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# Native *vs* photoinduced chemiluminescence in dimethoate determination

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## Abstract

The determination of dimethoate using either its native chemiluminescent (CL) properties or its photoinduced chemiluminescence obtained by irradiation with a 15 W low-pressure mercury lamp was studied. Thereby, two flow injection systems (FIA) with and without irradiation were exhaustively optimised and their analytical characteristics studied. Better sensitivity and selectivity was found in absence of irradiation, due to the enhancing effect of hexadecylpyridinium chloride (HPC), which acted as a sensitizer. In the developed FIA-CL system, the alkaline hydrolysis of dimethoate with NaOH was performed on-line in presence of HPC. The oxidation of the product of hydrolysis with Ce(IV) in hydrochloric medium induced chemiluminescence. The method provided a limit of detection of only 0.05 ng $mL^{-1}$ without any pre-treatment. However, the combination with solid phase extraction allowed the removal of some potential interferents as well as the preconcentration of the pesticide. Finally, the CL developed method was successfully applied to natural waters with recoveries between 95-108%.

**Keywors:** Dimethoate; photoreaction; Chemiluminescence; flow injection; water.

## 1. Introduction

Dimethoate (O,O-dimethyl-S-methylcarbamoyl methylphosphorothioate) (Figure 1) is a systemic and contact broad-spectrum insecticide and acaricide, used against numerous crops as well as to control houseflies. It belongs to the organophosphate family and, as most of these pesticides, is moderately toxic and readily absorbed through the skin and lungs, acting as a cholinesterase inhibitor, an enzyme essential for normal nerve impulse transmission. It is enzymatically converted in the intestine wall and liver enabling desulfuration to its oxon-derivative omethoate (O,O-dimethyl-S-methylcarbamoyl methylthiophosphate) causing enhanced neurotoxicity [1]. Hence, it is highly toxic to fish and aquatic invertebrates. The World Health Organisation (WHO) has set an Acceptable Daily Intake (ADI) value for dimethoate of 0.002 mg $kg^{-1}$ bodyweight. Dimethoate is very soluble in water. It hydrolyses very slowly at pHs between 2 and 7 and it is not photodegraded by sunlight; consequently it is persistent in the aquatic environment.

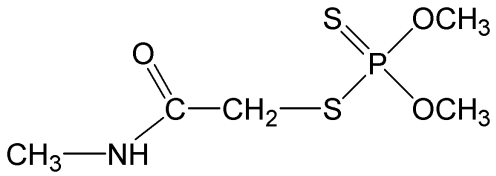


**Figure 1.** Molecular structure of dimethoate.

The most usual methods of determining dimethoate are based on gas chromatography (GC) coupled with a nitrogen-phosphorus detector [2,3], electron ionization mass spectrometry [4,5] or flame photometric detection [6,7]. Nevertheless, liquid chromatography/mass spectrometry (LC/MS) is the most advantageous approach for the analysis of polar, non-volatile or thermally labile compounds as dimethoate. The liquid separation combined with electrospray ionization followed by tandem-mass spectrometric detection (LC–ESI-MS/MS) [8-10] has been used to overcome the above-mentioned drawbacks. A further advance in the analysis with LC/MS/MS is the use of the isotope dilution method in conjunction with MS/MS, which allows the determination of contaminant residues at sub-picomole level [11,12].

Capillary electrophoresis with MS detection after ionization by inductively coupled plasma (CE-ICP-MS) or UV-detection (CEUV) has also been used [13,14] although less frequently. Y. Chen et al. [15] developed a capillary electrophoresis–electrochemiluminescence detection system, equipped with an electrically heated

$Ru(bpy)_3^{2+}$/multi-wallcarbon-nanotube paste electrode. The heated electrode provided some advantages over the conventional electrode at room temperature, such as higher sensitivity, lower RSD and decreasing width of the peak. In fact, the limit of detection (S/N) ranged from 230 to 80 μg $L^{-1}$ when the temperature increased from 22 to 49 ºC.

Other non-separative methods based on polarography [16], voltammetry [17] or amperometry [18] have also been developed.

The only chemiluminescent strategy developed for dimethoate determination, was based on the reaction between ozone and the nitric oxide produced by pyrolysis at 1050ºC of dimethoate and other N-compounds, performed in the effluent of a supercritical-fluid chromatography equipment [19]. The calibration graph was linear over the range 2.96-850 mg $L^{-1}$ of nitrogen and the detection limit was 60 pg.

On the other hand, a flow injection (FI) procedure was used coupled with a combination of ESI-MS/MS (FI-ESI-MS/MS) for dimethoate determination by H. John et al. [8], which allowed to increase the throughput. The extensive sample dilution required in that method (it was applied to plasma and urine) diluted also the matrix and consequently its potential interfering effect. Hence, similar validation characteristics as those provided by the chromatographic method (LC-ESI-MS/MS) were achieved.

