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Determination of Copper in Hydroponic Nutrient Solutions by UV-Visible Spectrophotometry and Flow Injection Analysis

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Abstract: Copper (Cu) is a micronutrient that plays an important role in the growth of plants. A spectrophotometric analysis method has been developed to determine Cu^{2+} in hydroponic nutrient solutions. 4-(2-pyridylazo)-resorcinol (PAR), 1-(2 pyridylazo)-2-naphthol (PAN) and 1-(2-thiazolylazo)-2-naphthol (TAN) ligands were studied as co-complexants that will enable determination of Cu^{2+}. Each microelement has an optimum pH value at which it forms a complex with the ligand. By studying the effect of pH on the metal-ligand complex, the optimum pH values at which maximum absorbances were obtained were determined. The interference effects of foreign ions in the nutrient solution were examined. 4-(2-pyridylazo)-resorcinol (PAR) complexes were found to be more resistant to foreign ion interference. For this reason, validation studies of the method developed for 4-(2-pyridylazo)-resorcinol (PAR) complexes were carried out. Afterwards, the developed method was adapted to the flow injection analysis system. The procedure is simple, rapid and reliable. This method was successfully applied to the determination of copper in hydroponic nutrient solutions.

Keywords: Micronutrients, Hydroponic nutrient solution, Spectrophotometry, Flow injection analysis.

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1. INTRODUCTION

Hydroponic farming is a type of soilless farming system, and the most distinctive feature that distinguishes it from conventional farming is that it directly uses mineral-rich solutions to feed plants (1). The nutrient supply notably influences plant growth in many ways. The growth of plants is influenced both by macronutrients such as N, P, and K and by micronutrients (2). For this reason, it is important that the nutrient solution, which is one of the primary factors in the growth of plants, contains all the necessary nutrients that plants need (2). Today, using nutrient solutions to optimize crop nutrition (fertilization or liquid fertilization) is a popular practice in soil-grown greenhouse crops as well as in hydroponic cultures (3). Agricultural research in the world is focused on "in situ and specific application". The aim of the studies is to increase the agricultural quality, capacity, and yield, to make an accurate and optimum application (such as fertilization, pesticide, irrigation, harvest time estimation), and thus to add value to the agricultural economy. In most national and international greenhouse enterprises, the ion control of the nutrient solution is done by automatic measurement of pH and electrical conductivity (EC), the solution content is followed according to the EC change (4). However, since EC is the total electrical conductivity value, it cannot give information about the concentration of each ion in the system. Therefore, it is not possible to make individual and real-time corrections for nutrients. With such a determination method, more or less fertilization will be made than the ion needed by the plant, in this case, sufficient yield will not be obtained from the plant.

Copper is one of the vital trace elements needed for plant development (5). It serves as a key component of various proteins. It plays several important roles, including in cell wall metabolism, photosynthetic electron transport, responses to oxidative stress, protein synthesis, hormone regulation, and mitochondrial respiration (6). Although copper is essential for plants in small amounts to support cellular functions as a micronutrient, excessive copper can be harmful, leading to negative impacts on plant growth and survival. Plants are capable of surviving and tolerating conditions with a deficiency in Cu^{2+}. However, when the concentration of Cu^{2+} surpasses the optimal level, it interferes with the plant's metabolism and becomes harmful. An excess of copper significantly hampers photosynthesis by stunting plant growth and development, limiting nutrient uptake from the soil, decreasing pigment levels, and inhibiting root growth and leaf expansion (7). Furthermore, excessive copper disturbs the proper functioning of essential cellular components (8).

Various techniques, including graphite furnace atomic absorption spectrometry (GF-AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and ion-selective electrode (ISE), have been established to measure copper (II) ions in solutions based on their concentration. However, many of these methods involve procedures that are either time-intensive or require complex equipment. Additionally, certain spectrophotometric approaches previously used for the determination of copper (II) present drawbacks, such as being time-consuming, labor-intensive, and relying on significant quantities of organic solvents, which can contribute to environmental pollution (9).

In recent years, flow injections have emerged as a popular analytical method, extensively utilized with spectrophotometric detection due to its widespread availability in laboratories worldwide. It offers advantages such as low cost, minimal reagent consumption, reduced waste generation, cost efficiency, small sample volume requirements, and ease of automation. Additionally, flow injections are widely applied in quantitative analysis and enable experiments under dynamically non-equilibrated conditions, thereby reducing analysis time and increasing sample throughput (10).

When planning a procedure (from sampling, extraction, purification to instrument analysis) in analytical chemistry, the green chemistry factor must be taken into consideration. There are various tools developed to assess the environmental impacts of analytical methods. These tools aim to increase the sustainability of analytical methods by assessing the toxicity of chemicals used in the analysis processes, energy consumption, waste amount and overall environmental impact (11).

The environmental sustainability of the method was assessed using the AGREE, AGREEprep and MoGAPI tools (12).

Many studies have developed methods for measuring copper in various samples (13-20). In this study, however, a spectrophotometric method was developed that will enable the measurement of copper elements in hydroponic nutrient solutions. With the developed method, the amounts of copper in hydroponic nutrient solutions can be determined specifically. In this way, faulty analyses based on electrical conductivity measurements made with EC meters in greenhouses will be prevented, correct and controlled fertilization will be made, and productivity and quality will be increased in production.

