

# Determination of labile $\text{Cu}^{2+}$ in fresh waters by chemiluminescence: interference by iron and other cations

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Received 4 July 2003; received in revised form 3 October 2003; accepted 14 October 2003

## Abstract

Analysis of labile  $\text{Cu}^{2+}$  in fresh waters using the  $\text{Cu}^{2+}$ -catalysed oxidation of 1,10-phenanthroline by superoxide anion radical has been investigated. It was found that certain metal ions, notably  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$  and  $\text{Pb}^{2+}$  enhance the chemiluminescence (CL) intensity of this reaction by up to an order of magnitude when present in the  $\mu\text{M}$  concentration range. This enhancement is considered to arise through coordination of the metal ion to 1,10-phenanthroline, which prevents free rotation of the benzene rings in the excited state intermediate thought to be responsible for light emission. This introduces a potential interference when analyzing fresh waters, which usually contain  $\text{Fe}^{3+}$  concentrations of this magnitude. However, the enhancement effect saturates at about  $4 \mu\text{M}$   $\text{Fe}^{3+}$ , so that reliable results can be obtained if the water sample is supplemented with  $\text{Fe}^{3+}$  to reach this level. Application of the enhanced technique to a river water, and a reservoir to which  $\text{CuSO}_4$  had been added to control algal growth, are described. In both cases, only a small fraction of total dissolved  $\text{Cu}^{2+}$  is labile with respect to the chemiluminescence method.

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**Keywords:** Trace labile copper; Fresh waters; Chemiluminescence spectrometry; Oxidation of 1,10-phenanthroline

## 1. Introduction

Trace metals are important factors in the biochemical functions of all living organisms because of their role in the catalytic centres of metalloenzymes. Many are also toxic because they interfere with the function of metalloenzymes and other biochemical pathways. In recent years, it has become clear that the biological availability of trace metals in aquatic systems is dominated by the formation of strong complexes with natural organic matter (NOM) ligands [1,2]. It is even possible that these ligands are specifically produced by some species of microalgae as a mean of controlling their chemical environment [3]. As a result, there has been considerable attention focused on the development of analytical methods that measure the so-called labile fraction of trace metals in natural waters in order to assess biologically-available forms of trace metals. The underlying assumption of this approach is that only labile metal complexes are able to dissociate rapidly enough to participate in the ion-transport system that

carries metal ions across the cell membrane. Useful techniques include electrochemical methods [1,2], ion-selective electrodes [4], and diffusion gradient in thin films (DGT) devices [5].

Chemiluminescence (CL), which involves the emission of light during a specific chemical reaction, is receiving increase attention as a method for measuring the labile fraction of several biologically-important trace metals in natural waters [6–8]. The method is extremely sensitive because the use of a photomultiplier detector allows measurement of very low light levels. Thus, measurement of labile metal concentrations below  $10^{-13}$  M is not difficult, which enables in situ study of biologically-important processes involving metal ions.

The oxidation of 1,10-phenanthroline to, 2'-dipyridyl-3,3'-dicarboxylic acid by superoxide anion radical  $\text{O}_2^{\bullet-}$  formed from the decomposition of  $\text{H}_2\text{O}_2$  in water is catalyzed by  $\text{Cu}^{2+}$  ions, and is accompanied by CL emission in the wavelength range 445–450 nm [9]. Key steps in the mechanism of the reaction are shown in Fig. 1.

The reaction is thought to proceed via an intermediate containing aldehyde or ketone excited states in the 3 and 3' positions (species X in Fig. 1), with relaxation of this

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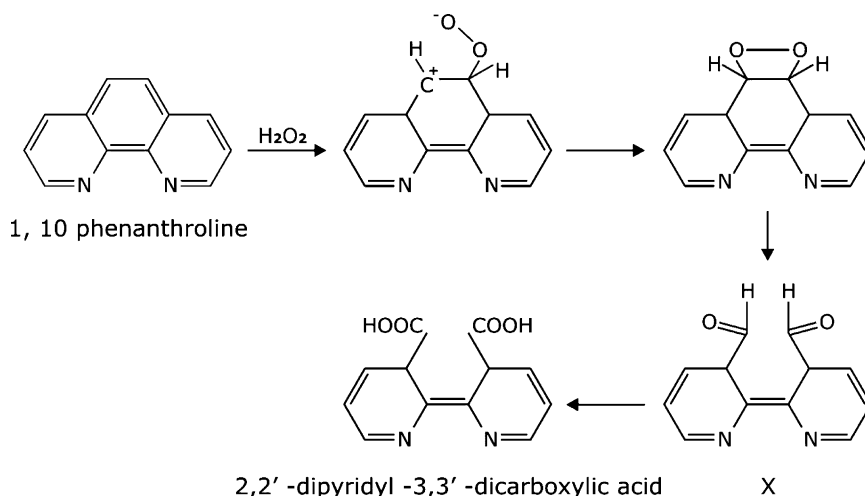


