

Development of a Derivative Spectrophotometric Method for the Determination of Fungicide Zinc Ethylenbisdithiocarbamate using Sodium Molybdate

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Neste trabalho foi desenvolvido um procedimento espectrofotométrico derivativo para a determinação de etilenobisditiocarbamato de zinco(II), Zineb, com base na formação do complexo azul de molibdato de sódio em meio ácido. A lei de Beer é obedecida até 40 $\mu\text{g mL}^{-1}$ de Zineb no comprimento de onda de 956 nm. O limite de detecção foi de 0,006 $\mu\text{g mL}^{-1}$ de Zineb para a razão S/N = 3 considerando diversos parâmetros como o efeito da concentração de ácido. A interferência de diversos íons na determinação de Zineb foi avaliada. A maioria dos metais alcalinos e sais metálicos não causou interferências. O procedimento tem boa sensibilidade e foi empregado para a determinação de Zineb em amostras comerciais de alimentos contendo Dithane Z 78 e Hexathane 75% W.P.. Os resultados foram comparados com procedimentos previamente propostos. Zineb foi determinado com sucesso sem a ocorrência de interferências na presença de outros ditiocarbamatos, tais como Ziram, Thiram, Ferbam etc.

A derivative spectrophotometric procedure was developed for the determination of zinc(II) ethylenbisdithiocarbamate, Zineb, after formation of its blue colored complex with sodium molybdate in acidic medium. The Beer's law is obeyed up to 40 $\mu\text{g mL}^{-1}$ of Zineb at 956 nm. The detection limit was 0.006 $\mu\text{g mL}^{-1}$ for Zineb when S/N ratio is 3 taking into account various parameters, such as the effect of acid concentration. The interference of a large number of ions on the determination of Zineb was evaluated. Most of the alkaline metals and metal salts did not interfere. The procedure presented proper sensitivity and it was applied for determining Zineb in food stuffs and commercial samples of Dithane Z 78 and Hexathane 75% W.P.. Results were compared with earlier reported methods. Zineb was successfully determined without any interferences in the presence of other dithiocarbamates like ziram, thiram, ferbam etc.

Keywords: derivative spectrophotometry, Zineb, sodium molybdate, commercial samples

Introduction

Zineb is a light colored ethylenbisdithiocarbamate (EBDC) fungicide (Figure 1) used to prevent crop damage in the field and to protect the harvested crops from deterioration during storage and transport. It is a non-systemic (surface acting) fungicide, commercially available as wettable powder, dust formulations and also in mixture with other fungicides under trade names like Dithane Z-78, Amitan etc. Like other EBDCs, it also shows strong metal binding properties^{1,2} and hence is capable of blocking enzymes and affecting biological systems. Even the breakdown products of Zineb, viz. ethylenethiourea (ETU) and ethylene thiuram monosulfide, are quite harmful. The US EPA has classified ETU as a probable

human carcinogen. Hence, the residues of Zineb in food and fodder are harmful and can also go through soil and leach into the ground water.

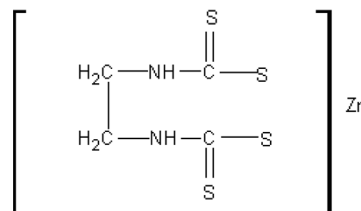


Figure 1. Structure of Zineb.

Many methods like spectrophotometry,^{1,3-8} flame atomic absorption spectrometry^{9,10} FT-IR,¹¹ gas chromatography,^{12,13} HPLC,¹⁴⁻¹⁶ polarography,^{17,18} biosensors,¹⁹ and enzyme linked immuno sorbent assay²⁰ are available for Zineb determination and other dithiocarbamates.

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Most of the applied methods use different techniques like spectrophotometry and gas chromatography³ based on analysis of CS_2 , H_2S or amines which are evolved by the decomposition of EBDCs. Dithiocarbamates have been determined in vegetables and food stuffs using HPLC,²¹ extraction voltammetry²² and titrimetry.²³ Dithiocarbamates (DTCs) can also be determined by methods like, iodometry^{24,25} and indirect complexometry²⁶ etc. Although, the methods discussed above have various disadvantages, such as methods other than gas chromatography are indirect, time consuming and less sensitive. Gas chromatographic methods are sensitive but suffer from lack of the selectivity since all dithiocarbamate pesticides evolve carbon disulphide on acid hydrolysis. Moreover, the dithiocarbamate decompositions are strongly dependent on temperature and on individual dithiocarbamates. The evolution of CS_2 requires more than 2 h for the decomposition of dithiocarbamate. Additionally, high performance LC methods are relatively expensive and may involve the use of the toxic and expensive solvents. DTCs are also determined using FTIR spectrometry but this method is less sensitive and time consuming.²⁷