2. EXPERIMENTAL SECTION

2.1. Instrumentation

Shimadzu-1800 model UV-Vis spectrophotometer (Kyoto, Japan) for absorbance measurements and wavelength scanning for method optimization and analysis of metal ligand complexes in still environment studies, KERN-PFB 1200 2 model precision balance (Balingen, Germany) for chemical weighing, ALEX Machine ultrasonic bath (Istanbul, Turkey) for solution mixing and dissolving processes, MILLIPORE- Direct Q UV 3 model deionized pure water device (Burlington, Massachusetts, USA) for the preparation of ultrapure water used in solution preparation and cleaning of glassware, WTW-7310 model desktop pH meter (Weilheim, Germany) for pH measurements of solutions, IKA-VORTEX 3 model vortex (Staufen, Germany) for mixing solutions, Eppendorf Research series 10 µL, 100 µL, 100-1000 µL, 1000-5000 µL automatic pipettes (Hamburg, Germany) were used for solution transfers. For flow injection analysis; Ismatec-REGLO ICC peristaltic pump (SA, Switzerland) to transfer the solutions to the flow system, Rheodyne sample injection valve (Northbrook, Illinois, USA) to allow the sample to enter the flow system, Ocean Optics flame spectrometer (Massachusetts, USA) to detect the formed species, Ocean Optics beam source (Massachusetts, USA) to provide the beam to be sent to the flow cell, Biotech Degassi Classic degaser (Kungsbacka, Sweden) to remove air from the mobile phase, Saint Gobain Tygon™ S3™ E-3603 tubes (Courbevoie, France) for the solutions to advance in the flow system, plexiglass flow cell (Istanbul, Turkey) to pass enough light through the path were used. AGILENT- 240 FS AA model Atomic Absorption Spectrophotometer (Santa Clara, United States) was used for method validation tests.

2.2. Materials and Reagents

4-(2-pyridylazo)-resorcinol (PAR) (≥97.5% purity) and 1-(2-pyridylazo)-2-naphthol (PAN) (99.0%) were purchased from Sigma-Aldrich (Munich, Germany). 1-(2-thiazolylazo)-2-naphthol (TAN) (≥97.5%) was purchased from Alfa Aesar (Massachusetts, USA). Potassium chloride (KCl) (≥ 99.5%), ammonium chloride (NH_4Cl) (≥ 99.8%), sodium acetate ($NaCH_3COO$) (≥ 99.0%), ammonium acetate (NH_4CHOO) (≥ 98.0%), ammonium sulfate ($(NH_4)_2SO_4$) (≥ 99.5%), boric acid (H_3BO_3) (99.5 - 100.5%), glacial acetic acid (CH_3COOH) (≥ 99.8%), hydrochloric acid fuming 37% (HCl) (36.5 - 38.0%), sodium hydroxide (NaOH) (≥ 97.0%), ethanol (≥99.9%), nitrate standard (1000 µg/mL) in water (H_2O), calcium standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), potassium standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), Triton X 100 (90.0 - 110.0%) were purchased Merck (Rahway, NJ USA). Zinc standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), cupper standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), iron standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), manganese standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), boron standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), phosphate standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3), magnesium standard (1000 µg/mL) in 0.5 M nitric acid (HNO_3) were purchased from Reagecon (Clare, Ireland). Ultrapure water (Milli-Q RG, Merck Millipore, Burlington, Massachusetts) was used throughout this study.

2.3. Preparation Stock and Standard Solutions

For the stock solution of 4-(2-pyridylazo)-resorcinol (PAR), 1-(2-pyridylazo)-2-naphthol (PAN) and 1-(2-thiazolylazo)-2-naphthol (TAN) were prepared in ethanol (≥99.9%) and were further diluted in ethanol (≥99.9%) as required. These solutions were freshly prepared. All standard solutions were diluted with ultrapure water.

2.4. Determination of pH Conditions

Complex formations Cu^{2+} ions with 4-(2-pyridylazo)-resorcinol (PAR), 1-(2-pyridylazo) 2-naphthol (PAN) and 1-(2-thiazolylazo)-2-naphthol (TAN) were studied at different pH values (pH 3-pH 11). For pH 3 buffer solution, 0.657 g of solid KCl is dissolved in some ultrapure water and 11.9 mL of HCl is added. Final volume is made up to 100 mL with ultrapure water. Acetate buffers (16 mL of 0.1 M sodium acetate and 84 mL of 0.1 M acetic acid, 67.8 mL of 0.1 M sodium acetate and 32.2 mL of 0.1 M acetic acid, respectively) were used for the pH 4 and pH 5. For pH 6 buffer solution 0.82 mL of glacial CH_3COOH was added to 20 g of solid NH_4CH_3COO salt and dissolved in a little amount of ultrapure water after it was made up to 100 mL with ultrapure water. For pH 7 41.3 mL of 1/15 M (9.073 g/L) KH_2PO_4 and 58.7 mL of 1/15 M (11.87 g/L) Na_2HPO_4 solution were mixed. Solution A: (0.05 M Boric acid); 1.237 g solid H_3BO_3 was dissolved with 1 M NaOH, and the final volume was made up to 100 mL with 1 M NaOH. Solution B: (0.1 M HCl); 0.2 mL of 37% HCl was made up to 25 mL with ultrapure water. 55.4 mL of solution A and 44.6 mL of solution B were mixed for pH 8. (0.25 M) 1.55 g of solid H_3BO_3 was dissolved in some 0.05 M KCl and made up to 100 mL with 0.05 M KCl for pH 9, for pH 10 2.64 g of solid H_3BO_3 was dissolved in 90 mL of ultrapure water. The pH was adjusted to 10 with 10 M NaOH. The final volume was made up to 100 mL with ultrapure water. 6.75 g of solid NH_4Cl was dissolved in ammonia and made up to 100 mL with ammonia for pH 11. When necessary, the pH of the buffers was adjusted to the required values with HCl and NaOH solutions.