In this paper FI analysis, which provides high throughput and reproducibility, has been coupled with the detection of the chemiluminescence (CL) generated by strong oxidants, in order to obtain a highly sensitive and selective method. The use of the UV light, as a derivatization tool to obtain photoproducts with better chemiluminescent properties, was also studied. However, despite the fact that photoinduced chemiluminescence (PICL) often improves sensitivity and selectivity [20], the best results were achieved in this case from the CL generated by the hydrolysis products obtained in the absence of irradiation, and the signal was greatly increased by hexadecylpyridinium chloride (HPC).

## 2. Materials and methods

### *2.1. Reagents*

All solutions were prepared from analytical-grade reagents in Milli-Q water (18 MΩ-cm) from Millipore, Bedford, MA, USA, provided with a fiber filter of 0.22 μm pore-size. Dimethoate was purchased from Fluka (99.4% purity). Other reagents were: $Ce(NH_4)_2(NO_3)_6$, from Panreac; HCl and NaOH from Scharlau; $CH_3COOH$ from J.T.

Baker; and hexadecylpyridinium chloride monohydrate from Sigma. Other pesticides used were: amitrole, metazachlor, metalaxyl, thiacloprid and cyromazine (99.9%), 2,4-D and pirimicarb (99.6%), diquat monohydrate (99.4%), glyphosate and quinmerac (99.2%), fenamiphos (97.7%), diuron (99.5%), imazalil (99.8%), MCPA (98.7%), malathion (97.3%), omethoate (98.5%) and methidathion (96%), all of them from Riedel-de Haën; methomyl (99.5%) from Chem Service; Chlorpyrifos from Sigma-Aldrich (99.9%); and, diphenamide (99.9%) from Fluka.

### *2.2. Flow injection procedure*

The FIA manifolds optimized for the CL and PICL determination of dimethoate are depicted in Figure 2. Connections between the different parts of the flow assemblies were carried out with PTFE coil of 0.8 mm id from Omnifit. Gilson (Worthington, OH, USA) Minipuls 2 peristaltic pumps, provided with tygon pump tubes from Restec, were used for flow control. The laboratory-made photoreactor included PTFE tubing (0.8 mm id x 400 cm) tightly coiled around a 15 W low-pressure mercury lamp (Sylvania) for germicidal use. A 6-port medium pressure (Upchurch Scientific, Model V-450) injection valve was used. The photodetector package P30CWAD5F-29 Type 9125 photomultiplier tube (PMT) was supplied by Electron Tubes operating at 1280 V and located in a laboratory-made light-tight box. The solutions merged in a T-piece placed inside close to the flow-cell, a flat-spiral glass tube of 1 mm id and 3 cm total diameter. The output was fed to a computer equipped with the CT2 counter-timer board, also supplied by Electron Tubes.

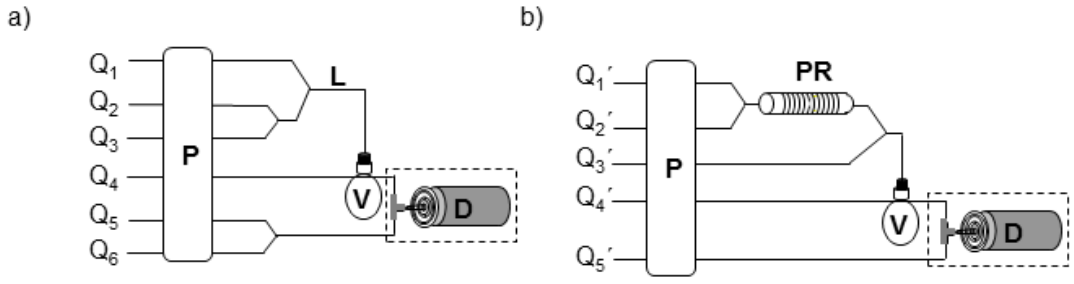


**Figure 2.** Manifold configurations for dimethoate determination:
a) CL system: $Q_1$: dimethoate (1.6 mL $min^{-1}$); $Q_2$: NaOH 0.34 M (0.8 mL $min^{-1}$); $Q_3$: hexadecylpyridinium chloride 0.4% (0.8 mL $min^{-1}$); $Q_4$: water (14.3 mL $min^{-1}$); $Q_5$: Ce(IV) 1.8 $10^{-3}$ M (2.2 mL $min^{-1}$); $Q_6$: HCl 2.2 M (2.2 mL $min^{-1}$); V: 659 μL; L: 4.5 m; hydrolysis time: 42 s; b) PICL system: $Q_1$´: dimethoate (2.1 mL $min^{-1}$); $Q_2$´: NaOH 2.0 M (0.5 mL $min^{-1}$); $Q_3$´: hexadecylpyridinium chloride 0.36% (0.5 mL $min^{-1}$); $Q_4$´: water (12.2 mL $min^{-1}$); $Q_5$´: Ce(IV) 1.15 $10^{-3}$ M in acetic acid 2.5 M (4.2 mL $min^{-1}$); V: 809 μL; Irradiation time: 46 s.
P: peristaltic pump; PR: Photoreactor; V: injection valve; D: luminometer; L: PTFE tubing of 0.8 mm i.d.

