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FI-On line Chemiluminescence Reaction for Determination of MCPA in Water Samples

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Abstract

This paper reports an economic, simple and rapid FI-CL method for the determination of MCPA. This method requires simple instrumentation and it is fast enough to be used in routine analyses. Chemiluminescence signal is generated by reaction between photodegraded MCPA and ferricyanide solution in alkaline medium. All physical and chemical parameters in the flow injection chemiluminescence system were optimized in the experimental setting. To eliminate the interferences a solid phase extraction stage with SDB-1 cartridges and ethanol elution is applied. The signal-MCPA concentration relation is linear in concentration intervals between 0.0015 $\mu g \cdot mL^{-1}$ and 0.6 $\mu g \cdot mL^{-1}$. The calibration lines are statistically similar in different working conditions: standards with ethanol without extraction and standards with ethanol and extraction, allowing standards to be excluded from the extraction step, which simplifies the process. The detection limit (DL) is 0.5 $ng \cdot mL^{-1}$, which is the same order as the maximum limit established in legislation regarding pesticide limits in water destined for human consumption. A DL of 0.13 $ng \cdot mL^{-1}$ can be reached if a sample of 100 mL is preconcentrated. The interday variance coefficient is 3% and the sample throughput is 90 $hour^{-1}$. The water analysis method is efficient with relative error percentages lower than 5% with respect to the added concentration.

Keywords: MCPA, chemiluminescence, Flow Injection, solid-phase extraction, SDB-1, water samples, SPE

1 Introduction

MCPA (4-chloro-2-methylphenoxyacetic acid) is a systemic phenoxy herbicide used to control annual and perennial weeds in cereals, grasslands and trees. It is a Restricted Use Pesticide used extensively in Spanish agriculture due to its relatively low cost and high efficiency even at low concentrations. It is extensively used in lemmon-growing [1] and rice cultivation [2,3].

Due to its high solubility in water, it goes easily into surface or ground waters through natural drainage or infiltration [4]. In fact, it has been widely detected in ground water and surface water sources [3-9]. As a consequence of its toxicity, monitoring possible pesticide contamination in water is an essential task in environmental protection [10, 11]. Spanish legislation establishes in human consumption areas, a maximum total concentration of pesticides of 0.50 $ng \cdot mL^{-1}$ and a maximum of 0.10 $ng \cdot mL^{-1}$ for any single pesticide [12]. The European Union Water Framework Directive (EU-WFD) aims to achieve good status of the European surface waters and groundwater by 2015. In that framework, MCPA has been included in the study to assess the risk of 500 organic substances in four European river basins [13].

The standard procedure recommended for this substance by the AOAC (Official Methods of Analysis) involves the saponification of MCPA esters in situ and conversion of amine salts into a water-soluble potassium salt of MCPA. Then, ion suppression in reverse phase bonded in microparticulate column separates isomers and impurities. Ionic MCPA moiety is protonated by acidic mobile solvent, forming nonionic MCPA moiety, which greatly increases partitioning into stationary phase. Small changes in mobile solvent pH significantly affects retention time and MCPA is detected using HPCL with a pH 2.83 mobile phase with internal standard [14].

Nonetheless, in recent years many chromatographic methods have been developed, which are adapted to a mass detector to determine the MCPA. Thus, for example, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) [3, 5-9] is used to determine this pesticide in water samples, always performed after carrying out a solid phase extraction step (SPE) on-line or off-line. For MCPA extraction, columns with silica-bonded sorbent C18 [4,10] and divinylbenzene polymers (SDB and Oasis HLB) [6,9] have been used, achieving better results with polymeric resins [15,16].

Although it is obvious the advantage provided by chromatographic methods because of their very low detection limits for MCPA, it is important to note that the cost of the instrumental equipment is very high, and the time of analysis is usually very long, between 25 and 45 min per sample. Therefore, simple, inexpensive and fast methods are required for routine analysis of this polar herbicide.

Alternatives to chromatographic methods are ELISA and immunoassay methods. A great variety of simple immunoassay methods have been described for the chlorophenoxyacetic acid herbicide 2,4D [17, 18, 19, 20]. These methods are selective enough, being the cross-reactivity of MCPA between 0.3 and 13.8%. Moreover, in recent years static [21, 22] and flow injection methods [23, 24] have been described for the determination of chlorophenoxyacid herbicides with fluorescence detection that provides detection limits for MCPA in the range of 74 $\mu g \cdot mL^{-1}$.

