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Particular Amino Acid Detection Of By Henna Leaf Extract Mediated Copper Nanoparticles.

Kaushik Roy[†, §] *, Srijoni Malakar [§], Soumik Podder[§], and Koushik Pal[§].

[†]Jadavpur University, Kolkata-700 032, India.
[§]Department of ECE, Guru Nanak Institute of Technology, Panihati, Kolkata-700 114, India.

ABSTRACT

Here, we described an easy, eco-benign synthetic protocol for copper nanoparticles from copper sulphate employing extract of henna leaves (*Lawsonia inermis*) along with the evaluation of their activities for selective detection of amino acids. UV-Vis spectrometry was utilized to monitor the preparation of colloidal nano-copper in reacting medium. Standard characterization tools like X-ray powder diffraction (XRD) and transmission electron microscopy (TEM) were performed to realize the micro-structure and crystalline phases of biogenic nanoparticles. Finally, the amino acid detectability of these copper nanostructures was examined and the actual mechanism behind the rapid detection of a particular amino acid has been demonstrated.

Keywords: Biogenic copper nanoparticles, *Lawsonia inermis* leaf extract, UV-Vis spectroscopy, HRTEM, XRD, Amino acid detection

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**Corresponding author*

INTRODUCTION

Metallic nanoparticles and nanostructures feature lucrative electronic, optical and biochemical properties. These properties make the nanoparticles an attractive choice to develop newer technologies [1]. Specifically, nanoparticles of noble metals are gaining popularity in last few years for having unparallel merits like pollutant detecting properties [2], photo-catalytic activities [3], antibacterial properties [4] and reduced toxicity [5]. Mostly these diverse properties depend on size and shape of the particles and the capping agents on their surface [6]. Synthesis of nanoparticles is a primary step for nano-research. Biological synthesis or rather green synthetic protocols are always preferable over chemical or physical procedures as they are simple, cost-effective and thoroughly eco-benign too [7,8]. Biological eco-benign synthesis procedures for preparation of gold or silver nanoparticles are quiet available in literature [9,10], On the other hand, biosynthesis of copper nanostructure is regarded still as a challenge for the researchers [11]. The reason is the tendency of copper to get oxidized easily [12]. In current literature, fewer articles have reported to produce Cu nanoparticles from copper salt directly, involving extracts of plants [13,14]. In this report, *Lawsonia inermis* or henna leaves have been selected for preparing the extract because it can be regarded as potential bioactive source that contains reducing and stabilizing compounds such as eugenol and ascorbic acid which possess the ability to reduce metal ions and form nano-copper in the reacting solution [15]. In addition to that, it is crucial to identify a specific type of amino acid in a particular sample of food for nutritional information [16]. Here, the ability of extract-mediated copper nanoparticles for sensing amino acids was assessed to develop a cost-effective and easy-to-use chemical sensor for selective detection of amino acid.

MATERIALS AND METHODS

Synthesis of copper nanoparticles

Fresh green leaves of *Lawsonia inermis* (henna) were collected, cleaned and authenticated from the nursery for preparing leaf extract (shown in Fig.1). Next, 50 g of leaves were taken and ground in standard grinder and then filtered to make pure *Lawsonia inermis* leaf extract. Pure copper sulphate (analytical grade) was procured from Merck and its stock solution (concentration-20 mM) was obtained by dissolving 0.5 g copper sulphate into hundred millilitre of deionized (DI) water. The stock solution was preserved then at ambient temperature for further experimental use. Metallic nano-copper was produced through reduction of copper cations present in copper sulphate aqueous solution after addition of equivalent quantity of leaf extract of henna into reacting solution (keeping medium half-diluted). Then reacting solution containing copper salt and extract of henna was kept at room temperature for experimental observation. After twenty four hrs, the reacting solution turned reddish from colorless suggesting the formation of biogenic Cu nanoparticles in the medium. After 48 hrs of incubation, the reaction rate possibly saturated and colloidal particles were separated out of the medium through centrifuging the reacting solution at 5000 rpm for 15 minutes. Obtained soup was discarded and the pellets found at the bottom of centrifuge tube were carefully collected, cleaned and overnight dried to obtain pure dried powder of Cu nanoparticles.

