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Research Article

NEW SPECTROPHOTOMETRIC METHOD FOR THE ANALYSIS OF COMMERCIAL MALATHION FORMULATION AND ITS RESIDUES ON SOME CROP PRODUCES AND ENVIRONMENTAL SAMPLES

Devender Kumar Sharma[1], Niharika Thakur[1], Ashima Sharma[2], Pushap Raj*[1]

[1]*Department of Chemistry, Himachal Pradesh University, Shimla, Himachal Pradesh, India*

[2]*Department of Physiology, Indira Gandhi Medical College (IGMC), Shimla, India*

**Corresponding author: pushap.hp@gmail.com*

ABSTRACT

Malathion is the most widely used insecticide globally. Because of the toxicity of this insecticide its presence in residues in crop produces, drinking water and environmental samples is of serious health and environmental concern thus necessitating its analysis in these substrates. The analysis of its marketed formulations is equally important to obtain reliable residue data and also in curbing adulteration problems. A new simple and rapid spectrophotometric method based on the reaction of dimethyl dithiophosphate formed from the alkaline hydrolysis of malathion with chloranil in 4:1 molar ratio to form yellowish brown colored dichlorodithiophosphato-1,4-benzoquinone complex has been developed for the purpose. The color showed λ_{max} at 420 nm and was stable for at least 60 min. The stoichiometry of the color reaction has been well supported by potentiometric and photometric titrations. Malathion solution obeyed the Beers law in the range 2.4-66.0 µg mL^{-1}. The method has successfully been applied to the analysis of malathion in residues on some grains, apple and water samples with high recoveries in the range 80.3-99.0% with low relative standard deviation in the range 0.55-2.29%. The method has also been applied for the assessment of its risk of ground water contamination.

Keywords: Malathion, Spectrophotometric method, Commercial formulation, Environmental samples

1. INTRODUCTION

Malathion (Fig. 1) is a synthetic ester of the thiophosphoric acid and is amongst the top ten organic phosphorus insecticides used globally [1]. After excessive application in agricultural fields the residues of this insecticide on crop produce, drinking water and soil samples is a subject of serious health and environmental concern owing to its toxic nature. For taking effective measures against above ill-effects and to ensure its safe and effective use the analysis of malathion in commercial formulation and in soil, water and crop produce is essential which necessitates the development of methods for its analysis.

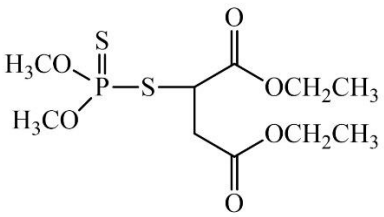


Fig. 1: Chemical structure of malathion

Chromatography is considered as a powerful analytical technique in the analysis of malathion but the methods based on this technique require not only initial long analysis time but also good experimental skill to obtain reproducible and reliable results [2-11]. The high cost of the technique also comes in the way of their wide adoption especially by the laboratories of limited means. Methods based on spectrophotometry find wide acceptance due to their sensitivity, simplicity and relatively low cost and also permitting the preparation and processing of a large number of samples in a shorter time. Existing spectrophotometric methods [12-16] for the determination of malathion require number of steps with strict adherence to experimental conditions. In the present work a simple, sensitive and rapid spectrophotometric method has been developed for the determination of malathion. That dimethyl dithiophosphate (DMDTP) formed from alkaline hydrolysis of malathion reacts with acetonitrile solution of chloranil in 4:1 molar ratio to form yellowish brown colored product in a microwave oven showing maximum absorbance at 420 nm has been formed the basis of the proposed method. The method has also been validated for its determination in commercial formulation, grains and apple, water and soil samples. In the latter context

soil adsorption study has been carried out to know its ground water contamination risk.

2. MATERIAL AND METHODS

2.1. Instruments

Varian-Cary100 Bio UV-Visible spectrophotometer with 1cm matched quartz cells was used for absorption measurements. Incubator shaker PT-422 (Popular Traders, Ambala Cantt.) was used for equilibration of insecticide with soil and Sigma 3-30KS model centrifuge was used for the centrifugation of the soil samples.

