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ARTICLE TYPE

A facile route to synthesize casein capped copper nanoparticles: An effective antibacterial agent and selective colorimetric sensor for mercury and tryptophan†

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A simple method was developed for the synthesis of copper nanoparticles (CuNPs) with relatively high concentration by using casein, hydrazine, and ascorbic acid as a stabilizing, reducing and antioxidant agents, respectively. The obtained nanoparticles were characterized with UV-Vis spectroscopy, transmission electron microscope, FTIR and X-ray diffraction. The synthesized CuNPs exhibited good antibacterial activity against Gram positive and Gram negative bacterial strains. Notably, it exhibited excellent antibacterial activity against the multi drug resistant *Staphylococcus aureus* ATCC 43300 strain. Additionally, the optical properties of CuNPs for the first time to our knowledge has been utilized for the selective colorimetric sensing of tryptophan amino acid and Hg²⁺ metal cations in aqueous solution at ppm and ppb level, respectively.

1. Introduction

Metallic nanoparticles have been exploited for application in material science, catalysis, sensors, bioanalytical, medicine and so forth¹. Among the metal nanoparticles, coinage metals (gold, silver and copper) are known for attractive optical properties, arising from localized surface plasmon resonances in the visible and the near infrared frequencies². Hence, a substantial amount of efforts have been devoted towards the development of synthesis protocols for the production of nanoparticles³. In contrast to silver and gold, it is difficult to synthesis stable copper nanoparticles (CuNPs) because they are prone to surface oxidation upon exposure to air or aqueous medium⁴. Despite the difficulty, several methods have been developed to prepare CuNPs *via* reduction of copper salts under an inert atmosphere⁵. There have been very few reports exist on CuNPs synthesis without inert atmosphere⁶. Moreover, eco-friendly method to synthesis CuNPs is still in a rudimentary state of development relative to what has been achieved for both silver and gold. Copper is easily viable due to its low-cost, when compared with metals (silver and gold), therefore, developing a simple method to prepare CuNPs would be more economical for the development of day-to-day usage nanotechnology products.

To prepare nanoparticles by environmentally clean and sustainable way, researchers have begun to use biomolecules such as biopolymers, DNA and proteins⁷. Recently, Chatterjee et al., reported green route for the CuNPs synthesis and its worth to

mention the simplicity, versatility, and robustness of the process and material. This method involved the formation of copper-ammonia complex upon mixing copper chloride (CuCl₂) and concentrated ammonia in a gelatin medium, followed by the introduction of hydrazine (a reductant)⁶. Usman et al., reported chitosan stabilized CuNPs from copper sulfate with size range from 35 – 75 nm⁸. Jia et al., reported on the preparation of cellulose film coated CuNPs for antibacterial application⁹. In a recent study, Rastogi and Arunachalam reported on bovine serum albumin-copper nanocomposites. It consists of embedded CuNPs with a diameter of 28 nm and exhibit excellent bactericidal activity against both Gram positive and Gram negative bacterial strains¹⁰. Despite these reports, it remains a challenging task to produce CuNPs with well-defined and controllable shapes, under ambient conditions, as well as with desirable quality and quantity for further investigation.

Micelles forming surfactants have been widely used as a template for the stabilization of different types of nanostructures¹¹. Casein is the major phosphoprotein of bovine milk and constitutes about 80% of the total milk. There are four main types of casein, namely α -s1 casein, α -s2 casein, β -casein, and κ -casein¹². Casein self assembles into micelles in aqueous solution, but these micelles are completely different from surfactant type micelles. The interior of the casein micelles are highly hydrated. This property was successfully used for encapsulating both hydrophilic and hydrophobic molecules such as vitamins, polyphenols, lipophilic drugs¹³. Despite a great

amount of interest in the scientific community to applying casein in food and drug system, there are only limited studies on utilizing the micellar property of casein in the synthesis of inorganic nanoparticles¹⁴. In this paper, we report a simple chemical reduction method for the preparation of CuNPs using casein as a stabilizing agent and ascorbic acid as an antioxidant at atmospheric conditions without need of inert environment. The prepared CuNPs can be stored in open air for longer time without considerable oxidations. Besides having exceptional stability, casein stabilized CuNPs showed excellent antibacterial activity against Gram positive and Gram negative bacteria, as good as standard antibiotic. In addition, our studies revealed that even *Staphylococcus aureus* ATCC 43300 strain resistant to standard antibiotics (methicilin, ampicillin and kanamycin) was killed upon treatment with casein stabilized CuNPs.