Figure 1: Leaves of *Lawsonia inermis*

Characterization tools

To follow up the formation of Cu nanoparticles in reacting medium, the solution was scanned using ultraviolet visible spectrometer (Perkin Elmer, USA) for recording the absorption spectra at regular intervals. X-ray diffraction curves of dried nanoparticles were achieved by means of Rigaku Ultima-III (Japan) X-ray diffractometer. For high resolution TEM study, the dried nano-particles were suspended in de-ionized water with concentration- 50 µg/ml. A couple of drops of this suspension were then placed on ultra-clean copper mesh (grid) and dried followed by scanning using ultra-high resolution electron microscope (JEOL-2010, operating volt.- 200 kV). This dark red nano-suspension would be deployed as visual colorimetric sensor in this study later.

Amino acid detection by Cu nanoparticles

The amino acid detecting ability of these green synthesized Cu nanoparticles was examined by adding small concentrations of various amino acids to dark suspension of copper nanoparticles. Six essential amino acids- methionine, leucine, isoleucine, tryptophan, valine and theonine were ordered and purchased from Merck India Ltd. (Mumbai). The aqueous solutions (conc.-0.1%) of these amino acids were mixed drop wise to the nano-suspension of copper particles separately and the dark red suspension of nanoparticles turned fully colorless after addition of tryptophan only. Minimum detectable concentration was measured by applying gradually lower concentration of tryptophan to the nano-suspension and monitoring the absorption spectra for experimental record.

RESULTS AND DISCUSSION

Green synthesis of copper nanoparticles

Lawsonia inermis leaves are used traditionally for hair-care in Ayurveda as they are abundant source of many bioactive molecules such as ascorbic acid and eugenol [17]. Figure 2 shows the visual change of mixture coloration after 24 hrs of incubation. The colorless mixture became reddish after 24 hours and gradually intensified and turned dark red after 48 hours. This specific color- *dark red* of the mixture indicates plasmonic resonance of green synthesized copper nanoparticles produced in liquid reacting medium [18]. The absorption spectra of reacting solution recorded by UV-Vis spectroscope suggest a distinct peak near 560 nm wavelength, which may be justified as plasmonic peak of metallic copper (shown in Figure 3).

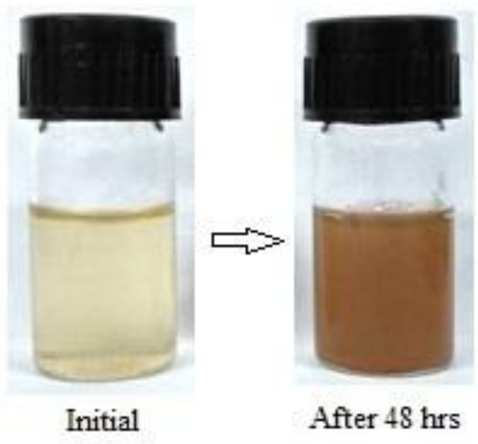

Figure 2: Change of color for reacting mixture

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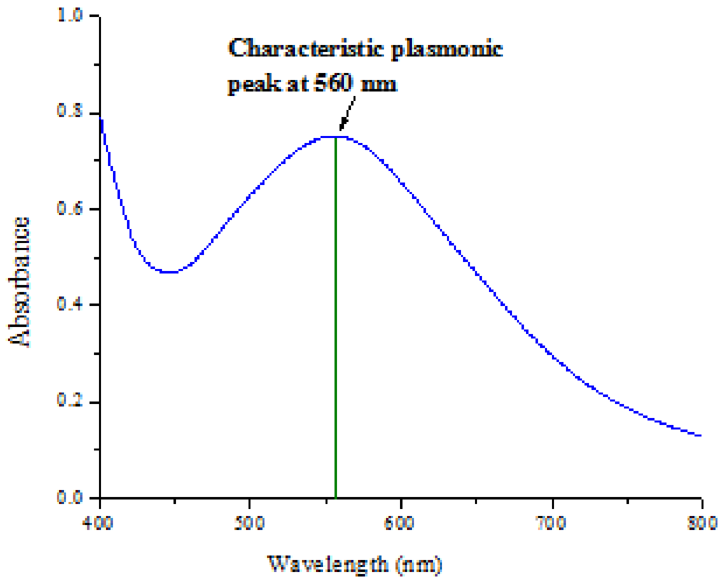

Figure 3: UV-Vis curve recorded after 24 Hrs of incubation

Structural analysis of nanoparticles

X-ray diffraction curve of these copper nanoparticles (Fig.4) consists of three clear peaks at 2θ = 43°, 51° and 74° which can easily be correlated to (111), (200) and (220) planes of metallic copper (JCPDS file card no.-04-836) [19].