In the work here described, a relatively fast, simple, sensitive and selective derivative spectrophotometric method is presented for the analysis of Zineb by converting it into a molybdenum complex. Zineb reacts with sodium molybdate under heating to form a blue colored complex. Zineb and sodium molybdate formed a 1:2 complex. Zineb releases Zn^{2+} and its dithiocarbamate unit, the latter forms a complex with sodium molybdate which is then extracted into methyl isobutyl ketone (MIBK) and determined by derivative spectrophotometry. The significant advantage of this method compared to the gas chromatographic methods is that it can be applied for the direct determination of Zineb in the presence of other dithiocarbamates like Ziram, Thiram and Ferbam.

Materials and Methods

Equipment and reagents

An Elico SL-164, double beam UV-Vis spectrophotometer was used. Zineb standard was obtained from Riedel de Haën, Germany. A stock solution was prepared by dissolving 100 mg of Zineb in 100 mL of 0.1 mol L^{-1} NaOH. Further dilutions were carried out with 0.1 mol L^{-1} NaOH as required. A 2% m/v solution of sodium molybdate was prepared in doubly distilled water.

Absorption spectra

A volume of 2 mL of 2% m/v sodium molybdate solution and 1 mL of 2 mol L^{-1} H_2SO_4 were added to an

aliquot containing 100 μg of Zineb. The mixture was boiled for 5 min, cooled and water was added to make the volume up to 5 mL. The blue complex formed was extracted after shaking into 5 mL of MIBK (methyl isobutyl ketone). The organic layer was collected into a test tube containing fused CaCl_2 to remove any moisture. The suspension was then decanted into a 1 cm cell and the spectrum was taken against a reagent blank. The molybdenum complex shows peaks at 670 nm and 956 nm (Figure 2), but the peak at 956 nm has much higher absorbance; hence all the measurements were made at this wavelength. The first derivative, second derivative, third derivative and fourth derivative curves were given in Figures 3-6 respectively.

In Derivative Spectrophotometry, DS, the wavelength reproducibility and signal to noise ratio are quite important. The features like peak height and noise level depend on parameters chosen like order of derivative, scan speed and integration time during recording of spectra. The use of optimum parameter will give better resolution

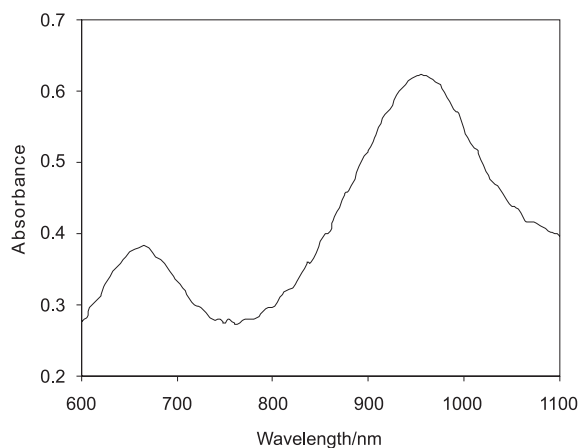


Figure 2. Absorption spectrum of molybdenum ethylenebis(dithiocarbamate) complex: Zineb (2 mL) with sodium molybdate (2% m/v) in presence of H_2SO_4 (2 mol L^{-1}), extracted with MIBK compared to a reagent blank.

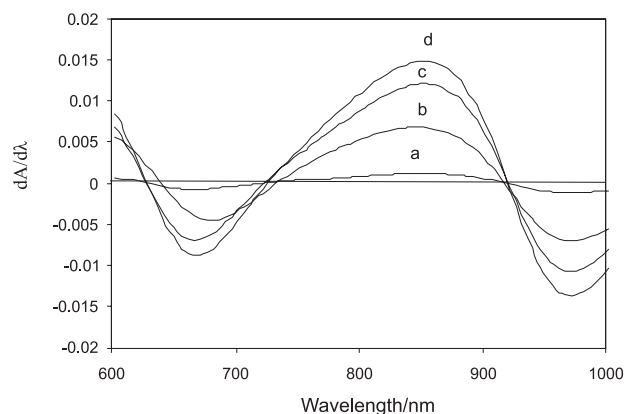


Figure 3. 1st derivative curves of molybdenum-ethylenebis(dithiocarbamate) complex: a, b, c, d representing the amount of Zineb in final solutions (a: 2, b: 10, c: 20, d: 40, all values in $\mu\text{g mL}^{-1}$).

