

Development of Time-Resolved Chemiluminescence for the Determination of Antu in River Water, Wheat, Barley, and Oat Grain Samples

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A novel chemiluminescence method for the determination of antu has been developed based on the reaction between potassium permanganate in acid medium with this rat-poison in the presence of formaldehyde as an emission enhancer. The main feature of the system used is that the recording of the whole chemiluminescence intensity-vs-time profiles can be obtained, using the stopped-flow technique in a continuous-flow system. This enables the use of three quantitative parameters adjustable via software settings, one of them a typically kinetic parameter, such as rate of the light-decay reaction, and the others conventional parameters, such as maximum emission intensity and total emission area, which are proportional to the analyte concentration. The optimum chemical conditions for the chemiluminescence emission were investigated. The effect of common emission enhancers, such as formic acid, formaldehyde, glutaraldehyde, acetaldehyde, quinine, fluorescein, rhodamine B, and rhodamine 6G, was studied. The parameters selected were sulfuric acid 4.0 mol L⁻¹, permanganate 0.1 mmol L⁻¹, and formaldehyde 1.0 mol L⁻¹. The calibration graphs obtained with each one of the measurement parameters were linear for the concentration range from 0.05 to 3.00 μg mL⁻¹. The detection limits ranged from 0.005 to 0.010 μg mL⁻¹, and RSD values (*n* = 10) of 0.99–1.79% at a 0.30 μg mL⁻¹ concentration level and 1.71–2.22% at a 1.0 μg mL⁻¹ concentration level were obtained. The present chemiluminescence procedures were applied to the determination of antu in different kinds of samples, such as river water, wheat, barley, and oat grain samples. Recovery values not significantly different from the spiked amount were found for these determinations.

KEYWORDS: Antu; pesticide; toxicity; rodents; wheat; barley; oats.

INTRODUCTION

Antu (1-naphthylthiourea) (**Figure 1**) is a pesticide the toxicity of which to rodents was described by Richter (1). Tolerance to it is developed in rats by repeated administration of sublethal doses (2). In some countries it has been withdrawn from use because of the carcinogenicity of the 1-naphthylamine that is usually present as an impurity. It is stable on exposure to air and sunlight.

Only a few methods for the determination of antu have been reported. Bromine has been used in the colorimetric determination of antu residues (3). Later, Vachek (4) described a colorimetric method based on reaction with sodium nitroprusside. Voltammetric methods have been given by Smyth and Osteryoung (5) and by Stará and Kopanica (6). The native fluorescence of antu has also been used for its detection by HPLC (7). Fluorescence detection is also used in another HPLC

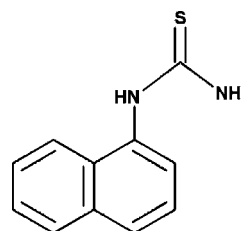


Figure 1. Chemical structure of antu.

method for the determination of antu together with other pesticides; this involves postcolumn ultraviolet photolysis and reaction with *o*-phthalaldehyde-2-mercaptoethanol (8). A fluorimetric procedure has been described (9) which is based on alkaline hydrolysis of the rodenticide. This provides a limit of detection of 10 ng mL⁻¹ for the determination of antu, and that is applicable to residues in wheat grain. Another method has been described for the determination of antu together with 1-naphthylamine and naptalam in river-water by HPLC (10). Finally, antu has been determined in water, together with other organic compounds, by means of liquid chromatography and

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mass spectrometry (11). A detection limit of 31 ng mL⁻¹ is achieved using solid-liquid extraction in short columns as a preconcentration step. This combination HPLC-mass spectrometry is the most recent choice for the determination of this rodenticide as this provides high sensitivity and selectivity; however, this requires expensive equipment and skilled operators (12, 13). No chemiluminescent procedures have been found in the literature search.

CL analysis has been used to determine a variety of substances in various fields (14, 15). Analytically CL reactions are attractive due to the potential for excellent limits of detection because of the absence of source noise and scatter, high selectivity due to the limited number of available reactions, and a wide linear range; all that can generally be achieved with simple, robust, and relatively inexpensive instrumentation suitable to both batch and flow analytical techniques. It requires no excitation source (as does fluorescence and phosphorescence), only a single light detector such as a photomultiplier tube, and no monochromator and often not even a filter.