2.5. Spectra and The Complex Formation

The reagent and standard solutions were prepared according to the descriptions below. Shortly, 1.2 mg 4-(2-pyridylazo)-resorcinol (PAR) dissolved in a little amount of ethanol was transferred to an amber volumetric flask and made up to 25 mL with ethanol. The solution was stored at 4°C until analysis and prepared freshly for daily investigation. In the same way, 1 mg of 1-(2 pyridylazo)-2-naphthol (PAN) in 25 mL ethanol and 1 mg of 1-(2-thiazolylazo)-2-naphthol (TAN) in 25 mL of ethanol solution were prepared. Prior to analysis, standard solutions of Cu^{2+} diluted from 1000 ppm to 10 ppm with ultrapure water. To prepare the calibration chart of Cu-PAR complexes, appropriate volumes were taken from 10 ppm Cu^{2+} solution with final concentrations 0.125; 0.25; 0.5; 0.75, and 1 ppm. 2.23×10^{-4} M, 1.2 mL 4-(2-pyridylazo)-resorcinol (PAR), 25 µL of 10% Triton X-100, and 1 mL of buffer solution were added in this order. The final volume was made up to 5 mL with ethanol. Afterward, standard solutions were mixed for 10 seconds with a vortex. Absorbance values were read against the blank solution at a wavelength of 512 nm. The same procedure was applied to prepare the calibration graphs of TAN and PAN complexes. For TAN complexes, 1.56×10^{-4} M 4.4 mL 1-(2-thiazolylazo)-2-naphthol (TAN) was used and absorbance values were read at 576 nm wavelength. For PAN complexes, 1.6×10^{-4} M 1-(2-pyridylazo)-2 naphthol (PAN) was used and absorbance values were read at 553 nm wavelength.

2.6. Flow Injection Analysis

Figure 1 shows the absorbance schematic diagram of the flow system. The four-channel peristaltic pump was fitted for pumping the solutions. The tubes with 0.76 mm i.d. were used for delivery of the solutions. The ultrapure water was used as carrier. Sample and standard solutions were injected into a carrier stream with a sample injector. While the flame spectrometer was used to detect the species formed, the beam source was used to send the beam to the flow cell. The degasser removed air from the mobile phase and the plexiglass 2 mm flow cell to pass enough light through the path used. Three channels of the pump were used for the analysis of Cu^{2+} microelement in the flow injection analysis system. Sample/standard (2 mL/min), carrier (2 mL/min) and 4-(2 pyridylazo)-resorcinol (PAR) / buffer (1 mL/min) pass through these channels, respectively. The liquids that move along the line and mix in the reaction coil are detected (512 nm) by moving towards the flow cell after turning into the detectable species here.

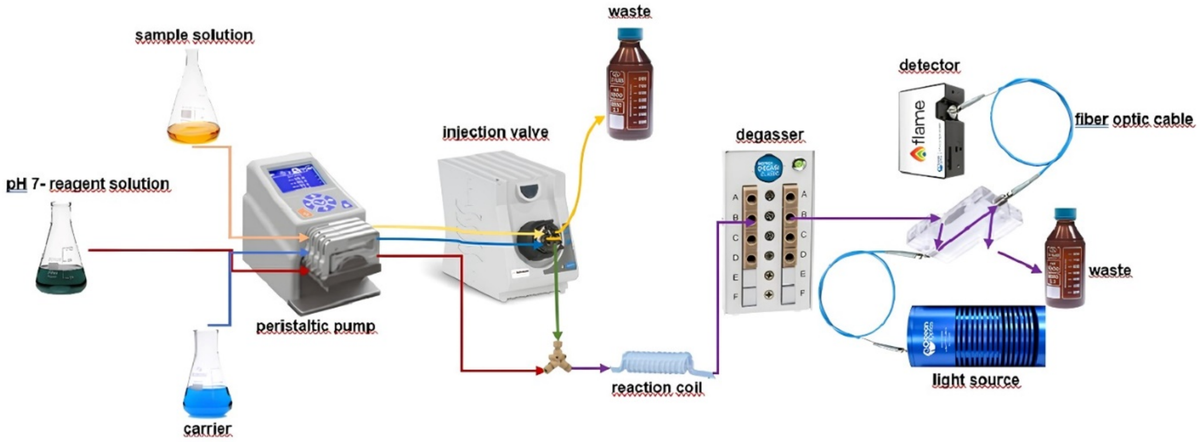

Figure 1: Schematic diagram of the flow injection system used for spectrophotometric determination of copper (II). Sample solutions / standard solutions, carrier (deionized water), pH7-R; reagent solution (pH 7 buffer solution - 4-(2-pyridylazo)-resorcinol (PAR) - deionized water), peristaltic pump, injection valve, waste, sample loop (200 µL), reaction coil (300 cm), degasser, flow cell (2 mm), source of light, detector (λ:512 nm).

3. RESULTS AND DISCUSSION

3.1. Effect of pH

Firstly, the effect of the pH on 4-(2-pyridylazo)-resorcinol (PAR), 1-(2-pyridylazo)-2 naphthol (PAN) and 1-(2-thiazolylazo)-2-naftol (TAN) complexes of Cu^{2+} have been investigated individually. Metal ligand complexes were formed using buffer solutions between pH 3 and pH 11 to determine the optimum pH range. The pH values and wavelengths at which Cu^{2+} gave maximum absorbance were determined. Table 1 shows the wavelengths at which Cu^{2+} gives maximum absorbance and Figure 2 shows absorbance values of the Cu^{2+} complexes between pH 3 and pH 11. Considering the spectrum results, the complex formed by Cu^{2+} and 4-(2-pyridylazo)-resorcinol (PAR) gave maximum absorbance between pH 7 and pH 9. However, 1-(2-pyridylazo)-2 naphthol (PAN) complexes give maximum absorbance at pH 8 and 9, while 1-(2-thiazolylazo)-2-naphthol (TAN) complexes give maximum peak at pH 5. pH values at which Cu^{2+} gives maximum absorbance with the 4-(2-pyridylazo)-resorcinol (PAR), 1 (2-pyridylazo)-2-naphthol (PAN) and 1-(2-thiazolylazo)-2-naphthol (TAN) were selected for further studies.

