ISSN: 2578-8760

Journal of Nanomedicine

Open Access | Research Article

Biosynthesis and Haemolytic Activity of Ag-Cu Alloy Nanoparticles by Butea monosperma Plant Extract

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Received: Aug 02, 2021

Accepted: Sep 14, 2021

Published Online: Sep 16, 2021

Journal: Journal of Nanomedicine

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Reduction; Ag-Cu ANPs; Butea monosperma extract; Reduction; Structure.

Abstract

Objective: Metal or metal oxide nanoparticles have attracted the recent researchers much for their suitable applications and easy way of synthesis. This synthesis is based on biological reduction that uses plant extract as it is simple and it can integrate the chemical technology. An attempt has been made to understand the synthesis of Ag-Cu ANPs material by biological reduction of silver salt and copper salt using *Butea monosperma* leaf extract.

Methods: Easily available plant leaves are used for the simultaneous reduction of metal salts in an eco-friendly system. Biological reduction of silver salt and copper salt is applied for the synthesis of Silver-Copper alloy nanoparticles (Ag-Cu ANPs) using *Butea monosperma* plant leaf extract. Haemolytic activity study for the prepared alloy sample is also undertaken to know its toxicity.

Results: Crystalline structural of the Ag-Cu ANPs sample is analysed by powder X-ray diffraction (XRD) tool and it supports by EDX study. Morphology of the sample is studied by scanning electron micrograph (SEM) as well as transmission electron microscope (TEM) tool. Enhanced morphology and nano sized particle of the alloy sample is confirmed. Likewise, Fourier Transform Infrared (FT-IR) spectral study is undertaken in order to view the bonding in the sample. Haemolytic activity study shows that, haemolysis is within the permissible limit and also shows less toxicity.

Conclusion: It is found that using *Butea monosperma* plant leaf extract for biosynthesis of Ag-Cu ANPs is a simple method, efficient and eco-friendly system. Moreover, this method can be of good use in the other metal alloys/nanocomposite particles synthesis. This is one of the economically viable methods in the nanoparticles synthesis.

Cite this article: Lagashetty AK, Shashishar, Preeti RK, Ganiger SK. Biosynthesis and Haemolytic Activity of Ag-Cu Alloy Nanoparticles by Butea Monosperma Plant Extract. J Nanomed. 2021; 4(2): 1044.

Introduction

Nanotechnology is an emerging technology of the era due to the new properties it has and their applications [1-2]. Hence, It is hoped that the technology during next generation will be probably based on tailoring the blocks of many other applications. A high specific surface area and high fraction of surface atoms could be found in metal or metal oxide nanoparticles. They have been drawing the attention of scientists due to their novel methods of synthesis and the unique physicochemical characteristics of nanoparticles that include catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties [3-4]. The nanomaterials obtained from the technique of several plant extractions have shown enhanced, unique properties and applications with proper manipulation than its bulk materials [5-7]. Research on the synthesis of metal nanoparticles through biological method especially the plant extract has been developing rapidly due to its simple experimentation. This is because of the biological method as the synthesis of materials at nanoscale is based on eco-friendly system that avoids the usage of fuel in burning. Indeed there is a growth in developing environmentally benevolent synthesis processes of nanoparticles that use no toxic chemicals in the protocol of synthesis [8-10]. Thus, the need of environmental friendly approach of synthetic protocols for nanoparticles synthesis further increases the interest in biological approaches as they are free from toxic chemicals as by products. It is vivid that, the demand for green nanotechnology has been increasing day by day to satisfy the needs of the society without any harming the natural resources [11-12]. Till date, both for extracellular and for intracellular nanoparticles synthesis many biological approaches have been reported. In this process researchers have used microorganisms, plants, bacteria and fungi in the synthetic process. The most adopted method of green and eco-friendly production of nanoparticles has been carried out by plant extracts. Moreover, it has a special advantage as the plants are distributed widely, easily available, safer to handle and for several metabolites sources [13-14]. The silver and copper metal alloys or metal nanoparticles prepared biologically have attracted the modern researchers as they reveal extensive applicability in various areas like drug delivery system, electronics, chemical engineering, energy efficient system and even in the field of medicine [15-17]. Nanoparticles composed of two different metal elements show greater interest compared to single metal nanoparticles due to its properties and applications [18]. In addition to this, the research record proves that, the Ag-Cu ANPs shows antimicrobial activity, high thermal stability and localized surface Plasmon behavior [19-20]. These properties of the metal alloy nanoparticles encapsulate the development of applications to the recent field of science and technology.

