SIMPLIFIED CHEMILUMINESCENT FIA FOR OXIDIMETRIC DETERMINATION OF CAPTAN

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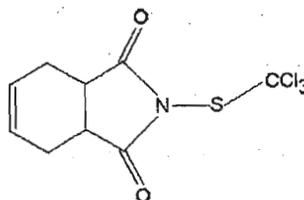
Abstract

A new chemiluminescent reaction for captan-hydrogen peroxide in alkaline medium is described and applied through a FIA manifold to a determination of captan. The FIA assembly consists of a channel in which the oxidant (hydrogen peroxide) and the medium (NaOH solution) are mixed in situ and then the resulting mixture merges with the carrier (NaOH solution) where an aliquot of 582.1 µl of the sample solution was inserted. The calibration graph was studied over the range 0.9-400 mg l^{-1}, with a rsd and limit of detection of 1.8% (30 replicates) and 0.9 mg l^{-1}, respectively. The sample frequency was 160 h^{-1}.

Keywords.- Pesticide, captan, chemiluminescence, FIA, continuous-flow.

Introduction

Captan or N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide is a broad-spectrum fungicide of wide use. Captan prevents a number of infections in citrus fruits, grape vine, strawberry bed or olive tree. It is also useful in soil, seed and foliar applications to control damping-off diseases. Moreover, it stimulates the vegetation and improve the

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appearance of fruits. The fungitoxic properties of captan were first discovered by

Kittleson in 1952 and its value for the control of fugal diseases was reported by Daines in 1953. In the begining it was called "Kittleson's killer" (1).

The chemical method commonly employed for its determination in formulations involves a selective hydrolysis followed by the determination of halide (2). The analytical literature contains some examples of determination. In a method developed by Carabias et al. (3) captan and other fungicides were preconcentrated with Triton X-100 by the cloud-point procedure and then it was electrochemically determined. Gas chromatographic procedures have been developed for determination of captan in different kind of samples like vegetables and fuits, milk, animal tissues, water and commercial formulations (4, 5). Other techniques published involved inmunaossays (6, 7) or titrimetric procedures (8) for commercially available formulations.

Solution-phase chemiluminescence (CL) reactions have considerable analytical potential because of the low limits of detection and wide dynamic range that can be achieved with relative simple instrumentation. The absence of a light source reduces the background noise and detection limits are generally governed by the chemical noise of the system. The potential of CL-based analytical techniques is attested by the number of monographs and

reviews covering various aspects of its application (9, 10). The determination of pesticides in flow-injection assemblies (including CL detection) has been recently reviewed (11).

CL reactions have been defined as direct or indirect depending on the origin of the CL emission. With the direct CL the analyte itself is the responsible of the emitted light; the analyte generates the first excited-state molecule when it is treated by a strong oxidant.

In solution, CL finds many applications in analytical chemistry for the determination of many analytes in a variety of environmental matrices (12,13). On the other hand, chemiluminescent reactions are usually fast: the emission of light can occur over several seconds and their intensity is generally weak For reproducible monitoring of them, merging zones FI manifolds are very successful, because they permit rapid and reproducible mixing of sample and reagents and monitoring emission in a fixed time. Moreover, their simplicity make them suitable for in situ measurements (14).

The main goal of this work is to seek chemiluminescent behaviour of the pesticides themselves. After an study of the application of molecular connectivity to

chemiluminescent prediction (studied 143 molecules) the obtained discriminant function was able to predict the chemiluminescent behaviour of several substances, captan between them (15). When the chemiluminescence was experimentally tested in a continuous flow manifold, captan itself emitted light when it reacted with hydrogen peroxide in alkaline medium. This fact was the start of a new FIA method of direct chemiluminescence developed for the captan determination in some common formulations dealing with a quick, accurate and inexpensive chemiluminescent procedure.

Experimental

Reagents

Oxidation of the captan, from Supelco (purity 98%, certified) was performed with the aid of hydrogen peroxyde (from Scharlau, 33% w/v) in sodium hydroxide medium (from Panreac). Captan was dissolved in acetone (from Guinama) / water, 1:1.