Of late, the optical properties of the coinage metal nanoparticles such as silver and gold nanoparticles have been widely exploited as colorimetric sensor. For example, silver nanoparticles appended with different chemical functionalities have been used as media for detecting Ni²⁺, Co²⁺, Hg²⁺, Pb²⁺ and Cu²⁺¹⁵. Natural bifunctional gallic acid based silver and gold nanoparticles have shown selective colorimetric detection of Pb²⁺¹⁶. Although CuNPs exhibit optical property but till-date there are no report on the use of CuNPs as a colorimetric sensor. To the best of our knowledge, this is the first report on selective colorimetric sensor studies for hazardous metal ion and biologically important amino acid using CuNPs. Addition of Hg²⁺ selectively converts the wine red colour of CuNPs to dark red-coloured precipitate due to the amalgamation. Similarly additions of tryptophan selectively decolourise the CuNPs wine red colour. The detection limit of tryptophan and Hg²⁺ ions by CuNPs was found to be ppm and ppb level, respectively. High selectivity of CuNPs for Hg²⁺ and tryptophan was confirmed from the interference studies carried out in the presence of other amino acid/metal cations.

2. Materials and Methods

2.1. Materials

Copper chloride, ammonium hydroxide, and hydrazine were purchased from Merck, India. Casein (from bovine milk) was purchased from Alfa Aesar, India. Luria broth (LB), Nutrient broth (NB), *Staphylococcus aureus* ATCC 43300 strain, ampicillin, kanamycin and ofloxacin were purchased from Himedia chemicals India Pvt. Ltd (Mumbai, India). Millipore water with a resistivity more than 18.0 MΩ was used for the preparation of all aqueous solutions. All the glass wares were cleaned with freshly prepared aqua regia solution (3:1, HCl: HNO₃) and rinsed thoroughly with water. Then, they were dry sterilized using hot air oven at 160 °C for 3 h, prior to use. All other chemicals and solvents used in this work are of analytical grade obtained from the local supplier.

2.2. Copper nanoparticles synthesis

Aqueous casein solution was prepared by dissolving 50 mg casein in 50 ml of water containing 4 mg of ascorbic acid. To the

sparingly solubilized protein solution, aqueous CuCl₂ (1 mM, 8.5 mg, final concentration) were added slowly under vigorous stirring. To this mixture 200 μL of concentrated ammonia solution was added under constant stirring. The reaction was allowed to proceed under gentle stirring at room temperature for 5 min. The colour changes from greenish blue to blue due to the formation of copper-ammonia complex. After 5 min of gentle mixing, 400 μL of hydrazine hydrate was added as a mild reducing agent. After stirring the solution for 5 min, the reaction was allowed to proceed at room temperature for 6 h. The formation of copper nanoparticles was easily noticeable due to the change in the color of the solution to wine red – the characteristic color of the copper nanoparticles (CuNPs).

2.3. Characterization

Optical absorbance of synthesized CuNPs was monitored by UV-Vis spectrophotometer (Thermo Scientific Evolution 201) between a wavelength of 400 to 900 nm at a resolution of 1 nm. Size and crystallinity of CuNPs was measured by using high-resolution transmission electron microscope (TEM). X-ray diffraction measurements of CuNPs were done on a XRD-Bruker D8 Advance X-ray diffractometer using monochromatic Cu Kα radiation. The FT-IR spectra were recorded using a PerkinElmer FT-IR spectrometer with 1 cm⁻¹ resolution. Casein capped CuNPs was layer on a glass plate and dried in open air and scratched and grinded with KBr to obtain pellet for recording FT-IR analysis.