In order to increase sensitivity of fluorescence methods, chemiluminescence detection (CL) is an alternative that can be used in the determination of different compounds in a great variety of matrices. This detection technique allows the determination of compounds which do not exhibit native chemiluminescence if they, or their fragments obtained after

photolysis, participate in the chemiluminescence reaction as precursors, catalysts, inhibitors, oxidants, etc. Moreover, chemiluminescence provides wide dynamic range with cheap and simple instrumentation. Recently, the coupling of Flow Injection Analysis (FIA) techniques and CL detection has been used to carry out the determination of several pesticides with low limits of detection, short time analysis and high throughputs [25-32]. Up to now, to the author's knowledge no FI-CL method has been reported for MCPA.

This paper reports an economic and novel FI-CL method for the determination of MCPA. This method requires simple instrumentation and it is fast enough to be used in routine analysis. Chemiluminescence signal is generated by reaction between photodegraded MCPA and ferricyanide solution in alkaline medium. The method has been successfully applied to the determination of MPCA in water samples, after solid phase extraction (SPE) with SDB-1 columns.

2 Experimental

2.1 Reagents

All experiments were carried out by using analytical reagent grade chemicals and Milli-Q water (Millipore, Bedford, MA, USA).

The reagents used were as follows: MCPA, 2,4-D, H_2SO_4, H_2O_2, ethanol, and acetonitrile (Merck); $KMnO_4$, $K_3Fe(CN)_6$, NaCl , H_3PO_4, acetone, and Triton X-100 (Panreac); HNO_3, $HClO_4$, KIO_4, and $CeSO_4{\cdot}4H_2O$ (Scharlau); NaOH, HCl, and acetic acid (J.T. Baker).

The following salts were used for testing the potential inorganic interferences of cations: chloride salts of Na^+ and K^+ (Panreac), Ca^{2+} (Probus), Mg^{2+} (Prolabo), Zn^{2+} (Scharlau), Mn^{2+} (D'Hernio), sulfate salt of Cu^{2+} (Probus) and nitrate salt of Pb^{2+} (Probus). For testing the potential interference of anions, sodium salts of NO_2^- (Probus), SO_4^{2-}(Panreac) and HCO_3^- (Guinama) and potassium salts of $H_2PO_4^-$ (Panreac) and NO_3^-(Probus) were used.

As enhancer were tested 8-hydroxyquinoline, Rodamine B (Merck), dioxane (Scharlau); quinine hydrochloride, acridine orange (Sigma); sodium sulfite anhydrous (Panreac); *beta*-cylodextrin, sodium dodecyl sulfate, and hexadecylpyridinium chloride (Fluka).

2.2 Apparatus

The flow injection manifold is shown in fig. 1. It consisted of PTFE coil of 0.8 mm i.d., a Gilson (Worthington, OH, USA) minipuls peristaltic pump provided with pump tubing from Omnifit, and a Model 161T031 valve (NRearch, Horthboro, MA, USA). The flow cell, in the chemiluminescence detector, was a flat-spiral quartz tube of 1 mm i.d. and 3 cm total diameter backed by a mirror for maximum light collection. The photodetector package was a P30CWAD5 type 9125B photomultiplier tube supplied by Electron Tubes (Uxbridge, United Kingdom); it was located in a laboratory-made light-tight box to avoid light input. The output was fed to a computer equipped with a counter-time, also supplied by Electron Tubes. In order to photodegradate the MCPA sample, a photoreactor is used. It consisted of a

400 cm length and 0.8 mm i.d. PTFE tubing helically coiled around a 15 W low-pressure mercury lamp (Sylvania, Raunheim, Germany) from germicidal use.

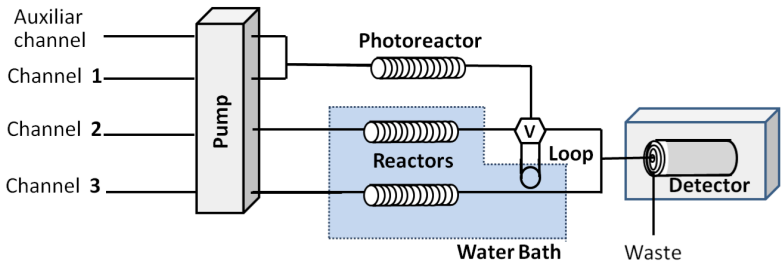

Fig. 1 Flow injection chemiluminescence manifold. Channel 1: sample stream; channel 2: carrier stream; channel 3: oxidant stream; V: injection valve. The auxiliar channel was only used during the optimization procedure

2.3 Flow injection procedure

In the final FI-CL assembly (fig. 1) both, standard and sample, flow at 3.68 mL·min^{-1} along the photoreactor through channel 1. In the photoreactor, MCPA is degraded obtaining some photofragments that can later react with the oxidant and generate the chemiluminescence signal.

