


FULL PAPER

Attenuation of incident light sources (two flow tubes in one geometrical flow cell assembly: First with eleven sources while the second is covered by six sources) in CFIA for the determination of Copper (II) ion

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A highly accurate, simple, sensitive and fast developed method was used for the determination of copper (II) ion using a homemade NAG-ADF-300-2 system with continuous flow injection analysis system. This method is based on reaction between copper (II) ion and rubeanic acid to obtain a black precipitate for the ion-pair complex. Turbidity was measured via the attenuated of incident light by precipitated at 0-180°. Optimum parameters were studied to increase the sensitivity for newly developed methodology of copper (II) ion -rubeanic acid system. The linear range for calibration graph was 1-25 mmol.L⁻¹ for cell A and 1-30 mmol.L⁻¹ for cell B, and LOQ 27.0538 µg /78.5 µL for cell A and 18.5539 µg /78.5 µL for cell B respectively with correlation coefficient (r) 0.9979 for cell A and 0.9992 for cell B, RSD % was lower than 0.5%, (n=8) for the determination of copper (II) ion at concentration 18, 23 mmol.L⁻¹ for cell A and 12, 19 mmol.L⁻¹ for cell B, respectively. A comparison was made between the newly developed method with UV-Spectrophotometric method as a classical method by tow type of statistical analysis for comparison the means and variance from different methods. The comparison of data revealed that long distance chasing photometer (NAG-ADF-300-2) was the choice with excellent extended detection, wide application and more sensitive.

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KEYWORDS

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Introduction

The alloys of copper are utilized in car radiators, warm exchangers, domestic warming frameworks and boards for retaining sun powered vitality. Copper (II) ion may be a naturally dynamic metal; its compounds influence crucial movement of plant and creature life forms. Copper is one of basic elements required for typical human digestion

system [1]. Copper (II) ion is known to play noteworthy part in organic frameworks additionally as a pharmaceutical agent [2]. Its antibacterial properties have been known for thousands of a long time. Manufactured copper (II) complexes have been detailed to act as a potential anticancer and cancer repressing specialists and number of copper complexes have been found to be dynamic both in vitro and in vivo [3,4]. Hence it is

essential to control that the substance of copper (II) ions particles within the natural objects is inside the reasonable concentrations.

Several methods depend on continuous flow injection analysis [5-12]. Different spectrophotometric methods have been proposed for the determination of copper substance of the different samples [13-15] and several analytical methods have been reported for determination of copper (II) ion including, HPLC-MSIS-ICP-OES [16], voltammetry [17], FAAS [18], capillary zone electrophoresis [19], and Ion-selective electrode [20].

In this work using Continuous flow injection analysis method, was measured via precipitation of copper (II) ion by rubeanic acid, a black precipitate product was obtained which was determined at angle 0-180° using homemade long-distance chasing photometer (NAG-ADF-300-2) [21].

Chemicals and apparatus

Reagents and chemicals

All chemicals were used of the highest purity available and all the solutions dissolved by distilled water. A standard solution of 250 mmol.L⁻¹ of $Cu(NO_3)_2 \cdot 3H_2O$ (BDH), molecular weight 241.60 g.mol⁻¹, was prepared by weight of 6.04 g and dissolving in 100 mL conical flask. A series of *rubeanic acid* (BDH) solutions were prepared from the dilution of standard solution 50 mmol.L⁻¹ with distilled water. Two alloys samples preparation for the conducted research *High purity copper* (B.C.S.NO.197e) (99.95%) and *low alloy steel* (B.C.S.NO.255/1) (0.75%): 1 g of first alloy and 10 g of second alloy were weighed, transferred to 400 mL beaker followed by the addition of 1:1 HCL: HNO₃ then heated gently with constant agitation until all brown fume was ceased. The remainder was diluted to 100 mL. The washed filter paper was washed few

times, and the solution was collected in volumetric flask (100 mL).

Apparatus

Using the manifold homemade NAG-ADF-300-2 instrument is a multi-purpose photometric device including the offer of multi measurement individually or simultaneously or combine or separated whether 0-180° or 0-90°. This applies to clear solution or colored or precipitated reaction product whether colloidal or crystalline colored or white or even clear precipitate. It is a long-distance chasing photometer as a flow cell with 300 mm as a distance with 2 mm as a path length to chase and to accumulate the output resulted from attenuation of incident light 0-180° and diverged or fluorescence light at 0-90° via a flow cell. The first flow cell is of 110 mm length covered with 11 white snow LED (WSLED) followed by uncovered distance of 100 mm length then attached to another with 2 solar cell at each side of (0-180° and 0-90°) cell (cell no. 2) which was covered by 6 WSLED and a single photo cell (solar) of 60 mm length at each side was used with peristaltic pump (Ismatec, Switzerland) and six-port medium pressure injection valve (IDEX Corporation, USA) with sample loop (1 mm i.e. Teflon, variable length). Potentiometric recorder was used to estimate the output signals (Siemens, Germany). UV-Vis. Spectrophotometric (RF-1501, Shimadzu, Japan) was use for classical methods.

Methodology

Using a manifold of two lines coupled NAG-ADF-300-2 instrument to determine Copper (II) ion via its reaction with rubeanic acid as shown in Figure 1. It is composed of two lines. The first line is the carrier stream of ammonium chloride (3.8 mL.min⁻¹) flow rate will pass through the injection valve to carry the sample segment (78.5 µL for both cell) of 20 mmol.L⁻¹ initial concentration Copper (II)

ion to mix with second line at flow rate 3.8 mL.min⁻¹ by Y-junction point that carries the reagent of rubeanic acid (17 mmol.L⁻¹) for cell A and cell B before it is introduced to the NAG-ADF-300-2 analyzer, and then leading to measure using both cell A and cell B. The reaction product is black particles from the ion pair complex. The obtained signals which resulted from the attenuation of the incident light by the precipitating particles are agglomerated at 0-180°. It can be noticed that the results obtained from cell A are higher in

sensitivity than the output signals from cell B. The higher sensitivity (i.e.; 46% enhanced signal (S/N-Y_z (mV) compared with cell A) might be attributed to the completion of the formation of particulate, rearrangement of precipitated particulate or re-crystallization effect which might be occurs in the cell B.

Scheme 1 shows a proposed expected mechanism for the reaction of copper (II) ion with rubeanic acid in aqueous medium [22,23].

