SHORT COMMUNICATION

DEVELOPMENT OF AN INDIRECT SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF METHAMIDOPHOS INSECTICIDE IN SOIL, WATER AND VEGETABLE SAMPLES

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ABSTRACT. A simple and rapid indirect spectrophotometric method for determination of methamidophos in water, soil and vegetable samples has been described. Methamidophos reacts with acid produced from *p*-dimethylaminobenzaldehyde (*p*-DMAB) as a result of Cannizaro's reaction. The resultant adduct undergoes condensation reaction in acidic medium forming a yellow colored product. Absorbance of the colored product was measured at 405 nm and pH 3 against a reagent blank. The Beer's law range is obeyed in the range 1-30 µg mL^{-1} with molar absorptivity of 2.8×10^3 L mol^{-1} cm^{-1}. The limit of detection and quantification were found to be 0.20 ± 0.03 and 0.60 ± 0.04 µg mL^{-1} respectively. The proposed method was effectively applied for determination of methamidophos in various samples with percent recoveries in the range of 96 ± 0.08 to 102 ± 0.06 %.

INTRODUCTION

Methamidophos (O,S-dimethylphosphor-amidothiolate) is a broad spectrum, water soluble, systemic organophosphorus insecticide and acaricide [1]. It is used to control pests of cotton, rice, tobacco, brassica, head lettuce, sugar beet, corn and potatoes (e.g., leaf hoppers, spider mites, trips, aphids, etc.) [2, 3]. It has substantial effects on non-target organisms, humans, animals, plants, and insects. Methamidophos has been reported to exhibit acute and chronic toxicity and has an acute oral and dermal LD$_{50}$ of 10-50 and 50-110 mg (a.i.) kg^{-1} (b.w.) respectively in different test species [3]. Maximum residue level (MRL) of methamidophos in vegetables is 1.0 µg g^{-1} for cauliflower and 2.0 µg g^{-1} for tomato [4].

Methamidophos having high solubility in water (200 g L^{-1} at 20 ^{o}C) may contaminate ground and surface water and pose potential threats to human health via drinking water and foods [2, 3]. Due to its potential risk to human it is necessary to establish a selective and sensitive approach for determination of methamidophos in environmental samples. A number of analytical methods have been reported for determination of methamidophos. These include gas chromatography-mass spectrometry [5], high performance liquid chromatography [6], adsorptive stripping square wave voltammetry [7], differential pulse stripping voltammetry [8] and acetylcholine enzyme sensor [9]. Some of these methods like GC and HPLC being highly sensitive and specific, need complicated pre-treatment and clean-up steps [10] and are, therefore, unsuited for rapid assay of samples. In some cases the instrumentations employed is very expensive and can hardly be equipped due to their high setup costs and skills [5].

The present method is based on reaction of methamidophos with *p*-dimethylaminobenzaldehyde (*p*-DMAB) in alkaline media followed by acidification and subsequent spectrophotometric detection of the yellow colored condensation adduct formed, against a reagent blank. The proposed method was successfully applied for determination of methamidophos in vegetable and environmental samples. The novelty of the present method lies

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in the fact that it has substantial sensitivity, does not need complicated pre-treatment and clean-up steps and is suitable for rapid assay of samples especially for screening purposes. The instrumentation employed is not very expensive and can be easily operated with little laboratory expertise.

EXPERIMENTAL

Instruments. The absorption spectra and absorbance of solutions were measured with Spectrophotometer (SP-300, optima Inc., Japan) at 405 nm using matched glass cells with an optical path length of 1.0 cm.

Reagents. All reagents were of analytical reagent-grade purity or similar grade. *p*-DMAB (Merck-Schuchardt; Eduard-Buchner-Str. 14-20, 85662 Hohenbrunn Germany), sodium hydroxide, hydrochloric acid, and sodium chloride were obtained from Merck (Germany). Methamidophos standard was obtained from DrEhrenstorfer GmbH, Augsburg, Germany. Methanol and dichloromethane (Merck Germany) were used as solvents.