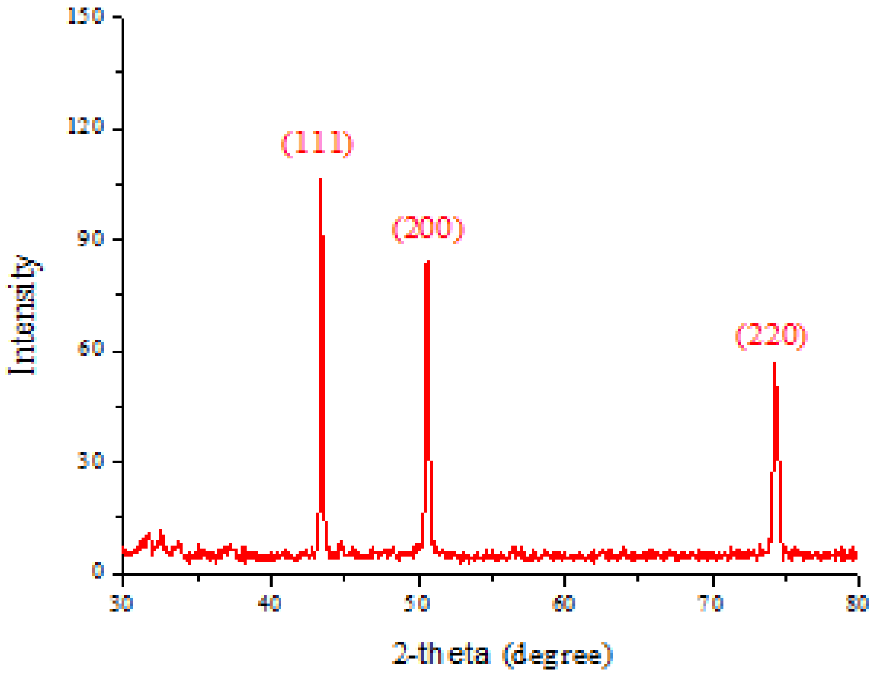

Figure 4: XRD graph of extract-mediated Cu nanoparticles

The electron microscopy image shows that these biogenic nanoparticles are almost spherical in shape and their diameter lies between 8 to 10 nm as shown in Fig. 5 [20].

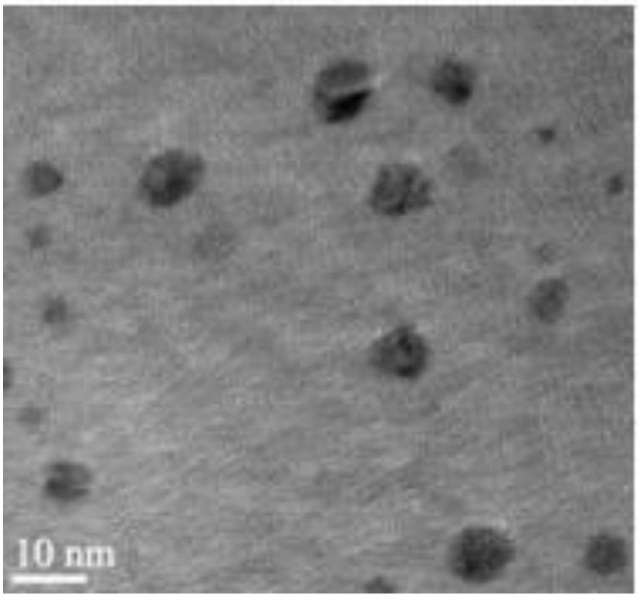

Figure 5: TEM image of nanoparticles

Study of Amino acid detection

Here, the suspension of extract-mediated copper nanoparticles has been examined as visual detector for selective sensing of amino acid. The dark suspension of nanoparticles decolorized immediately when only tryptophan was added to it. Other amino acids did not produce any significant changes as shown in the Figure 6.

