

Analysis and Cloud Point Extraction of Trace Copper (II) in Urine of Occupational Workers

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For pre-concentration, cloud-point extraction method is used to extract and determine the trace amount of copper element present in urine samples and analyzed by using UV-visible spectrophotometry and flame atomic absorption spectrometry techniques, when copper reacted with salicylaldoxime by using Triton X-114 as non-ionic surfactant to form the complex. The most important factors studied in present work are the order of additions, pH values, the concentration of salicylaldoxime, temperature and appropriate time of extraction process. Other factors studied were the effect of types and concentration of Triton X-114 as non-ionic surfactant. The scatter curve was in the range of 0.50-16.00 $\mu\text{g/L}$ with $r^2 = 0.9979$ for UV-visible spectrophotometer ($\lambda_{\text{max}} = 380 \text{ nm}$). The LOD was 0.103 $\mu\text{g/L}$. The RSD for six replicates was found to be 4.917 %. This method was applied successfully to determine copper(II) contents in 44 urine samples of occupational workers.

Keywords: Cloud point extraction, Copper, Triton X-114, Urine samples.

INTRODUCTION

The pre-concentration methods include cloud point extraction, membrane filtration, solid phase extraction and liquid-liquid extraction [1]. The cloud point extraction (CPE) method was used for several reasons as high recovery efficiency and high concentration coefficient [2]. The CPE method can be considered as alternative to usual extraction systems for several reasons, the most important is the low cost and simplicity of high efficiency and is one of the ways in which the safety conditions are available [3]. This technique can be considered an excellent method which is compatible with green chemistry [4-6].

Copper is one of the basic elements which have several important roles in the human body [7]. Copper has important and effective roles, including in maintaining the function of the immune system [8]. Either decrease or increase in the concentration of copper in the body has negative effects on human health and results in anemia, leucopenia, liver and brain formation system [8,9]. The literature survey of cloud point extraction of Cu(II) by coupling with different analytical technique includes UV-visible spectrophotometer [10-12], flame atomic absorption spectrometry [13-17], inductively coupled plasma mass spectrometry (ICP-MS) [18,19], etc.

The pre-concentration of iron(III) in urine samples of occupational workers prior measured by using flame atomic absorption spectrometry and UV-visible spectrophotometer [20] and determination of bismuth(III) based on the reaction with fuchsine reagent and sodium dodecyl sulfate as a surfactant compound to form red ion-pair complex as turbid phase by using batch turbidimetric method [21] are also reported. The aim of this study was to develop of friendly spectrometric method of determination of Cu(II), using salicylaldoxime as a complexing agent and cloud point extraction. UV-visible spectrophotometer and flame atomic absorption spectrometry used as detection technique and the proposed method is also applied to determine copper(II) concentration in urine of occupational worker samples.

EXPERIMENTAL

The detection system used to determine copper(II) ion was Shimadzu double beam UV-VIS spectrophotometer, UV-1800, Japan equipped with Deuterium and Tungsten Lamps and using 1 cm quartz cells (1 mL) for all the determinations. Flame atomic absorption spectrophotometer (FAAS) (Buck scientific model 210 VGP, USA) with deuterium background correction equipped with 10 cm of air/acetylene flame burner

head and hollow-cathode lamp that can be changed with metal ion. The pH value in this study was adjusted and measured by using a pH meter WTW (model 720) with a combined glass electrode. A Hettich centrifuge (model EBA-20, Germany) with 10 mL calibrated centrifuge tubes was used for phase separation at 3600 rpm for 5 min. A Lab Line Super mixer (model 129) Hitech Trader, U.S.A. was used to mix the solutions.

All the chemicals used in this work were of high purity. The deionized water was used to prepare all chemical solutions. Salicylaldehyde (1 % w/v) reagent was prepared by dissolving 1 g salicylaldehyde in 5 mL of ethanol followed by the addition of hot deionized water. Solutions of non-ionic surfactant Triton X-114 have been prepared at 20 % (v/v) by diluting 20 mL of Triton X-114 to 100 mL hot deionized water in a volumetric flask. A stock solution of $1000 \mu\text{g}/\text{mL}^{-1}$ of Cu(II) was prepared by dissolving 0.3800 g of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ in 100 mL deionized water.

Samples: A 44 urine samples were collected from occupational workers associated with several work such as dyeing, welding, casting alloys, workers in oil refineries. The urine sample was collected in a new polyethylene bottles (120 mL) and stored 0-5 °C in the fridge [21,22].