Solutions. A standard stock solution of methamidophos (1000 µg mL^{-1}) was prepared by dissolving 0.1 g of the authentic standard in 20 mL distilled water and diluted to 100 mL with distilled water. Working standards were prepared by diluting appropriate volumes of the stock solution with distilled water. *p*-DMAB stock solution (0.2 mol L^{-1}) was prepared by dissolving 1.49 g of the reagent in 30 mL methanol and diluted to 50 mL with methanol. Sodium hydroxide (2.5 mol L^{-1}) and hydrochloric acid (3.0 mol L^{-1}) solutions were prepared by dissolving appropriate amounts in distilled water and diluted to 100 mL with distilled water.

Procedures

Analysis procedure. In a 200 mL conical flask, 2.0 mL of methamidophos standard solution (100 µg mL^{-1}) was taken and 0.5 mL of sodium hydroxide solution (2.5 mol L^{-1}) was added to it. After thorough mixing 1.0 mL of *p*-DMAB solution (0.2 mol L^{-1}) was added to the mixture followed by the addition of 1.0 mL of hydrochloric acid solution (3.0 mol L^{-1}). The reaction mixture was mixed thoroughly followed by the addition of 2.0 mL methanol to dissolve the turbidity formed. After shaking, absorbance of the resultant yellow colored product was measured against a reagent blank at wavelength of maximum absorbance, i.e. 405 nm. In the same way different volumes of the stock solution (100 µg mL^{-1}) of methamidophos were taken and each one treated as above. Different solutions having different concentrations of the analyte (1, 3, 5, 10, 15 and 30 µg mL^{-1}) were obtained after dilution. The absorbance of each solution was measured against a blank at 405 nm and calibration curve was constructed and linear range determined.

Investigation of sample interference effect. For extraction purpose, easily manageable amounts of control samples of water (30 mL), soil (10 g), tomato (50 g) and cauliflower (25 g) were extracted with dichloromethane and the filtrates were analyzed by the proposed method. No detectable spectrophotometric response was observed and the extraction and analysis process was decided to be free of interferences and applicable to percent recovery determination of methamidophos.

Extraction and preconcentration of methamidophos from water samples. For extraction and preconcentration of methamidophos from water, 20 mL control water sample, previously salted with 10% sodium chloride solution (to provide a salting-out effect) was spiked with three different concentrations of methamidophos (4, 8 and 12 µg mL^{-1}) and extracted with 2.0 mL dichloromethane under mechanical shaking for five min in a separating funnel. The phases were

then equilibrated for 15 min. The organic phase was separated from aqueous phase and the funnel was washed with 3.0 mL dichloromethane. The organic phase was concentrated to dryness and reconstituted in 2.0 mL water.

Extraction and preconcentration of methamidophos from soil samples. Dry control samples (10 g) of soil was spiked with three different concentrations of methamidophos (2, 4 and 6 µg mL^{-1}) and extracted with 30 mL dichloromethane after thoroughly shaking for 10 min on shaker. The samples were filtered and the filtrate was salted with 10% sodium chloride solution and extracted with 3.0 mL dichloromethane under mechanical shaking for five min in a separating funnel. The same procedure as employed for water samples was used for separation of phases.

Extraction and preconcentration of methamidophos from vegetable samples. Tomato sample (50 g) was ground, spiked with 250 µg methamidophos and washed with 30 mL water after thoroughly shaking for 10 min on shaker. Then the samples were filtered and filtrate salted with 5.0 mL of 10% sodium chloride solution and extracted with 3.0 mL dichloromethane under mechanical shaking for five min in a separating funnel. The phases were then equilibrated and separated as described above.

Fresh cauliflower (25 g) and tomato (50 g) samples were purchased from the local market, cleaned, ground and spiked with 100 µg and 250 µg methamidophos respectively and washed with 30 mL water after thoroughly shaking for 10 min on shaker. Then the samples were filtered and filtrate salted with 10% sodium chloride solution and extracted with 3.0 mL dichloromethane under mechanical shaking for five min in a separating funnel. The phases were then equilibrated and separated as described above. The extraction was done in triplicate in all cases and percent recovery of methamidophos was determined by applying the proposed method.

Analysis of sample extracts. In a series of 100 mL separate conical flasks extracts from different samples were taken and 0.5 mL of sodium hydroxide solution (2.0 mol L^{-1}) was added to each flask. After thorough mixing, 1.0 mL of *p*-DMAB solution (0.2 mol L^{-1}) was added to the mixtures followed by the addition of 1.0 mL of hydrochloric acid solution (3.0 mol L^{-1}). The reaction mixtures were mixed thoroughly and 2.0 mL methanol was added to each to dissolve the turbidity formed. After shaking the absorbance of the yellow colored product formed was measured against a reagent blank at 405nm in each case.