2.4. Antibacterial activity

The antibacterial activity of the casein stabilized CuNPs were evaluated by zone of inhibition (ZOI) using disc diffusion method. *Klebsiella pneumoniae* MTCC 109, *Pseudomonas aeruginosa* MTCC 1688, *Salmonella typhimurium* MTCC 98, *Shigella flexneri* MTCC 1457, *Bacillus thuringiensis* MTCC 869, and *Staphylococcus aureus* MTCC 3160 were used in this study. The paper disc (Whatman No.1) was cut down into small disc (4mm diameter) and sterilized in an autoclave. Approximately 20 ml of molten Nutrient agar was poured in sterilized Petri plates and plates were left overnight at room temperature. Meanwhile, the sterilized discs were impregnated with the aqueous solution of CuNPs (20 μg) and positive control drug. Then the disc was dried in sterile condition. The pure cultures of bacteria were subcultured on nutrient agar medium. Inoculum (10⁵ cfu/mL) of each strain was swabbed uniformly over the individual plates using sterile cotton swabs. The CuNPs impregnated discs were placed on the plates and incubated at 37°C for 24 h. After incubation, the different levels of zone of inhibition of bacteria were measured. Ofloxacin and kanamycin was used as control antibacterial drugs. The diameter of the inhibition zones was measured in mm and the mean values were presented.

2.5. Sensor studies

Metal cations and amino acids for sensor studies were prepared by mixing the requisite amount of salt in Mill-Q water. Sensor studies were performed by adding different metal ions or amino acids into CuNPs (1 mM) and monitoring colour and change in absorption.

3. Result and Discussion

3.1. Preparation of Copper nanoparticles

Casein, a protein commonly found in mammalian milk, have the ability to self-assemble into spherical micelles (ca. 50 – 500 nm in diameter) owing to their hydrophobic and hydrophilic amino acid residues¹⁷. In this work, we exploited the micellar property of casein and developed a strategy for the synthesis of CuNPs with relatively high concentration, and remarkable optical response in the visible region. Hydrazine was used as a reducing agent, casein as a stabilizer and ascorbic acid as an antioxidant. The main advantage of this method is that the syntheses were carried out at room temperature in open air with no need for inert atmosphere. Although ascorbic acid has often been demonstrated to be a good reducing agent for silver and gold nanoparticles formation¹⁸, it is not likely to be sufficiently strong for reducing copper salts, and thus hydrazine, a stronger reducing agent, was utilized. However, ascorbic acid was observed to play a significant role in preventing CuNPs surface oxidation. In fact, the particles prepared in absence of ascorbic acid lead to bigger aggregates with poor optical property.

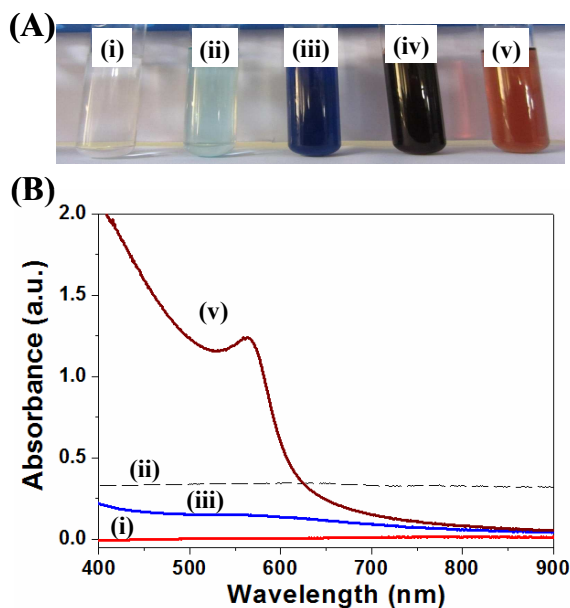


Fig. 1: (A) Photographs taken at various stages of CuNPs synthesis. Casein in ascorbic acid (i), copper chloride solution (ii), ammonical copper chloride solution (iii), highly concentrated casein-CuNPs (iv) and casein-CuNPs after dilution with milliQ water (v). (B) UV-Vis spectra of the corresponding photographs. Due to the high absorption intensity, UV-Vis spectra of image (iv) is not shown in (B)