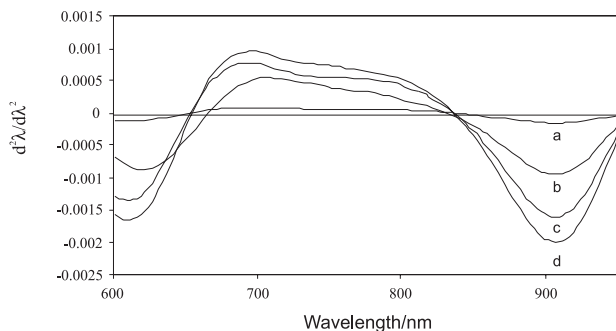


Figure 4. 2nd derivative curves of molybdenum-ethylenebisdithiocarbamate complex: a, b, c, d representing the amount of Zineb in final solutions (a: 2, b: 10, c: 20, d: 40, all values in $\mu\text{g mL}^{-1}$).

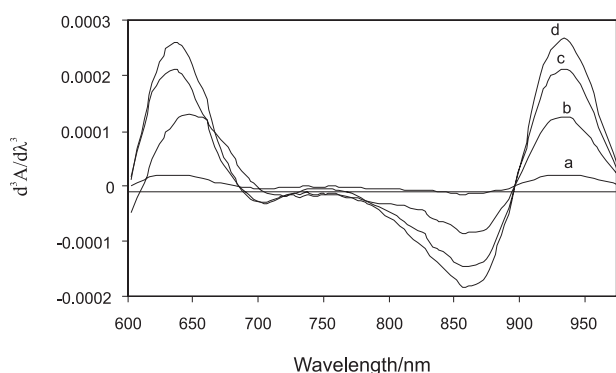


Figure 5. 3rd derivative curves of molybdenum-ethylenebisdithiocarbamate complex: a, b, c, d representing the amount of Zineb in final solutions (a: 2, b: 10, c: 20, d: 40, all values in $\mu\text{g mL}^{-1}$).

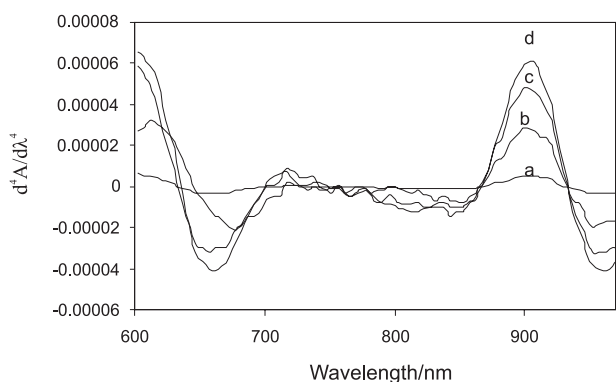


Figure 6. 4th derivative curves of molybdenum-ethylenebisdithiocarbamate complex: a, b, c, d representing the amount of Zineb in final solutions (a: 2, b: 10, c: 20, d: 40, all values in $\mu\text{g mL}^{-1}$).

and improved sensitivity. For the 4th derivative spectra, $\Delta\lambda = 9 \text{ nm}$ was found to be ideal.

Preparation of calibration curve

Solutions containing from 10 to 250 μg of Zineb were analysed applying the proposed procedure and the

derivative spectra were obtained against a reagent blank prepared under similar conditions. The characteristics of zero order and derivative spectra are summarized in Tables 1 and 2.

Table 1. Different parameters of zero order spectrum

Sample No.	Parameter	Zero derivative spectrum of Zineb-sodium molybdate complex
1	molar absorptivity/ ($\text{L mol}^{-1} \text{cm}^{-1}$)	1.4×10^4
2	specific absorptivity/ ($\text{mL g}^{-1} \text{cm}^{-1}$)	35.2
3	Sandell's sensitivity/ ($\mu\text{g mL}^{-1}$)	2.84×10^{-2}
4	analytical sensitivity/ ($\mu\text{g mL}^{-1}$)	2.80×10^{-2}
5	linear range/($\mu\text{g mL}^{-1}$)	2 to 40

Results and Discussion

Beer's law and sensitivity

The optimum wavelength interval was found to be 9 nm for improve both resolution and sensitivity. The wavelength range to obtain the spectra was selected from 600 to 1100 nm. The calibration curve was obtained by measuring the peak height between wavelengths from 850 to 938 nm. Absorbance of sodium molybdate complex with Zineb recorded against a reagent blank was linear over a concentration range from 2 to 40 $\mu\text{g mL}^{-1}$. The estimated detection limit is 0.006 $\mu\text{g mL}^{-1}$ for Zineb when S/N ratio was 3.