Urine sample digestion: Digestion of urine samples were carried out according to the reported methods [23,24]. In a 100 mL beaker, 25 mL of urine sample accurately measured, was treated with a mixture of 5 mL H_2O_2 and 2.5 mL of conc. HNO_3 and then heated on a hot plate until the dark color turns into light color. Continue the process of heating the samples until the sample become completely dried. Thereafter, a dry residuum having dark colour is added 2.5 mL of conc. HNO_3 and are heated again to the point of drought, this step is repeated several times until a white ashes are obtained. The white ashes are dissolved in the final step by using 2.5 mL of 3M of HCl.

Cloud point extraction procedure: An aliquots of 10 mL of $10 \mu\text{g}/\text{mL}$ of Cu(II) was added to a 0.1 mL of 1 % (w/v) of salicylaldehyde, a light green colour solution having λ_{max} 380 nm. A 0.5 mL of 20 % (v/v) Triton X-114 was added and diluted to about 10 mL by deionized water, and then finally mix the solution thoroughly. The mixture was kept in a water-bath at 75 °C for 10 min for equilibrium and the separation into two phases was achieved by centrifugation for 5 min at 3500 rpm and cooled in an ice-bath at 0-5 °C for 5 min to increase the viscosity of the surfactant rich phase which can be separated easily by separating funnel. The surfactant rich phase was dissolved and diluted to 0.75 mL by using absolute ethanol and transferred into 1.0 cm quartz cell (1 mL). The absorbance of solution was measured against a blank solution prepared in the same method.

Absorption spectra of Cu(II)-SAO complex: The maximum absorption wavelength for salicylaldehyde was shown at λ_{max} 303 nm while copper (II) solution has 296 nm, upon the absorbance spectra of Cu (II)-SAO complex was 380 nm. It is shifting of about < 80 nm as compared to salicylaldehyde and copper(II) solution spectra.

RESULTS AND DISCUSSION

Optimization of experimental conditions: To achieve the best performance for cloud point extraction procedure, the

effects including order addition, pH, concentration of reagent, types of surfactant, concentration of surfactant, equilibrium temperature, time of incubation, salt out and interfering ions were evaluated and optimized.

Effect of order of addition: The effect of order of additions of copper complex formation by using 10 mL of $10 \mu\text{g}/\text{mL}$ of Cu(II) solution and 0.1 mL of 1 % w/v of salicylaldehyde with 0.5 mL of 20 % v/v Triton X-114 was mixed as shown in Table-1. It is clear that the first test showed the greatest absorbance value.

Type of complex	Order addition	A_{aq}	A_{s}
Cu(II) complex	M + R + T	0.036	2.071
	R + M + T	0.099	1.823

Effect of pH: The extraction of copper(II) was completed by using pH range from 3.50 to 11.50. Fig. 1 shows the effect of pH on the absorbance of Cu(II)-salicylaldehyde complex. The best result of absorbance on Cu(II)-salicylaldehyde complex was achieved at pH 4.2, which is without acid or base added to complex solution. While the complexation reaction at pH values lower than 4.2 is incomplete reaction between copper (II) and salicylaldehyde oxime reagent may be attributed due to the decomposition of complex formation because of the protonation of salicylaldehyde but at pH value higher than 4.2 resulted in the hydrolysis of Cu(II).

