Bull Environ Contam Toxicol (2008) 80:85–89
DOI 10.1007/s00128-007-9321-5

Spectrophotometric Determination of Endosulfan Using Thionin and Methylene Blue as Chromogenic Reagents

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Received: 5 November 2007 / Accepted: 6 November 2007 / Published online: 6 December 2007

Abstract A simple, selective and sensitive spectrophotometric method is proposed for the determination of widely used organochlorine pesticide endosulfan using thionin and methylene blue as chromogenic reagents. The method is based on the liberation of sulfur dioxide from endosulfan by adding acid reagent and alcoholic potassium hydroxide. The liberated sulfur dioxide is passed through potassium iodate solution and the iodine so liberated bleaches the violet color of thionin and blue color of methylene blue and is measured at 600 nm and 665 nm respectively. This decrease in absorbance is directly proportional to the endosulfan concentration. The Beer's law is obeyed in the range of 0.4–7.0 and 0.2–9.0 μg mL^{-1} of endosulfan using thionin and methylene blue as reagents respectively. The molar absorptivity and Sandell's sensitivity were found to be 1.05×10^5 and 5.03×10^4 L mol^{-1} cm^{-1}, 3.85×10^{-3} and 8.10×10^{-3} μg cm^{-2} of endosulfan using thionin and methylene blue as reagents respectively. The method has been applied for the determination of endosulfan in water, soil and vegetables.

Keywords Endosulfan determination · Spectrophotometry · Iodate · Thionin · Methylene blue

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Endosulfan (6,7,8,9,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4-benzodioxa thiepin-3-oxide) is a toxic organochlorine pesticide. It has smell like turpentine, does not occur naturally in the environment (ATSDR 2000). It is used to control the pests in vegetables and fruits. It is widely used on cauliflower, rice, potato, coffee, maize grapes, spinach, strawberries etc. It reaches the body through contaminated water and food. It is highly toxic and major route for the endosulfan absorption in application tasks are dermal and respiratory (Dikshit et al. 1988). The toxic symptoms in human beings caused by endosulfan are hyperexcitability, dypsnea, salivation and reduction of protein, carbohydrate and lipid content in liver etc. It is reported to be a carcinogenic compound (Sax 1981; Edward 1973). Endosulfan has been shown to be highly toxic to freshwater fish, promoting metabolic and reproductive disorders (Mishra and Shukla 1997). Additionally the accumulation of endosulfan in marine species has been reported (Naqvi and Vaishnavi 1993). For these reasons due care must be exercised when using this product in close proximity to surface water; the environmental protection agency recommends that the level of endosulfan in lakes and rivers should not exceed 74 ppb. Endosulfan may be absorbed via the gastrointestinal tract, by inhalation or by contact with the skin. The exposed workers are reported to suffer from irritability, convulsions and related neurological disorders (Lemes et al. 1993). The oral LD_{50} = 90–100 mg kg^{-1} for rats and dermal LD_{50} = 35 mg kg^{-1} (Grudev et al. 1983).

Various analytical methods proposed for the determination of endosulfan were gas chromatography (Lentza et al. 2001), high performance liquid chromatography (Galeano et al. 1992), visual spectrophotometry (Zweig 1964), stripping voltametry (Prabu and Manisankar 1999) and infrared spectroscopy (Guillermo et al. 2005). The

spectrophotometric method for the determination of endosulfan is based on its liberation of sulfur dioxide by using acid reagent which is then absorbed in an absorbing medium and subsequently estimated by a suitable reagent. Some of the reported reagents for the spectrophotometric determination of endosulfan are *p*-aminoazobenzene and formaldehyde (Jaishree and Gupta 1991), *p*-rosaniline methylsulfonic acid (Dangwal and Mithbavkar 1995) and leucocrystal blue (Asthana et al. 2002). In the present communication new reagents thionin and methylene blue are used for simple, selective and sensitive spectrophotometric determination of endosulfan. This method is based on the liberation of sulfur dioxide from endosulfan by *p*-toluene sulfonic acid. The liberated sulfur dioxide is passed through potassium iodate and N-chlorosuccinimide solution and the iodine so liberated bleaches the violet color of thionin or blue color of methylene blue and is measured at 600 and 665 nm respectively. This decrease in absorbance is directly proportional to the endosulfan concentration. The proposed method has been successfully applied for the determination of endosulfan in water, soil and vegetables.