Other used reagents were: $K_3Fe(CN)_6$ (from Panreac); $Ce(SO_4)_2 \cdot 4H_2O$ (from Fluka); $KMnO_4$ (from Panreac). Other acids used reagentsand alkalis like H_2SO_4 (from

Scharlau); H_3PO_4 (from Panreac). Substances tested as interferences were: Lindane (from Guinama), Carbendazime (from Afrasa) Zineb (from Afrasa) sodium dodecyl sulfate, SDS (from Panreac); sodium dodecylbenzene sulfonate, DBSS (from Fluka); sodium dioctylsulfosuccinate, DOSS (from Scharlau); and, Triton X-100 (from Scharlau).
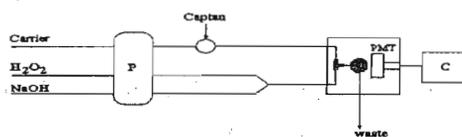

Figure 1. Flow injection assembly for captan determination. For details see text. Carrier flows at 5.9 ml/min, H_2O_2 and NaOH streams at 1.6 ml/min. The distances of injection valve-detector and (NaOH-H_2O_2 confluence)-detector were 48 and 66 cm, respectively. P: peristaltic pump, PMT: photomultiplier tube, C: computer.

Materials and apparatus

The proposed flow assembly is shown in Figure 1 and consisted of a peristaltic pump Gilson Minupuls 2 which pumped solutions (with a PTFE tubing of 0.5 mm internal diameter. Sample solution was inserted by means of a valve from Rheodyne
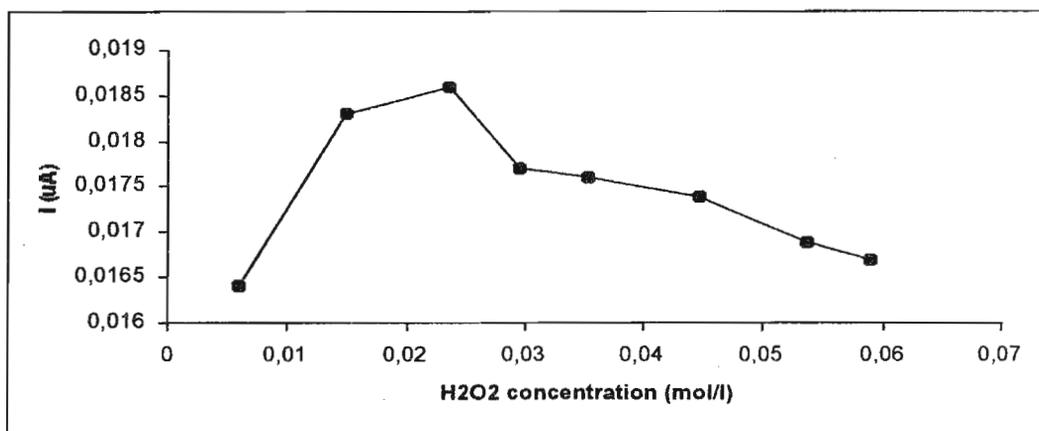
Figure 2. Influence of H_2O_2 concentration.

(model 5041). For pumping sample solution, a solvent resistant tube was used. Oxidant solution and carrier merged in a T-piece's type junction positioned 2 cm from the entrance the home-made flow cell. This cell was a flat-spiral coiled quartz tubing of 1.0 mm of internal diameter and 3 cm of the spiral total diameter.

The chemiluminescence emission was measured by a home-made luminometer which comprised an absolutely light-tight box containing the photomultiplier tube operated at 800 volts from a programmable photomultiplier high voltage power supply. The detection electronics allow monitoring of peak intensity (in μA); the analytical signal was always calculated as sample output minus blank.

Procedures

The sample solution (captan in acetone/water, 1:1) is injected in a carrier stream of 5×10^{-3} M and merges with the oxidant solution, formed by confluence of 2.3×10^{-2} M H_2O_2 and 5×10^{-3} M Na OH in a T-piece much close to the flow-cell.

