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Synthesis and Characterization of Copper Nanoparticles by Using a Protein as a Reducer and as well as a Stabilizer

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ABSTRACT

Synthesis of Copper nanoparticles (CuNPs) by a chemical reduction method is very difficult in aqueous medium than preparation of metallic nanoparticles like Pt, Ag and Au because it (Cu) easily converts into its oxidized form i.e. cupric oxide (CuO). Synthesis of stable CuNPs is described in this present article which is produced by the reaction of few drops hydrazine dihydrate and copper sulfate solution solution followed by catalytic amount of 0.01% β-lg. The whole reaction was carried out in nitrogen environment evolved during this reaction which is a very good advantage of this reaction. Bovine β-lg is utilized here to play dual function of a reducer as well as a stabilizer. Colliodal CuNPs were produced by in this process are characterized by some modern methods like powdered XRD, UV-Vis spectroscopy, field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). One of the main outcome of this present articles is that the synthesis of colloidal CuNPs by a reducer β-lg is not hazardous and environment friendly. This nanoparticles also could be applied in the field of nanomedicines and drug delivery.

Keywords: Copper nanoparticles, Reduction, Inert environment, β-lg, XRD, FE-SEM, TEM.

INTRODUCTION

Cu is a 3d transitional metal element of group 11 in the periodic table. Copper nanoparticle has a vast application in synthetic organic chemistry as a catalyst.[1] Its antibacterial activity is huge.[2] It is used in the field of agriculture, medicines, cosmetics, wound dressing, sensors, microelectronics, conversion of heavy petroleum fractions, transformation of solar energy, for the preparation of up-to-date lubricants, composite materials.[3–12] Various available methods for synthesis of CuNPs are chemical reduction, thermal decomposition, electron beam irradiation, laser ablation, synthetic route via an *in situ* and polyol.[13–18] But chemical reduction method is one of the best method for synthesizing CuNPs. Chemical reduction of copper salt using nontoxic ascorbic acid is a very good green approach where ascorbic acid is used as capping and reducer.[19] CuNPs was prepared by reducing Cupric (Cu^{+2}) salt using bovine serum albumin (BSA) where BSA plays the role of a partially reducer.[20] Thiol group (–SH) of cystein in BSA was utilized to reduce silver ions to AgNP.[21] This is where some opportunity arises to use protein like β-lg having –SH group could be applied to reduce metal ions in production of metal

nanoparticles. Bovine β-lg of molecular weight 18.4 kDa is a suitable protein for this purpose. It consists of eight anti parallel calyx structure and a free –SH group in cys-121. Here in this article it is shown that probably this free –SH group of β-lg play crucial role for reduction of Cu^{+2} to produce CuNPs in alkaline medium.

Materials

Copper sulfate ($CuSO_4$. $5H_2O$), hydrazine dihydrate (N_2H_4,$2H_2O$), ammonium hydroxide (NH_4OH) were purchased from Merk (India). Catalytic amount bovine β-lg was used. The isolation and purification of bovine β-lg from cow's milk was done as mentioned by Aschaffenburg and Drewry.[22] De-ionized water was used for making solutions in all experiments. All the reagents were used as received without any further purification. All the chemical reagents used here were obtainable in maximum pure form.

Synthesis of CuNPs

Freshly prepared 5 mM copper sulfate solution was stirred for 5 minutes. The pH of this solution was kept at 10 by adding ammonium hydroxide with continuous stirring. The colour of the solution became blue in the basic medium.[23] Then few drops of newly prepared 0.01 β-lg was added to it and the solution stirred for next 30 minutes. After this few drops of hydrazine dihydrate solution was added to this solution slowly. The colour changes to yellow and then brown and after 2 h it became brown red coloured which indicates the formation of CuNPs.[20] The whole reaction was performed in tightly closed bottle with nonstop stirring with the help of small magnetic bar. The following are the probable reactions during the synthesis of CuNPs.

$$CuSO_4 + H_2O \rightarrow Cu^{+2}\,(aq)$$

$$Cu^{+2}\,(aq) + NH_4OH \rightarrow [Cu(NH_3)2]^{+2}\,(aq)$$

$$[Cu(NH_3)_2]^{+2}\,(aq) + e^{-}\,(\text{source } \beta\text{-lg}) \rightarrow [Cu(NH_3)_2]^{+}\,(aq)$$

$$[Cu(NH_3)_2]^{+}\,(aq) + NH_2NH_2 + 2OH^{-} \rightarrow Cu^0\,(\text{embedded on } \beta\text{-lg}) + N_2\uparrow + 2NH_4OH$$