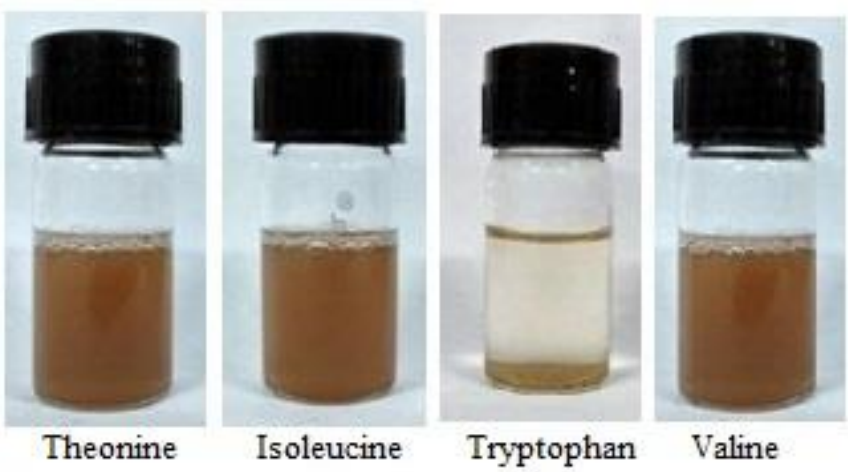

Figure 6. Colorimetric detection of tryptophan by suspension of nano-copper

Here, the mechanism of selective sensing of amino acid may be elucidated with the help of copper-amine complex formation theory [21]. When Tryptophan (molecular formula- $C_{11}H_{12}N_2O_2$) was added drop wise to the suspension of nano-copper, copper possibly reacts with the large molecules of tryptophan and produces copper (II) tryptophan complexes resulting into the decolorization of the dark red suspension. The reaction is as follows:

$$\mathbf{Cu + 2C_{11}H_{12}N_2O_2 \rightarrow C_{22}H_{22}CuN_4O_4}$$

Minimum detectable limit of tryptophan was measured to be as low as 0.01%. Hence the real food samples can also be examined to verify the presence of tryptophan following this procedure.

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CONCLUSION

The environment-friendly green synthetic protocols for metal nanoparticles and nanostructures are gaining attention recently due to their simplicity, cost-effectivity and eco-friendly features. In this report, we demonstrated biosynthesis of Cu nanoparticles from low-cost copper salt involving leaf extract of *Lawsonia inermis*. The nanoparticles were found to have crystalline phases and spherical shape with average diameter- 8 to 10 nm. Evaluation of amino acid sensing by extract mediated copper nano-particles was carried out meticulously and result of this study evidently proved desired efficacy of these extract-mediated copper nanoparticles for selective and instant detection of tryptophan.

REFERENCES

[1] K. Cheirmadurai, S. Biswas, R. Murali and P. Thanikaivelan, "Green synthesis of copper nanoparticles and conducting nanobiocomposites using plant and animal sources," RSC Adv. 4, 2014, pp. 19507-19511.

[2] K. Roy, C.K. Ghosh and C.K. Sarkar, "Rapid detection of hazardous H_2O_2 by biogenic copper nanoparticles synthesized using *Eichhornia crassipes* extract," 'Micro-2016' conference proceedings, ISBN: 978-93-80813-45-5, 2016, pp. 203-207.

[3] C. Mcmanamon, P. Delaney and M.A. Morris, "Photocatalytic properties of metal and non-metal doped novel sub 10 nm titanium dioxide nanoparticles on methyl orange," J. Colloid. Interf. Sci. 411, 2013, pp. 169-172.

[4] A.J. Kora and R.B. Sashidhar, "Antibacterial activity of biogenic silver nanoparticles synthesized with gum ghatti and gum olibanum: a comparative study," J. Antibiot. 68, 2014, pp. 88-97.