Fig. 1. Proposed mechanism for oxidation of 1,10-phenanthroline by superoxide anion radical [9].

excited state giving rise to CL [9]. In a situation where 1,10-phenanthroline and  $\text{H}_2\text{O}_2$  are present in excess over  $\text{Cu}^{2+}$ , the amount of CL is proportional to the concentration of labile  $\text{Cu}^{2+}$  in solution, i.e. to the combined concentrations of those complexes that readily dissociate to form free  $\text{Cu}^{2+}$  ions on the time scale of the CL reaction. This labile fraction, denoted  $[\text{Cu}']$ , will include all complexes formed with inorganic ligands (e.g.  $\text{Cl}^-$ ,  $\text{OH}^-$ ,  $\text{CO}_3^{2-}$ ) and those with small organic ligands that might be present [1].

This CL process forms the basis of a very sensitive method for measurement of labile  $\text{Cu}^{2+}$  in seawater [7,8,10]. A surfactant, cetyltrimethylammonium bromide (CETAB) is normally added which enhances the CL output through a surface catalytic effect.

This may arise because  $\text{O}_2^-$  is attracted to the positively charged CETAB micellar surface, forming a dioxetane intermediate which initiates the reaction with 1,10-phenanthroline [10]. A further enhancement is the addition of tetraethylenepentamine (TEPA), which minimizes the effects of  $\text{Cu}^{2+}$  impurities present in the reagents.

Although this ligand binds strongly to  $\text{Cu}^{2+}$ , it does not interfere appreciably with analysis of  $\text{Cu}^{2+}$  in the sample

because the kinetics of its reaction with  $\text{Cu}^{2+}$  are very slow compared to the time scale of CL [6].

$\text{Cu}^{2+}$  is one of the most abundant trace metals in fresh waters that is toxic to algae, and is frequently used to control algal growth in potable water reservoirs. Accordingly, much effort has been devoted to determining the speciation and bio-availability of  $\text{Cu}^{2+}$  in fresh water systems. Here, we report on the results of applying the above CL method to the determination of labile  $\text{Cu}^{2+}$  in fresh waters.

## 2. Materials and methods

The experimental method used was based on flow injection [10] in which the sample solution was injected into a stream of reagents and, after the required time for reaction, pumped into a coil of clear plastic in contact with a photomultiplier tube.

The flow system is shown schematically in Fig. 2.

Teflon tubing was used for all flow lines except for pump tubes (C-flex) and the detection flow cell (Tygon). Teflon

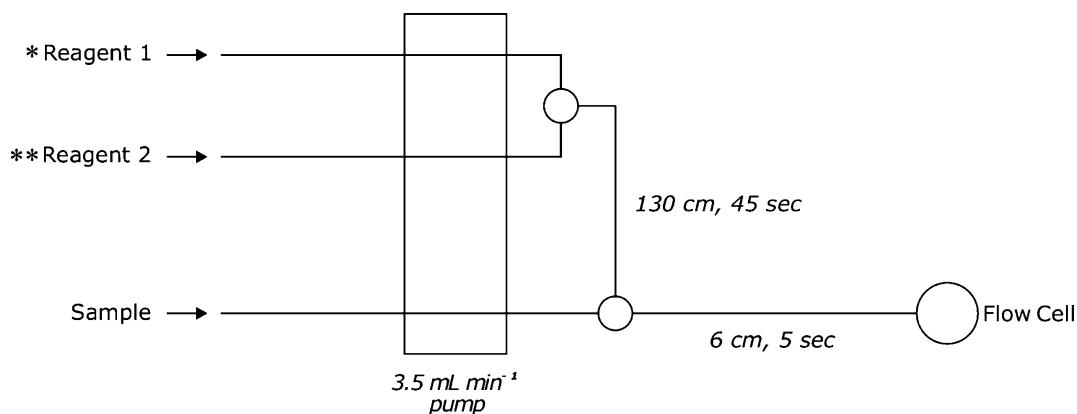


Fig. 2. Schematic diagram of flow injection system used for CL measurements.

tubing was chosen to minimize  $\text{Cu}^{2+}$  adsorption following the results of initial experiments. The sample flow lines and two reagent flow lines were connected to the pump separately. The two reagent streams were merged at a 3-way Teflon connector (Omnifit, 0.8 mm bore, A 4247) with cones (Omnifit, 3.2 mm i.d., A 1671) and mixed together in a 130 cm length of Teflon tubing (1.6 mm i.d) which permitted a necessary 45 s period for complete mixing before introduction of the sample.