*2.3. Sample preparation*

Water samples from different origins, namely: irrigation, ground, spring, mineral and tap waters were tested. They were collected in plastic flaks at 4 ºC and analysed before 48 h. In order to remove sand and other suspended solid matters, the samples were pretreated by filtering over a 0.45 μm membrane filter (Sartorius, Goettongen, Germany). Spiking was done by adding a proper amount of 100 mg $L^{-1}$ of dimethoate stock solution to 50 mL of sample, in order to obtain 0.2, 0.5 and 1.0 μg $L^{-1}$ of pesticide. Three replicates of each concentration were prepared.

A solid phase extraction (SPE) of the 50 mL of spiked samples at a flow-rate of 2-3 mL $min^{-1}$ was performed off-line using a vacuum system and cartridges Bond Elut-$C_{18}$, 200 mg, from Varian, in order to improve the selectivity and sensitivity of the method. 3.0 mL of methanol, 3.0 mL of acetonitrile, 3.0 mL of methanol and 9.0 mL of water, were used to precondition the cartridges. Once the samples have been passed, the washing was performed with 9 mL of water and, next, air was passed 20 min for drying. Dimethoate was eluted by means of gravity with 1.0 mL of acetonitrile and finally under vacuum. Then 1 mL of water was passed through the cartridge to recover the remainder. Both volumes were collected in a volumetric flask of 10 mL and filling up with deionised water.

## 3. Results and discussion

*3.1. Preliminary studies*

Molecular irradiation can led to the formation of minor fragments with smaller molecular weight (fotolysis) or to induce reactions of photocyclization, photoisomerization, photooxidation and photoreduction [20]. Hence, light has been used often as a “reactive” to induce the formation of photoproducts with improved analytical properties.

A FIA manifold on the basic lines of that shown in Figure 2b was employed for testing the influence of the irradiation on the CL signal, but removing the $Q_2$´ channel and adding a Y-shaped piece into $Q_5$´channel in order to mix the oxidant and its medium in situ (both flowing at 1.2 mL $min^{-1}$). 100 mg $L^{-1}$ of dimethoate solution was mixed with three different photodegradation media (water, NaOH 0.1 M and $H_2SO_4$ 0.1

M) (Q1´and $Q_2$´, both at 1.3 ml $min^{-1}$). The lamp was alternatively switched OFF and ON to obtain the CL signal from dimethoate and its photoproducts, respectively. Next, 508 μL of that mixture were injected into a water carrier (5 mL $min^{-1}$), and merged with each of the 9 oxidant systems tested, namely $KMnO_4$, Ce(IV), $KIO_4$ and $K_2S_2O_8$, all of them at $8.0 \cdot 10^{-3}$ M except $KMnO_4$, which was $1.4 \cdot 10^{-3}$ M, in $H_2SO_4$ 2 M; and $KIO_4$, $K_2S_2O_8$, $K_3Fe(CN)_6$, N-bromosuccinimide (NBS) and $H_2O_2$, all of them at $8.0 \cdot 10^{-3}$ M except NBS, which was $4.0 \cdot 10^{-2}$ M, in NaOH 2 M.

Table 1 shows the best results achieved. As can be seen, higher outputs were obtained when previous irradiation of dimethoate was performed. However, in the absence of irradiation a significant CL signal was also achieved.

**Table 1.** Preliminary study of the oxidant systems employing 100 mg $L^{-1}$ of dimethoate.

| **Oxidant system** | **Photodegradation medium** | **CL system I (kHz)[a]** | **PICL system I (kHz)[a]** |
|---|---|---|---|
| $KMnO_4$ $1.4 \cdot 10^{-3}$ M/ $H_2SO_4$ 2 M | $H_2O$ | 0.708 | 1.760 |
| | $H_2SO_4$ 0.1 M | 0.744 | 1.760 |
| | NaOH 0.1 M | 2.050 | 2.870 |
| Ce(IV) $8 \cdot 10^{-3}$ M/ $H_2SO_4$ 2 M | $H_2O$ | 0.257 | 0.390 |
| | $H_2SO_4$ 0.1 M | 0.265 | 0.367 |
| | NaOH 0.1 M | 0.410 | 0.890 |
| Nbromosuccinimide $4 \cdot 10^{-2}$ M/NaOH 2 M | $H_2O$ | 0.337 | 1.100 |
| | $H_2SO_4$ 0.1 M | 0.640 | 1.020 |
| | NaOH 0.1 M | 1.720 | 2.320 |