Most chemiluminescence methods involve only a few chemical components to actually generate light. Liquid-phase chemiluminescence reactions have been used as the basis for sensitive and selective detection for a range of analytical techniques including flow injection analysis (FIA), sequential injection analysis (SIA), high performance liquid chromatography (HPLC), and capillary electrophoresis. Various sample-reagent mixing modes have been used in combination with a detector to record the chemiluminescence signal, such as the continuous-addition-of-reagent technique (16), which is based on the continuous addition, at low pressure and constant rate, of one reaction ingredient (usually the reagent) over a vessel containing the analyte and the other reaction ingredients, and a stopped-flow mixing module coupled to a CL detector (17, 18). Recently, a kinetic-based chemiluminescence method using the stopped-flow technique in a continuous-flow system has been reported (19).

Lahuerta-Zamora et al. (20) presented a paper with the first attempt to apply molecular connectivity calculations to predict the chemiluminescent behavior of substances when reacted with common oxidants in a liquid phase. Potassium permanganate as a CL reagent has been extensively investigated, and many substances have been determined via their direct oxidation by potassium permanganate in acidic medium (21). Fluorescing compounds as the energy-transferred excitation in an acidic potassium permanganate system have been described in the literature (22-24).

In this work, the chemiluminescent behavior of antu, by reaction of this rodenticide with a common oxidant, potassium permanganate, in a liquid phase is described. It was found that a weak CL emission was emitted during its oxidation with potassium permanganate in acidic solution. In the presence of formaldehyde, this weak CL signal was greatly increased. A stopped-flow chemiluminescence method for the determination of antu has been proposed using a typically kinetic parameter, such as CL decay rate, in addition to conventional measured parameters (peak height and area under the CL response signal), both of which are directly related to the analyte concentration. Furthermore, this approach can be regarded as the basis for the development of kinetic-chemiluminescence methods without the use of a special and expensive stopped-flow reactor. The method was applied to the determination of antu in river water, wheat, barley and oat grain samples.

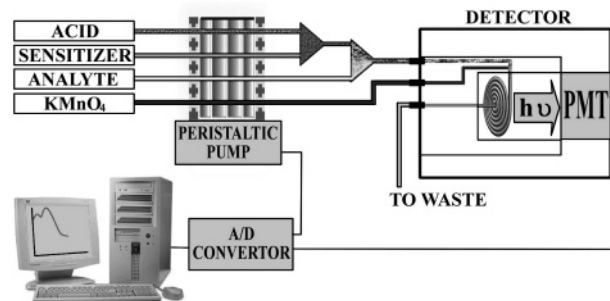


Figure 2. Schematic diagram of the continuous-flow manifold, using $\text{KMnO}_4\text{-H}_2\text{SO}_4$ as the oxidant and formaldehyde as the emission enhancer; the analyte is an aqueous solution of antu.

EXPERIMENTAL PROCEDURES

Reagents. All experiments were performed with analytical-reagent grade chemicals, pure solvents, and Milli-Q purified water. Antu was obtained from Riedel-de-Häen. Potassium permanganate and sulfuric acid were obtained from Panreac. Formaldehyde was supplied by Sigma.

A standard solution of antu (100 $\mu\text{g mL}^{-1}$) was prepared by dissolving 10 mg of antu in 1 mL of acetone and diluting to 100 mL with water. This standard solution of antu was stored at room temperature and protected from light. Under these conditions, it was stable for at least 4 weeks. Working standard solutions were then prepared by appropriate dilution of this standard solution. The working standard solution of antu was stable for at least 8 h at room temperature.

A standard solution of 1.0 mol L⁻¹ of formaldehyde was used. A standard solution of potassium permanganate (0.1 mmol L⁻¹) was prepared daily by dissolving 8 mg of KMnO_4 in 500 mL of water. The solution was protected from light.

Manifold and General Procedure. A simplified scheme of the continuous-flow manifold used to get the stopped-flow system is depicted in **Figure 2**.

