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Development and Analytical Evaluation of an L-Arginine-Based Optical Sensor for Spectrophotometric Detection of Cu(II) Ions in Water Samples

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تطوير وتقييم تحليلي لمستشعر ضوئي يعتمد على "إل-أرجينين" (L-Arginine) للكشف الطيفي الضوئي عن أيونات النحاس الثنائي Cu(II) في عينات المياه

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Abstract:
The arginine ligand's analytical performance as a photochemical sensor for the determination of Cu (II) ions was next assessed. The optimal response for the arginine sensor was obtained at pH 8.79 after the experimental circumstances influencing sensor's reaction were methodically tuned. The wavelength used for spectrophotometric measurements was 655 nm. With relative standard deviation (RSD) values of 0.01% and 0.04% for the arginine sensor, respectively, the sensor demonstrated great repeatability and high photostability, indicating a high degree of analytical precision. Additionally, the developed sensor showed a strong linear response within the analyzed concentration range of Cu (II) ions, together with adequate analytical sensitivity. The influence of potential interfering ions, including Fe (III) ,Ca (II), Ni (II), Co (II), and Cd (II), was tested using a 1:1 molar ratio between Cu (II) ions and the interfering ion, where the results revealed the sensor's strong selectivity toward Cu (II) ions. Finally, the proposed sensing system was effectively employed to assess the concentration of Cu (II) ions in water samples. The results showed good agreement with those obtained using the arginine sensor as well as with standard certified analytical procedures, indicating the reliability, efficiency, and practical usefulness of the developed photochemical sensing technology in the detection of Cu (II) ions.

Keywords: Arginine; Photochemical sensor; Spectrophotometric; water samples; analytical sensitivity; Selectivity.

الملخص
تم تقييم الأداء التحليلي للرابطة الأرجينينية كحساس كيميائي ضوئي لتقديرأيون النحاس بعد ذلك .تم الحصول على الاستجابة المثلى لحساس الأرجينين عند pH 8.79بعد ضبط الظروف التجريبية المؤثرة على تفاعل المستشعر بشكل منهجي .كان الطول الموجي المستخدم في القياسات الطيفية 655نانومتر .مع قيم الانحراف المعياري النسبي (RSD)بنسبة 0.01٪ و040.٪ لحساس الأرجينين على التوالي، أظهر المستشعر تكرارا عاليا واستقرارا ضوئيا عاليا، مما يشير إلى درجة عالية من الدقة التحليلية .بالإضافة إلى ذلك،

أظهر المستشعر المطور استجابة خطية قوية ضمن نطاق التركيز المحلل لأيونات النحاس، إلى جانب حساسية تحليلية كافية .تم اختبار تأثير الأيونات المحتملة المتداخلة، بما في ذلك الحديد (III)، الكالسيوم (II)، النيكل (II)، الكوبالت (II)، والكادميوم (II)، باستخدام نسبة مولية 1:1بين أيون النحاس (II) والأيون المتداخل، حيث كشفت النتائج عن انتقائية قوية للحساس نحو أيونات النحاس (II). وأخيرا، تم استخدام نظام الاستشعار المقترح بفعالية لتقييم تركيز أيونات النحاس (II) في عينات المياه .أظهرت النتائج توافقا جيدا مع تلك التي تم الحصول عليها باستخدام حساس الأرجينين وكذلك مع الإجراءات التحليلية المعتمدة القياسية، مما يشير إلى موثوقية وكفاءة وفائدة تقنية الاستشعار الضوئي المطورة في اكتشاف أيونات النحاس .

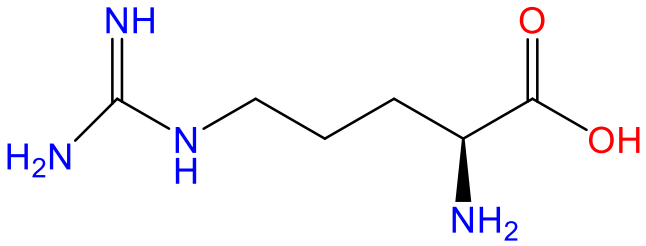
الكلمات المفتاحية: الأرجينين؛ الحساس الفوتوكيميائي؛ التحليل الطيفي؛ عينات المياه؛ الحساسية التحليلية؛ الانتقائية.

