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SPECTROFLUOROMETRIC DETERMINATION OF DIMETHOATE RESIDUES IN ENVIRONMENTAL SAMPLES

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Abstract

A simple, sensitive and extractive spectrofluorimetric method for the determination of dimethoate is described. Dimethoate was hydrolysed with sodium-ethoxide in to sodium dimethyl dithiophosphate (Na-DMDTP); the Na-DMDTP was extracted with cationic fluorescein dye as an ion pair in to 1-chloro 2-nitrobenzene which greatly increases the sensitivity. The fluorescence of the organic layer was measured at 528 nm after exitation at 485 nm under the optimal conditions. Beer's law is obeyed over the range from 5.0 mg to 60.0 mg of DMDTP. The limit of detection [LOD], variation co-efficient, the limit of quantification [LOQ] where 5.0 mg mL^{-1}, 1.0% and 60.0 mg mL^{-1} respectively. The proposed method has been applied successfully for the determination of dimethoate residues in water samples up to the ppb level with preconcentration on amberlite XAD-4.

Introduction

Dimethoate [O,O-dimethyl-S-(N-methyl carbomoylmethyl)-phosphoro-dithioate] is a systemic organophosphorous insecticide, widely applied on crops, trees and ornamental plants to control house flies around live stock pens, processing plants and human dwellings, grasshoppers on livestock forage and in extensive application the pesticide finds its way into the surface water bodies through agricultural runoff and into human being through food grains. Hence there is a need for a simple and sensitive method to determine its residue in water to check health the hazard to human beings.

Several analytical methods like chromatography[1], polorography[2] and spectrophotometry[3] has been reported for the determination of dimethoate residues. However, these suffer from serious drawbacks, like increasing color of blank with increase in concentration of reagent[4], incomplete acid hydrolysis, etc[5]. Since flourimetry offers high sensitivity in the analysis of organic compounds, several workers[6] have tried to develop a fluorometric method for the determination of pesticide residues. However, many of the pesticide are non or weakly fluorescent.

Here the authors reported an extractive spectrofluorometric method for the determination of the dimethoate insecticide in formulations and its residue in environmental samples. The method is based on the hydrolysis of dimethoate to sodium dimethyldithiophosphate (Na-DMDTP) and extraction as an ion pair with the cationic dye, Fluorescein into 1-chloro,2-nitrobenzene. The fluorescence of the organic layer was measured at 528 nm after excitation at 485 nm. The method has been extended for the determination of dimethoate residue in environmental samples. To enhance the sensitivity of the method, dimethoate residues was collected on Amberlite XAD-4 and eluted with dichloromethane. The dimethoate residue thus concentrated was hydrolysed and determine with fluorescein.

Experimental procedure

Apparatus

A HITACH (Tokyo, Japan) fluorescene spectrophotometer model 650. 10S with 10 mm glass cell and xenon source was employed. Glass columns, 2.5 X 50 cm, fitted with Teflon stop clocks were used.

Reagents and Solutions

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

1.053 g of 95% dimethoate (1 mg mL^{-1}) (Northern Mineral Ltd., New Delhi, India) was dissolved in 100 mL of dichloromethane. A 10 mL aliquot of this stock solution was diluted to 100 mL. The working standard solutions were prepared by appropriate solution of dimethoate solution with dichloromethane . 0.14659 g of 30% EC Dimethoate solution (Northern Mineral Ltd., India) was dissolved in 100 mL of dichloromethane. 5 mL of this solution is diluted to 100 mL. Working standard solutions were prepared by the appropriate solution of dimethoate in dichloromethane. Freshly cut sodium (5 g) was dissolved in 100 mL of ethanol for 5% solution. The solution was prepared afresh.

Fluorescein (2 g) in 100 mL of double distilled water for 2% solution. Buffer solution pH 6 was prepared by adding concentrated sulfuric acid acid (3.4 mL) to 250 mL distilled water in 500 mL standard flask, then monopotassium dihydrogen phosphate dihydrate 25 g was added and the flask shaken until dissolution was complete and diluted to 500 mL with double distilled water. Phenolphthalein indicator, 2 M sulfuric acid, dichloromethane, 1-chloro,-2-nitrobenzene, toluene, diethyl ether, (Glaxo, ExcelaR, Mumbai, India) n-hexane, cyclohexane, (SD fine chem Ltd., Mumbai, India) diisopropyl ether, Amberlite XAD-4 (Aldrich chemicals company, Inc., Bangalore, India) were employed.