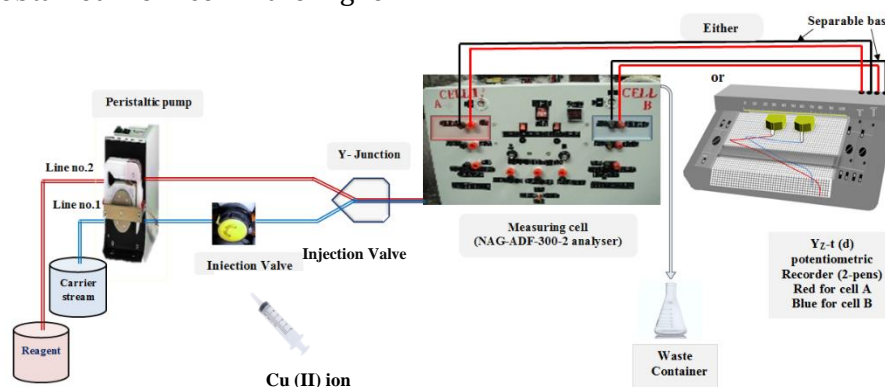
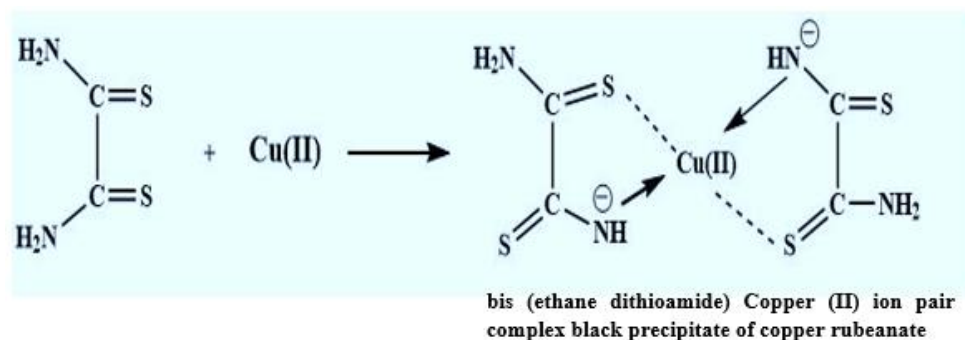


FIGURE 1 Diagram of manifold of flow injection analysis used for determination of copper (II) ion. Using sample volume 78.5 μL for each cell, [Copper (II) ion] = 20 mmol.L⁻¹, [Rubeanic acid] = (17 mmol.L⁻¹), flow rate of each line = 3.8 mL.min⁻¹, potentiometric scanning speed 60_{sec} (10_{mm}), intensity: I=1 for cell A and I=3 for cell B with enlarged NAG-ADF-300-2 unit (working keys). Responses were plotted simultaneously but with a time difference expressed by distance equivalent to 100 mm



SCHEME 1 Probable mechanism between Copper (II) ion with rubeanic acid as a precipitate agent

Results and discussion

Optimization of variables

Chemical variables

Rubeanic acid concentration

A series of the rubeanic acid solution ranging from 5-20 mmol.L⁻¹ were prepared using 78.5 μL sample volume that was injected into the carrier stream line (distilled water). 20 mmol.L⁻¹ of copper (II) ion was injected with 3 mL.min⁻¹ flow rate for carrier stream line and reagent line. The increase of rubeanic acid

concentration leads to an increase in the peak height expressed as an attenuation of incident light reaching 17 mmol.L⁻¹ obtaining highest sensitivity; this can be attributed to the nature of formed particulate (e.g., Colloidal, crystalline, or suspension) and its surfaces, also its tendency to obscure the direct light to the detector. Dealing with higher concentration (i.e., > 17 mmol.L⁻¹) leads to decrease of peak heights that were obtained

from both cells. This might have been probably caused by the increase of accumulation of particulate to prevent the optical fiber phenomenon, which might occur in the measuring flow cell that will increase the light intensity. These results were provided through with slope - intercept method as segment a₄ -a₆ (Figure 2) was the choice thus 17 mmol.L⁻¹ falls for both cells.

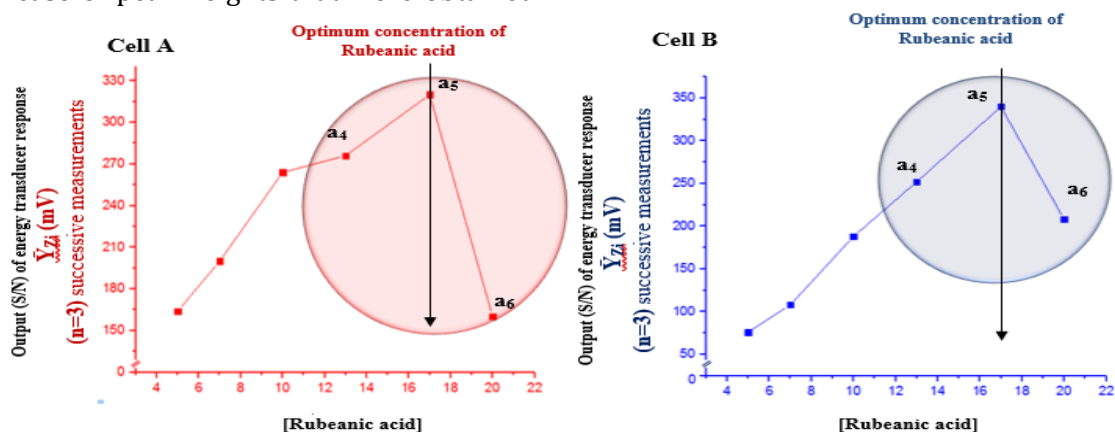


FIGURE 2 Output (S/N) of energy transducer response expressed as an average peak heights versus rubenic acid concentration. Three-data point as one segment their interaction and choice for both cells

Effect of different media (Selected salts and acids)

Copper (II) ion 20 mmol.L⁻¹ reaction with rubenic acid 17 mmol.L⁻¹ for both cells, 78.5 μ L sample volume and 3 mL.min⁻¹ flow rate for two line (rubenic acid line & carrier stream line) to form black color precipitate was studied in different medium at 50 mmol.L⁻¹ concentration (ammonium acetate, ammonium chloride, sodium nitrate, sodium sulfate, sodium chloride and potassium chloride) as a salt media, as well as the use of 50 mmol.L⁻¹ concentration (hydrochloric acid and sulphuric acid) as an acid media in addition to distilled water used as a carrier stream. From Figure 3, it was noticed that all media solution (salts & acids) used for cell A leads to an increase of (S/N)- response due to

the effect of formation of tiny solid particulate that might be the cause to decrease of inter spatial distances and an increase of attenuation of incident light, while for cell B was noticed that there is no clear significant effect from the data response obtained except ammonium chloride and ammonium acetate. In general, salts and its negative radicals helps to reconstruct and the accumulation of precipitated particulate which increase the prevention of light and then increase peak height. The same effect was noticed when we use NH₄Cl while acids in general helps peptize, precipitate and scatter them causing the spectral filtration which in turn causes decrease in sensitivity; therefore, acids were avoided. NH₄Cl was used as a transferring medium to improve the sensitivity of measurement to determination Cu (II) ion.