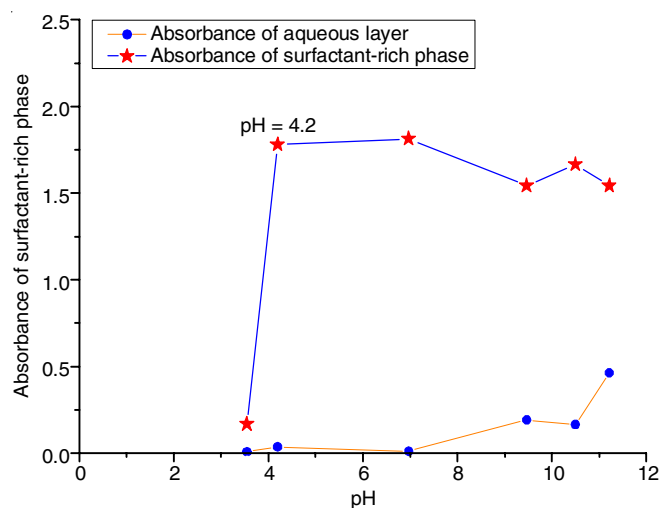


Fig. 1. Effect of pH on the absorbance of Cu(II)-SAO complex

Effect of reagent concentration: A series of salicylaldehyde (0.01-1.00 mL) of 1 % (w/w) of reagent were prepared. A 10 mL of $10 \mu\text{g}/\text{mL}$ of Cu(II) ion was used having pH 4.2. The results of the influence of salicylaldehyde concentration on cloud point extraction of copper(II) shows the best results at 0.688 mmol/L of salicylaldehyde oxime reagent (Table-2) while, other results were neglected for several reasons, including precipitate formation when added large quantities of salicylaldehyde.

TABLE-2
CONCENTRATION OF SALICYLALDOXIME EFFECT
ON THE ABSORBANCE OF EXTRACTING Cu(II)-
SALICYLALDOXIME COMPLEX

Volume added (mL)	SAO conc. (mmol L ⁻¹)	A _o	A _{aq}	A _s	D	E (%)
1.00	6.339	1.454	0.165	2.71	16.425	94.26
0.50	3.313	1.599	0.169	2.39	14.153	93.40
0.30	2.025	1.770	0.122	2.503	20.550	95.35
0.10	0.688	1.791	0.088	2.452	28.066	96.55
0.05	0.345	1.651	0.110	2.064	18.860	94.96
0.03	0.208	1.089	0.153	1.67	10.920	91.61
0.01	0.069	0.405	0.063	0.857	13.668	93.18

SAO = Salicylaldehyde

Type and concentration of surfactant influence: The effect of type of surfactant on Cu(II)-salicylaldehyde complex was studied by using different type of surfactant such as Triton X-100, Triton X-114 and sodium dodecyl sulfate (SDS). From Fig. 2, it can be seen the absorbance of Cu(II)-salicylaldehyde complex value increased with Triton X-114 surfactant. Therefore, Triton X-114 surfactant concentration effect on the performance of developed method was studied. A series of volume 0.05 to 1.00 mL of 20 % v/v of Triton X-114 was examined. The analytical signal of Cu (II)-salicylaldehyde complex is increased with increase of Triton X-114 amount (Fig. 3).

