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## **Colorimetric Assay for Copper Ion Based on Silver Nanoparticles Functionalized with 1,3-Dimethyl Benzotriazolium Iodide**

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Measuring the heavy metals in environmental and food samples to evaluate the decontamination of toxic pollutants in the environment is vital for human health. In the current project, a new colorimetric method based on the aggregation of 1,3-dimethyl benzotriazolium iodide Ionic Liquid (BTAIL) stabilized silver nanoparticles (AgNPs) for detection of copper (Cu<sup>2+</sup>) ion was developed. Aggregation of AgNPs induced by coordinative coupling between Cu<sup>2+</sup> and triazole ring of BTAIL. Cu<sup>2+</sup> AgNPs aggregates with a color change from yellow to red and a decrease in UV-Vis absorption peak at 400 nm. Under the optimized experimental conditions (pH = 9, reaction time = 3 min, BTAIL volume = 600 µl (0.01 M)) a linear range of 20-100 nM was obtained. Good selectivity and sensitivity toward Cu<sup>2+</sup> among the other ions were observed. Finally, real sample results such as green tea, Rosa damascene, Chamaemelum nobile, and Thymus vulgaris indicated that the proposed method could apply precisely for practical applications.

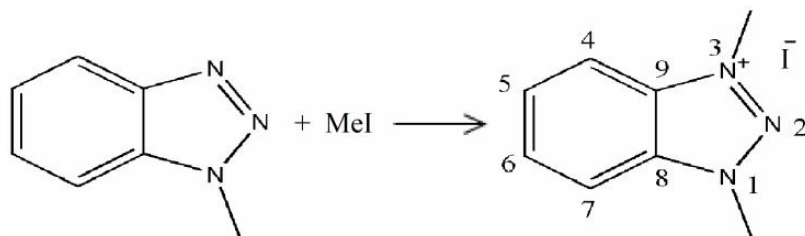
**Keywords:** Colorimetry, AgNPs, Copper, 1,3-Dimethylbenzotriazolium iodide, Green tea

### **INTRODUCTION**

Determination of heavy metal ions in water and food samples in decontamination of toxic pollutants in the environment is vital for human health. Heavy metals are the leading cause of excessive free radical activity, damaging the body's metabolism and depleting the body's immune system. After zinc and iron, copper, as an essential metal for the human body, plays a critical physiological role in most biological systems [1,2]. However, copper amounts higher than the required limit have toxicity on internal organs such as the cardiovascular, liver, and kidney and increase blood pressure and damage to the central nervous system [3,4]. Increasing concern about the potential danger of heavy metals is a significant motive for developing sensitive, simple and rapid analytical methods for detecting small amounts of these metals. So far, many ways were developed to determine the low amounts of copper ion. Most of these

methods used atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), electrochemical techniques [5] and spectroscopic fluorescence techniques [6]. Although these techniques are sensitive and selective, they are costly and need complicated instruments and time-consuming sample preparation methods [5]. Colorimetry is one of the simple, rapid, and sensitive methods used for measuring the trace amount of heavy metal ions [7,8]. Colorimetric methods recorded the optical absorption change by spectroscopy or the naked eye to follow the concentration changes. In these methods, a sensor records color changes when interacting with the analyte producing an optical signal that the intensity of the signal is proportional to the concentration of the analyte [9]. For this purpose, chemical sensors made up of metal nanoparticles such as gold and silver nanoparticles were significantly used [10,11]. The optical properties of silver nanoparticles depend on nanoparticles' size, shape, and aggregation in solution controlled by surface plasmon resonance (SPR). The AgNPs color change from yellow to

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**Fig. 1.** The structure and synthesis of BTAIL.

red upon aggregation. When the interparticle distance decreased, the effect of plasmonic coupling increased, in color changed and a displacement in the UV-Vis spectrum [12].

The selectively recognize metal ions by the AgNPs are controlled by the nature of the ligands and various functional groups placing on the nanoparticle surface. While the interaction of metal ions with ligands, the aggregation of or disassembly of nanoparticles cause to change the color [8,13]. Different capping agents are used for the stabilization of metal NPs. Ionic compounds such as carboxylates, fluorides and polyoxoanions are used to stabilize NPs by generating the electrostatic repulsion between NPs, following to prevent the aggregation of NPs [14,15]. Ionic liquids (ILs) are ionic compounds used to stabilize metal nanoparticles through electrostatic repulsion [16]. There are many reports about the feasibility of IL-stabilized metal nanoparticles in organocatalytic reactions [17,18] and biomass conversion [19]. Still, this is the first report of applying IL-stabilized metal nanoparticles as the colorimetric sensor to our best knowledge.

