# A Simple Flow Injection Spectrophotometric Procedure for Direct Determination of Copper(II) in Environmental Samples

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A new simple, rapid and sensitive flow-injection spectrophotometric detection method has been developed for the on-line determination of copper(II) in environmental water samples. The method is based on the measurement of the absorbance of the coloured complex formed by copper(II) with the Alizarin Red S (3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt) in an acidic medium. The optimum conditions for the reaction of Cu(II) with Alizarin Red S is studied and the complex is selectively monitored at $\lambda_{max}$ 510 nm. With the reagent carrier solvent ($3.5 \times 10^{-5}$ M Alizarin Red S solution and 0.1 M acetate buffer, pH 5.0) flow-rate of 1 mL min$^{-1}$. The calibration graph was linear in the Cu(II) concentration range 2-110 µg L$^{-1}$ with the detection limit of 0.6 µg L$^{-1}$ (RSD = 3.3 %, n = 6) was obtained at a sampling rate of 80 sample h$^{-1}$. The detailed study of various interferences confirmed the high selectivity of the developed method. The proposed method was successfully applied to determination of copper(II) in real samples including river water and sea water. The accuracy of the method was demonstrated by the analysis of standard reference material MBH-C31XB20.

Keywords: Flow-injection spectrophotometric method, Cu(II) determination, Alizarin Red S.

## INTRODUCTION

Copper is an essential element with an important role in living organisms and one of the main toxic elements in the environment. Except for natural sources like weathering of soil and mining operations.

Copper is released to environment also from anthropogenic ones: fertilizers, wood preservatives, fungicides, algicydes or corrosion of water delivery system made of copper. Increasing amount of copper compounds in environment is especially dangerous for water plants, fish and invertebrates which accumulate copper in organism and as the lower food chain link transfer copper to higher trophic levels increasing its uptake. Long-term exposition to high copper concentrations causes gastrointestinal or liver symptoms in humans[1]. Therefore, application of methods to control trace amounts of copper in environment is necessary.

Several methods such as graphite furnace atomic absorption spectrometr (GF-AAS)[2,3] inductively coupled plasma optical emission spectrometry (ICP-OES)[4,5] and ion selective electrode (ISE)[6,7,8] have been developed to determine the copper(II) ions in solutions depending on its concentration. However, some of them suffer from more or less time-consuming procedures and complicated instrumentation. Some of the previous

methods for spectrophotometric determination of Cu(II) have some disadvantages such as time-consuming, labor intensive and the use of large amounts of organic solvents that can increase the environmental pollution. The determination of metal ions in flow-injection systems have received a growing amount of attention in recent years since its simplicity, speed and versatility which allows the method to be used in conjunction with a variety of detection systems such as spectrophotometry[9,10] ISE[11], AAS[12-15], optical[16,17,9], electrochemical[18-20] and ICP-MS[21]. Copper(II) has been selectively determined in flow-injection systems by forming coloured complexes with chelating agents which often show a strong absorption at a characteristic wavelength. In analytical chemistry, anthraquinone derivatives have been widely used, mainly as strong chelating agents and chromophores. These chelates are then used in selective and sensitive determination of metals[22]. Therefore, an attractive alternative method that could determine trace amount of copper(II), rapidly, sensitive and conveniently, by using Alizarin Red S (ARS) chelating agent was studied. In this study, Alizarin Red S was used to react with Cu(II), forming a coloured, water-soluble complex. The reaction is very fast and the sensitivity of the reagent has been made selective by controlling the pH of the solution.