2.2. Chemical reagents and Standard solutions

The technical high purity malathion standard (95%) and its formulation Milthion 50% EC were procured from the manufacturer Insecticides (India) Ltd. The purity and maker's specification of the formulation was, however, checked by a known method [17]. Chloroform and acetonitrile (AR, Merck) were used as received. A standard solution of Chloranil (0.01 mol L^{-1}) was prepared by dissolving 0.24 g of Chloranil (AR, FlukaChemika) in 100 mL acetonitrile. Sodium hydroxide (0.02 mol L^{-1}, in water) was prepared by dissolving more than the calculated amount and standardized by titrating against 0.02 mol L^{-1} oxalic acid (0.25 g in 100 mL) using phenolphthalein as indicator. Hydrochloric acid (0.02 mol L^{-1}) was prepared by dissolving 0.17 mL in 100 mL acetonitrile.

2.3. Analytical Procedures

2.3.1. Preparation of calibration graph for pure malathion

A standard solution of malathion (0.001mol L^{-1}), was prepared by dissolving 0.069mL, of pure compound in 100mL acetonitrile. Aliquots (0.02-2.0 mL) of this standard solution were taken in 10 mL measuring flasks and diluted to 2 mL with acetonitrile. Each solution was mixed with 1 mL of sodium hydroxide (0.02mol L^{-1}in water) and kept in microwave oven for 60 sec. Each solution was then neutralized with 1 mL of hydrochloric acid (0.02mol L^{-1} in acetonitrile) followed by the addition of 1 mL of chloranil (0.01 mol L^{-1} in acetonitrile) and final volume made to 5 mL with acetonitrile. The absorbance of yellowish-brown color was measured at 420 nm (the absorption spectrum of colored product is shown in Fig. 2) against a reagent blank. The color was stable for at least one hour. The calibration curve was prepared by plotting absorbance values against concentration of malathion and is shown in Fig.3.

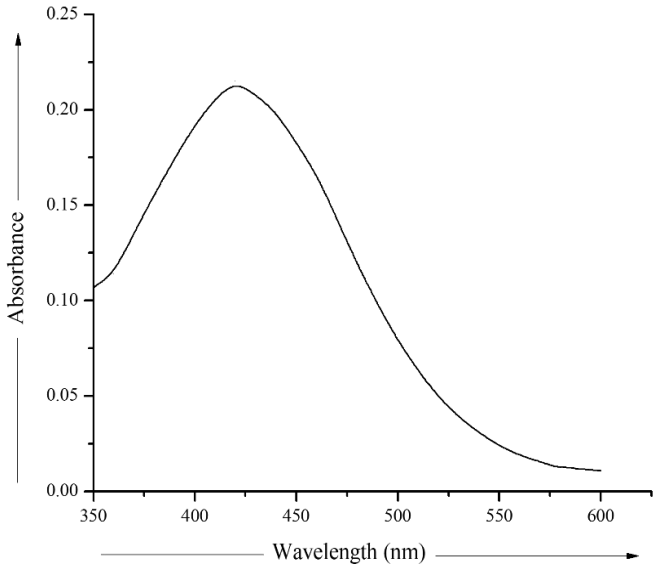


Fig. 2: Absorption spectrum of malathion-chloranil complex

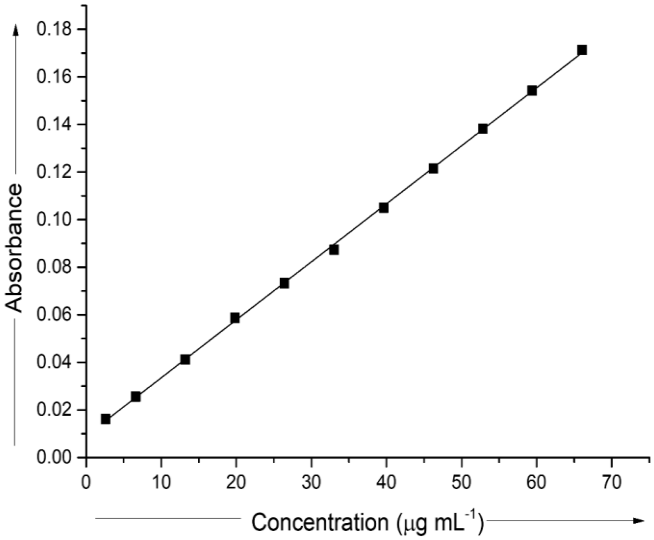


Fig. 3: Relationship between absorbance and concentration (calibration graph) of malathion as malathion-chloranil complex

2.3.2. Procedure for assay of malathion in commercial formulation

A single large sample of commercial formulation equivalent to 16.52 mg active ingredient of malathion was dissolved in acetonitrile and volume made to 50 mL with the same solvent to obtain a solution with working concentration of 0.001 mol L^{-1}. Suitable aliquots of acetonitrile extracts of malathion formulation were taken for analysis and processed in the same manner as described for pure compound.