The formation of CuNPs was accompanied by color change from greenish blue to blue and finally to wine red (Fig. 1A). The UV-Vis spectra recorded from the resulting solutions show the characteristic surface plasmon resonance (SPR) peak of CuNPs centred at about 565 nm⁶ (Fig. 1B). The absence of characteristic absorption peak for copper oxide at 800 nm¹⁹, suggest that the synthesized CuNPs is purely metallic and free of oxides. Fig. S1A† shows the UV-Vis spectra for the samples synthesized by

reduction of 10 mL of different concentrations of CuCl₂ with hydrazine. The digital photograph of the high concentration CuNPs are shown in Fig S1B†. As evident from Fig. S1A†, the intensity of the CuNPs SPR absorption shows a progressive enhancement with increasing concentration of CuCl₂. This clearly suggests that the increased content of metallic Cu in the solution. Time dependent absorption studies of CuNPs formation with casein revealed color intensification with time and the reaction was completed within 6 h (Fig. S2†). Analyzing the time dependent formation of NPs yielded the half time (182.10 min), the time required to convert half of the copper complex to CuNPs (Fig S2†, inset). The stability of the CuNPs was monitored by exposing the sample to open air for 3 days. UV-Vis spectra measured after 3 days hardly shows any difference in SPR peak, indicating that the samples are highly stable.

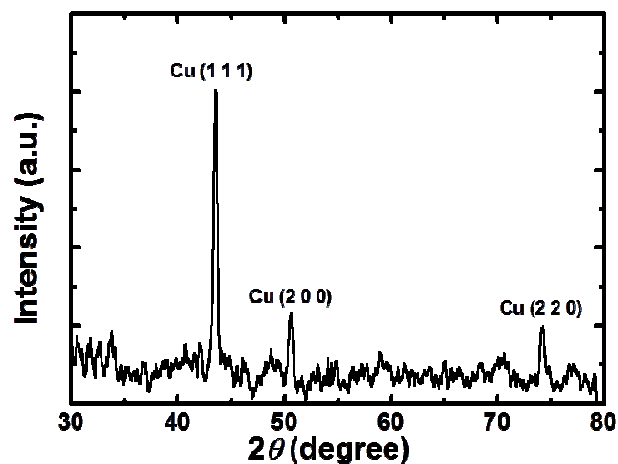


Fig. 2: XRD analysis of as-synthesized casein-CuNPs.

The XRD pattern of the synthesized CuNPs was shown in Fig 2. The characteristic diffraction peaks were observed at 43.55°, 50.67° and 74.32°, corresponding to the crystal facets of (1 1 1), (2 0 0) and (2 2 0), respectively, confirming that the synthesized material is composed of highly crystalline CuNPs and also matched with the standard diffraction pattern of metallic copper (JCPDS card no. 4-0836). The pattern was very clean, with no indication of impurities such as copper oxides (CuO, Cu₂O), revealing the high purity of the casein capped CuNPs.

Fig 3 shows the typical TEM images of the as-prepared copper nanoparticles. It is evident from Fig 3A and B that all the copper nanoparticles are spherical in shape with size ranging from 35-80nm. Each sphere is covered with a thin amorphous layer (Fig. 3C). This provides strong evidence for the formation of CuNPs inside the nanoscopic template of casein micelles. It is visible from HRTEM images that most of CuNPs exhibit approximately spherical in shape with low degree of agglomeration (Fig 3B). The images show an outer less-intense layer over a highly intense sphere; this signifies that a layer of casein covered the CuNPs (Fig.3C). HRTEM image show lattice fringes of the CuNPs, which indicates the crystallinity of the CuNPs (Fig. 3C). The interplanar spacing measured from Fig. 3C was around 0.212 nm. It is well matched to the two (1 1 1) planes of metallic copper

(0.208 nm). Selected area electron diffraction (SAED) pattern also confirms the formation of single crystalline CuNPs (Fig. 3D). The size-distribution of nanoparticles was determined using a particle-size analyzer and the average particle size was found to be 109.50 nm (Fig. S3[†]), which is higher than the values obtained from HRTEM. Since CuNPs are expected to be in nanoscopic template of casein micelles, we postulate that the average size of the casein micelles is around 110 nm, which stabilizes the CuNPs of size range from 35 – 89 nm.