In our previous studies[23-26] we developed simple flow-injection analysis methods for the selective and sensitive determination of copper(II) in sea water and river water by using different complexing agents. In the present study, an ultra-sensitive and highly selective, simple and economical flow-injection spectrophotometric procedure based on Alizarin Red S (an anthraquinone derivative) (also known as 3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt Alizarin Red S (ARS) has been described for the determination of copper(II). In the procedure, the Alizarin Red S in the reagent carrier solution, selectively reacts with copper(II) forming a coloured complex with absorption maxima at 510 nm. The chemical structure of Alizarin Red S is shown in Fig. 1.
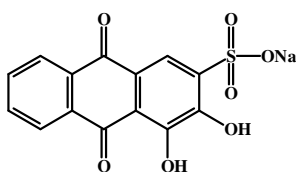


Fig. 1. Chemical structure of 3,4-dihydroxy-9,10-dioxo-2-anthracene-sulfonic acid sodium salt (Alizarin Red S)

The reagent itself is soluble in water and does not absorb in the visible region of the spectrum, therefore, might be well suited for flow-injection analysis of copper(II). The method was successfully applied for determination of copper in water samples and accuracy of the developed method was demonstrated by the analysis of certified reference materials.

## EXPERIMENTAL

All chemicals used in this experiment were of analytical grade. Deionized water was used throughout all experiments. A stock standard solution of copper(II) ion ($5 \times 10^{-3}$ mol L$^{-1}$) was prepared by dissolving 0.1247 g of CuSO$_4$·5H$_2$O (Merck) in 100 mL water. Copper working standard solutions were prepared daily by several dilutions of the stock standard solution with deionized water. Interference studies were carried out by using the chloride or nitrate salts of the metal cations. An alizarin Red S solution ($5 \times 10^{-3}$ mol L$^{-1}$) was prepared by dissolving 0.1711 g of the reagent (Merck) in 100 mL of water. The M-(ARS)$_n$ complexes were prepared by mixing equal volumes of 0.001 mol L$^{-1}$ standard solutions of each metal (M) in ethanol with 0.002 mol L$^{-1}$ Alizarin Red S in deionized water. Reagent carrier solution was composed of $3.5 \times 10^{-5}$ M Alizarin Red S in water 80 % and 0.1 mol L$^{-1}$ acetate buffer solution 20 %, pH 5.0. Standard reference material (C31XB20) was provided from MBH analytical Ltd. (UK).

A high-pressure liquid chromatography pump furnished with stainless-steel tubing was used to propel the samples and reagent carrier solutions. Samples (20 μL) were injected into the carrier stream by a rheodyne injection valve provided with a loop. The absorbance of the coloured complex formed ($\lambda_{max}$ = 510 nm) was measured with a UV-visible spectrophotometer equipped with a flow-through micro cell (Spectra SYSTEM UV 3000 HR, Thermo Separation Products, USA) and connected to a computer incorporated with a PC1000 software program.

UV-visible spectra of M-(ARS)$_n$ complexes were taken using a UV-visible spectrophotometer (GBC Cintra 20, Australia). A Perkin Elmer Model Analyst 700 flame atomic absorption spectrometer, equipped with a copper hollow cathode lamb operated at 30 mA, was used throughout this work. The wavelength and spectral band pass were set at 324.8 and 0.7 nm, respectively. An air-acetylene burner was used for the determination of copper(II). A Jenway 3040 model digital pH meter was used for pH measurements.

The flow injection analysis system used is simple and shown schematically in Fig. 2. The sample solution was introduced into the reagent carrier solution by the Rhodyne injection valve. The complex ($\lambda_{max}$ = 510 nm) was formed on passage of the reagent and copper(II) ion solution through the mixing coil. A PTFE tubing (50 cm long) was attached before the flow-through detection cell as a mixing coil. The absorbance of the coloured complex was selectively monitored in the flow-through spectrophotometric cell at 510 nm for complex. The transient signal was recorded as a peak, the height of which was proportional to the copper(II) concentration in the sample and was used for all measurements. Five replicate injections per sample were made.

For the determination of copper(II) in sea and river water samples, 50 mL of undiluted river water and sea water in nalgene plastics were filtered over a 0.45 mm filter from Millipore (Milford, MA) and then 20 μL of each filtered water sample was used as described in the procedure above.

