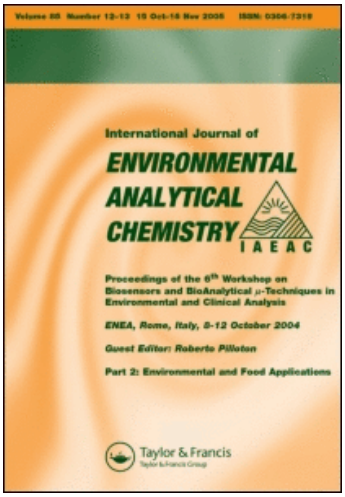

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Flow-injection spectrophotometric determination of chloramben

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A new protocol for the spectrophotometric determination of chloramben using online diazotization has been investigated. The diazotization reaction and coupling reaction were carried out online in flow assembly by injecting chloramben in a nitrite stream followed by coupling with β-naphthol. The resulting azo dye was measured at 475 nm. The calibration plot is linear in the concentration range investigated, with a molar absorptivity of $2.64 \times 10^4\,\mathrm{L\,mol^{-1}\,cm^{-1}}$ and sample throughput of $90\,\mathrm{h^{-1}}$. The proposed method is simple, rapid, and sensitive. The LOD and LOQ were calculated and were found to be 0.01 and $0.04\,\mu\mathrm{g\,mL^{-1}}$, respectively, with an RSD value of 8.3.

Keywords: Chloramben; Spectrophotometric; FIA; Diazotization; Acidic herbicide

1. Introduction

Chloramben (3-amino-2,5-dichlorobenzoic acid) is classified as a General Use Pesticide (GUP) by the US Environmental Protection Agency [1]. Chloramben is a selective, pre-emergence benzoic acid herbicide that is primarily applied to soil for the control of annual grasses and broadleaved weed seedlings. It is mostly used for soybeans, but it is also used for dry beans, peanuts, sunflowers, peppers, cotton, sweet potatoes, corn, tomatoes, squash, hardwood trees, shrubs, and some conifers. Chloramben inhibits seedling root development and causes plants to bend and die as they emerge from the soil.

Chloramben is active in the soil. It is moderately persistent. The wetter the soil, the greater the toxicity of this compound to target plants as it is water-soluble, and uptake by the plant is easier. Much of it is lost from the soil due to leaching, although some loss is due to breakdown by soil microorganisms. Leaching is greatest in sandy soil [2]. As the organic-matter content of the soil increases, binding of chloramben also increases, and leaching decreases. In general, chloramben activity lasts for about 6–8 weeks in soil [3, 4].

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Chloramben reacts with plant constituents to form a stable and nontoxic product (3-amino-2,5-dichlorobenzoic acid-*N*-glucoside) in both susceptible and tolerant plants [5]. The ease of formation of this metabolite seems to be the reason that the herbicide is not totally degraded within the plant. When soil-applied, plant roots are the primary site of absorption and chloramben action. Roots of resistant species, like soybeans, can absorb large amounts of the compound, but translocate very little of it to the above ground portions of the plant. In contrast, the susceptible barley plant translocates a significant amount into its leaves and thus dies [6].

Most of the residue studies reported for acidic herbicides have been carried out by using chromatographic techniques [7–10]. As chromatographic methods are relatively laborious and require a clean-up procedure, the aim of the present study was to develop a simple, fast, and reproducible method for residue analysis of chloramben in real samples compatible in sensitivity and accuracy to chromatographic techniques. Flow-injection analysis (FIA) proved itself to be a good and sensitive approach for determination of acidic herbicides [11, 12].

2. Experimental

2.1 *Instruments*

A UV/Vis spectrophotometer (UNICO UV-2100 United Products and Instruments, Dayton, NJ), peristaltic pump, PET tubing (i.d. 1.19 mm, Becton Dickson, Franklin Lakes, NJ), silicon tube (i.d. 1.71 mm), V-450 six-port injection valve (Upchurch Scientific, Oak Harbor, WA), and digital analytical balance (Sartorius handy H51, Göttingen, Germany) were used during this work. The layout of the flow injection (FI) system is given in figure 1.

2.2 *Reagents and chemicals*

All chemicals used were of analytical-reagent-grade purity. Sodium nitrite, β-nephthol, concentrated hydrochloric acid, ethanol, and sodium hydroxide were used during this work. Standard reference material (97% purity) was purchased from ACROS Organics (Geel, Belgium).