Reagent 1 was a 5% (w/v)  $\text{H}_2\text{O}_2$  solution prepared from reagent grade  $\text{H}_2\text{O}_2$  and distilled, deionised water from a Milli-Q system (hereafter termed Milli-Q water).

Reagent 2 consisted of a mixture containing 0.1 M NaOH, 60  $\mu\text{M}$  1,10 phenanthroline, 0.4  $\mu\text{M}$  TEPA and 2  $\text{g l}^{-1}$  of CETAB. Both reagent solutions were prepared fresh daily and stored in acid-cleaned high density polyethylene (HDPE) bottles.

The mixed reagent stream and the sample were then merged at another 3-way Teflon connector, followed by 5 s mixing time along a 6 cm length of Teflon tubing before entering the flow cell.

The flow cell consisted of a 110 cm long clear Tygon tubing (1.6 mm i.d., R 3606) coiled into a spiral fixed together with double sided sticky tape. This was mounted to the inside of the PMT housing so that the spiral flow cell faced the PMT in order to obtain the maximum detection of released light. To prevent ambient light reaching the PMT, it was mounted in a cardboard box, the outside of which was sealed by black tape. In addition, the cardboard box was covered with aluminum foil and sealed again with black tape. For further prevention of stray light, the sample and mixed reagent tubes were also covered with black tape. A Philips 56 UVP PMT with 41 mm diameter cathode tube was used to detect the emitted photons. The PMT signal was sent via an ORTEC 417 fast discriminator to an ORTEC 775 scaler for counting. An ORTEC 456 high voltage power supply adjusted to 1900 V was used.

Analysis of each sample solution was followed by a 5 min rinse with 10% (v/v) high-purity HCl (HCl double-distilled in a quartz sub boiling still [11], hereafter designated Q-HCL), and then several rinses with Milli-Q water. The CL count rate returned to baseline levels between each sample, indicating no carry-over of  $\text{Cu}^{2+}$ . A blank sample was measured at the start and the end of every experiment to ensure no baseline drift occurred.

Initial experiments showed that acid-cleaned fluorinate polyethylene (FLPE) bottles were necessary to minimize sample contamination and loss of  $\text{Cu}^{2+}$  by adsorption to the bottle walls. Before use, these were cleaned by soaking for at least 5 days in 50% aqua regia, then rinsed thoroughly with Milli-Q water and filled with 1% Q-HCl. After 3 days, the bottles were emptied, rinsed with Milli-Q water and dried within a Class 100 laminar flow cabinet. All sample handling and preparation of standards and blanks were also performed inside the laminar flow cabinet.

$\text{Cu}^{2+}$  standard solutions for calibration were prepared by dilution of a 1000 ppm spectroscopy standard (APS Ajax Finechem) with Milli-Q water in FLPE bottles, and were acidified with 2% (v/v) Q- $\text{HNO}_3$  ( $\text{HNO}_3$  purified as for Q-HCl described above).

Blank samples were prepared daily from acidified Milli-Q water.

Filtered fresh water samples were collected using a trace metal-clean technique [12] which involves extending a length of acid-cleaned 10 mm i.d. Teflon tubing upstream and below the water surface in the middle of the river using an aluminum pole. The sample is then pumped through the tubing using a peristaltic pump attached to a 0.45  $\mu\text{m}$  filtering capsule (Aqua Prep 600). The filtered water was directly collected into the acid-cleaned sample bottles, which were subsequently sealed in double plastic bags. The Teflon tubing, pump tubing and filtering capsules were cleaned using 50% HCl acid for at least 7 days, rinsed with Milli-Q water and filled with dilute Q-HCl (0.1%) for another week before use.

Total dissolved  $\text{Cu}^{2+}$  concentrations in all water samples were measured by graphite furnace atomic absorption spectrophotometry (GFAAS) using a Perkin-Elmer 4100 Z instrument with Zeeman background correction. The accuracy of the analyses was verified against a Canadian NRC Reference River water Standard (SLRS-4).  $\text{Fe}^{3+}$  in some water samples was determined by inductively-coupled plasma optical emission spectrometry (ICP-OES) using a Thermo Jarrell-Ash Atomscan 25.