2.3.3. Procedure for the assay of malathion in fortified grains and apple fruit

A known weight (10 g) of grains (rice and wheat) and apple fruit was mixed with acetonitrile solution of the malathion (0.001 mol L^{-1}). The samples were well mixed and extracted with 2 instalments of 5 mL of chloroform. The extracts were purified through the silica column. The eluate was collected and dried with nitrogen gas drier and the remainder was dissolved in 2 mL acetonitrile and analyzed in same manner as discussed for pure compounds.

2.3.4. Procedure for the assay of malathion in spiked water samples

About one liter of tap water was randomly collected from the local area. 25 mL of water sample was transferred into five 25 mL glass tubes and spiked with 0.2-1.8 mL of standard solution of malathion (0.001 mol L^{-1}). The spiked water samples were well mixed and extracted with 2 installments of 5 mL of chloroform. The chloroform extracts were purified and processed for analysis in same manner as described above.

2.3.5. Procedure for evaluation of leaching potential of malathion on three soils

Triplicate soil samples (2 g) of each soil type taken in 50 mL conical flask were equilibrated with 2 mL of acetonitrile solution of malathion in the concentration range from 6.6-59.4 µg mL^{-1} and 8 mL distilled water at 25±1°C and agitated by stirring mechanically at 150 rpm for 24 h equilibration time in the incubator shaker. Each sample was then centrifuged and the supernatant was extracted with 10 mL of chloroform and processed for the determination of the equilibrium concentration (C_e) as described above. Two adsorption models viz. Freundlich (equation 1 and 2) and Langmuir (equation 3) were used to evaluate various adsorption parameters on three Indian soils with different soil characteristics.

$$X = K_f \times c_e^{n_f} \quad (1)$$

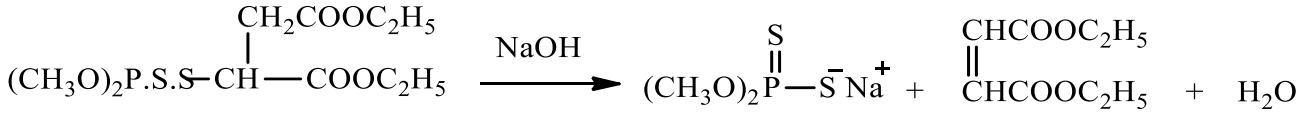
$$\log x = \log K_f + n_f \log C_e \quad (2)$$

$$\frac{C_e}{X} = \frac{1}{kb} + \frac{C_e}{b} \quad (3)$$

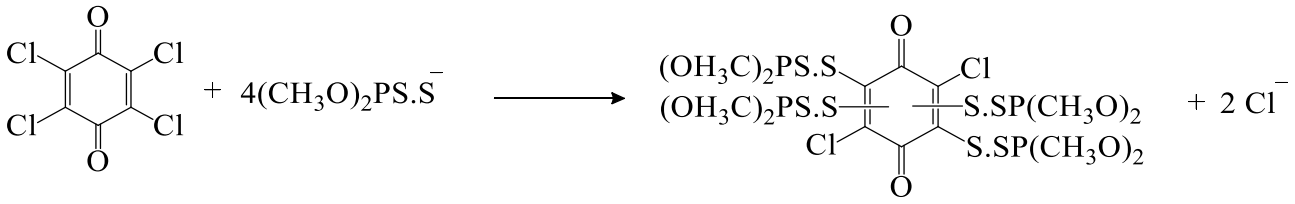
Where X is the amount of insecticide adsorbed (mg Kg^{-1}) on the adsorbent; C_e is the equilibrium solution concentration (mg L^{-1}); K_f and n_f are adsorption coefficients which are related to the adsorption capacity and adsorption intensity respectively, k is Langmuir constant (L mg^{-1}) and b is maximum adsorption capacity (µg g^{-1}).