It is certain that the rapid development in the field of nanotechnology would result in the exposure of nanoparticles to humans through numerous routes (e.g., inhalation, ingestion, skin, etc.). Of course, nanoparticles can translocate from an exposure route to the other vital organs as well as penetrate the cells. Therefore, the study of toxicity is required in order to regulate the deleterious effects of nanoparticles on the living cells. Indeed, there is literature that includes many techniques of synthesizing metal nanoparticles like chemical reduction of metal ions in aqueous solutions with or without stabilizing agents [21-22]. Further, at times the synthesis of biological nanoparticles using plants or parts of plants can prove to be advantageous over the other biological processes as they eliminate the elaborate processes of maintaining microbial cultures [23-24].

The review of literature survey has shown that, the different metals and metal oxide nanoparticles have been successfully prepared by biological route. However, the studies on green derived bimetallic alloy nanocomposite particles are reported to be very less. In this study, an attempt has been made to understand the synthesis of Ag-Cu ANPs material by biological reduction of silver salt and copper salt using *Butea monosperma* leaf extract. In situ and simultaneous bio reduction method is adopted for synthesis Ag-Cu ANPs material. The prepared sample was characterized the structure by X-ray diffraction (XRD), morphology by Scanning Electron Microscope (SEM) and bonding by Fourier Transform Infrared study (FT-IR) techniques. EDX analysis of the prepared sample is undertaken in order to confirm the presence of Ag-Cu ANPs formation.

Experimental

Materials and methods

The required chemicals used in the present experimentation are of AR grade and are perchaged from Merck (Mumbai, India). Double distilled waters is used in the preparation of *Butea monosperma* plant extract, silver nitrate and copper chloride solution. Properly rinsed glass wares with chromic acid are used in the experimentation. Biological reduction of silver nitrate and copper chloride using *Butea monosperma* leaf extract is adopted in the synthesis of Ag-Cu alloy nanocomposite at room temperature.

Preparation of *Butea monosperma* leaf extract

Butea monosperma leaves were collected freshly from the tree (Figure 1) then thoroughly washed repeatedly with double distilled water to remove complete wastes on leaves. Two grams of the leaves were cut into small pieces and placed them into a conical flask filled with 100mL double distilled water. Boil the content of the conical flask for about 15 minutes in order to get the aqueous leaf extract and is filtered through whatmann no. 40 filter paper. Cover the content of the eluent extract solution with proper cover and is stored in dark and low temperature.

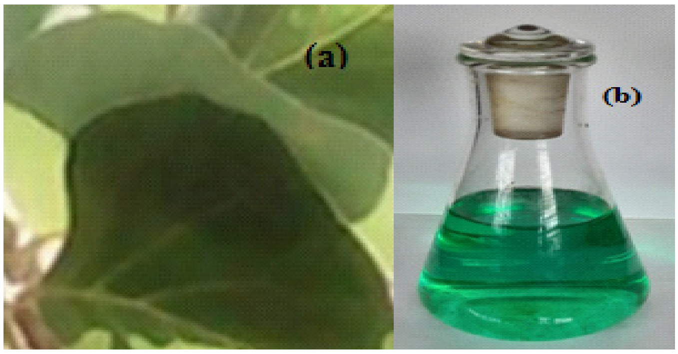

Figure 1: **(a)** *Butea Monosperma* plant leaves **(b)** *Butea Monosperma* plant leaves extract.

