Photodegradation and Flow-Injection Determination of Simetryn Herbicide by Luminol Chemiluminescence Detection

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A novel and simple flow injection chemiluminescence method is reported for the determination of simetryn, a common herbicide. The method is based on the direct oxidation of luminol by the photoproducts of the simetryn in alkaline medium in the absence of catalyst/oxidant. The linear concentration range was 0.01 – 2 $\mu\text{g mL}^{-1}$ simetryn with a correlation coefficient (r^2) of 0.9997 and relative standard deviations (RSD; $n = 4$) in the range of 0.9 – 2.3%. The limit of detection ($S/N = 3$) was 7.5 ng mL^{-1} with a sample throughput of 100 h^{-1} . The proposed method has been applied to determine simetryn in natural waters using Sep-Pak C_{18} cartridges for solid phase extraction (SPE) procedure. The recoveries were in the range of 97 ± 1 to $104 \pm 2\%$. The mechanism of chemiluminescence reaction has also been discussed briefly.

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Introduction

Herbicides are applied over millions of acres of farmland worldwide and are widespread water pollutants. A number of important herbicides contain three heterocyclic nitrogen atoms in ring structures and are therefore called triazines. The two most common triazines in natural waters are atrazine and simazine. Other important members of the triazine family include simetryn, ametryn, metribuzin, propazine, and terbuthylazine, which are widely used on soybeans, sugarcane, and wheat.¹ Triazine herbicides are applied as pre- and post-emergent weed control agents to improve crop yields and are one of the most widely used soil-applied herbicides in Europe. The half-lives of these herbicides vary from weeks to several months, and under environmental conditions they are usually degraded to compounds more soluble in water. The most important physicochemical properties of these pesticides and their degradation products are the solubility in water and the capacity to be retained by the organic matter of the soil.^{2,3}

Simetryn or *N,N'*-diethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine (Fig. 1) is a white crystalline solid with melting range of 82 – 83°C. Simetryn is stable in neutral, weakly acidic and weakly alkaline media, hydrolyzed by strong acids (pH 1) and alkalis (pH 13). It is highly soluble in water 450 mg L^{-1} at 20°C and is more soluble in organic solvents like acetone, ethanol, toluene and hexane.

Methods for triazine determination in water include separation by HPLC⁴⁻⁶ and GC^{7,8} after solid-phase extraction (SPE),⁹⁻¹¹ capillary electrophoresis,¹² micellar electrokinetic capillary chromatography,¹³ triazine multianalyte immunoassays,^{14,15} CL/ECL immunoassays,^{16,17} ELISA,^{18,19} ECL,²⁰ fluorometric methods with dansyl chloride²¹ and photochemically-induced fluorometric method with *o*-phthalaldehyde-2-mercaptoethanol derivatization.²²

These methods are sensitive and accurate; however, many of these involve expensive methods, and toxic solvents, are slow and require the development of extremely complex gradients for the separation. Table 1 reports a comparison of various methods for determination of triazines in terms of sample matrix, limit of detection and sampling rate. These methods are sensitive and accurate but involve relatively expensive and bulky instrumentation and have poor sample throughput.

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) is one of the most commonly used CL reagents. The analytical applications of luminol exploit the chemiluminescent oxidation of luminol to 3-aminophthalate with blue light emission (425 nm) by interacting directly with an analyte reagent, a catalyst, an enhancer or a quencher. When this is not possible, a previous chemical transformation of the analyte is required; in this context, photochemical reactions (irradiation with UV-Vis light) offer a powerful means for obtaining derivatives with improved chromophoric, fluorophoric or electrolytic properties.^{23,24}

This paper presents a simple photodegraded-chemiluminescent method for the determination of simetryn (methylthio-triazine) in water samples. The light from a low pressure UV lamp is used to produce a derivatized pesticide reagent in alkaline medium, and photo-products were reacted with luminol to generate intense CL.

Fig. 1 Molecular structure of simetryn.