After crossing the photoreactor, a carrier of Milli-Q water (channel 2), flowing at 5.74 mL·min^{-1}, collects the photodegraded standard or sample from the injection valve (V), which has a loop of 1000 mL. Finally, the oxidant stream (channel 3), an 8×10^{-4} M ferricyanide in 0.5 M solution flowing at 3.11 mL·min^{-1}, merges with the carrier stream just before the detector. The oxidant, the carrier and the loop were introduced in a water bath at 70 ºC.

2.4 Standard preparation

Standard solutions of 100 µg·mL^{-1} of MCPA were prepared in water and stored a 5ºC. This solution remained stable for at least one month.

The MCPA standard solutions were prepared with and without ethanol. The MCPA standard solutions in presence of ethanol were prepared by mixing 1 mL of ethanol with variable amount of MCPA stock standard solution (100 µg·mL^{-1}) and diluting up to 25 mL with Milli-Q water. The MCPA standard solutions without ethanol were prepared with variable amount of MCPA stock standard solution (100 µg·mL^{-1}) and diluting up to 25 mL with Milli-Q water.

2.5 Sample pretreatment

In general, solid-phase extraction (SPE) is the most commonly used sample pretreatment methods for the isolation and/or enrichment of pesticides. In this work, solid phase extraction with SDB-1 (Bakerbond SPE SDB 200 mg, J.T.Baker, The Netherlands) has been applied to water samples with the aim of avoiding interferences.

To carry out the extraction of MCPA in C18 columns cartridges, standards were prepared and were acidified with 0.1 M HCl, to give them a pH lower than 3. The columns were conditioned with 5 mL of methanol and 10 mL water, the solution was passed, the column was washed with 5 mL of water and dried with air for 5 minutes before eluting the pesticide with 1 mL of acetonitrile. Finally, the extract was brought to a volume of 25 mL with Milli-Q water.

To carry out the extraction of MCPA in SDB-1 cartridges, the cartridges were conditioned with 5 ml of methanol and 10 mL of water. Then, 25 mL of standard or water sample were transferred through the cartridge, which was further washed with 5 mL of Mili-Q water and dried under vacuum for 5 minutes. The analyte was eluted by adding 2 ml 50% ethanol solution. The eluate was diluted up to 25 mL with Mili-Q water prior to FI-CL analysis.

2.6 Sample preparation

Water samples from different sources were analyzed: well water from an inner region of Spain (Miguel Esteban, Toledo), well water from a coastal area (Castellón), well water from an inner region of Valencia (Villamarchante), water from the river Lucena (Castellón), water from the river Xuquer (Alzira, Valencia) and water from the river Bohilgues (Ademuz, Valencia).

Water samples were collected in plastic flasks and filtered in the lab with polyamide membrane filters of 0.45 mm to remove the suspended soil matter and stored in glass flasks protected from light at 4 ^{o}C in the refrigerator. Prior to analysis, water samples were spiked with at 0.3 $mg \times mL^{-1}$ MCPA concentration levels.

3 Results and discussion

3.1 Optimization of the flow system

This study was carried out at room temperature using the flow assembly depicted in fig. 1 and the auxiliary channel was used when it was necessary. A 10 μg·mL^{-1} MCPA solution was used.

Preliminary study

A preliminary study was carried out to determine whether the MCPA when oxidised with permanganate ($7 \cdot 10^{-4}$ M in 2M H_2SO_4) presented chemiluminescence in the presence of or in the absence of light, and what the nature of the photodegradation medium should be. To do this, the assembly shown in fig. 1 was used: a 10 μg·mL^{-1} MCPA solution (channel 1) was mixed with 0.1M H_2SO_4 solution, 0.1M NaOH solution or water (auxiliary channel). Drug insertions were performed with and without previous irradiation in order to study the induced and natural chemiluminescence (CL) of MCPA, respectively. Only when the lamp was on chemiluminescence was detected (induced chemiluminescence) and the best signal was obtained with the basic photodegradation media.