Materials and Methods

A Secomam Anthelie NUA 002 UV–Visible spectrophotometer with 1 cm quartz cell was used for the absorbance measurements and a WTW pH 330, pH meter was used. Impingers, a vacuum pump and a flow rate adjustable calibrated rotameter were used for the liberation and absorption of sulfur dioxide from endosulfan. All chemicals used were of analytical reagent grade or chemically pure grade and double distilled water was used throughout the study. A stock solution of endosulfan (1 mg mL^{-1}) was prepared in ethanol and working standard was prepared by appropriate dilution of the stock. N-Chlorosuccinimide (NCS) was prepared by adding 500 mg of N-chlorosuccinimide in a 500 mL volumetric flask containing 5 g of succinimide, 50 mL of water was added to dissolve the solids and the solution was finally diluted to 500 mL with water. Acid reagent was prepared by dissolving 15.2 g of *p*-toluene sulfonic acid in 50 mL of isoproponol, to this 10 mL of water was added. A 0.1 N potassium iodate and 2% alcoholic potassium hydroxide were used. A 0.1% solution of thionin was prepared by dissolving 0.1 g of thionin in 25 mL methanol and made up to 100 mL with distilled water. A 0.05% solution of methylene blue was prepared by dissolving 0.05 g of methylene blue in distilled water and made up to 100 mL with distilled water. Absorbing solution for sulfur dioxide was prepared by mixing 7 mL of potassium iodate solution, 1 mL of NCS, 1 mL of phosphate buffer and 0.2 mL of phosphoric acid followed by dilution to 10 mL with water.

An aliquot of working standard solution of endosulfan containing 0.4–7.0 μg mL^{-1} was taken in an impinger, 5 mL of acid reagent and 1 mL of alcoholic potassium hydroxide were added. It was placed in a hot water bath maintained at 90°C. This impinger was connected to two other impingers of the same capacity containing 10 mL of absorbing solution connected to a suction pump. Purified air was passed through the solution at a rate of 1 L min^{-1} for 30 min. The sulfur dioxide was liberated and absorbed in the absorbing solution. The solutions of two impingers were mixed and pH was adjusted to 4.0 ± 0.2, 1 mL of 0.1% of thionin was added to the solution and diluted to 25 mL. The solution was kept aside for 10 min before taking absorbance and absorbance was measured at 600 nm against reagent blank. The absorbance corresponding to the bleached color which inturn corresponds to the analyte (endosulfan) concentration was obtained by subtracting the absorbance of the blank solution from that of test solution. The amount of the endosulfan present in the volume taken was computed from the calibration graph. The same procedure with different aliquots of the solution of endosulfan containing 0.2–9.0 μg mL^{-1} was used with 1 mL of 0.05% of methylene blue as reagent and the absorbance was measured at 665 nm against reagent blank.

Water samples (10 mL), 10 g of finely ground soil samples and vegetable samples (10 g) were spiked with known amount of the working standard solution of endosulfan. These samples were kept for 30 min and then extracted with 2 × 10 mL portions of ether. The extracts from soil and vegetables sample were decanted and washed with 2 × 5 mL, portions of water. The extract were dried over anhydrous sodium sulfate and made up to the mark by ether in a 50 mL volumetric flask. Aliquots of the washed extracts of endosulfan were evaporated off under suction. To the residue, 5 mL of acid reagent and 1 mL of alcoholic potassium hydroxide solutions were added. Sulfur dioxide so liberated was absorbed and analyzed by the proposed method. Suitable volume of aliquot was analyzed according to the proposed and reference method. The recovery of endosulfan for water, soil and vegetables varied from 97%–99.7% (Tables 2 and 3).