Synthesis of Ag-Cu alloy nanoparticles (Ag-Cu ANPs)

Prepare 0.001M silver nitrate ($AgNO_3$) and copper chloride ($CuSO_4$) solutions in double distilled water in separate container. 15 ml of these prepared solutions were mixed with each other in another container. Further, added the above prepared 15 mL of *Butea monosperma* aqueous leaf extract to the above salt solution mixture followed by heating the solution on hot plate at 60 ^{0}C for about15 minutes. Cool the solution in room temperature for about 24 hours. Dark brownish colour is obtained due to complete reduction of silver and copper ions in to Ag-Cu ANPs (Figure-2). During the synthesis process, the Ag^+ and Cu^{2+} ions reduces to its Ag and Cu metals by use of organic content of the plant leaf extract. Then centrifuged the solution at 10000 rpm for about ten minutes and is re-dispersed in water so as to get the yield 0.1g of the sample. The colours of prepared samples are given in figure-2 and its preparative scheme is shown in figure. 3. Possible reactions taking place in the synthetic scheme are given below.

$Ag^{+}NO_{3}^{-}.4H_{2}O$ + Plant extract → Ag^{0}NPS + Biproducts

$Cu_{2}^{+}SO_{4}^{2-}.4H_{2}O$ + Plant extract → Cu^{0}NPS + Biproducts

Ag^{0}NPS + Cu^{0}NPS + Plant extract → Ag^{0}-Cu^{0} ANPS

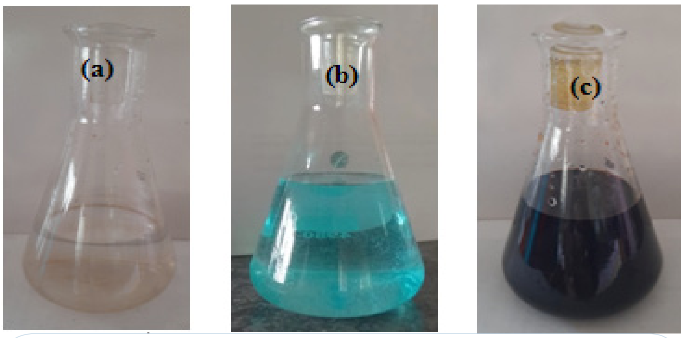

Figure 2: Colour of **(a)** $AgNO_3$ solution **(b)** $CuSO_4$ **(c)** solution (c) Ag-Cu ANPs solution.

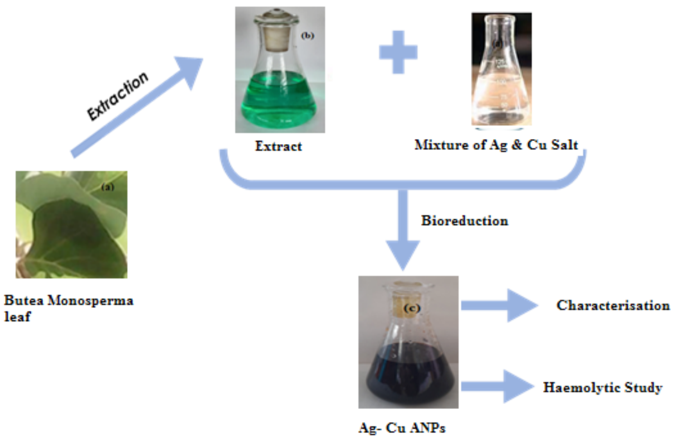

Figure 3: Experimental scheme for Ag-Cu ANPs sample.

Determination of average crystallite sizes from X-ray line broadening

Following Scherrer equation is used to calculate the average crystallite size (D) of the alloy sample is carried out from broadening of X-ray lines.

$$D = \frac{0.9\,\lambda}{\beta_{1/2}\cos\theta}$$

Where λ is the wavelength of the X-ray beam; $\beta_{½}$ is the angular width at the half-maximum intensity and θ is the Bragg angle. Quartz is considered as internal standard [25-27].