Procedure

Calibration

Standard dimethoate solution (5.0 to 60.0 µg) in 10 mL dichloromethane was placed in separating funnel. 1 mL sodium ethoxide was added and the contents were swirled. Double distilled water (10 mL) was added followed by vigarous shaking, the

layers were allowed to separate and dichloromethane layer was discarded. The aqueous layer was washed with twice with 10 mL portion of dichloromethane and neutralised with 2 M sulfuric acid. A 2 mL of aliquot of buffer solution was added followed by 5 mL of fluorescein and 10 mL 1-chloro, 2-nitrobenzene and the mixture was shaken vigarously. The organic layer was collected and dried over anhydrous sodiumsulphate, transferred into spectro-photo fluorometric cell and the fluorescence was measured at 528 nm after excitation at 485 nm. Plot of mean fluorescence readings against the concentration of dimethoate yielded in calibration. The LOD is 5.0 µg and the LOQ is 5.0 µg to 60.0 µg.

Determination of dimethoate in formulation

30% EC dimethoate solution equivalent to (5.0 to 60.0 µg of active ingredient) was placed in a separating funnel, hydrolysed and then determined by the procedure described above.

Recovery of dimethoate residues from spiked water samples

Spiked water samples were prepared by adding known amounts of dimethoate formulations (5.0 to 60.0 µg) to 2 L each of pesticide free water and then allowing them to stand overnight.

A glass column was filled with Amberlite XAD-4 resin up to a height of 10 cm. The column was washed successively with 50 mL ethanol, diethyl ether and double distilled water, then the spiked water was allowed to percolate through the column at the average rate of 10 mL min^{-1}. When all the water had passed through, the column was allowed to drain for 10 min. The tap was closed and about 90 mL of diisopropyl ether was added to columns and left to condition for 10 min. After this time the column were drained into 250 mL beaker. The solution was dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue was dissolved in 10 mL dichloromethane and the pesticide content determined by the procedure described earlier.

Results and Discussion

The proposed reaction mechanism of hydrolysed dimethoate and formulation of an Na-DMDTP - fluorescein ion pair is shown in Fig.1.

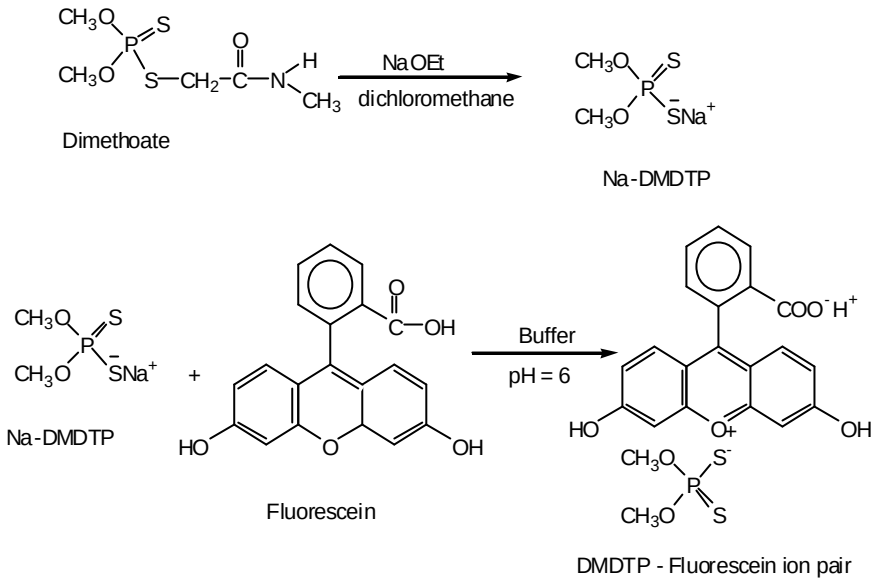


Fig. 1 : Proposed reaction mechanism of hydrolysed dimethoate and DMDTP-Fluorescein ion pair