Oxidant selection

In order to select the best oxidant to obtaine chemiluminescence from MCPA, different oxidants usually employed in direct CL methods were tested. The oxidants used were $KMnO_4$ in a concentration range between $5 \cdot 10^{-5}$ and $2 \cdot 10^{-3}$ M,

in 1.8 M H_2SO_4; Ce(*IV*) in a concentration range between $1{\cdot}10^{-5}$ to $1{\cdot}10^{-3}$ M, in 1.8M H_2SO_4; $K_3Fe(CN)_6$ in a concentration range between $5{\cdot}10^{-5}$ and $2{\cdot}10^{-3}$ M, in 0.5 M NaOH; KIO_4 in a concentration range between $1{\cdot}10^{-5}$ and $5{\cdot}10^{-3}$ M, in 1.8 M H_2SO_4; KIO_4 in a concentration range between $1{\cdot}10^{-5}$ and $5{\cdot}10^{-3}$ M, in 1.8 M H_2SO_4 and $2{\cdot}10^{-5}$ M Ag^+; and H_2O_2 in a concentration range between $1{\cdot}10^{-3}$ to $8{\cdot}10^{-3}$ M, in NaOH 1 M.

The obtained results are shown in fig.2. Chemiluminescence signal was only detected with $KMnO_4$, Ce(*IV*) and $K_3Fe(CN)_6$. $K_3Fe(CN)_6$ provided a much higher CL-signal than $KMnO_4$ and Ce(*IV*) over the whole range of concentration tested, and the best result was obtained with a $8{\cdot}10^{-4}$ M $K_3Fe(CN)_6$ solution. Therefore, $K_3Fe(CN)_6$ $8{\cdot}10^{-4}$ M was selected as the oxidant.

Given that this oxidant requires a basic medium, several concentrations of NaOH (0.1M, 0.3M, 0.5M, 0.6M y 1M) were tried. Only a concentration with 0.5 M NaOH showed a slightly higher value, which led to this concentration being selected. Then, $K_3Fe(CN)_6$ $8{\cdot}10^{-4}$ M in NaOH 0.5M was selected for further research as the oxidant solution.

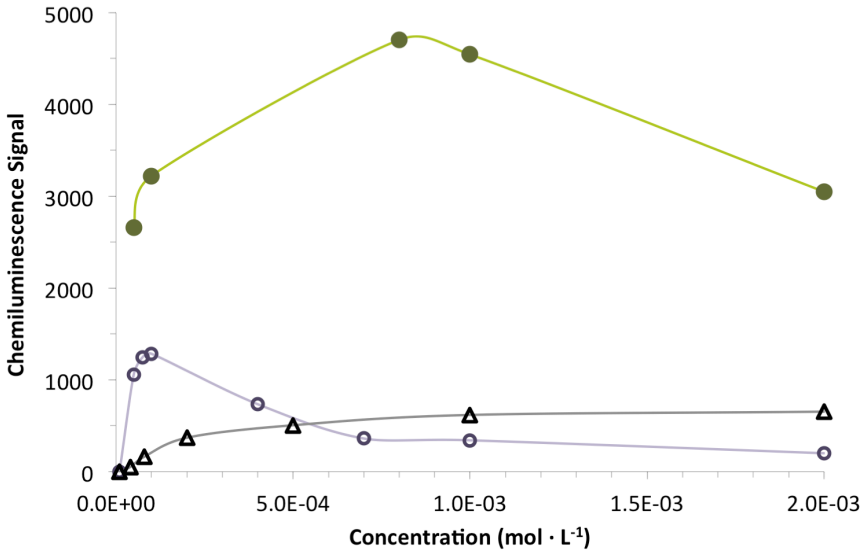

Fig. 2 Chemiluminescence signal for three oxidants. (o) Permanganate, (▵)Cerium and (•)Ferricyanide. Lines are just for visual aid.

Photodegradation medium

A study was carried out to determine the most appropriate degradation medium, which was passed through the auxiliary channel as in the assembly in fig. 1. Water, 0.1M solution, 0.1 M, H_2SO_4 solution, glycerin buffer solutions (pH 2.5, 3.5 8.6 and 9.6), phosphate buffer solutions (pH 6, 7 and 8) and acetate buffer solutions (pH 4 and 5) were tested. The best results were obtained from the solutions with a pH between 4 and 10, even though they were always inferior to those achieved with distilled water as the photodegradation medium. Therefore, in view of the results, distilled water was selected as a medium of photodegradation

Given the experiences as a whole, it could be deduced that the presence of ions affects the chemiluminescence signal, tested later in the interferences study (Section 3.2)

Effects of chemiluminescence enhancers and organized media

There is a group of substances that, in some cases, can improve the chemiluminescence signal, such as the so-called enhancers and organized media. The following compounds were studied: $0.01 \cdot 10^{-3}$ M fluoresceine in 10^{-4} M NaOH, $0.1 \cdot 10^{-3}$ M rhodamine B, $0.1 \cdot 10^{-3}$ M quinine, $0.1 \cdot 10^{-3}$ M eosin, $0.1 \cdot 10^{-3}$ M riboflavin, 0.5% formic acid, 1.2% β-cyclodextrins, 1.2% SDS, 0.6% triton X-100, 0.14% CTAB, 20% ethanol, 20% acetonitrile. The influence of these compounds in the photodegradation process and the chemiluminescence stage was studied.