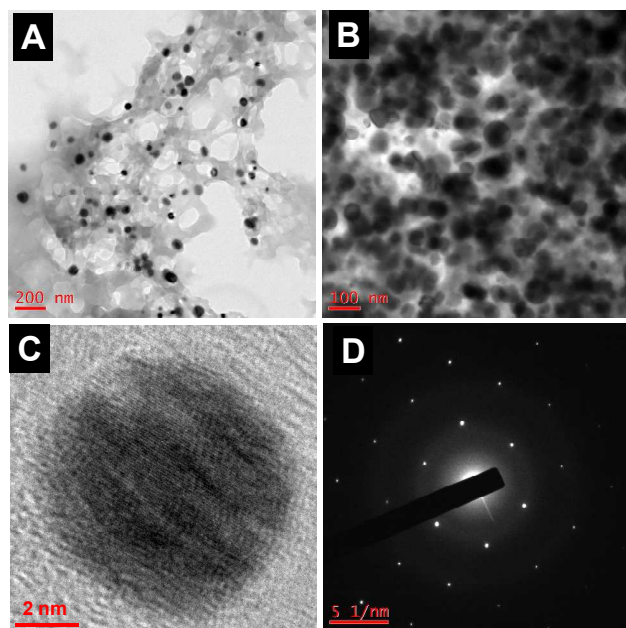


Fig. 3: TEM images of as-synthesized CuNPs (A, B), HRTEM (C) and SAED pattern (D).

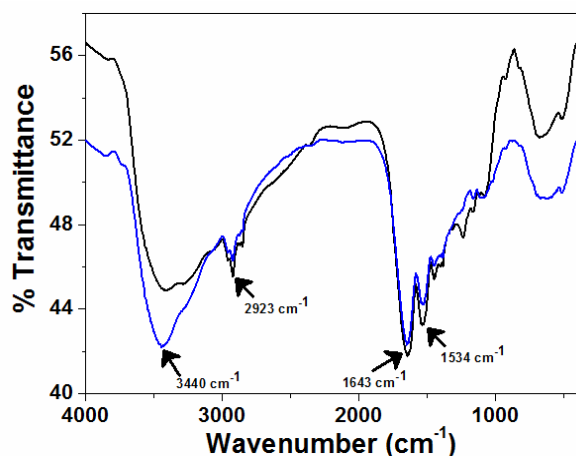


Fig. 4: FTIR spectra of pure casein (blue line) and as-synthesized casein-CuNPs (black line).

Further to confirm the presence of casein around the CuNPs we performed FTIR (Fig. 4). The broad band at 3440 cm⁻¹ is attributed to the –NH, –OH stretching vibrations and a peak at 2923 cm⁻¹ is assigned to C-H stretching. Presence of IR peaks at 1643 cm⁻¹ and 1534 cm⁻¹, which are characteristic of amide I and amide II are present in both casein and the synthesized casein-CuNPs. This clearly indicated that the structure of the protein is

conserved following the reduction of copper ions and further suggest the presence of protein around the NPs. It is well known that, peptides with more than three amino acids can form chelate complex with alkaline copper ions, popularly known as biuret reaction²⁰. Copper ions can form metal complex through the four peptide bonds and get reduced to NPs by hydrazine. Since there is no shift in IR peaks, we postulate that the formed NPs are surrounded by peptide bond and stabilized through weak interactions. Similar observation was made with bovine serum albumin (BSA)-copper nanocomposite¹⁰, BSA capped gold and silver nanoparticles²¹.

3.2. Antibacterial studies

The identification of bacterial strain resistance towards the potent antibiotic becomes a serious threat to modern medicine. In search for new antibacterial drugs with wide spectrum of activity, a substantial amount of research has been devoted to exploit the coinage metal nanoparticles as antibacterial agent and some achievements have been obtained. Different from silver and gold, copper is an essential element for living organism, and it might be more suitable for antibacterial application than silver and gold. The antimicrobial activities of the synthesized CuNPs were evaluated by disc diffusion method.

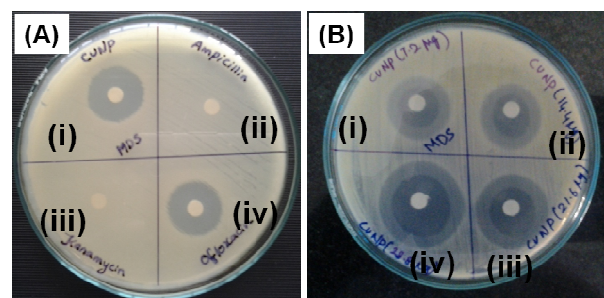


Fig. 5: (A) ZOI against methicillin resistant *S. aureus* strain, (i) Casein CuNPs, (ii) ampicillin, (iii) kanamycin and (iv) ofloxacin. About 7.2 µg of materials is added to the respective disk. (B) ZOI against MR-SA at different concentration of Casein CuNPs, (i) 7.2 µg, (ii) 14.4 µg, (iii) 21.6 µg and (iv) 27.8 µg.