3. RESULTS AND DISCUSSION

The four chlorine groups of Chloranil serve as good leaving groups during nucleophilic substitution. The interaction of chloranil and donor organic compounds leading to the formation of colored molecular complexes and/or substitution products has served as the basis for the identification and determination of donor compounds [18-21]. DMDTP being a nucleophile can also form such complexes prompted us to extend the above use of chloranil in the analysis of malathion. When a standard solution of chloranil (in acetonitrile) was added to a colorless solution of DMDTP, the solution became increasingly yellowish brown and color intensity was maximum at malathion to chloranil molar ratio of 4:1. It has been reported [22] that when concentration of chloranil is high, the charge transfer complexes are formed and get stabilized and when the concentration of donor is high the substitution products are formed. In view of this it is proposed that of the four DMDTP molecules as per observed 4:1 molar ratio, firstly two molecules substitute two chlorides of chloranil molecule (at this stage DMDTP nucleophile in excess) and with further addition of chloranil the other two DMDTP molecules participate in a charge transfer complexation by donating electrons and resulting into the formation of a dichlorodithiophosphato 1, 4-benzoquinone. That two dithiophosphato groups forming the charge- transfer complex were loosely held by disubstituted chloranil molecule has been established by titrating the end product i.e. dichlorodithiophosphato 1, 4-benzoquinone with iodine reagent where volume of reagent consumed corresponded to the presence of only two dithiophosphato groups. The most plausible course of reaction is shown below:



The smooth and quantitative formation of DMDTP upon alkaline hydrolysis has also been established separately by titrating malathion with standard sodium hydroxide visually and pH metrically and the volume of alkali corresponded to above reaction.



The above stoichiometry of the color reaction has also been established by monitoring the color reaction using potentiometric and photometric titration of DMDTP with chloranil. Whereas in potentiometric titrations performed with Pt-SCE assembly, sharp jump in potential was observed at 4:1 molar ratio while in photometric titrations performed at 420 nm the absorbance increased corresponding to the formation of colored complex till 4:1 molar ratio and there after attained almost constant values.

3.1. Optimization of experimental conditions

The effect of microwave heating time, reagent concentration and volume were carefully studied in terms of the development of maximum color intensity to get accurate and reproducible results. The microwave heating time of 60 sec and 1 ml of 0.01 mol L^{-1} chloranil concentration gave best results. To get clear solution, addition of 1mL of hydrochloric acid (0.02 mol L^{-1} in acetonitrile) was also optimized. The yellowish brown dichlorodithiophosphato 1,4-benzoquinone complex showed λ_{max} at 420 nm where the chloranil reagent (λ_{max} 365 nm) has negligible absorbance. The color was found to be stable for 60 min based on the kinetic stability measurements.

3.1.1. Linearity

The linearity of the developed method was evaluated by analyzing the different concentration of insecticide under optimized conditions.

The linearity between absorbance and concentration (calibration graph) was in the range of 2.4-66.0 µg mL^{-1} for malathion (Fig. 3). The high values of the correlation coefficient (r^2) and the small values of the *y*-intercepts of the regression equation proved the linearity of calibration graph. The various optical and calibration parameters viz. Beer's law range, Sandell's sensitivity, molar absorptivity, slope and intercept values have been calculated and are summarized in Table 1.

Table 1: Spectrophotometric determination of malathion with chloranil: Optical and calibration characteristics

Optical characteristics	Optimised value
λ_{max}(nm)	420
Beer's law range (µg $mL^{-1)}$	2.4-66.0
Molar absorptivity (L mol^{-1} cm^{-1})	1.71×10^3
Sandell's sensitivity (µg cm^{-2})	0.1928
Stability (min)	60
Slope	0.0023
Intercept	0.0093
Correlation coefficient (r^2)	0.9964
Limit of detection (µg mL^{-1})	1.60
Limit of quantification (µg mL^{-1})	2.40

3.1.2. Accuracy and precision

The high values of % recoveries evaluated by performing five replicate analyses at five different concentration levels of malathion over the concentration range 3.3-59.4 µg mL^{-1} with low RSD show the good accuracy and precision of the method (Table 2).