Effect of heating time

It was observed that the absorbance of the complex increased up to certain extent when heating time was also increased. Therefore, the reaction mixture was heated in different intervals of time. It was observed that a 5 min heating time was enough to obtain maximum absorbance. Increasing the heating time beyond this did not increase the absorbance showing that complete complex formation was achieved.

Effect of acid concentration

Maximum absorbance was observed when acid volume added varied from 1 to 1.5 mL. Greater acid volumes caused a decrease in absorbance signals, thus higher acid concentrations did not improve the complex formation.

Table 2. Comparison of calibration curves of Zineb using different derivative spectra

Zineb complex	Order of derivative	λ/nm	Regression equation	R^2	RSD (%)	SD of slope	SD of intercept	Analytical sensitivity/ ($\mu\text{g mL}^{-1}$)
1	1 st	852	$y = 6 \times 10^{-5}x + 0.007$	0.9997	± 1.9	1.01×10^{-6}	1.4×10^{-4}	1.20
2	2 nd	907	$y = 6 \times 10^{-6}x + 0.009$	0.9937	± 1.5	8.16×10^{-8}	1.6×10^{-4}	0.16
3	3 rd	937	$y = 8 \times 10^{-7}x + 9 \times 10^{-5}$	0.9992	± 1.9	8.16×10^{-9}	8.16×10^{-7}	0.20
4	4 th	902	$y = 3 \times 10^{-7}x - 9 \times 10^{-6}$	0.9994	± 1.3	5.03×10^{-9}	8.16×10^{-8}	0.006

Effect of other ions

Sample solutions containing $5 \mu\text{g mL}^{-1}$ of Zineb and various amounts of different alkaline metal salts or metal ions were prepared and the general procedure was applied. It was observed that the addition of the following foreign anions did not interfere in the determination of Zineb: bromide (11 mg), acetate (15 mg), chloride (3.5 mg), fluoride (2 mg), citrate (20 mg) and EDTA (0.06 mg). Among the metal ions examined, Zn(II) (0.066 mg), Mo(IV) (0.14 mg), Ni(II) (0.30 mg), Co(II) (0.009 mg) did not interfere. It was clear that several ions like Fe(II and III), Mn(II), Hg(II), interfered in Zineb determination. Therefore, 1 mL of 5% m/v NaF solution was used to mask Fe(II) and Fe(III) ions whereas 2 mL of 5% m/v sodium citrate was used to mask Hg(II) and Cu(II). Manganese(II) was masked by adding 1.5 mL of 1% m/v potassium bromide solution.

Effect of standing time

It was observed that the solution absorbance became constant after 2-3 min. So, after extracting into MIBK, a 5 min standing time was selected. The absorbance of the complex remained practically constant for about

30 min with absorbance RSD values varying from 0.69 to 1.6% ($n = 3$) at analytes concentrations between 2 to $40 \mu\text{g mL}^{-1}$.

Applications

Determination of Zineb in fortified samples of wheat grains, cabbage and rice

The developed procedure was applied to the determination of Zineb in fortified samples of wheat grains, cabbage and rice. A mass of 20 g of the foodstuff was finely crushed and a solution containing a known amount of Zineb was added and mechanically shaken with 100 mL of ACN acetonitrile, ACN, for 1 h. This mixture was filtered and the residue was washed with $3 \times 10 \text{ mL}$ portions of ACN. The extracts were evaporated in a water bath (70-90 °C). Residual solvent were removed using a current of dry air. The Zineb content in the residue was determined using the developed procedure and the results indicated good recoveries in all cases (Table 3).

