

A New Spectrophotometric Determination of Endosulfan and its Application

¹Kalpana Wani¹, M. K Rai², Raisa Khaton³

^{1,2,3}School of Studies in Chemistry, Pt. Ravishankar Shukla University Raipur

Abstract: A new spectrophotometric method has been developed based on the modification of Fujiwara reaction for the determination of endosulfan in sub ppm level. Endosulfan on its alkaline hydrolysis gives chloroform, which can be reacted with pyridine to produce pink colour. In the present method the pink colour obtained in the above reaction is discharged with a few drops of acetic acid followed by the addition of p-aminoazobenzene reagent which gives an orange coloured dye. The absorbance maxima of orange coloured dye formed is measured at 490 nm. Beer's law is obeyed over the concentration range of 1- 36 µg in a final solution volume of 25ml. The molar absorptivity and Sandell's sensitivity were found to be $6.71 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.22 \times 10^{-3} \mu\text{g cm}^{-1}$ respectively. The standard deviation and relative standard deviation for the absorbance value were found to be 0.0148 and 4.05% respectively. This method is free from interference of other organochlorines pesticides and can be successfully applied for the determination of endosulfan in various environmental samples.

Keywords: spectrophotometry, endosulfan, p-aminoazobenzene reagent, pyridine, samples.

1. Introduction

Endosulfan is an organochlorine insecticide and acaricide [1]. Endosulfan(6,7,8,9,10 hexa chloro -1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4-enzodioxo thiepin-3-oxide) is a toxic organochlorine pesticide [2]

Endosulfan was introduced into the earth's environment in 1956. It possesses a relatively broad spectrum of activity. Technical grade endosulfan is a mixture of two stereoisomer, alpha and beta endosulfan in a ratio of 7: 1. It also acts as a poison to a wide variety of insects and mites ion contact [3]

Endosulfan a well known pesticide belongs to the organochlorine group under the cyclodiene subgroup. It is used as a fumigant for vegetables, fruits, paddy, cotton, cashew, tea, coffee, tobacco and timber crops. It is also used as wood preservatives and to control tse- tse flies and termites [4]. The various disorders caused from endosulfan in humans due to toxicity are savalation, less carbohydrate and lipid content etc [2]

The impact of endosulfan and its toxic metabolites in the environment result in contamination and persistence leading to bioaccumulation and biomagnifications [5]. Residues of this pesticide have been detected in cotton seed, cotton lint, milk, drinking water and other food stuffs [6].

Because of its threats to human health and the environment, a global ban on the manufacture and use of endosulfan was negotiated under the Stockholm Convention in April 2011. The ban will take effect in mid 2012, with certain uses exempted for 5 additional years. More than 80 countries including the European Union, Australia and New Zealand, several West African nations, the United States, Brazil and Canada had already banned it or announced phase outs by the time the Stockholm Convention ban was agreed upon. It is still used extensively in India, China, and few other countries⁽⁴⁾. The persistence of endosulfan in environment and toxic effects on biota necessitate its removal [7].

2. Experimental

a) Apparatus

A Systronics UV-Vis spectrophotometer model – 104 with matched silica cell was used for all spectral measurements. A Systronic pH meter model – 335 was used for pH measurements.

b) Reagents

All reagents used were of Anala. R. grade and Double Distilled water was used throughout.

c) Standard solution of endosulfan

Supplied by SHINE METAL INDUSTRIES: A stock solution of 1mg/ml solution of endosulfan is prepared in water. Working standard solution is prepared by appropriate dilution of the stock solution.

d) Sodium hydroxide

Supplied by OSTER CHEMICAL AND PHARMACEUTICAL WORKS PRIVATE LTD., CALCUTTA: A 5.0 mol L⁻¹ aqueous solution is used.

e) P- aminoazobenzene reagent

Supplied by LOBA CHEMIE PVT. LTD.: 0.25 % (W/V) solution of PAAB in 25% of ethanol is prepared.

f) Hydrochloric acid

- 10 M aqueous solution was used
- Pyridine and acetic acid of A.R grade were used.