### 3. Results and discussion

In preliminary experiments, a filtered river water sample was collected from the Water of Leith, a small stream (annual mean flow 0.79  $\text{m}^3 \text{s}^{-1}$ ) flowing adjacent to our laboratory building. This sample was split in two, with the first portion being analyzed for labile  $\text{Cu}^{2+}$  by CL within 20 min of collection, with a calibration curve prepared using  $\text{Cu}^{2+}$  standards in acidified Milli-Q water. The second portion was acidified to 1% (v/v) with Q- $\text{HNO}_3$  and analyzed by CL 24 h later. Both portions were then analyzed for total dissolved  $\text{Cu}^{2+}$  by GFAAS. The initial aim of the acidification was to break down some of the inert  $\text{Cu}^{2+}$  complexes formed with natural organic matter ligands that might be present in the water.

The results, presented in Table 1, indicate different results depending on the method used. In the unacidified sample, the apparent labile  $[\text{Cu}^{2+}]$  measured by CL is almost twice the total  $[\text{Cu}^{2+}]$  measured by GFAAS, whereas in the acidified sample it is about 10 times as great. Clearly, something is interfering with the CL measurement producing apparent concentrations much higher than the actual ones. The interference also seems to be associated with acidification of the sample, which was confirmed by measuring the CL

Table 1  
Concentrations of Cu measured by GFAAS and CL in acidified and unacidified aliquots of a filtered river water sample

Samples	Concentration (nM)	
	GFAAS	CL
River water	31 ± 2	50 ± 3
Acidified river water	42 ± 2	415 ± 20

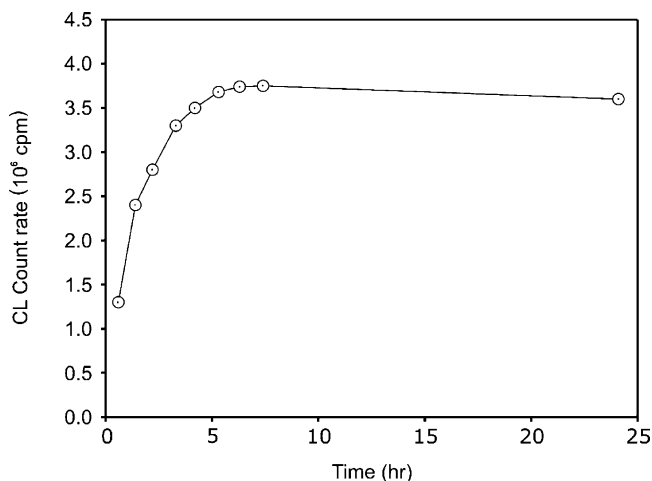


Fig. 3. CL intensity ( $10^6$  counts per minute = cpm) as a function of time after initial acidification of a filtered Water of Leith sample.

intensity periodically in a freshly-acidified aliquot of river water (Fig. 3).

We identified  $\text{Fe}^{3+}$  as a potential interferent, since this metal ion is considerably more abundant in freshwaters (typically in the  $\mu\text{M}$  range) than in seawater (typically nM range) in which the present CL technique is reported to work well [6–8].

Moreover, both  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  are reported to give a response to the CL method, but at a much lower sensitivity than  $\text{Cu}^{2+}$  [10]. Therefore, we next investigated the effect of both  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  on labile  $\text{Cu}^{2+}$  determination by CL.

Table 2 shows the results of measuring CL intensity on solutions containing 30 nM  $\text{Cu}^{2+}$  and 2  $\mu\text{M}$   $\text{Fe}^{3+}$  either separately or combined together. It is seen that for a 2  $\mu\text{M}$  concentration,  $\text{Fe}^{3+}$  exhibits a small CL intensity compared to that of 30 nM  $\text{Cu}^{2+}$ . At equal concentrations of either metal, the response for  $\text{Fe}^{3+}$  is 370 times smaller than that of  $\text{Cu}^{2+}$ . This relative response agrees well with values reported in [10]. However, when both ions are combined in

Table 2  
CL intensity in counts per minute (cpm) of 30 nM  $\text{Cu}^{2+}$  and 2  $\mu\text{M}$   $\text{Fe}^{3+}$  separately and combined in the same solution