[a] Results after subtracting the blank

On the other hand, the best results were obtained when the sample was merged with NaOH, which indicated the important role played by alkaline hydrolysis in the determination of dimethoate. The main degradation products were probably *O*-desmethyl-dimethoate and *O,O*-dimethyl hydrogen phosphorothioate acid [21]. In general, hydrolysis of organophosphorus pesticides is faster under alkaline conditions, what suggests that the reaction is more effectively catalyzed by $OH^-$ than by $H_2O$ or $H_3O^+$ [22].

*3.2. Optimization process*

An univariate strategy was employed for the optimization process of the FIA-CL and FIA-PICL manifolds. Different parameters were studied in the following order:

1) Oxidant concentration
2) Oxidant medium
3) Flow rate in the detector
4) Hydrolysis and/or irradiation media
5) Irradiation time in PICL
6) Effect of sensitizers and organized media
7) Insertion volume
8) Temperature

Table 2 shows the ranges considered and the optimal values found for each manifold, CL and PICL (lamp OFF and ON respectively). The concentrations of dimethoate used were decreasing throughout the optimization process, as the signals achieved were increasing.

Permanganate and Ce(IV), which gave the highest outputs, were chosen for further investigation. NBS was discarded because of its irreproducible results. It is interesting to point out the great enhancing effect observed when acetic acid was employed as oxidation medium in the PICL system, especially with Ce(IV), providing signals between 7.3 and 13.4 times higher than the other media.

The effect of the oxidation time was studied by changing the carrier and oxidant system flow rates keeping constant their ratio.

With a view to establish the optimum conditions for the hydrolysis in the CL system, different concentrations of NaOH were tested (Table 2). As a result, a two-fold increase in the signal was found when Ce(IV)/HCl was employed; consequently, this oxidant system was chosen for further investigation. On the other hand, acetic acid and NaOH were considered as photoreaction medium in the PICL system but no substantial differences were found in the signals achieved. In order to optimize the irradiation time on the PICL system, both media were considered and, although similar outputs were found, NaOH 0.75 M, flowing at 1.3 mL $min^{-1}$ (irradiation time 46 s), was finally selected, bearing in mind the negligible blank and lower irradiation time required.

**Table 2.** Optimization: ranges studied and optimal values.

| Variable | CL system | | PICL system | |
|---|---|---|---|---|
| | **Range assayed** | **Optimum** | **Range assayed** | **Optimum** |
| **Oxidant concentration (M)** | | | | |
| $KMnO_4$ | $2\cdot10^{-4}$-$1.4\cdot10^{-3}$ | $9\cdot10^{-4}$ | $2\cdot10^{-4}$-$1.4\cdot10^{-3}$ | $6\cdot10^{-4}$ |
| Ce(IV) | $4\cdot10^{-4}$-$8\cdot10^{-3}$ | $1.2\cdot10^{-3}$ | $4\cdot10^{-4}$-$8\cdot10^{-3}$ | $1.5\cdot10^{-3}$ |
| NBS[a] | $10^{-3}$-$5\cdot10^{-2}$ | $4\cdot10^{-3}$ | $10^{-3}$-$5\cdot10^{-2}$ | $4\cdot10^{-3}$ |
| **Oxidant medium** | | | | |
| With $KMnO_4$ | $H_2SO_4$, HCl, $HClO_4$, $HNO_3$, $H_3PO_4$ and $CH_3COOH$ | $HClO_4$ | $H_2SO_4$, HCl, $HClO_4$, $HNO_3$, $H_3PO_4$ and $CH_3COOH$ | $CH_3COOH$ |
| With Ce(IV) | $H_2SO_4$, HCl, $HClO_4$, $HNO_3$ and $CH_3COOH$ | HCl | $H_2SO_4$, HCl, $HClO_4$, $HNO_3$ and $CH_3COOH$ | $CH_3COOH$ |
| **Oxidant medium concentration (M)** | | | | |
| With $KMnO_4$ | $[HClO_4]$:0.5-6.0 | $[HClO_4]$=6.0 | $[CH_3COOH]$:0.25-6.0 | $[CH_3COOH]$=4.0 |
| With Ce(IV) | [HCl]:0.5-3.0 | [HCl]=1.5 | $[CH_3COOH]$:0.5-6.0 | $[CH_3COOH]$=4.0 |
| **Flow-rate in the detector (mL $min^{-1}$)** | | | | |
| With $KMnO_4$ | 6.7-14.3 | 13.6 | - | - |
| With Ce(IV) | 6.7-17.2 | 14.5 | 6.6-17.7 | 16.2 |
| **Hydrolysis or irradiation media concentration (M)** | | | | |
| Acetic acid | - | - | 0.1-3.0 | 1.0 |
| NaOH [with Ce(IV)] | 0.1-0.5 | 0.17 | 0.1-2.0 | 0.75 |
| NaOH [with $KMnO_4$] | 0.1-5.0 | 3.0 | - | - |
| **Irradiation time (s)** | | | | |
| With acetic acid 1.0 M | - | - | 38-134 | 67 |
| With NaOH 0.75 M | - | - | 38-67 | 46 |
| **Insertion volume (μL)** | | | | |
| | 407-860 | 659 | 508-1111 | 809 |
| **Temperature (ºC)** | | | | |
| | - | Room temperature | 21-60 | Room temperature |

