
Spectrofluorimetric Determination of Copper (II) at Parts per Billion Levels and Its Application to Real and Environmental Samples.

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ABSTRACT:

A very simple, rapid, highly selective and ultrasensitive spectrofluorimetric method for accurate determination of copper (II) has been developed. The method is based on the instantaneous quenching action of Cu(II) on the native fluorescence of Bathophenanthrolinedisulphonate solution (4, 7- diphenyl -1, 10- phenanthroline disulphonate) having excitation and emission wavelength maxima at 288nm and 444.8nm respectively in the optimum pH-range of 2.5 - 4.2. The fluorescence intensity quenching maintains a rectilinearity in the range of 2 ppb-1ppm Cu(II). The S.D. and R.S.D. are ± 0.008 and 7.27% (11 determinations; 0.1ppm Cu) respectively. This method is rendered virtually Cu(II)- specific by using masking agents and has found remarkable success when applied to various real and environmental samples of certified analysis.

Copper is industrially important, environmentally pollutant, occupationally hazardous and biologically toxic and micronutrient¹. Copper toxicity causes sporadic fever, hypotension, haemolytic anaemia, cardiovascular collapse and even death². It is an essential part of several enzymes, e.g., phenolase, hemocyanin, ferroxidase and cytochrome oxidase. The enzyme phenolase and hemocyanin are capable of carrying oxygen as haemoglobin does. Therefore, its trace and ultra trace analysis is of paramount importance. Review on previous fluorimetric determination methods of Cu reveals that these are low sensitive, unselective and encountered with high interferences.³⁻⁶ The fluorimetric method being presented here is simpler, more selective and more sensitive than the previous ones.

Keywords – Spectrofluorimetry, Fluorescence quenching, Environmental Samples, Copper (II), Toxicity.

EXPERIMENTAL

Apparatus – A Shimadzu spectrofluorophotometer (Model RF-5000) fitted with a video accessory was used to record the uncorrected spectra and to measure the fluorescence intensity. A pH meter, Model pH 5651 (Electronic Corporation of India Ltd.), was used to measure pH. A Hanovia U.V lamp set was used for the qualitative visual study of fluorescence intensity changes.

REAGENTS

Copper (II) standard solution – A stock standard solution of copper (II) (1ng/ml) was prepared by dissolving 0.3929gm. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (BDH, Analar Grade) in water, adding 1ml of dilute sulphuric acid and diluting to 100ml in a 100ml volumetric flask. It was standardized by EDTA titration method.⁷ Bathophenanthrolinedisulphonate solution: A 0.1% (W/V) corresponding to $1.693 \times 10^{-3}\text{M}$ bathophenanthrolinedisulphonate) (Loba – Chemie Wein – Fischamend) solution was prepared in deionised water. This solution was stable for more than a month if preserved in refrigerator. From this stock solution, more diluted solutions were prepared as and when needed.

Other solution – A 1% (W/V) $\text{Na}_2\text{-EDTA}$ (Pro-analysis, E. Merck) was prepared in 100ml volumetric flask with deionised water. A 100ml solution of 0.2 N H_2SO_4 (95 – 98%, E. Merck) was also prepared in deionised water. A buffer solution of pH- 3.4 was prepared by mixing 5ml of 0.2M sodium acetate and 95ml of 0.2M acetic acid. A large number of solutions of inorganic ions and complexing agents were prepared from their Analar grade or equivalent grade water soluble salts. In case of insoluble substances, special dissolution methods were adopted.⁷ Doubly distilled demineralised water was used throughout the study.

PROCEDURE

To 1ml sample solution containing 0.02-0.2ug or 0.2-2.0ug or 2.0-10ug of copper (II) in a 10ml volumetric flask, were respectively added 0.1ml of 10^{-4} M or 0.3ml of 10^{-4} M or 1ml of 10^{-3} M bathophenanthrolinedisulphonate solution and 2ml of acetate buffer (pH 3.4). The volume was then made up to the mark with deionised water. Fluorescence intensity of the complex was then measured against a corresponding reagent blank at 444.8nm, keeping the excitation wavelength maximum at 288nm. The concentration of copper (II) in the unknown sample was determined with the help of a concurrently prepared calibration graph from standard copper (II) solution.

RESULTS AND DISCUSSION

Spectral characteristics – The uncorrected excitation and emission spectra of the bathophenanthrolinedisulphonate solution are shown in Fig.1. The wavelength maxima of excitation and of emission were found to occur at 288nm and 444.8nm, respectively.