Characterization

X-ray diffraction study

Powdered X-ray diffraction (XRD) experiment was performed in department of Chemistry, Jadavpur University by a Bruker AXS diffractometer (Model D8, WI, USA). Monochromatic Cu Kα radiation (1.5409 Å) was used, running with a scan speed of 5° min^{-1} at 40 kV and 30mA. XRD was recorded in the range two thetta from 30°–80°. A very small amount dry CuNPs were used to prepare the sample for XRD purpose. During this procedure the various intensities of the diffracted X-rays were recorded.[24]

UV-Visible spectroscopy

The UV-Vis measurement of the synthesized spherical colloidal CuNPs was done in department of Chemistry, Jadavpur UniversityJadavpur by Shimazdu TCC 240A. A quartz cubate cell of path length 1cm was used in this experiment which contains 2 mL colloidal CuNPs. The UV measurement was done at room temperature in the range from 350nm to 800nm.

Field emission scanning electron microscopy (FE-SEM)

FESEM (Hitachi S-4800, JAPAN) was utilized with an operating voltage of 20 kV at School of Material Science and Nanotechnology, Jadavpur University. Sample for this study was set by placing a droplet of colloidal CuNPs on carbon tape. Then it was air dried at room temperature. Finally it was gold coated for imaging purpose.

Transmission electron microscopy

The morphological of synthesized CuNPs was also recorded in CRNN (Centre for Research in Nanoscience and Nanotechnology) of University of Calcutta by the instrument (Jeol-HRTEM-2011, Tokyo, Japan) with an accelerating voltage 80–85 Kv. A droplet of colloidal CuNPs (50 times diluted) was taken by a micropipette and then it was casted on a carbon coated copper grid of mesh size 30^0C (Pro Sci Tech). After 20 second it was soaked by using filter paper. Then it was dried for about 5 h in a vacuum desiccator carefully for imaging purpose.

RESULT AND DISCUSSION

X-ray diffraction study

Powder XRD is a familiar characterizan method for nanoparticles. To identify the phase and morphology of the studied sample XRD is reliable and a very good spectroscopic process. It also helps to know the purity of the sample and size as

well as crystalline pattern. The powdered XRD of the synthesized CuNPs was recorded (Fig. 1). The corresponding main peak values for the planes 111, 200 and 220 are shown in Table 1. The peaks are sharp in nature which informs that the synthesized nanoparticles are crystalline. Geometrically CuNPs are fcc which is fitted with JCPDS card no. is: 85–1326.[25] This XRD values were shown by the Table1.

Table 1: The different values of 2θ versus hkl

2θ (degree)	43.5	50.9	75
Hkl (planes)	111	200	220

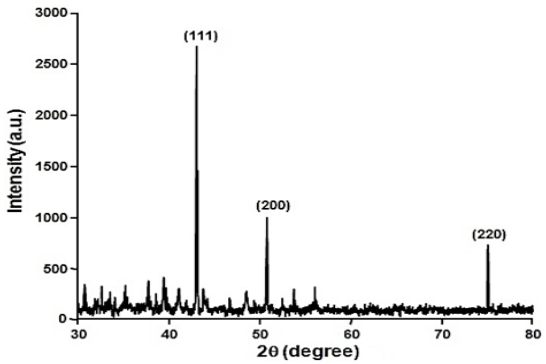

Fig. 1. XRD spectrum of CuNPs

UV-Visible spectroscopy

UV-Visible spectroscopy shows a clear absorption peak at wave length 573nm Fig. 2 suggests the formation of colloidal CuNPs. The inset picture of Fig. 2 shows brown red coloured colloidal solution appeared due to production of CuNPs.[20] The absorption band arises in between 550 nm to 600nm which confirms the formation of CuNPs.[1] The absence of absorption band at 800nm informs the absence of copper monoxide i.e. cupric oxide.[26] This information suggests the formation of spherical and purely metallic CuNPs which is free from cupric oxide. Little amount N_2 produced during the reaction creates sufficient inert atmosphere which cancels the formation of cupric oxide by oxidation process.

