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FI automatic method for the determination of copper(II) based on coproporphyrin I–Cu(II)/TCPO/H_2O_2 chemiluminescence reaction for the screening of waters

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Abstract

In this paper, an automatic method for the screening of water samples containing Cu(II) was proposed, based on peryoxalate chemiluminescence reaction using coproporphyrin I as fluorophor compound to provide selectivity and a simple flow injection (FI) chemiluminescence detector (CLD). FI system conditions were chosen in order to distinguish samples over or under legislation limit established (50 $\mu g\,l^{-1}$) with high reliability. The detection limit found was 9 $\mu g\,l^{-1}$ and the linear dynamic range was 15–125 $\mu g\,l^{-1}$ of Cu(II). Repeatability and reproducibility studies gave good precision and accuracy with recovery near 100%. Under these conditions, the method resulted selective and only Fe(II), Fe(III) and Pb(II) could interfere, but at a concentration level higher than their normal concentration in waters. The proposed method was found to be highly reliable for screening purposes and it was successfully applied to the screening of a variety of real water samples.

Keywords: Copper(II); Waters; Chemiluminescence; Direct screening

1. Introduction

The chemiluminescence technique provides methods for trace analysis that are attractive because of their high sensitivity and fast kinetics. This technique, combined with flow injection (FI) systems, provides good accuracy and precision.

Peryoxalate chemiluminescence reaction (TCPO/H_2O_2 system), has been extensively used in recent years for liquid chromatography of fatty acids [1], synthesis, characterisation and analytical evaluation of oxamide reagents [2], determination of amphetamines in urine samples [3], immunoassays [4], detection of proteins separated by capillary zone electrophoresis [5], resolution of carboxylic acids by HPLC [6], determination of cholesterol [7], detection of retinoids using normal-phase chromatography [8] and the study of emission of spectroscopic properties of water soluble porphyrins with d- and f-electron metals [9,10]. The use of porphyrins can be remarked by their high selectivity to metal ions [10].

Metal ions are usually introduced into water cycle by industrial dumping and many of them have effected toxic for human life. Therefore, their maximum tolerated concentrations have been fixed by legislation: 50 $\mu g\,l^{-1}$ for copper(II) [11]. In order to control these values in real water samples, fast automatic systems are required. These type of systems are called screening systems [12,13] and they provide a binary yes/no response that leads to distinguish samples containing Cu(II) over or under legislation level with good reliability, fast speed, versatility and low cost.

Igarashi et al. [10] proposed a static method for Cu(II) determination based on coproporphyrin I–Cu(II)/TCPO/H_2O_2 chemiluminescence reaction. Based on their studies, we are proposing an automatic FI method in this paper for the screening of Cu(II) in water samples.

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2. Experimental

2.1. Apparatus

Flow injection chemiluminescence measurements were carried out with a firefly chemiluminescence detector (Global FIA Inc., P.O. Box 480, Fox Island, WA 98333) designed specially for use in flow through systems. Light generated in a liquid core waveguide is internally reflected and transmitted to a small but powerful photo multiplier. FI registers were monitored by an Hercule Lite–chromatography interface connected on line to a personal computer.

The flow system consisted of two Gilson Minipuls 3 peristaltic pumps furnished with polyvinyl chloride pumping tubes, two Rheodyne (Cotati, CA) 5041 injection valves, PTFE tubing of 0.5 mm i.d., and a water bath with a heater and magnetic stirrer (Raypa, Spain).

Static chemiluminescence measures were registered with a Jasco FP-750 (Tokio, Japan) fluorescence spectrophotometer. For the kinetic measures, the light emission was monitored at 622 nm. The chemiluminescence was measured with a 1-cm path length quartz cell and the last reagent (H_2O_2) was injected using a Hamilton Digital syringe (Nevada, USA).