Haemolytic activity

The hemolysis study of the Ag-Cu ANPs was performed with acid citrate dextrose human blood to know the toxicity of the sample. Prepared Ag-Cu ANPs sample is taken in different test tubes, and about 10 mL phosphate buffer solution is added into the same test tubes. It is observed that, the production of positive and negative controls when 0.2 ml human blood is added to 4 ml distilled water and 10 mL phosphate buffer saline (PBS) solution. These mixture solutions are under desiccated for about 30 min at 40°C and add 0.2 mL of acid citrate dextrose blood to each test tube. In addition, these test tubes were incubated for about at 40°C and are centrifuged at 5000 rpm for 8-10 minutes. Mean time optical density (OD) of sample solution, positive and negative control treated samples was calculated at 550 nm from 1 mL supernatant solution. Then calculated the percentage of haemolysis as follows [28].

$$\%\text{ of hemolysis} = \frac{\text{OD of the sample} - \text{OD of the negative control} \times 100}{\text{OD of the positive control} - \text{OD of the negative control}}$$

(OD of the sample = optical density of the sample, OD of the negative control = optical density of the PBS solution, OD of the positive control = optical density of the water)

Characterization

The structural formation of the prepared Ag-Cu ANPs sample was studied through X - ray diffraction using X' Pert Pro X-ray diffractometer with Cu Kα as a source of radiation in a configuration of θ-2θ. Similarly, In order to study the particle morphology with metal confirmation of the sample was analysed by using JEOL JSM-6380 LA Scanning electron microscope with energy dispersive microanalysis of X-Ray (EDAX). Bonding nature of the prepared alloy sample was studied by Perkin–Elmer 1600 spectrophotometer in KBr medium. The absorption behaviour of the sample was carried out by UV visible spectrophotometric measurements using Elico spectrophotometer. Technai-20 Philips transmission electron microscope operated at 190 KeV in order to carry out TEM images.

Further discussion and the results

Visual observation of colour change

Physical confirmation of the synthesized sample Ag-Cu ANPs (the reduction process Ag^{+} and Cu^{2+} to Ag^{0} and Cu^{0} nanoparticles) was done by visual observation of the color change in the solution. Figure 1 shows that, the *Butea Monosperma* plant extract is in green colour where as figure 2(a-b) shows the colourless silver salt solution and blue color for copper salt solution. Mixture of these two solutions shows light blue color solution. In addition, figure 2© shows dark brown color solution when plant extract is mixed with mixture of these metal salt solutions indicates the reduction reaction leading to the formation of Ag-Cu ANPs.

X-ray diffraction

The XRD pattern of the prepared *Butea Monosperma* plant extract and Ag-Cu ANPs sample are shown in Figure 4(a-b). The absence of Bragg's reflections in the XRD pattern of plant extract indicates the amorphous or very partially crystalline nature. Whereas the Ag-Cu ANPs pattern shows presence of the Bragg's reflections as it has the composite's crystalline nature. It is also observed that the d-spacing values of the sample matches well with the standard 87-0720 JCPDS file of Ag, however, some of the d-spacing values match with standard 04-0836 JCPDS file of Cu. Unit cell parameters of the Ag-Cu ANPs samples was observed by the XRD data's of least–square refinement. Likewise, it was observed that the presence of both the Ag and Cu peaks in single indexed pattern indicated the formation of Ag-Cu ANPs and is supported by EDX results. Further, the average crystallite sizes of the particles are calculated using Scherrer equation and are approximately in 100-120 nm range [29].