Introduction

Heavy metals make up a heterogeneous group of elements characterized by varied chemical properties and biological roles. They can be classified broadly as essential and non-essential elements; essential metals such as molybdenum (Mo), manganese (Mn), copper (Cu), nickel (Ni), iron (Fe), and zinc (Zn) are needed in trace amounts for multiple physiological and biochemical processes, while non-essential metals including cadmium (Cd), arsenic (As), mercury (Hg), and lead (Pb) lack any established biological function and can produce toxic effects even at low concentrations [1]. In addition, they are prominent environmental contaminants that can reach groundwater via anthropogenic activities, with their mobility and persistence in soil and groundwater governed by a range of physicochemical and environmental factors [2]. Consequently, determining and characterizing the pollutant sources that enter riverine systems, together with evaluating their likely environmental impacts and formulating effective mitigation and management strategies, represents a vital and ongoing focus of research within the field of environmental environmental [3].Copper (II) is an indispensable trace element and a redox-active transition metal that is required to sustain normal biological function. Because of its characteristic physicochemical properties, copper is involved in a wide range of biochemical pathways and is a necessary constituent of many copper dependent enzymes and metalloproteins. These biomolecules are central to cellular energy metabolism, oxidative stress control, iron homeostasis, connective tissue development, hemoglobin synthesis, and cellular respiration. Consequently, maintaining an adequate intracellular copper level is critical for preserving normal metabolic and physiological activity. However [4], excessive exposure to Cu (II) may trigger substantial toxicological effects, especially when concentrations surpass physiological tolerance limits. Extended or high-dose exposure has been linked to harmful health outcomes, including metabolic disorders, anemia, renal impairment, hepatic damage, and when exposure is severe potentially fatal toxicity [5].

L-arginine (Arg) is classified as a conditionally essential amino acid in adults and as an essential amino acid in juvenile mammals, birds, and carnivores. Arginase converts it into L-ornithine, a vital constituent of the urea cycle and a precursor to polyamines and urea. Arg also serves as a precursor to creatine, which is required for protein synthesis and for the production of agmatine, as well as for the energy metabolism of muscle, neurons, and the testis. It influences immunological function by enhancing the release of growth hormone. In humans and animals, typical plasma Arg concentrations fall within 95 to 250 μmol/l, depending on developmental stage and nutritional status [6]. L-Arginine is among 20 amino acids found in proteins that play an important role in cellular metabolism. Its guanidine group provides five potential hydrogen bond donors arranged to support favourable interactions with biological hydrogen bond acceptors and it carries a positive charge at neutral pH [7,8] In healthy individuals, it is considered dispensable because it can be generated from other amino acids. In seriously ill or injured patients, however, larger amounts of L-Arginine are needed, since the body may be unable to synthesize it in sufficient quantity [9]. Some cancers are auxotrophic for a specific amino acid, and amino acid deprivation is one strategy used to treat these tumours. It has been proposed that arginine-degrading enzymes could be useful in the management of arginine-dependent cancers [10]. Accordingly, it is important to establish a way to monitor the level of this amino acid in biological fluids [11], nutritional supplements, and most recently in cosmetics, owing to the widespread use of this molecule. Sources of arginine can be either exogenous, meaning dietary intake, or endogenous, originating from whole-body protein turnover, intestinal synthesis, or de novo synthesis. The majority of plasma arginine is obtained from protein metabolism and turnover. The kidney is the principal site of net arginine synthesis, contributing approximately 60% of these stores. Citrulline functions as the main substrate, being generated in the small intestine from the metabolism of dietary amino acids such as glutamine and glutamate. After leaving the intestine, citrulline is transported to the proximal tubules, where it is converted to arginine and released into the bloodstream [12]. De novo arginine synthesis provides only 5–15% of the endogenous supply and does not make a major contribution to arginine homeostasis in adult humans. One location of arginine synthesis is the liver's urea cycle[13]. A range of detection methods for L-Arginine analysis has been developed; most rely on time-consuming, expensive, and complex approaches, including high-performance liquid chromatography [14]. Other examples include spectrophotometry-based methods [15], fluorometry [16], atomic absorption spectrometry [17], catalytic–thermometric titrametry [18], capillary electrophoresis [19], and enzymatic assays [20], [13]. There remains a need for easy-to-use, cost-effective, and decentralised diagnostic tools for clinical practice[21]. There are two ways to get L-arginine: intravenously (IV) or orally (capsules or tablets) R-Gene 10® is