The zone of inhibition (ZOI) obtained around the disk reflects susceptibility of bacterial strain towards the tested agent. Fig. S4[†] shows the presence of ZOI around the disk loaded casein capped CuNPs against *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium*, *S. flexneri*, *B. thuringiensis*, and *S. aureus*. The results are presented in the Table 1. It is evident from Table 1, that the casein capped CuNPs has an ability to inhibit the growth of Gram positive and Gram negative bacteria. For comparison, the inhibitory activity of standard antibiotic ofloxacin and kanamycin are also presented. From Table 1, it is evident that the casein capped CuNPs shows significant ZOI, as good as ofloxacin and kanamycin. The observed difference in ZOI among the bacterial species can be attributed to different cell wall composition. As noted, the capping agent, casein were found to produce no zone of inhibition.

Table 1: Antibacterial activity evaluated by zone of inhibition

Bacteria	Zone of Inhibition (mm)		
	CuNPs	ofloxacin	Kanamycin
<i>K. pneumoniae</i>	18	25	21
<i>P. aeruginosa</i>	23	19	17
<i>S. typhimurium</i>	20	21	19
<i>S. flexneri</i>	20	21	19
<i>B. thuringiensis</i>	25	23	18
<i>S. aureus</i>	21	18	15
MR <i>S. aureus</i>	20	18	0

Further, we tested the antibacterial properties of casein stabilized CuNPs against methicillin-resistant pathogenic *S. aureus* ATCC 43300 strain. This strain is also resistance to multiple antibiotics like ampicillin and kanamycin. Figure 5 shows the plate of *S. aureus*. The plates were incubated overnight with three antibiotics (ampicillin, kanamycin and ofloxacin) and CuNPs. From Fig. 5, it is evident that the CuNPs show the inhibition of growth and multiplication of the *S. aureus*. The ZOI was found to increase with increasing CuNPs concentration indicating the dose dependent inhibition of bacterial growth (Fig. 5B). The diameter of ZOI was almost similar to that in the case of the antibiotic-sensitive *S. aureus* cells (Table 1). The obtained result suggests that the casein capped CuNPs has high degree of antimicrobial indentions. Further, the use of casein for stabilization of CuNPs may render the nanoparticles with negligible toxicity for safer clinical trials.

3.3. Mercury sensor

The selective colorimetric sensing of CuNPs were explored for a series of heavy metal ions such as Cr^{3+} , Mn^{2+} , Cd^{2+} , Ca^{2+} , Cu^{2+} , Hg^{2+} , Zn^{2+} , Mg^{2+} , Ni^{2+} , Pb^{2+} , Fe^{3+} and Co^{3+} in aqueous solution. CuNPs showed wine-red color with an absorption at 565 nm. Addition of Hg^{2+} selectively changes the wine-red color to dark red colour opaque solution (Fig. 6A). The opaque nature of the solution indicates formation of precipitate. Addition of other metal ions did not show any significant colour change. The absorption studies also showed selective disappearances of CuNPs absorption at 565 nm by the addition of Hg^{2+} . But there is a weak absorption at 570 nm. We postulate that the formation of dark-red color and Mie-scattering effect of the precipitate is responsible for the base line shift²². These data are consistent with calorimetric sensing of Hg^{2+} using peptide functionalized AuNPs and mercaptopropionic modified AuNPs²². Addition of Pb^{2+} also showed clearly the appearance of cloudiness in the solution and showed small shift in absorption maximum. However, a clear color change was observed only with Hg^{2+} . Absorption spectra of CuNPs with other metal ions exhibited either little intensity reduction or no change in the spectra (Fig. 6B). Fig S5† shows the TEM image of CuNPs in presence of Hg^{2+} . It is clear from the TEM image that the addition of Hg^{2+} leads to the aggregation of the CuNPs, augmenting the observed absorption spectral changes in Fig 6.

