide residue causes serious health hazard, its ill effect can be reduced to minimum by detecting and then controlling it [5]. A number of methods have been developed in the last few years for the detection of organophosphorus pesticides. The most widely used methods are gas chromatography (GC) [6-8], high-performance liquid chromatography (HPLC) [9-10], gas chromatography-mass spectrometry (GC-MS) [11], immune assay and fluorescence [12-13] and chemiluminescence (CL) [14-16].

Different methods like HPLC, GC and GC-MS have been reported but despite the precision and accuracy of these methods, analyses are restricted to laboratory facilities, are time-consuming and expensive due to its analytical cost, limiting the operation of these instruments to highly qualified laboratory persons while spectrophotometry is considered the most convenient analytical technique because of its inherent simplicity, low cost, and wide availability in most of the laboratories. Some spectrophotometric methods have also been reported in which most of the methods involve the determination of organophosphorus pesticides by total phosphorous measurements based on the formation of molybdenum blue using various reducing agents [17-19] and some are based on the oxidation of pesticide and determination of unconsumed oxidant by bleaching of dye [20]. Some of the methods suffer from interference, poor sensitivity, instability of color or involve extraction whereas other suffers from blank absorption or longer time required for the color development. To overcome these drawbacks a selective and sensitive method has been proposed for the determination of the studied organophosphorus pesticides.

In the present study a validated, rapid, sensitive, selective kinetic spectrophotometric method for the determination of organophosphorus pesticides is developed. The developed method is based on the catalytic effect of organophosphorus pesticides on the oxidation of LCV by potassium iodate in acidic medium. The proposed method is specific for thion compounds containing P=S bond like Malathoin, Dimethoate, Phorate. Now days these pesticides are increasingly being used in agriculture.

**EXPERIMENTAL**
Apparatus
A systronics spectrophotometer 166 was used for spectral measurements. pH measurements were made with systronics digital pH meter 335.

Reagents
All chemicals used were of analytical grade, double deionized water has been used for preparation of solutions.

Stock solutions of organophosphorus pesticides (malathion, phorate, dimethoate) [Northern Minerals Ltd., India]:
Stock solution of organophosphorus insecticides (malathion, phorate, dimethoate) were prepared by dissolving 100 mg of insecticide (technical forms and formulations) in minimum amount of glacial acetic acid [Merck, Mumbai, India] and then diluted to 100 mL with distilled water. Working standards were prepared by appropriate dilution.

Potassium iodate (Merck, Mumbai, India)
An aqueous solution of 0.01% (w/v) was prepared. This was stored in amber colored bottle.

LCV (Leuco crystal violet) (Merck, Mumbai, India)
To a 1 L volumetric flask 200 mL of water, 3 mL 85% phosphoric acid, 250 mg of LCV were added and shaken gently until the LCV dissolved. The content of the flask were then diluted to 1 L with distilled water. The solution was stable for several months.

Hydrochloric acid (Merck, Mumbai, India)
2 M aqueous solution was prepared.

Sodium hydroxide solution (Merck, Mumbai, India)
2 M aqueous solution was prepared.
Procedure

Preparation of calibration curve
To a series of 25 mL volumetric flasks, 1.0 mL of $6.7 \times 10^{-4}$ mol L$^{-1}$ LCV solution, 1.0 mL of 2.0 mol L$^{-1}$ hydrochloric acid and 1.0 mL of different concentration of organophosphorus pesticides (malathion, dimethoate and phorate) were added in sequence. Then 1.0 mL of $4.7 \times 10^{-3}$ mol L$^{-1}$ potassium iodate solution was added and pH was adjusted by adding 0.2 mol L$^{-1}$ NaOH then the solution was diluted to the mark with deionized water. The zero time was taken as the moment at which the last drop of potassium iodate solution was added and the solution was mixed well. Then the volumetric flasks were placed in a boiling water bath for 15 min at 35°C for completion of the reaction. The content was then cooled under tap water for 3 min to stop the catalytic reaction. After that, a portion of the solution was transferred into a quartz cell and the absorbance was measured against double distilled water at 592 nm. The blank reaction was performed according to the same procedure without addition of organophosphorus pesticides and the change in absorbance was measured. The standard curve was constructed between the difference between blank and sample absorbance verses time. The absorbance of catalyzed and non- catalyzed reaction was measured simultaneously.