Benzotriazole (BTA) is a heterocyclic organic compound. Having nitrogen atoms in triazole ring can easily coordinate with copper, so it is widely used as a copper corrosion inhibitor [20-22]. Because of this characteristic, we used a BTA based IL as a ligand in synthesizes of AgNPs. Then, this IL-stabilized AgNPs were utilized for colorimetric detection of copper ions in different herbal drink samples.

## MATERIALS AND METHODS

### Materials

Methyl iodide, silver nitrate ( $\text{AgNO}_3$ ), sodium

borohydride ( $\text{NaBH}_4$ ), hydrochloric acid (HCl) and sodium hydroxide (NaOH) purchased from Merck (Darmstadt, Germany). Benzotriazole, acetone, hexane, ethyl acetate, acetonitrile, and tetrahydrofuran (THF) purchased from Sigma-Aldrich (USA). Copper standard solution ( $\text{Cu}(\text{NO}_3)_2$  in  $\text{HNO}_3$  0.5 M, 1000  $\text{mg l}^{-1}$  Cu) was purchased from Merck. Deionized water (Shahid Ghazi pharmaceutical Company, Tabriz, Iran) was used for all the sample preparations. All experiments were carried out at room temperature.

### Instruments

UV-Vis spectra were recorded by a spectrophotometer (Thermo, USA). The FTIR spectrum was measured by the GX FTIR system (PerkinElmer). For the transmission electron microscopy (TEM) analysis, the solution of AgNPs was dropped onto the carbon-coated copper grid, and a CM30 microscope was used.

### Methods

**Synthesis of 1,3-dimethyl benzotriazolium iodide Ionic Liquid (BTAIL).** The 1-methyl-1,2,3-benzotriazole was synthesized using the method described in the literature [23]. The second alkylation was done using the procedure described by Mudzakir [24]. Briefly, 0.5 g (3.72 mmol) 1-methyl-1,2,3-benzotriazole in 4 ml dry acetonitrile was folded in a necked tow flask. Then 0.31 ml (5 mmol) methyl iodide was added to this mixture. After stirring the mixture at 60 °C for 5 h, the solid product was washed several times by dry THF. The structure and synthesis of BTAIL is shown in Fig. 1.

**Synthesis of BTAIL-AgNPs.** The AgNPs were prepared by a previously reported method with a few

changes [10]. In a sterilized flask, 2 ml of 0.01 M AgNO<sub>3</sub> was added to 98 ml double distilled water. Then 8.8 mg NaBH<sub>4</sub> was added to the above mixture. After incredibly stirring at room temperature for 30 min, 800 µl of BTAIL (0.01 M) was added into the above aqueous solution. The attained yellowish solution was stirred in a dark room for 2 h. Finally, the as-prepared silver colloidal solution was stored at 4 °C under dark before use.

### Colorimetric Detection of Cu<sup>2+</sup> Ions

For detection of copper ions, 600 µl of BTAIL-AgNPs mixed with 300 µl of different concentrations of Cu<sup>2+</sup> solution then the mixture diluted to 1000 µl with deionized water, and the pH was adjust to 9. After shaken and incubating the mixture for 3 min at room temperature, the solution color changed from golden yellow to red. The intensity of color change was monitored by a UV-Vis spectrophotometer. The power of color change depends on the Cu<sup>2+</sup> concentration.

### Sample Pretreatment of Herbal Drinks

Herbal drink samples, including Rosa damascene, Chamaemelum nobile, Thymus vulgaris, and green tea, were applied to our colorimetric assay. Each sample (3 g) was mashed and transferred into a porcelain crucible and heated to smokeless. Then the samples were transferred to the furnace and heated up to 500 °C for 6 h. The ashing samples were dispersed in 30 ml of HNO<sub>3</sub> 0.5 mM, and this mixture was heated to remove acid until the volume reached 10 ml. The solution was diluted by double distilled water to 250 ml. Finally, samples were filtered through 0.2 µm membranes and measured by the above detection procedure.

## RESULTS AND DISCUSSION

### Characterization of BTAIL Capped AgNPs

For BTAIL, the bands at 2986 and 3087 cm<sup>-1</sup> are related to C-H aliphatic and aromatic stretching vibration. The aromatic C=C stretching vibration was observed at bands between 1600-1646 cm<sup>-1</sup>. The band at 1442 cm<sup>-1</sup>, is related to the stretching vibration of the triazole ring (Fig. 2).

Figure 3 shows the TEM images of AgNPs in the absence and presence of Cu<sup>2+</sup>. The photos showed that BTAIL-AgNPs were mono-dispersed. These nanoparticles

have an urchin-like morphology which confirms the successful functionalization of silver nanoparticles with ionic liquid. The aggregation of BTAIL-AgNPs after adding copper-containing solution (0.5 µM) was observed by TEM image (Fig. 3b).