Determination of Zineb in commercial samples

The method was applied for determination of Zineb in commercial samples, Dithane Z 78 and Hexathane 75% W.P. The formulated product sample solutions were

Table 3. Determination of Zineb in fortified samples (mean values and standard deviations, $n = 5$)

Sample	Crop	Zineb Added/ (μg)	Zineb Found/ (μg)	Recovery (%) \pm RSD (%)	Rangaswamy <i>et al.</i> ³⁰ method	
					Zineb found/ (μg)	Recovery (%)
Zineb	Wheat	10	9.7	97.0 ± 2.4	9.6	96.0
		15	14.8	98.6 ± 2.3	14.7	98.0
		20	19.8	99.0 ± 1.9	19.8	99.0
	Rice	8	7.8	97.5 ± 2.5	7.6	95.0
		10	9.9	99.0 ± 2.3	9.6	96.0
		12	11.8	98.3 ± 2.2	11.6	96.6
	Cabbage	12	11.8	98.3 ± 2.3	11.7	97.5
		14	13.8	98.5 ± 2.4	13.7	97.8
		18	18.1	100.5 ± 2.2	17.6	97.8

Sample mass: 10 g.

Table 4. Determination of Zineb in commercial samples (mean values and standard deviations, n = 5)

Commercial Samples	Zineb taken/(μg)	Zineb found/(μg)	RSD(%)	Rangaswamy <i>et al.</i> ³⁰ method	
				Zineb found/(μg)	Recovery (%)
Dithane Z-78	8	7.7	2.1	7.6	95.0
	20	19.9	2.3	19.8	99.0
Hexathane 75% W.P.	5	4.9	1.9	4.7	94.0
	15	15.1	2.2	14.6	97.3

Table 5. Determination of Zineb and Ziram in synthetic mixtures (mean values and standard deviations, n = 5)

Zineb added/(μg)	Ziram added/(μg)	Zineb found/(μg)	Ziram found/(μg)	Recovery (%) \pm RSD (%)	
				Zineb	Ziram
60	40	59.0	39.5	98.3 \pm 2.3	98.7 \pm 2.7
50	50	49.7	49.0	99.4 \pm 2.8	98.0 \pm 2.5
30	70	29.5	69.5	98.3 \pm 3.1	99.3 \pm 2.4

prepared as discussed earlier and determined using the proposed procedure. Results are presented in Table 4.

Sequential determination of Zineb and Ziram in synthetic mixtures

The procedure was applied for the simultaneous determination of Ziram and Zineb in synthetic mixtures. Ziram forms a yellow complex with sodium molybdate in cold solution which absorbs at 420 nm²⁸, whereas Zineb forms complex under heating, all other conditions remaining the same. Synthetic mixtures of Zineb and Ziram were made in different proportions. To the binary mixture, 0.15 mL of 2 mol L⁻¹ H₂SO₄ and 2 mL of 2% m/v sodium molybdate were added. Molybdenum-Ziram complex was extracted into a volume of 5 mL MIBK and Zineb remained in aqueous phase. The absorbance of Ziram-molybdenum complex was measured at 420 nm. Afterwards, it was added 1 mL 4 mol L⁻¹ H₂SO₄ and 2 mL of 2% m/v sodium molybdate solution to the aqueous phase containing Zineb. This solution was

boiled for 5 min, cooled and extracted into 5 mL MIBK. The spectrum of blue complex of Zineb was taken between 600 and 1100 nm. Ziram was determined from the standard calibration curve.²⁸ Results are shown in Table 5.

Conclusions

The proposed procedure is more sensitive than the carbon disulphide evolution methods. Using this procedure 0.006 $\mu\text{g mL}^{-1}$ of Zineb can be determined which is equivalent to 0.0033 $\mu\text{g mL}^{-1}$ of evolved carbon disulphide. Additionally, direct sequential determination of Ziram and Zineb is also possible. The sensitivity of the present procedure is comparable to other spectrophotometric methods, however its selectivity was better (Table 6). This comment is in disagreement with the remark pointed out in Table 6. The broad applicability of this method makes it suitable for dithiocarbamate analysis in foodstuffs and in commercial samples.

Table 6. Molar absorptivities of Zineb complexes

Procedure	Molar absorptivity/ (L mol ⁻¹ cm ⁻¹)	Remarks	Reference
Cupric acetate	Not reported	Low sensitivity, long tedious procedure	30
Molybdenum	0.62 $\times 10^4$	Less sensitive	28
Phenylfluorone + Cetylpyridinium bromide	6.9 $\times 10^4$	Selective and sensitive	31
1-(2-Pyridylazo)-2-naphthol	5.06 $\times 10^4$	Column pre-concentration is required	29, 32
Molybdenum (at 956 nm) and derivative spectrophotometry	1.4 $\times 10^4$	Similar molar absorptivity and improved selectivity	This work

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Received: June 11, 2008

Web Release Date: March 31, 2009