Reverse flow Injection Spectrophotometric Determination of Thiram and Nabam Fungicides in Natural Water Samples

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Summary: A simple reversed flow injection analysis (rFIA) method is reported for the determination of thiram and nabam fungicides in natural water samples with spectrophotometric detection. It is based on the reduction of iron(III) in the presence of thiram/nabam in acidic medium at 60°C and formation of iron(II)-ferricyanide complex with an absorbance maxima at 790 nm. The limits of detection (3σ blank) were 0.01 and 0.05 μg mL^{-1} for thiram and nabam respectively with a sample throughput of 60 h^{-1}. Calibration graphs were linear over the range of 0.02 – 8.0 μg mL^{-1} (R^2 = 0.9999, n = 8) and 0.1 – 30 μg mL^{-1} (R^2 = 0.9982, n = 10) for thiram and nabam with relative standard deviations (RSDs; n = 3) in the range of 0.8 – 1.6% respectively. Experimental parameters and potential interferences were examined. The method was applied to determine thiram and nabam in natural water samples using Sep-Pak C18 cartridges for solid-phase extraction procedure. The recoveries were in the range of 93±3 – 105±2% and 87±4 – 102±3% for thiram and nabam respectively and the results obtained were not significantly different compared with a HPLC method.

Key words: Reverse flow injection analysis; thiram; nabam; natural water samples; spectrophotometry.

Introduction

Dithiocarbamate fungicides are the most widely used organic fungicides and have a wide spectrum of activity as foliar sprays for fruits, vegetables, and ornamentals and as seed protectants [1]. Thiram and nabam are dithiocarbamate fungicides used to protect seeds, fruits, vegetable ornamental and turf crops from a variety of fungal diseases [2]. Several toxic effects of thiram have been reported including skin lesions [3], hepatic dysfunctions [4], neurotoxicity [5] and citotoxicity in rat [6]. The European Union set maximum residue limits for dithiocarbamates (expressed as carbon disulphide) in the range of 2–7 mg kg^{-1} [7]. Table-1 describes chemical name, structure and physicochemical properties of thiram and nabam fungicides.

Various analytical techniques have been reported for the determination of dithiocarbamates fungicides in diverse samples. These include spectrophotometry / colorimetry [8–13], fluorimetry [7, 14], polarography [15], electrophoresis [16], electrochemical [17], gas chromatography with electron capture detector, flame photometry and mass spectrometry detectors [18–20], high performance liquid chromatography with ultraviolet, fluorescence and mass spectrometry detectors [21–24] and chemiluminescence [25, 26].

Spectrophotometric methods are the most commonly used techniques due to availability of the instrumentation, simplicity of procedures, speed, precision and accuracy. Spectrophotometric methods of analysis are more economic and simpler, when compared with methods such as chromatography and electrophoresis [27]. Sharma *et al*. [28] reported a comprehensive review on thiram degradation, applications and analytical methods applied for its analysis in commercial formulations, synthetic mixtures in grains, vegetables and fruits.

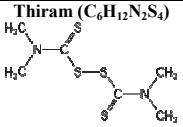
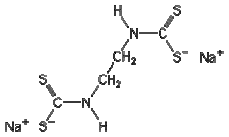
This paper reports a simple rFIA–spectrophotometric method for determination of thiram and nabam in natural water samples. Iron(III) was reduced with thermally degraded products of thiram/nabam in aqueous acidic medium at 60°C. Potassium ferricyanide reagent was then injected into the incubated reaction mixture stream which resulted in the formation of iron(II)-ferricyanide complex monitored at 790 nm.

Results and Discussion

Optimization Studies

To establish the optimum experimental conditions for the determination of thiram and nabam fungicides, the influence of key chemical and physical variables on the peak absorbance was examined using a univariate approach. The key variables were iron(III), HCl, potassium ferricyanide, ethanol and various surfactants concentrations (Fig. 1) and flow rates, sample injection volume, reaction coil length and reaction mixture temperature (Table-2). All of these studies were performed with thiram and nabam standard solution (0.25 μg mL^{-1}) and all measurements were made in triplicate.

Table-1: Chemical names, structures and physicochemical properties of thiram and nabam fungicides.