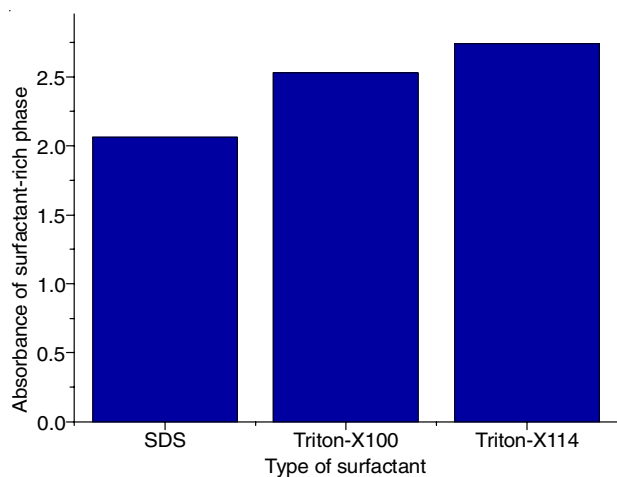


Fig. 2. Effect of type of surfactant on Cu(II)-SAO complex

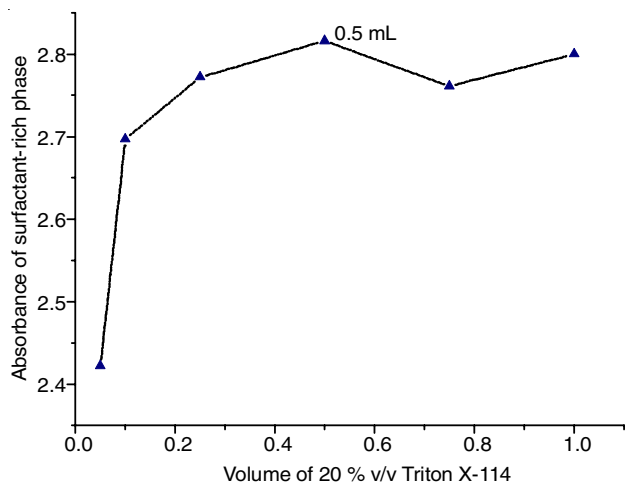


Fig. 3. Effect the amount of Triton X-114 surfactant on Cu(II)-SAO complex

Effect of equilibrium temperature and time: The effect of temperature increasing on the surfactant rich phase of Cu(II) complex separation was done by varying the temperature of water bath from 50 to 85 °C (Table-3). It can be noted that the absorbance signals increased in an increase of temperature at 75 °C. In order to achieve easy phase separation and efficient pre-concentration in cloud point extraction processes, it was desirable to employ the shortest incubation time. The effect of incubation time was investigated in the ranges 5- 25 min and the results demonstrated that incubation time of 10 min was chosen for further experiments. Fig. 4 showed the effect of incubation time on Cu(II)-salicylaldehyde complex formation.

TABLE-3
EFFECT OF EQUILIBRIUM TEMPERATURE ON
Cu(II)-SALICYLALDEHYDE OXIME COMPLEX

Temp. (°C)	A _o	A _{aq}	A _s	D	E (%)
50	1.792	0.386	2.710	7.047	87.57
55	1.792	0.246	2.711	11.063	91.71
60	1.792	0.079	2.614	331.29	99.69
65	1.792	0.128	2.661	20.804	95.41
70	1.792	0.047	2.721	58.020	98.30
75	1.792	0.001	2.727	2753.3	99.96
80	1.792	0.045	2.677	59.615	98.35
85	1.792	0.011	2.707	246.24	99.59

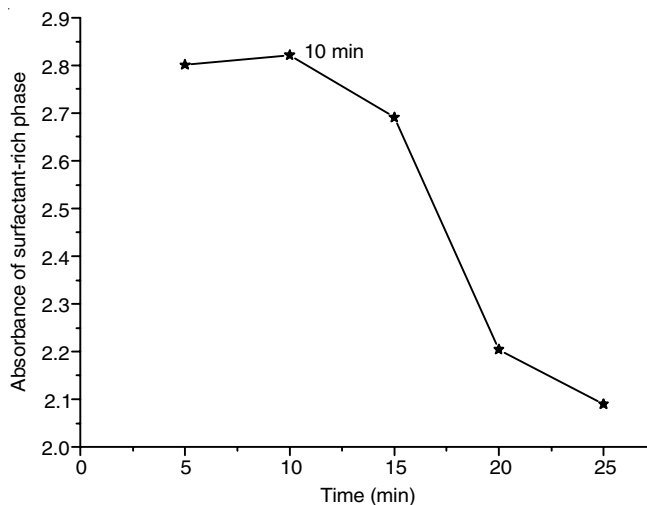


Fig. 4. Effect of incubation time on the Cu(II)-SAO complex

Effect of salt out: In cloud point extraction, addition of salt to sample solution helps to phase separation and increased the mass transfer of analyte from aqueous phase to surfactant rich phase. The effect of salts on CPE were studied by adding 0.5 mL of 15 % w/v of NaCl, KCl, Na₂CO₃ and Na₂SO₄ to the solution of Cu(II) and subjected to complete the procedure. The results (Table-4) showed that the presence of some salts lead to increase the absorbance value due to increase in CPE efficiency because of these salts, water molecules will destroy the hydration shell of Cu(II) ion which leads to increase the bonding between salicylaldehyde oxime reagent and copper(II) ions.

Effect of interference: The interference of foreign ions on the extraction of 10 µg/mL of Cu(II) were studied. The cations either react with salicylaldehyde or species that react with analytes may decrease the extraction efficiency. The interfering

Type of added salt	A_o	A_{aq}	A_s	Recovery (%)	E_{re} (%)
Without salt	1.692	0.0010	1.769	—	—
NaCl	1.743	0.1012	2.473	139.79	39.79
KCl	1.761	0.0103	2.349	132.78	32.78
Na ₂ CO ₃	1.801	0.6860	2.304	130.02	30.24
Na ₂ SO ₄	1.695	0.2420	2.740	154.88	54.88

ions (100 µg/mL) were added to a solution of 10 µg/mL of Cu(II) and subjected to complete the procedure. The results (Table-5) showed that the presence of large amounts of species commonly present in water samples have significant effect on the cloud point extraction efficiency.

Stoichiometry study (Job's method): A solution of reagent and copper(II) ion (1×10^{-3} M) were prepared and mixed in continuous variation then diluted to 10 mL of deionized water after that measurement of solution by UV-visible spectrophotometer at 380 nm. The stoichiometry of the complex determined Job's method was found to be 1:1. Fig. 5 showed the Job's plot of absorbance at 380 nm *versus* mole fraction of Cu (II) ion.