RESULTS AND DISCUSSION

The proposed reaction mechanism described in Scheme 1 shows that *p*-dimethylamino-benzaldehyde molecule undergoes Cannizaro's reaction in basic medium. The disproportionation product, i.e. carboxylic acid derivative reacts with methamidophos molecule. The electron rich nitrogen atom of phosphoramido group in methamidophosis supposed to attack the electron deficient carbonyl carbon of carboxylic acid derivative, forming an unstable intermediate. In acidic medium condensation results in the formation of yellow colored product which is stable for 5-10 min. The resultant product shows maximum absorbance at 405 nm.

The effects of chemical variables such as sodium hydroxide, *p*-DMAB, and hydrochloric acid concentration on the reaction were investigated. The results for the sodium hydroxide concentration are given in Figure 1. The absorbance was found to increase from 0.25-2.5 mol L^{-1} sodium hydroxide solution beyond which no increase in the absorbance was observed. It means that at 2.5 mol L^{-1} of sodium hydroxide, the point of optimum pH for the reaction, is reached and rise in pH beyond optimum, dissociates the product formed.
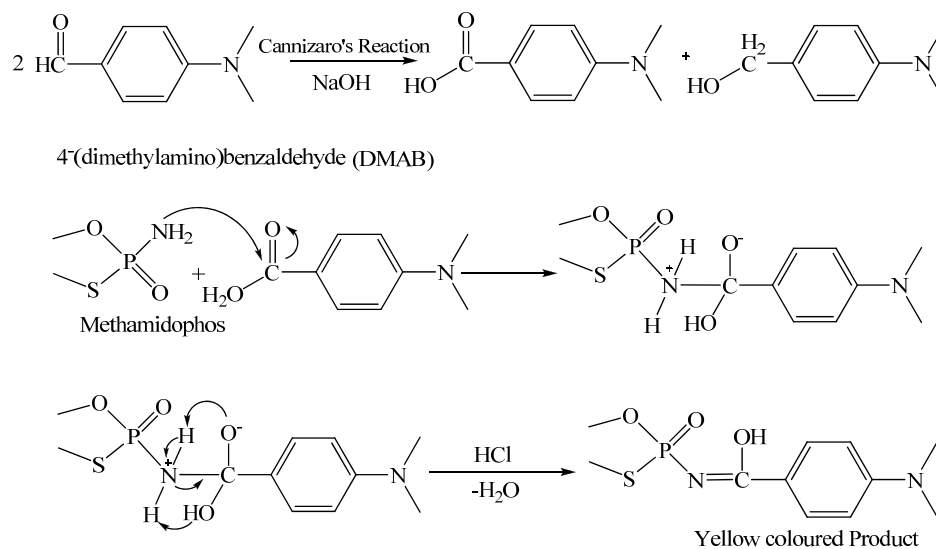
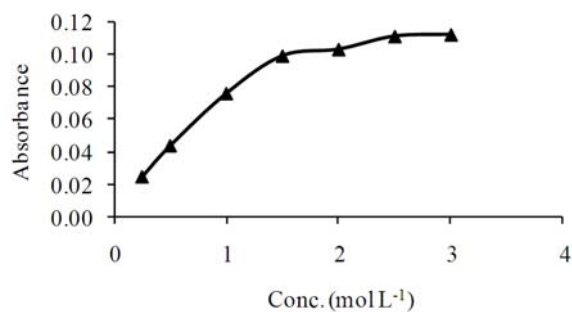Scheme 1. Proposed reaction mechanism for spectrophotometric determination of methamidophos.

Figure 1. Effect of sodium hydroxide concentration on the reaction.

p-DMAB reacts with methamidophos in alkaline media and the effect of its concentration was studied in the range of 0.05-0.25 mol L^{-1}. Reaction starts with 0.05 mol L^{-1} *p*-DMAB solution (Figure 2) and maximum absorbance of the product was observed at 0.25 mol L^{-1} solution of *p*-DMAB beyond which turbidity appears in the solution which cannot be made clear upon addition of methanol. This can be attributed to the excess of aldehyde which is insoluble in the medium. Hence 0.25 mol L^{-1} solution of *p*-DMAB was found to be optimum.