the brand name for IV-form l-arginine. L-arginine is used to cure a number of ailments, such as: pressure or pain in the chest (angina). erectile dysfunction. heart disease. hypertension, or elevated blood pressure. and migraines [6]. In the design of such a ligand, (Arg) in Figure 1, it is recognized that Cu (II) tends to coordinate in pairs; specifically, the two donor atoms (N and O) and the two intervening carbons (Cα and the carbonyl carbon) form a five-membered chelate ring with copper.



Arginine

Figure 1: The chemical structure of arginine is characterised by its unique composition, which includes a central carbon atom bonded to a carboxyl group, an amino group, and a side chain that contains a guanidinium group.

Material and methods

All chemicals and reagents used I n this study were analytical grade and were used as received, without any further purification. Arginine (Arg) was obtained from Sigma-Aldrich. The remaining chemicals were likewise purchased from Sigma and applied without additional purification. Stock solutions of the metal ions were prepared using analytical-grade reagents, and their concentrations were confirmed by titrimetric methods [22]. A standard Cu(II) solution with a molarity of 0.2 M was prepared by dissolving a suitable mass of 3.409 g in 100 mL of deionised water. The standard stock solution of Arginine (Arg) at a concentration of 0.2 M was obtained by dissolving the required amount of 3.484 g in 100 mL of deionised water. In the present study, the reagents used include $CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $NiCl_2$, $CdCl_2.H_2O$, and $Fe_2(CO_3)_3$, in combination with a phosphate buffer (remaining), hydrochloric acid (HCl), sodium hydroxide (NaOH), and H_2O as the reference. All of the aforementioned chemical substances were utilised without undergoing any purification processes.

Measurements

The electronic spectra of the Cu (II) complexe were recorded with a The SPECORD R 210 PLUS spectrophotometer (Analytik Jena, Germany), which was configured to operate over a wavelength span of 200 to 800 nm. All solutions were prepared immediately, using deionized water for both the metal and the ligand. The concentrations of the ligand and the metal were maintained at 0.01 M. Measurements were performed in aqueous media over the pH range 2.00 to 12.1, using 10 mm pathlength cuvettes in the wavelength ranges of 250 to 800 nm. An uncoordinated ligand served as the blank reference.

Optimisation of the Reaction Condition Between Ligand and Cu(II) Ion

The effect of the concentration of (Arg) on Cu (II) was examined by combining an equal concentration of Cu (II) solution with different amounts of L-Arginine(Arg) at various concentrations spanning 1×10^{-4} to 0.2 M within the spectrum. After that, all samples were subjected to analysis via a UV-visible double-beam spectrophotometer for the purpose of measuring absorption. The reproducibility of the sensor response was determined by performing seven independent trials using a Cu (II) concentration of 0.01 M. The photostability of the sample was also evaluated. For this study, the concentration of (Arg) was set to 0.1 M. This diffrent concentration was maintained for six hours, and the absorption intensity was recorded at 30-minute intervals. The absorption spectrum of each mixture was measured using a UV-Visible spectrophotometer. The calibration curve for Cu (II) concentration was constructed by plotting absorption against Cu (II) concentration. Solutions containing Cu (II) at varying concentrations from 1×10^{-5} M to 0.2 M were combined with reagents at a concentration of 0.1 M of (Arg). The influence of different foreign ions was investigated separately by using different amounts of interfering ions to determine the Cu (II) concentration at 0.01 M . The interference levels of Ni (II), Fe (III), Ca (II), Cd (II) and Co (II) ions were evaluated in both the presence and absence of these interfering ions, employing an optimisation strategy.