3. Procedure

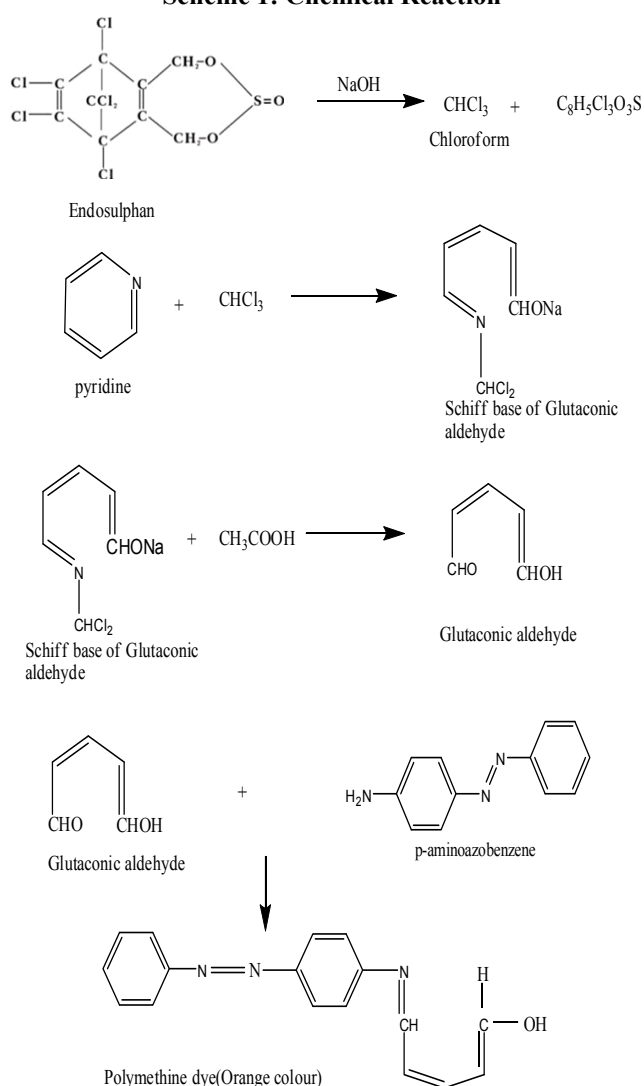
An aliquot containing 1.0 to 36.0 µg of endosulfan was taken in a 25 ml of calibrated test tube. Solution of test tube was evaporated off up to 0.5 ml on a water bath. To this 1ml of pyridine followed by 2ml of 5 M sodium hydroxide were added and the test tube was kept in a boiling water bath for 2-5 minutes. The pink coloured dye obtained was cooled in ice cold water and then decolourised with few drops of glacial acetic acid. Then 1ml of p- aminoazobenzene reagent

and 10 M HCl was added and the solution was kept for 10 minutes for full colour development. The volume of test tube was made up to 10 ml with distilled water and absorbance of the orange coloured dye was measured at 490 nm against a reagent blank.

3.1 Chemical Reaction (colour reaction)

The reaction takes place in four steps. In the first step endosulfan is hydrolysed by NaOH to form chloroform (I) which is now further used in the reaction. In the second step chloroform react with pyridine in alkaline medium to form schiff's base of glutamic aldehyde (II). In the third step on addition of glacial acetic acid, the pink colour of schiff's base of glutamic aldehyde (II) is converted into glutamic aldehyde (III), which forms an orange coloured dye (IV) with p-aminoazobenzene reagent in the fourth step (Scheme 1).

Scheme 1: Chemical Reaction



4. Result and Discussion

4.1 Spectral characteristics

The absorption spectrum of orange colour dye shows maximum absorbance at 490 nm. The reagent blank had negligible absorbance at this wavelength. fig. (1). All spectral

measurements carried out against double distil water as the reagent blank shows negligible absorption at this wavelength. The colour system obeys the Beer's law in the range of 1-36 μg of endosulfan in 25 mL of final solution at 490 nm fig. (2). The molar absorptivity and Sandell's sensitivity were found to be $6.71 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.22 \times 10^{-3} \mu\text{g cm}^{-1}$ respectively.