In order to study the influence of the selected compounds in the chemiluminescence process, these compounds were passed through a carrier channel (channel 2, fig. 1). This resulted in an increase in the chemiluminescence signal only when quinine, cyclodextrin and riboflavin were used, but in all cases the signal increased by less than 10%. No significant increase was observed either in the chemiluminescence signal, when using these 3 sensitizers at different concentration levels (riboflavine between $1 \cdot 10^{-3}$ M and $0.1 \cdot 10^{-3}$ M; β-cyclodextrin between 0.35% and 0.75%; and quinine between $0.01 \cdot 10^{-3}$ M and $0.5 \cdot 10^{-3}$ M), and therefore the use of sensitizers in the chemiluminescence process was rejected.

In order to study the influence of these compounds, added during the photodegradation stage, the assembly shown in fig. 1 was used. The blanks, in all cases, were high and unstable. Of all the compounds tested, only acetonitrile showed exaltation at around 15%. Nevertheless, having achieved such an irregular blank signal, repeatability of the method is highly affected, and therefore the use of this compound was discarded as enhancer.

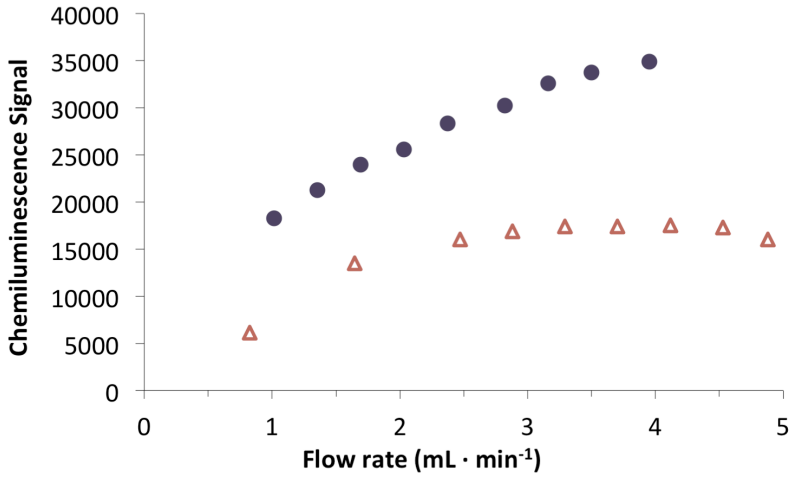

Fig. 3 Variation of the chemiluminescence signal with the flow rate: (▵)Photodegradation flow rate and (•) Oxidant flow rate

Optimization of FIA parameters: flow rates and sample volume

Next, the photodegradation flow rate, the oxidant flow rate, the carrier flow rate and the sample volume were optimized.

With the optimization of the photodegradation flow rate, the time the sample is irradiated with the lamp for its photodegradation process is optimized. Research was done on a flow rate between 0.84 $mL \cdot min^{-1}$ and 4.88 $mL \cdot min^{-1}$, corresponding to an irradiation time between 25 and 143 *s*. A maximum signal for short irradiation times was observed at between 29 and 36 s; 33 s was chosen as optimum time for the photodegradation flow rate which corresponds to a flow rate of 3.68 $mL \cdot min^{-1}$. Fig. 3 (▵) shows the variation of the chemiluminescence signal with the variation of the photodegradation flow rate.

The influence of the oxidant flow rate and the carrier flow rate were studied together: the oxidant flow rate varied between 1.08 $mL \cdot min^{-1}$ and 3.77 $mL \cdot min^{-1}$ and the carrier's between 2.54 $mL \cdot min^{-1}$ and 6.33 $mL \cdot min^{-1}$. The chemiluminescence signal increased when both flow rates were increased. A flow of 3.11 $mL \cdot min^{-1}$ was selected for the oxidant and a flow of 5.74 $mL \cdot min^{-1}$ for the carrier. Fig. 3 (•) shows the variation of the chemiluminescence signal with the variation of the oxidant flow rate.