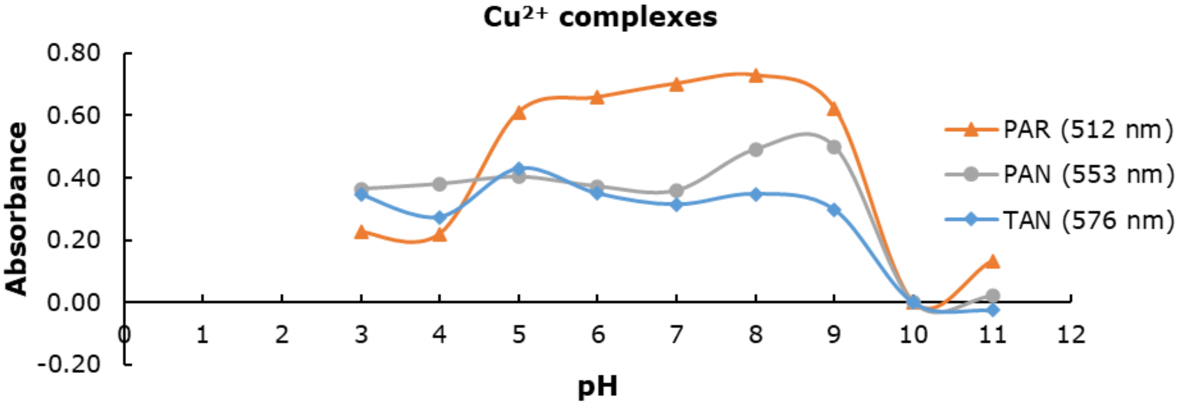

Figure 2: Between pH 3 - pH 11, absorbance values of the Cu^{2+} complexes. Cu-PAR complex, $C_{Cu\,=}$ 1 µg/mL, $C_{PAR=}$ 4.4 x10^{-5} M, Λ: 512 nm). Cu-PAN complex ($C_{Cu\,=}$ 1 µg/mL, $C_{PAN=}$ 7.4 x10^{-5} M, Λ: 553 nm). Cu-TAN complex ($C_{Cu\,=}$ 1 µg/mL, $C_{TAN=}$ 3.9 x10^{5} M, Λ:576 nm).

Table 1: Summarized data of absorption maxima of Cu^{2+} with PAR, PAN and TAN complexes.

Ligand	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11
PAR	540	520	513	514	513	510	512	513	513
PAN	561	559	555	553	551	553	554	554	-
TAN	583	579	577	575	574	583	583	-	-

3.2. Spectrophotometric Analysis of Cu^{2+} Ions

Absorption spectra of PAR, PAN, and TAN ranging from 300 to 800 nm were first scanned, and the absorption maxima were determined under all pH conditions. Then, the formation of complexes between the Cu^{2+} ions and PAR, PAN, and TAN were tested. Absorption spectra versus the blank were recorded over the same wavelength range. The blank for the metal complex was composed of the buffer, water, ethanol, and chromogenic reagent solution. 10 mm path length, 3500 µL quartz glass high performance macro cells (Hellma Analytics, Müllheim, Germany) and A UV-1800 model UV–vis spectrophotometer equipped with UV Probe 2.43 version (Shimadzu, Kyoto, Japan) were used for analysis. Full scan spectra were recorded from 800 to 300 nm with a slit width of 1.0 nm and fast scanning speed. Spectral properties of the complexes formed by Cu^{2+} with PAR, PAN, and TAN are given in Figure 3. The rapid reaction between the metal and the ligand allows the reaction circle to be shorter in flow injection analysis, thus allowing more sample analysis in a shorter time. In this sense, all three ligand sources are suitable for use in flow injection analysis for Cu^{2+} analysis.