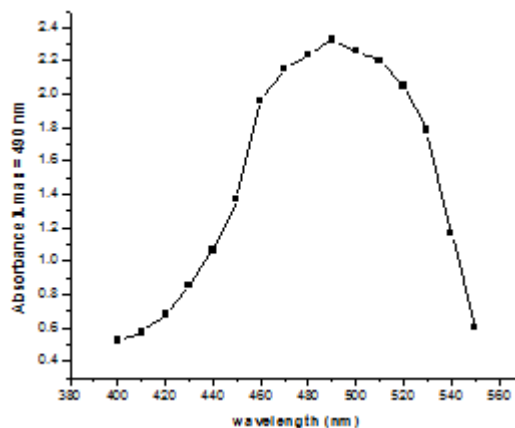


Figure 1: Absorbance Curve of the Endosulfan

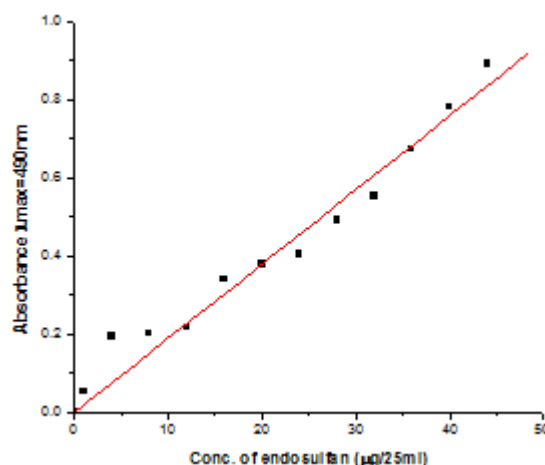


Figure 2: Calibration curve for the determination of endosulfan

4.2 Optimization of condition

Hydrolysis of endosulfan to chloroform and the reaction of chloroform with pyridine to form pink coloured dye was studied at different temperature and alkalinity. It was found that 5 M, 2ml NaOH was sufficient for full colour development. Higher amount of these reagent decreases the absorbance value. It was found that 2-5 minutes in a water bath at temperature range 50°C were sufficient for the complete colour development. Further heating and increasing temperature of the solution increases the absorbance value. It was observed that 0.5 ml of dye was sufficient for complete colour development fig. (3). It was found that 1 ml of pyridine was sufficient for complete colour development fig. (4).

Precision of the method was checked by the replicate analysis of working standard solution containing 4 μg of endosulfan in 25 mL final solution over a period of 7 days. The standard deviation and relative standard deviation for

the absorbance value were found to be 0.0148 and 4.05% respectively.

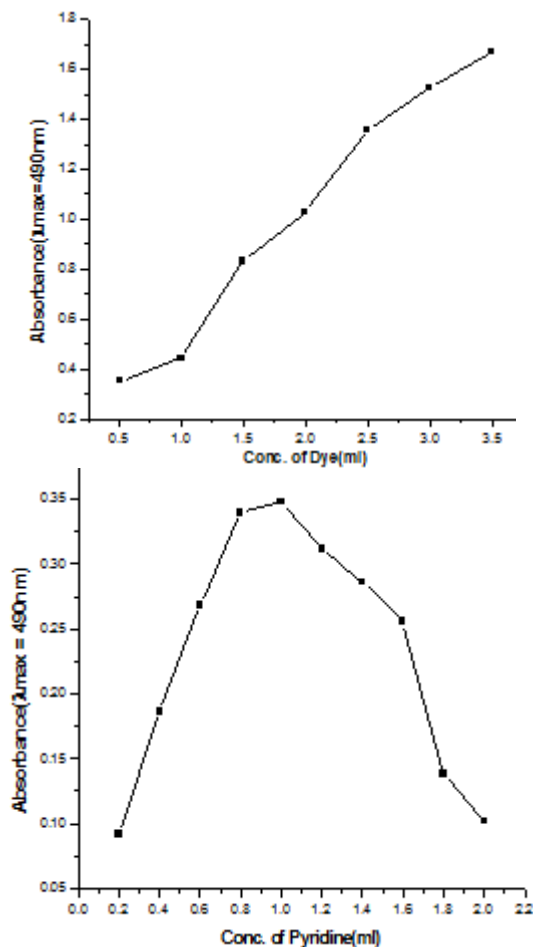


Figure 3: Effect of dye on colour development Fig 4: Effect of Pyridine on colour development