[a] NBS: N-bromosuccinimide

*3.2.1 Sensitizers and organized media*

A wide variety of compounds described in the literature as potential CL enhancers were examined [23]. Some of these compounds act as a promoters of photodegradation step; others generating or stabilising free radicals; others can provide organized media and structural rigidity to the medium, which increases the lifetime of the emitting species; finally, some of them, act as sensitizers enhancing the emission intensity due to the energy transfer.

To study the effect of these substances on the CL and PICL systems, the manifolds were modified being the configurations employed as the basic lines showed in figures 2a and 2b respectively, but with an additional Y-shaped piece into channel

$Q_2'$ in order to study the effect of the studied substances both, in the photoreaction and in the CL step.

The following substances were tested: ethanol, acetone, acetonitrile, a mixture of acetonitrile and acetone, 2-propanol, 1,4-dioxane, formic acid, sodium sulfite, quinine, 8-hydroxyquinoline, fluorescein, eosin yellowish and rhodamine B, $\beta$-cyclodextrin, sodium dodecyl sulfate, hexadecyltrimethylammonium bromide, Triton X-100 and hexadecylpyridinium chloride (HPC).

In the CL system, HPC 0.25% provided an increase in the signal (1059%) much higher than that of other sensitizers. Consequently, the effect of the concentration of HPC (0.15 - 0.65%) on 0.2 mg $L^{-1}$ of dimethoate was tested. As a result, a signal of 36.7 kHz was registered for 0.5% of HPC, whereas without sensitizer only 0.57 kHz for a concentration 10 times higher of dimethoate was found. Consequently it was decided to work with this surfactant. On the other hand, 1.6 mL $min^{-1}$ for sample and 0.8 mL $min^{-1}$ for both, NaOH and HPC, were chosen for further work, in order to avoid a low throughput, despite the signal increase observed for low flow rates.

With regard to PICL system, 0.25% HPC, $10^{-4}$ M quinine, $10^{-4}$ M riboflavin and 30% propanol provided the best results, with increases in PICL signal of: 380, 601, 454 and 192%, respectively. HPC provided better results when it was introduced after the lamp, which could mean that the sensitizing effect took place on the CL reaction more than on the photoreaction. The following ranges of concentrations (dimethoate 1 mg $L^{-1}$) were tested (optimal values between brackets): 0.15-1.0% HPC (0.35%); $10^{-5}$-$4\cdot10^{-4}$ M quinine ($4\cdot10^{-5}$ M); $10^{-5}$-$1.5\cdot10^{-4}$ M riboflavin ($5\cdot10^{-5}$ M); 15-40% 2-propanol (20%). Several combinations between these substances were also assayed, but no improvements were observed. The best results are presented in figure 3. As can be observed, after subtracting the blank the obtained signals were very similar. Bearing in mind the high blanks yielded by quinine and riboflavin, 0.35% of HPC was finally selected. The results showed a very high increase in the intensity emissions (about 11-fold) when HPC was employed.

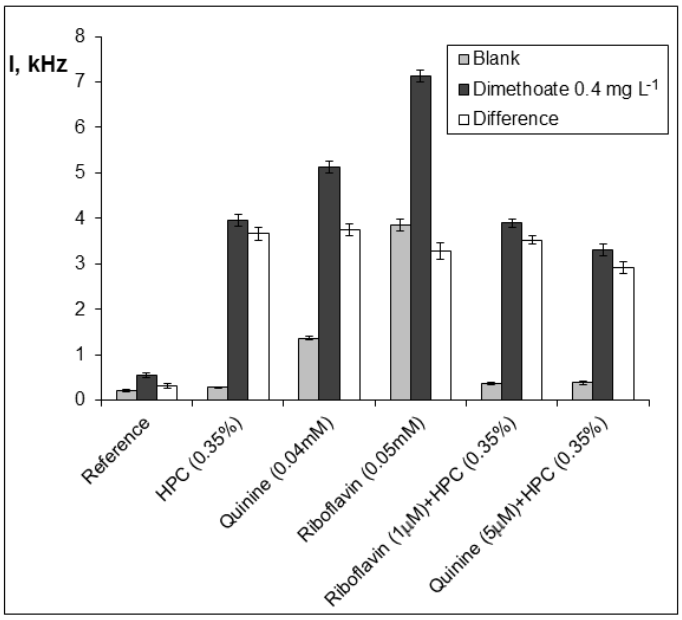


**Figure 3.** Effect of some sensitizers on the PICL of dimethoate 0.4 mg $L^{-1}$. HPC: hexadecylpyridinium chloride; Uncertainty (±2SD ) is indicated by error bars.