Samples between 510 mL and 1515 mL were tested and, in this interval, the sample volume had no noticeable influence on the signal. A sample volume of 1000 mL was selected due to the signal being slightly higher.

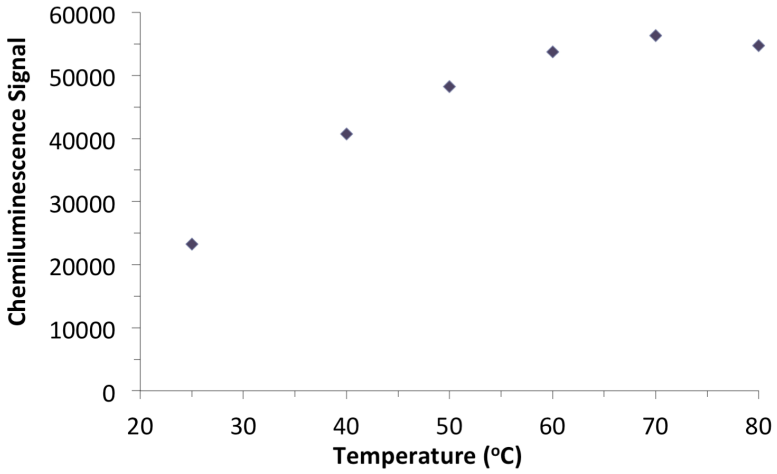

Fig. 4 Variation of the chemiluminescence signal with the temperature.

Temperature of the FI-CL system

The temperature could be an important parameter in the chemical processes which occur in the system, which is why experiments were carried out by introducing different reactors (fig.1) in a thermostatized bath, between 25 ºC y 80 ºC.

On one hand, it was observed that the temperature of the photodegradation flow did not influence the chemiluminescence signal, given that the sample was heated due to being exposed to UV radiation through the teflon tube during the 33 s.

Nevertheless, having placed the oxidant, the carrier and the sample loop in the water bath, the temperature became a significantly important parameter. As can be seen in fig. 4, the chemiluminescence signal increases as the temperature increases, with the maximum signal being between 60ºC y 80ºC, which is why 70ºC was chosen as the optimum temperature for the water bath.

Reoptimization

To conclude the research into optimum conditions for the FI-CL system to determine MCPA, the composition of the oxidizing solution was revised in short intervals around the value considered as optimum. To achieve this, different concentrations of oxidant at different MCPA concentration levels were tested (0.02 $\mu g \cdot mL^{-1}$, 0.2 $\mu g \cdot mL^{-1}$ and 2 $\mu g \cdot mL^{-1}$). These tests showed the optimum concentration of ferricyanide to be $8 \cdot 10^{-4}$ M in 0.5 M NaOH. On the other hand, it was observed that in all MCPA concentrations, this was the most appropriate concentration of ferricyanide, thus showing there is no significant dependency between oxidant and pesticide concentrations.

3.2 Study of interferences and their treatment

Interferences study

Research was done on how the presence of 2,4-D and common anions and cations influence in the MCPA chemiluminescence signal of water samples.

In order to study the influence of the presence of the pesticide 2,4-D in the chemiluminescent signal of the MCPA, standards of 0.1 $\mu g \cdot mL^{-1}$ MCPA in presence of 0.025, 0.05, 0.075 and 0.1 $\mu g \cdot mL^{-1}$ of 2,2-D were prepared.

These signals were compared to those obtained from a standard solution of 0.10 $\mu g \cdot mL^{-1}$ MCPA, and the percentage of relative error was calculated ($E_r(\%) = \frac{signal_{MCPA+Interferent} - signal_{MCPA}}{signal_{MCPA}} \times 100$). The percentage of relative error was less than 5% for concentrations of 2,4-D equal or lower than 0.05 ppm, so it can be concluded that there is no interference of 2.4-D in these conditions.

Solutions containing a MCPA concentration of 0.50 $\mu g \cdot mL^{-1}$, and each of the ions under study at a maximum concentration of 2000 $\mu g \cdot mL^{-1}$ were prepared. These signals were compared to those obtained from a standard solution of 0.50 $\mu g \cdot mL^{-1}$ MCPA. In those cases in which the relative error percentage was greater than 5%, the concentration of the interfering ion was reduced.

Table 1 shows a summary of the results obtained. Most of the ions did not interfere in the habitual water concentrations. However, some ions commonly found in water, such as Ca^{2+}, Mg^{2+} and CO_3^{2-}, interfered at concentrations of 20 $\mu g \cdot mL^{-1}$, 20 $\mu g \cdot mL^{-1}$ and 5 $\mu g \cdot mL^{-1}$, respectively.