Results and discussion

To enhance sensor responsiveness, this study conducts multiple trials. Arginine (Arg) is used as a substrate to evaluate the sensor's performance by examining its dynamic range, stability, ideal conditions, and detection limit. In the UV–vis spectrum of an arginine solution, Cu (II), and the combined system of arginine solution with Cu (II) (complex), three bands appear at 0.0414 nm, 0.0682 nm, and 0.2291 nm. These bands correspond to π-π*, n-π*, and d-d transitions. As shown in Figure 2, the absorption spectra of the ligand are shifted to higher wavelengths in the UV–vis spectra of the complex; this comparison is made at 1 × 10-2 M. The findings show an increase in peak emergence attributed to the d-d transition, which is also supported by the amine group, along with the appearance of a new peak.

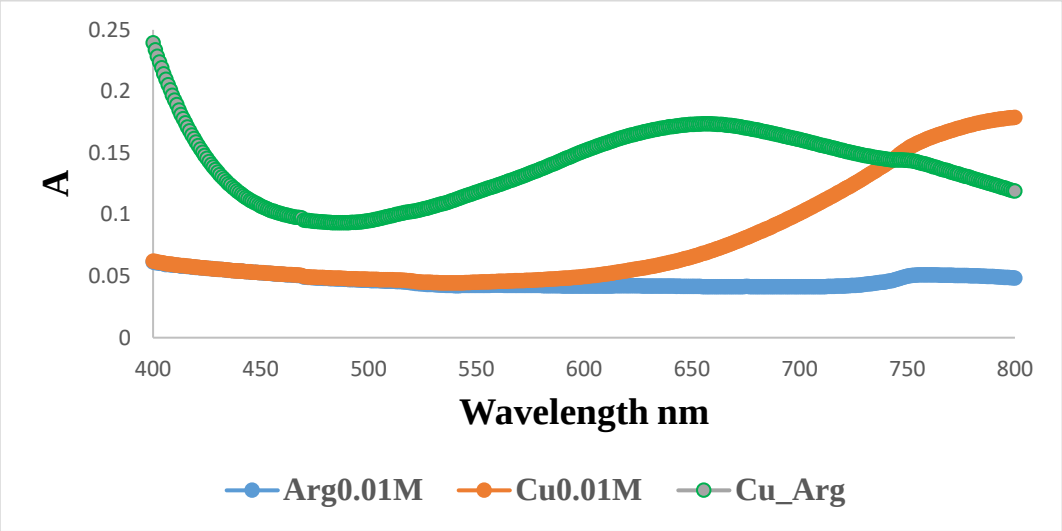


Figure 2: The Arg , Cu (II) , and complex at 1 × 10^{-2}M by 1:1 have their UV–Vis absorption spectra shown with a λ_{max} of 655 nm.

As illustrated in Figure 3 , the effect of pH is further examined by using various pH values in a 0.25 M phosphate buffer. In addition, the stability and shelf life of the product depend on pH, making it a key factor for extraction and storage [23]. Proper understanding of the pH effect is essential for effective sensor performance in real-world applications. Consequently, the copper solution, a reagent containing a arginine analyte, has a pH of 8.79.