Although a very high enhancing effect was observed in both, CL and PICL systems, when HPC was employed, it was particularly market in the absence of irradiation. It could mean that the sensitizing effect took place more on the hydrolysis than on the photoproducts.

*3.2.2 Effect of temperature and re-optimization of the main variables*

In order to study the effect of the temperature in the determination of dimethoate, the sample loop and two 1.5 m sections of teflon tube (0.8 mm i.d.), corresponding to the carrier (located immediately prior to the injection valve, to minimize sample dispersion) and to the oxidant channel, were submerged in a water bath. System outputs fell quickly when temperature increased in the studied range (see Table 2); consequently, room temperature was selected.

Finally, the main variables (concentrations and flow rates) were re-optimized studying values around the previously selected. The optimal conditions are showed in the caption of Figure 2.

*3.3. Analytical characteristics*

Two different linear ranges were found for the CL system, whereas a double-logarithmic relationship fitted better the relation between the CL intensity and the concentration in the PICL system. Table 3 shows the ranges and equations average for five calibrations obtained on different days with fresh solutions.

**Table 3.** Analytical figures of merit.

| | **CL system** | **PICL system** |
|---|---|---|
| Application range (μg $L^{-1}$) | (1) 0.5 – 100<br>(2) 100 - 800 | 50-1000 |
| Equation (n=5)<br>I: Intensity (kHz);<br>C: concentration (μg $L^{-1}$) | (1) I=(0.269±0.012)·C-(0.20±0.07)<br>$r^2$=0.9992<br>(2) I=(0.372±0.017)·C-(17±3)<br>$r^2$=0.9975 | log I=(1.71±0.02)·logC - (3.62±0.10)<br>$r^2$=0.9967 |
| Limit of detection (μg $L^{-1}$) | 0.05 | 10 |
| Reproducibility (interday, n=5) | (1) 4.5%<br>(2) 4.6% | 1.2% |
| Repeatability (intraday, n=21) | (1) 2.8% (10 μg $L^{-1}$)<br>(2) 1.5% (400 μg $L^{-1}$) | 3.8% (200 μg $L^{-1}$) |
| Throughput | 120 $h^{-1}$ | 105 $h^{-1}$ |

The detection limit (LOD) was experimentally determined. It was defined as the lowest dimethoate concentration giving a signal equal to or greater than the blank peak plus three times its standard deviation (SD). The inter-day reproducibility of the proposed method was determined from the above-mentioned series of 5 calibrations and the relative SD was below 5% for both systems, as it is shown in Table 3. The intra-day repeatability was determined as the relative SD of a series of 21 injections of dimethoate. The throughput, calculated from the same series of peaks, was 120 and 105 $h^{-1}$ in CL and PICL systems respectively.

The interfering effect of the ions commonly present in natural waters at relevant concentrations was investigated in order to compare the tolerance of the proposed methods. The relative errors showed in Table 4 were obtained using a pure dimethoate solution (200 and 10 μg $L^{-1}$ for PICL and CL systems respectively) as reference. The effect of other pesticides from different chemical groups [24] was also studied. As can be observed in Table 5, the CL system exhibited a higher selectivity against ionic species and other pesticides. Other organothiophosphate (chlorpyriphos, malathion, methidathion and omethoate) and the carbamate methomyl showed the strongest interfering effect, as they provided a CL signal themselves. This fact makes the

proposed method, if coupled with a separative technique as LC, highly promising for the simultaneous determination of these substances with a high sensitivity and selectivity.