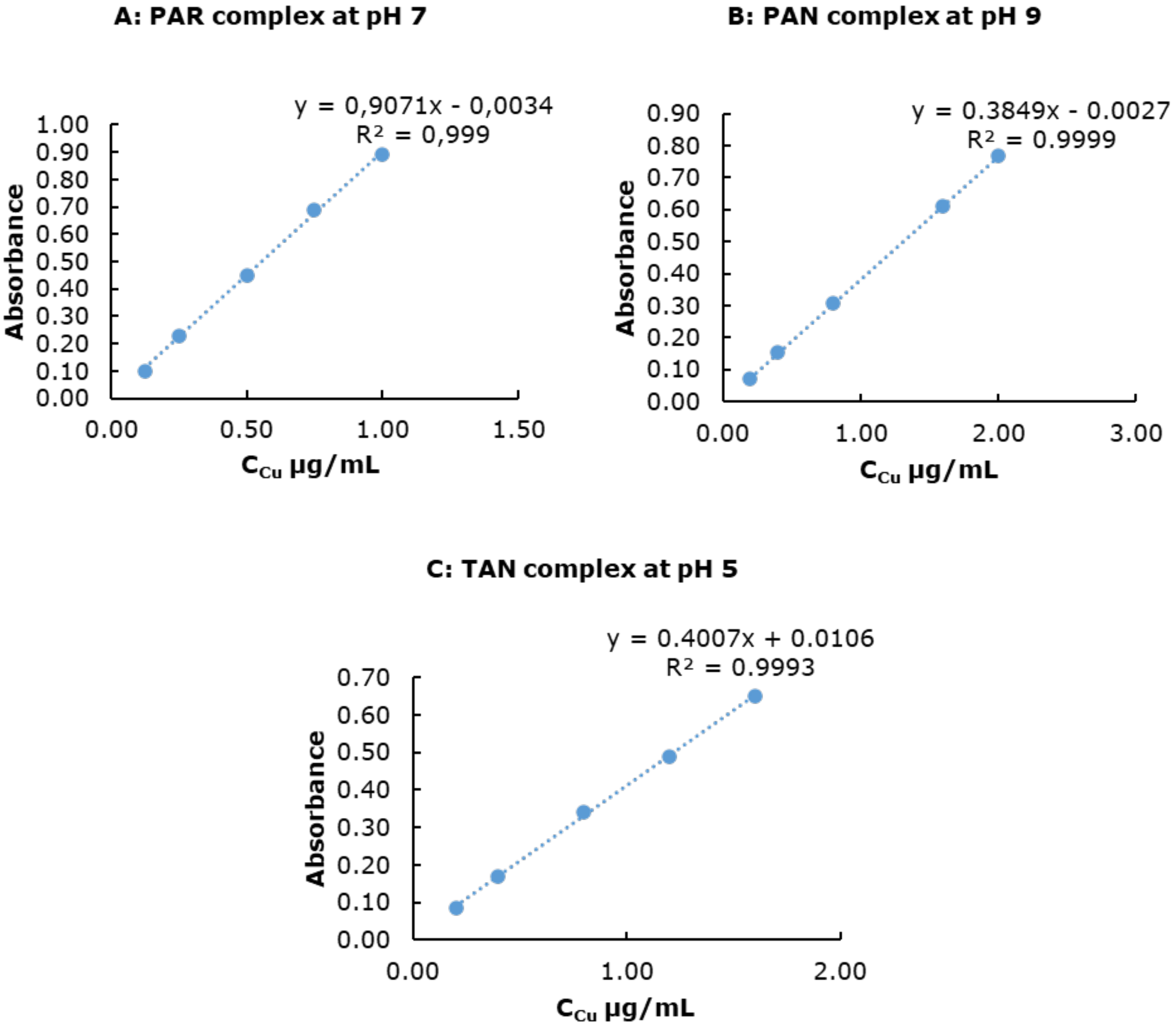

Figure 3: Calibration charts of Cu^{2+}. A) PAR complex at pH 7, Λ: 512 nm B) PAN complex at pH 9, Λ: 553 nm C) TAN complex at pH 5, Λ: 576 nm.

3.3. Interference Studies

The interference effects of K^+, NH_4^+, Zn^{2+}, Fe^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, BO_3^{3-}, NO_3^-, SO_4^{2-} and PO_4^{3-} ions for each metal ligand complex of other anions and cations in the matrix were investigated. While forming a fixed concentration metal-ligand solution, foreign ion solution at varying concentrations was added to the environment. The absorbance value with the least interference effect was determined by measuring the absorbance against the blank. PAR complexes were found to be more resistant to foreign ion interference. The % relative error values calculated from the determined absorbance value are given in Table 2.

Table 2: The effects of matrix ions on the determination of PAR, PAN and TAN complexes (n=3, 1 μg/mL Cu^{2+}).

Interferent	**Supply of ion**	**Ligand of complex**	**Add ion concentration, μg/mL**	**Recovery, % Cu^{2+}**
K^+	Standard KNO_3	PAR	210	-9.6
		PAN	400	-10.4
		TAN	50	-19.9
NH_4^+	NH_4CH_3COO	PAR	50	-5.6
		PAN	20	-2.5
		TAN	50	-9.9
Ca^{2+}	Standard Ca^{2+}	PAR	200	-9.4
		PAN	200	-7.2
		TAN	25	-13.8
Mg^{2+}	Standard Mg^{2+}	PAR	25	-4.8
		PAN	200	-1.4
		TAN	25	-16.6
Fe^{2+}	Standard Fe^{2+}	PAR	3	+5.3
		PAN	2.5	+54.1
		TAN	5	-74
Mn^{2+}	Standard Mn^{2+}	PAR	0.5	+0.1
		PAN	1	-1.0
		TAN	4	-3.3
Zn^{2+}	Standard Zn^{2+}	PAR	5	+0.1
		PAN	5	+0.3
		TAN	5	+0.4
BO_3^{3-}	Standard BO_3^{3-}	PAR	10	+4.4
		PAN	10	+0.2
		TAN	20	-5.4
NO_3^-	Standard NO_3^-	PAR	100	+9.1
		PAN	200	+10.5
		TAN	200	-7.2
SO_4^{2-}	Standard SO_4^{2-}	PAR	50	+4.2
		PAN	50	+5.2
		TAN	50	-6.3
PO_4^{3-}	Standard PO_4^{3-}	PAR	50	+1.3
		PAN	200	+6.7
		TAN	50	-4.7

3.4. Optimization of Spectrophotometric FIA System

It was determined that PAR complexes were more resistant to foreign ion interference compared to PAN and TAN complexes, therefore adaptation of the method to flow injection analysis was performed only for 4-(2-pyridylazo)-resorcinol (PAR) complexes. The effect of PAR concentration, carrier and reagent flow rates, sample injection volume, and reaction cell length on analytical signals was investigated and optimized one by one, to create optimum conditions for determination of Cu^{2+} by FIA method.