Determination of organophosphorus pesticide in pesticide free samples
To check the recoveries of organophosphorus pesticides vegetable samples free from organophosphorus pesticide were taken and treated with a known amount of the organophosphorus pesticide and kept for ~24 h. The samples were then washed with ethanol [20] different proportions of ethanol were tested for extraction best result was obtained with 85 % ethanol. Then washings were collected and evaporated and residue is dissolved in 0.1% acetic acid. Aliquots of these washings were used for the determination of organophosphorus pesticide by the proposed method. The proposed method was successfully applied for the determination of organophosphorus pesticide in vegetable samples. The recovery range is summarized in table 3.
RESULTS AND DISCUSSION

LCV (Leuco crystal violet) is a compound which can be oxidized with potassium iodate at very slow reaction rate. It has been reported that organophosphates have an induction effect on iodine azide reaction [22], similarly in the proposed method organophosphorus pesticides act as a catalyst in the reaction between LCV and potassium iodate in the presence of hydrochloric acid medium. An organophosphorus pesticide increases the rate of this reaction at ultra trace level, therefore by measuring the increase in absorbance of CV (Crystal violet) which is the oxidized product of LCV for a fixed time of 15 min of the reaction the organophosphate content in the sample can be measured. It was observed that such type of catalytic activity was mainly exhibited by thiophosphoryl compounds and depends on the nature of the P=S bond so the organophosphorus pesticide like monocrotophos which does not have P=S bond does not show the catalytic activity. The probable mechanism of the catalytic reaction is given in reaction scheme 1.

Spectral characteristics and method validation

The absorption spectra of final colored product showed a maximum absorbance at 592 nm (Fig. 1). The reagent blank had negligible absorbance at this wavelength. Beer’s law was obeyed over the concentration range of 0.02-0.2, 0.032-0.32 and 0.03-0.3 µg mL\(^{-1}\) for malathion, dimethoate and phorate respectively (Fig. 2). The curve was linear with different slopes for malathion, dimethoate, phorate and has a good correlation. The molar absorptivities and Sandell’s sensitivity of malathion, dimethoate and phorate are given in table 1. The slope, intercept, and the correlation coefficient were calculated by least square regression analysis (Table 1). The detection limits (DL = 3.3 \(\sigma\)/S) and quantitation limits (QL = 10 \(\sigma\)/S) [where ‘\(\sigma\)’ is the standard deviation of blank and ‘S’ is slope of the calibration curve], SD of slope and intercept calculated are given in table 1. The precision of the method was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of opp (within the working limits) were analyzed in seven replicates during the same day (intra-day precision) and seven consecutive days (inter-day precision). The RSD (%) values range of intra-day and inter-day studies showed that the precision was good for the method (Table 1).
**Effect of acid concentration**
The effect of various acids of same concentration such as sulphuric acid, acetic acid, nitric acid has been studied. The results show that hydrochloric acid gives greater sensitivity. The effect of hydrochloric acid concentration on obtaining maximum sensitivity was investigated with $4.7 \times 10^{-3}$ mol L$^{-1}$ potassium iodate, and $6.7 \times 10^{-4}$ mol L$^{-1}$ LCV for catalyzed and uncatalyzed reaction at 35 °C (Fig. 3).

**Effect of potassium iodate concentration**
The effect of potassium iodate concentration in the reaction rate was studied with 2 mol L$^{-1}$ hydrochloric acid and $6.7 \times 10^{-4}$ mol L$^{-1}$ LCV at 35 °C. The results show that by increasing the potassium iodate concentration up to $4.7 \times 10^{-3}$ mol L$^{-1}$ the sensitivity increases, whereas a greater amount of reagent decreases sensitivity. Thus $4.7 \times 10^{-3}$ mol L$^{-1}$ potassium iodate was selected throughout the study (Fig. 4).

**Effect of Leuco crystal violet concentration**
The influence of LCV concentration on the reaction rate was studied in the range of $1 \times 10^{-4}$ to $10 \times 10^{-4}$ mol L$^{-1}$ with 2 mol L$^{-1}$ hydrochloric acid and $4.7 \times 10^{-3}$ mol L$^{-1}$ potassium iodate at 35 °C. The results show that by increasing the LCV concentration up to $6.7 \times 10^{-4}$ mol L$^{-1}$, the sensitivity increases, whereas a greater amount of reagent decreases sensitivity, thus $6.7 \times 10^{-4}$ mol L$^{-1}$ LCV was selected for the study (Fig. 5).