**Table 4.** Study of ionic interfering compounds, in the presence of 200 and 10 μg $L^{-1}$ of dimethoate, for PICL and CL systems respectively.

| Interference | CL system | | PICL system | |
|---|---|---|---|---|
| | Concentration (mg $L^{-1}$) | Error (%) | Concentration (mg $L^{-1}$) | Error (%) |
| $Na^+$ | 1000 [a] | -1.2 | 500 | +5.0 |
| $Cl^-$ | 1540[a] | -1.2 | 770 | +5.0 |
| $K^+$ | 1000[a] | -3.4 | 800 | +5.3 |
| $Ca^{2+}$ | 600 | -2.9 | 250 | +4.4 |
| $Mg^{2+}$ | 10 | +4.5 | 100[a] | -4.7 |
| $NH_4^+$ | 100[a] | -2.6 | 0.2 | -5.1 |
| $SO_4^{2-}$ | 1000[a] | -5.0 | 1000[a] | +0.8 |
| $CH_3COO^-$ | 100[a] | +0.9 | 15 | -4.0 |
| $H_2PO_4^-$ | 10 | +4.9 | 60 | -2.6 |
| $HCO_3^-$ | 500[a] | +1.3 | 500[a] | -3.3 |
| $NO_2^-$ | 1.5 | +2.6 | 0.2 | -4.8 |
| $NO_3^-$ | 100[a] | -4.3 | 0.9 | -1.1 |
| Urea | 15 | +4.6 | 35 | -4.9 |

[a] Maximum assayed concentration

**Table 5.** Effect of other pesticides in the PICL and CL signals, in the presence of 200 or 10 μg $L^{-1}$ of dimethoate respectively.

| Interference | Chemical group | CL system | | | PICL system | | |
|---|---|---|---|---|---|---|---|
| | | Conc.(μg $L^{-1}$) | I/D[b] | Error(%) | Conc.(μg $L^{-1}$) | I/D[b] | Error(%) |
| Amitrole | Triazole | 1000[a] | 100 | +3.8 | 30 | 0.15 | -2.5 |
| Cyromazine | Triazine | 1000[a] | 100 | +4.9 | 1000[a] | 5 | -2.4 |
| 2,4-D | Alkylchlorophenoxy | 600 | 60 | +1.1 | 400 | 2 | +5.0 |
| Diphenamid | Alkanamide | 1000[a] | 100 | +1.6 | 1000[a] | 5 | -1.5 |
| Diquat monohydrate | Bipyridylium | 800 | 80 | +3.5 | 400 | 2 | +5.0 |
| Diuron | Phenylurea | 600 | 60 | +2.0 | 1000[a] | 5 | +0.9 |
| Glyphosate | Phosphonoglycine | 1000[a] | 100 | -2.0 | 1000[a] | 5 | -2.6 |
| Imazalil | Imidazole | 1000[a] | 100 | -0.1 | 1000[a] | 5 | +4.8 |
| MCPA | Aryloxyalkanoic acid | 1000[a] | 100 | -3.0 | 1000[a] | 5 | +5.0 |
| Metalaxyl | Phenylamide | 1000[a] | 100 | -0.3 | 600 | 3 | -0.4 |
| Metazachlor | Chloroacetamide | 1000[a] | 100 | -2.5 | 600 | 3 | +1.5 |
| Methomyl | Carbamate | 5 | 0.5 | +4.7 | 50 | 0.25 | +4.2 |
| Pirimicarb | Carbamate | 1000[a] | 100 | +1.5 | 400 | 2 | -2.5 |
| Quinmerac | Quinoline | 500 | 50 | +2.6 | 300 | 1.5 | -2.0 |
| Thiacloprid | Neonicotinoid | 1000[a] | 100 | +3.6 | 20 | 0.1 | +4.8 |
| ***Organophosphate pesticides*** | | | | | | | |
| Chlorpyrifos | | 3.5 | 0.35 | +2.8 | Saturated | - | -4.9 |
| Fenamiphos | | 60 | 6 | +5.0 | 100 | 0.5 | +5.0 |
| Malathion | | 1 | 0.1 | +2.9 | 200 | 1 | +2.1 |
| Methidathion | | 1 | 0.1 | +4.8 | 100 | 0.5 | +4.7 |
| Omethoate | | 1 | 0.1 | +4.9 | 100 | 0.5 | +1.1 |

[a] Maximum assayed concentration

[b] I/D= relation [interference]/[dimethoate]

In summary, as a result of the great sensitizing effect of HPC on the CL signal of dimethoate, a much more sensitive system was obtained in the absence of previous irradiation (CL-system). That system resulted also to be more selective than the PICL system. Consequently, it is recommended to carry out the determination of this pesticide using its native CL rather than its photoinduced CL.

### *3.4. Development of the SPE method*

The use of solid phase extraction (SPE) was studied in order to improve the sensitivity and selectivity of the method. The commonly organic solvents employed for elution in SPE used to affect negatively CL reactions. Hence, in order to avoid the tedious procedures for their elimination, preliminary investigation was carried out to check the effect of these substances on the CL response of dimethoate. Calibration graphs were obtained using 10 and 20% of methanol, ethanol and acetonitrile. The variation found for the slopes were: -63.3 and -71.8 for methanol; -21.4 and -38.3 for ethanol; +8.0 and +9.7% for acetonitrile. Hence, acetonitrile was chosen to carry out the elution.