Sample	CL count rate (cpm)
30 nM $\text{Cu}^{2+}$	152000
2 $\mu\text{M}$ $\text{Fe}^{3+}$	27200
30 nM $\text{Cu}^{2+}$ + 2 $\mu\text{M}$ $\text{Fe}^{3+}$	359000

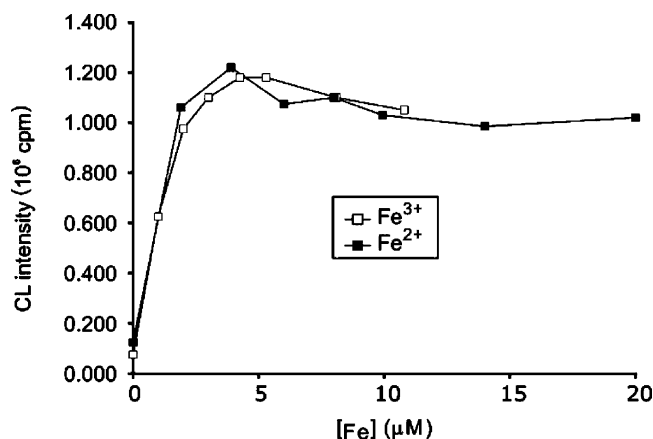


Fig. 4. CL intensity as a function of iron concentration in the presence of 30 nM  $\text{Cu}^{2+}$ .

the same solution, the overall CL intensity is about double that expected from adding together the separate responses, showing that it is not a simple additive effect of both ions contributing to CL separately.

Next, the effect of  $\text{Fe}^{3+}$  on CL intensity was investigated using a solution containing 30 nM  $\text{Cu}^{2+}$  and different  $\text{Fe}^{3+}$  concentrations in the range 0–20  $\mu\text{M}$ , which spans that normally encountered in river waters [13]. The results, shown in Fig. 4, reveal that  $\text{Fe}^{3+}$  enhances the CL intensity at constant  $[\text{Cu}^{2+}]$  up to about 4  $\mu\text{M}$   $\text{Fe}^{3+}$ , after which the effect plateaus and tends to decrease very slightly at higher  $[\text{Fe}^{3+}]$ .

Interestingly, Fig. 4 also shows that much the same enhancement of CL intensity is observed with  $\text{Fe}^{2+}$ , which is not normally present in river waters.

Fig. 5 compares CL calibration graphs for  $[\text{Cu}^{2+}]$  in the absence of  $\text{Fe}^{3+}$  and in the presence of 4  $\mu\text{M}$   $\text{Fe}^{3+}$ , the level at which the enhancement effect plateaus in Fig. 4. This comparison shows that in the presence of 4  $\mu\text{M}$   $\text{Fe}^{3+}$ , the CL calibration graph is still linear, but has a significantly increased slope (i.e. sensitivity).

The slope in the presence of  $\text{Fe}^{3+}$  is 11.4 times greater.

The fact that the calibration graph in the presence of 4  $\mu\text{M}$   $\text{Fe}^{3+}$  in Fig. 5 remains linear with  $[\text{Cu}^{2+}]$  over the range 0–28 nM, and passes through the origin, shows that  $\text{Fe}^{3+}$  does not simply replace  $\text{Cu}^{2+}$  as a catalyst for the CL process. If this were so, then the slope of the graph would remain constant and its intercept at zero  $[\text{Cu}^{2+}]$  would be non-zero and equal to the CL intensity of the plateau level in Fig. 4.

It follows therefore that the role of  $\text{Fe}^{3+}$  in the process is different from  $[\text{Cu}^{2+}]$ . In addition, it seems that this enhancement role is shared by  $\text{Fe}^{2+}$  which has nearly the same effect.

Therefore, we investigated the effects of other metal ions on the CL process. The next most effective was  $\text{Pb}^{2+}$  (Fig. 6). Fig. 7 compares the enhancement effect of different ions. The results are presented in terms of the measured ratio of the slopes of the  $\text{Cu}^{2+}$  calibration graph in the presence of 4  $\mu\text{M}$  of each metal ion to that in its absence.  $\text{Cr}^{3+}$ ,  $\text{Zn}^{2+}$  and  $\text{Co}^{2+}$