S. No.	Common name & structure	IUPAC Name	Mol. weight	Vapor Pressure at 25°C (mPa)	K_{ow} log *P*	Water Solubility mg L^{-1} (20°C)	Half life in soil Days)
1	**Thiram ($C_6H_{12}N_2S_4$)** 	Tetramethylthiuram disulfide	240.43	2.3	1.73	16.5	15.2
2	**Nabam ($C_4H_6N_2Na_2S_4$)** 	Disodium ethylene bis (dithiocarbamates)	256.35	1.26×10^{-07}	-4.24	200000	3.5

Sources: C. Tomlin, The pesticide manual, British Crop Protection Council, 2000; PPDB: Pesticides: http://sitem.herts.ac.uk/aeru/index.htm

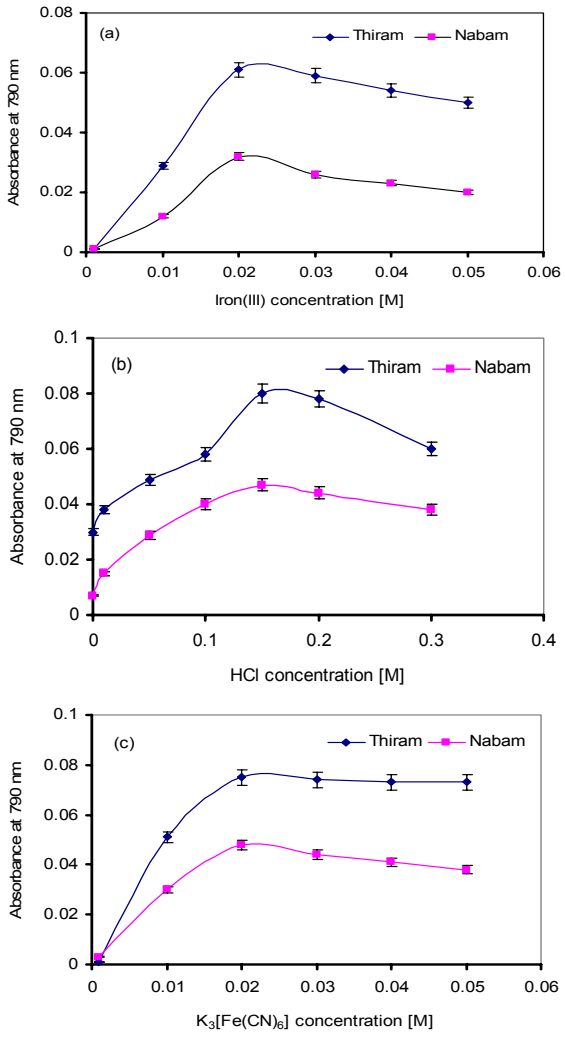


Fig. 1: Variation of absorbance with; (a) ammonium iron(III) sulfate; (b) hydrochloric acid and (c) potassium ferricyanide concentrations.

The effect of iron(III) concentration in the incubation mixture (thiram and or nabam standard solution (0.25 µg mL^{-1}) in ethanol (1% v/v) containing HCl (0.15 M) and temperature 60°C) was examined over the range of 0.001 – 0.05 M and the maximum absorbance was achieved at 0.02 M and therefore was selected as an optimum concentration for further studies. The pH is a key factor for the hydrolysis of dithiocarbamates and releasing of CS_2 and the optimum HCl concentration for hydrolysis was 0.15 M, and at higher acid concentrations, the absorbance decreased. Potassium ferricyanide solution (prepared in optimized HCl solution (0.15 M)) was injected via rotary injection valve into the incubated mixture steam for iron(II)-ferricyanide complex formation. The effect of potassium ferricyanide concentration was examined over the range of 0.001 – 0.05 M. Maximum peak height absorbance was achieved at 0.02 M and therefore was selected as an optimum for subsequent studies. The effect of ethanol (0.1 – 15%, v/v) and various surfactants including CTAB, SDS, Brij-35 and Trion X-100 (0.001 – 1.0%, v/v or w/v) were examined on the determination of thiram and nabam (results not shown). These reagents were added separately in the incubation mixture ($NH_4Fe(SO_4)_2 \cdot 12H_2O$ (0.02 M) in HCl solution (0.15 M), temperature 60°C) and no increase in peak height absorbance was observed and therefore the use of ethanol and surfactants were abandoned subsequently.