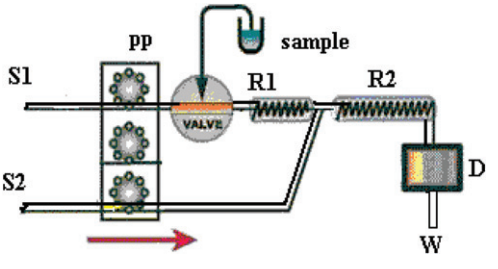

Figure 1. Schematic diagram of the FI-manifold, showing: (S1) nitrite stream, (S2) β-naphthol stream (R1) diazotization coil, (R2) coupling coil, (pp) peristaltic pump, (S) sample injected, (V) valve, (W) waste, (C) cell, (D) detector, and (RD) readout device.

2.3 *Solution preparation*

Nitrite solution (2 mg mL^{-1}) was prepared by dissolving 0.3 g of sodium nitrite in 0.4 M HCl in a 100-mL volumetric flask. β-Naphthol solution (1 mg mL^{-1}) was prepared by dissolving 0.1 g of β-naphthol in 1.5 M sodium hydroxide solution in a 100-mL volumetric flask. Chloramben solution (1 mg mL^{-1}) was prepared by dissolving 0.103 g of standard in 50 mL of ethanol and diluted to 100 mL with distilled water as chloramben have no shelf-life limitations. Through stepwise dilution, working standards in the range of 2×10^{-4} to 1×10^{-3} mg mL^{-1} were prepared from concentrated solution.

2.4 *Procedure*

For batch analysis, 1–4 mL of chloramben (1×10^{-3} mg mL^{-1}) from standard stock solution was taken in 50-mL volumetric flasks followed by the addition of optimum volume (4 mL) of 2 mg mL^{-1} nitrite solution and allowed to equilibrate for 5 min to complete the diazotization reaction. To these solutions, the optimum volume (4 mL) of 1 mg mL^{-1} of β-napthol was added and diluted to 50 mL with distilled water. The absorbance of the resulting azo dye was measured at 475 nm using a UNICO UV-2100 spectrophotometer.

In the case of FIA, 1 mL of sample was injected in a double-channel flow-injection (FI) assembly in a nitrite carrier stream for the diazotization reaction, which was allowed to flow downstream and mixed with coupling reagent (β-naphthol) to form the azo dye. The azo dye produced was measured at 475 nm against the corresponding blank. The proposed reaction mechanism is shown in figure 2.

2.5 *Sample preparation*

The method described in [13] was adopted for extraction, and 25 g of tomato paste, corn, and cornflour were taken in a titration flask. To each flask, 50 mL of 1 : 1 acetone and petroleum ether was added and equilibrated for 2 h with periodical shaking. The extract was filtered and evaporated on a boiling water bath because chloramben is unaffected by temperature to its boiling point. The residue remaining was dissolved in 5 mL of ethanol. For batch analysis, 2 mL of the extracted sample was taken, and the colour was developed in the same way as in the standard and measured at optimum wavelength. For FI analysis 1 mL of extracted sample was injected in the reagent stream, and the absorbance of the resulting azo dye was measured at 475 nm against the blank. The residue of chloramben in the real sample were determined by using the standard calibration curve.

2.6 *Recovery*

The percentage recovery for chloramben was calculated by extraction from samples fortified with 1×10^{-2} mg mL^{-1} of herbicides in the same manner as described above.