The reaction reagents (acid medium, formaldehyde, antu sample solution, and potassium permanganate) were pumped through the four-line manifold by a peristaltic pump (Gilson, Minipuls-3), which was controlled by a microcomputer. The flow rate of the reagents was 15.2 mL min⁻¹, and the reagents went through poly(tetrafluoroethylene) (PTFE) flow tubes (Tygon, 0.8 mm i.d., resistant to acid). In the flow-through system, the first reaction components, sulfuric acid 4.0 mol L⁻¹ and formaldehyde 1.0 mol L⁻¹, were transported through channels to the point where they were mixed. Then, the resultant mixed solution was added to the antu sample solution channel. The emergent solution and the potassium permanganate, 0.1 mmol L⁻¹, were carried to the flow-cell, located in front of the photomultiplier tube (PMT), to allow both solutions to mix completely in the detector cell. The flow-through system was stopped for 30 s. In this way, the reaction took place in the flow-cell. The chemiluminescence-time data pairs were acquired using a Camspec chemiluminescence detector CL-2 (photosensor module Hamamatsu 45773-20; spectral response from 300-900 nm; spiral-type flow cell, volume 120 μL ; Sawston, Cambridge).

Finally, the pump was restarted to operate for at least 30 s before the flow was stopped again. All measurements were made at room temperature (18-20 °C).

The detector was connected to a computer by a digital analogical converter; data were acquired using the Chromatography Station for Windows CSW32 software (Data Apex Ltd, Prague, Czech Republic). In addition to conventional parameters such as the peak height or area under the CL response signal, we used a typical kinetic parameter, such as the CL decay rate, both of which are directly related to the analyte concentration. This kinetic parameter can be easily determined from the straight segment of the falling portion of the response curve, using data processing software developed by the authors. Each solution was assayed in triplicate. The resulting throughput was 45 measurements per hour.

The concentration of antu present in the sample was determined by using three plots of as many parameters related to the transient CL

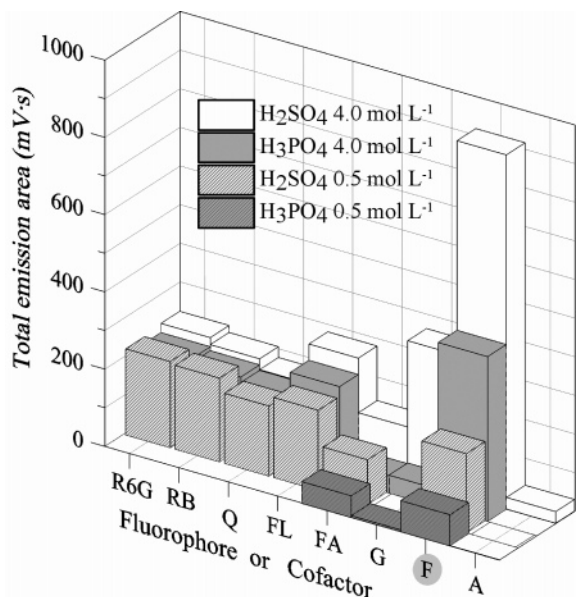


Figure 3. Effect of emission enhancers on the total emission area of the permanganate-induced antu CL reaction in phosphoric acid and sulfuric acid both at two concentrations levels. [antu] = 1.0 $\mu\text{g mL}^{-1}$; [KMnO₄] = 0.15 mmol L⁻¹; [rhodamine 6G, R6G] = [rhodamine B, RB] = [quinine, Q] = [fluorescein, FL] = 0.5 mmol L⁻¹; [formic acid, FA] = [glutaraldehyde, G] = [formaldehyde, F] = [acetaldehyde, A] = 0.25 mol L⁻¹.