4.3 Effect of foreign species

The effect of common foreign species and pesticides were studied to assess the validity of the method. Known amount of foreign species and pesticides were added to the standard solution containing 4 μg of endosulfan prior to hydrolysis, and the solution was analysed by the proposed method. The method was found to be free from interferences of most of the foreign species and pesticides (Table 1).

Table 1: Effect of foreign species

Foreign Species	Tolerance Limit μg mL ⁻¹	Foreign Species	Tolerance Limit μg mL ⁻¹
Butachlor	400	Zn ²⁺	950
Glyphosphate	850	Cu ²⁺	900
Fenvalerate	750	Ni ²⁺	1000
Dicofol	800	Ba ²⁺	1000
Dichlorovos	600	SO ₄ ²⁻	750
Baygon	250	Cl ⁻	800

5. Application

5.1 Determination of Endosulfan in Water Sample

River water samples, receiving run-off water from agricultural fields sprayed with endosulfan, were collected. 100 ml of water sample was taken and fortified with known amount of endosulfan and kept for 3 – 4 hours. Endosulfan was determined by the proposed as well as reported method. The recoveries are shown in table 2.

5.2 Determination of Endosulfan in Vegetables, Fruits, Grains and Soil

Various samples of vegetables, carrots, rice, paddy and soil each of 5gm were taken, collected from agricultural field, where endosulfan had been sprayed as an insecticide. The samples were crushed and spiked with known amount of endosulfan and kept for 3-4 hours. Endosulfan was determined by the proposed as well as reported method. The recoveries are shown in table 2. The recoveries range from 90 – 97% by the present method.

Table 2: Recovery of Endosulfan in Environmental samples

Sample	Endosulfan originally found*	Endosulfan added (μg)	Total Endosulfan found by proposed method	Difference (z - x)	Recovery
	Proposed method (μg)X	Y	z	(z - x)	z-x/y × 100
Water**	1.62	4.0	5.32	3.7	93%
Potato***	1.92	4.0	5.51	3.59	90%
Carrot***	1.30	4.0	4.94	3.64	91%
Rice***	1.79	4.0	5.73	3.94	98%
Soil***	2.03	4.0	5.89	3.86	97%
Cotton	1.05	4.0	4.91	3.86	97%

* Mean of three replicate Analysis

** Amount of sample 100 ml

*** Amount of sample 5 gm

Table 3: Comparison of the proposed Endosulfan method with some other methods

S.No	Technique	Reagent	λ max (nm)	Determination Range	Remarks
1.	Spectrophotometric	Hydrogen Peroxide ⁽³⁾	605	0.5- 9.0	Time consuming
2.	Gas chromatography and mass spectrometry	Acetonitrile ⁽⁴⁾	-	0.1 – 50	Instrument is highly expensive
3.	Spectrophotometric	Perchloric acid ⁽⁴⁾	214	0.1 – 10	Less sensitive
4.	Proposed Method	p-aminoazobenzene	490	1 – 36	Free from interference, cheapest, more sensitive

6. Conclusion

The present method is cheapest, simple and more sensitive than the other spectrophotometric methods reported for the determination of endosulfan. It can be successfully applied

for the determination of endosulfan in various environmental samples.

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Authors Profile



Kalpana Wani, Research Scholar, Pursuing M.Phil, School of Studies in Chemistry, Pt Ravishankar Shukla University, Raipur (C.G)



Dr. M.K Rai, Associate Professor, School of Studies in Chemistry, Pt Ravishankar Shukla University, Raipur (C.G), Ph.D from Pt Ravishankar Shukla University, Raipur (C.G)



Raisa Khatoon, Research Scholar, Pursuing Ph.D, School of Studies in Chemistry, Pt Ravishankar Shukla University, Raipur (C.G)