The minimum detectable concentration of Hg^{2+} ions was determined by adding different volume of 10^{-9} M Hg^{2+} ions into CuNPs. At initial additions, only reduction of CuNPs absorption intensity was observed that was completely disappeared by the addition of 560 μl of Hg^{2+} at ppb level (Fig. S6A†). The presence

of other metal ions in CuNPs had very little influence on the selective colorimetric sensing of Hg^{2+} that confirmed the high selectivity (Fig. S6B†). The mechanism for the selective colorimetric sensing of Hg^{2+} by CuNPs is due to the amalgamation process²³. Hg^{2+} is reduced to Hg^0 by Cu^0 that lead to the formation of Cu-Hg amalgam, and Cu^{2+} is released to the corresponding amount of Hg bound to the elemental Cu:

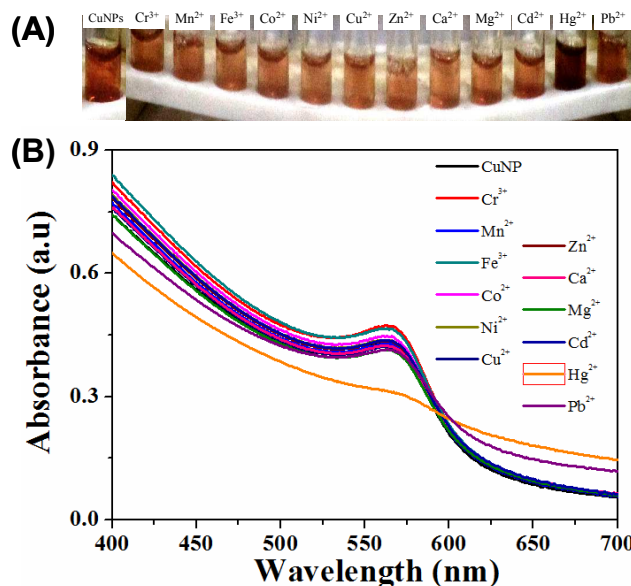
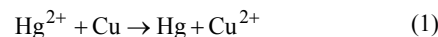


Fig. 6: (A) Digital images and (B) absorption spectra of CuNPs with different metal ions. Addition of Hg^{2+} selectively decreases the SPR peak of CuNPs.



The Gibbs energy for the reaction 1 can be calculated using standard potential from electrochemical series as shown below,



$$E^0_{\text{cell}} = E^0_{\text{reduced species}} - E^0_{\text{oxidized species}} = 0.5117\text{V}$$

$$\Delta G^0 = nFE^0_{\text{cell}} = (-2)(96500\text{C/mole})0.5117\text{J/C} = -98.75\text{KJ}$$

ΔG = Gibbs energy, n = valence, F = Faraday constant and E^0_{cell} = standard potential difference. The resulting, large negative Gibbs energy shows that the Hg^{2+} reduction as shown in eq. 1 is favoured reaction inducing the amalgamation.

3.4. Amino acid sensor

The synthesized CuNPs were also subjected to colorimetric sensing of different amino acids (glycine (Gly), alanine (Ala), serine (Ser), valine (Val), leucine (Leu), phenylalanine (Phe), tryptophan (Trp), histidine (His), cysteine (Cys), methionine (Met), tyrosine (Tyr) and glutathione (GSH)) in aqueous solution and monitored the colour change and absorption. Interestingly tryptophan addition selectively showed decolourisation of CuNPs wine red color with the formation of precipitate (Fig. 7).