2.2. Reagents and standard solutions

All chemical reagents were of analytical grade or better. Copper(II) sulphate pentahydrated, hydrogen peroxide 30%, nitric acid (Trace pur) 69% and sodium carbonate were purchased from Merck (Germany), coproporphyrin I dihydrochloride was from Aldrich Chemistry (Germany), bis(2,4,6-trichlorophenyl) oxalate (TCPO; Fluka Chemika, Switzerland) was prepared in tetrahydrofuran (Scharlau, Barcelona, Spain) : acetonitrile (J.T.Baker, Holland). Hexadecyltrimethylammonium chloride 25% (CTAC; Fluka Chemika, Switzerland) was prepared in 0.1 M pH 7.4 sodium tetraborate decahydrated (Panreac, Barcelona, Spain) and ammonium acetate was also from Panreac (Barcelona, Spain). Metal interference solutions were prepared from nitrate or chloride salt (Probus, Spain).

2.3. Sample preparation

Five mililitres (for samples 1–4), 9 ml (for sample 5) or 3.65 ml (for sample 6) of water samples were added to 0.25 ml of borax buffer 0.2 M pH 7.4 and 0.1 ml of 10^{-4} M coproporphyrin I and diluted up to 10 ml. Sample 1 was a synthetic natural water sample, prepared carrying 26.7 mg l^{-1} of Ca(II), 106.5 mg l^{-1} of bicarbonate, 4.3 mg l^{-1} of Mg(II), 5 mg l^{-1} of chloride, 12 mg l^{-1} of sulphate and 1 mg l^{-1} of fluoride.

For standard reference material (SRM®) 1640, 3.72 ml of SRM® containing 85.2 μg l^{-1} of Cu(II) (reference value) was added to 0.1 ml of 10^{-4} M coproporphyrin I and 1.9 ml of 1 M ammonium acetate. The pH was adjusted to 5.35 and the mixture was diluted up to 10 ml.

Water samples were measured directly and fortifying sample with 31.77 μg l^{-1} of Cu(II) for samples 1–4 and SRM; and with 50 μg l^{-1} for samples 5 and 6.

2.4. Screening procedure

In order to analyse copper(II) in waters, a FI automatic system was employed. It can be seen in Fig. 1. Two peristaltic pumps were used. Pump 1 consisted of two carrier streams; in one of them, standards from 15 to 125 μg l^{-1} of copper(II), in 10^{-6} M coproporphyrin I at 2.2 ml min^{-1}, pass through a 400-μl reactor placed on a water bath at 100 °C where flow was stopped for 5 min. After that, pump 1 started for 25 s in which sample was cooled passing through the first 200-μl coil and further mixed with the CTAC/H_2O_2 stream (at 0.7 ml min^{-1}) in the second 200-μl coil to fill the 50-μl sample loop. Pump 2 consisted of two water carrier streams. One of them at 5 ml min^{-1} passes through sample loop (IV_1), and the other at 2.9 ml min^{-1} passes through

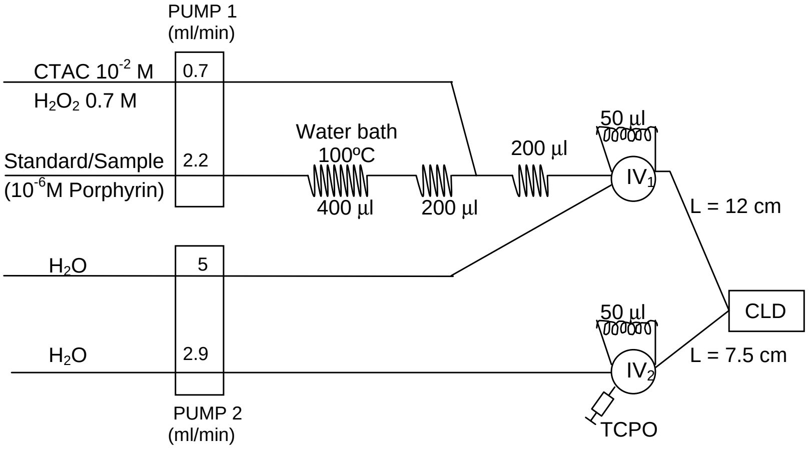

Fig. 1. FI system designed for the screening of water samples for copper(II) using a quenching chemiluminiscence determination. IV, injection valve; CLD, chemiluminiscence detector.