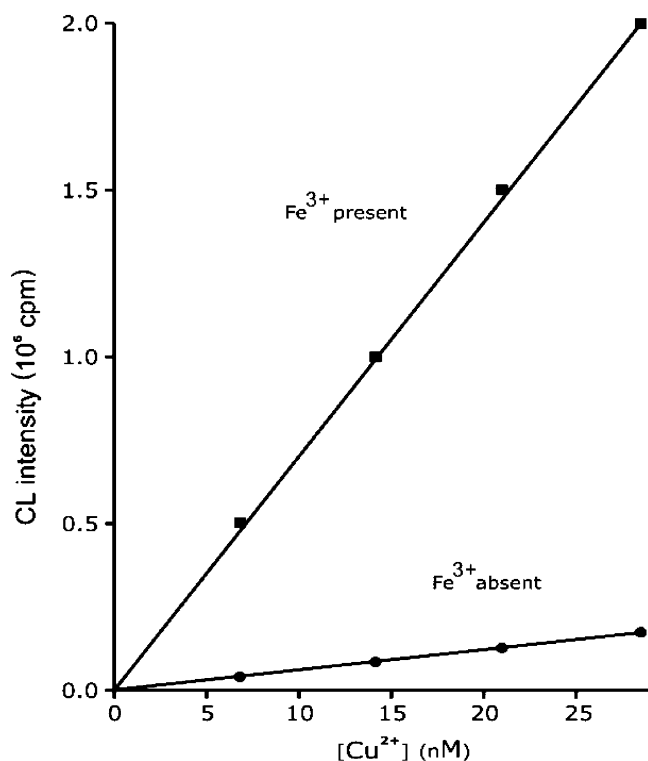


Fig. 5. CL intensity as a function of  $\text{Cu}^{2+}$  concentration in the presence and absence of  $4 \mu\text{M}$   $\text{Fe}^{3+}$ .

showed very little enhancement, while  $\text{Mn}^{2+}$  depressed the CL intensity to about 50% of that observed with  $\text{Cu}^{2+}$  only.

We propose that the enhancement effect we have observed results from binding of the secondary metal ion to the donor N atoms in the 1,10-phenanthroline ring. The mechanism shown in Fig. 1 shows that under normal circumstances, once the central ring has opened to form the intermediate containing aldehyde or ketone excited states in the 3 and 3' positions (species X in Fig. 1), the remaining benzene rings are free to rotate. However, if the molecule is bound to a

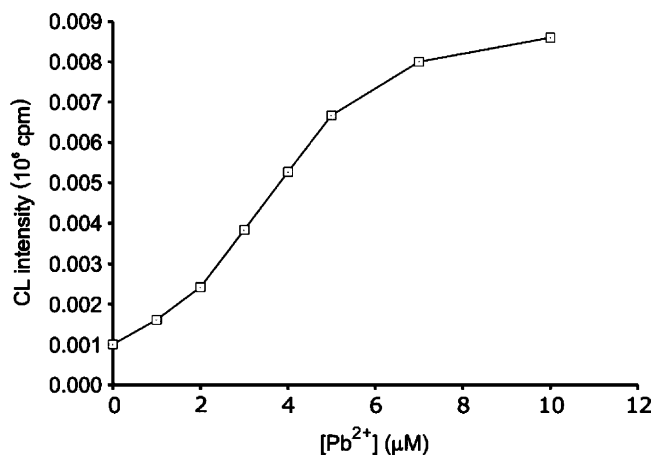


Fig. 6. CL intensity as a function of  $\text{Pb}^{2+}$  concentration in the presence of  $21 \text{ nM}$   $\text{Cu}^{2+}$ .

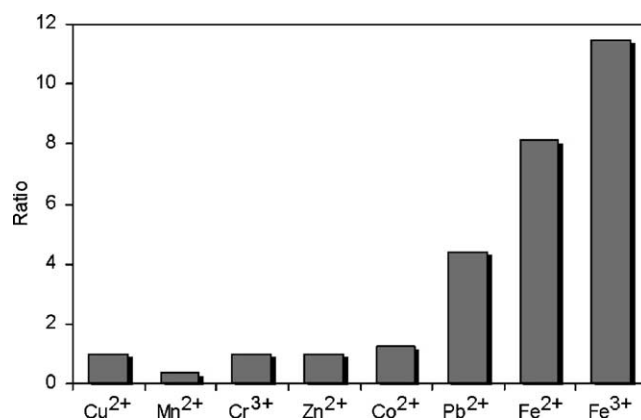


Fig. 7. Ratio of calibration graph slopes in the presence and absence of  $4 \mu\text{M}$  of different metal ions for the region  $0\text{--}30 \text{ nM}$   $\text{Cu}^{2+}$ .

metal ion, such as  $\text{Fe}^{3+}$ , this rotation is not possible and the excited state complex is locked in a flat conformation, at least on the time scale of dissociation from the metal ion. We speculate that this locked structure enhances the probability of the reaction proceeding to the 2, 2'-dipyridyl-3, 3'-dicarboxylic acid product via a chemiluminescent pathway.