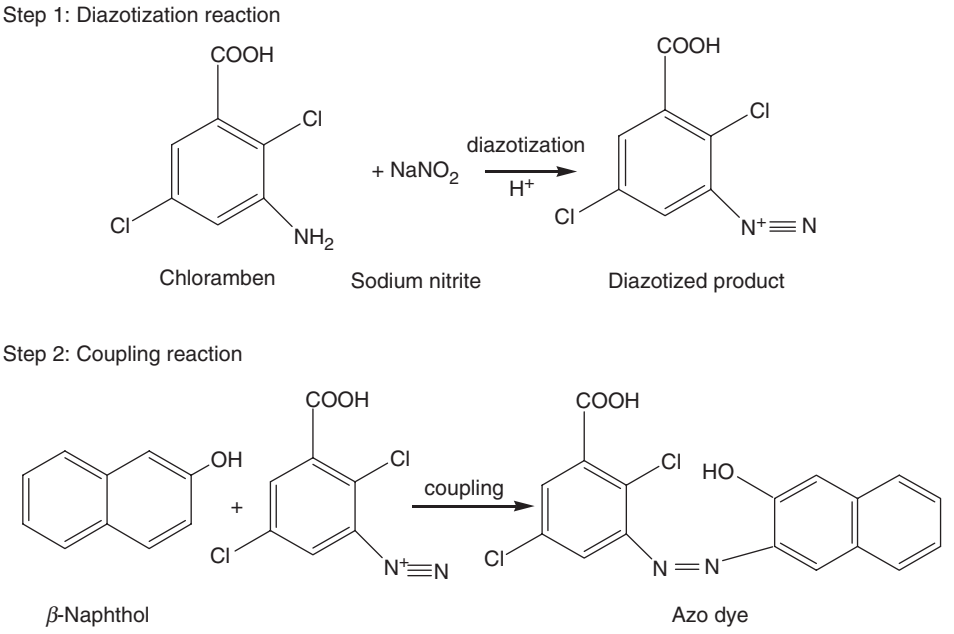

Figure 2. Reaction mechanism for the spectrophotometric determination of chloramben.

3. Results and discussion

3.1 *Optimization of reaction conditions*

The reaction conditions for diazotization and coupling were first optimized for batch analysis. All optimizations were done in triplicate. The concentration of nitrite (diazotizing reagent) was optimized in the range of 0.5–3 mg L^{-1} in acidic media. It was found that 2 mg mL^{-1} of nitrite in 0.4 M HCl media is sufficient for maximum diazotization reaction. For coupling, the concentration of β-naphthol (coupling reagent) was optimized in the range of 0.5–3 mg mL^{-1}. It was observed that 1 mg mL^{-1} of β-naphthol in 1.5 M sodium hydroxide was found to be sufficient for maximum formation of azo dye (figure 3). Similarly, the effect of the volume of nitrite (2 mg L^{-1}) was studied in the range of 2–10 mL for the diazotization reaction, and 4 mL of 2 mg L^{-1} nitrite solution was found to be the optimum. For the coupling reaction, a volume of β-naphthol (1 mg mL^{-1}) was optimized in the range of 2–10 mL. It was observed that 4 mL of β-naphthol is the optimum volume for the coupling reaction (figure 4).

3.2 *Optimization of reaction time*

The kinetics study of diazotization and the coupling reaction (figure 5) shows that the diazotization reaction is spontaneous with a slight increase in the absorbance signal up to 5 min. Further, the diazonium ion formed was stable at room temperature, and no

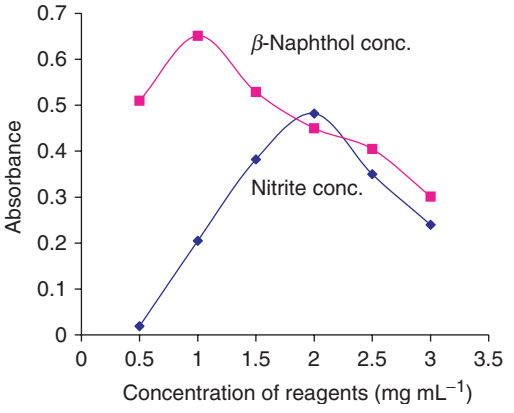

Figure 3. Investigation of the effect of nitrite and β-naphthol concentration for the spectrophotometric determination of chloramben.

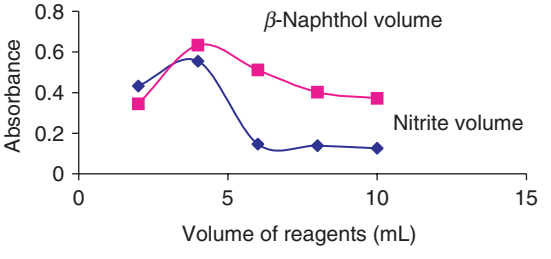

Figure 4. Investigation of the effect of nitrite and β-naphthol volume for the spectrophotometric determination of chloramben.