**Spectrophotometric studies:** The reaction mechanism of the present method was reported as earlier[27]. Job's method of continuous variation and the molar ratio method were applied to ascertain the stoichiometric composition of the complex. A Cu(II)-ARS (1:2) complex was indicated by both methods. The reaction was very fast and highly selective for Cu(II). Copper(II) ions react with Alizarin Red S in aqueous medium in the pH range 3.8-5.5 forming coloured complexes with different stoichiometry. Absorption spectra corresponding to solutions of $2 \times 10^{-5}$ M of copper(II)-complex were recorded against all the other metal-Alizarin Red S complexes (Fig. 3). The average molar absorption coefficient of $4.15 \times 10^{4}$ L mol$^{-1}$ cm$^{-1}$ for Cu(II) was obtained.

As can be seen from Fig. 3, only copper(II) efficiently reacted with Alizarin Red S to give the copper(II) Alizarin Red S complex which exhibit an absorbance maxima at 510 nm. At this wavelength, the Alizarin Red S molecule itself has no absorption while the Alizarin Red S complexes of all the metal ions with the anions tested show a negligible absorption.

In order to develop an flow injection analysis method based on the above phenomenon, The flow injection analysis setup shown in Fig. 2 was used. In the flow injection analysis system, a coloured complex was formed with an absorption maximum at 510 nm, in agreement with the value obtained in the spectrophotometric study.

**Effect of concentration of Alizarin Red S:** The effect of concentration of Alizarin Red S in the reagent carrier solution on the peak height was examined in the flow injection analysis system using 0.85 and 4.80 mg L$^{-1}$ copper(II) solutions. The concentration of Alizarin Red S was varied over the range $1 \times 10^{-4}$ to $1 \times 10^{-6}$ M, at pH 5.0 and a flow rate of 1 mL min$^{-1}$. For each concentration level of copper(II) ion, peaks were maximal at a concentration of $3.5 \times 10^{-5}$ M. Therefore, the concentration of $3.5 \times 10^{-5}$ M of Alizarin Red S as colour developing reagent in the carrier solution was chosen.
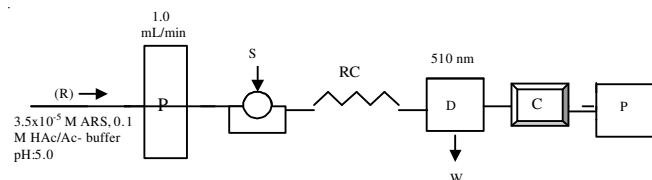
Fig. 2.   Flow diagram of the flow injection analysis system used. R; reagent carrier solution ($3.5 \times 10^{-5}$ M ARS in 0.1 M acetic acid-acetate-buffer (pH:5.0), P, LC pump, S; Rheodyne sample injection valve, RC; reaction coil (50 cm long, 0.5 mm i.d), D; spectrophotometric detector ($\lambda_{max}$ = 510 nm), W; waste, C; computer, P; print
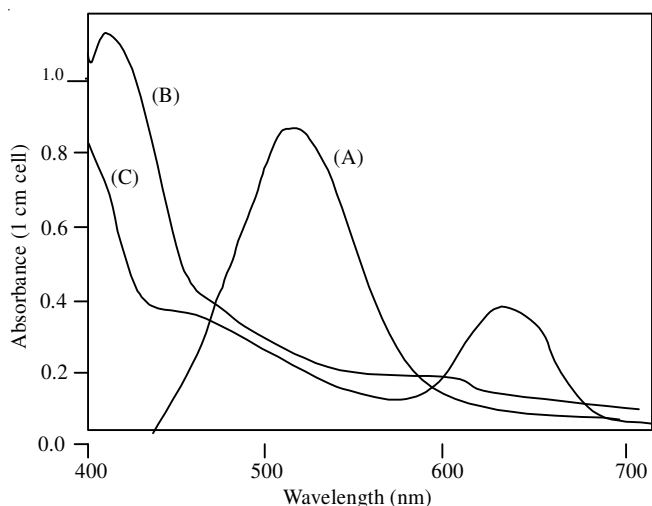


Fig. 3.   Absorption spectra of (A) Cu(II)-ARS complex against the reagent blank (B) Alizarin Red S (ARS) against deionized water (C). M-(ARS)$_n$. The concentration of the ARS and the metal ions (M) were $2 \times 10^{-5}$ and $1 \times 10^{-5}$ mol L$^{-1}$ respectively. [M: Fe(II), Fe(III), Zn(II), Pb(II), Cd(II), Ni(II), Co(II), Mn(II), Hg(II), Ag(I), Ca(II), Ba(II), Ag(I), Cr(III), Al(III)]

**Effect of flow-rate:** The flow-rate of reagent carrier solution was changed from 0.5 to 1.5 mL min$^{-1}$. The peak height decreased with increasing flow-rate. Taking into consideration of the stability of the pump, peak shape and sampling time, the flow-rate of the reagent carrier solution was adjusted to 1 mL min$^{-1}$.