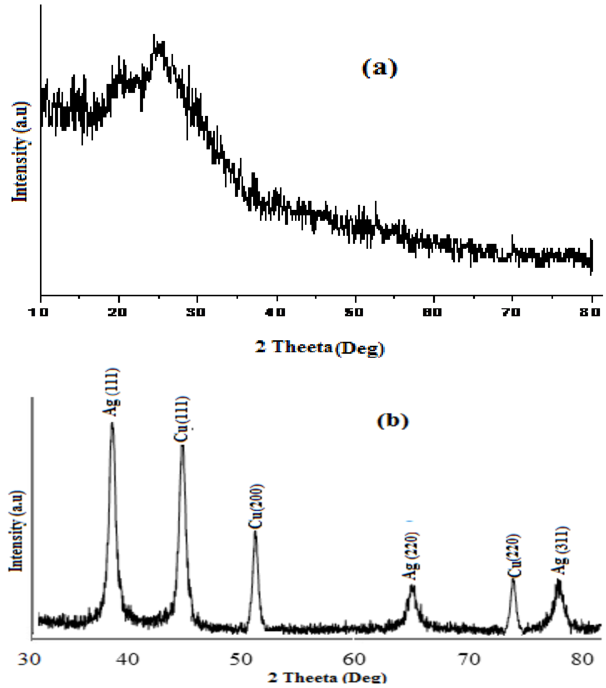

Figure 4: XRD pattern - **(a)** *Butea Monosperma* plant extract **(b)** Ag-Cu ANPs.

Scanning electron microscopy (SEM)

The SEM images of the prepared *Butea monosperma* plant extract and Ag-Cu ANPs sample was shown in Figure 5 (a-b). The extract image (Figure 5(a)) shows that, the fine particles with self- assembling form the poor crystalline nature of the sample. It is also viewed that, the various particles having different shape and sizes in the image. The SEM image of the prepared Ag-Cu ANP's sample is shown below in Figure-5(b), which reveals the crystalline nature with fine spherical particles that have different particle sizes and shape. In addition to this, it is observed the close compact arrangement and also most of the particles being closely assembled. This kind of morphology finds applications in various fields like medicine, as a good adsorbents etc.

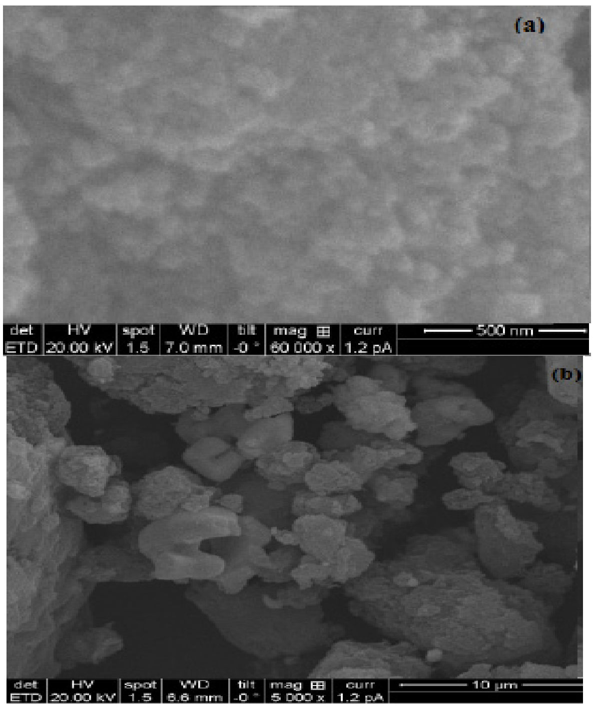

Figure 5: SEM image of (a) *Butea Monosperma* plant extract (b) Ag-Cu ANPs.