This is consistent with the result that the metal ions that give the greatest CL enhancement ( $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Pb}^{2+}$ ) are of the hard acid type that prefers to bind to relatively hard donor atoms like N.

### 3.1. Application to fresh waters

The fact that the enhancement effect for  $\text{Fe}^{3+}$  plateaus at about  $4 \mu\text{M}$  (Fig. 4) implies that measurements of labile  $\text{Cu}^{2+}$  can still be made in fresh waters containing  $\text{Fe}^{3+}$  provided that the method is calibrated using  $\text{Cu}^{2+}$  standard solutions containing  $4 \mu\text{M}$   $\text{Fe}^{3+}$ , and that the  $\text{Fe}^{3+}$  concentration of the sample is checked so that if necessary, it can be supplemented with  $\text{Fe}^{3+}$  to bring the concentration of the latter up to  $4 \mu\text{M}$ .

Table 3 shows the results of a series of measurements made on a sample from the Water of Leith following this principle. The first row shows an apparent labile  $\text{Cu}^{2+}$  concentration of  $0.1 \text{ nM}$  (this time calibrated with standards

Table 3

Measurements of labile  $\text{Cu}^{2+}$  by CL, total  $\text{Cu}^{2+}$  by GFAAS and total  $\text{Fe}^{3+}$  by ICP-OES in a Water of Leith river water sample after various treatments

Sample	$[\text{Cu}^{2+}]$ (nM) CL	$[\text{Cu}^{2+}]_{\text{T}}$ (nM) GFAAS	$[\text{Fe}^{3+}]$ ( $\mu\text{M}$ ) ICP-OES
River water	$0.10 \pm 0.03$	17	0.56
+4 $\mu\text{M}$ $\text{Fe}^{3+}$	$0.20 \pm 0.03$	17	4.6
Acidified	$10.2 \pm 1.5$	23	2.8
Acidified, +4 $\mu\text{M}$ $\text{Fe}^{3+}$	$9.2 \pm 1.5$	23	6.8
Acidified, +1.5 $\mu\text{M}$ $\text{Fe}^{3+}$	$11 \pm 2$	23	4.3

Error values for CL measurements are based on three to four replicate analyses.

Table 4  
Measurements of labile  $\text{Cu}^{2+}$  by CL, total  $\text{Cu}^{2+}$  by GFAAS and total  $\text{Fe}^{3+}$  by ICP-OES in a Ross Creek Water Reservoir sample after various treatments

Sample	$[\text{Cu}^{2+}]$ (nM) CL	$[\text{Cu}^{2+}]_{\text{T}}$ (nM) GFAAS	$[\text{Fe}^{3+}]$ ( $\mu\text{M}$ ) ICP-OES
Original sample	$9 \pm 2$	280	0.23
+4 $\mu\text{M}$ $\text{Fe}^{3+}$	$16.4 \pm 2.5$	280	4.23
Acidified	$25.8 \pm 2.5$	360	1.05
Acidified, +4 $\mu\text{M}$ $\text{Fe}^{3+}$	$93 \pm 15$	360	5.05
Acidified, diluted 20 $\times$ , +4 $\mu\text{M}$ $\text{Fe}^{3+}$	$16.7 \pm 1.5$	18	4.05

containing 4  $\mu\text{M}$   $\text{Fe}^{3+}$ ) which is only 0.6% of the measured total dissolved  $[\text{Cu}^{2+}]$ , of 17 nM. However, ICP-OES analysis shows that the unacidified sample contains only 0.56 nM  $\text{Fe}^{3+}$ , which is not enough to saturate the  $\text{Fe}^{3+}$  enhancement effect, so this result will still be too low. After addition of 4  $\mu\text{M}$   $\text{Fe}^{3+}$  to the sample (second row of Table 3), the labile  $\text{Cu}^{2+}$  concentration increases to 0.2 nM, now 1.2% of  $[\text{Cu}^{2+}]_{\text{T}}$ .