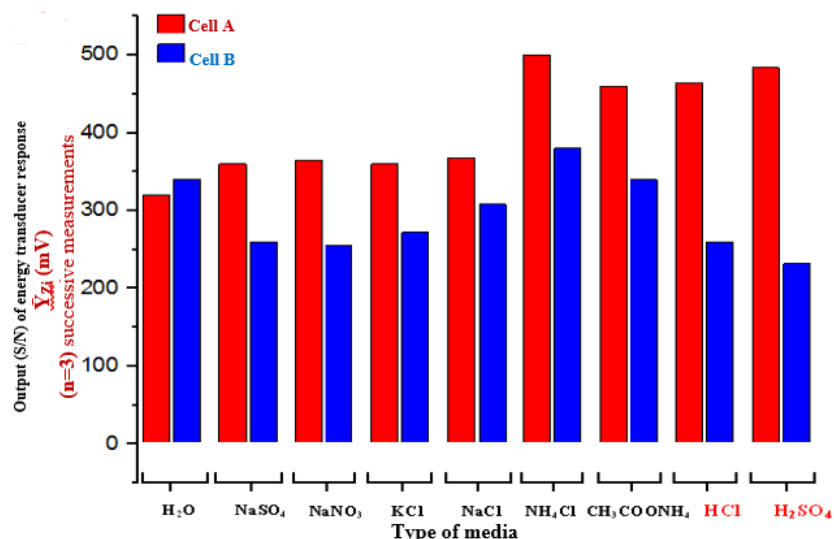


FIGURE 3 Effect the different media on (S/N) energy transducer response versus time using $3\text{mL}\cdot\text{min}^{-1}$ flow rate for both carrier stream and reagent, irradiation intensity for cell A was $I=1$ while for cell B was $I=3$. Sample volume of $78.5\ \mu\text{L}$ was in use, and potentiometric scanning speed $60_{\text{sec}} (10_{\text{mm}})$.

Effect of $[\text{NH}_4\text{Cl}]$

The effect of NH_4Cl was investigated by using Copper (II) ion ($20\ \text{mmol}\cdot\text{L}^{-1}$)- Rubenic acid ($17\ \text{mmol}\cdot\text{L}^{-1}$) system for both cells, $78.5\ \mu\text{L}$ sample volume at $3\ \text{mL}\cdot\text{min}^{-1}$ flow rate for carrier stream and reagent. Variable concentrations of ammonium chloride at ranging $10\text{-}60\ \text{mmol}\cdot\text{L}^{-1}$ as a carrier stream were used. From Figure 4, it was noticed that an increase of ammonium chloride concentration for the range $30\text{-}50\ \text{mmol}\cdot\text{L}^{-1}$ leads to an increase the output signal for both cells. This might be attributed to the ability or the tendency of the salt in growing the nuclei

to crystals followed by the formation of compact granules with each other, thus causing the surface area that obscure the generated light which coming from W.S.LEDs i.e., it makes a more compact barrier compared with the rest of the solution. When dealing with concentration more than $50\ \text{mmol}\cdot\text{L}^{-1}$, a decrease in peak heights was noticed. This could be attributed to the dispersion of precipitate to a smaller tiny scattered without affecting on obstruction of incident light and this is called spectral filtering. A $50\ \text{mmol}\cdot\text{L}^{-1}$ was chosen as the most favorite concentration to be used on all over this work for both cells.

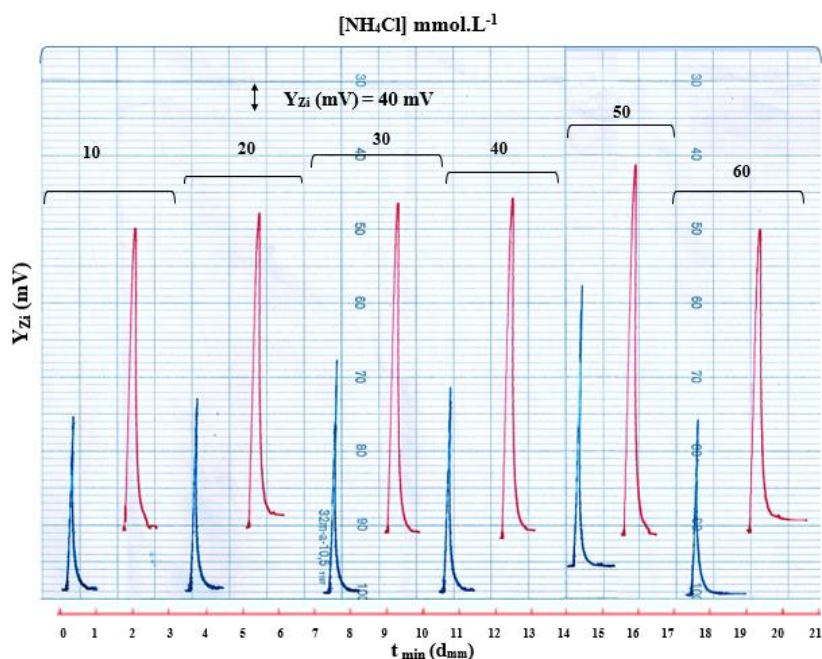


FIGURE 4 Effect of $[\text{NH}_4\text{Cl}]$ on (S/N) energy transducer response expressed as average peak heights in mV versus concentration of $[\text{NH}_4\text{Cl}]$ solution, potentiometric scanning speed 60_{sec} (10_{mm}). Responses were plotted simultaneously but with a time difference expressed by distance equivalent to 100_{mm}

Physical variables

Flow rate

Variable of flow rates ($0.5\text{--}5$) $\text{mL}\cdot\text{min}^{-1}$ for two lines (50 $\text{mmol}\cdot\text{L}^{-1}$ of NH_4Cl solution as a carrier stream and Rubeanic acid as reagent line) for cell A and cell B was used at copper(II) ion (20 $\text{mmol}\cdot\text{L}^{-1}$)-rubeanic acid (17 $\text{mmol}\cdot\text{L}^{-1}$) - NH_4Cl 50 $\text{mmol}\cdot\text{L}^{-1}$ system, 78.5 μL sample volume for both cells. The obtained response profile is shown in Figure 5. It can be observed the obtained responses are distorted especially at low flow rates, in the meantime, enough time is available for successive growth of crystals to large particulate which in turn

will be affected by the motion of the peristaltic pump in case of relaxed and pressed mode. In case of pressed mode, the formed precipitated particulate will be pressed causing an accumulative compactness leading to obscure the incident light and distorted for the obtained responses, while at high speed, a more uniform response is due the unavailability of enough time for crystal growth. Also, no spreading of precipitated particulate on a wider tube length causing loose of sensitivity and response time will suffer from extension. Therefore, the 3.8 $\text{mL}\cdot\text{min}^{-1}$ flow rate for both cells will be the choice within it to obtain a regular response.

