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Flow Injection Spectrophotometric Determination of Copper in the environmental and biological samples using iodine-starch complex as chromogenic agent

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Abstract

A simple and facile flow injection spectrophotometric method was developed for the determination of copper (II) in aqueous and biological samples. The method is based on the oxidation – reduction reaction between copper (II) and iodide ion in acidic medium to form iodine, the released iodine reacts with soluble starch solution to form iodine starch blue complex which is detected at 600 nm wavelength .Chemical and physical parameters were investigated. The calibration curve obeyed Beer's law between (1.0– 25 µg.ml^{-1}) with a limit of detection 0.6 µg.ml^{-1}.The relative standard deviation was 0.62% for 10.0 µg.ml^{-1} copper solution (n=8) with sampling rate of 35 samples / h

الخلاصة

طريقة طيفية ضوئية جديده بسيطة وسهلة لتقدير ايون النحاس الثنائي في العينات المائية والبايلوجية باستخدام تقنية التحليل بالحقن الجرياني المستمر.ان طريقة التقدير تعتمد على تفاعل الاكسدة والاختزال بين ايون النحاس الثنائي وايون اليوديد في وسط حامضي حيث يقود التفاعل الى تكوين محلول اليود الاحمر والذي بدوره يتفاعل مع محلول النشأ الذائب لتكوين معقد النشأ – يود الازرق الذي يتم كشفه عند الطول الموجي 600 نانومتر.جميع المتغيرات الكيمياوية والفيزياوية المستخدمة في هذا التفاعل تم دراستها تماماً.منحنى المعايرة يخضع الى قانون بير – لامبرت ضمن المدى 1.0 – 25 مايكروغرام . مل$^{-1}$ وبحد كشف يصل الى 0.6 مايكروغرام . مل$^{-1}$. الانحراف القياسي النسبي كان 0.62 % عند تركيز مقداره 10.0 مايكرو غرام . مل$^{-1}$ من محلول ايون النحاس الثنائي لـ ـ ـ ن=8 . ان معدل قياس عدد النماذج بالساعة كان 35 نموذج.ساعه$^{-1}$.

Introduction

Copper is available in nature in a free state in the form of sulfides, chlorides and carbonates. Copper is utilized in electric industries and in industrially useful alloys (1-3).Biologically, copper plays an important role in carbohydrate and lipid metabolism (4-6) and is vital and toxic for many biological systems depending on it's concentration (4,7).It's essential in small amounts for synthesis of hemoglobin and is essential constituents of about thirty enzymes and glycoprotein and it also promotes iron absorption in the gastrointestinal system. Copper is involved in the transportation of iron from tissues into plasma. It helps to maintain the myelin in the nervous system which is important in the formation of bone and brain tissues and it's necessary for other many important functions. Copper deficiency causes disease such as anemia while it's excess causes "Jaundice" and "Wilson Disease". As a pollutant, copper is of particular concern because of its high degree of toxicity of aquatic organisms. because of that, determination of trace amounts of copper is becoming increasingly in environmental pollution(8-12).

Several analytical techniques have been monitored for the determination of copper(II) in different matrices, they include spectrophotometry (14-17), chemiluminescence (18), atomic absorption spectrometry (19), Inductive coupled plasma –atomic emission spectrometry (20), Neutron activation analysis (21) and flow injection analysis (22,23).

In this paper, a simple, sensitive and rapid flow injection spectrophotometric method for the determination of copper in different matrices. It is based on the oxidation of potassium iodide (KI) in acidic medium to form iodine and the latter reacts with soluble starch solution to form iodine starch blue complex which is detected at 600 nm.

Experimental

Chemicals

Distilled water and analytical –grade reagents were used throughout without further purification.

- The standard stock solution of copper (II) 1000 µg ml^{-1} was prepared by dissolving 3.9280 g of $CuSO_4.5H_2O$ (BDH, UK) in distilled water in a 1000- ml volumetric flask. Working solutions were prepared by appropriate dilution of the stock solution with distilled water.
- Potassium Iodide 1 mol. L^{-1} was prepared by dissolving 16.6 g of KI (BDH, UK) in distilled water in a 100-ml volumetric flask .working solutions were prepared by appropriate dilution of the stock solution with distilled water.
- Hydrochloric acid 1.0 mol. L^{-1} was prepared by dilution 93 ml of concentrated hydrochloric acid (BDH-UK) in distilled water in 1-Liter volumetric flask. Working solutions were prepared by appropriate dilution of the stock solution with distilled water.
- Soluble starch solution 1% W/V (SIGMA-USA) was prepared by dissolving 1.00 g of starch in 100 ml hot distilled water.