Table 1
Comparison of sensitivity of detectors for Cr(III)/luminol/H_2O_2 chemiluminescence reaction

Reference	$\log a \pm s_{\log a}$	$b \pm s_b$	Detection limit ($\mu g\,l^{-1}$)	Detector
[14]	1.91 ± 0.03	1.19 ± 0.03	1.3	Hitachi F4500
[15]	1.97 ± 0.07	1.25 ± 0.07	1.2	Hitachi F4500
[16]	1.84 ± 0.03	1.16 ± 0.03	1.6	Hitachi F4500
This work	1.04 ± 0.06	1.25 ± 0.06	1.4	Firefly chemiluminescence detector

TCPO loop (IV_2), previously filled by means of a syringe. Injection valve positions were activated manually. When injection valves were in position 1 (loading), only nanopure water was reaching the chemiluminescence detector (CLD). Both injection valves must be switched to position 2 (injection) at the same time, in order to allow both sample and reagent plugs reaching detector simultaneously. The height of the FI signal obtained must provide the binary (yes/no) response.

3. Results and discussion

3.1. Checking the firefly chemiluminescence detector

The detector was checked following the FI procedure described by Tortajada et al. [14] for Cr(III)/luminol/H_2O_2 reaction. Table 1 shows log–log calibration curves and detection limits obtained by this research group in recent publications [14–16] using a Hitachi F-4500 spectrofluorimeter and the same obtained with this new detector.

3.2. Optimisation of the flow system

Emission chemiluminescence spectrum was obtained for a blank solution following static conditions (1.12 μM coproporphyrin I, 0.0096 M CTAC, 1.795 mM TCPO and 0.743 M H_2O_2). In Fig. 2a, it can be seen that there is an emission maximum at 622 nm. Reaction kinetics curve at 622 nm was also obtained. Fig. 2b shows that the kinetics of this reaction was fast, it took place in about 10 s.

Table 2
Optimisation of the chemical and physical variables of the FI system

Variable	Range studied	Optimum condition
CTAC concentration	2×10^{-5} to 9×10^{-2} M	1×10^{-2} M
H_2O_2 concentration	0.1–1 M	0.7 M
TCPO concentration	1–10 mM	5 mM
MeCN:THF ratio	0%:100%–100%:0%	75%:25%
pH	4.7–10.3	7.4
Flow rate pump 2	10–48 rpm	40 rpm
Loop volume	50–500 μl	50 μl
Length from injection valve (IV) to CLD	L_{IV_1-CLD} = 12–24 cm L_{IV_2-CLD} = 7.5–15 cm	L_{IV_1-CLD} = 12 cm L_{IV_2-CLD} = 7.5 cm
Length liquid core waveguide	17–50 cm	17 cm
Coproporphyrin–Cu(II)		
Reaction time	1–10 min	5 min

Optimisation of the flow system was carried out with a blank solution using only pump 2 and injecting both coproporphyrin I 10^{-5} M/H_2O_2 variable/CTAC variable/borax 0.1 M pH 7.4 (IV_1, loop 160 μl) and variable TCPO (variable ratio MeCN:THF) (IV_2, loop 200 μl) with a syringe (see Fig. 1).

Table 2 shows the variables optimised, the range studied on each case and the optimum variable was selected. First, the addition of CTAC was studied based on previous studies [10]. Hydrogen peroxide and TCPO concentrations were varied according to recent studies of the chemiluminescence reaction [2,10]. These two reagents must reach the detector separately in order to achieve the maximum sensitivity.