The study of infrared

Infrared study is exercised to know the bonding nature of the prepared Ag-Cu ANPs sample and its spectral record is shown in Figure 6. Further, the centrifuged and dispersed Ag-Cu ANPs sample obtained has removed any free residual biomass. FTIR spectrum shows that, the band between 3490-3500 cm^{-1} (corresponds) and O-H stretching H-bonded phenols and alcohols. A stretch for C-H bond was shown by the peak found at around 1500-1550 cm^{-1}, and the bond stretch for N-H was shown by the peak at around 1450-1500 cm^{-1}. Whereas, around 500-550 cm^{-1} the stretch for Ag or Cu NPs were found. Further, the surrounded synthesized nanoparticles were observed by proteins and metabolites like terpenoids with functional groups. Likewise, it is confirmed from the analysis of FTIR spectrum that, the carbonyl groups, the amino acid residues and proteins have the stronger ability to bind the metal. It also emphasise that, the possible formation of the metal nanoparticles by the proteins to prevent agglomeration and thereby stabilizing the medium. Further, it is learnt that possibly the biological molecules could perform the dual functions of forming and stabilizing of the Ag-Cu alloy nanoparticles in the aqueous medium. Similarly, the groups of carbonyl proved the absorption of flavanones or terpenoids on the surface of metal nanoparticles. Moreover, in the absence of other strong ligating agents in sufficient concentration is possibly by the interaction of carbonyl or π-electrons. For the reduction of metal ions, the presence of reducing sugars in the solution and the formation of the corresponding metal nanoparticles could be responsible. In similar way, the terpenoids also play a vital role in reducing the metal ions by oxidizing the groups of aldehydic in the molecules to carboxylic acids [30-34].

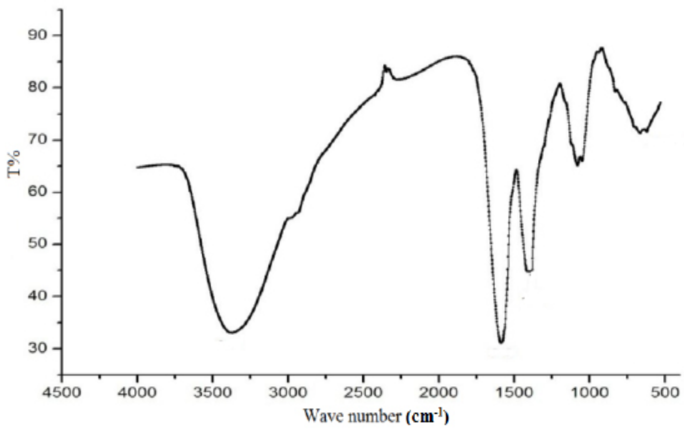

Figure 6: FT-IR spectra of Ag-Cu ANPs.

Analysis of EDX pattern

In order to conformational analysis of the sample, the EDX technique has been used for knowing the presence of silver and copper in the synthesized Ag-Cu ANPs sample. Signals originated for the sample from the grid is used for the complete analysis. The EDX pattern of the synthesized Ag-Cu ANPs sample is shown in figure-7, which shows the presence of both the Ag and the Cu atom signals at respective binding energy. Likewise, such characteristic absorption peaks of Ag and Cu confirm the alloy nanoparticle formation. Impurity peaks are not observed throughout the scanning range of the binding energies and hence, the pure sample.

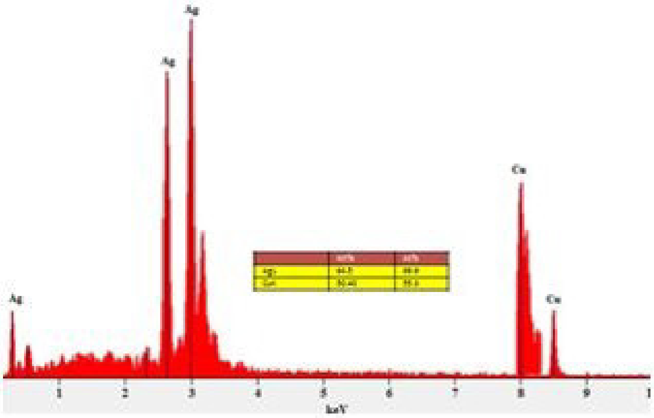

Figure 7: EDX pattern of as synthesized Ag-Cu ANP's.