Results and Discussion

The method involves the liberation of sulfur dioxide from endosulfan by using *p*-toluene sulfonic acid. The liberated sulfur dioxide is passed through potassium iodate and N-chlorosuccinimide solution and the iodine so liberated bleaches the violet color of thionin and blue color of methylene blue and is measured at 600 and 665 nm respectively. This decrease in absorbance is directly proportional to the endosulfan concentration and obeys Beer's law in the range

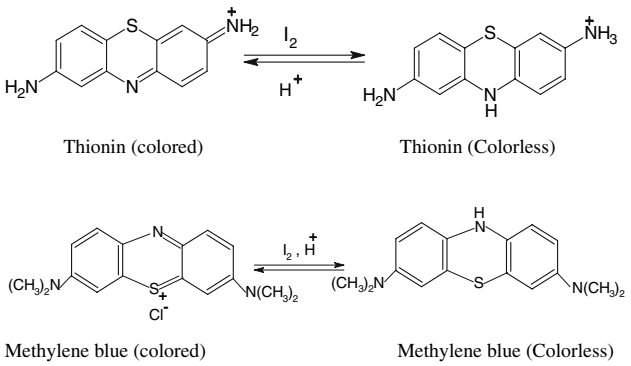

Fig. 1 Oxidation of thionin and methylene blue

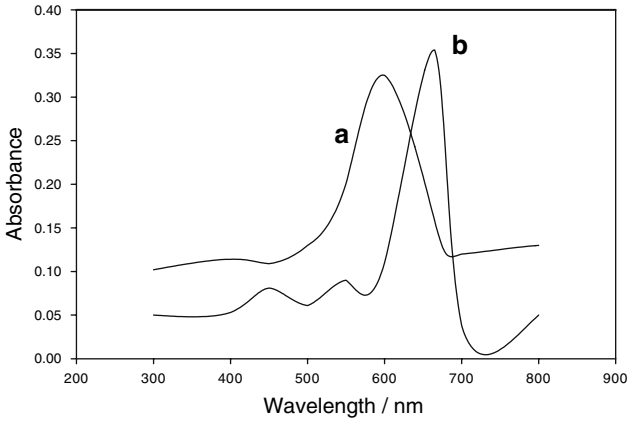

Fig. 2 Absorption spectra of colored species of (a) Thionin (b) Methylene blue

of 0.4–7.0 and 0.2–9.0 μg mL^{-1} of endosulfan using thionin and methylene blue as reagents respectively. Absorption spectra of thionin and methylene blue (Fig. 2) and the reaction systems (Fig. 1/Scheme 1) are presented. A linear calibration graph was obtained for 0.4–7.0 and 0.2–9.0 μg mL^{-1} of endosulfan with thionin and methylene blue in a final volume of 25 mL. The slope and intercept of endosulfan determination were 0.217 and 0.068, 0.104 and 0.043 respectively and correlation coefficient was 0.968 and 0.972. The limit of detection (LOD = 3.3 σ/S) and limit of quantification limit (LOQ = 10 σ/S) for the endosulfan determination were found to be 0.13 and 0.39 μg mL^{-1}, 0.27 and 0.82 μg mL^{-1} respectively. The molar absorptivity (ε) and Sandell's sensitivity (s) of the reaction system were found to be 1.05×10^5 L mol^{-1} cm^{-1}, 3.85×10^{-3} μg/cm^2, 5.03×10^4 L mol^{-1} cm^{-1}, 8.10×10^{-3} μg cm^{-2} respectively.

For the generation of sulfur dioxide from endosulfan solution, 3 mL of *p*-toluene sulfonic acid and 1 mL of alcoholic potassium hydroxide were sufficient. The temperature required for the liberation of sulfur dioxide from endosulfan was found to be between 90–100°C. Sulfur dioxide was effectively absorbed in an absorbing solution containing 5 mL of potassium iodate, 1 mL of NCS, 1 mL of phosphate buffer and 0.2 mL of phosphoric acid. It was found that 1 mL of 0.1% thionin was used for subsequent decolorization and 1 mL of 0.05% methylene blue was used for subsequent decolorization. Under optimum reaction condition, reaction system was found to be stable about 5 h. The effect of pH on the color reaction was studied and the optimum pH was found to be 4.0 ± 0.2. Above and below this pH the sensitivity and stability of the dye was affected.