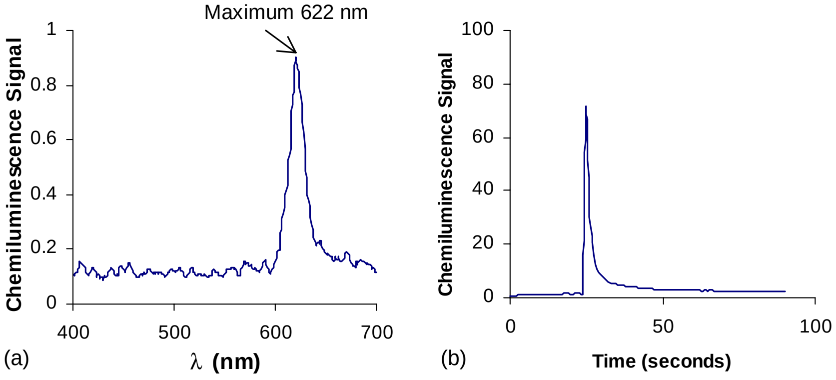

Fig. 2. (a) Emission chemiluminescence spectrum for a blank. (b) Reaction kinetic curve. Conditions: 1.12×10^{-6} M coproporphyrin I, 0.0096 M CTAC, 0.743 M H_2O_2, 1.795 mM TCPO.

TCPO reagent was prepared in acetonitrile:tetrahydrofuran at different ratios from 0:100 to 100:0. Best results were obtained for 75% acetonitrile:25% tetrahydrofuran avoiding TCPO destruction and obtaining the best sensitivity.

CTAC/H_2O_2 reagent pH was studied using different buffer solutions at 0.1 M concentration, pH 4.7 was studied in acetic/acetate buffer, pH 6.5 and 10.3 were studied with carbonate/bicarbonate buffer and pH 7.4 was studied with borax buffer. Best results were obtained at pH 7.4 with borax buffer.

Concerning the flow conditions, flow rate in pump 2 was varied from 10 to 48 rpm, and 40 rpm selected as optimum, providing 5 ml min^{-1} for coproporphyrin I stream and 2.9 ml min^{-1} for TCPO stream. Loop volume was also studied, and 50 μl were selected because signal was more reproducible and pressure problems were avoided.

Due to the fast speed of the chemiluminescence reaction, another parameters were optimised: distance from injection valves to chemiluminescence detector and length of the liquid core waveguide. The length from injection valves (IV_1 and IV_2) to chemiluminescence detector (see Fig. 1) was selected as the minimum possible. This condition avoided reagent dispersion and improved sensitivity by 10 times. The length of the liquid core waveguide was also selected as the minimum possible for the detector, and it improved the sensitivity by about 2.5 times.

With those changes, coproporphyrin I concentration could be reduced to 1×10^{-6} M (lower coproporphyrin concentrations did not give reaction with Cu(II) with the established conditions). Then, pump 1 was added to the FI system and copper(II) was introduced using the best conditions established for the optimisation of the Cu(II)-coproporphyrin reaction time in the water bath; it was studied from 1 to 10 min and it was observed that quenching effect was better when reaction time increased, and 5 min were selected.

3.3. Sensitivity and selectivity of the method

Calibration curves for Cu(II) were obtained in different conditions of pump 1 flow rate and reaction time. Analytical figures of merit for the conditions assayed can be seen on Table 3. When pump 1 flow rate was 0.7 ml min^{-1} for coproporphyrin carrier and 0.5 ml min^{-1} for CTAC/H_2O_2 carrier, calibration curves were obtained stopping the flow for 5 min in the heating bath (t_1) and 2 or 5 min in the second reactor of the flow system (t_2) in order to cool the reaction mixture. Good reproducibility for the same conditions can be observed comparing the first and second calibration curve, being the recovery 97%. Increasing t_2 from 2 to 5 min, increased the sensitivity 1.65 times.

When pump 1 flow rate was fixed at 2.2 ml min^{-1} for coproporphyrin carrier and 0.7 ml min^{-1} for CTAC/H_2O_2 carrier, the flow was not halted in the second coil in order to eliminate a dispersion phase. Calibration curves were obtained for $t_1 = 4$ or 5 min in the interval 0–32 μg l^{-1}. Results showed that sensitivity was increased near two times for $t_1 = 5$ min, obtaining the best detection limit at the level of 8.7 μg l^{-1}, level that was 5.8 times lower than limit established by legislation for Cu(II) in waters (50 μg l^{-1}). Calibration curve was also obtained with that conditions in the range 0–100 μg l^{-1} which was the linear dynamic range because signals remained constant at higher Cu(II) concentrations. FI registers obtained can be seen on Fig. 3. Limits of detection, which are shown in Table 3, were calculated as 3 s_{blank}/b, being $s_{\mathrm{blank}} = 105.96$ ($n = 9$).