The UV-Vis spectrum of BTAIL capped AgNPs is shown in Fig. 3. As can be seen, an SPR peak is visible at 400 nm that confirms the reduction of Ag<sup>+</sup> ions to Ag<sup>0</sup> and the presence of spherical AgNPs. It was found that there is no color change of NPs solution even after one week, and also, the intensity of peak at 400 nm remained almost the same as before (see Fig. 4). It indicates that the BTAIL stabilized AgNPs are principally stable.

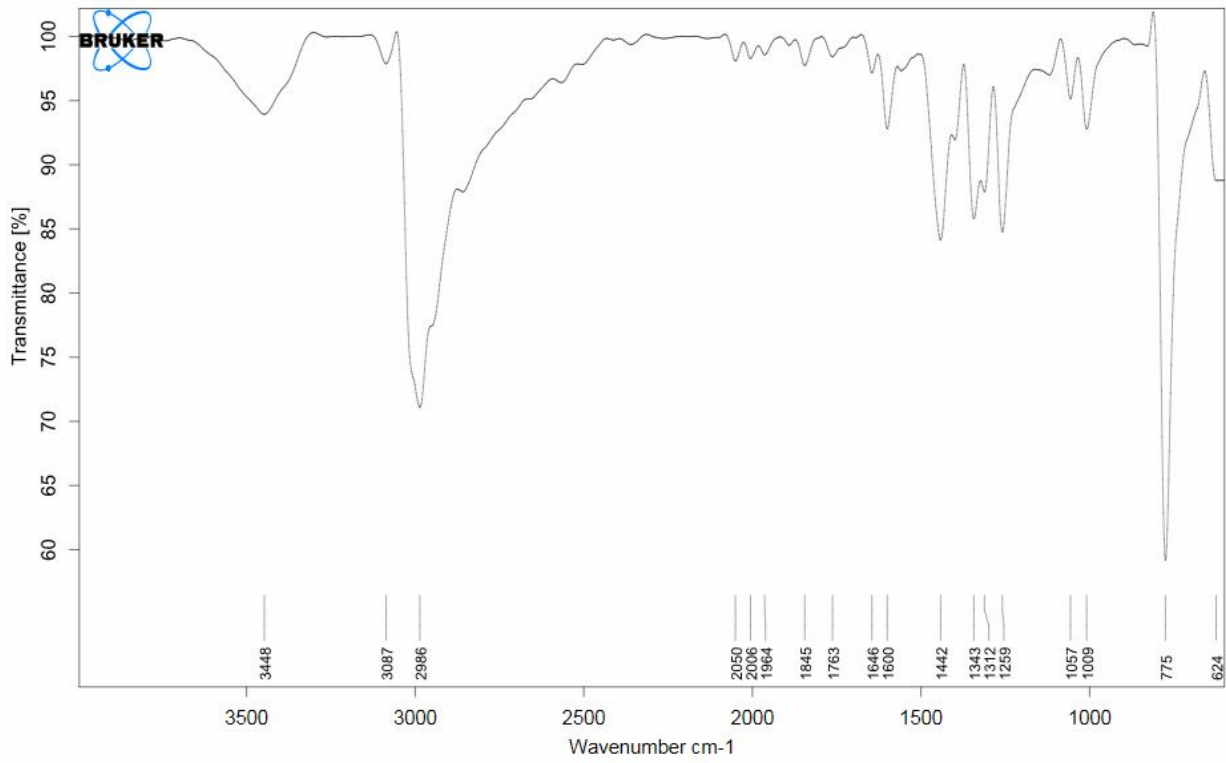
### Mechanistic Investigation of Colorimetric Detection of Cu<sup>2+</sup>

In our study 1,3-dimethyl benzotriazole iodide ionic liquid was used to functionalize and stabilization of AgNPs. Stabilization is achieved through electrostatic repulsion, which prevents the aggregation of metal NPs [16]. This IL capped NPs was used to sensing of Cu<sup>2+</sup> ions in different samples. As shown in Fig. 4, BTAIL-AgNPs are yellow, and after adding Cu<sup>2+</sup> ion-containing solution, the color changed to red. As it is well known, Cu<sup>2+</sup> can bridge two benzotriazole molecules through nitrogen atoms on the triazole ring [20,21]. As a result, BTAIL-AgNPs attach by BTA-Cu<sup>2+</sup>-BTA coordinative coupling. Both color change and intensity reduction of the absorption peak at 400 nm demonstrate this claim.