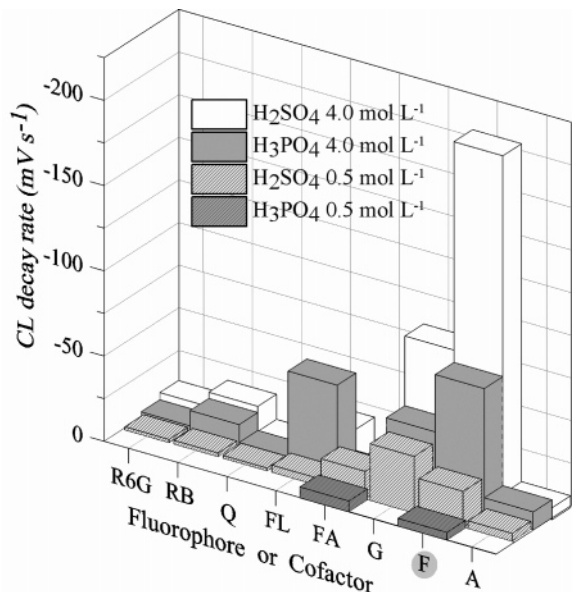


Figure 4. Effect of emission enhancers on the maximum CL decay rate of the permanganate-induced antu CL reaction in phosphoric acid and sulfuric acid both at two concentrations levels. [antu] = 1.0 $\mu\text{g mL}^{-1}$; [KMnO₄] = 0.15 mmol L⁻¹; [rhodamine 6G, R6G] = [rhodamine B, RB] = [quinine, Q] = [fluorescein, FL] = 0.5 mmol L⁻¹; [formic acid, FA] = [glutaraldehyde, G] = [formaldehyde, F] = [acetaldehyde, A] = 0.25 mol L⁻¹.

signal (viz. the maximum emission intensity, total emission area, and CL decay rate) against the analyte concentration.

Procedure for River Water. Aliquots of river water (500 mL) were taken from Guadiana river (Ciudad Real), decanted, and, since a preliminary study showed that this rodenticide was initially absent in these water samples, a known quantity of a standard aqueous solution of antu (20 $\mu\text{g mL}^{-1}$) was added, in such a way that the final concentration was included in the calibration graphs. Water samples were adjusted to pH 8.0 and passed on vacuum through Sep-Pak C18 plus cartridges, previously conditioned with 25 mL of acetone and later

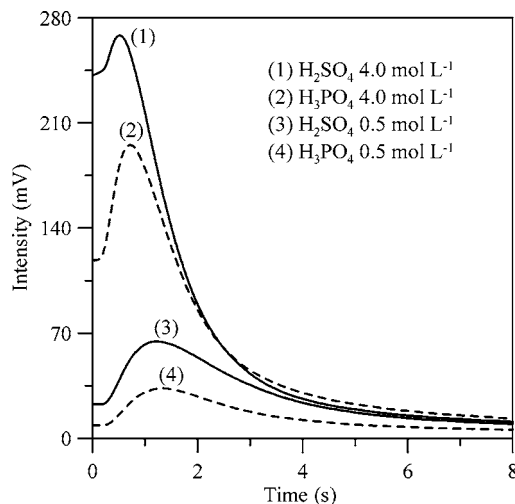


Figure 5. Time course of the CL reaction of antu using different acid media. [antu] = 1.0 $\mu\text{g mL}^{-1}$; [KMnO₄] = 0.15 mmol L⁻¹; [formaldehyde] = 0.25 mol L⁻¹.

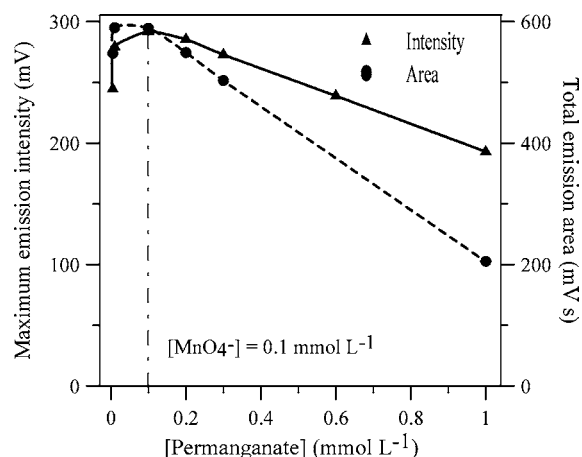


Figure 6. Effect of potassium permanganate concentration on the maximum emission intensity and total emission area in the CL reaction with antu. [antu] = 1.0 $\mu\text{g mL}^{-1}$; [H₂SO₄] = 4.0 mol L⁻¹; [formaldehyde] = 0.25 mol L⁻¹.

with 0.1 M phosphate buffer solution at pH 8.0. The analyte was then eluted with 10 mL of acetone. The organic solvent was evaporated to dryness using a stream of nitrogen. The residue was dissolved and diluted to 100 mL with water, and then the general procedure was followed.

Procedure for Wheat, Barley, and Oat Grain Samples. Ten-gram portions of wheat or barley grain samples were transferred to 250 mL beakers, and to each portion 2.0 mL of standard aqueous solution of antu (20 $\mu\text{g mL}^{-1}$) was added. After thorough mixing, the doped grains were left at room temperature for 30 min before extraction to ensure binding of the rodenticide to the matrix. The antu was extracted into 15 mL of acetone by sonication for 5 min. The solution was filtered on a Whatman filter paper, transferred into a 50 mL standard flask, and diluted to volume (50 mL) with deionized water. This solution was used for the analytical determination as described above.