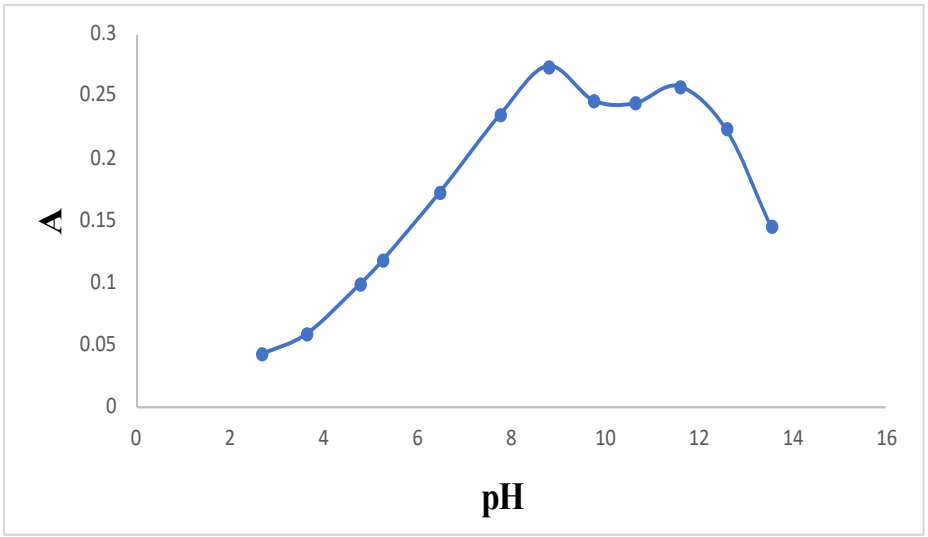


Figure 3: The effect of working pH on the absorbance of arginine upon reaction with 0.01 M Cu (II) solution.

By measuring absorbance at a fixed wavelength of 655 nm, the impact of glycine concentration on the formation of the Cu (II)-Arginnie complex is investigated in detail. As depicted in Figure 4, the optimal absorbance for the Cu (II)-Arginine complex increases proportionally with (Arg) concentrations within the range of 1 × 10^{-4} M to 0.2 M. Preliminary experiments indicate a marked rise in the absorbance signal as concentration increases; however, the response gradually levels off at higher concentrations, reaching saturation at 0.1 M. A stronger signal is

obtained when higher concentrations of arginine are added, enabling more extensive interactions between arginine ions and Cu (II) in the nearby region. Ultimately, the absorbance signal becomes nearly constant once Cu (II) ions occupy most of the arginine binding sites.

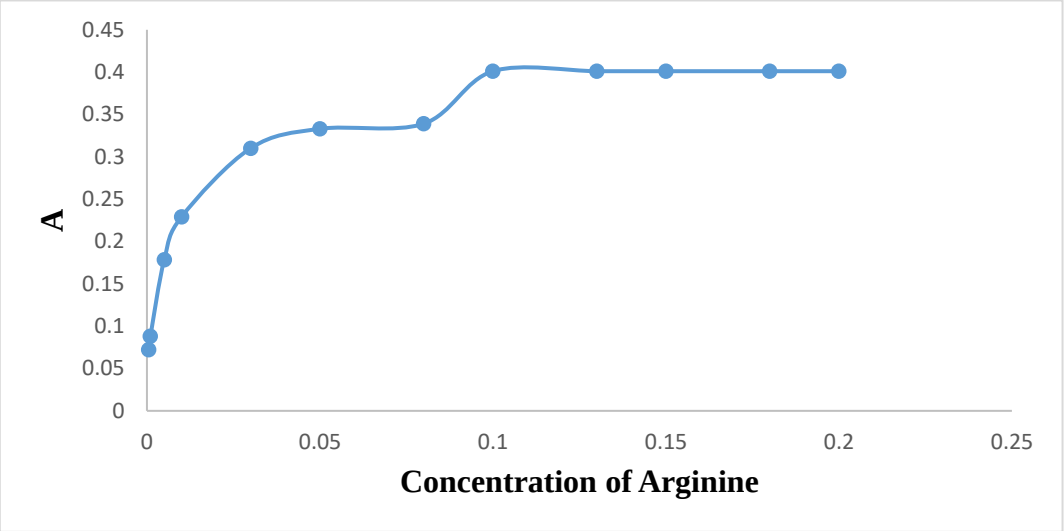


Figure 4: The effect of Arginine concentration on the response of the Cu(II)-Arg complex when Cu(II) concentration used was fixed at 0.01 M with a buffer solution of pH 8.79 and λ_{max} is 655 nm.