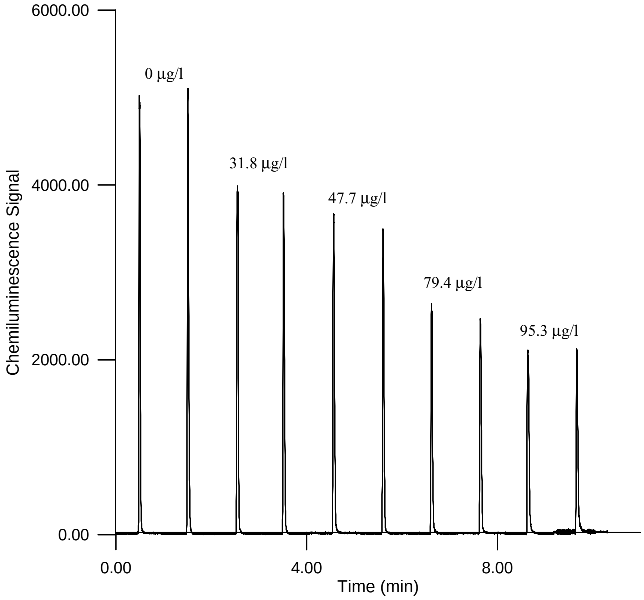

Fig. 3. Cu(II) calibration curve FI registers.

Table 3
Cu(II) calibration curves and figures of merit in different conditions

v_{pump1} (ml min^{-1}, porphyrin carrier/CTAC/H_2O_2 carrier)	t_1 (min)	t_2 (min)	$a \pm s_a$	$b \pm s_b$	LI (μg l^{-1})	LD (μg l^{-1})	$s_{y/x}/b$	r^2	n
0.7/0.5	5	2	4300 ± 40	−18.3 ± 0.7	0–125	17	5.38	0.984	12
0.7/0.5	5	2	4370 ± 110	−18.9 ± 1.3	0–125	17	8.96	0.972	8
0.7/0.5	5	5	6770 ± 30	−30.7 ± 0.4	0–125	10	1.32	0.999	5
2.2/0.7	4	0	3840 ± 20	−19.0 ± 1.0	0–32	17	1.22	0.994	4
2.2/0.7	5	0	4255 ± 18	−36.6 ± 1.0	0–32	8.7	0.58	0.999	4
2.2/0.7	5	0	5010 ± 50	−30.7 ± 0.8	0–100	10	2.83	0.994	10

LI: linear interval; DL: detection limit.

Table 4
Interference study

Interference ion	Legislation limit ($\mu g\, l^{-1}$)	Concentration in natural waters ($\mu g\, l^{-1}$)	Limit of tolerance ($\mu g\, l^{-1}$)
Al(III)		10	3000
Fe(III)	2000	500	2000
Fe(III)	2000	500	2000
Sn(II)			300
Pb(II)	50	1	30
Hg(II)	1	0.07	300
Cd(II)	5	0.03	30
Co(II)		0.05	30
Cr(III)	50	1	3000

Repeatability and reproducibility studies were carried out for standards analysed in triplicate. Repeatability studies were obtained for a standard of 15.9 $\mu g\, l^{-1}$ with a R.S.D. of 16%. Results for reproducibility studies were obtained for a standard of 17.3 $\mu g\, l^{-1}$ of Cu(II), and R.S.D. 12%. The recoveries obtained for repeatability and reproducibility studies were 93% and 109%. These values are in accordance with the required accuracy in trace analysis.

Results obtained with the standard reference material SRM® 1640 were analysed which demonstrated the accuracy of the method, giving a copper(II) concentration of (96 ± 14) $\mu g\, l^{-1}$ (reference value of SRM 1640 : 85.2 $\mu g\, l^{-1}$). The percentage of recovery was 113 ± 17% ($n = 3$).