Table 1. Study of potential interferents: maximum allowed concentrations and percent relative errors

Interferent	Maximun allowed concentration ($\mu g \cdot mL^{-1}$)	Relative error (%)
Ca^{2+}	20	1.4
Cd^{2+}	100	3.7
Co^{2+}	1	-2.2
Cr^{3+}	4	-1.8
Cu^{2+}	100	-1.0
Fe^{3+}	0.32	-3.7
K^{+}	400	0.3
Mg^{2+}	20	3.5
Na^{+}	1500	0.8
NH_4^{+}	2000	-1.3
Ni^{2+}	0.08	0.3
Pb^{2+}	20	1.6
Zn^{2+}	800	0.4
CN^{-}	5	2.6
Cl^{-}	2000	3.8
CH_3COO^{-}	2000	1.4
CO_3^{2-}	5	4.1
HCO_3^{-}	2000	1.3
I^{-}	0.08	2.4
$H_2PO_4^{-}$	2000	1.7
NO_3^{-}	5	1.1
NO_2^{-}	5	0.6
SO_4^{2-}	2000	2.1

Therefore, it is necessary to implement a treatment strategy that permits elimination of ionic interferences.

Sample pretreatment

Different sample treatment strategies were tested in order to select the most simple and which would give the best results in determining the pesticide: standard addition method, use of ionic interchange resins (*Duolite C206A* and *Amberlite IRA-400*) and solid phase extraction (SPE) with different fillings (C18 and SDB-1). Complete elimination of interferences was only possible with SPE.

Two types of habitual SPE cartridges were studied in the MCPA extraction [15,16]: C18 cartridges and SDB-1 cartriges, specific to polar compound extraction. In both cases the elution step was optimized.

In the elution step with C18, 2.5 mL of different solvents were tested: acetonitrile, ethanol and methanol. The recuperation percentages of a standard solution of 0.3 $\mu g \cdot mL^{-1}$ MCPA with SPE, compared with those prepared with the same final concentration of solvent, were 102.5% with ethanol, 95% with acetonitrile and 84% with methanol.

On the other hand, the presence of these solvents reduced the chemiluminescence signal (CS) compared with a standards solution in absent of solvent. Specifically, for a solution of 10% solvent, the reduction of the CS was 35% with acetronitrile, 54% with ethanol and 93% with methanol. As the acetronitrile had a suitable recuperation value and a lower loss of signal, the necessary amount of acetronitrile for the extraction was optimized, being 1 mL as the optimum volume.

In the elution step with SDB-1 different eluents were tested: acetronitrile, ethanol and mixtures. Table 2 shows recovery values obtained in each of the elution conditions tested for a standard of 0.3 $\mu g \cdot mL^{-1}$ MCPA. The best results were obtained from a mixture of ethanol-water at 50% (recovery 100.3%), which is why this composition was chosen for the MCPA elution.

As has been shown, both columns were suitable for MCPA extraction. However, after applying it to real samples (section 3.4), columns SDB-1 were selected to determine MCPA.

Table 2 MCPA recovery with a SDB-1 SPE cartridge and different solvents

Extractant solution	**Recovery (%)**
1 mL 100% Acetonitrile	< 30%
1.5 mL 100% Acetonitrile	< 30%
1 mL 50% Acetonitrile	90 %
2 mL 50% Acetonitrile	93 %
2 mL 100% Ethanol	52%
2 mL 50% Ethanol	100%

3.3 Analytical characteristics

The signal-MCPA concentration relation is linear in a wide interval of concentrations. To obtain the analytical parameters, a range of concentrations between 0.0015 $\mu g \cdot mL^{-1}$ and 0.6 $\mu g \cdot mL^{-1}$ was selected.

Table 3 shows the analytical parameters obtained in different calibration conditions: without extraction (Condition 1), without extraction with 4% ethanol (Condition 2) and with extraction by means of an SDB-1 column (Condition 3).

When working without extraction and without ethanol (Condition 1, table 3) the detection limit (DL), calculated based on the standard deviation of the blank ($3s_{blank}/slope$), was 0.2 $ng \cdot mL^{-1}$ and the quantification limit (QL) was 0.7 $ng \cdot mL^{-1}$. With a solution of 0.02 $\mu g \cdot mL^{-1}$ MCPA, the repeatability of the method (n=5) gave a coefficient variation (CV) of 3.3% and reproducibility (n=5) gave a CV of 9.8%.

Table 3 Figures of merit for calibration curves obtained in different conditions.