The photostability of the sensing materials is assessed by exposing them to continuous illumination for six hours and recording the absorption intensity at 30-minute intervals. The absorption spectrum for each mixture is obtained using a UV-visible spectrophotometer, producing a calculated (RSD) value of 0.01 % shown in the Figure 5 . A comparable study was documented by Ahmad & Yusof [24], where a sensor photostability investigation was conducted to evaluate the potential for photobleaching or photodegradation of the reagent phase during sustained exposure to a light source for extended durations. The findings revealed that the reagent phase maintained stability with no leaching observed over a 7-hour interval. The Cu (II) complex formed with arginine shows good repeatability, with an estimated relative standard deviation (RSD) of 0.04 % measured at a Cu (II) concentration of 0.01 M. Over nearly six hours, the sensor demonstrates excellent stability Figure 6. A robustness value of 0.6 indicates reliable and long-lasting sensor components, thereby ensuring accurate measurements across a range of applications. A robustness value of 0.6 corresponds to a maximum percentage error of 0.27% and a minimum value of zero, thus confirming high accuracy in resistive sensor readings relevant to biomedical applications [25].

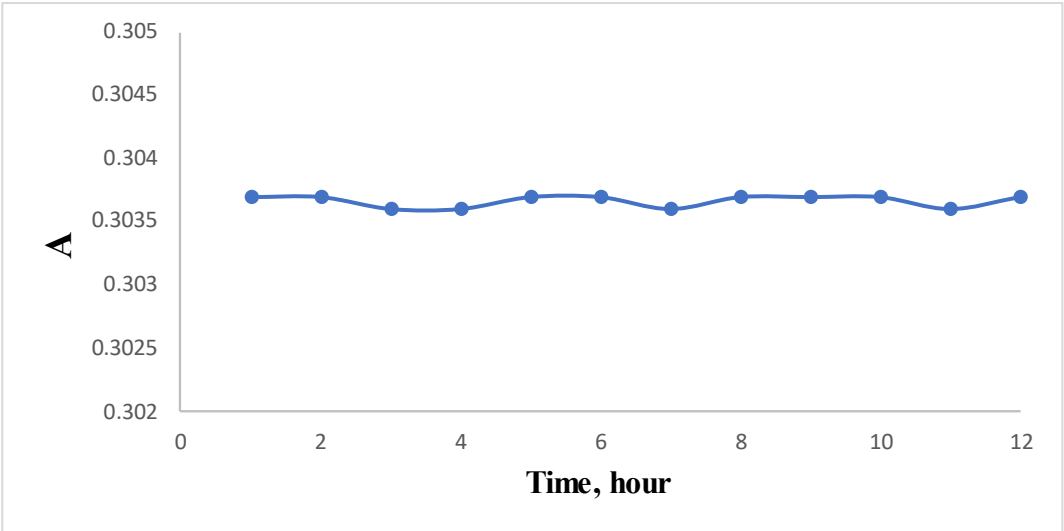


Figure 5: Time-dependent photostability of the arginine with concentration of 0.1 M.

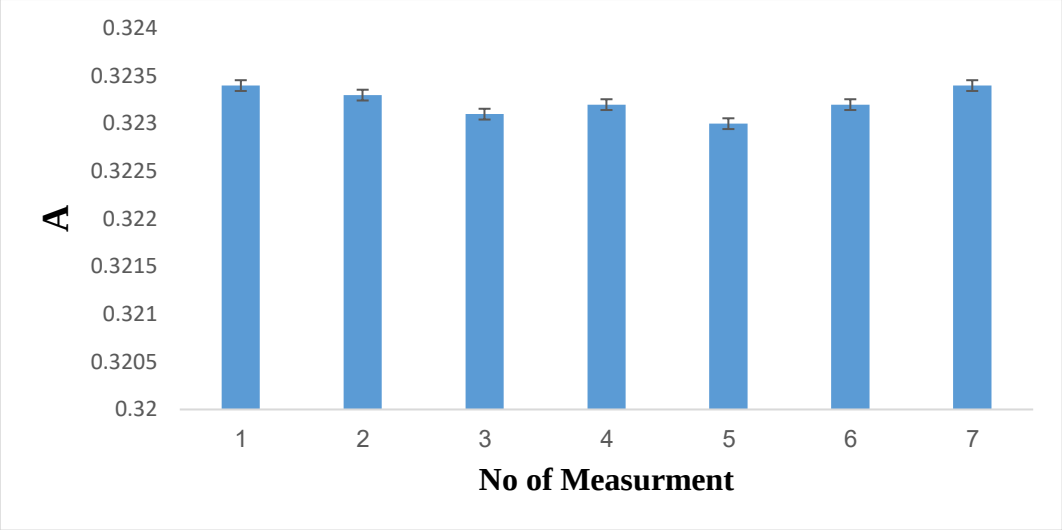


Figure 6: The reproducibility of the reagent arginine 0.1 M Cu (II) analyte.