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Table 1 Comparison among several methods for the determination of triazines

Detection technique	Analyte	Matrix	Calibration range	Limit of detection	R value	Sample rate/h	Ref.
Ion chromatography	Triazines	Environmental samples	0.6 – 10 mg L ⁻¹	100 ng L ⁻¹	> 0.995	NG	4
SPE-HPLC	Triazines, dinitroanilines	Water	0.4 – 20 ng	0.03 – 0.1 µg L ⁻¹	0.9967	NG	5
HPLC	Triazines	Water	0 – 200 ng L ⁻¹	15 – 32 ng L ⁻¹	0.940 – 0.996	NG	6
GC-MS	Triazines	Water	0.05 – 10 µg L ⁻¹	1 – 10 ng L ⁻¹	0.9970	NG	8
SPME-GC	Triazines	Environmental samples	0.03 – 30 ng mL ⁻¹	0.001 – 0.09 ng L ⁻¹	0.991 – 0.999997	NG	10
SPE-GC-MS, CE-UV	s-Triazines	Environmental samples	1 – 2000 µg L ⁻¹ , 0.2 – 10 mg L ⁻¹	1 µg L ⁻¹ , 0.2 – 0.5 mg L ⁻¹	0.996 – 0.999	NG	12
Immunosensor-CL FIIA, Disc Systems	Atrazine	Surface water	0.8 – 8 µg L ⁻¹	0.59 µg L ⁻¹ , 0.038 µg L ⁻¹	NG	10 – 15	17
Immunoassay-ECL	Atrazine	Standards	0 – 10 ppb	0.1 ppb	NG	NG	20
FI-CL	Simetryn	Water	0.01 – 2 µg mL ⁻¹	7.5 ng mL ⁻¹	0.9997	120	This work

NG, not given; SPME-GC, solid phase micro-extraction gas chromatography; CE, capillary electrophoresis; FIIA, flow injection immunoassay; CL, chemiluminescence; ECL, electrochemiluminescence; FI-CL, flow injection chemiluminescence.

Fig. 2 Flow injection chemiluminescence manifold for the determination of photodegraded simetryn.

Experimental

Reagents and solutions

All chemicals were of analytical reagent grade; they were supplied by Merck (Darmstadt, Germany), unless stated otherwise and were used without further purification. All pieces of glassware used during the experiments for storage of reagents and standards were pre-cleaned with 20% HCl and thoroughly rinsed with ultra-high-purity (UHP) deionized water (18.2 MΩ/cm, Elgastat-Maxima, UK). Simetryn, atrazine (Dr. Ehrenstorfer GmbH, Germany) stock solution 500 mg L⁻¹ was prepared in ethanol and stored at -4°C in brown bottles in the dark. Subsequent standard solutions were prepared daily by serial dilution of the stock solution with UHP water. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma, St. Louis, MO) stock solution (1.0 × 10⁻² mol L⁻¹) was prepared by dissolving 0.177 g in dilute sodium hydroxide solution followed by sonicating for 30 min. A working luminol solution (5.0 × 10⁻⁵ mol L⁻¹) was prepared by diluting the required volume in borate buffer (0.05 mol L⁻¹, pH 11). Sodium hydroxide solution was prepared freshly before use by dissolving the required amount of the compound in UHP water.

Instrumentation and procedures

The flow injection chemiluminescence manifold used for this work is shown in Fig. 2. A peristaltic pump (Ismatec Reglo, 4 channels, Switzerland) was used to deliver the sample carrier and reagent solutions at a flow rate of 2 mL min⁻¹. A rotary injection valve (Rheodyne 5020, Anachem, Luton, UK) was used to inject standards 120 (µL) into an aqueous stream that was merged with a stream of NaOH (5 × 10⁻³ mol L⁻¹ prepared in UHP water). This stream was passed through the photo reactor and then merged at a T-piece with the luminol reagent stream. The merged stream traveled 1.5 cm before passing through a glass spiral flow cell (1.5 mm i.d., 300 µL internal volume) placed directly in front of an end window photomultiplier tube (PMT, 9798B, Electron Tubes, Ruislip, UK). The PMT, glass coil and T-piece were enclosed in a light-tight housing. The PMT was kept at 830 V via a 2 kV power supply (Burle, PF1053, USA). The detector output was recorded using a chart recorder (Kipp & Zonen BD 40, Holland).

Construction of photo-reactor

The photo reactor consisted of PTFE tubing (0.75 mm i.d., 2 m long, Fisher, UK) wound around a thin glass plate with exposed area (4.5 × 5.5 cm) placed on a compact 4 W UV lamp (Model UVGL-25, UVP, Upland, USA). The plate was placed at a distance of 1 cm from the lamp, which produces short wavelengths of 254 nm. The lamp, glass plate and PTFE tubing were covered with aluminum foil. The operation is carried out at room temperature.