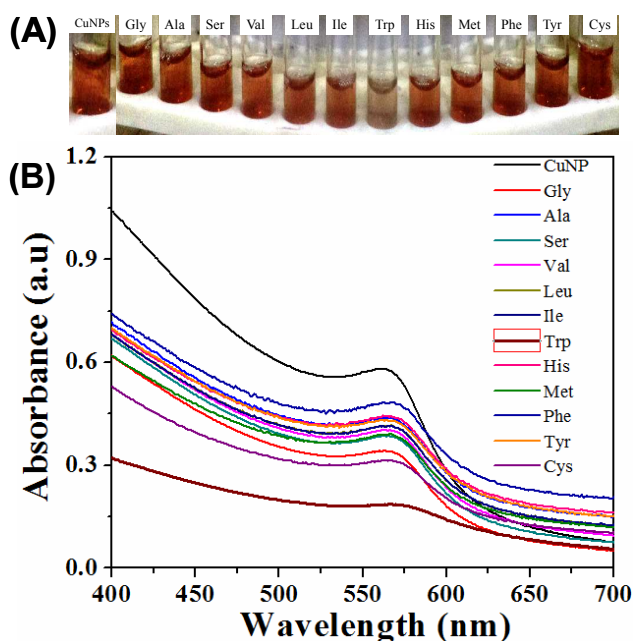


Fig.7: (A) Digital images and (B) absorption spectra of CuNPs with different amino acids. Addition of Trp changes the color of casein-CuNPs and considerably decreases the intensity of the SPR peak.

Addition of other amino acids did not show any significant colour change. It is noted that Cys, Met and smallest tripeptide GSH are all having thiol functionality that often showed preferential binding with coinage metal NPs such as Ag and AuNPs did not show any colour change. Similar to Hg²⁺ sensor studies, the absorption spectra of CuNPs with tryptophan showed the disappearance of 565 nm peak. The other amino acids only exhibited reduction of absorption peak. Particularly, Gly and Cys addition showed strongest reduction in absorption intensity. However, there is no significant colour change was observed visually compared to tryptophan addition. The concentration dependent studies showed gradual decrease of absorption intensity with tryptophan addition (10⁻⁶ M). The absorption peak was completely disappeared with the addition of 500 µl of tryptophan (Fig. S7A†). The interference studies in presence of other amino acids on the selectivity of CuNPs clearly confirmed the high selectivity for tryptophan (Fig. S7B†). The mechanism of tryptophan sensing is not known with certainty, however, the decolourisation process indicates that tryptophan addition destabilize the surface resonance properties of the CuNPs. We speculate that the CuNPs might be forming a supramolecular complex with tryptophan. Addition of CuNPs shows a concentration dependent decrease in the Trp fluorescence (Fig. S8†). Quenching of fluorescence emission of Trp may occur by several mechanisms: energy transfer, surface bound complexation, equilibrium attraction and surface absorption. The change in the fluorescence intensity decreases with increasing CuNPs concentration (Fig. S8†, inset). The displayed saturation behaviour suggested the complex formation between the Trp and CuNPs. However, further studies are required to confirm the reason for the formation of complex selectively with tryptophan.

4. Conclusions

The present study describes a simple and robust method to synthesize relatively high concentration of non-agglomerated CuNPs in the presence of casein as capping agent and ascorbic acid as antioxidant. UV-Visible, XRD, TEM and FTIR measurements were used to characterize the resulting CuNPs. This approach for CuNPs synthesis involved advantages like open atmosphere, room temperature, cost-effective, longer stability, and reproducibility. More importantly, the use of biocompatible protein as stabilizing agents renders the synthesized CuNPs for safer use in biological and biomedical applications. The CuNPs were found to be inhibitory to the Gram positive and Gram negative bacteria. Moreover, methicillin resistant strain of *S. aureus* is also sensitive to CuNPs and killed while exposed to casein-CuNPs. Our results indicated that casein-CuNPs has an ability to selectively sense the environmentally hazardous Hg²⁺ at the concentration of ppb. Besides, casein-CuNPs also selectively sense tryptophan amino acids at the concentration of ppm. So far, upto our knowledge we are the first to report the colorimetric sensing property of CuNPs. These results are expected to motivate researchers to explore the optical property of CuNPs as colorimetric sensor and also an alternate to the expensive silver and gold nanoparticles in nanotechnology application.

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Notes and references

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† Electronic Supplementary Information (ESI) available: The concentration and time dependent UV-Visible spectra of Casein-CuNPs; Zone of inhibition against different bacterial strains; Mercury and tryptophan sensor interference studies and concentration dependent absorption of CuNPs; Trp fluorescence quenching studies. See DOI: 10.1039/b000000x/

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A facile route to synthesize casein capped copper nanoparticles: An effective antibacterial agent and selective colorimetric sensor for mercury and tryptophan

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Graphical Abstract