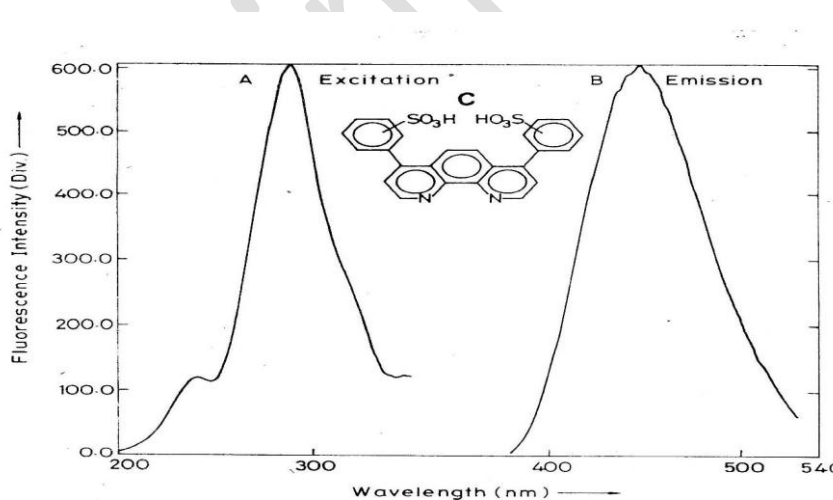


Fig. 1 Excitation (A) and emission (B) spectra of Bathophenanthrolinedisulphonate (4, 7-diphenyl -1, 10-phenanthrolinedisulphonate). (C) Structure of Bathophenanthrolinedisulphonate.

Effect of time – The maximum fluorescence quenching intensity was achieved just after 5 min of making up of the volume and remained unchanged for 24 hrs, longer than this time was not studied.

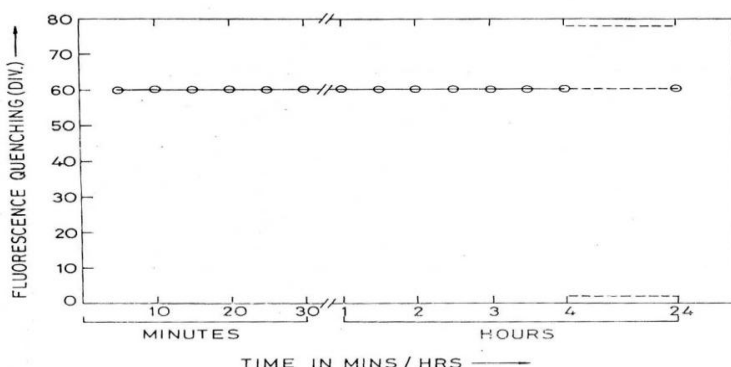


Fig. 2 Effect of time on the fluorescence quenching intensity of Cu(II) – Bathophenanthrolinedisulphonate system.

Effect of buffer solution – The study of the effect of buffer for range of 1ml to 5ml of acetate buffer added to the total 10ml volume of the metal – reagent system revealed that the fluorescence intensity remained unaltered. Consequently, 2ml of the buffer solution was used throughout the study.

Effect of pH – The study of the effect of pH while other conditions in the procedure remained the same, showed that the maximum and constant fluorescence quenching intensity occurred in the pH range 2.5 – 4.2 (Fig. 3).

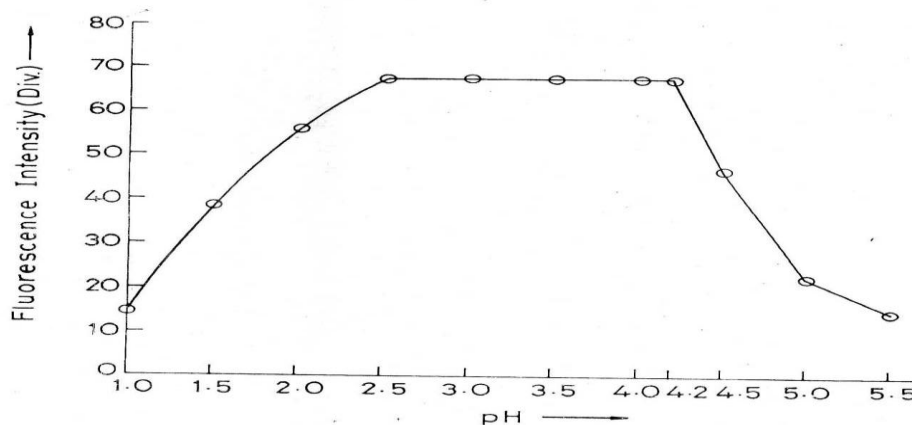


Fig. 3 Effect of pH on the fluorescence quenching intensity of Cu (II) – Bathophenanthrolinedisulphonate system.