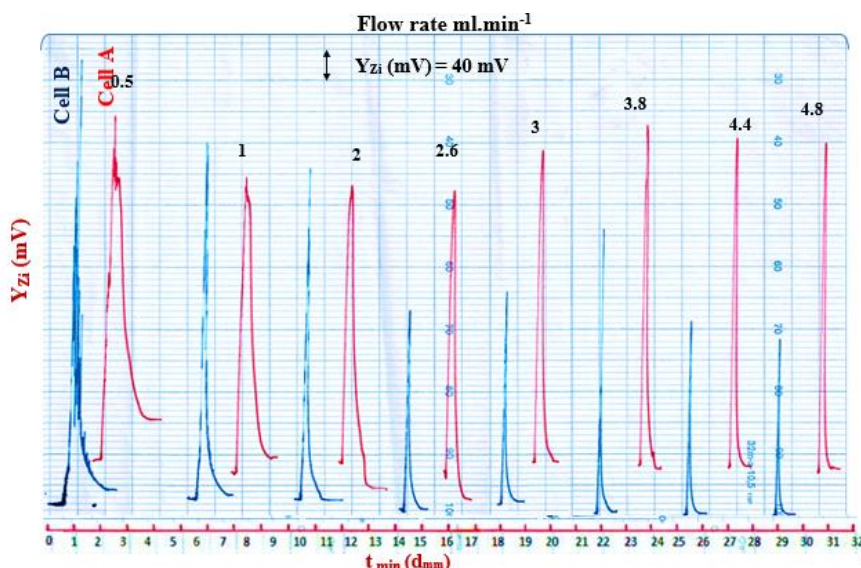


FIGURE 5 The effect of flow rates on (S/N) energy transducer versus $t_{\min}(\text{dmm})$, and potentiometric scanning speed 60_{sec} (10_{mm})

Sample volume

Sample volumes variations ($40\text{--}281$) μL were studied at optimum flow rate $3.8 \text{ mL}\cdot\text{min}^{-1}$ for both lines (NH_4Cl line & reagent line), with selected concentration ($20 \text{ mmol}\cdot\text{L}^{-1}$) of copper (II) ion, rubeanic acid of ($17 \text{ mmol}\cdot\text{L}^{-1}$) and NH_4Cl of $50 \text{ mmol}\cdot\text{L}^{-1}$ were used. It can be seen from the reported results in Table 1 there is an increase in sensitivity with the increase of sample segment (loop) up to $78.5 \mu\text{L}$ and obtaining symmetric responses reflects the form of crystal formed. Here the crystals are spherical; in addition to aid in moving with the carrier stream, the speed will be higher (more than) and falls within the effect of convection.

While at volume larger than $78.5 \mu\text{L}$, responses are lower and a width at the apex and base width that will be obtained from cell A. This might be attributed to the nature of formed crystals and its morphology i.e., non-spherical form which causes a slow movement and falls on the effect of diffusion. This effect will cause the transparency for certain or some of the particulate and obtaining spectral filtering; therefore, the responses are smooth and losses in sensitivity are minimized. Based on the above statement, and in order to compromise with the economy of sample usage, $78.5 \mu\text{L}$ was chosen as it could give the highest sensitivity.

TABLE 1 Effect of variation of sample volume on the attenuation of incident light. Responses were plotted simultaneously but with a time difference expressed by distance equivalent to 100 mm .

Length Of Sample Segment Cm $r=0.5\text{mm}$	Sample Volume $\mu\text{L}(\text{Ca.})$ $V=\pi r^2 h$	Output (S/N) of energy transducer response expressed as an average peak heights($n=3$) $\bar{Y}_{zi} (\text{mV})$		Reliability (two tailed) at 95% $\bar{Y}_{zi} (\text{mV}) \pm t_{0.05/n-1} \sigma_{n-1}/\sqrt{n}$		arrival time to cells unit t^*_{sec}	Base Width Δt_b (sec)	V_{add} (mL) at flow cell	Concentration ($\text{mmol}\cdot\text{L}^{-1}$) at flow cell	D_f at flow cell
		RSD%								
Cell A										
Cell B										
5.10	40	472	0.15	472 ± 1.7888	6	24	3.080	0.2597	77.01	
		308	0.20	308 ± 1.5403	12	18	2.320	0.3448	58.00	
10	78.5	540	0.08	540 ± 1.1179	9	30	3.879	0.4047	49.42	
		456	0.17	456 ± 1.9378	15	30	3.879	0.4047	49.42	
14.40	113	480	0.07	480 ± 0.8695	10	36	4.673	0.4836	41.36	
		428	0.17	428 ± 1.8136	18	33	4.293	0.5264	37.99	
17.20	135	496	0.13	496 ± 1.5403	12	39	5.075	0.5320	37.59	

		444	0.15	444±1.6149	21	36	4.695	0.5751	34.78
17.90	140	504	0.15	504±1.9378	14	42	5.460	0.5128	39.00
		420	0.17	420±1.8136	24	42	5.460	0.5128	39.00
19.50	153	512	0.11	512±1.3913	16	48	6.233	0.4909	40.74
		424	0.15	424±1.5403	27	48	6.233	0.4909	40.74
35.80	281	516	0.08	516±1.0435	18	51	6.741	0.8337	23.99
		420	0.14	420±1.4409	27	54	7.121	0.7892	25.34

$t_{0.05/2,2} = 4.303$, Df: Dilution factor at flow cell, (Ca): calculated volume

Delay reaction coil

In general, the aim of this study was to decide whether a reaction (direct attachment) or delay coil necessary for maximum reaction rate or precipitate particulate formation or growing to form a dense of particulate or granules to increase a high sensitivity and taking into account a compromise should be made between reaction completion and avoiding excessive dilution. Effect of variable coil lengths of 10,15, 25 and 30 cm were studied which was attached after Y- junction point, conducted on the use of copper (II) ion (20 mmol.L⁻¹) – rubeanic acid - (17 mmol.L⁻¹)- NH₄Cl (50 mmol.L⁻¹) system, 78.5 μL sample volume and 3.8 mL.min⁻¹ flow rate for both cells. The increase of reaction coil volume for both cells (Cell A & Cell B) led to decrease of response height with increase of base width (Δt_B); these might be attributed to diffuse and

dispersion of precipitate particles, causing increase of the dispersion regions and leading to decrease in the (S/N) energy transducer response which in turn gave less sensitivity of response. Table 2 tabulates all these results. In addition to the tabulated results of doing the precipitation reaction in a dynamic system, the presence of delay reaction coils will give an enough time to growth crystals in addition to increasing the effect of dilution and dispersion of the large particulate on a wider area causing the loss of sensitivity, also measurements of time will be longer. Due the presence of water with the body of precipitated particles, photons will suffer from internal refraction and dispersion leading to divergence of incident light and decrease of sensitivity. Therefore, there is no need for delay reaction coils and direct attachment is regarded as the most suitable for both cells.