[5] P.V. Asharani, G.L.K. Mun, M.P. Hande and S. Valiyaveettil, "Cytotoxicity and genotoxicity of silver nanoparticles in human cells," ACS Nano 3(2), 2009, pp. 279-290.

[6] N. Li, P. Zhao and D. Astruc, "Anisotropic gold nanoparticles: synthesis, properties, applications, and toxicity," Angewandte Chemie Int. Edit. 53(7), 2014, pp. 1756–1789.

[7] V.V. Makarov, A.J. Love and N.O. Kalinina, "Green nanotechnologies: Synthesis of metal nanoparticles using plants," Acta Naturae 6(1), 2014, pp. 35-44.

[8] K. Roy and C.K. Ghosh, "Biological Synthesis of Metallic Nanoparticles: A Green Alternative," Chapter-7, Nanotechnology: Synthesis to Applications, 2017, ISBN: 978-1138032736, pp.153-168.

[9] M.V. Sujitha and S. Kannan, "Green synthesis of gold nanoparticles using Citrus fruits (*Citrus limon*, *Citrus reticulata* and *Citrus sinensis*) aqueous extract and its characterization," Spectrochim. Acta Part A 102, 2013, pp. 15-23.

[10] K. Vijayaraghavan, S.P.K. Nalini, N.U. Prakash and D. Madhankumar, "Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*," Mater. Lett. 75, 2012, pp. 33-35.

[11] S. Priyadarshini, V. Gopinath, N.M. Priyadharsshini, D.M. Ali and P. Velusamy, "Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application," Colloid. Surf. B Biointerfaces 102, 2013, p. 232-237.

[12] Z. Chen, S. Ye, I.E. Stewart and B.J. Wiley, "Copper Nanowire Networks with Transparent Oxide Shells That Prevent Oxidation without Reducing Transmittance," ACS Nano 8(9), 2014, pp. 9673-9679.

[13] K. Roy, C.K. Sarkar and C.K. Ghosh, "Rapid colorimetric detection of Hg^{2+} ion by green silver nanoparticles synthesized using *Dahlia pinnata* leaf extract," Green Process. Synth. 4(6), 2015, pp. 455-461.

[14] Z. Fan, X. Huang, C. Tan and H. Zhang, "Thin metal nanostructures: synthesis, properties and applications," Chem. Sci. 6, 2015, pp. 95-111.

[15] P. Pattanayak, P. Behera, D. Das and S.K. Panda, "*Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview," Pharmacogn. Rev. 4(7), 2010, pp. 95-105.

[16] R. Nielson and Z. Yang, "Likelihood Models for Detecting Positively Selected Amino Acid Sites and Applications to the HIV-1 Envelope Gene," Genetics 148, 1998, pp. 929-936.

[17] T.H. Zohourian, A.T. Quitain, M. Sasaki and M. Goto, "Extraction of Bioactive Compounds from Leaves of *Lawsonia inermis* by Green Pressurized Fluids," Separation Sci. Technol. 47(7), 2012, doi: 10.1080/01496395.2011.641056.

[18] G.H. Chan, J. Zhao, E.M. Hicks, G.C. Schatz and R.P.V. Duyne, "Plasmonic properties of copper nanoparticles fabricated by nanosphere lithography," Nano Lett. 7(7), 2007, pp. 1947-1952.

[19] E.K. Plyer, "Infrared Spectra of Methanol, Ethanol, and n-Propanol," J. Res. National Bureau Standards 48(4), 1952, pp. 281-286.

[20] K. Roy, C. K. Sarkar and C. K. Ghosh, "Semiconducting Behaviour of Metal Thin Film composed of Green Synthesized Silver Nanoparticles," 2019 Devices for Integrated Circuit (DevIC), 2019, pp. 480-483, doi: 10.1109/DEVIC.2019.8783229.
[21] V. Chakrapani, K.B.A. Ahmed, V.V. Kumar, V. Ganapathy, S.P. Anthony and V. Anbazhagan, “A facile route to synthesize casein capped copper nanoparticles: an effective antibacterial agent and selective colorimetric sensor for mercury and tryptophan,” RSC Adv. 4, 2014, pp. 33215-33221.