Solid phase extraction procedure

Sep-Pak C₁₈ cartridges (Waters Associates, USA) were conditioned with 5 mL of UHP water and 5 mL of methanol, and then air-dried over 2 min. Each sample is passed by positive displacement with a luer-tip syringe having a flow rate of 1 mL min⁻¹. Once the retention step has been completed, the cartridges were dried by passing 10 mL of air. Elution of the retained simetryn was achieved with 5 mL of acetone and 5 mL of methanol, and then the organic phase was brought near to dryness under a nitrogen stream. The dry residue was dissolved in an appropriate amount of UHP water and injected into the manifold.

Fig. 3 UV spectra of simetryn in NaOH, (a) without and (b) with UV irradiation.

Results and Discussion

Photo transformation studies

It has been demonstrated in studies of model organosulfur compounds such as thioamides²⁵ and thioureas²⁶ that photolysis, photo-oxidations and photosensitized oxidations (singlet oxygen processes) readily occur in these systems and that the products depend on the substituents attached to the sulfur functional group. The nature of the photoproducts of *s*-triazine (simetryn) pesticides in aqueous solution after irradiation with UV-Vis light was investigated in detail by Pape and Zabik.^{27,28}

To assess the effect of UV irradiation on the simetryn, we obtained UV-Vis spectra in water and NaOH with lamp ON and OFF. UV-Vis scanning is performed in the range of 200 – 300 nm using a UV-Vis spectrophotometer (PharmaSpec UV-1700, Shimadzu, Japan). A two-channel FIA manifold was used, merging two streams with T-piece before passing through the flow cell (80 μ L, Hellma GmbH, Germany). An aqueous solution of simetryn (5 mg L⁻¹) was allowed to flow in one channel and NaOH solution in the other. The flow rate of both channels was 2 mL min⁻¹. Figure 3 shows the UV spectral differences when the mixture was irradiated. The simetryn has shown one distinct absorption band at $\lambda_{\text{max}} \approx 222$ nm and two bands at $\lambda_{\text{max}} \approx 243$ and 265 nm as shoulders. These bands were still present after irradiation; however the absorption intensity was much lower, this change shows the conversion of simetryn into photoproducts. The spectra were the same for both water and NaOH with an absorption maximum at 222 nm.

Possible CL mechanism

The reaction mechanism of the luminol system has been extensively studied. The excited state of 3-aminophthalic acid ion has been confirmed as an emitter.²⁹ The luminol radical formed by one-electron oxidation of luminol^{30,31} and the azaquinone formed by two-electron oxidation of luminol³² have also been proposed as two possible reaction intermediates.

The testing solution, luminol solution and sodium hydroxide solution were purged with nitrogen or oxygen for 5 min. When the dissolved oxygen was removed from the solutions by the purge of nitrogen, the CL intensity decreased about 20% for simetryn. In contrast, when the solutions purged with oxygen were used, the CL intensity increased about 50%. The results indicated that the dissolved oxygen is necessary for the luminol chemiluminescence reaction with simetryn. In order to examine if the reactive oxygen species participated in the CL reaction, we added the scavengers of reactive oxygen species, such as

Fig. 4 CL spectra of the reaction of luminol (a) without and (b) with simetryn (1 μ g mL⁻¹) (with UV lamp ON).

ascorbic acid, sodium benzoate and mannitol, into the reaction system. The CL intensity was decreased greatly in the presence of these scavengers of radicals. These results showed that there are reactive oxygen species (ROS) in the CL reaction and that they participated in this reaction.

It was reported that sulfide and some thiol-containing compounds can reduce dissolved oxygen to superoxide anion radical in alkaline solution, and that superoxide anion radical itself can react with luminol to generate CL in alkaline solution.³³ But no chemiluminescence was detected when luminol reacted with these pesticides without the application of UV light; possibly due to the fact that the thermodynamic equilibrium constant of reaction of these pesticides with dissolved oxygen is smaller. It led to a small amount of superoxide anion radical being produced. Upon the application of UV light, the strong CL signal was observed, possibly due to the generation of excess of superoxide anion radicals and its transformation to hydroxyl radicals, such hydroxyl radicals could be used as a strong oxidant for the luminol system.³⁴ Hydroxyl radical was a typical one-electron oxidant; it could oxidize luminol to form intermediates of luminol radicals. The luminol radical formed in the reaction was further oxidized by dissolved oxygen or ROS to yield an electronically excited 3-aminophthalate acid ion, which returns to the ground state, producing CL.