Figure 7 clarifies the response curve related to the sensitive material. The signal measured by the spectrophotometer showed a marked increase as the Cu (II) concentration rose during the initial phase of the study; this was followed by a gradual stabilization at higher concentrations and, ultimately, saturation at 0.14 M Cu (II). Increasing the Cu (II) concentration promoted more interactions between the reactant and analyte molecules, leading to a stronger observed signal. Throughout these experiments, the concentration of (Arg) was kept constant at 0.1 M. Figure 7 (a) presents the variation in Cu (II) concentration across the range from 1×10^{-5} M to 0.2 M. In addition, Figure 7(b) demonstrates a linear relationship between absorbance and Cu (II) concentration over the interval of 1×10^{-5} M to 0.05 M, with a linear regression giving a relative absorbance of R^2 = 0.99. E. Yuhana-Ariffin ORCID obtained a comparable response curve during the design phase. The optical reflectance sensor, based on Tetraaza/SBA15, demonstrated a detection limit (LOD) of 1.02 x 10^{-7} M (R^2 = 0.99) and a rapid reaction time of one minute. It exhibited both sensitivity and selectivity towards Cu (II) ions, maintaining a linear response across a concentration range from 1 x 10^{-7} M to 2 x 10^{-5} M, with notable repeatability indicated by a relative standard deviation (RSD) of 0.47%. By utilising a regeneration solution of 0.1 M EDTA at a pH of 6, this optical sensor could be employed for up to five successive detections of Cu (II) ions, displaying a reversible (RSD) value of 0.79%. The developed optical sensor offers a rapid and sensitive method for detecting Cu (II) ions in teabag samples, aligning with findings obtained from the ICP-MS standard method [26].

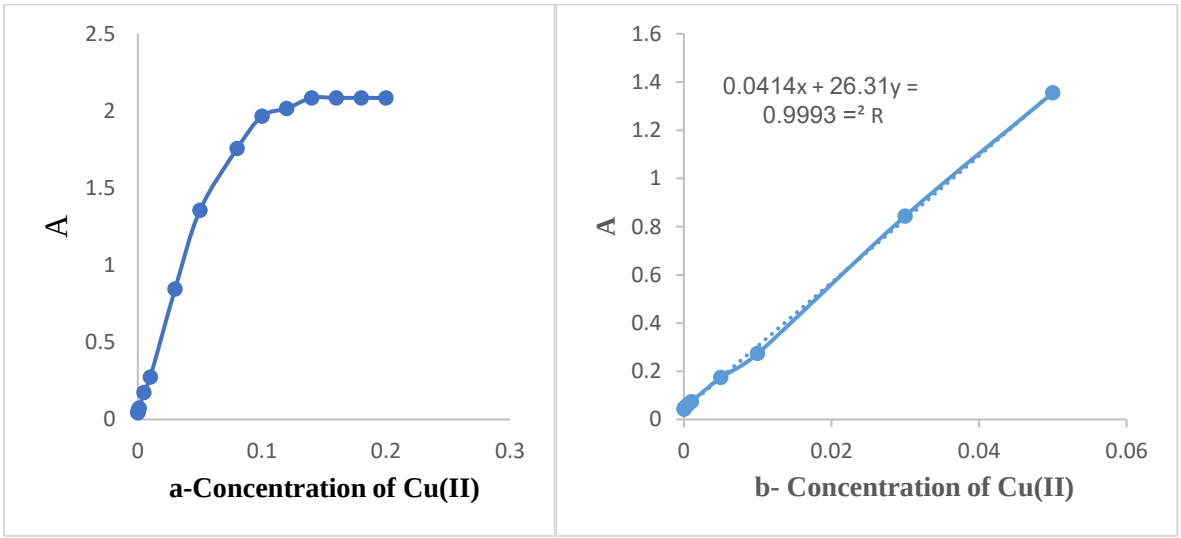


Figure 7: The response curve of the Arginine at different concentrations of Cu(II) **(a)** and is the linear dynamic range of the Cu(II) concentration **(b).**