Apparatus

- UV-Visible Spectrophotometer Computerized (Optima, Japan) with 10.mm flow through cell (Helma, USA) was used for absorbance measurements at 600 nm.
- 2500 FIAlab Toolkit was equipped by Fialab Corporation (Washington , USA)
- V-450 Upchurch 6-Port Injection Valve (Upchurch , USA)
- Peristaltic Pump Reglo Analog ,4-Channals (ISMATEC – Switzerland)

Procedure for the digestion of river water sample(16)

The river water sample (150 ml were stored at 0–5 °C in polyethylene bottles. The river water sample was digested by 10 ml of aqua regia and heated to near dryness. Then the sample was mixed with 5 ml of concentrated sulfuric acid and heated for 30 min. The solution was then diluted and neutralized with NaOH. The water sample was filtered through a Whatman filter paper No. 41 to remove the suspended solids and the clean solution was collected into a 250 ml beaker. The contents were diluted up to the mark with double distilled water. 15 ml of this solution was further diluted to obtain a working solution for the determination of Cu(II) as described in the proposed method and compared with the reference method (25).

Procedure for the digestion of blood serum sample(24)

A 5.0 ml of the blood serum was digested by 5.0 ml of concentrated HNO_3 and heated to near dryness and then sample was diluted with 50 ml distilled water and boiled for 15 min and the residue of the solution was diluted and neutralized with NaOH .The sample was filtered through a Whatman filter paper No.41 and 15 ml of this solution was further diluted to obtain a working solution for the determination of Cu(II) as described in the proposed method and compared with the reference method(25).

Methodology

Depending on the flow diagram manifold shown in figure 1, sample loop of Upchurch injection valve loaded with copper sample with volume (75 µl).The carrier stream was hydrochloric acid 0.8 mol. L^{-1} at flow rate of 1.5 $mL.min^{-1}$,the latter passed through injection valve to load copper sample meet with potassium iodide (KI) to form iodine (I_2) and mixed in the reaction coil (1) for completion of the reaction and released iodine meets with starch to form iodine starch blue complex and they are mixed at reaction coil (2) for completion of formation iodine starch blue complex and passed through the flow through cell detected at 600 nm.