The CL spectra of luminol in alkaline medium with and without photodegraded simetryn were also examined using the FI-CL manifold shown in Fig. 2. No CL signal enhancement was observed when the sample was introduced with UV lamp when in OFF, however, the signal markedly increased when the lamp was turned ON with wavelength maximum at 425 nm (Fig. 4). It is well known that the peak at 425 nm is the typical CL spectra of luminol. The possible emission species is excited 3-aminophthalate. Therefore it may be concluded that the photocatalyzed product of simetryn played a role of an enhancer in the reaction. A kinetic profile of luminol-simetryn CL reaction with UV lamp ON is shown in Fig. 5. Without simetryn, the luminol shows a slow reaction process and only emits weak CL (as background). However, when simetryn is introduced the CL intensity increased markedly.

Selection of FI-manifold

Various manifolds were reported in the literature^{35,36} for luminol-CL to check the susceptibility of the pesticide to generate CL. No CL signal was observed without photolysis of simetryn (20 mg L⁻¹); however, when the sample was irradiated with UV light, a strong CL signal was generated. Figure 2

Fig. 5 Time course of the kinetic profile of the luminol CL reaction (a) without and (b) with simetryn ($0.1 \mu\text{g mL}^{-1}$).

Fig. 6 Effect of NaOH concentration on photodegradation of simetryn ($60 \mu\text{L}$, $0.5 \mu\text{g mL}^{-1}$).

Table 2 Ranges investigated and optimized for FI-CL manifold parameters ($n = 4$)

Parameter	Range studied	Optimal value
Borate buffer pH (0.05 mol L^{-1})	9.5 – 12	11.0
Luminol/ mol L^{-1}	5.0×10^{-6} – 1.0×10^{-4}	5.0×10^{-5}
NaOH/ mol L^{-1}	1.0×10^{-3} – 0.5	5.0×10^{-3}
Flow rate/ mL min^{-1}	0.5 – 2.5	2.0
Injection volume/ μL	30 – 180	120

shows the FI-CL manifold for the photodegraded determination of simetryn. This was adapted to check various oxidants *e.g.*, hydrogen peroxide, potassium permanganate, persulfate, and periodate, in the range of 5×10^{-3} to $5 \times 10^{-5} \text{ mol L}^{-1}$ and all of these generated CL signals with noisy/high background. However, in the absence of these oxidants, a steady baseline with reproducible CL signals was achieved. For use as the photodegradation medium, various buffers were tested, including sodium carbonate, sodium phosphate buffer, borate buffer (0.1 mol L^{-1} , pH 11), sodium hydroxide (0.1 mol L^{-1}) and UHP water. Carbonate and phosphate buffers generated very high background signals, whereas borate buffer and water generated a weak CL signal, when compared with NaOH. Therefore NaOH was used as photodegradation medium.

In order to establish the optimum conditions for the determination of simetryn, we investigated various parameters using a univariate approach. The key parameters optimized were NaOH and luminol concentrations, sample volume, flow rate of sample carrier and reagent streams (Table 2). All these studies were performed with $0.5 \mu\text{g mL}^{-1}$ simetryn standard solution.

The effect of sodium hydroxide on the photodegraded determination of simetryn was investigated in the range 5×10^{-5} – $5 \times 10^{-1} \text{ mol L}^{-1}$. The maximum CL response was at $5 \times 10^{-3} \text{ mol L}^{-1}$ NaOH concentration, as shown in Fig. 6. Therefore, NaOH solution of $5 \times 10^{-3} \text{ mol L}^{-1}$ was used for subsequent studies.

The efficiency of luminol chemiluminescence is highly dependent on reaction pH. Borate/NaOH buffer has been found to give higher CL intensity with luminol as compared to the other buffers listed above. Therefore, in the proposed system, the effect of borate concentration was studied in the range of 0.01 – 0.1 mol L^{-1} , adjusting the pH to 11 with NaOH solution. However, the maximum CL signal was observed at a borate concentration of 0.05 mol L^{-1} . The effect of borate buffer pH (0.05 mol L^{-1}) was investigated in the range of 9.5 – 12 and the

maximum CL response was observed at pH 11. Sodium hydroxide solutions of different concentrations were also investigated to check the response of luminol ($5.0 \times 10^{-6} \text{ mol L}^{-1}$) for enhancement process. Maximum CL was observed for 0.05 mol L^{-1} sodium hydroxide, but this CL signal was lower as compared to borate buffer (0.05 mol L^{-1} , pH 11). Therefore, borate buffer (0.05 mol L^{-1} , pH 11) was selected and used for subsequent studies.