The extent to which specific ions interfered with the Cu(II) measurement in our study is illustrated in Figure 8. Several foreign ions were identified at a 1:1 molar ratio of Cu(II) to foreign ion, including Fe(III), Co(II), Ni(II), and Ca(II). The curve indicates that the Ca(II) and Ni(II) ions yielded acceptable results and did not hinder the assessment of the Cu(II) ion. For the Co(II) ion, the outcome was almost the same as that obtained when estimating

the Cu(II) ion. II. The earlier research was intended to detect ultra-trace Al(III) ions with extremely high selectivity and sensitivity. The results of the investigation into the effect of the interfering ion are presented. The sensor shows:

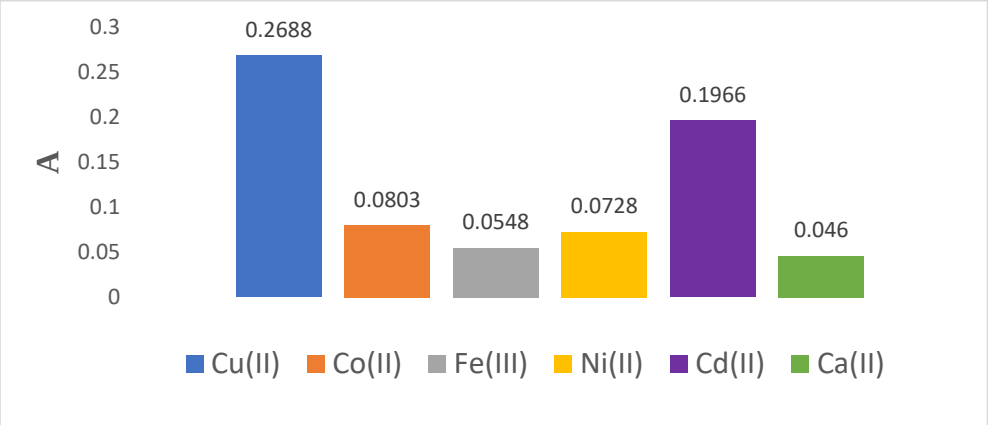


Figure 8: The degree of interference of the Arginine at 1:1 molar ratio of Cu (II): foreign ion**.**

An additional experiment was carried out with the fabricated sensor to determine the Cu(II) concentration in lake water samples, thereby confirming its practical suitability for real- sample analysis. The sensor's results were compared with those obtained from the standard flame atomic absorption spectrometry (AAS) method, demonstrating strong agreement with the established analytical procedure.

Table 1: The determination of Cu(II) was conducted using the standard method of flame atomic absorption spectroscopy (AAS) and an optical Cu(II) sensor based on

SN.	Standard Cu(II) solution (ppm)	Cu(II) determined by flame AAS (ppm)	Cu (II) determined by optical sensor (ppm)
1	**Real Sample Arginine**	**0.118**	**0.0003**

Conclusion

The usage of arginine as ligands in the creation of an optical sensing material for the detection of Cu (II) ions was thoroughly examined in the current study. Effective sensor responsiveness was demonstrated by the fact that the color intensity of the resultant complexes increased proportionately with increasing Cu (II) concentrations. Under the experimental conditions, the sensor demonstrated exceptional photostability and reproducibility. Cu (II) concentrations were kept at 0.01 M throughout the trials, and optimal performance was noted at pH values of 8.79. Positive relative standard deviation (RSD) values of 0.01% were obtained from reproducibility analysis. The stability of the complexes and the lack of significant photobleaching during ambient light exposure were confirmed by photostability studies, which showed (RSD) values of 0.04% for arginine. the potential interference from other metal ions, especially Fe (III), Co (II), Ni (II), and Cd (II), was assessed. The results demonstrated low interference, supporting the selectivity of the arginine based sensor for Cu (II). Notably, the sensor constructed employing arginine exhibited significant potential for reliably assessing Cu (II) in real-world environmental samples.

Acknowledgements

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