3.4.1. Influence of the PAR concentration

For Cu-PAR complexes, PAR in amounts ranging from 39 μM to 781 μM was added to 0.5 μg/mL Cu^{2+} at pH 7, and absorbance values were read at 512 nm. Previous studies on determining the ligand concentration in stagnant media were effective in determining this range. In Figure 4, absorbance values corresponding to PAR concentration are given for Cu^{2+} ions. It was decided to use 156 μM PAR in subsequent studies.

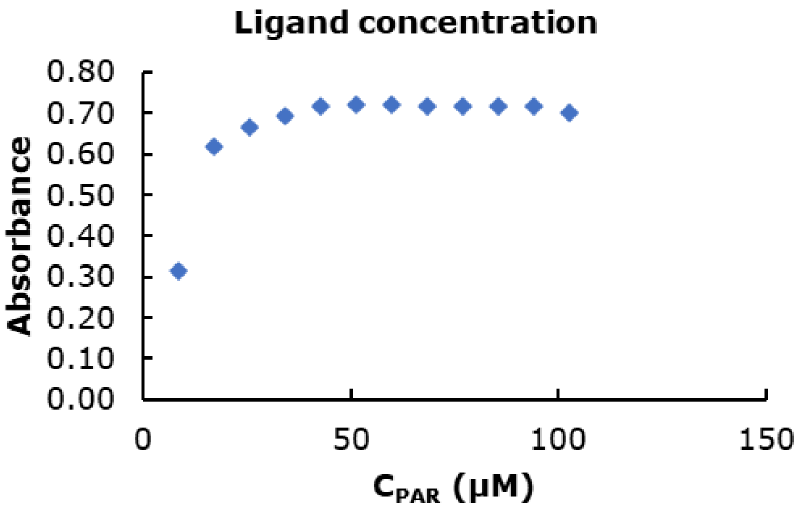

Figure 4: Plot of absorbance versus ligand concentration in flow injection analysis.

3.4.2. Determination of carrier and reagent flow rates, sample injection volume, and reaction cell length

The experiments in which PAR was used as the reagent for Cu^{2+}, 0.5 µg/mL Cu^{2+} in the sample channel, 156 µM PAR, 10 % Triton X-100, and pH 7 buffer solution in the reagent channel and deionized water in the carrier channel were passed through the system and absorbance measurements were made at 512 nm. Working ranges and selected values for each parameter are given in Table 3. When determining the working ranges, the reaction time of the analytes and the ligand in the stationary environment studies were considered. In stationary environment studies, it was observed that the analyte and the ligand reacted immediately and changed color. We can list the advantages of this situation for flow injection analysis such as keeping the reaction circle short, keeping the flow rates high, and allowing more samples to be read in a shorter time.

Table 3: Working ranges and selected values for FIA parameters.

Optimized parameters	Worked range	Max. value for pH 7
mixing coil length	100-500 cm	300 cm
carrier flow rate	1-3 mL/min	2 mL/min
reactant flow rate	0.5-1.5 mL/min	1 mL/min
injected sample volume	100-500 µL	200 µL

3.5. Method Validation

In order to determine the suitability of the method for the purpose, precision, linear range, limit of detection (LOD), limit of quantification (LOQ) parameters was examined. For the linear range, 10 standard solutions were prepared at increasing concentrations and the absorbance values read against these solutions were transferred to the calibration chart. Calibration curve for Cu^{2+} at pH 7 had a good linear relationship in the range 0.125-1 µg/mL with a correlation coefficient R^2 of 0.999. For Cu^{2+} the LOD (S/N = 3) and LOQ (S/N = 10) were 0.04 µg/mL and 0.1 µg/mL respectively. The proposed method has been applied to the determination of Cu (II) hydroponic nutrient solutions. The results obtained compared favorably with those obtained by FAAS. The paired T-test verified the accuracy. T-test value (1.89) less than the critical value at a confidence level of 95% (P value of 0.118). There is agreement between the results obtained with the proposed method and the FAAS method. Five repeated measurements were taken with Cu working solutions of known concentration on the same day, and the same process was repeated on three different days. The calculated intraday and interday precision values, relative standard deviations were ≤ 4.0%. Precision and accuracy evaluation was performed to verify the accuracy of the LOQ (Limit of Quantification) value of the method. The standard deviation (SD) and the relative standard deviation (RSD, %) for repeatability of LOQ were 0.01 and 1.39 respectively. (RSD, %) is 12.4 for intermediate precision. To test the accuracy at LOQ level, recovery was calculated at 95%. The absolute t value (5.9) calculated according to the t test (95% confidence level) result is greater than the critical t value (2.26) for d.f=8.9≈9. There is a statistically significant difference between the blank and LOQ values.

3.6. Determination of Copper in Hydroponic Nutrient Solutions

The recommended method was applied to hydroponic nutrient solutions. The results are shown in Table 4.

Table 4: Accuracy of the proposed method compared with FAAS method for determination of Cu^{2+} in hydroponic nutrient solutions samples.

Sample	Spiked, μg/mL	Found, μg/mL	Recovery, %	FAAS	Recovery, %
S1	0.20	0.19±0.02	96.48	0.20±0.01	100.00
	0.40	0.39±0.01	96.57	0.42±0.01	105.00
S2	0.20	0.20±0.02	100.35	0.21±0.00	105.00
	0.40	0.38±0.00	95.36	0.42±0.00	105.00
S3	0.20	0.21±0.00	104.27	0.19±0.01	95.00
	0.40	0.38±0.02	95.97	0.41±0.01	102.5

The analytical efficiency reported in the present study was also compared to other studies in the literature that investigated copper determination spectrophotometric methods. This comparison is shown in Table 5.