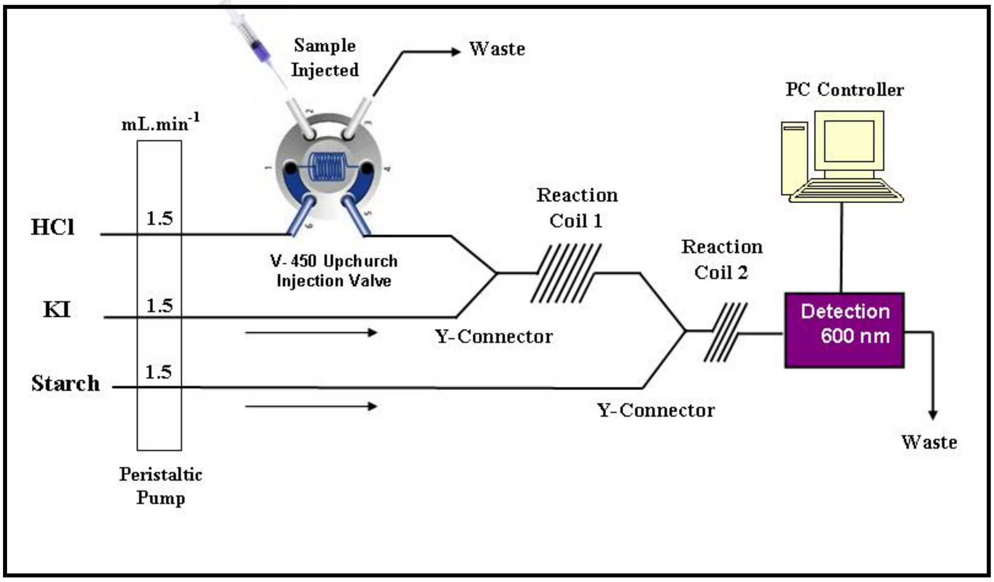

Fig 1: Flow diagram manifold used for the determination of Copper

Results and Discussion

The overall reaction for the determination of Copper is

$$2CuSO_4 + 4KI\ (excess) + H^+ \longrightarrow 2CuI + I_2 + 2K_2SO_4$$

In this reaction, Cu(II) is oxidizing iodide in acidic medium to form iodine and the released iodine reacts with starch to form iodine starch blue complex .It gives a broad band of absorption with λ_{max} is 600 nm .Fig 2 shows the absorption spectra of iodine starch blue complex formed from the system Cu (II) – I^- - H^+ - starch. By studying the chemical and physical parameters that affecting the determination of copper, higher intensities and best response can be obtained.

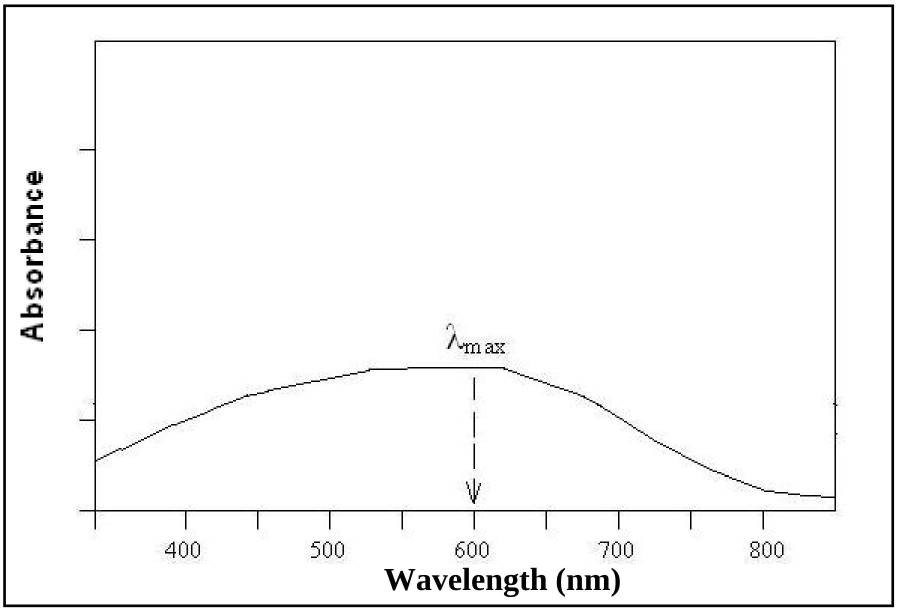

Fig 2: Absorption Spectrum of the iodine starch complex that it formed from the system (Cu (II) - I^- - H^+ - starch)

Choice of acid and effect of it concentration

A series of inorganic and organic acids (HCl, H_2SO_4 and CH_3COOH) were used at various concentrations 0.2, 0.4, 0.6 and 0.8 mol. L^{-1} using 0.5 mol . L^{-1} of KI , 1% W/V starch solution and 50 µl of copper sample volume. A concentration of Cu(II) was injected at flow rate of 1.5 $mL.min^{-1}$. Nitric acid was avoided as it affected the release of iodine .Fig. 3 shows the variation of absorbance versus concentration of acids (mol. L^{-1}) and because of hydrochloric acid gives high values of intensities; therefore, hydrochloric acid can be selected as a best acid and gives best concentration for this system.