RESULTS AND DISCUSSION

Most CL reactions have small quantum efficiencies and thus show weak luminescence. It has been reported that the CL intensity from several of these reactions can be enhanced by energy transfer procedures by some sensitizer, normally a highly fluorescent compound. Energy is transferred from the excited species to the sensitizer, emitting the characteristic radiation of the sensitizer. Since the sensitizer has higher quantum efficiency,

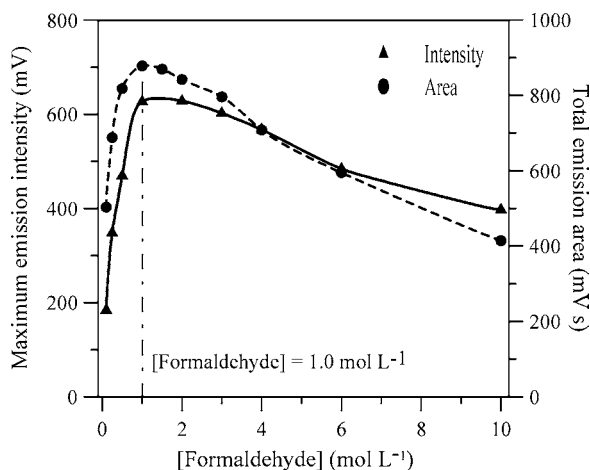


Figure 7. Effect of formaldehyde concentration on the maximum emission intensity and total emission area of the permanganate-induced antu CL reaction. [antu] = 1.0 $\mu\text{g mL}^{-1}$; $[\text{H}_2\text{SO}_4]$ = 4.0 mol L^{-1} ; $[\text{KMnO}_4]$ = 0.1 mmol L^{-1} .

more photons are emitted, facilitating measurement (25). In the bibliography, others common emission enhancers of CL reactions, namely reaction cofactors, have been examined (26).

In this work, it was found that a weak CL emission was emitted during the oxidation of antu with potassium permanganate in acidic solution. Therefore, an exhaustive chemiluminescence study has been carried out, with the object of investigating the influence of the presence of sensitizers or cofactors on the chemiluminescence emission of the antu–permanganate reaction in acidic medium, using the stopped-flow technique in a continuous-flow system. In this regard, four sensitizers, rhodamine 6G, rhodamine B, quinine, and fluoresceine, at a concentration of 0.5 mmol L^{-1} , have been assayed. On the other hand, formic acid, glutaraldehyde, formaldehyde, and acetaldehyde, at a 0.25 mol L^{-1} concentration, were selected and examined, according to the literature, as possible enhancers on the present CL reaction. All these studies were carried out using phosphoric acid and sulfuric acid both at two concentration levels, 0.5 and 4.0 mol L^{-1} . Some results obtained in this study are shown in **Figures 3** and **4** which are related to a conventional parameter (total emission area) and a kinetic parameter (CL decay rate), respectively. No useful enhancing effect was noticed with most sensitizers used. As can be seen, the best CL emission conditions were obtained using formaldehyde as the enhancer, a greater emission intensity and total emission area, and too the higher value of the decay rate of the CL signal; and then this emission enhancer was selected.

With the object of investigating the effect of acid media in the reaction between antu and permanganate, a comparative

study of the CL emission signal of the oxidant reaction was performed, in the presence of sulfuric acid and phosphoric acid. For the studies, 0.5 and 4.0 mol L^{-1} concentrations of sulfuric acid or phosphoric acid were maintained. The typical profile of a CL transient signal (a plot of CL intensity vs time) is a kinetic response curve that corresponds to a first-order sequence of two consecutive steps, namely: (1) generation of the light-emitting product by mixing of the chemical ingredients (the substrate and oxidant), and (2) formation of the end product. The rate at which each step takes place depends on the formation and decay rate constant, which correspond to the rising and falling portion, respectively, of the transient signal. **Figure 5** shows the profiles of the transient signals obtained with sulfuric acid and phosphoric acid, resulting in different developments for each one of these acids. Consequently, the parameters of the CL signals to be used to determine antu (viz. the maximum emission intensity, total emission area, and CL decay rate) were heavily dependent on the particular acid used. This experiment allows us to conclude that, in the presence of formaldehyde, all the parameters used to quantify the CL emission increased with increasing acid concentration and with H_2SO_4 the signal showed the best sensitivity; therefore this acid was selected for subsequent studies.