Optimization of experimental parameters was carried out by a sequential procedure of successive optimizations. After preliminary optimization of several parameters (concentration of the oxidant, concentration of the alkaline medium), the Modified Simplex Method (MSM) was used for optimization of the manifolds parameters. The Modified Simplex program for the work was based on the method of Nedler and
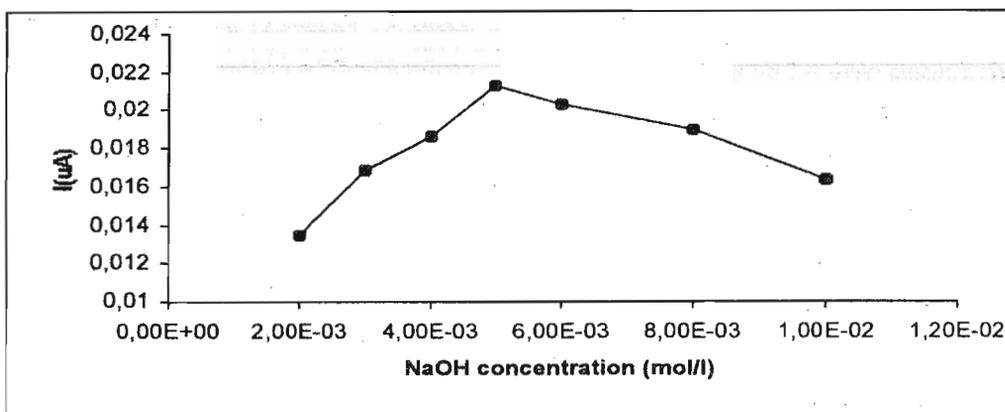
Figure 3. Influence of NaOH concentration

Mead (16). After the first set of 7 experiments within the MSM, a new MSM was applied (25 experiments) by adjusting new limits for every tested parameter according to results obtained in the first set. Then, in order to obtain the best compromise of sensitivity (peak height), reproducibility (rsd, %) and sample feeding throughput, four points from the Simplex producing the highest transient signals were selected and compared by recording a series of 30 injections. Final optimization of chemical parameters was achieved by the univariate method.

Results and discussion

Preliminary experiments

Different oxidative reagents in several media were tested to find chemiluminescent emission from the reaction with captan. Tested oxidants were 10^{-3} M $K_3Fe(CN)_6$ in 10^{-2} M NaOH; 10^{-4} M $KMnO_4$ in 0.1 M H_2SO_4; id. in 2 M H_3PO_4 ; 10^{-3} M Ce(IV) in 0.1 M H_2SO_4; and 0.2% H_2O_2 in 10^{-3} M NaOH. Some small emission outputs were observed, however only hydrogen peroxide resulted in a suitable analytical signal. The presence of metal ions (Ni(II), Co(II), Mn(II) and Cu(II)) usually proposed as cataliysts for oxidation reactions did not result in observed increases of the analytical output.

We performed some preliminary tests to select a set on initial conditions that would be subsequently refined. For this purpose the continuous flow device asosociated with Fig.1 was used with a solution of 100 mg l⁻¹ of captan in acetone/water (1:1) flowing at

4.8 ml/min. The flow rate of H_2O_2 and NaOH streams were 2.1 ml/min.

At the first, we studied the influence of H_2O_2 concentration in the CL response; 3×10^{-2} M was selected. NaOH concentration was also tested; the best results were obtained with 5×10^{-3} M.

Flow injection experiments

Further experiments were performed with the FIA system proposed (see Figure 1).

The first parameter to be studied was the total flow-rate, measured at the waste of the manifold. The selected value was 8.3 ml/min.

Next, we tested the influence of flow-rate ratio between carrier and H_2O_2/NaOH reagents. The confluence of H_2O_2 – NaOH channels is necessary due at the lack of stability of H_2O_2 in alkaline medium. The flow-rate ratio (carrier vs reagent) major than 1 produced the best results than could be obtained.

We also assayed different concentration ratios of NaOH in carrier and solution that merges with H_2O_2: the outputs were higher with a ratio of 1. Table 1 shows the results obtained in this stage.