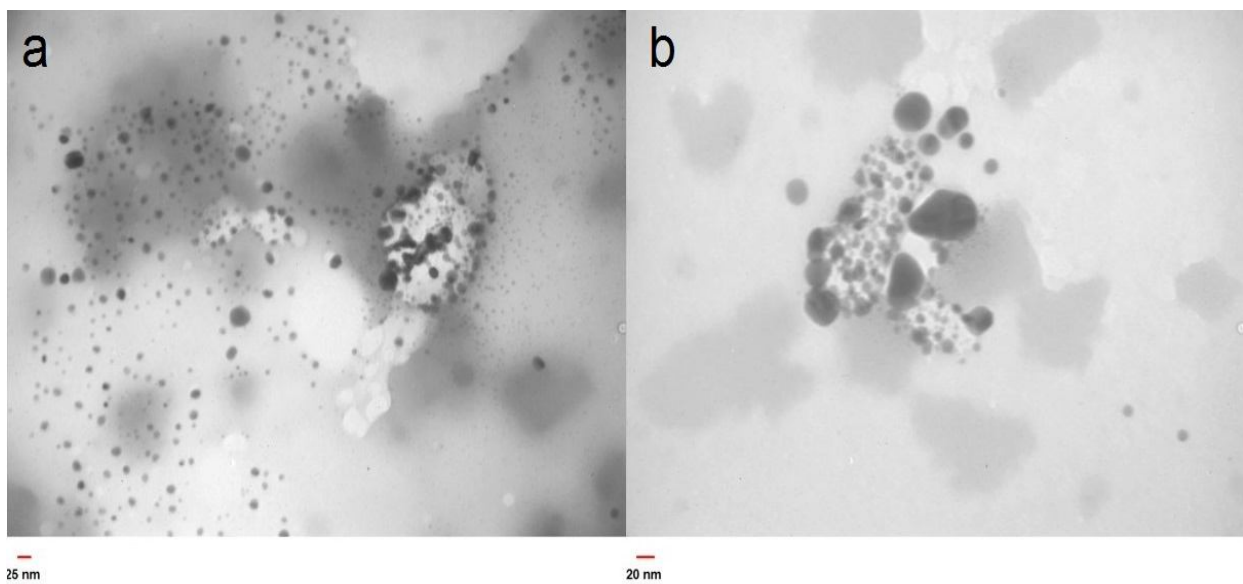
### Optimization the Method

The current colorimetric method is based on the aggregation of BTAIL-AgNPs and their color change, which corresponds to the absorption spectrum at 400 nm. However, the BTAIL-AgNPs aggregation can be affected by several experimental factors such as solution pH, reaction time, and BTAIL volume, so these factors were investigated to ensure having robust sensing. Parameters could be optimized by computational models [25,26] or simply by one parameter in which we select the second method.

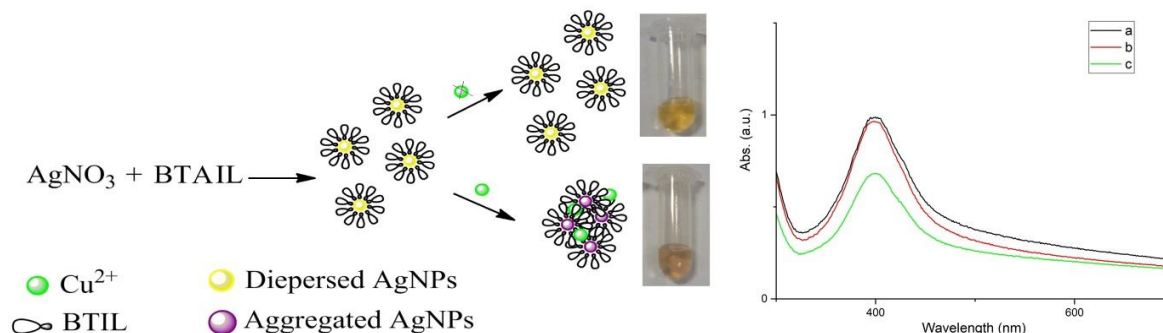
Because metal oxides might be formed at pH upper 10 and can cause some loss of analyte, higher pH is not suitable for testing. Also, metal NPs fully aggregated at pH