Mole ratio: By using the mole ratio method at constant volume of 1×10^{-3} M Cu(II) and varying volume of 1×10^{-3} M salicylaldehyde, the absorbance of Cu(II)-salicylaldehyde complex was measured at λ_{max} 380 nm. Fig. 6 observed at 1:1 mole ratio of salicylaldehyde and Cu(II).

Calibration curve: The linearity curve of Cu(II) ion was constructed from 0.50 to 16.00 µg/mL. Table-6 and Fig. 7 showed the scattering curve for determination of Cu(II) ion. Also, the results of linear regression analysis of the scatter curve of copper(II) ions are tabulated in Table-7.

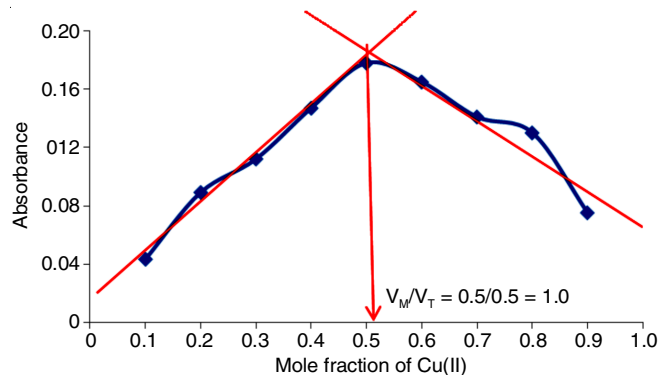


Fig. 5. Job's plot of absorbance at 380 nm *versus* the mole fraction of Cu(II) ion. (V_M : Volume of Cu(II) mL, V_T : Total volume (volume metal + volume reagent))

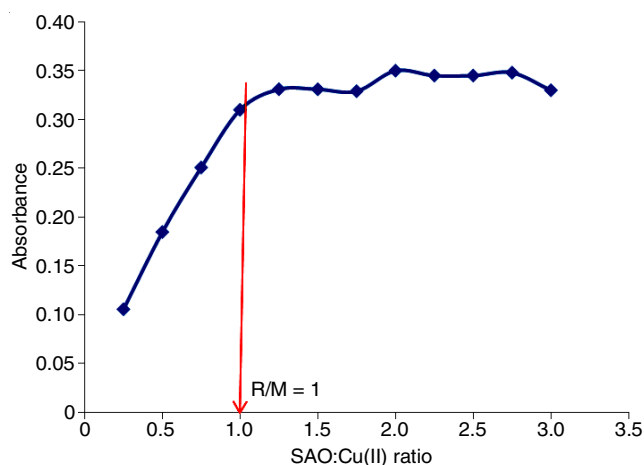


Fig. 6. Molar-ratio plots of Cu(II)-SAO complex. R: mole of SAO reagent, M: mole of metal Cu(II)

Interferent	Composition divers ions compound	Interferent conc./ metal conc.	A_s of Cu(II)	Cu(II)-SAO complex	
				Recovery (%)	E_{re} (%)
Co(II)	Co(NO ₃) ₂	50	2.810	535.20	435.23
Ni(II)	Ni(NO ₃) ₂	50	2.512	478.47	378.47
Fe(III)	FeCl ₃	50	2.925	557.14	457.10
Cr(III)	Cr(NO ₃) ₃	50	0.622	118.47	18.47
Al(III)	Al(NO ₃) ₃ ·9H ₂ O	50	0.212	40.38	59.61
Pb(II)	Pb(NO ₃) ₂	50	0.538	102.47	2.476
Zn(II)	ZnCl ₂	50	1.214	231.23	131.23