TABLE 2 Effect volume of delay reaction coil on output (S/N) of energy transducer response expressed as an average peak height (mV) for estimation copper (II) ion

Delay reaction Coil Length (Cm) r=1mm	Volume of delay reaction coil (μL) V _{RC}	Output (S/N) of energy transducer response expressed as an average peak heights (n=3) \bar{Y}_{Zi} (mV)	RSD%	Reliability (two tailed) at 95% \bar{Y}_{Zi} (mV) ± $t_{0.05/n-1} \sigma_{n-1}/\sqrt{n}$	arrival time to cells unit t* _{sec}	Base Width Δt_B (sec)	V _{add} (mL) at flow cell	Concentrati on (mmol.L ⁻¹) at flow cell	D _f at flow cell
				Cell A					
Direct attachment	Direct attachment	540	0.08	540±1.0683	15	30	3.879	0.4048	49.42
		456	0.17	456±1.9378	9	30	3.879	0.4047	49.42
10	314	520	0.12	520±1.5403	18	36	4.639	0.3389	59.10
		324	0.22	324±1.7888	12	18	2.359	0.6655	30.05
15	471	520	0.14	520±1.8136	21	37.2	4.791	0.3277	61.03
		352	0.24	352±2.0621	15	22.8	2.967	0.5292	37.79
25	785	520	0.16	520±2.0869	24	39	5.019	0.3128	63.94
		352	0.22	352±1.8882	18	24	3.119	0.5034	39.73
30	942	480	0.13	480±1.5652	27	40.8	5.247	0.2992	66.84
		400	0.15	400±1.4658	21	35	4.512	0.3479	57.49

$t_{0.05/2,2} = 4.303$, Df: Dilution factor at flow cell

The study of the applied voltage expressed as an intensity used for supplying the White Snow Light Emitting Diodes (WSLEDs) in NAG-ADF-300-2 analyzer

A study was carried out to determine the effect of intensity of incident light of the irradiation sources on the (S/N)- response of the energy transducer response via the selector switch. Using the control of the light intensity knob (they are two), the increase will be maximized clockwise at four stages (level of irradiation I=1-2-3-4 i.e., 3.0 VDC, 3.2 VDC, 3.4 VDC and 3.5 VDC, respectively). In addition to the off position, an indication of LED is controlled electrically for intensity operation i.e.,

indication lamp for cell A attached with eleven sources of WLED plus and indication lamp for cell B parallelly attached with the six WLED. This study was conducted with physical and chemical parameters achieved in previous sections which were kept constants for both cells. 3.4 VDC is the optimum for both cells (Even 11 WLED were used in cell A while 6 WLED for cell B), It was noticed from figure 6 that a selection of position 3 (i.e.; I=3) was very convenient intensity for cell A (cell no.1) (larger number of the selector switch means more light intensity) while position 2 (I=2) of the selector switch was a convenient intensity for cell B (cell no.2).

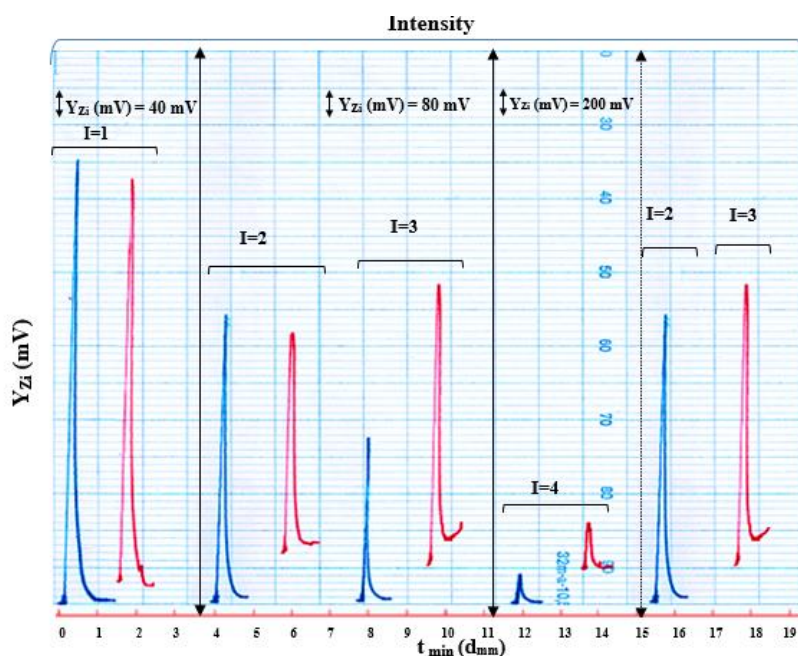


FIGURE 6 Effect of intensity of incident light on (S/N)-response of the energy transducer versus t_{\min} (d_{mm}), potentiometric scanning speed 60_{sec} (10_{mm}). Responses were plotted simultaneously but with a time difference expressed by distance equivalent to 100 mm

Variation of concentration with obtained response leading to calibration range

The steps involved concentration (measured as t_{sec} (d_{mm}) as the x- axis versus the response Y_{zi} (mV)) and this falls in the range i.e., the extent to which or the limit between which variation is possible. i.e., obtaining a peak which mean a mountain with a pointed summit, and this prevents noise (electrical disturbance). A series of solutions ranging 1-

50 mmol.L^{-1} were prepared leading to the scatter plot as shown in Figure 7A & B in which a correlation of Y_z (mV) versus t_{sec} (d_{mm}) of 0.9333 and 0.9420 with coefficient of determination of 0.8711 and 0.8882 is given and the chosen equation represents 87.11% and 88.82% of the obtained results for cell A and cell B consequently which is fair enough. Therefore, both cells were able to distinguish various ranges of concentration. Table 3

summarizes all results for both cells (A & B). Ordinary spectrophotometer will never reach this level of concentration. If under any circumstances higher concentration was

required for analyte dilution, it can be used to make the responses with the range available in the text above.