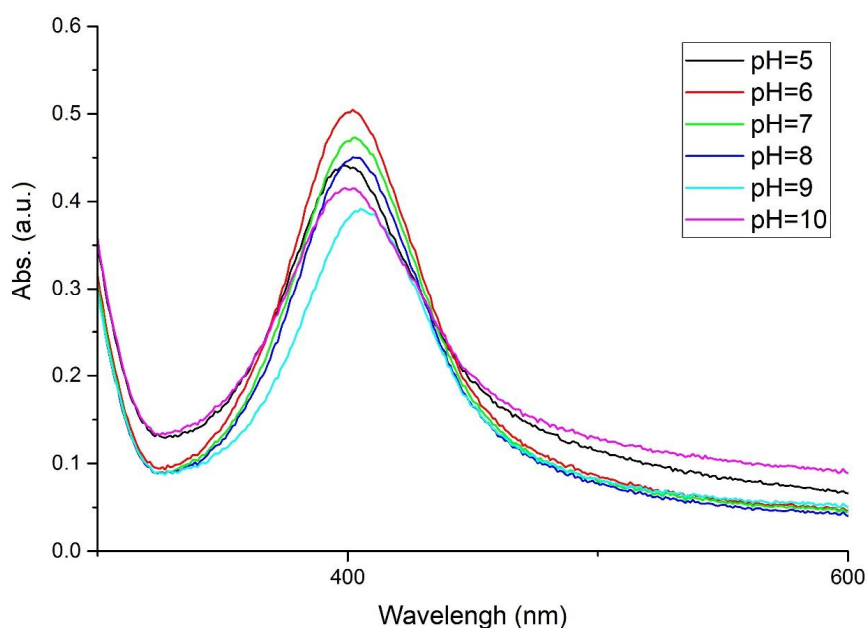
**Fig. 2.** The FTIR spectra of BTAIL.



**Fig. 3.** TEM image of dispersed BTAIL-AgNPs (a), aggregated BTAIL-AgNPs (b).



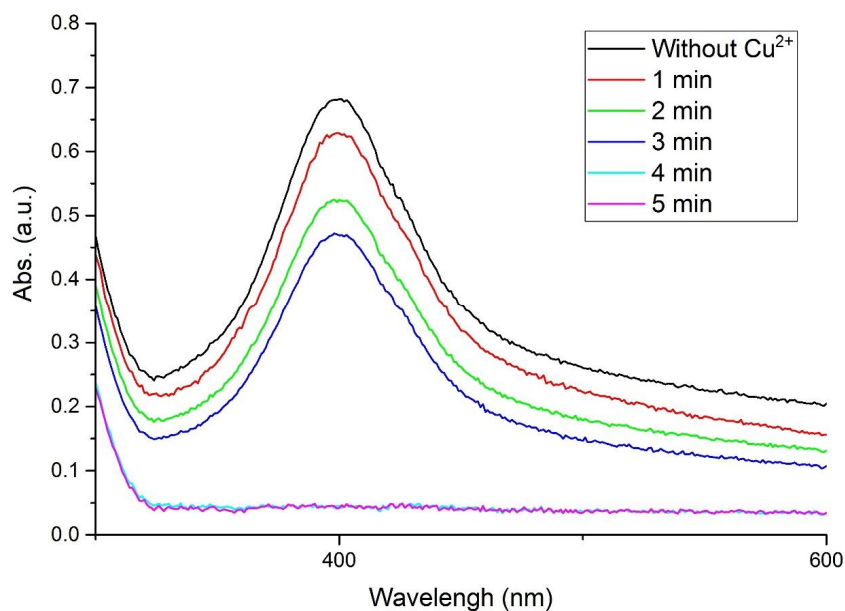
**Fig. 4.** Schematic representation of visual colorimetric sensing of  $\text{Cu}^{2+}$  using BTAIL-AgNPs and UV-Vis absorption spectra of synthesized AgNPs after 24 h (a) after a week (b) in the presence of  $0.1 \mu\text{M}$   $\text{Cu}^{2+}$  (c).



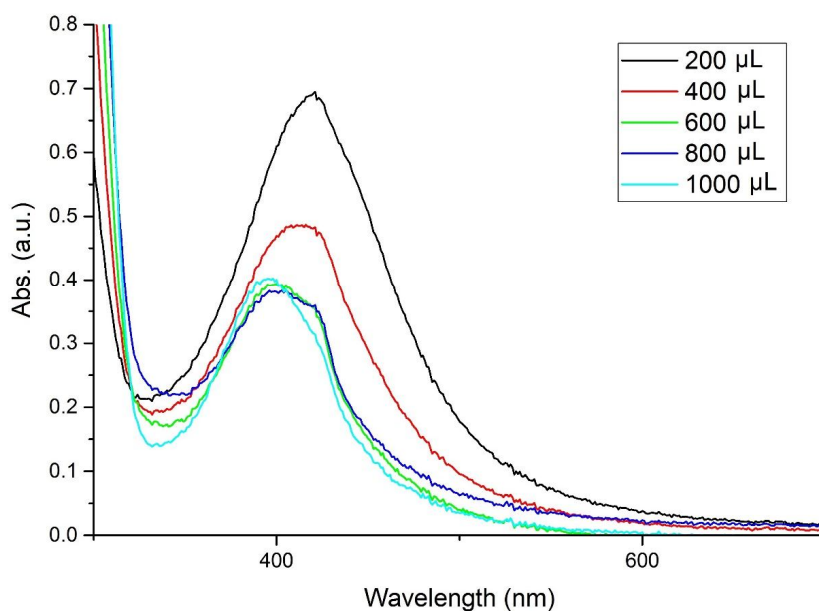
**Fig. 5.** UV-Vis absorption spectra of the BTAIL-AgNPs suspension containing  $0.1 \mu\text{M}$   $\text{Cu}^{2+}$  at different pH.