The acidified sample aliquot shows a much increased  $\text{Fe}^{3+}$  concentration, presumably from the dissolution of colloidal  $\text{Fe}^{3+}$  that passed through the 0.45  $\mu\text{m}$  filter. It also shows an increase in  $[\text{Cu}^{2+}]_{\text{T}}$ , which we ascribe to adsorptive loss of some  $\text{Cu}^{2+}$  to the sample bottle of the unacidified sample. The acidified aliquot also shows a greatly increased apparent labile  $\text{Cu}^{2+}$  concentration, 10.2 nM which is 44% of  $[\text{Cu}^{2+}]_{\text{T}}$ , a result that implies that a significant fraction of the labile  $\text{Cu}^{2+}$  has been released from complexes with natural organic matter by acidification. Although the  $[\text{Fe}^{3+}]$  in this sample (2.8  $\mu\text{M}$ ) is below the threshold for saturation of the enhancement effect, the last two lines in Table 3 show that both addition of 4  $\mu\text{M}$   $\text{Fe}^{3+}$ , and more exact matching of  $[\text{Fe}^{3+}]$  to the desired threshold value of 4  $\mu\text{M}$ , both produce apparent labile  $\text{Cu}^{2+}$  concentrations that are the same, within experimental uncertainties.

Table 4 shows the application of the method to a very different fresh water sample. This was taken from a local water supply reservoir (Ross Creek Reservoir) 2 weeks after the reservoir had been supplied with  $\text{CuSO}_4$  to control algal growth. The original unacidified sample (first line of Table 4) showed a much higher apparent labile  $\text{Cu}^{2+}$  of 9 nM, and very high  $[\text{Cu}^{2+}]_{\text{T}}$  of 280 nM, consistent with the  $\text{CuSO}_4$  dosing. However, the  $\text{Fe}^{3+}$  concentration in this sample was too low for saturation of the enhancement effect, and after addition of 4  $\mu\text{M}$   $\text{Fe}^{3+}$  to the sample the labile  $\text{Cu}^{2+}$  concentration increased to 16 nM, about 6% of the measured  $[\text{Cu}^{2+}]_{\text{T}}$ . Acidification of this water sample produced a much smaller increase in labile  $\text{Cu}^{2+}$ , and again an increase in  $[\text{Cu}^{2+}]_{\text{T}}$  attributable to adsorption by the bottle of the unacidified sample (third row of Table 4). When the  $[\text{Fe}^{3+}]$  was adjusted to achieve saturation of the CL enhancement, a very high apparent labile  $\text{Cu}^{2+}$  concentration (93 nM) was observed.

We were concerned that this had such a high CL intensity that the counting system may have been saturated, so we diluted the acidified sample by twenty times, added 4  $\mu\text{M}$   $\text{Fe}^{3+}$  and re-analysed the diluted sample by CL. The result, shown in the last line of Table 4, indicates that most of the  $\text{Cu}^{2+}$  is labile and that the 93 nM result may be too low. However, we cannot preclude additional dissociation of  $\text{Cu}^{2+}$ -organic matter complexes as a result of the dilution. In any case, the results indicate a small labile fraction in the original sample (6%), but nevertheless one that is significantly larger than that observed in the Water of Leith. This may result from the much higher  $[\text{Cu}^{2+}]_{\text{T}}$  as a result of  $\text{CuSO}_4$  supplementing, which has elevated  $[\text{Cu}^{2+}]$  beyond the range that would normally be as readily complexed by natural organic matter ligands.

To date, we have only been able to make limited comparisons of our  $\text{Fe}^{3+}$ -enhanced CL method with other  $\text{Cu}^{2+}$  speciation techniques. Application of a cathodic stripping voltammetry technique using salicylaldehyde oxime as a competing ligand [14] showed that the Water of Leith sample in Table 3 contained <0.4 nM labile  $\text{Cu}^{2+}$ , which is at least consistent with our result of 0.2 nM. Sangi et al. [15] used the diffusion gradient in thin films method to study labile metals in the Water of Leith, showing that on average 5% of  $[\text{Cu}^{2+}]_{\text{T}}$  was labile according to DGT. Although there is no reason to expect different speciation techniques to give exactly the same answer, our method is consistent with the results of these and other studies which show that most  $\text{Cu}^{2+}$  in river and lake waters is bound to natural organic matter, and that the labile fraction is very small [16–19].

In summary, we have shown that several metal ions provide a useful enhancement to the chemiluminescence intensity of the  $\text{Cu}^{2+}$ -catalysed oxidation of 1,10-phenanthroline. Provided that the  $\text{Fe}^{3+}$  concentration of the water sample is controlled, this method can be applied to the measurement of labile  $\text{Cu}^{2+}$  in fresh waters.

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