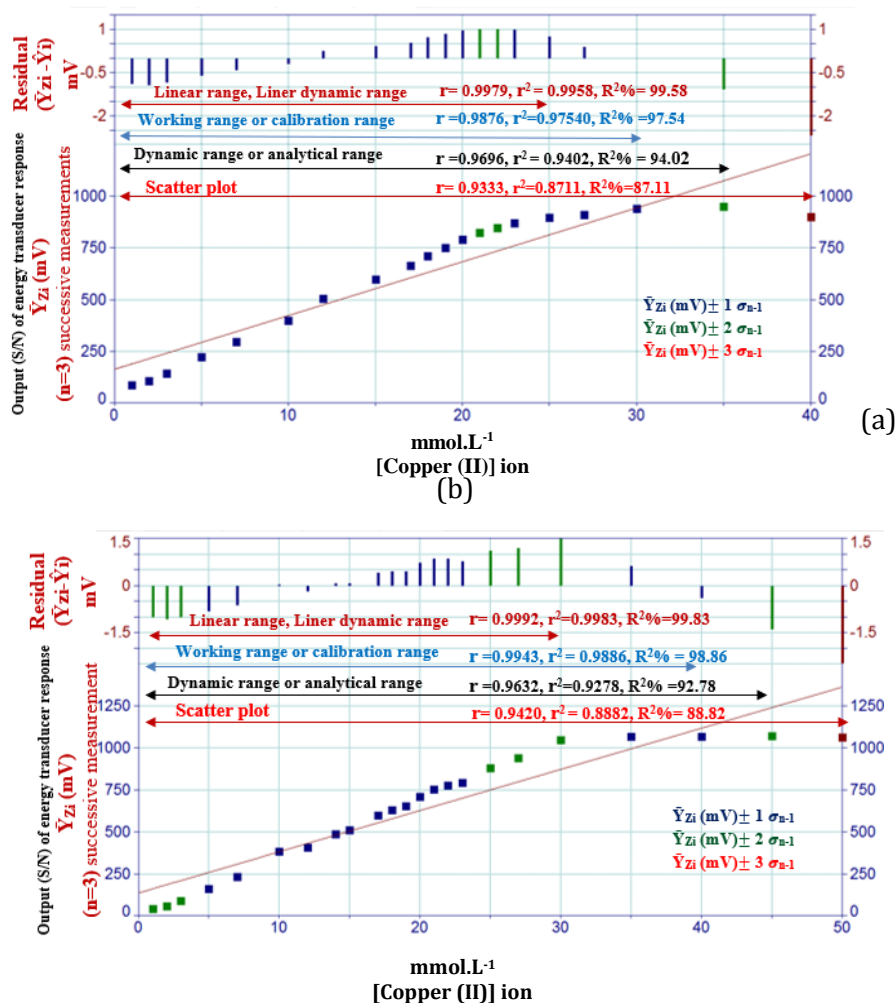


FIGURE 7 Different range for the effect of copper (II) ion concentration on attenuation of incident light using NAG- ADF-300-2 analyzer. a: For cell A & b: For cell B

TABLE 3 Summary of results for linear regression for the variation of (S/N) energy transducer response with copper (II) ion concentration using first degree equation of the form $\hat{Y} = a + b x$ at optimum conditions

Type of mode	Range of [Copper (II)] ion mmol.L ⁻¹ (n)	$\hat{Y}_{Zi}(\text{mV}) = a_{\text{mV}} \pm S_a t + b (\Delta y_{\text{mV}/\Delta x_{\text{mmol.L}^{-1}}}) \pm S_b t$ [Cu (II)] mmol.L ⁻¹ at confidence level 95%, n-2	r, r ² , R ² %	t _{tab} at 95%, n-2	Calculated t-value $t_{\text{cal}} = r / \sqrt{1 - r^2}$
Linear range or linear dynamic range	1-25 (16)	$47.7018 \pm 21.2389 + 36.1944 \pm 1.3416$ [Copper (II)] mmol.L ⁻¹	0.9979, 0.9958, 99.58	2.145	$<< 57.6141$
	1-30 (19)	$-7.1615 \pm 13.1056 + 35.3748 \pm 0.7478$ [Copper (II)] mmol.L ⁻¹	0.99915, 0.9983, 99.83	2.110	$<< 99.6276$
Working range or calibration range	1-30 (18)	$77.1064 \pm 49.4140 + 33.1700 \pm 2.7918$ [Copper (II)] mmol.L ⁻¹	0.9876, 0.9754, 97.54	2.120	$<< 25.1875$
	1-35 (20)	$13.1357 \pm 33.6584 + 33.6584 \pm 1.7915$ [Copper (II)] mmol.L ⁻¹	0.9943, 0.9886, 98.86	2.101	$<< 39.5098$
Dynamic range or	1-35 (19)	$112.4339 \pm 3.8513 + 30.0954 \pm 3.8855$ [Copper (II)] mmol.L ⁻¹	0.9696, 0.9402, 94.02	2.110	$<< 16.3481$

analytical range	1-45 (22)	91.8782±79.1816+27.6075±3.5929 [Copper (II)] mmol.L ⁻¹	0.9632, 0.9278, 92.78	2.086 << 16.0257
	1-40 (20)	163.9513±101.8163+25.98857±4.949 3 [Copper (II)] mmol.L ⁻¹	0.9333, 0.8711, 87.11	2.101 << 11.0306
Scatter plot	1-50 (23)	136.0480±94.5680+24.5230±3.9497 [Copper (II)] mmol.L ⁻¹	0.9424, 0.8882, 88.82	2.080 << 12.9104

n: no. of measurement, \hat{Y}_{zi} (mV); estimated value of cell A and cell B in mV for developed method, r: correlation coefficient, r²: coefficient of determination, R²% (percentage capital R-squared): explained variation as a percentage / total variation and $t_{tab} = t_{0.05/2, n-2}$.

Three approaches were used to describe the limit of detection (L.o.D). Any one of the described definition could be used depending on the practical research need. While Limit of

quantitative (L.o.Q) will be decided by the research to decide the comfortable concentration to deal with and always L.o.Q is higher than L.o.D (Table 4).