Akmese et al. (9) reported a flow injection analysis which was able to determine copper in river water and sea water. However, this work focused only on low metal ion concentrations. Although the linear working range values reported in the study of Granado-Castro M.D. et al. (16) are similar to our research, the method includes sample preconcentration as an extra analysis step. Despite its simplicity, the spectrophotometric flow injection analysis method developed by Purachat B. et al. (17) does not have the desired low LOD level and linear working range for our sample. The developed system by Youngvises N. et al. (18) was utilized for the analysis of Cu (II) ions in natural water. However, in hydroponic nutrient solutions, the matrix environment is formed by more types of ions at higher concentration levels. For example, macronutrients (N, P, K, Ca, Mg) are at much higher concentrations in hydroponic solutions because they are the main growth elements of plants. Ni Y. et al. (19), who used PAR as a chromogenic reagent in their studies, achieved a similar LOD level and linear working range. However, in our study, the analytical performance of PAN and TAN reagents was evaluated together with PAR as a chromogenic reagent. Optimization studies of the flow injection analysis system were also carried out for the method developed with PAR reagent. The disadvantage of the technique developed by Ghasemi et al. (20) is that it allows the detection of trace amounts of analyte.

Table 5: Findings reported from studies that examined the analysis of Cu^{2+}.

Analyte	Sample	Technique	Reagent	LOD	Linear range	Ref.
Cu(II)	River water and Sea water	FIA	Alizarin Red S	0.6 μg/L	2-110 μg/L	9
Cu(II)	Saline water	FIA	PABH	1.8 μg/L	6.9-984.5 μg/L	16
Cu(II)	Waste water, copper ore samples	FIA	Nitroso-R	0.68 μg/mL	1.0-7.0 μg/mL	17
Cu(II), Mn(II), Fe(II), Fe(III)	River water	FIA	Zincon	12 μg/L	0.050–1.0 mg/L	18
Fe(II), Cu(II), Zn(II), Mn(II)	Pharmaceutical preparations, chicken feedstuff, water samples	UV-VisS	PAR	0.015 μg/mL	0.05–0.8 μg/mL	19
Cu(II), Zn(II), Hg(II)	Natural waters	MCPE, UV-VisS	PAR	9.8 μg/L	0.02-0.10 mg/L	20
Cu(II)	Hydroponic nutrient solution	UV-VisS and FIA	PAN, TAN, PAR	0.04 μg/mL	0.125-1 μg/mL	This study

Abbreviations: FIA, flow injection analysis; Alizarin Red S, (3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt); PABH, pyridine-2-acetaldehyde benzoylhydrazone; Nitroso-R, disodium 1-nitroso-2-napthol-3,6-disulfonate; Zincon, 2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene; MCPE, micro cloud point extraction; UV-VisS, UV-*vis* spectrophotometry.

3.7. Green Profile Assessment

The environmental sustainability of the method was assessed using the AGREE, AGREEprep and MoGAPI tools.

3.7.1 Modified green analytical procedure index (MoGAPI)

MoGAPI visually assesses the environmental impact of a method, while also allowing direct comparison of different methods by assigning a total score. In the MoGAPI index, the different stages of the chemical analysis process are shown in a pentagram, with each section marked in green, yellow or red (12).

The data obtained because of the evaluation of the method with the MoGAPI tool are shown in Table 6 and Figure 5.

Table 6: Evaluation of the method with the MoGAPI tool.

Category	**Method**	**Color (Point)**
Collection (1)	Online or at-line	Yellow (2)
Preservation (2)	None	Green (3)
Transport (3)	Required	Yellow (2)
Storage (4)	Under normal conditions	Yellow (2)
Type of method: direct or indirect (5)	Simple procedures, e.g., filtration and decantation	Yellow (2)
Scale of extraction (6)	Not applicable	-
Solvents/reagents used (7)	Non-green solvents/reagents used	Red (1)
Additional treatments (8)	None	Green (3)
Reagents and solvents		
Amount (9)	<10 mL(<10 g)	Green (3)
Health hazard (10)	Moderately toxic; could cause temporary incapacitation; NFPA=2 or 3	Yellow (2)
Safety hazard (11)	Highest NFPA flammability or instability score is 2 or 3, or a special hazard is used.	Yellow (2)
Instrumentation		
Energy (12)	≤0.1 kWh per sample	Green (3)
Occupational hazard (13)	Emission of vapors to the atmosphere	Red (1)
Waste (14)	1–10 mL (1–10 g)	Yellow (2)
Waste treatment (15)	Degradation, passivation	Yellow (2)

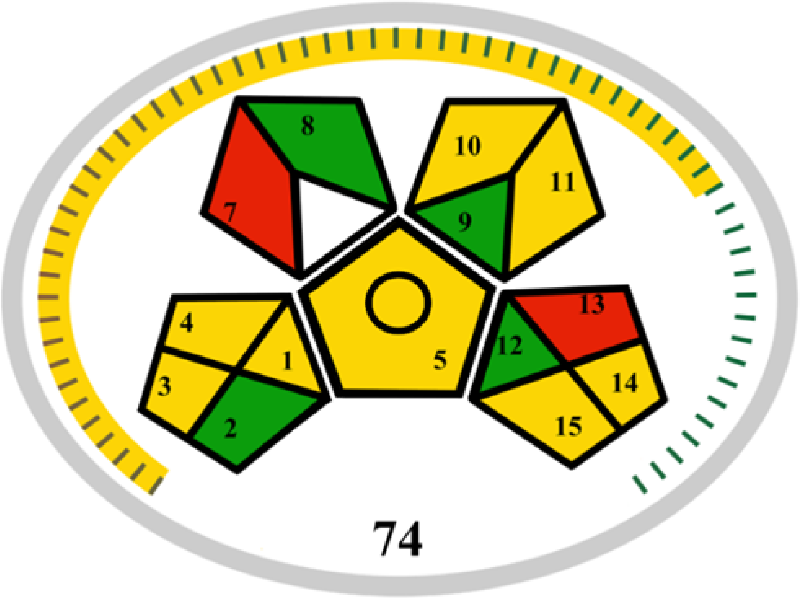

Figure 5: MoGAPI assessment scores for developed method.