**Effect of temperature and time**
The effect of temperature on the catalytic reaction was studied in the range of 10 °C to 70 °C with the optimum of the reagent concentrations. The results showed that as the temperature increases up to 35 °C the sensitivity increases, whereas higher temperature values decreases the sensitivity ($\Delta A_r - \Delta A_b$). Therefore, 35 °C was selected for further study and 15 min time was suitable for the study of catalytic reaction (Fig. 6).

**Interference studies**
The effect of foreign ions and pesticides expected to exist in field samples of organophosphorus pesticide were studied by adding known amount of different foreign ions to the test solution containing 0.1 µg mL$^{-1}$ organophosphorus pesticide.
The method was found to be free from most of the interferents. The tolerance limits shown in table 2 are the concentration of interfering species that may cause \( \leq (\pm) 2\% \) variation in the absorbance value.

**APPLICATIONS**

**Determination of organophosphorus pesticides in real vegetable samples**

Vegetable samples were collected from agricultural field where organophosphorus pesticide (malathion) was sprayed (1 to 5 pints per acre). The samples were weighed, macerated with ethanol and then filtered through a thin cotton cloth. The filtrate was centrifuged at 1850 g for 10 min. An aliquot of supernatant was taken and evaporated then residue is dissolved in 0.1% acetic acid and analyzed as described above. The filtrate which was greenish yellow due to the presence of organic matter from plant was passed through a silica gel column (10 × 1 cm) to remove chlorophyll and other interfering materials. The column was washed with 10 mL of 0.1% acetic acid. Washings were collected and analyzed as recommended above by the proposed as well as the conventional ascorbic acid method [21] and the results are shown in table 4.

Ascorbic acid method involves the reaction of orthophosphate with molybdate in acid solution, which forms a yellow-colored phosphomolybdate. The phosphomolybdate complex is then reduced by ascorbic acid, causing a characteristic molybdenum blue species which is measured at 700 nm.

**CONCLUSION**

The proposed method is more sensitive, simple and selective as compared with other spectrophotometric methods for determination of organophosphorus pesticides. The reported spectrophotometric methods are indirect, lengthy, time consuming, suffer from interference, poor sensitivity, instability of color, blank absorption or involve extraction. The rapid color development, stability and easy availability of the reagent and freedom from a large group of interfering species are some advantages of the method. As the presented method is based on catalytic effect on rate of reaction it can
be further applied for simultaneous determination of these pesticides by applying initial rate and partial least square analysis method.

**Reaction scheme 1**

\[
\begin{align*}
\text{IO}_3^- + H^+ + \text{LCV (reduced)} & \rightarrow \text{I} + \text{CV (oxidized)} \quad (1) \quad \text{[Slow reaction]} \\
\text{IO}_3^- + H^+ + \text{OPP} & \rightarrow \text{I} + \text{OPP (oxidized)} + H_2O \quad (2) \quad \text{[Fast reaction]} \\
\text{IO}_3^- + H^+ + \text{I} & \rightarrow I_2 + H_2O \quad (3) \quad \text{[Fast reaction]}
\end{align*}
\]
\[ I_2 + H^+ + \text{LCV (reduced)} \rightarrow I^- + \text{CV (oxidized)} \quad (4) \]

\[ \text{Leuco crystal violet} \]

\[ \text{Crystal violet} \]