TABLE 4 Limit of detection (L.o.D) for copper (II) ion at optimum achieved parameters using 78.5 µl as an injection sample for both cells, and flow rate for each line 3.8 mL.min⁻¹

Type of cell	General dilution for the minimum concentration in scatter plot	Based on the value of slope $x=3S_B/\text{slope}$	Linear equation $\hat{Y} = Y_b + 3S_B$	$\hat{Y} = Y_b + 10S_B$
Cell A	0.005 mmol.L ⁻¹	0.2196 µg/sample	8.1162	27.0538
	24.9418 ng/sample		µg/sample	µg/sample
Cell B	0.009 mmol.L ⁻¹	0.2669 µg/sample	5.5662	18.5539
	44.8952 ng/sample		µg/sample	µg/sample

To study the efficiency of the newly developed methodology using NAG-ADF-300-2 analyzer for determination of copper (II) ion, it was compared with an available literature method of UV-spectrophotometric which was based on the measurements of absorbance for the range of concentration 1-37 mmol.L⁻¹ at $\lambda_{max} = 300$ nm [24] using quartz cell. The

scatter plot and the best linear range extended from 1-27 mmol.L⁻¹ with (r) of 0.9979 and % capital R square= 99.59 %, n=12 (no. of measurements). Table 5 shows the variable data treatments. The limit of detection was 0.01 mmol.L⁻¹ equivalent to 0.6355 µg/sample.

TABLE 5 Different ranges for the copper (II) ion concentration versus absorbance using spectrophotometer (classical method)

Type of mode	Range of [copper (II)] mmol.L ⁻¹ (n)	$\hat{Y}_{zi} = a \pm S_a t + b (\Delta y / \Delta x_{\text{mmol/L}}) \pm S_b t$ [copper (II)] mmol.L ⁻¹ at confidence level 95%, n-2	r, r ² , R ² %	t_{tab} at 95%, n-2	Calculated t-value $t_{cal} = r / \sqrt{(n-2) / \sqrt{1-r^2}}$
Linear range or linear dynamic range	1-27 (12)	-0.0194± 0.0429 + 0.0592 ± 0.0027 [copper (II)] mmol.L ⁻¹	0.9979, 0.9959, 99.59	2.228	<49.2862
Working range or calibration range	1-32 (14)	0.0223±0.0765 + 0.0549± 0.0040 [copper (II)] mmol.L ⁻¹	0.9932, 0.9865, 98.65	2.179	<29.6018
Dynamic range or analytical range and Scatter plot	1-37 (16)	0.0883± 0.1219 + 0.0491 ± 0.0056 [copper (II)] mmol.L ⁻¹	0.9808, 0.9620, 96.20	2.145	<18.8254

n: number of injection, \hat{Y}_{zi} : estimated value without unit on spectrophotometric, r: correlation coefficient, r²: coefficient of determination, R² % (percentage capital R- squared): explained variation as a percentage / total variation and $t_{tab} = t_{0.05/2, n-2}$.

Assessment of NAG-ADF-300-2 analyzer using both cells for the determination of copper (II) ion in alloys

A comparative study was done between the developed method and conventional method to determine copper in two types of alloy from different manufactures with different contents. On this basis, the quoted value is the absolute reference value that will be compared with the values from the table 6 A & B. The values of the copper (II) ion were obtained using copper (II) ion – rubeanic acid (17 mmol.L⁻¹) -NH₄Cl (50 mmol.L⁻¹) system, flow rate 3.8 mL.min⁻¹ of each line, 78.5 μL sample volume for cell A and cell B respectively, in addition to direct attachment delay reaction coil for cell A and cell B, I=3 for cell A and I= 2 for cell B which form a black precipitate. The standard addition method for both methods (i.e., developed method and classical spectrophotometric method) were applied by preparing a series of solutions from sample no.1 via transferring 1.27 mL of (0.157 mol.L⁻¹) to each volumetric flask (25

mL), followed by the addition of (0, 0.5, 1, 2 and 3 mL) from 100 mmol.L⁻¹ of standard solution to obtain 0, 2, 4, 8 and 12 mmol.L⁻¹ and 16.95 mL from sample no.2 of (0.0118 mol.L⁻¹) to volumetric flask (25 mL), followed by the addition of (0, 0.5, 1, 2 and 3 mL) from 100 mmol.L⁻¹ of standard solution to obtain 0, 2, 4, 8 and 12 mmol.L⁻¹. Table 6B represents the form of two types of statistical analysis for comparison the means and variance from different methods.

The first comparison was based on the one-way ANOVA (F-test) [25,26], which was carried out at $\alpha = 0.05$ (95 % confidence level) for compare between four different methods (i.e., Cell A, Cell B (using NAG-ADF 300-2), UV-spectrophotometry and reference method). This test (i.e., ANOVA) (Table 6. B and scheme 2 summed up the obtained results) depends on the calculated F- value for comparing three or more means. The first estimate is called between group variance while second estimate is based on the within variance.

The hypothesis should be used as follows:

H₀ (Null hypothesis): $\bar{W}_{\text{Cell A}} = \bar{W}_{\text{Cell B}} = \bar{W}_{\text{UV-spectrophotometry}} = \bar{W}_{\text{reference quoted method}}$

Against

H₁ (Alternative hypothesis): At least one mean is different from the other

i.e., $\bar{W}_{\text{Cell A}} \neq \bar{W}_{\text{Cell B}} \neq \bar{W}_{\text{UV-spectrophotometry}} \neq \bar{W}_{\text{reference quoted method}}$

From the results obtained that were tabulated in Table 6B (Column 5), the kind of two samples of alloys shows no significant difference between the means since the value of $F_{\text{cal}} (5374.6940) \gg F_{\text{tab}} (5.99)$; therefore, null hypothesis will be accepted and the alternative hypothesis will be rejected.

As for the second comparison, we used paired t-test to compare the newly developed method

i.e., $H_0 = \bar{W}_i (\text{NAG-ADF-300-2 analyser}) = \bar{W}_i (\text{classical - spectrophotometry})$

(for either of the two alloys)

Against

i.e., $H_1 : \bar{W}_i (\text{NAG-ADF-300-2 analyser}) \neq \bar{W}_i (\text{classical - spectrophotometry})$

for either of the two alloys

i.e.; NAG-ADF-300-2 analyzer for both cells with the classical spectrophotometric method at $\lambda_{\text{max}} = 300 \text{ nm}$. The obtained results summed up in Table 6.B (column 6) and scheme 2, in which a hypothesis can be estimated as follows:

Null hypothesis: There is no significant difference between the means obtained from both methods

Alternative hypothesis: There is a significant difference in the means of both method for two samples

And, from the results, it was concluded that there was no significant difference between

the two methods at 95% confidence level as the calculated t- value ($t_{\text{cal}} = 0.9855$ (cell A), 0.9956 (cell B)) is less than critical t- value (12.706).