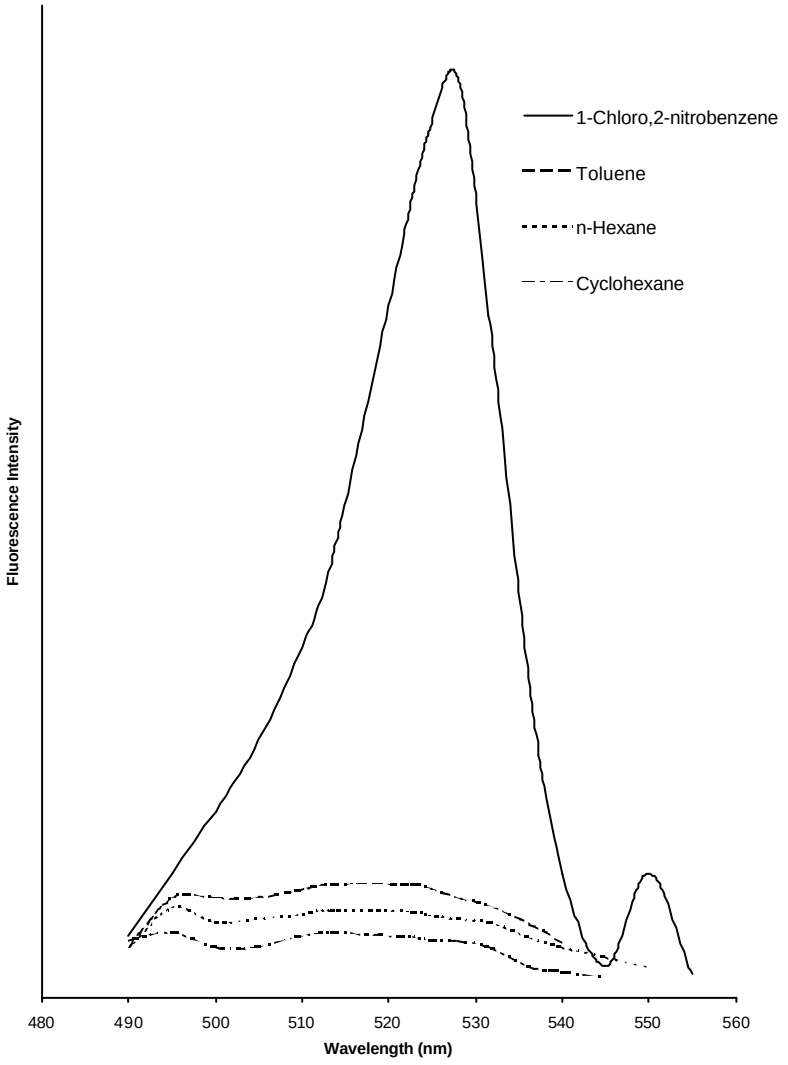
Effect of solvents on extraction

Extraction of ion pair was carried out in different organic solvents such as 1-chloro,2-nitrobenzene, toluene, n-hexane and cyclohexane and their fluorescence spectra was observed as 1-chloro,2-nitrobenzene>toluene>n-hexane>cyclohexane. Hence 1-chloro,2-nitrobenzene was used a most suitable solvent for extraction of DMTP-fluorescein ion pair because maximum absorbance was recorded.

Effect of pH and various buffer solutions on extraction

Extraction of ion pair fluorescein was carried out in different buffer solutions of pH 6 such as sulfuric acid-potassium dihydrogen phosphate, acetic acid-sodium acetate, ammonia-hydrochloric acid and borax-hydrochloric acid, the fluorescent spectra is shown in Fig.2. The fluorescence intensity was greater in sulfuric acid- potassium dihydrogen phosphate of pH 6 is greater than in acetic acid-sodium acetate, ammonium-hydrochloric acid and Borax-hydrochloric acid.

Fig. 2: Fluorescence Spectrum of ion pair (NaDMDTP - Fluorescencein) Extracted with different solvents



The effect of foreign species and of other pesticides

The effect of foreign species and of other pesticides on the determination of dimethoate were studied. A known amount of a variety of foreign species and a pesticides were added to a standard solution containing 30 µg of dimethoate in 10 mL. The solutions were analysed by the proposed method. The results obtained are shown in Table-1. The results shows that the foreign species and pesticides tested with the proposed procedure do not interfere in the analysis under the reported condition. This indicates the validity of the method for the determination of dimethoate.