Influence of Experimental Variables. Several variables, including sulfuric acid, potassium permanganate and formaldehyde concentrations, and flow rate, could affect the kinetic of the proposed CL reaction for the determination of antu. Then, the optimum chemical conditions for the chemiluminescence emission were investigated and the optimum value for each variable was calculated from the resulting intensity vs time plots.

The influence of the sulfuric acid concentration over the range of 0.1–6.0 mol L^{-1} on the CL emission of the permanganate-induced antu CL reaction was then examined. The results obtained with all the parameters used to quantify the CL emission increased, with an increase of H_2SO_4 concentration. A concentration of 4.0 mol L^{-1} H_2SO_4 was selected for subsequent investigations.

The influence of potassium permanganate concentration over the range of 0.005–1.00 mmol L^{-1} upon the CL emission was studied. In **Figure 6**, the variations of the maximum intensity and total area of CL reaction with the permanganate concentration are represented. KMnO_4 concentrations over the range of 0.05–0.2 mmol L^{-1} provided the highest CL emission. Thus, 0.1 mmol L^{-1} was selected for application of the proposed method to the chemiluminometric determination of antu. The CL signal decreased above 0.2 mmol L^{-1} , likely as the result of permanganate absorbing the emitted light (26–28).

The influence of the formaldehyde concentration was studied in the range between 0.1 and 10.0 mol L^{-1} . As can be seen in

Table 1. Statistical Parameters

| | measurement parameter (concentration interval) | | |
|--------------------------------------|--|---|---|
| | maximum emission intensity (0.05–2.00 $\mu\text{g mL}^{-1}$) | total emission area (0.05–1.40 $\mu\text{g mL}^{-1}$) | CL decay rate (0.05–3.00 $\mu\text{g mL}^{-1}$) |
| intercept on the y-axis (<i>a</i>) | 15.5 | 51.02 | 6.246 |
| standard deviation of intercept | 4.2 | 9.9 | 5.1 |
| slope (<i>m</i>) | 550.3 | 921.0 | −511.2 |
| standard deviation of slope | 4.5 | 14 | 3.9 |
| standard deviation of estimation | 15 | 32 | 20 |
| determination coefficient (r^2) | 0.998 | 0.995 | 0.998 |
| precision | | | |
| relative standard error (RSE, %) | 0.3 $\mu\text{g mL}^{-1}$ | 2.42 | 2.84 |
| | 1.0 $\mu\text{g mL}^{-1}$ | 0.88 | 1.38 |
| relative standard deviation (RSD, %) | 0.3 $\mu\text{g mL}^{-1}$ | 0.99 | 1.14 |
| | 1.0 $\mu\text{g mL}^{-1}$ | 1.92 | 1.71 |
| | | | 2.22 |

Table 2. Detection Limits (LOD, $\mu\text{g mL}^{-1}$)

| | maximum emission intensity | total emission area | CL decay rate (mV min^{-1}) |
|-------------|----------------------------|---------------------|--|
| LOD (IUPAC) | 0.005 | 0.005 | 0.010 |
| LOD (30) | 0.024 | 0.025 | 0.023 |
| LOD (31) | 0.038 | 0.040 | 0.043 |

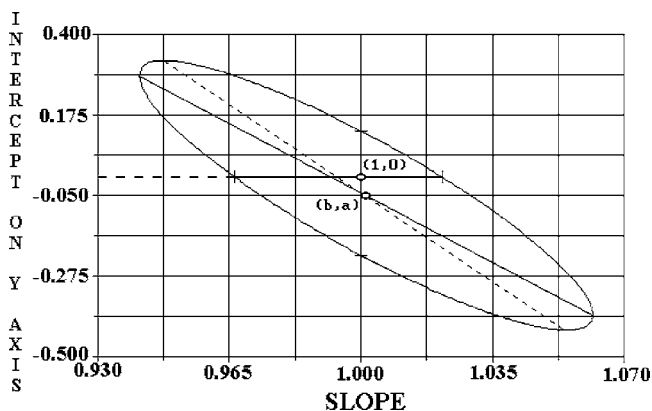


Figure 8. Comparison between the proposed method and the HPLC method. The ellipse is the 95% confidence region for the true slope and the intercept on the *y*-axis estimated from the overall least-squares regression performed with the concentration calculated in reverse through both. The point (*b*,*a*) is the center of the ellipse corresponding with the true intercept and slope estimated. The point (1,0) means the null intercept and unitary slope.