SAO = Salicylaldehyde

Conc. of Cu(II) (µg mL ⁻¹)	Abs. y_i	Mean \bar{y}	SD σ_{n-1}	RSD (%)	C.I
					$\bar{y} \pm t_{0.05/2} \frac{\sigma_{n-1}}{\sqrt{n}}$
16	2.598, 2.508, 2.399	2.501	0.099651	3.9833	2.501 ± 0.247
12	1.966, 1.895, 2.100	1.987	0.104101	5.2391	1.987 ± 0.258
10	1.782, 1.619, 1.670	1.690	0.083381	4.9327	1.690 ± 0.206
8	1.470, 1.040, 1.218	1.242	0.216059	17.3866	1.242 ± 0.536
6	1.018, 0.972, 0.700	0.896	0.171864	19.1670	0.896 ± 0.426
4	0.569, 0.56, 0.561	0.563	0.004933	0.8756	0.563 ± 0.012
2	0.321, 0.279, 0.273	0.291	0.026153	8.9874	0.291 ± 0.064
1	0.143, 0.120, 0.21	0.157	0.046758	29.656	0.157 ± 0.116
0.5	0.092, 0.020, 0.091	0.067	0.041284	61.010	0.067 ± 0.102

TABLE-7
RESULTS OF LINEAR REGRESSION ANALYSIS OF COPPER(II) USING UV-VIS-CPE

	Range of conc. ($\mu\text{g mL}^{-1}$)	Linear regression equation	R r^2 r^2 (%)	t_{table} at 95 % confidence level, n-2	Calculated t-value $t_{\text{cal}} = \frac{(r \sqrt{n2})}{(\sqrt{1-r^2})}$	Limit of detection ($\mu\text{g mL}^{-1}$)
Cu(II) n=9	0.50 – 16.00	$Y = 0.162 X - 0.0284$	0.9979 0.996 99.60	2.365	$\lll 41.74$	0.100

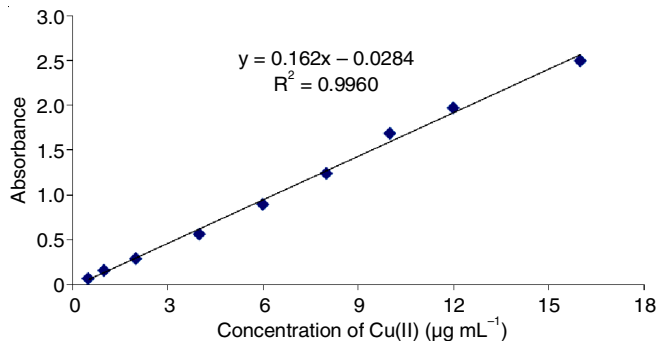


Fig. 7. Calibration curve for Cu(II) - SAO complex

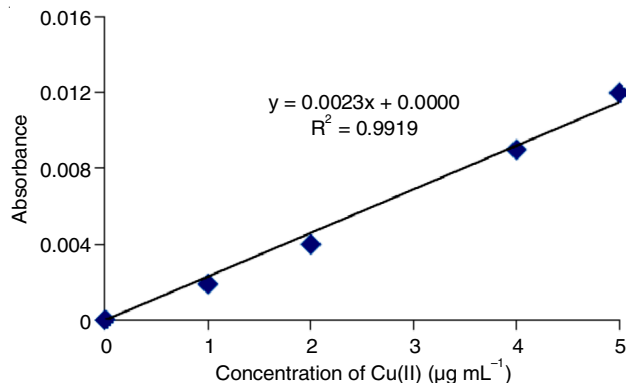


Fig. 8. Calibration graphs for determination of Cu(II) by FAAS

Precision and Accuracy

Repeatability: The precision test of UV-visible-CPE method for copper(II) ion determination is applied by calculation of the relative standard deviation (RSD %) values for the resulting of repeatability test. The concentration varies from 4.0 to 8.0 $\mu\text{g/mL}$ of Cu(II) was considered. The relative standard deviation (RSD %) value for Cu(II) ion is shown in Table-8. The percentage relative standard deviation less than 5 % can be achieved by this method.

Calibration curve of Fe(III) by FAAS: A series of standard iron(III) solutions ranging from 0-5 $\mu\text{g/mL}$ were used at λ_{max} 324.7 nm to determine the calibration curve for Cu(II) by FAAS technique. Fig. 8 shows the range of concentration of Cu(II) $\mu\text{g/mL}$ and the results of absorbance for linear regression analysis using FAAS.

Applications: After the digestion of urine sample pre-concentrated by CPE method and determine Cu(II) concentration in urine of 44 occupational worker samples by two methods *viz.* UV-visible-CPE and FAAS-CPE at the optimum conditions.