Field emission scanning electron microscopy (FE-SEM)

FE-SEM study was involved to evaluate the morphology of the surface of the synthesized CuNPs. The exterior morphology of the synthesized colloidal CuNPs is shown by Fig. 3a and Fig. 3b. The FE-SEM images displayed that CuNPs were deposited on protein surface. A very close look displayed that the shape of the synthesized nanoparticles is spherical in nature.

Transmission electron microscopy

TEM is one of the best tools to characterize the morphological structure of the nanoparticle because it provides some very important practicle information of visualization in authentic space. The geometric shape of the synthesized CuNPs is of spherical which is very clear in Fig. 4c–e. The diameter of the CuNPs is of aboute (10 ± 2nm). The bright SAED pattern (inset picture of Fig. 4f) indicates the synthesized nanoparticles are of crystalline and metallic in naure.[27]

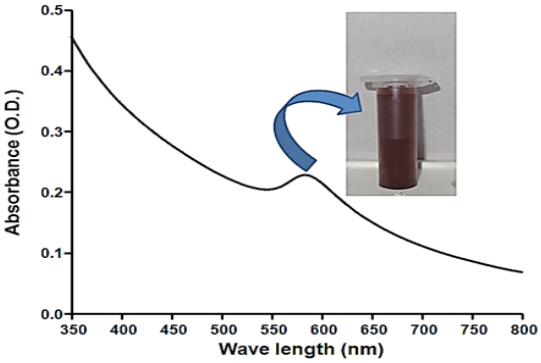

Fig. 2. UV-Visible spectra of synthesized colloidal CuNPs

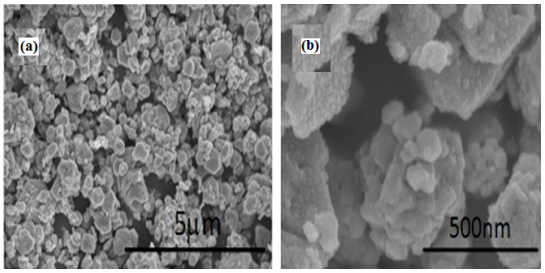

Fig. 3. Field emission scanning electron microscopic images of synthesized CuNPs with different magnifications (a and b)

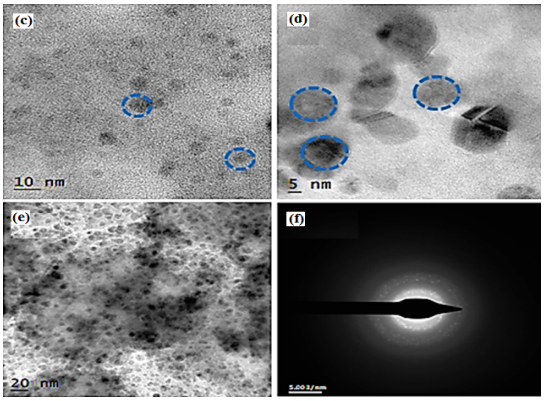

Fig. 4. High resolution transmission electron microscopic images of synthesized CuNPs with different magnifications are represented by (4c–e) and Fig. 4f represent the SAED pattern of CuNPs

Challenges

The synthesis of stable CuNPs is very challenging work because easy oxidation of these nanoparticles into oxide form (particularly CuO) takes place during production if it comes in contact with air or oxygen. The synthesis of spherical CuNPs is mentioned throughout the article which is very much difficult if one should not precisely take care about parameters of the reaction like the adjustment of the pH during the reaction, temperature and duration. All these parameters play very important role to synthesize desired shaped CuNPs which make them essential in specific requirement or formation of important product. Biocpatibility of the synthesized CuNPs must be checked before using these nanoparticles produce according to this article as toxicity is another challenge of CuNPs.

CONCLUSION

The synthesized metallic and crystalline copper nanoparticles were confirmed by XRD studies. Initially the colloidal CuNPs were established by UV-Visble spectroscopic measurement. Further SEM and TEM analysis gives solid proof of the generated CuNPs.The CuNPs were produced by this chemical reduction process have some advantages. Firstly the reagent copper sulfate is very cheap available in the market. Secondly there is no need of inert atmosphere production from outside. Thirdly catalytic amount bovine β-lg is acting here as a reducer as well as a stabilizer. Fourthly the total reaction is carried out in aqueous medium which is very economic solvent than the costly organic solvent. Thus by considering the mentioned advantages this present article must be valuable and interesting in preparation of stable and spherical shaped colloidal CuNPs in the field nanochemistry.

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Conflict of Interest

The author speak outs that no conflict of interest.

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