Calibration curve $S=(b \pm s_b)\cdot C_{MCPA} + (a \pm s_a)$; (r^2, n)	**Detection Limit** ($ng\cdot mL^{-1}$)	**Linear interval** ($\mu g\cdot mL^{-1}$)
Condition 1: Calibration curves without extraction and without ethanol		
$S=(51800 \pm 1200)\cdot C_{MCPA} + (11 \pm 250)$; (0.99, 10)	0.2	0.0025 a 0.50
Condition 2: Calibration curves with 4% ethanol, without extraction		
$S=(38600 \pm 1300)\cdot C_{MCPA} + (20 \pm 60)$; (0.99, 7)	0.5	0.0015 a 0.60
$S=(40600 \pm 1500)\cdot C_{MCPA} + (30 \pm 70)$; (0.99, 6)	0.9	0.0015 a 0.60
$S=(37900 \pm 1400)\cdot C_{MCPA} + (50 \pm 60)$; (0.99, 7)	0.6	0.0015 a 0.60
$S=(39500 \pm 1100)\cdot C_{MCPA} + (20 \pm 50)$; (0.99, 6)	0.7	0.0015 a 0.60
Condition 3: Calibration curves with extraction. The final concentration of ethanol is 4%		
$S=(36000 \pm 1300)\cdot C_{MCPA} + (50 \pm 70)$; (0.999, 5)	0.5	0.02 a 0.60
$S=(38300 \pm 1100)\cdot C_{MCPA} + (70 \pm 80)$; (0.998, 5)	0.45	0.02 a 0.60

Table 3 shows that calibrations with ethanol without SPE (Condition 2) gave a 20% reduction in sensitivity, with the DL being in the region of 0.6 $ng\cdot mL^{-1}$. The variation coefficient of the slopes in these conditions was 3%.

Both measurements taken with extraction via SDB-1 cartridges (Condition 3, table 3) were statistically similar to those obtained in Condition 2, as can be deduced by applying this comparison test to the slopes: $t_{tabulated}$ (using 12 degrees of freedom and 95% probability) was 1.8589, while the $t_{calculated}$ for both calibrations was lower in all cases (1.41 y 0.17, respectively). With these results, it was concluded that calibrations without extraction but with ethanol can be used to determine MCPA in real samples, which have been submitted to extraction.

To test the method's preconcentration capacity, with the extraction cartridges SDB-1, solutions with 3.75 mg of MCPA at different final volumes were prepared, and the extraction process was carried out. The process was done in triplicate. Recoveries close to 100% were obtained for volume standards of up to 100 mL, which implies the possibility of preconcentrating the sample up to 4 times. With this, the DL of the method was reduced to 0.13 $ng\cdot mL^{-1}$

3.4 Real samples

Three well water samples and three river water samples from different sources were analyzed, which did not contain MCPA.

For the study of the real samples, the two extraction procedures in solid phase described in section 3.2 were applied. The determinations were done in triplicate.

The C18 cartridges showed an 80% recovery of the added MCPA (0.3 $\mu g\cdot mL^{-1}$), which is why the use of this cartridge was ruled out for the analysis of the real samples.

Using extraction cartridges SDB-1, the real samples were spiked with MCPA (0.3 μg·mL^{-1}). The relative error percentages were also lower than 5%, which validates the accuracy of the method for determining this pesticide in environmental water samples (Table 4).

Table 4 Analysis of water from different sources

Samples	Relative Error (%)
Lucena's river water (Castellón)	-4.0
Xuquer's river water (Alzira)	-3.6
Bohilgues' river water (Ademuz)	-3.0
Ground water (Villamarchante)	-5.0
Ground water (Miguel Esteban)	-1.5
Ground water (Castellón)	-1.8

4 Conclusions

The proposed FI-CL method allows the determination of MCPA in water samples in a new, fast, economic and simple way. This is the first time that a chemiluminescence method for the determination of this pesticide has been proposed.

The calibration curves with and without applying SPE are statistically similar, allowing standards to be excluded from the extraction step, which simplifies the process. It is only necessary to apply the SPE procedure to real samples to eliminate the interferences. The method has resulted efficient in the analysis of water from different sources (ground and river water) via extraction of the pesticide with SDB-1 cartridges and ethanol elution. The method's detection limit is 0.5 ng·mL^{-1}, which is in the same order as the legal limit established by law for total amounts of pesticide in water for human consumption. Furthermore, a DL of 0.13 ng·mL^{-1} can be reached if a 100 mL sample is preconcentrated, with a recovery percentage close to 100%.

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