lower than 4 [27]; therefore, the UV-Vis absorption spectrum of BTAIL-AgNPs was investigated at different pH range from 5-10. The pH was adjusted by 0.1 M HCl and 0.1 M NaOH solutions. The pH effect on SPR bond of BTAIL-AgNPs was investigated in the presence of  $0.1 \mu\text{M}$  of  $\text{Cu}^{2+}$ . The absorption 400 nm was plotted toward pH shown in Fig. 5. Based on the finding, pH = 9 was selected as optimum pH for reproducible results, and further experiments were done at this pH.

The influence of incubation time upon the interaction between  $\text{Cu}^{2+}$  and BTAIL-AgNPs was investigated. For this purpose, the change in absorption spectra of BTAIL-AgNPs after adding  $300 \mu\text{l}$  of  $0.1 \mu\text{M}$   $\text{Cu}^{2+}$  solution was recorded, and the absorption 400 nm was plotted toward reaction time (1-5 min). As shown in Fig. 6, the NPs aggregation is completed within 3 min; therefore, this reaction time was selected to do further experiments. When the reaction time increased up to 3 min, all NPs sedimented, and the



**Fig. 6.** Time-dependent UV-Vis absorption spectra of ABTIL-AgNPs suspension containing the 0.1 μM Cu<sup>2+</sup>.

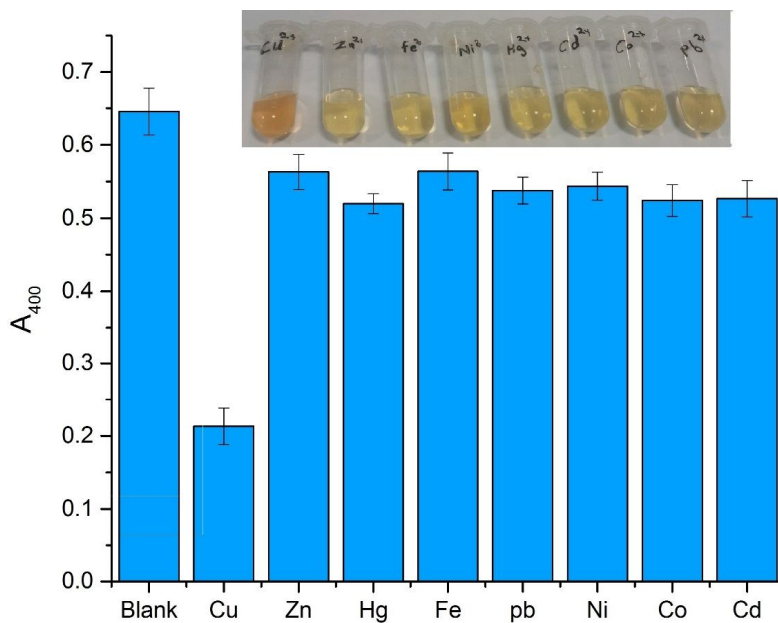


**Fig. 7.** UV-Vis absorption spectra of synthesized BTAIL-AgNPs (using different BTAIL volume (0.01 M)) in the presence of 0.1 μM Cu<sup>2+</sup>.