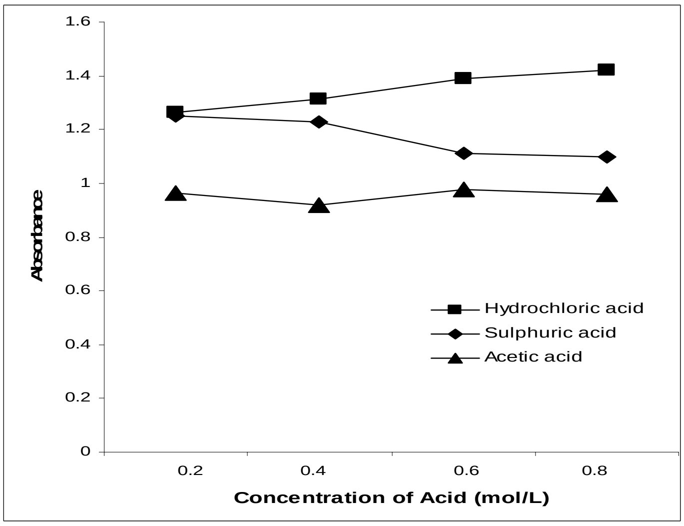

Fig 3: The variation of absorbance versus concentration of acid (mol. L^{-1}) using Cu (II) – I^- - H^+ - starch

Effect of Concentration of Potassium Iodide

Various concentrations 0.2 mol. L^{-1} – 1 mol. L^{-1} of KI were investigated at 1.5 ml.min^{-1} flow rate of carrier stream using 20 µg. mL^{-1} of Cu (II), 0.8 mol. L^{-1} of HCl and 1% W/V soluble starch solution. The Cu(II) solution was injected at 50 µl sample loop; therefore, 1.0 mol. L^{-1} of KI was selected as an optimum value. Fig 4 shows the variation of absorbance versus concentration of KI (mol. L^{-1}) by using Cu (II) – I^- - H^+ - starch system for the determination of copper.

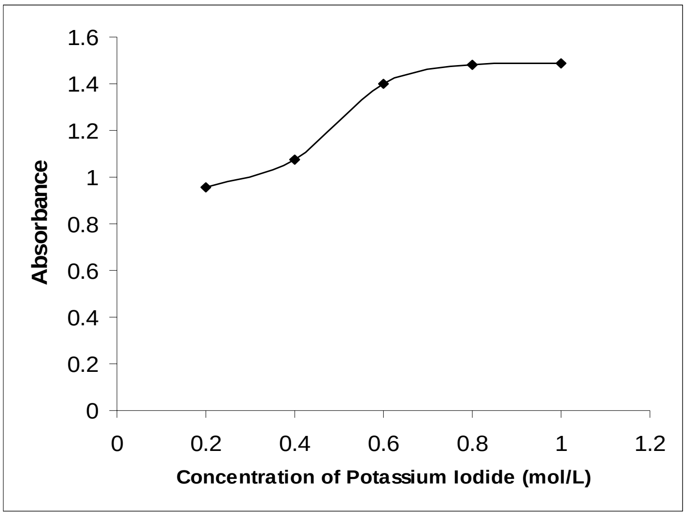

Fig 4: The variation of absorbance versus concentration of KI (mol. L^{-1}) by using Cu (II) – I^- - H^+ - starch system

Effect of flow rate

The effect of flow rate was investigated in the range 0.5 mL.min^{-1} – 2.0 mL.min^{-1} using 20 µg. mL^{-1} of Cu (II), 0.8 mol. L^{-1} of HCl, 1.0 mol. L^{-1} of KI and 1% W/V soluble starch solution. The flow rate of 1.5 mL.min^{-1} gave the highest response .Fig 5 shows the variation of absorbance versus the total flow rate for the system.

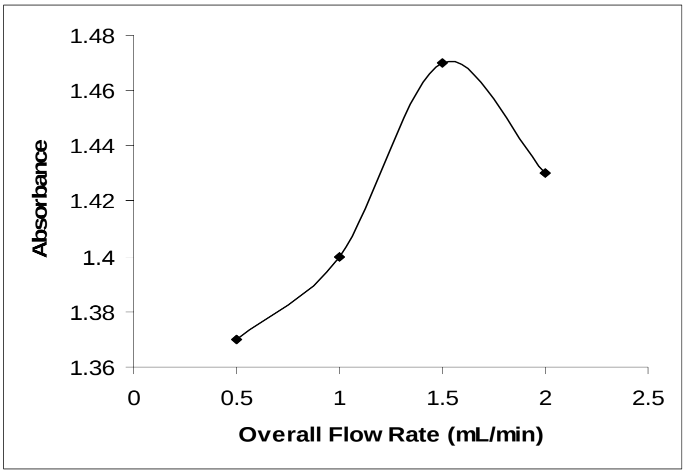

Fig 5: The variation of absorbance versus the flow rate of the carrier stream using Cu (II) – I^- - H^+ - starch system for the determination of copper.