**Table 1. Spectral characteristics and precision data**

| Parameters                      | Malathion | Dimethoate | Phorate |
|---------------------------------|-----------|------------|---------|
| \( \lambda_{\text{max}} \) (nm) | 592       | 592        | 592     |
| Range of Beer’s law (\( \mu g \text{ mL}^{-1} \)) | 0.02-0.2  | 0.032-0.32 | 0.03-0.3 |
| Stability of color(hours) | 48   | 48   | 48   |
|--------------------------|------|------|------|
| Molar absorptivity (L mol\(^{-1}\) cm\(^{-1}\)) | \(1.2 \times 10^6\) | \(5.2 \times 10^5\) | \(6.3 \times 10^5\) |
| Sandell’s sensitivity (µg cm\(^{-2}\)) | 0.0002 | 0.0004 | 0.0004 |
| Relative standard deviation (%) |
| Intra-day | 0.466-3.278 | 0.46-2.6 | 0.53-2.7 |
| Inter-day | 1.11-3.37 | 0.65-1.48 | 0.59-4.5 |
| Limit of Detection | 0.004 | 0.008 | 0.007 |
| Limit of Quantification | 0.01 | 0.02 | 0.02 |
| SD of slope | 0.084 | 0.064 | 0.059 |
| SD of intercept | 0.006 | 0.016 | 0.017 |
| Regression equation (y = bx+a) |
| Correlation coefficient | 0.999 | 0.999 | 0.999 |
| Slope (b) | 4.37 | 2.73 | 2.86 |
| Intercept (a) | -0.0033 | 0.0065 | -0.0044 |

x-Concentration in µg mL\(^{-1}\)

Table 2. Effect of foreign species (concentration of organophosphorus pesticide 0.1 µg mL\(^{-1}\))
Table 3. Determination of organophosphorus pesticide in pesticide free samples

| Samples | Malathion | Dimethoate | Phorate |
|---------|-----------|------------|---------|
|         | Amt. added (µg) | Amt. Found (µg) | Rec. % | Amt. Added (µg) | Amt. Found (µg) | Rec. % | Amt. Added (µg) | Amt. Found (µg) | Rec. % |

\(^a\)Causing (±) 2\% variation in absorbance value

\(^b\)Masking with 0.1\% EDTA solution

\(^c\)Removed by addition of nitric acid as well as boiling the solution
|                  | 20 | 40 | 60 | 20 | 40 | 60 | 20 | 40 | 60 |
|------------------|----|----|----|----|----|----|----|----|----|
| Cauliflower      | 18.5 | 38.2 | 59.1 | 18.92 | 39.12 | 59.13 | 18.75 | 38.33 | 58.75 |
|                  | 92.5 | 95.5 | 98.5 | 94.6 | 97.8 | 98.55 | 93.75 | 95.82 | 97.91 |
| Cabbage          | 19.4 | 39.2 | 58.5 | 19.16 | 38.75 | 58.93 | 19.06 | 39.16 | 58.55 |
|                  | 97  | 98  | 97.5 | 95.8 | 96.87 | 98.21 | 95.3  | 97.9  | 97.58 |
| Spinach          | 19.2 | 38.8 | 58.1 | 19.77 | 39.43 | 58.84 | 18.88 | 38.46 | 59.31 |
|                  | 96  | 97  | 96.83 | 98.85 | 98.06 | 98.85 | 94.4  | 96.15 | 98.85 |

*e Amount of sample: 25 g  
*f Mean of three replicate analysis*
Fig. 1 Absorption spectra of colored product: A-blank; B-0.1 µg ml\(^{-1}\); C-0.14 µg ml\(^{-1}\)

Fig. 2 Calibration data for the determination of organophosphorus pesticide: A-Malathion; B-Dimethoate; C-Phorate
Fig. 3 Effect of HCl concentration on the sensitivity. condition- opp: 0.12 µg ml\(^{-1}\); LCV: 6.7×10\(^{-4}\) mol l\(^{-1}\); potassium iodate: 4.7×10\(^{-3}\) mol l\(^{-1}\); temperature: 35 °C

Fig. 4 Effect of iodate concentration on the sensitivity. condition- opp: 0.12 µg ml\(^{-1}\); hydrochloric acid: 2 mol l\(^{-1}\); LCV: 6.7×10\(^{-4}\) mol l\(^{-1}\); temperature: 35 °C
Fig. 5 Effect of LCV concentration on the sensitivity. condition: opp: 0.12 µg ml⁻¹; hydrochloric acid: 2.0 mol l⁻¹; potassium iodate: 4.7×10⁻³ mol l⁻¹; temperature: 35 °C.

Fig. 6 Effect of temperature on the sensitivity. conditions: opp: 0.12 µg ml⁻¹; LCV: 6.7×10⁻⁴ mol l⁻¹; hydrochloric acid: 2 mol l⁻¹; potassium iodate: 4.7×10⁻³ mol l⁻¹.
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