Statistical treatment of results: Applying a Grubbs-test to outlier of the sample concentrations after estimating by UV-visible spectrophotometry and FAAS techniques, the results were excluded as shown in Table-9. This test was applied to 44 samples as shown in Table-10. The significance tests described

so far are used for comparing means, and hence for detecting systematic errors. The F-test was used to compare the variances between the concentration of copper by two methods UV-visible-CPE and direct FAAS to appear have significant or not between them and the comparison of a sample mean with true mean must be based on Student's t test to compare mean of copper concentration by two methods as shown in Table-11.

One-way ANOVA test: One-way ANOVA is used to separate and approximate the diverse sources of variation, rather than to test whether several sample means differ significantly [24]. Table-12 shows the results one-way ANOVA test applied for comparison between different groups to determine of Cu(II) by using UV-visible spectrophotometer and FAAS, whereas the results for P-value showed that there is no significant difference between these groups for copper(II) concentration in the same way of determination.

On the other hand, Table-13 shows the comparison results of Cu(II) determinations by using UV-visible and FAAS methods. Depending on p-value, it was found that there is no significant difference in the estimation of trace elements using both methods. It was also found that when using UV-visible coupled with cloud point extraction has increased the efficiency, accuracy and sensitivity of this technique and can use this method as an alternative technique for FAAS to estimate trace elements.

TABLE-8
REPEATABILITY OF Cu(II) COMPLEX AT OPTIMUM PARAMETERS

	No. of repetition	Conc. of metal ($\mu\text{g mL}^{-1}$)	Absorbance	Mean \pm SD	RSD (%)
Cu(II)	6	4	0.500, 0.520, 0.509, 0.560, 0.561, 0.542	0.532 ± 0.026	4.917
	5	8	1.260, 1.246, 1.292, 1.101, 1.218	1.223 ± 0.073	6.002

TABLE-9
RESULTS OF GRUBBS-TEST APPLIED ON Cu(II) IONS

No. of sample	Readings excluded		C.F.	Range of concentration ($\mu\text{g mL}^{-1}$)	
	UV-Vis-CPE	Direct FAAS		UV-Vis-CPE	Direct FAAS
44	0	0	22.5	0.052-0.382	0.088-1.15

TABLE-10
RESULTS OF COPPER(II) CONCENTRATION IN URINE SAMPLES BY DIFFERENT TECHNIQUES

Occupation	Method	No. of sample	Mean \pm SD	C.I $t_{0.05/2} \frac{\sigma_{n-1}}{\sqrt{n}}$
Oil refineries	UV-Vis-CPE	16	0.1838 \pm 0.0830	0.0442
	Direct AAS	16	0.1757 \pm 0.0661	0.0366
Welding	UV-Vis-CPE	12	0.1767 \pm 0.0790	0.0501
	Direct AAS	12	0.2116 \pm 0.0780	0.0495
Casting alloys	UV-Vis-CPE	5	0.1994 \pm 0.1381	0.1714
	Direct AAS	5	0.1936 \pm 0.0367	0.0580
Terminals to provide fuel	UV-Vis-CPE	6	0.1766 \pm 0.0715	0.0750
	Direct AAS	6	0.1765 \pm 0.1055	0.1107
Dyeing	UV-Vis-CPE	5	0.1883 \pm 0.0615	0.0764
	Direct AAS	5	0.1498 \pm 0.0546	0.0670
Total	UV-Vis-CPE	44	0.1832 \pm 0.0813	0.0055
	Direct AAS	44	0.1847 \pm 0.0718	0.0223

TABLE-11
APPLICATION OF F-TEST AND t-TEST ON Cu(II) CONCENTRATION IN URINE SAMPLES TO COMPARE THE RESULTS BETWEEN TWO METHODS

Occupation	Type of method	d.f = n-1	F-test			t-test	
			Variance σ^2	F-calculate $F = \frac{\sigma^2}{\sigma^2}$	F-critical value	t-calculated $t = \bar{y} - \mu \frac{\sqrt{n}}{\sigma_{n-1}}$	P-value
Oil refineries	UV-Vis-CPE	15	0.0068	1.5767	2.54	8.1909	0.00001
	Direct AAS	15	0.0044			9.8059	0.00001
Welding	UV-Vis-CPE	11	0.0062	1.0258	2.80	7.3840	0.00001
	Direct AAS	11	0.0061			9.0317	0.00001
Casting alloys	UV-Vis-CPE	4	0.0191	14.1597	6.39	3.0944	0.01820
	Direct AAS	4	0.0014			11.290	0.000175
Terminals to provide fuel	UV-Vis-CPE	5	0.0051	2.1771	4.39	5.7645	0.00110
	Direct AAS	5	0.0111			3.9047	0.00567
Dyeing	UV-Vis-CPE	4	0.0038	1.2687	6.39	6.5451	0.00140
	Direct AAS	4	0.0030			5.7947	0.00220
Total	UV-Vis-CPE	43	0.0066	1.2817	1.69	14.269	0.00001
	Direct AAS	43	0.0052			16.298	0.00001