The influence of physical parameters, *i.e.*, flow rate, mixing coil length, reagent injection volume and temperature of reaction mixture were then investigated (Table-2). A flow rate of 2.8 mL min^{-1} gave maximum peak height absorbance with a steady baseline and reproducible signals and was therefore used for further studies. A reagent ($(NH_4Fe(SO_4)_2 \cdot 12H_2O$ (0.02 M) in HCl solution (0.15 M)) volume of 60 µL, a reaction coil length of 20 cm and wavelength of 790 nm gave almost

maximum absorbance signals and were therefore used for all further studies. The effect of temperature was examined over the range of 20 – 80°C by immersing the reaction mixture ($NH_4Fe(SO_4)_2 \cdot 12H_2O$ (0.02 M) in HCl solution (0.15 M) and analyte standard solution (0.25 µg mL^{-1} thiram and or nabam in ethanol (1% v/v)) in a circulating water bath (Type JB1, Grant Instruments, Cambridge, UK). The optimum peak height absorbance was achieved at 60 °C, above which non-reproducible signals and decrease in peak absorbance were observed probably due to the evaporation of reaction mixture.

Table-2: Effect of key physical variables on the determination of thiram and nabam (0.25 µg mL^{-1}, n = 4) using the FIA–spectrophotometric manifold. For optimizing each parameter, the optimized experimental conditions for all other parameters were used. At optimum, variable peak absorbance was observed for thiram and nabam fungicides.

Variables	Range studied	Optimum value
Flow rate (mL min^{-1})	0.5 – 4.8	2.8
Reagent volume (µL)	30 – 300	60
Reaction coil (cm)	0 – 200	20
Reaction mixture temperature (°C)	20 – 80	60

Analytical Figures of Merit

Under the optimized experimental conditions described above, calibration graphs for thiram and nabam were linear over the range of 0.01 – 8 µg mL^{-1} and 0.1 – 30 µg mL^{-1} with regression equations y = 0.2415x – 0.0013 (R^2 = 0.9999, n = 8), and y = 0.0205x – 0.0018 (R^2 = 0.9982, n = 10), [y = absorbance and x = concentration in µg mL^{-1}] after subtraction of the blank value and the RSDs (n = 3) was 0.8 – 1.6% over the range studied, respectively. The limits of detection (3σ blank) for thiram and nabam were 0.01 and 0.05 µg mL^{-1} respectively with a sample throughput of 60 h^{-1}.

Interference Study

The effect of some possible inorganic ions and pesticides such as antu, asulam, diazinon, malathione, maneb, phoxime, terbofos, thiabendazole and thiobencarb, at concentration level of 0.5 µg mL^{-1} (5 fold) and 1 µg mL^{-1} (10 fold) was examined on the blank and on the determination of thiram and or nabam (0.1 µg mL^{-1}). The tolerable concentration of interference was defined as that giving a relative bias of ≤ ±5%. All the above mentioned pesticides did not interfere on the blank and on the determination of thiram and or nabam except antu >5 fold had an enhancive effect on the peak absorbance. There was no effect from cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Co^{2+}) and anions (PO^{3-}, SO_4^{2-}, and NO_3^-) at 75 and 100 fold on the determination. Therefore, the developed method may find application in real samples containing the above foreign species.