Maximum adduct formation requires acidic media. The concentration of hydrochloric acid was optimized in the range 2.5-5.0 mol L^{-1} as shown in Figure 3. The product formation starts at 3.0 mol L^{-1} HCl and goes on decreasing with increase in concentration because a lower pH is reached at which the product is unstable and gets dissociated. Therefore further analysis was performed using 1.0 mL of 3.0 mol L^{-1} solution of hydrochloric acid.
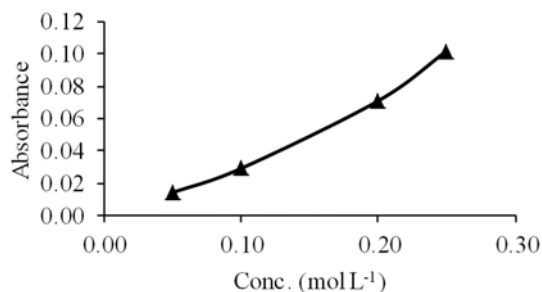
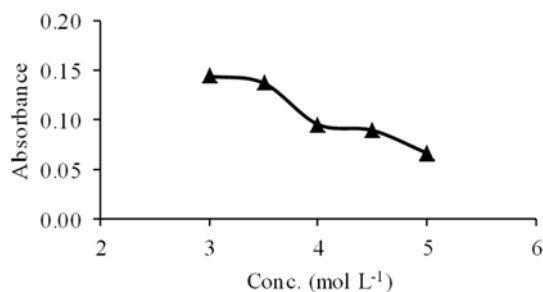Figure 2. Effect of *p*-DMAB concentration on the reaction.

Figure 3. Effect of hydrochloric acid concentration on reaction.

The effect of physical variables like temperature and time on the stability of the colored product was also investigated. The product formed was heated at different temperatures in the range of 22-70 $^{\circ}$C. The results shown in Figure 4 indicate that as the temperature increases, the absorbance of the product decreases due to dissociation at higher temperature. The effect of time on the stability of the final adduct was studied in the range of 1.0-30 min and it was observed that its absorbance remains constant till 5 min after formation beyond which it goes on decreasing steadily due to certain possible side reactions (Figure 5). This indicates that the product formed is unstable at room temperature and vanishes away with time but can easily be used for analysis purposes.
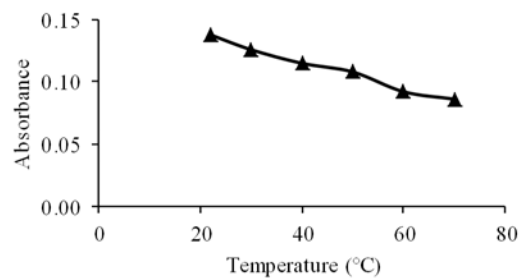

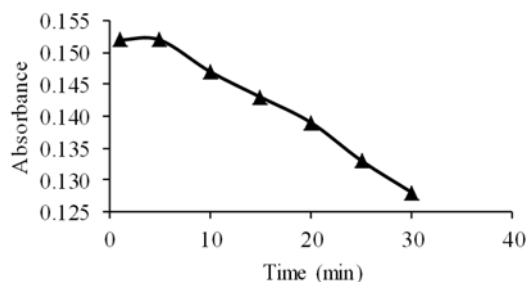Figure 4. Effect of temperature on the stability of the product.

Figure 5. Effect of time on the stability of the product.

Analytical characteristics determined under optimized conditions are given in Table 1. The applicability of Beer's law to the proposed method was found out. A linear relation was observed in the concentration range of 1.0-30 μg mL^{-1} (Figure 6). The molar absorptivity of the resulting adduct was found to be 2.8×10^3 L mol^{-1} cm^{-1}. The limit of detection (3S) and limit of quantification (10S) was calculated at lower concentration that can be measured with acceptable precision and accuracy and found to be 0.20±0.03 and 0.60±0.08 μg mL^{-1}, respectively.

Table 1. Analytical characteristics of the proposed method.