**Effect of pH:** Using Alizarin Red S ($3.5 \times 10^{-5}$ M) in acetate buffer (as 0.1 M in the reagent carrier solution) system, the effect of the pH was examined over the range 3.8-5.5. In more acidic or more alkaline solutions, the peak height decreased because of incomplete complex formation and hydrolysis of the complex. As the peak height was not strongly affected by the pH at lower concentrations of Cu(II), the pH of the reagent carrier was adjusted to 5 to obtain maximum peak height. Therefore, a buffer solution of acetic acid-acetate solution (pH:5.0) was chosen throughout the study.

## RESULTS AND DISCUSSION

The absorbance maximum of the Cu-(ARS)$_2$ coloured complex at 510 nm can be applied for the selective determination of Cu(II) in the flow injection analysis system. Reagent carrier solution ($3.5 \times 10^{-5}$ M Alizarin Red S in water 80 % and 0.1 M acetate buffer solution 20 %, pH:5.0) was used. The introduction of Cu(II) ion to this reagent carrier solution from the injection valve produced a positive response on the

spectrophotometric detector, which was free from background absorption and the signals for Cu(II) ion show good reproducibility. The detector response to 20 μL injections of 2-110 μg L$^{-1}$ solution of Cu(II) varied linearly with concentration. Most of the ions examined did not interfere with the copper(II) concentrations up to at least 500-fold excesses.

**Applications:** To evaluate the applicability of the proposed flow-injection method, it was applied to the determination of copper(II) in river water and sea water samples. Seawater samples were collected from the coastal and harbor regions of the Black sea, river water samples were obtained from the Mert river and the Kürtün river in Samsun, Turkey. The water samples were collected in pre-washed polyethylene bottles. The samples were filtered through a 0.45 mm pore-sized millipore cellulose nitrate membrane. The samples were filtered and directly injected into the flow line. Both calibration curve and standard methods were carried out. The values obtained from the calibration curve and the standard addition methods are in good agreement with each other. The results for Cu(II) in water samples were also in good agreement with results obtained by the FAAS (Table-2).

The accuracy of the method was confirmed by analyzing C31XB20 standard reference material. The standard sample contains 0.18 % Sn, 0.21 % Pb, 0.035 % Fe, 0.21 % Ni, 0.15 % Al, 0.10 % Si, 0.20 % Mn, 0.115 % As, 0.09 % Bi, 0.05 % Sb, 60.8 % Cu. The results obtained were in good agreement with the certified Cu(II) concentration (Table-2).

**Effect of foreign ions:** The interference effects of many metal ions and anions by standard addition on the determination of 30 mg L$^{-1}$ copper(II) at fixed concentration were examined. The results were summarized in Table-1. The tolerance limits given in Table-1 are the concentrations that cause an error of less than ± 4 % in the peak height of a 30 mg L$^{-1}$ copper(II) solution. Most of the ions examined did not interfere with the Cu(II) determination up to at least 500 fold excesses. Ni(II), Co(II), Fe(III), Pb(II), Cr(III), Citrate, NO$_3^-$ ions interfered in some degree owing to the reaction with the reagent and those complexes show negligible absorption at 510 nm. Fe(III) and Cr(III) at the amounts of 900 μg L$^{-1}$, Ni(II), Co(II) and Pb(II) at the amount of 1000 μg L$^{-1}$, citrate and nitrate ions at the amounts of 800 mg L$^{-1}$ gave positive interference. The levels of this metals ions and anions, normally present in sea water and river water samples, were tolerable.