Table-1 : Effects of natural water contaminants and other pesticides on determination of dimethoate residues (concentration of dimethoate 20 mg 25 mL^{-1})

	Foreign species	Tolerance limit mg 25 mL^{-1}	Other pesticides	Tolerance limit mg 25 mL^{-1}
1.	Ca^{2+}	1000	BHC	300
2.	Mg^{2+}	200	Phenol	250
3.	Zn^{2+}	500	Carbaryl	200
4.	Cu^{2+}	450	DDT	250
5.	Fe^{2+}	3950	Ethylparathion	250
6.	Al^{3+}	450	Quinolphos	200
7.	NH_4^+	480	Monocrotophos	150
8.	CO_3^{2-}	400		
9.	SO_4^{2-}	400		
10.	Cl^-	500		

Applications

The method was applied to the determination of dimethoate active ingredient in dimethoate formulations. In order to study the interference of various ingredients such as emulsifiers and solvents. 30% emulsifiable concentrate dimethoate formulations (30% EC : Commercial product, 30% m/m active dimethoate and 30% m/m solvent and emulsifier) was analysed. The results are shown in Table-2. Thus, the obtained results demonstrate the suitability of the method for the determination of pesticide in formulation. The minimum detection limits for determination of dimethoate with proposed method is 5.0 to 60.0 µg with a relative standard deviation of 5.22.

Table-2 : Determination of dimethoate active ingredient in formulations (30% emulsion).

	Amount of pesticide (active ingredient) / mg			
	Taken	**Found**	**% Recovery**	**RSD[a]**
1.	10	9.8	98.0	5.22
2.	20	19.55	97.75	4.91
3.	30	29.20	97.33	5.41
4.	40	39.80	97.00	5.84
5.	50	48.38	96.76	4.84
6.	60	58.0	96.66	4.70

[a] n = 5.

Determination of dimethoate in water samples

The method was applied to the determination of dimethoate residues in spiked water samples. The results relating to the recoveries of dimethoate residues from spiked water samples are shown in Table-3. The recoveries presented in Table-3 suggest that the percentage of pesticide recovery from fortfied water ranges from 89.60% to 92.80%. The results showed that dimethoate residues up to 12.5 ppb level can be determined from 2 L of water samples with a relative standard deviation of 4.95 to 6.10.

Table-3 : Recovery of dimethoate residues from spiked water samples

	Added / mg $2L^{-1}$	**Found / mg $2L^{-1}$**	**% Recovery[a]**	**RSD[a]**
1.	25	22.50	90.0	6.10
2.	75	67.25	89.6	5.70
3.	125	114.0	91.2	5.92
4.	175	162.50	92.8	4.95
5.	225	206.25	91.66	5.79
6.	275	255.10	92.7	5.28

[a] n = 5.

Conclusion

Determination of dimethoate with fluorescein is very simple and convenient. Fluorescence intensity is two fold increased with a buffer of pH 6 (sulfuric acid potassium dihydrogen phosphate) employed in these studies, is more sensitive and selective than other methods. The proposed method has very high sensitivity. Its sensitivity is much better than that of other reported methods for the determination of dimethoate. Nearly all the anions and cation do not interfere with the our reagent.

Thus, the proposed method can be routinely used for the determination of dimethoate in water samples and other environmental samples.

References

1. P. Delgado, T. Albi and M.A. Navas. *Grasas Aceites (Seville)*, 1987, 38, 1.
2. Y.Y. Yuan, *Fen His Hua Hsuesh* 1981, 9, 77, *Chem Abstr*, 1981, 95, 165.
3. J.V. Das, K.N. Ramachandran and V.K. Guptha, *Analyst*, 1994, 119, 1387.
4. G. Zweig, *Analytical methods for pesticides, plant growth regulators and food additives, Vol.2, Insecticides (Academic Press, New York),* 1964, pp 172-174.
5. A. Coly and J.J. Aaron, *Analyst*, 1994, 119, 1205.
6. A. Coly and J.J. Aaron, *Talanta*, 1994, 41, 1475.