Application to Natural Water Samples

The proposed method based on solid-phase extraction and iron(III)-ferricyanide reaction was applied for analyzing thiram and nabam in natural water samples. Tap water, lake water and irrigation water samples from various locations of Quetta valley were collected in acid washed (10%, HCl), high density polyethylene bottles, filtered through a cellulose membrane filter (pore size, 0.45 mm, 47 mm, diameter, Whatman, Maidstone, UK) to remove suspended particles and stored at 4°C. The recovery experiments were carried out with spiked water samples using 0.5 – 3.0 µg mL^{-1} spikes for thiram and nabam. The results are given in Table-3. Recoveries were in the range of 93±3 – 105±2 and 87±5 – 102±2% for thiram and nabam respectively. The results obtained were in good agreement with a HPLC method [22]. For extraction of spiked thiram and nabam, Sep-Pak C18 cartridges (Waters Associates, USA) were conditioned with 5.0 mL UHP water, 5.0 mL ethanol and then air dried over 2 minutes [29]. Each sample was passed through SPE cartridge under vacuum at a flow rate of 10 mL min^{-1}. Once the retention step has been completed, the cartridges were washed with 0.5 mL UHP water and then dried by passing air for 10 minutes. Elution of the retained fungicide was achieved with 5.0 mL ethanol, and then the organic phase was brought near to dryness under a nitrogen stream. The residue was dissolved in the incubation mixture and was analyzed using the proposed rFIA-spectrophotometric manifold (Fig. 2).

Table-3: Results of recovery tests of thiram and nabam fungicides in natural water samples (n = 4).

Sample matrix	Spiked (µg mL^{-1})	Recovery (%)			
		Proposed method		HPLC method [22]	
		Thiram	Nabam	Thiram	Nabam
Tap water	0.50	98 ± 5	92 ± 4	100 ± 5	96 ± 4
	1.00	94 ± 3	102 ± 3	98 ± 7	98 ± 3
	1.50	96 ± 2	101 ± 2	92 ±4	94 ± 5
Irrigation water	1.00	105 ± 2	99 ± 3	102 ± 4	102 ± 3
	2.00	95 ± 4	102 ± 3	93 ± 6	95 ± 6
	3.00	97 ± 5	100 ± 2	105 ± 2	97 ± 4
Lake water	1.00	96 ± 7	88 ± 5	98 ± 4	93 ± 5
	2.00	102 ± 5	90 ± 4	100 ± 3	95 ± 6
	3.00	93 ± 3	87 ± 5	98 ± 3	92 ± 3

The analysis of thiram and nabam was also carried out on LC–10AT liquid chromatograph (Shimadzu, Corporation, Tokyo, Japan) equipped with double pumps, Rheodyne injector with 20 µL loop and an SPD–10A UV–Vis detector based on the work of Aulakh *et al*. [22]. An analytical column

bonda pack C18 (250 x 4.6 mm i.d., 10 μm) was used for analysis at 45°C. Acetonitrile–water (70:30, v/v), at a flow rate of 0.7 mL min^{-1} was used as the mobile phase and the analytes were monitored at 254 nm. The retention time for thiram and nabam was found 2.6 and 6.1 min respectively.

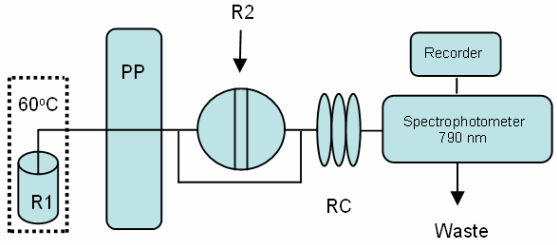


Fig. 2: Reverse FI manifold for determination of thiram and nabam. R1 = Ammonium iron(III) sulfate solution (0.02 M) in HCl (0.15 M) containing thiram and or nabam standard, temperature 60°C; R2 = potassium ferricyanide solution (0.02 M, 60 μL); PP = peristaltic pump (flow rate 2.8 mL min^{-1}); RC = reaction coil (0.8 x 200 mm).

Experimental

Reagents and Solutions

All plastic ware was cleaned in a nutrient-free detergent (2%, v/v) (Neutracon, Decon Laboratories, UK), rinsed with ultra high purity (UHP) water (0.067 μS/cm, Purelab Option, Elga, UK), soaked overnight in HCl (10%, v/v), thoroughly rinsed with UHP water and stored in zip-lock plastic bags to avoid contamination. All of the reagents were of analytical grade, obtained from Merck (Darmstadt, Germany), unless stated otherwise, and all the solutions were prepared in UHP water.