In order to study the selectivity of the method, several metal ions were studied with this FI system. Na^+, Mg^{2+}, K^+, Ca^{2+}, Ag^+, Mn^{2+}, Ni^{2+} and Zn^{2+} only interfere at high levels, between 5 and 500 $mg\, l^{-1}$ which are not usual in water samples. Al(III), Sn(II), Pb(II), Hg(II), Fe(II), Fe(III), Cd(II), Cr(III) and Co(II) were also studied. Their limit of tolerance (concentration at which signal was modified more than 10%) was lower, but this was over the limit established by legislation. Only Pb(II), Fe(II) and Fe(III) had their limit of tolerance under limit established by legislation, but it was over its usual concentration in waters. Therefore, we concluded that the method was selective to Cu(II). These results can be observed on Table 4.

3.4. Reliability of the screening method for Cu(II) determination

The confidence level of the proposed sample screening system can be established on the basis of the percentage of false positives and false negatives through a simple chemometric study. The cut-off level was set at a concentration corresponding to two times the detection limit (2DL). A false positive corresponded to a water sample containing a Cu(II) concentration lower than the cut-off but giving a positive response. A false negative is obtained when a water sample with an analyte concentration higher than that of the cut-off provides a negative response in the detector. About 65 standards were measured at concentrations near detection limit: 0.5DL, 1DL, 1.5DL, 2DL, 3DL and 3.5DL. In Fig. 4 it can be seen that the percentage of false positive was 0% at 0.5DL, increasing at 9% for 1DL and 25% for 1.5DL. The percentage of false negatives was 6% for 3DL and 0% at 3.5DL. Considering that this value was far away from legislated concentration (50 $\mu g\, l^{-1}$), the proposed procedure can be used for the screening of Cu(II) in water samples.

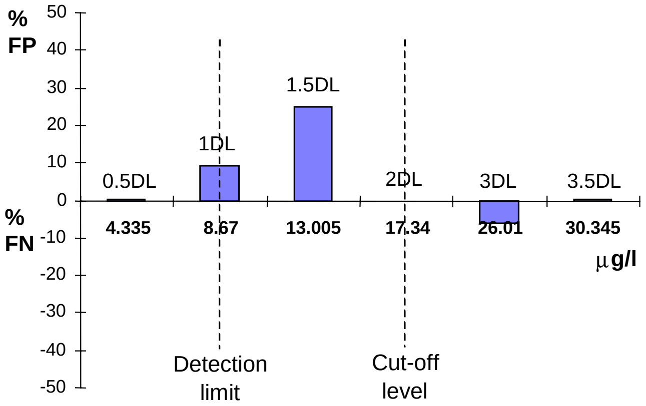

Fig. 4. Reliability of the screening method. Standard solutions ($n = 65$) of Cu(II) at concentration near detection limit (DL). %FP = percentage of false positives; %FN = percentage of false negatives.

3.5. Application to real water samples

The method was applied to the determination of Cu(II) in real water samples (see Table 5). A synthetic water sample (S1), three natural water samples (S2, S3 and S4), tap water sample (S5) and a fountain water sample (S6) were analysed and only fountain water sample gave a positive response. The recoveries obtained for all samples demonstrated the accuracy of the method being between 83% and 119% of recovery.

Table 5
Results obtained in the Cu(II) determination in real water samples

Sample	Screening response	Concentration found ($C \pm s$, $\mu g\, l^{-1}$)	Concentration added ($\mu g\, l^{-1}$)	%Rec ± s
S1, synthetic sample	No	–	31.77	92 ± 16 ($n = 6$)
S2, natural water	No	–	31.77	102 ± 19 ($n = 3$)
S3, natural water	No	–	31.77	100 ± 13 ($n = 4$)
S4, natural water	No	–	31.77	117 ± 7 ($n = 4$)
S5, tap water	No	–	50	83 ± 11 ($n = 3$)
S6, fountain water	Yes	(61 ± 5, $n = 8$)	50	119 ± 12 ($n = 3$)

4. Conclusions

The proposed method can be used as an automatic system for screening of Cu(II) in water samples providing good results in terms of sensitivity, repeatability, reproducibility, precision, accuracy and selectivity. It allows filtering sample set of study with good reliability, low cost, versatility, speed and minimisation of operator errors, providing the analytical information of client interest.

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