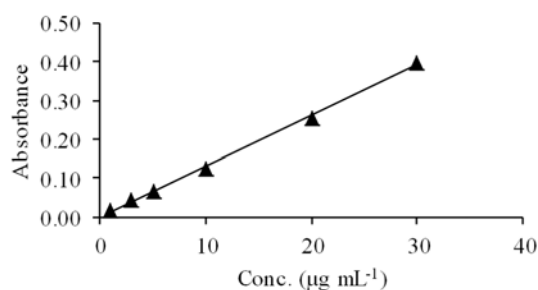| Parameter	Value
λ $_{max}$ (nm)	405
Molar absorptivity (L mol^{-1} Cm^{-1})	2.8×10^3
Beer's law range (μg mL^{-1})	1-30
Slope	0.013
Intercept	0.00
Correlation coefficient (r^2)	0.998
RSD (%)	4.9
SD	± 0.062
LOD (μg mL^{-1})	0.2 ± 0.03
LOQ (μg mL^{-1})	0.6 ± 0.08

Figure 6. Effect of concentration of methamidophos on absorbance of the product.

The recovery studies were carried out using standard addition method. Different concentrations in the range of 2-15 µg mL^{-1} of standard methamidophos (µg mL^{-1} or µgg^{-1}) were added to control samples of water, soil, tomato and cauliflower. Different extraction strategies were followed for different samples and the extracts were analysed by the proposed method.

Recoveries from water, soil, tomato, and cauliflower were found to be 96±0.08%, 102±0.06%, 101±0.24% and 102±0.08 %, respectively (Table 2). The LOD and LOQ for water samples were found to be 0.18±0.01 µg mL^{-1} and 0.56±0.03 µg mL^{-1}, respectively. In case of soil, tomato and cauliflower samples, LOD and LOQ were found to be 0.15±0.03 µg g^{-1}, 0.5±0.1 µg g^{-1}, 0.17±0.02 µg g^{-1}, 0.56±0.1 µg g^{-1}, and 0.13±0.04 µg g^{-1}, 0.43±0.08 µg g^{-1}, respectively.

Table 2. Application of the proposed method for percent recovery determination of methamidophos from various samples.

Sample	µg mL^{-1} or µg g^{-1} added	µg mL^{-1} or µg g^{-1} found	% Recovery	Average % Recovery ± SD
Water	4.0	3.8	95.0	96.5±0.1
	8.0	7.7	96.3	
	12.0	11.8	98.3	
Soil	2.0	2.1	105	102±0.1
	4.0	4.3	107	
	6.0	5.7	95.0	
Tomato	5.0	5.1	102	102±0.2
	10.0	10.3	103	
	15.0	15.1	101	
Cauliflower	4.0	4.1	103	102±0.1
	8.0	8.2	103	
	12.0	12.2	102	

The above results reflect that compared with other methods like GC and HPLC for the analysis of methamidophos, the present method overcomes tedious and time consuming sample pre-treatment steps. As well as, the method is sensitive enough for the analysis of lower concentration of methamidophos as low as 0.6 µg mL^{-1}. The proposed method is also a good analytical method for the determination and monitoring of methamidophos in fortified and real agricultural samples of water, soil, tomato and cauliflower with acceptable recoveries.

CONCLUSIONS

A simple and rapid spectrophotometric method is proposed for determination of methamidophos in water, soil and vegetable samples. The variables effecting the formation of colored product have been studied. Compared with other methods like GC and HPLC for the analysis of methamidophos, the present method overcomes tedious and time consuming sample pre-treatment steps like filtration, rinsing, washing and preconcentration. As well as, the method is sensitive enough for the analysis of lower concentration of methamidophos as low as 0.6 µg mL^{-1}. The LOD and LOQ for water samples were found to be 0.18±0.01 µg mL^{-1} and 0.56±0.03 µg mL^{-1}, respectively. In case of soil, tomato and cauliflower samples, LOD and LOQ were found to be 0.15±0.03 µg g^{-1}, 0.5±0.1 µg g^{-1} 0.17±0.02 µg g^{-1}, 0.56±0.10 µg g^{-1}, and 0.13±0.04 µg g^{-1}, 0.43±0.08 µg g^{-1}, respectively. The proposed method is also a good analytical method for the determination and monitoring of methamidophos in fortified and real agricultural samples of water, soil, tomato and cauliflower with acceptable recoveries.

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