Effect of Sample Volume

The effect of sample volume was studied as a set of injected volumes of copper (II) varied between 40 – 150 µl by changing the length of sample loop. Volume of 75 µl of copper (II) sample was selected as an optimum value while flow rate at 100 µl and 125 µl were avoided because they gave a broad peaks .Fig 6 shows the variation of absorbance versus copper (II) volume.

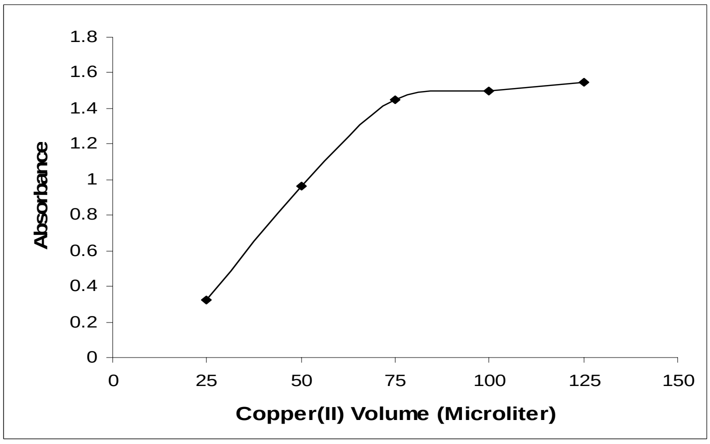

Fig 6 : The variation of absorbance versus copper (II) volume (µl) using Cu (II) – I^- - H^+ - starch system for the determination of copper.

Calibration Curve and Statistical Data

Under optimum chemical and physical conditions described in the previous sections , the calibration curve for the determination of copper shown in fig.7 obeys Beer's law between (1.0 – 25 µg.ml^{-1}) Cu(II) with correlation coefficient 0.9944 and the sampling rate was 35 sample / hour. The calibration curve can be represented by a linear regression equation(26) Y = 0.0646 [X] - 0.0155 Where Y and X refer to absorbance and concentration of copper (II), respectively. Fig 7 shows the variation of absorbance versus the concentration of copper (II) (µg.ml^{-1}).

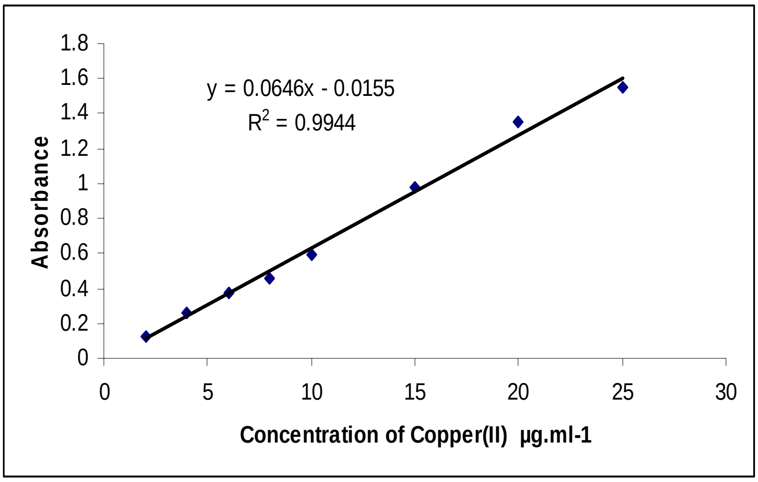

Fig 7: The calibration curve for the determination of copper (II)

The limit of detection(26) (based on 3 δb of the blank) was 0.6 µg.ml^{-1}.The relative standard deviation was found to be 0.62 % (N= 8) at 10.0 µg.ml^{-1} of copper (II) concentration. Table 1 tabulates the average, standard deviation and the confidence limit at 95% (26) for (n-1) for the repeatability of the copper (II)

Table 1: Mean, standard deviation and confidence Limit at 95% for the determination of Copper using proposed method.