Calibration Graph – The copper (II) – concentration versus fluorescence intensity calibration curve was rectilinear for a wide overall range of 2ppb – 1ppm Cu(II), distributed into 3 different sets (2-20ppb; 20-200ppb; 0.2-1ppm) for convenience of fluorescence intensity measurements. Typical calibration graphs after reproduction from the printer recorder are shown in Fig. 4.

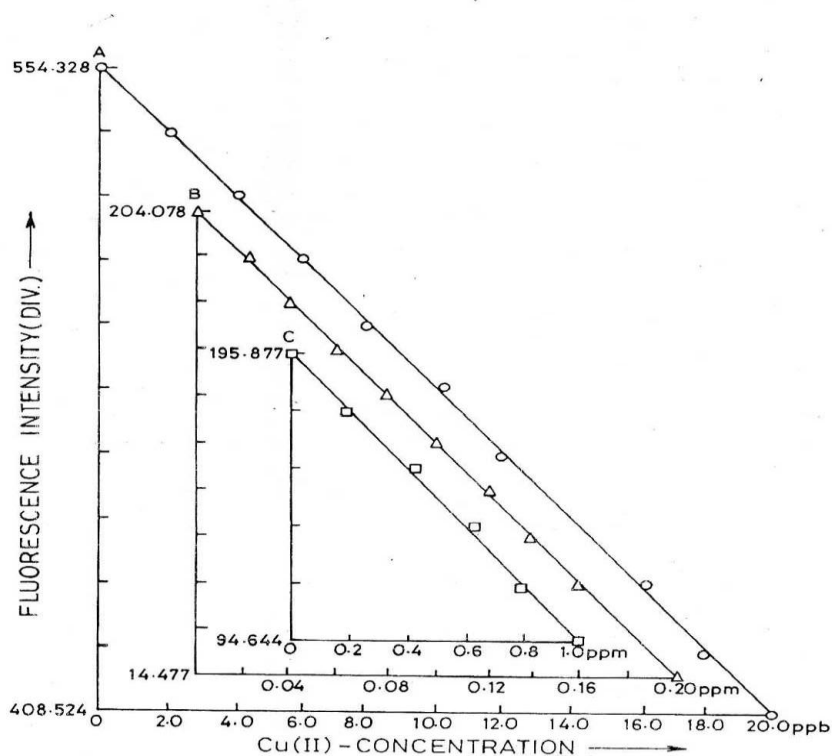


Fig. 4 Calibration graphs; (A) 2-20ppb, Cu(II); (B) 0.02-0.20ppm, Cu(II); (C) 0.20-1.0ppm, Cu(II). Band width: Ex.=3nm, Em.=3nm. Response (sec.): Auto; Sensitivity High; Degree L 1; Conc. Output Factor: 1.000

Interference study – The individual effects of over 60 cations, anions and some common complexing agents on the determination of 50ng/ml of copper(II) were examined separately by the proposed method. Less than 3% error by (W/W) ratio of foreign ions to Cu(II) added is the criterion of non-interference. Thus 1000-fold excess of alkali and alkaline earth metals, Mn (II), Se (IV) U (VI), Ta (IV), Al (III), Ag (I), Ce (IV), citrate, oxalate, tartarate, halides, thio-urea, ascorbate, chlorate, thiocyanate bisulphate, H₂O₂, biphosphate, sulphate and nitrate do not interfere. This is not the maximum tolerance limit of these foreign ions but the actual

amount studied. The actual maximum tolerance limits of the following ions are; 500-fold excess, Zn (II), Bi (III), nitrite; 200-fold excess, Th (III), Sn (II), Sn (IV), Nb (V), thiosulphate; 100-fold excess, As(III), Ti (III), W (VI) (in presence of tartarate), Mo (VI) (in presence of H₂O₂); 50-fold excess, Zr (IV), Tl (I), Sb (III), Sb (V), Tl (III), (in presence of citrate); 20-fold excess, Fe (III), Fe (II), V (V), La (III), Cr (III), Cr (VI); 15-fold excess, Co (II), Cd (II), Pb (II) (in presence of thiosulphate), Hg (II) (in presence of thiosulphate), Ni (II) (in presence of DMG).

APPLICATIONS

The proposed method was successfully applied to the determination of copper (II) in a series of synthetic mixture of various compositions, certified reference materials and environmental water samples. The results of analyses were in excellent agreement with their certified values. In case of environmental water samples of unknown compositions, the analyses were done in both Cu(II) –spiked and – unspiked conditions. These results are in agreement with those reported by AAS method. Tables I, II, III, & IV show these results.