The effect of various ions on the determination of endosulfan using thionin and methylene blue was examined. The tolerance limits of interfering species were established at those concentrations that do not cause more than ±2.0% error in absorbance values of endosulfan at 2 μg mL^{-1}. The tolerance limits of foreign ions are listed (Table 1). The results indicated most of the cations like Al^{3+}, Co^{2+}, Sr^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, In^{3+}, Zn^{2+}, Cr^{3+}, Ca^{2+}, etc. do not interfere. Fe^{3+} and As^{3+} were found to interfere.

Table 1 Effects of diverse ions on the determination of (2.0 μg mL^{-1}) of endosulfan

Foreign ion	Tolerance limit in μg mL^{-1} Thionin as reagent	Tolerance limit in μg mL^{-1} Methylene blue as reagent
Ni^{2+}, In^{3+}, Al^{3+}, Tartarate, Oxalate, Acetate	1,000	850
Zn^{2+}, Sr^{2+}, Ca^{2+}, Nitrate	750	700
Co^{2+}, Cr^{3+}, Cd^{2+}, Mn^{2+}	500	500
As^{3+} *, Fe^{3+} *	≤20	≤10

Scheme 1 Reaction system of endosulfan

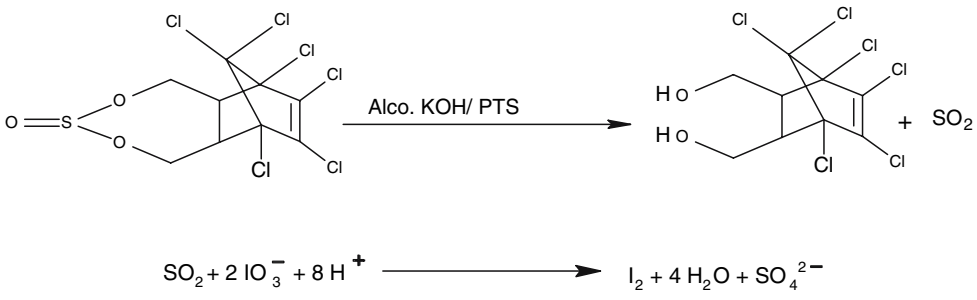

Table 2 Determination of endosulfan in water, soil and vegetables

Samples	ES added*	Proposed method		Reference method		*t*-Test[b]	*F*-Test[c]
		ES found ± SD[a]	% of Recovery	ES found ± SD[a]	% of Recovery		
Water	2.0	1.97 ± 0.01	98.5	1.96 ± 0.02	98.0	1.00	4.00
	4.0	3.94 ± 0.02	98.5	3.97 ± 0.03	99.2	1.87	2.25
	6.0	5.98 ± 0.03	99.7	5.96 ± 0.02	99.3	1.25	2.25
Soil	2.0	1.96 ± 0.02	98.0	1.98 ± 0.03	99.0	1.25	2.25
	4.0	3.96 ± 0.02	99.0	3.94 ± 0.04	98.5	1.00	4.00
	6.0	5.98 ± 0.04	99.7	5.95 ± 0.05	99.2	1.07	1.56
Vegetables	2.0	1.98 ± 0.04	99.0	1.96 ± 0.03	98.0	0.71	1.78
	4.0	3.95 ± 0.02	98.7	3.92 ± 0.03	98.0	1.87	2.25
	6.0	5.97 ± 0.05	99.5	5.94 ± 0.06	99.0	0.86	1.44

[a] Mean ± Standard deviation (n = 5). [b] Tabulated *t*-value for 8 degrees of freedom at *p* (0.95) is 2.306. [c] Tabulated *F*-value for (4, 4) degrees of freedom at *p* (0.95) is 6.39. ES – Endosulfan, *Concentration is expressed in terms of μg mL^{-1}