[Cu^{++}] µg. ml^{-1}	N	$\overline{X}$	σ_{n-1}	% RSD	Confidence Limit at 95% $\overline{X} \mp t_{0.05} \frac{\sigma_{n-1}}{\sqrt{n}}$
4	8	0.266	0.0022	0.82	0.266 ± 0.0018
10	8	0.596	0.0037	0.62	0.596± 0.0031

Effect of Interfering Ions

The effect of cations and anions for the determination of copper (II) at 5 µg.ml^{-1} was studied. Table 2 shows the effect of foreign ions on the determination of copper (II). Alkali, earth alkali and some transition metals not interfere with determination of copper and some metals that do have oxidizing or reducing properties will interfere with determination of copper. These interfering ions can be removed by anion or cation exchange resin or by using masking agents where the interfering ions are masked by adding 2.0 ml of EDTA solution with the addition of tartrate or citrate at pH 8-9.

Table 2: The effect of interfering ions on the determination of copper (II)

[Ion] µg.ml^{-1}	**% Interference**										
	Na^+	**Ca^+**	**Ni^{2+}**	**Fe^{3+}**	**Bi^{3+}**	**Pb^{2+}**	**ClO_4^-**	**$S_2O_3^=$**	**$SO_4^=$**	**Cl^-**	**NO_3^-**
2-Fold	0. 23	1.3	5.47	108.3	59	73	148.3	-66.5	4.2	2.35	129.2

Application of Proposed Method in Environmental and Biological Samples

The proposed method was applied for the determination of copper in environmental and biological samples, the recoveries of known concentrations of copper (II) added to these samples were investigated. To 10 ml aliquots of clear river water and 5 ml of whole blood , 4.0 and 10.0 µg.ml^{-1} were added then these samples were passed through anion exchange resin to remove anion oxidizing agents (NO_3^-, NO_2^-,ClO_4^-) and then 2ml of EDTA added with tartarate and citrate at PH 8-9 to remove effect of interferences Fe^{3+} , Bi^{3+} and Pb^{2+} .The concentration of copper (II) was determined by proposed method and compared with reference method (25) .Table 3 shows the results obtained from proposed and reference method for the determination of copper in environmental and biological samples. Table 4 shows the statistical treatments for the results for proposed and reference methods.

Table 3: The comparison of results of proposed method with reference method (25)

Sample	$[Cu^{2+}]$ added µg.ml^{-1}	Proposed Method			Reference Method		
		$[Cu^{2+}]$ Found µg.ml^{-1}	% Error	%Recovery	$[Cu^{2+}]$ Found µg.ml^{-1}	% Error	%Recovery
River Water	4	4.22	5.5	105.5	4.13	3.25	103.25
	10	10.09	0.9	100.9	10.3	3	103
Whole Blood	4	3.9	-2.5	97.5	3.5	-12.5	87.5
	10	10.43	4.3	104.3	10.21	2.1	102.1

Table 4: The statistical characteristics[(26)] between proposed method and reference method

Sample	$[Cu^{2+}]$ added µg.ml^{-1}	Proposed Method	Reference Method	di	$\overline{X}$ d	Sd	$\frac{\overline{X}d\sqrt{n}}{Sd}$	t_{tab} at 95% Confidence Limit
		$[Cu^{2+}]$ found µg.ml^{-1}	$[Cu^{2+}]$ found µg.ml^{-1}					
River Water	4	4.22	4.13	0.09	0.125	0.256	0.976 << 3.182	
	10	10.09	10.3	-0.21				
Whole Blood	4	3.9	3.5	0.4				
	10	10.43	10.21	0.22				

Conclusions

The proposed spectrophotometric flow injection method is simple and precise and has high sensitivity for the determination of copper in environmental and biological samples. Student's t- test shows there is not any significance difference between two methods and has less standard deviation; therefore, it can be used as an alternative method for the determination of copper. The proposed method has additional advantages over the reference method due to low consumption of reagents, low cost and speed of analysis and minimal interferences after removal them by cation and anion exchange resins or masking them by adding know amount of EDTA solution with the addition of tartrate or citrate at pH 8-9.

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