On this basis, two methods (cell A & cell B) were added to determine the copper (II) ion in addition to the traditional method.

TABLE 6A Standard addition results for the determination of copper (II) in two alloy of using NAG-ADF-300-2 analyzer for cell A, cell B and UV-spectrophotometric (Classical method)

No. of Sample	Type of alloy	Theoretical content of Cu (II) ion in 1 g (Sample 1) and 10 g in (sample 2) (g) mol.L ⁻¹	Type of method					Equation of standard addition at 95% for n-2	r
			Newly developed method						
			Cell A						
			Cell B						
			Classical method at $\lambda_{\text{max}} = 300 \text{ nm}$						
			Volume (ml)						
			[Cu (II)] mmol.L ⁻¹						
			0 ml	0.5 ml	1 ml	2 ml	3 ml	$\hat{Y}_{zi} (\text{mV}) = a \pm S_a t + b \pm S_b t [\text{Cu (II)}] \text{ mmol.L}^{-1}$	r
			0	2	4	8	12		r ²
			$\bar{y}_{zi} (n=3)$					$\hat{Y}_{zi} = a \pm S_a t + b \pm S_b t [\text{Cu (II)}] \text{ mmol.L}^{-1}$	R ² %
1	High purity copper. B.C.S. No. 197e 99.95 %	0.9995 g	290	355	412	580	720	$281.9138 \pm 24.2593 + 36.4397 \pm 3.5925 [\text{Cu(II)}] \text{ mmol.L}^{-1}$	0.9986 0.9971 99.71%
		(0.157 mol.L ⁻¹)	223	293	360	482	580	$232.6983 \pm 25.2704 + 29.7888 \pm 3.7422 [\text{Cu(II)}] \text{ mmol.L}^{-1}$	0.9977 0.9953 99.53%
		1.27 ml (8 mmol.L ⁻¹)	0.42	0.60	0.73	0.97	1.17	$0.4591 \pm 0.0768 + 0.0613 \pm 0.0114 [\text{Cu (II)}] \text{ mmol.L}^{-1}$	0.9949 0.9899 98.99%
2	low alloy steel B.C.S. No. 255/1 0.75%	0.075 g	252	308	380	510	640	$248.3276 \pm 8.7767 + 32.6293 \pm 1.2997 [\text{Cu (II)}] \text{ mmol.L}^{-1}$	0.9998 0.9995 99.95%
		0.0118 mol.L ⁻¹	132	175	198	280	340	$134.6724 \pm 14.4812 + 17.3707 \pm 2.1445 [\text{Cu (II)}] \text{ mmol.L}^{-1}$	0.9977 0.9955 99.55%
		16.95 ml (8 mmol.L ⁻¹)	0.36	0.41	0.51	0.68	0.87	$0.3405 \pm 0.0371 + 0.0434 \pm 0.0055 [\text{Cu (II)}] \text{ mmol.L}^{-1}$	0.9976 0.9953 99.53%

\hat{Y} : Estimated response in mV for developed and absorbance for UV-Sp. method, r: correlation coefficient, r²: coefficient of determination, R² % (percentage capital R-squared): explained variation as a percentage/ total variation, $t_{0.05/2, \infty} = 1.96$ at 95 %, $t_{0.05/2, 3} = 3.182$ for n=5

TABLE 6B Summary of results for practical content, efficiency (Rec %) for determination of copper (II) ion in two different samples of (high purity copper)

No. of Sample	Type of method							
	Newly developed method							
	Cell A							
	Cell B							
	(Classical method) UV- Spectrophotometric at $\lambda_{max}= 300 \text{ nm}$							
	Practically concentration in 25 ml (mmol.L ⁻¹) in 100 ml (mol.L ⁻¹)	Practically weight of Cu (II) ion \bar{W}_i (mg) \bar{W}_i (mg) \pm 4.303 σ_{n-1}/\sqrt{n} in 1 g (sample 1) and 10 g in (sample 2) Practically weight of Cu (II) ion in alloy (100 mg) (g)	Efficiency of determination Recovery % E. O. D	ANOVA- one way			Paired t –test Compared between two methods	
				Source			$t_{cal} = \frac{\bar{X}d}{\sigma_{n-1}}$	t_{tab} at 95% confidence level
					Betw een	Within (error)	Cell A	
1	7.736 0.152	0.9677 \pm 0.0325 96.77 \pm 3.25	96.82%	SS _q	18540 .928	20.698	$\bar{X}d = 1.5635$ $\sigma_{n-1} = 2.2436$ $t_{cal} \text{ Cell A} \ll t_{tab}$ 0.9855 \ll 12.706	
	7.812 0.154	0.9772 \pm 0.0823 97.72 \pm 8.23	97.77%					
	7.484 0.147	0.9362 \pm 0.0423 93.62 \pm 4.23	93.67%	d. f	K-1 1	(N-K) 6		
2	7.6106 0.0112	0.0713 \pm 0.00421 0.713 \pm 0.0421	95.07%	S ²	18540 .928	3.4496	Cell B $\bar{X}d = 2.0455$ $\sigma_{n-1} = 2.9055$ $t_{cal} \text{ Cell B} \ll t_{tab}$ 0.9956 \ll 12.706	
	7.753 0.0114	0.0727 \pm 0.00512 0.727 \pm 0.0512	96.93%					
	7.853 0.0116	0.0736 \pm 0.00721 0.736 \pm 0.0721	98.14%	results $F_{cal} \gg F_{tab} (F_6^1)$ 5374.6948 \gg 5.99				

μ : quoted value, $\bar{X}d$: average of difference between two type of method (developed & classical), n (no. of sample) = 2, σ_{n-1} : standard deviation of different, \bar{w}_i : practically weight in mg, $t_{tab} = t_{0.05/2,1} = 12.706$ (paired t-test). $F_{cal} = S_B^2 / S_W^2$, SS_q : Sum of squares, S_B^2 : between group variance S_W^2 : within group variance, d.f: degree of freedom, N: Sum of sample (measurements), K: number of groups, ANOVA : Analysis of variance, $F_{0.95, \nu_1, \nu_2} = F_{0.95, K-1, N-K} = F_{\nu_1}^{\nu_2} = F_6^1 = 5.99$

Conclusion

The unchallenged precision and accuracy gained in this research work using NAG-ADF-

300-2. Shows that an attenuation method that can be used to determination Copper (II) ion in a well trusted measurements, which is the

complete agreement with the cited values. No other turbidimetric method is available in the literature that can follow the same mode of working with the simplicity of the manifold used. Therefore, an alternative method is available with extended linearity and excellent L.o.D.

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