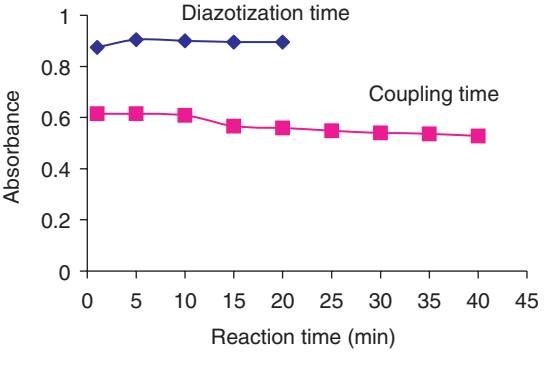

Figure 5. Investigation of the effect of time for diazotiozation and the coupling reaction for the spectrophotometric determination of chloramben.

further change in signal was observed. While the coupling reaction was also faster, and a maximum amount of azo dye was formed instantaneously, a small decrease in the signal was observed after 5 min possibly due to slight decomposition of azo dye. However, this does not affect the results, as only 45 s is required for online analysis.

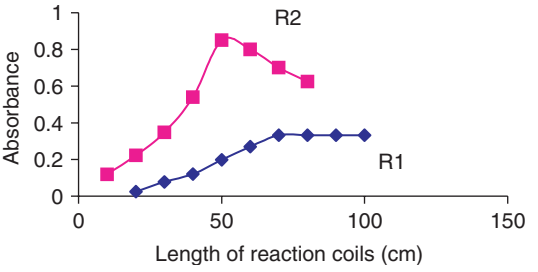

Figure 6. Investigation of the reactor coil length R1 and R2 for FI spectrophotometric determination of chloramben.

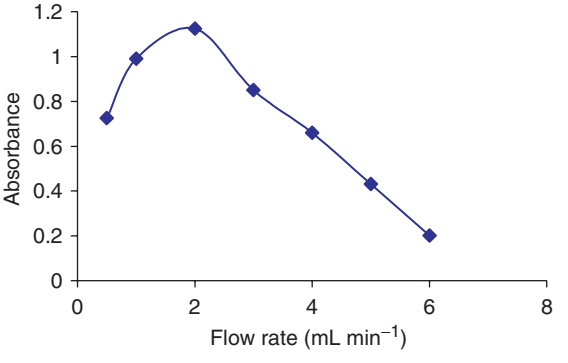

Figure 7. Investigation of flow rate for FI spectrophotometric determination of chloramben.

3.3 *Optimization of physical parameters for the FI assembly*

Keeping the order of reagents addition the same as mentioned for batch analysis, the FI assembly was tested for formation of azo dye. Physical parameters like reactor coil lengths (R1 and R2), flow rate, and injection volume were studied. The effect of reactor coil length (R1) on diazotization was studied in the range of 20–100 cm. It was observed (figure 6) that the diazotization reaction was completed in the 70-cm reactor coil. Above 70 cm, there was no change in the signal of the diazotized product. Similarly, the influence of the reactor coil length (R2) for the coupling reaction was studied in the range of 10–80 cm. The results show (figure 6) that a coil of 50-cm length was sufficient for the coupling reaction. This indicates that the coupling reaction is faster than the diazotization reaction. Any further increase in the coil length decreases the signal as dilution of the azo dye takes place. The injection volume was investigated in the range of 1–4 mL. An injection volume of 1 mL was selected to obtain a sharp absorbance peak. Above this volume, peak broadening takes place. The flow rate was also studied in the range of 0.5–6 mL min^{-1}. The maximum signal was found at 2 mL min^{-1} (figure 7). Above this rate, a decrease in signal was observed because the sample came out before the reaction had finished. With this flow rate, a sampling throughput of 90 samples per hour was achieved. Optimum parameters both for batch and FI analysis of chloramben are given in table 1.

Table 1. Optimum parameters for spectrophotometric determination of chloramben.