3.7.2 Analytical GREEnness Metric approach (AGREE) and AGREEprep

The Agree model responds to the 12 principles of green analytical chemistry. It includes many parameters that have not been considered before, and it is complete compared to other methods. The software interface has 12 windows where green analytical chemistry is explained. Thanks to an algorithm that evaluates all inputs, a pictogram of the procedure is obtained.

Similar to AGREE, AGREEprep is based on the 10 principles of Green Sample Prepare. Therefore, the software is divided into 10 windows with different sizes related to the weighting of the sectors in the pictogram (11).

Table 7 shows the principles of Green Analytical Chemistry and Green Sample Preparation (11).

The total scores of AGREE and AGREE prep shown in Figure 6.

Table 7: Principles of green analytical chemistry and green sample preparation.

	Green Analytical Chemistry (GAC)	**Green Sample Preparation (GSP)**
1	Direct analytical techniques should be applied to avoid sample treatment	Favor in situ sample preparation
2	Integration of analytical processes and operations saves energy and reduces the use of reagents	Use safer solvents and reagents
3	Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided	Target sustainable, reusable, and renewable materials
4	Minimal sample size and minimal number of samples are goals	Minimize waste
5	Automated and miniaturized methods should be selected	Minimize sample, chemical and material amounts
6	Reagents obtained from renewable sources should be preferred	Maximize sample throughput
7	The safety of the operator should be increased	Integrate steps and promote automation
8	In situ measurements should be performed	Minimize energy consumption
9	Derivatization should be avoided	Choose the greenest possible post-sample preparation configuration for analysis
10	The use of energy should be minimized	Ensure safe procedures for the operator
11	Multi-analyte or multi-parameter methods are preferred versus methods using one analyte or parameter at a time	
12	Toxic reagents should be eliminated or replaced	

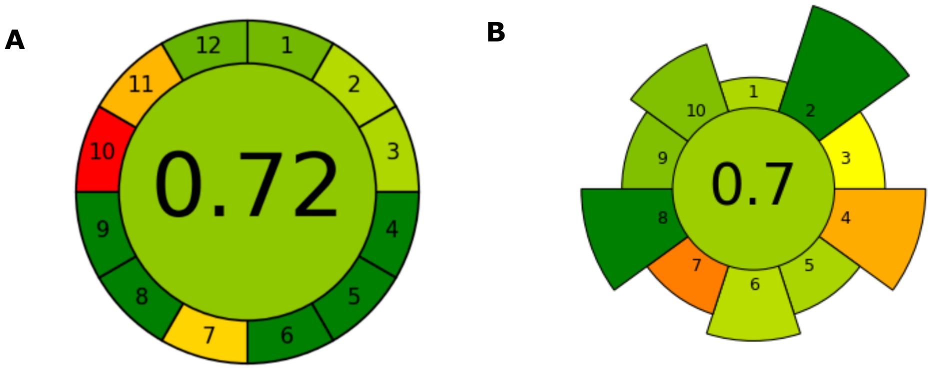

Figure 6: AGREE(A) and AGREE prep (B) assesment scores for developed method.

4. CONCLUSION

The present work deals with the spectrophotometric determination of copper using a UV spectrophotometer and flow injection analysis. This method uses PAR, PAN, and TAN, depending on the ambient pH based on complexities. These ligand types were studied to determine the complexing agent to copper microelement that gave maximum absorbance (Table 1). Complexes formed by Cu^{2+} ions with PAR, PAN and TAN ligands give characteristic absorption peaks in the UV-Vis region. The wavelength of the complexes generally shows a maximum absorption in the region between 500-600 nm. Parameters important for complex formation studies (ligand type, concentration, pH, etc.) have been optimized. The effect of pH on the metal-ligand complex was studied and the optimum pH at which maximum absorbance was obtained was determined. While PAR complexes give maximum absorbance at pH 7 and 8, PAN complexes give maximum absorbance at pH 9 and TAN complexes at pH 5. At the view of these results, the interaction between PAR and Cu^{2+} ions is pH dependent. While PAR's hydroxyl groups can be protonated (H^+ bonded) in acidic environments, they can be deprotonated at higher pHs and bind more strongly with the Cu^{2+} ion. Neutral or slightly basic pH ranges are generally preferred for complex formation. The complexation between PAN and Cu^{2+}

ions is pH sensitive. In acidic medium, the naphthol group can be protonated and this can prevent complex formation. In slightly basic medium (pH 8 and 9), the naphthol group is deprotonated and forms a stronger bond with the Cu^{2+} ion. The interactions between TAN and Cu^{2+} ions are weaker than those between PAR and PAN ligands. According to the results of interference analyses for metals (Table 2), the complexes formed with PAR are more resistant to foreign ion interference compared to PAN and TAN complexes. Detection limits of 0.04 µg/mL were reached for copper. The method uses EtOH as the reagent solvent, significantly reducing contamination and health hazards associated with the use of concentrated acids. Another advantage of this method is that it is suitable for determining the concentrations of copper ions in the nutrient solution, which are of great importance in hydroponic nutrient solutions. However, it should be emphasized that there are very few studies examining the spectrophotometric analysis of copper in hydroponic nutrient solutions. Although this study is simpler and faster compared to some of the existing methods, it also filled a gap in literature.

5. CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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