Figure 7, the parameters used to quantify antu (viz. maximum emission intensity and total emission area) increased with the concentration of formaldehyde up to 1.0 mol L^{-1} and then decreased for values higher than 1.0 mol L^{-1} . A similar representation was obtained using the CL decay rate as the measurement parameter. Consequently, a concentration of 1.0 mol L^{-1} was selected as optimum.

The flow rate is an important parameter in CL detection because the time taken to transfer the excited product into the flow cell is critical for maximum collection of the emitted light (29). Too low or too high flow rates result in a decrease or even absence of CL in the flow cell. The optimum flow rate was found to be 15.2 mL min^{-1} .

When the profile of the transient signal obtained with the selected chemical conditions was taken into account, the straight segment of the rising portion of this response curve was considered with the purpose of estimating whether the CL

formation rate could be an additional measurement parameter. This was realized by a similar study of the experimental variables using the CL formation rate as the measurement parameter, and then the corresponding calibration using this parameter was obtained. The results were not satisfactory because the straight segment of the rising portion of the signal was too short. Consequently, the CL formation rate was not included as parameter to quantify antu.

Calibration Curves and Analytical Parameters. Under the conditions selected, and using the above-described manifold, the chemiluminescence–time signals were recorded between 0 and 30 s, for solutions containing different amounts of antu. The time course of the CL reaction of permanganate in sulfuric acid and antu in the presence of formaldehyde shows an initial sharp rise in the signal; the maximum emission intensity was attained within 0.8 s and then decayed slowly. The calibration graphs were constructed, with 5 replicates per point, by measuring the maximum intensity, area under the CL response signal (total emission area, 10 s), and decay rate of the CL signal.

By application of the least-squares method, different calibration equations of CL reaction decay rate vs antu concentration were analyzed. Different interval times for the measurements, comprising between 0.8 and 3.0 s, were used. The most favorable statistical parameters were obtained in the range between 0.889 and 0.968 s for the CL decay rate.

Statistical parameters of the calibration graphs of the determination of antu with the proposed method are summarized in **Table 1**. The linearity of standard curves over the usable concentration range and the negligible scatter of the experimental points are clearly evidenced in the high determination coefficient obtained. The statistical treatment of the results revealed the absence of significant differences among the concentrations calculated using the different parameters of the profile for the CL transient signal, viz. maximum emission intensity, total emission area, and CL decay rate.

The precision achieved with each measurement parameter was assessed by preparing two series of 10 solutions, each one containing 0.3 and $1.0 \mu\text{g mL}^{-1}$ antu, and recording the time courses of the CL reaction of the analyte with $0.1 \text{ mmol L}^{-1} \text{ KMnO}_4$, $4.0 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$, and 1.0 mol L^{-1} formaldehyde on the same day, using the above-described manifold. By use of the theory of error propagation, the relative standard errors and the relative standard deviations for replicate samples were obtained with the maximum emission intensity, total emission area, and CL decay rate as measurement parameters. The results obtained at this study are summarized in **Table 1**.

Table 3. Results Obtained by the Application of the Proposed Time-Resolved Chemiluminescence Method (First-Order Calibration) to Determine Antu in River Water and Cereal Samples

| parameter | added ($\mu\text{g mL}^{-1}$) | samples | | | | | | | |
|----------------------------|---------------------------------|---------------------------------|------------------------------------|---------------------------------|------------------------------------|---------------------------------|------------------------------------|---------------------------------|------------------------------------|
| | | river water | | wheat | | barley | | oats | |
| | | found ($\mu\text{g mL}^{-1}$) | recovery \pm SD ^a (%) | found ($\mu\text{g mL}^{-1}$) | recovery \pm SD ^a (%) | found ($\mu\text{g mL}^{-1}$) | recovery \pm SD ^a (%) | found ($\mu\text{g mL}^{-1}$) | recovery \pm SD ^a (%) |
| maximum emission intensity | 0.8 | 0.799 | 99.9 \pm 3.6 | 0.787 | 98.4 \pm 1.9 | 0.839 | 104.9 \pm 1.0 | 0.738 | 92.3 \pm 4.0 |
| total emission area | 0.8 | 0.803 | 100.4 \pm 4.8 | 0.789 | 98.6 \pm 3.8 | 0.811 | 101.4 \pm 3.0 | 0.732 | 91.5 \pm 1.0 |
| CL decay rate | 0.8 | 0.805 | 100.7 \pm 3.1 | 0.732 | 91.4 \pm 4.3 | 0.754 | 94.2 \pm 1.7 | 0.749 | 93.6 \pm 3.7 |

^a For $n = 3$.