Table 3 Determination of endosulfan in water, soil and vegetables

Samples	ES added*	Proposed method		Reference method		*t*-Test[b]	*F*-Test[c]
		ES found ± SD[a]	% of Recovery	ES found ± SD[a]	% of Recovery		
Water	1.0	0.98 ± 0.02	98.0	0.97 ± 0.03	97.0	0.62	2.25
	3.0	2.97 ± 0.03	99.0	2.96 ± 0.04	98.6	0.45	1.78
	5.0	4.95 ± 0.05	98.0	4.98 ± 0.07	99.6	0.79	1.96
	8.0	7.97 ± 0.03	99.6	7.94 ± 0.05	99.2	1.15	2.78
Soil	1.0	0.97 ± 0.02	97.0	0.98 ± 0.02	98.0	0.79	1.00
	3.0	2.98 ± 0.04	99.3	2.95 ± 0.05	98.3	1.04	1.56
	5.0	4.97 ± 0.02	99.4	4.95 ± 0.03	99.0	1.25	2.25
	8.0	7.96 ± 0.03	99.5	7.98 ± 0.04	99.7	0.91	1.78
Vegetables	1.0	0.98 ± 0.01	98.0	0.97 ± 0.02	97.0	1.00	4.00
	3.0	2.98 ± 0.02	99.3	2.97 ± 0.03	99.0	0.62	2.25
	5.0	4.96 ± 0.02	99.2	4.95 ± 0.03	99.0	0.62	2.25
	8.0	7.95 ± 0.04	99.4	7.92 ± 0.05	99.0	1.07	1.56

[a] Mean ± Standard deviation (n = 5). [b] Tabulated *t*-value for 8 degrees of freedom at *p* (0.95) is 2.306. [c] Tabulated *F*-value for (4, 4) degrees of freedom at *p* (0.95) is 6.39. ES – Endosulfan, *Concentration is expressed in terms of μg mL^{-1}

However, the interference of Fe^{3+} and As^{3+} can be masked by the addition of 1 mL of 1% sodium fluoride and 1 mL of 2% 2,3 dimercaptopropanol (Sastri 1996) respectively. The proposed method was applied to the quantitative determination of endosufan in water, soil and vegetables. The results of the analysis of the above samples (Tables 2 and 3) compare favorably with those from a reference method (Raju and Gupta 1992). Statistical analysis of the results by the use of *t* and *F*-tests showed there was no significant difference between the accuracy and precision of the proposed and reference method. The reliability of the method to analyze real samples were checked by recovery experiments, which gave quantitative results with good reproducibility. The reproducibility of the proposed method was evaluated by five replicate analysis of samples containing endosulfan at different concentrations.

The optical parameters include Beer's law range, molar absorptivity, Sandell's sensitivity and other parameters (Table 4). The precision and accuracy of the method was studied by analysing the solution containing known amounts cited reagents within the Beer's law limit.

For the first time, thionin and methylene blue have been used as a chromogenic reagent for the spectrophotometric

Table 4 Optical characteristics and precision data

Parameters/Characteristics	Thionin	Methylene blue
Color	Violet	Blue red
λ max (nm)	600	665
Beer's law range (μg mL^{-1})	0.4–7.0	0.2–9.0
Molar absorptivity (L mol^{-1} cm^{-1})	1.05×10^5	5.03×10^4
Sandell's sensitivity(μg cm^{-2})	3.85×10^{-3}	8.10×10^{-3}
Slope (*a*)	0.217	0.104
Intercept (*b*)	0.068	0.043
Correlation coefficient (*r*)	0.968	0.972
Detection limit (μg mL^{-1})	0.13	0.27
Quantitation limit	0.39	0.82

determination of endosulfan. The proposed method is simple, selective, sensitive and rapid, offers the advantage of high sensitivity and has wide analytical range of determinations without the need for extraction or heating and has less interference from the common metal ions such as Al^{3+}, Co^{2+}, Sr^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, In^{3+}, Zn^{2+}, Cr^{3+}, Ca^{2+} etc. The developed method does not involve any stringent reaction conditions and offers the advantages of high stability of the reaction system (more than 5 h). The proposed method has been successfully applied to the determination of endosulfan in water, soil samples and vegetables.

Acknowledgments Chand Pasha thanks the Principal and Management of P. A. College of Engineering, Mangalore, for their constant encouragement and technical support.

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