Table 2: Spectrophotometric determination of malathion with chloranil

Amount taken (µg)	Amount found[a] (µg)	RE	RSD	t-value[b]
3.3	3.27±0.059	0.91	1.80	1.14
16.5	16.53±0.224	0.18	1.36	0.30
33.0	32.82±0.285	0.54	0.87	1.41
46.2	46.21±0.295	0.02	0.64	0.07
59.4	59.37±0.298	0.05	0.50	0.22

SD: standard deviation, RE: relative error, RSD: relative standard deviation, [a]Values expressed as mean±SD (n=5), [b]Theoretical t-value at 95% confidence level is 2.776

3.1.3. Limits of Detection (LOD) and quantification (LOQ)

The limits of detection (LOD) and quantification (LOQ) listed in Table1 were calculated using the following equation [23].

$$LOD=3S_a/b$$

$$LOQ=10S_a/b$$

Where, 'S_a' is the standard deviation of the response and 'b' is the slope of the calibration curve.

3.2. Application of the proposed method for determination of malathion in commercial formulations, crop produces and environmental samples

The proposed spectrophotometric method has employed for the routine analysis of malathion formulation in residues in crop produces (grains and apple) and spiked water samples for monitoring health hazards. When applied to the analysis of its commercial formulation, the recovery of active ingredient was 98.9-99.7 % of the normal content with RSD in the range 0.63-1.53 % (Table 3). In the determination of malathion in residue on grains, apple and water samples, the recoveries were in the ranges 88.7-93.4%, 80.3-83.9 and 97.7-99.0% with RSDs in the ranges 0.63-1.45%, 0.55-1.46% and 1.08-2.29 % respectively (Tables 4 and 5).

Table 3: Assay results of commercial formulation of malathion containing 50 % active ingredient[a]

Active ingredient added (µg)	Active Ingredient[b]	
	Found (µg)	Recovery (%)
6.6	6.57±0.101	99.5±1.53
19.8	19.61±0.299	99.0±1.51
33.0	32.62±0.392	98.8±1.19
46.2	45.70±0.501	98.9±1.08
59.4	59.21±0.373	99.7±0.63

[a]Maker's specifications established by an independent method[17], [b]Values expressed as mean±SD (n=5)

Table 4: Recovery of malathion from fortified grains and fruit samples

Active ingredient added (µg)	Active ingredient					
	Wheat		Rice		Apple	
	Found (µg)	Recovery (%)	Found (µg)	Recovery (%)	Found (µg)	Recovery (%)
6.6	5.90±0.096	89.4±1.45	5.85±0.080	88.7±1.21	5.30±0.094	80.3±1.42
19.8	18.30±0.232	92.4±1.17	18.15±0.174	91.7±0.88	16.15±0.256	81.6±1.29
33.0	30.83±0.314	93.4±0.95	30.05±0.245	91.0±0.74	27.56±0.483	83.5±1.46
46.2	42.98±0.350	92.0±0.76	41.85±0.486	90.6±1.05	38.76±0.358	83.9±0.77
59.4	55.06±0.374	92.7±0.63	53.41±0.573	90.0±0.96	49.50±0.325	83.3±0.55

Table 5: Recovery of malathion from spiked water samples

Active ingredient added(µg)	Active ingredient	
	Found (µg)	Recovery (%)
6.6	6.53±0.151	98.9±2.29
19.8	19.34±0.386	97.7±1.95
33.0	32.58±0.606	98.7±1.83
46.2	45.71±0.605	98.9±1.31
59.4	58.83±0.642	99.0±1.08

Table 6: Adsorption parameters for the adsorption of malathion on three different soils

Soils samples	K_d	K_{oc}
I	6.33	633
II	4.88	610
III	4.85	809

The method has also been applied to evaluate leaching behavior of malathion. To accomplish this aspect its adsorption on three soils has been studied by Freundlich and Langmuir isotherms.

The observed values of coefficients of determination were high (r^2> 0.85) for Freundlich isotherm than Langmuir isotherm (r^2> 0.51) and former has been used to evaluate the leaching potential of malathion. The leachability is related to adsorption coefficient (K_d) and soil organic carbon partition coefficient (K_{oc}) and have been calculated by reported method [24].

The observed values in the ranges 4.85-6.33 and 610-809 respectively (Table 6) suggest moderate adsorption of malathion in all three soil types and may have a leaching risk to contaminate groundwater.

4. CONCLUSION

The rapid and well-established hydrolysis of malathion in a microwave, stoichiometry of color reaction supported by potentiometric and photometric titrations, non-extraction of colored product and the high recoveries of malathion from its commercial formulations, fortified crop produces and water samples are some salient features of the method. The proposed method is simple, sensitive and fast having promise as an excellent economical alternative for the routine analysis of malathion in environmental samples.

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