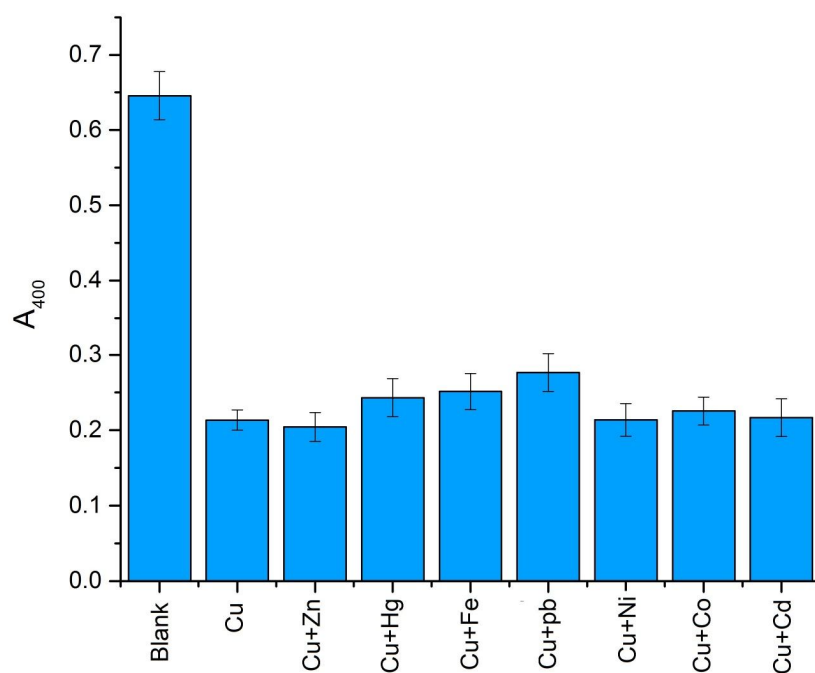
absorption peak at 400 nm disappeared.

The interaction between Cu<sup>2+</sup> and benzotriazole makes the aggregation of IL-capped AgNPs. The AgNPs were

synthesized in different concentrations of 0.01 μM BTAIL solution (200-1000 μl). The UV-Vis spectrum of aggregated IL capped NPs was recorded (Fig. 7). The results confirmed



**Fig. 8.** Selectivity of the colorimetric assay for  $\text{Cu}^{2+}$  using BTAIL-AgNPs.



**Fig. 9.** Interference evaluation of other metal ions towards this assay for  $\text{Cu}^{2+}$ .  $\text{Cu}^{2+}$ : 0.5  $\mu\text{M}$ ,  $\text{Zn}^{2+}$ : 0.2  $\mu\text{M}$ ,  $\text{Hg}^{2+}$ : 0.2  $\mu\text{M}$ ,  $\text{Fe}^{2+}$ : 0.2  $\mu\text{M}$ ,  $\text{Pb}^{2+}$ : 0.2  $\mu\text{M}$ ,  $\text{Ni}^{2+}$ : 0.2  $\mu\text{M}$ ,  $\text{Co}^{2+}$ : 0.2  $\mu\text{M}$ ,  $\text{Cd}^{2+}$ : 0.2  $\mu\text{M}$ .

that the optimized BTAIL volume is 600  $\mu\text{l}$ , wherein in the presence of 0.1  $\mu\text{M}$   $\text{Cu}^{2+}$ , the AgNPs show the lowest absorption peak at 400 nm.

### Method Validation

Under the above optimal condition, the  $\text{Cu}^{2+}$  analysis investigated in water, and the calibration curve in different  $\text{Cu}^{2+}$  concentration was plotted. The linear range was obtained from 20-100 nM with a correlation coefficient ( $R^2$ ) of 0.998. The lower limit of quantification (LLOQ) was 20 nM.

To study the  $\text{Cu}^{2+}$  detection ability of BTIL-AgNPs, the response was evaluated in the presence of  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Cd}^{2+}$ . The color change of NPs is shown in Fig. 8. As it is evident, the test zone color changed from yellow to red only in the presence of  $\text{Cu}^{2+}$  and the absorbance value in the presence of 0.5  $\mu\text{M}$  of these ions was remained almost unchangeable. These results demonstrated that this method is not only selective but also sensitive to  $\text{Cu}^{2+}$  ions. In addition, measuring of 0.5  $\mu\text{M}$   $\text{Cu}^{2+}$  in the absence and presence of each of all above ions (0.2  $\mu\text{M}$ ) was evaluated. It can be seen from Fig. 9; no interference exists between  $\text{Cu}^{2+}$  and other ions.

### Real Herbal Drink Samples Analysis

This developed method was applied to detect  $\text{Cu}^{2+}$  in four herbal drinks. Real samples were pretreated and measured by the above colorimetric sensing assay.

The accuracy of the developed method was tried by the standard addition method and calculating the recovery. The results showed the recoveries between 90.0% and 104.8%, and the relative standard deviations from 1.1 to 7.2%. Responses to real samples are very similar to those in double-distilled water. According to our findings, this colorimetric method to detect  $\text{Cu}^{2+}$  is applicable in different environments.