Table I – Analysis of Synthetic Mixture.

Sample No.	Composition of the mixture (ppm)	Copper(II) content (ppm)	Copper(II) found ^a (ppm)	Recovery (%)
A	Cu ²⁺ (0.1), Zn ²⁺ (10), Mn ²⁺ (20), Ca ²⁺ (30), Mg ²⁺ (10)	0.100	0.101±0.000	101
B	As in A, S ₂ O ₃ ²⁻ (20), Pb ²⁺ (1), As ³⁺ (10)	0.100	0.101±0.001	101
C	As in B, Tartarate (50), W ⁶⁺ (2), Cr ⁶⁺ (1)	0.100	0.099±0.001	99
D	As in C, V ⁵⁺ (1), Al ³⁺ (10)	0.100	0.102±0.002	102
E	As in D, Fe ³⁺ (1), Ag ⁺ (50)	0.100	0.104±0.002	104

^aMean ± Standard deviation (n = 3)

Table II – Cu (II) determination in certified reference materials.

Composition of CRM (%)	Cu(II) content (%)	Cu(II) found ^a (%)	Recovery (%)
BAS 48bG Mo – ore Mo = 55.7 Cu = 0.56	0.56	0.60 ± 0.001	107
BAS 20b Cu = 4.10, Fe = 0.43, Mn = 0.19, Ni = 1.93 Si = 0.24, Mg = 1.61, Al = 90.50	4.10	4.43 ± 0.001	108
BCR 151 Cd = 0.021, Cu = 2.23, Fe = 11.8, Pb = 1.0, Hg = 0.0094	2.23	2.26 ± 0.001	101

^aMean ± Standard deviation (n = 5)

Table III – Analysis of Environmental waters

Sample	Composition by AAS (ppm)	Added	Amount of Cu(II), (ppm) found ^a	Recovery (%)
Factory Drain water	As ³⁺ = 0.01, Hg ²⁺ = 0.001, Pb ²⁺ = 0.01, Cr ³⁺ = 0.09,	0.00	0.035 ± 0.004	
A	Ni ²⁺ = 0.20, Ag ⁺ = 0.02,	0.05	0.087 ± 0.002	104

	Zn ²⁺ = 0.19, Na ⁺ = 56, Cu ²⁺ = 0.03, Cl ⁻ = 108, SO ₄ ²⁻ = 25	0.10	0.137 ± 0.002	102
B	As ³⁺ = 0.01, Hg ²⁺ = 0.01, Pb ²⁺ = 0.01, Cr ³⁺ = 0.04, Ni ²⁺ = 0.18, Ag ⁺ = 0.008, Zn ²⁺ = 0.19, Na ⁺ = 52, Cu ²⁺ = 0.02, Cl ⁻ = 105, SO ₄ ²⁻ = 23	0.00	0.023 ± 0.002	
		0.05	0.073 ± 0.001	100
		0.10	0.126 ± 0.004	103
C	As ³⁺ = 0.01, Hg ²⁺ = 0.01, Pb ²⁺ = 0.01, Cr ³⁺ = 0.01, Ni ²⁺ = 0.15, Ag ⁺ = 0.005, Zn ²⁺ = 0.32, Na ⁺ = 54, Cu ²⁺ = 0.01, Cl ⁻ = 106, SO ₄ ²⁻ = 24	0.00	0.015 ± 0.001	
		0.05	0.061 ± 0.001	92
		0.10	0.144 ± 0.003	99
D	As ³⁺ = 0.01, Hg ²⁺ = 0.01, Pb ²⁺ = 0.01, Cr ³⁺ = 0.005, Ni ²⁺ = 0.1, Ag ⁺ = 0.03, Zn ²⁺ = 0.12, Na ⁺ = 53,	0.00	0.014 ± 0.002	
		0.05	0.062 ± 0.001	96
		0.10	0.113 ± 0.001	99

	Cu ²⁺ = 0.01, Cl ⁻ = 105, SO ₄ ²⁻ = 23			
Pond water		0.00	0.005 ± 0.001	
		0.05	0.054 ± 0.001	98
		0.10	0.102 ± 0.001	97
Deep tube well water		0.00	0.013 ± 0.001	
		0.05	0.066 ± 0.004	106
		0.10	0.117 ± 0.002	104

^aMean ± Standard deviation (n = 3)

CONCLUSION

The method is simple, specific, ultra-sensitive, very accurate and precise. It is applicable to complex matrices. It is a single-step quick method that requires no clean-up.

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