In addition to statistical parameters of the calibration graphs summarized in **Table 1**, a second-order calibration of each measurement parameter vs concentration of antu was obtained in the range of 0.01–4.00 $\mu\text{g mL}^{-1}$ antu with 12 standards involved and 3 replicates per sample. The regression equations were $I_{\text{CL}} = -0.942 + 611.121C - 30.388C^2$ with a determination coefficient of 0.9998, $A_{\text{CL}} = 28.495 + 1032.251C - 92.294C^2$ with a determination coefficient of 0.9997, and $R_{\text{CL}} = 10.717 - 538.516C + 14.820C^2$ with a determination coefficient of 0.9992, for maximum emission intensity (I_{CL}), total emission area (A_{CL}), and CL decay rate (R_{CL}), respectively. The ANOVA method was applied to the data obtained using the three measurements parameters. The results of this study showed that there were not statistically significant differences between the results obtained.

The detection limits obtained with the different measurement parameters are shown in **Table 2**. Detection limits were calculated in accordance with IUPACs definition, which considers the standard deviation of the blank only. If the theory of error propagation is considered, then the values of detection limits are consistent with the reliability of the blank measurements and for the signal measurements of the standards. **Table 2** shows the results obtained by calculating the detection limits according to Long and Winefordner (30) and to Clayton et al. (31) with provision for false positive and negative values.

The selectivity of the proposed method for antu was examined by using it in the presence of other pesticides. Compounds such as chlorfenac, 2,4-dichlorophenoxyacetic acid, 2-(2,4-dichlorophenoxy)propionic acid, isoproturon, propham, diuron, neburon, linurom, napropamide, naphthalam, thiobendazole, and fuberidazole exhibited no interference in a proportion of 1:1 (a substance was assumed to interfere when the recovery obtained in the determination of antu in its presence was 95–105%).

The determination of antu was validated by least-squares regression (32). The performance of the proposed method in the determination of antu in water was compared with that of an HPLC method (10) by analyzing 10 samples containing the pesticide at levels within the application range. The concentrations, provided by the currently accepted method and the proposed method were subjected to least-squares pair analysis. This procedure considers the effects of various types of error. The presence of random of errors in the test method causes points to scatter around the least-squares line and the calculated slope and intercept to slightly depart from unity and zero, respectively. The random error can be estimated from the standard deviation in the y -direction (also called the standard deviation of the estimate of y on x). A proportional systematic error leads to a change in b , so the difference between b and unity provides an estimate of the proportional error. A constant systematic error shows up in a nonzero value for the intercept. If both methods provided identical concentrations, in the same samples, then the least-squares analysis would give a zero intercept and a unit slope. **Figure 8** shows the 95% confidence region for the true slope and estimated intercept. As can be seen, the point corresponding to the zero intercept and unity slope falls within the joint confidence region, which means that the accuracy of the proposed method and the currently accepted method is not significantly different.

Determination of Antu in River Water, Wheat, Barley, and Oat Grain Samples. The proposed time-resolved chemiluminescence method was applied to the determination of antu in river water and cereal samples, using maximum emission intensity, total emission area, and CL decay rate as measurement parameters. All of these samples either were free of antu

contamination or perhaps were contaminated at concentrations lower than the detection limits. Therefore, a recovery study was carried out with standard solutions of antu. All the samples were spiked with an amount of antu to test the procedure. The recovery ratios, of known amount of antu added to the samples (river water and cereals) were obtained by using calibration graphs (maximum emission intensity vs concentration, total emission area vs concentration, and CL decay rate vs concentration). **Table 3** shows the assay results, as a percentage of recovery, from the average of 3 determinations. The recoveries agree well enough with the added amount, and the precision is quite satisfactory.

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