Parameters	Batch analysis	FIA
λ_{max} (nm)	475	475
Nitrite concentration ($mg\,mL^{-1}$)	2	2
β-Naphthol concentration ($mg\,mL^{-1}$)	1	1
Nitrite volume (mL)	4	–
β-Naphthol volume (mL)	4	–
Diazotization time (min)	5	
Coupling time (min)	1	
Reactor length R_1 (cm)		70
Reactor length R_2 (cm)		50
Flow rate ($mL\,min^{-1}$)		2
Injection volume (mL)		1
Sample/h		90

3.4 *Validation of assay procedures*

3.4.1 Concentration ranges and linearity. The applicability of Beer's law for the batch and FIA system was checked. A linear relationship from 2×10^{-4} to $1 \times 10^{-2}\,mg\,mL^{-1}$ and 4×10^{-4} to $1 \times 10^{-2}\,mg\,mL^{-1}$ was observed for FI and batch analysis, respectively. Calibration plots revealed a very small intercept (−0.106 and 0.000) and a good linear relation ship both for batch and FI procedures.

3.4.2 Sensitivity and precision. The sensitivity of the proposed method was evaluated by calculating the limits of detection (LOD) as $3 \times SD$ and quantification (LOQ) as $10 \times SD$. The LOD and LOQ found were 0.01 and $0.04\,\mu g\,mL^{-1}$ in the case of FIA and 0.05 and $0.17\,\mu g\,mL^{-1}$ for the batch analysis. The lower values of LOD indicate the sensitivity of the method, and the low value of the RSD for the six replicates (table 2) indicates the precision of the method. The method is particularly selective for commercial preparations, as it is directly based on diazotization of the amine group. There is no danger of interference for the degradation product as photodissociation can only take place if it is converted into a vapour form, which is a remote possibility because of its very high boiling point.

3.5 *Application to real samples*

The method was successfully applied for the determination of chloramben in real samples of tomato, corn, and cornflour. A sample analysis were carried out in triplicate. The mean values for chloramben in tomato, corn, and cornflour were found to be $0.12 \pm 0.110\,\mu g\,g^{-1}$, $0.10 \pm 0.33\,\mu g\,g^{-1}$, and $0.09 \pm 0.07\,\mu g\,g^{-1}$ in the FI analysis and $0.13 \pm 0.130\,\mu g\,g^{-1}$, $0.103 \pm 0.09\,\mu g\,g^{-1}$, and $0.1 \pm 0.22\,\mu g\,g^{-1}$ in the batch analysis. The residue levels for chloramben found in these samples are given in table 3. A percentage recovery test was performed for real samples, and the results show 88–96% recovery (table 4). As can be seen from table 4, the results of both the batch and FIA are comparable. The *F*-test values for residue determination and percentage recovery are less than the tabulated value, i.e. 9.26, which indicates that there is no significant difference between the two methods.

Table 2. Statistical data for spectrophotometric determination of chloramben.

Data	Batch analysis	FIA
RSD	10.6	8.3
Limit of detection ($\mu g\,mL^{-1}$)	0.05	0.01
Limit of quantification ($\mu g\,mL^{-1}$)	0.17	0.04
Molar absorptivity ($l\,mol\,cm^{-1}$)	2.45×10^4	2.64×10^4
Correlation coeffiecent	0.993	0.999
Slope	0.0961	0.1007
Intercept	−0.1016	–

Table 3. Percentage recovery of chloramben in real samples ($10\,\mu g\,25\,g^{-1}$).

Name	Batch analysis (%)	Batch analysis (%)	*F*-test values
Tomato paste	88.0 ± 0.03	89.6 ± 0.09	9
Corn	94.1 ± 0.09	96.0 ± 0.10	1.2
Cornflour	94.0 ± 0.01	95.0 ± 0.008	1.5

Table 4. Residue levels of chloramben in real samples.

Name	Batch analysis ($\mu g\,g^{-1}$)	FIA ($\mu g\,g^{-1}$)	*F*-test values
Tomato paste	0.130 ± 0.130	0.12 ± 0.110	1.3
Corn	0.14 ± 0.09	0.10 ± 0.33	5.5
Cornflour	0.100 ± 0.22	0.09 ± 0.10	4.8

4. Conclusion

A simple, rapid, and sensitive spectrophotometric method has been investigated for the analysis of chloramben. The procedure was successfully automated for use with the FI assembly. The results of both the batch and FI analysis are comparable. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. A high sampling frequency and good sensitivity make the method versatile and useful. The method could be used for determination of active ingredients in formulation and for residue analysis in samples of environmental and biological interest.

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