Iron(III) stock solution (0.1 M) was prepared by dissolving 4.822 g of $NH_4Fe(SO_4)_2{\cdot}12H_2O$ in HCl solution (0.15 M). A working solution (0.02 M) was prepared by diluting 20 mL of the stock solution to 100 mL with HCl (0.15 M). Potassium ferricyanide stock solution (0.1 M) was prepared by dissolving 3.293 g of $K_3[Fe(CN)_6]$ in UHP water. A working solution (0.02 M) was prepared by diluting 20 mL of the stock solution to 100 mL with HCl (0.15 M).

Thiram and nabam stock solutions (100 mg L^{-1}) were prepared by dissolving 10 mg of each compound (Dr Ehrenstorfer GmbH, Germany) in 100 mL of absolute ethanol (HPLC-grade) in brown bottles and stored at −4°C in the dark. Working standards were prepared by diluting aliquots of the stock solutions daily in $NH_4Fe(SO_4)_2{\cdot}12H_2O$ solution (0.02 M) containing HCl (0.15 M).

For the interference study, stock solutions (100 mg L^{-1}) of antu, asulam, diazinone, malathion, maneb, phoxime, terbofos, thiabendazole, and thiobencarb pesticides were prepared by dissolving 10 mg of each compound in 100 mL of absolute ethanol (HPLC grade). Cation and anion stock solutions (250 mg L^{-1}) including sodium, potassium, magnesium, calcium, cobalt, manganese, nitrate, sulfate and phosphate ions were prepared in UHP water and subsequent standard solutions of each were prepared by serial dilution of the stock solutions with HCl (0.15 M).

Flow Injection Manifold and Procedure

The rFIA manifold used in this work is shown in Fig. 2. A peristaltic pump (Ismatec, Switzerland) was used to propel the incubated mixture (ammonium iron(III) sulfate (0.02 M) in HCl (0.15 M) containing thiram and nabam standards, temperature (60°C) at a flow rate of 2.8 mL min^{-1}. A rotary injection valve (Rheodyne 5020, Anachem, Luton, UK) was used to inject potassium ferricyanide solution (0.02 M, 60 μL) into the incubated mixture stream. The stream was then passed through a reaction coil (20 cm) forming a soluble Prussian blue complex that was monitored at 790 nm with a spectrophotometer (Jenway 6505, UK) equipped with a 1 cm path length glass flow through cell (80 μL, Hellma Analytics, Germany). The detector output was recorded using a chart recorder (Kipp & Zonen, BD40, Netherlands). All manifold tubing was PTFE (0.8 mm i.d., Fisher, Loughborough, UK).

Conclusions

The reported reverse flow injection spectrophotometric method for the determination of thiram and nabam in natural water samples is simple with low limits of detection (0.01 and 0.05 μg mL^{-1} for thiram and nabam respectively). The method has a high sample throughput (60 h^{-1}) with relative standard deviations (n = 3) of 0.8 – 1.6% in the range studied. The method was applied to natural water samples and the recoveries were in good agreement with a HPLC method. Table-4 summarizes a comparison between presented approach and previously reported methods for the determination of thiram. The proposed method is better in terms of low detection limit, good precision (RSD 1.6%) and high sample throughput all compare favorably with previously reported spectrophotometric methods.

Table-4: Comparison of the proposed method with other spectrophotometric methods for determination of thiram fungicide.

Technique	Matrix	Linear range (μg mL^{-1})	LOD (μg mL^{-1})	R^2 value	RSD (%)	Ref.
Spectrophotometry	Commercial samples	0 – 24	0.3	0.9754	1.9	8
Spectrophotometry	Aqueous solution	0.5 – 2.5	0.33	NR	NR	9
Colorimetry	Natural waters and plant seeds	0.048 – 2.404	0.041	0.9990	<3.7	10
Spectrophotometry	River water	0 – 20	0.161	NR	NR	11
Spectrophotometry	Natural waters and plant seeds	0.025 – 1.0	0.0115	0.9985	1.1 – 2.7	12
Spectrophotometry	Grains and vegetables	0.44 – 13.25	0.0147	NG	0.86	13
FI–Spectrophotometry	Natural water	0.01 – 8.0 thiram 0.1 – 30 nabam	0.01 0.05	0.9999 0.9985	0.8 – 1.6	This work

NR, not reported

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