The effect of luminol concentration was then studied in the range 5×10^{-6} – $1 \times 10^{-4} \text{ mol L}^{-1}$ using the optimized borate buffer (0.05 mol L^{-1} , pH 11). The maximum CL response was observed at $5 \times 10^{-5} \text{ mol L}^{-1}$ of luminol concentration and further increase resulted in non-reproducible CL signals with high blank values. Therefore, a luminol solution of $5 \times 10^{-5} \text{ mol L}^{-1}$ was selected for further studies. The CL response varied with the age of the luminol solution and so, it was always prepared 24 h before use.

The effects of key physical parameters are given in Table 2. Flow rates for each of the three channels were simultaneously investigated over the range 0.5 – 2.5 mL min^{-1} in terms of sensitivity, sample throughput and reagent consumption. A flow rate of 2 mL min^{-1} gave the maximum CL response with a steady baseline and reproducible peak height. The optimum flow rate (2 mL min^{-1}) depends on the distance from the T-piece to the glass coil placed in the front of PMT which in this case is 1.5 cm . Similarly, a sample injection volume of $120 \mu\text{L}$ gave almost the highest CL signal and was used for economy of sample consumption.

Analytical figures of merit

Under the optimum conditions described above, a linear calibration graph of CL intensity *versus* simetryn concentration over the range 0.01 – $2 \mu\text{g mL}^{-1}$ was obtained ($r^2 = 0.9997$, $n = 10$) with the regression equation $I = 616.31c + 0.4374$ (I = intensity (mV), c = concentration ($\mu\text{g mL}^{-1}$)). The relative standard deviation (RSD) was 0.9 – 2.3% ($n = 4$) over the range studied. The limit of detection (3σ blank) was 7.5 ng L^{-1} with sample throughput of 100 h^{-1} .

Interferences

The influences of common ions present in water were studied by preparing solutions containing $0.1 \mu\text{g mL}^{-1}$ simetryn. The tolerable concentration ratios for interference at 5% level were over 2500-fold for SO_4^{2-} , HCO_3^- , CO_3^{2-} and Cl^- ; 1000-fold for atrazine, simazine, and Ca^{2+} ; 100-fold for Mg^{2+} , Zn^{2+} , NH_4^+ and NO_3^- ; 10-fold for PO_4^{3-} , NO_2^- , S^{2-} and Fe^{3+} ; and 1.0-fold for Fe^{2+} , Cu^{2+} and Mn^{2+} , respectively. Positive interference was observed from anions like PO_4^{3-} , NO_2^- , S^{2-} and from cations like

Table 3 Determination of simetryn in water samples ($n = 5$)

Type of water	Spiked/ $\mu\text{g mL}^{-1}$	Recovery, %
Tap water	0.1	99 ± 2
	0.5	100 ± 1
	1.0	103 ± 1
Irrigation water	0.1	104 ± 2
	0.5	103 ± 1
	1.0	102 ± 1
Lake water	0.1	97 ± 1
	0.5	98 ± 4
	1.0	100 ± 2

Fe^{2+} and Fe^{3+} and suppressive interference was observed due to Cu^{2+} and Mn^{2+} .

To eliminate these interferents it is necessary to pass water samples through appropriate exchange resins. After being spiked with simetryn standards, water samples were passed through Sep-Pak C_{18} cartridges (Waters Associates, USA) that were already conditioned with 5 mL of UHP water, 5 mL of methanol, and air-dried over 2 min.

Application to water samples

The applicability of the proposed method to water samples was checked by using an optimized system based on photo-reaction and CL by analyzing the natural water samples by spiking experiments. Lake water (from Hanna valley, Quetta), tap water (from university) and irrigation water samples (from apple orchards, Hanna valley) were collected into acid-washed (10%, v/v HCl) polypropylene bottles. After collection, samples were filtered through a cellulose membrane filter (cellulose acetate, pore size $0.45 \mu\text{m}$, 47 mm diameter, Whatman, Maidstone, UK) to remove the suspended solids, if any; then the samples were kept refrigerated in the dark at 4°C . After we used the SPE procedure, the samples were injected into FI-CL system. The results are shown in Table 3. The recoveries are in the range of 97 ± 1 to $104 \pm 2\%$.

Conclusion

The proposed photocatalyzed FI-CL method for the determination of simetryn is simple and rapid (100 h^{-1}) and the recoveries are within the acceptable range for pesticide residue analysis. The method has the limit of detection 7.5 ng mL^{-1} . The method is better in term of sensitivity and sample throughput than the methods reported previously.

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