P-value < 0.05 sig. difference, p-value > 0.05 non sig. difference, True value of copper $\mu = 8.3$ ng mL [Ref. 25]. $t_{0.05(n-1)}$, n = 3 (4.303), n = 4 (3.182), n = 5 (2.776), n = 6 (2.571), n = 11 (2.228), n = 12 (2.201), n = 14 (2.160), n = 15 (2.145), n = 16 (2.131), n = 37 (2.028), n = 44 (2.016).

TABLE-12
ONE-WAY ANOVA TEST APPLICATION OF COMPARISON BETWEEN THE CONCENTRATION OF Cu(II) IN DIFFERENT OCCUPATION WORKERS GROUPS

Group	Conc. ($\mu\text{g mL}^{-1}$)		d.f. between d.f. within d.f. total	SS between SS within SS total	MS between MS within	F = $\frac{MS_{\text{between}}}{MS_{\text{within}}}$	P-value
	UV-Vis-CPE	FAAS					
Group A	0.18388	0.1757					
Group B	0.17670	0.2116	1	0.00003	0.00003	0.099	0.760
Group C	0.19942	0.1936	8	0.00247	0.00030		
Group D	0.17660	0.1765	9	0.00250			
Group E	0.18831	0.1498					

p-value < 0.05 sig. difference, p-value > 0.05 non sig. difference, A: Oil refineries, B: Welding, C: Casting alloys, D: Terminals to provide fuel and E: Dyeing

TABLE-13
COMPARISON OF ONE-WAY ANOVA TEST FOR THE CONCENTRATION OF Cu(II) IN UV-VIS-CPE AND FAAS

Method	Source	SS	df	MS	F	p-value
UV-Vis-CPE	Between-treatments	0.0022	4	0.0006	0.0745	0.98950
	Within-treatments	0.2893	39	0.0074		
	Total	0.2915	43			
FAAS	Between-treatments	0.0168	4	0.0042	0.8210	0.5197
	Within-treatments	0.1999	39	0.0051		
	Total	0.2167	43			

Effect of age factor estimation: Histogram of copper(II) concentration in urine sample by two methods (UV-Vis-CPE and FAAS-CPE) with age of sample is represented. It can be seen from Fig. 9, concentration remained steady between 20 and 40 years, and suddenly dropped to 45 years after that the concentration of copper went up to 55 years old. It is due to the lack or poor absorption of copper by the body as a result of changes in metabolic processes as a result of progressing age, leading to increase the concentration of Cu(II) in urine [25].

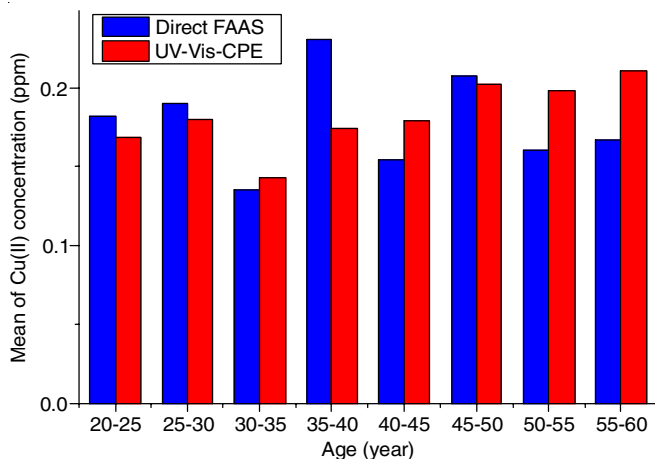


Fig. 9. Histogram of copper(II) concentration with the age of occupation workers samples

Conclusion

This study involves then the development of a sensitive, selective and environmentally friendly spectrometric method of determination of low concentrations of Cu (II), using salicylaldehyde (SAO) as a complexing agent and cloud point extraction. UV-Vis spectrophotometer and flame atomic absorption spectrometry were used as detection technique and the proposed method was applied for a number of samples.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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