Simplex optimization

The pre-optimization step allowed development of the MSM optimization pro-

Table 1. Results of pre-optimization in the continuous-flow system

Parameter assayed	Selected value
Flow-rate of carrier	4.3 ml/min
Flow-rate of H_2O_2	2.0 ml/min
Flow-rate of NaOH	2.0 ml/min
[NaOH] (carrier) / [NaOH] (reagent)	1

Table 2. Simplex Method of Optimization for the flow-injection assembly

Parameter	Studied range		Selected value
	1rst series	2nd series	
Carrier flow-rate (ml/min)	2.4 - 6.2	3.7 - 6.2	5.9
Sample volume (μl)	142.4 - 417.7	311.7 - 735.3	583.7
Flow-rates of H_2O_2 and NaOH (ml/min)	0.8 - 2.2	1.4 - 2.8	1.6

cedure by using initial chemicalconditions close to the optimum values. The range of variables considered is shown in Table 2. After 7 experiments it was decided to test a new simplex by considering a new range of variables, which are also shown in Table 2. After 25 experiments it was decided that the system did not merit further experimentation. In order to obtain the best compromise of sensitivity (peak-height), reproducibility (rsd, %) and sample feeding, four points

producing the highest transient signals were selected and compared by recording series of 30 injections. The results from the optimization are given in Table 2.

At a subsequent stage, chemical variables were re-optimized. Figures 3 and 4 show the results obtained on the study of the influence of H_2O_2 and NaOH concentrations: 2.36×10^{-2} M and 5×10^{-3} M were selected for further work.

Other tested chemical parameters were the media for the carrier and oxidant reagent solutions: KOH and phosphate buffer were tested and compared with NaOH medium. The outputs were higher when NaOH was used.

Analytical applications

With the optimized FIA manifold the dynamic calibration graph was studied over the range 0.9-400 mg l^{-1} of captan, with the equation:

$$\text{Intensity } (\mu A) = -3.1 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.00077;$$

were c is the captan concentration in mg l^{-1}. The correlation coefficient was 0.9988.

The reproducibility of the method and the sample frequency were studied by using a sample solution containing 100 mg l^{-1} of captan. The calculated rsd (30 replicates) was 1.8 % and sample frequency was 160 h^{-1}.

Table 3. Calibration equations for the day-to-day reproducibility study

Equation
Intensity $(\mu A) = -3 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.001$
Intensity $(\mu A) = -3 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.001$
Intensity $(\mu A) = -3 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.0012$
Intensity $(\mu A) = -3 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.0005$
Intensity $(\mu A) = -3 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.0006$
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Intensity $(\mu A) = -3 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.0009$
Intensity $(\mu A) = -3 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.0007$
Intensity $(\mu A) = -4 \cdot 10^{-7} c^2 + 0.0002 \, c - 0.0016$

The day-to-day reproducibility was studied by performing each day (9 days) with freshly prepared solutions. Table 3 shows the obtained equations.

The presence of other compounds which can change the analytical output was tested by preparing solutions containing 75.2 mg l^{-1} of pure captan and different concentrations of the foreingn subtance; results were compared against the solution containing only 75.2 mg l^{-1} of pure captan Different tensoactive agents were tested; Triton X-100, sodium dodecylsulfate, hexadedylpyridinium chloride, sodium diocthysulfosuccinate an sodium diocthylbenzenesulfosuccinate. Obtained results showed no relevant variations vs the pure captan solution; the different tested influences were the corresponding to

pesticides zineb, lindane and carbendazime; zineb (up to 160 mg 1 $^{-1}$ and lindane (up at 100 mg 1 $^{-1}$) give no serious errors; 0.1 % and 2.0 %, respectively. Carbendazine give serious errors at similar concentrations; with lower concentrations (20 mg 1 $^{-1}$) resulted in a relative error (%) of 1.3 . The proposed procedure was applied to different commercial available formulations (*Botrin C-48* and *Captamur 50*); relative errors for the results were, respectively, -2.5% and 0.7%.

Conclusions

The direct FIA-chemiluminescence reaction of the pesticide captan is reported by first time. The chemiluminescence emission is obtained by reaction with hydrogen peroxide in alkaline medium. The procedure is very simple. The method is applied to captan determination in industrial formulations.

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