| TABLE-1 TOLERANCE LIMIT OF FOREIGN IONS ON THE DETERMINATION OF 30 mg L$^{-1}$ OF COPPER(II) IN SOLUTION | | | |
|---|---|---|---|
| Concentration (μg L$^{-1}$) | Cation | Concentration (μg L$^{-1}$) | Anion |
| > 5000 | Cr(III), Al(III), Cd(II), Mn(II), K(I), Na(I), g(I), Ca(II), Mg(II), Ba(II), Hg(II) | > 5000 | CN$^-$, NO$_2^-$, SO$_4^{2-}$, F$^-$, CO$_3^{2-}$, Cl$^-$, Br$^-$, PO$_4^{3-}$, NH$_4^+$, SCN$^-$, tartrate, oxalate, citrate |
| > 800 | Ni(II), Fe(III), Cr(III), Pb(II), Co(II) | > 700 | Citrate, nitrate |

**Analytical performance characteristics:** Analytical performance characteristics of the method were evaluated under optimum conditions. Under the optimum working conditions,

| TABLE-2 |
| --- |
| DETERMINATION OF Cu(II) IN RIVER AND SEA WATER SAMPLES[a] |

| Sample | Copper(II) in sample[b] (µg L$^{-1}$) | | | |
| --- | --- | --- | --- | --- |
| | Found[c] | Found[d] | Certified | FAAS |
| Seashore water | | | | |
| Seaport | 84.12 (0.03) | 83.22 (0.10) | | 86.25 (0.05) |
| Atakum | 23.76 (0.12) | 23.45 (0.22) | | 23.95 (0.07) |
| River water | | | | |
| Mert | 43.32 (0.06) | 42.12 (0.18) | | 44.37 (0.14) |
| Kurtun | 28.47 (0.08) | 28.96 (0.24) | | 30.15 (0.09) |
| Certified reference material | | | | |
| MBH-C31XB20 | 40.25 (0.61) | 40.12 (0.18) | 40.00 | |
| (a) Samples were collected in Samsun, Turkey, (b) Values in paranthesis are the relative standard deviation for n = 6 with a confidence of 95 %, (c) Calibration curve method, (d) Standard addition method | | | | |

calibration graphs were prepared from the results of triplicate measurements of copper(II) standard solutions of increasing concentration. The calibration graph showed a good linearity from 2-110 mg L$^{-1}$. The correlation coefficient of 0.9929 with the regression equation $Y = 0.2926X + 0.5896$, where Y is the peak height (cm) and X is the concentration of copper(II) in mg L$^{-1}$ showed that this calibration curve had good linearity. The detection limit (LOD) and the limit of quantitation (LOQ) 28 were found to be 0.6 mg L$^{-1}$ and 2 mg L$^{-1}$, respectively. Relative standard deviation (RSD) of the method based on six measurements of 50 mg L$^{-1}$ copper(II) was 3.3 % for a 20 mL injection volume and obtained at a sampling rate of 80 sample h$^{-1}$. To evaluate the applicability of the proposed flow-injection spectroscopy method, it was applied to the determination of Cu(II) in river water and sea water. The standard reference material (MBH-C31XB20) was also used for method validation. After dissolving reference material sample with concentrated mineral acid, their aqueous solutions were prepared. The results was obtained were shown in Table-2. Good agreement was found between analytical value and certified reference value. Therefore, the proposed technique may be applied to the determination of Cu(II) in real samples.

## Conclusion

Alizarin Red S is a good spectrophotometeric reagent for the determination of copper(II). The method is fast, simple, sensitive and more selective than most reported methods. The proposed method allows to measure copper(II) concentrations directly in the sample without a matrix separation step. The method was successfully applied to the determination of copper(II) in different types of water samples including river, sea and industrial waste water samples. In addition to the simplicity of the method, the reagent used is commercially available and does not have a risk of serious toxicity, thus enhancing the potential applicability of the method for copper(II) analysis in real samples, having good recovery, sensitivity, selectivity and rapidity. The results obtained by the recommended method are in excellent agreement with those determined by FAAS and standard reference material. An automatic procedure should be attractive for routine analysis.

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