Three different cartridges were tested: Bond Elut-$C_{18}$, and Bond Elut-ENV 200 mg from Varian, and Chromabond 500 mg from Macherey-Nagel. SPE of six solutions of 50 mL of dimethoate between 0.1 and 12 μg $L^{-1}$ was carried out with the three cartridges according with the procedure described in section 2.3. Chromabond provided very small recoveries (below 30%) for concentrations of dimethoate above 3 μg $L^{-1}$, whereas with Bond Elut-ENV recoveries between 190-390% were achieved; it was also observed more than a two-fold increase in the blank signal. However, $C_{18}$ cartridges provided very good recoveries within the whole range of concentrations tested, with a mean value of (102±10)%; this cartridge was therefore selected.

To study the effectiveness of the SPE strategy, the procedure was applied to samples containing 2 μg $L^{-1}$ of dimethoate together with the substances that showed the strongest interfering effect. The highest concentrations initially tested were assayed, namely 1000 mg $L^{-1}$ of $Ca^{2+}$, 500 mg $L^{-1}$ of $H_2PO_4^-$, 200 mg $L^{-1}$ of $Mg^{2+}$ and urea. Nitrite was not included in the study since it is present in natural waters at lower concentrations than those that interfere due to its low stability. Chlorpyrifos with a concentration corresponding to the relation of concentrations present in the commercial formulations from Spain [25], which also is higher than the commonly used in insect control studies [26], was also tested. The results proved that SPE allowed the successful removal of these species since the errors were, in all cases, below 5%.

*3.5. Application to water samples*

Finally, the proposed SPE-FIA method was applied to the determination of dimethoate in water samples. With a view to test a variety of matrixes, samples from different origins were chosen. Hence, waters with high (irrigation water) and low (mineral, ground and tap waters) organic matter concentration were tested. On the other hand, they were also collected in different locations in which the chemical composition used to be significantly different. Hence waters collected in Valencia region (tap and irrigation waters) had a high concentration in ionic species, particularly calcium and magnesium. As shown in Table 6, recovery factors ranging from 95% to 108% were achieved for samples spiked in triplicate with dimethoate at three different concentrations (0.2, 0.5 and 1.0 μg $L^{-1}$). Bearing in mind that the acceptable range for recoveries in water samples is usually set between 70 and 110%, with a maximum permitted RSD of 20% [27], it can be considered that the analytical performance of the proposed CL method was successful.

**Table 6.** Recoveries of dimethoate in water samples.

| **Sample** | **Location** | **0.2 μg $L^{-1}$** | **0.5 μg $L^{-1}$** | **1.0 μg $L^{-1}$** | **Recovery[a]** |
|---|---|---|---|---|---|
| Mineral water | Segovia | (100±8) | (96±5) | (98±3) | (98±5) |
| Ground water | Huesca | (94±9) | (113±15) | (113±3) | (107±13) |
| Tap water | Gandía | (106±10) | (90±11) | (88±7) | (95±12) |
| Spring water | Zaragoza | (93±14) | (97±7) | (108±2) | (99±11) |
| Irrigation water | Gandía | (115±22) | (100±7) | (107.7±0.5) | (108±13) |

[a] Average of the three concentrations of dimethoate tested

## 4. Conclusions

Two methods have been developed for the determination of dimethoate. One of them was based on the CL generated by the hydrolysis products of the pesticide and the other on the PICL generated by UV light.

A great enhancing effect on the CL system was observed in presence of HPC. Consequently, the CL method resulted to be much more sensitive than the PICL method, providing a detection limit of 0.05 ng $mL^{-1}$, which is under the maximum permitted concentration, established by European Community, of 0.1 μg $L^{-1}$ for individual pesticides and of 0.5 μg $L^{-1}$ for total pesticides in drinking water [28] and 1–3 μg $L^{-1}$ in surface water [29].

On the other hand, the proposed system is competitive with most of developed methods for the determination of dimethoate in waters. Only some chromatographic methods coupled with MS provided a smaller LOD [30-32]. With regard to the only FI method proposed for determination of dimethoate, based on ESI-MS/MS [8], it exhibited a significantly higher LOD (0.12 and 0.78 mg $L^{-1}$ for plasma and urine matrix, respectively) than that reported in the present method.

The FIA-CL system showed also a high selectivity against ionic species and most of the other pesticides tested. Additionally, its analytical performance was significantly improved using SPE with $C_{18}$ cartridges.

In the other hand, in the FIA-PICL strategy, the light increased the number of pesticides which exhibited CL and consequently the potential interfering species.

In conclusion, it is recommended to carry out the determination of dimethoate using its native CL rather than its photoinduced CL, since a much more sensitive and selective system was obtained in the absence of previous irradiation. In addition, the reported method, coupled with an appropriate separative technique, as LC, can be highly promising for the determination of other organothiophosphate pesticides.

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