### CONCLUSIONS

A simple, sensitive and straightforward colorimetric probe for  $\text{Cu}^{2+}$  ions was implemented by a colorimetric probe consisting of BTAIL stabilized AgNPs. The assay of  $\text{Cu}^{2+}$  ions performed by the color change of yellow to the red that was vividly visible *via* the naked eye. The LLOQ

was 20 nM and could be detected with the naked eye in the aqueous solution. Moreover, the developed probe presented no significant interference to  $\text{Cu}^{2+}$  ions assay in the presence of other probable co-existing ions. It was used successfully to analyze  $\text{Cu}^{2+}$  ions in herbal samples with acceptable recoveries. Therefore, the simple and sensitive colorimetric probe for  $\text{Cu}^{2+}$  ions determination may be performed on different matrices such as environmental and biological samples on the bench and in real-time.

### ACKNOWLEDGMENTS

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### Conflicts of Interest

There are no conflicts to declare.

### REFERENCES

- [1] M. Angelova, S. Asenova, V. Nedkova, R. Koleva-Kolarova, T.J.S. 9 (2011) 88.
- [2] A. Jeevika, D.R. Shankaran, Colloid Surface A 461 (2014) 240.
- [3] Y. Dou, X. Yang, Z. Liu, S. Zhu, Colloid Surface A 423 (2013) 20.
- [4] L.P. Zhang, Y.P. Xing, C. Liu, X.h. Zhou, H.C. Shi, Sens. Actuators B Chem. 215 (2015) 561.
- [5] M.R. Hormozi-Nezhad, S. Abbasi-Moayed, Talanta 129 (2014) 227.
- [6] S. Wang, X. Wang, Z. Zhang, L. Chen, Colloid Surface A 468 (2015) 333.
- [7] E. Oliveira, C. Nunez, H.M. Santos, J. Fernandez-Lodeiro, A. Fernandez-Lodeiro, J.L. Capelo, C. Lodeiro, Sens. Actuators B Chem. 212 (2015) 297.
- [8] E. Priyadarshini, N. Pradhan, Sens. Actuators B Chem. 238 (2017) 888.
- [9] Q. Shen, W. Li, S. Tang, Y. Hu, Z. Nie, Y. Huang, S. Yao, Biosens. Bioelectron. 41 (2013) 663.
- [10] W. Jin, P. Huang, F. Wu, L.-H. Ma, Analyst 140 (2015) 3507.
- [11] J. Yang, Y. Zhang, L. Zhang, H. Wang, J. Nie, Z. Qin, J. Li, W. Xiao, Chem. Commun. 53 (2017) 7477.



- [12] W. Zhao, W. Jia, M. Sun, X. Liu, Q. Zhang, C. Zong, J. Qu, H. Gai, *Sens. Actuators B Chem.* 223 (2016) 411.
- [13] E. Priyadarshini, N. Pradhan, *Sci. Rep.* 7 (2017) 9278.
- [14] P. Raveendran, J. Fu, S.L. Wallen, *JACS.* 125 (2003) 13940.
- [15] D. Astruc, *Nanoparticles and Catalysis*, John Wiley & Sons, 2008.
- [16] K. Manojkumar, A. Sivaramakrishna, K. Vijayakrishna, *J. Nanopart. Res.* 18 (2016) 103.
- [17] W. Zhu, Z. Hou, *Curr. Inorganic Chem.* 2 (2012) 213.
- [18] K. Kumari, P. Singh, G.K. Mehrotra, *Adv. Energy Mater.* (2014) 529.
- [19] K. Luska, P. Migowski, W. Leitner, *Green Chem.* 17 (2015) 3195.
- [20] M. Antonijevic, M. Petrovic, *Int. J. Electrochem. Sci.* 3 (2008) 1.
- [21] M. Finšgar, I. Milošev, *Corros. Sci.* 52 (2010) 2737.
- [22] P.F. Khan, V. Shanthi, R.K. Babu, S. Muralidharan, R.C. Barik, *J. Environ. Chem. Eng.* 3 (2015) 10.
- [23] A.R. Katritzky, W. Kuzmierkiewicz, J.V. Greenhill, *Recl. Trav. Chim. Pays-Bas* 110 (1991) 369.
- [24] A. Mudzakir, *Infrared Spectroscopy* 3 (2008) 2.
- [25] A. Barzegar, S.J. Mousavi, H. Hamidi, M. Sadeghi, *Physica E* 93 (2017) 324.
- [26] A. Barzegar, H. Hamidi, *J. Theor. Comput. Chem.* 16 (2017) 1750038.
- [27] S. Basu, S.K. Ghosh, S. Kundu, S. Panigrahi, S. Praharaj, S. Pande, S. Jana, T. Pal, *J. Colloid Interface Sci.* 313 (2007) 724.