UV-visible study

It is the most widely used technique for the sample analysis by identifying the possible absorption. For the successful synthesis process, this stands a testimony for robust evidence. The reaction progress between Ag and Cu metal ions and leaf extract was monitored by UV spectral study in aqueous alloy solution. Figure 8 shows the UV spectrum of synthesised Ag-Cu ANP's sample. This figure indicates the presence of single surface plasmon absorption band at 430 nm and is the only maximum absorption band in the spectrum corresponds to Ag-Cu ANPs sample. It also proved the alloy like nature of the bimetallic nanoparticles but not of Ag and Cu metals physically embedded monometallic nanoparticles. The forming of colloidal gel solution that contains both Ag and Au is indicated by the shifting towards red side. Reduction of metal ions and formation of stable alloy nanoparticles occurred rapidly in short span of time.

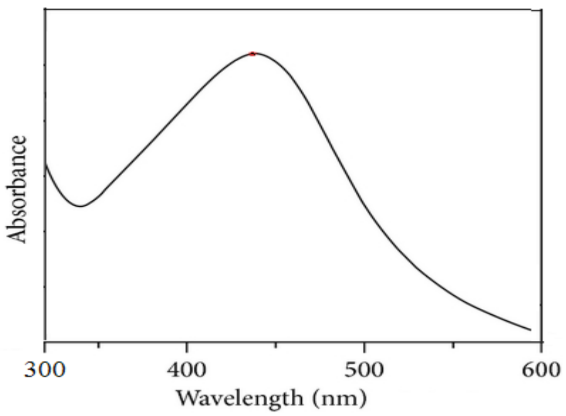

Figure 8: UV Spectrum of Ag-Cu ANPs.

Transmission electron micrograph (TEM)

TEM tool has been used for studying the morphology and particles size of the prepared Ag-Cu ANPs sample and image is represented in figure 9. This image shows the crystalline nature of the sample and most of the particles are in spherical with nano range diameters. However, the image also reviles that, some particles have shown particle agglomeration with compact arrangement. The obtained crystal size of the sample is recorded using this image and is in 100nm range and is very similar with calculated the particle size from XRD data.

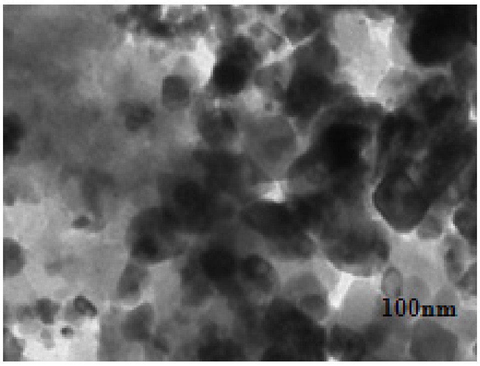

Figure 9: TEM image of Ag-Cu ANPs.

Haemolytic activity

The study of the haemolytic activity of the prepared Ag-Cu ANPs has been tested against normal human erythrocytes and recorded the obtained results in the table-1. The sample used to study the haemolytic activity is stated in percentage haemolysis and conveyed as mean ± standard deviation. The toxicity of Ag-Cu ANPs sample was well studied by haemolysis activity. Also observed that this haemolysis execute when the red blood cells are made to be in close contact with water and also checked before its use the implant material. Then realized the ACD blood haemolysis percentage with Ag-Cu ANPs sample was 3.5%. Likewise, it has been reported in the literature as in all the cases for biomedical materials the permissible limit of haemolysis should be less than 5%. This shows that haemolysis is within the permissible limit and shows less toxicity [35-36].

Table 1: Haemolytic activity of Ag-CuANPs.

Sample	Optical density OD)	Standard Deviation	Haemolysis percentage
Ag-Cu ANPs	0.089	± 0.002	3.5%
- ve sample	0.044	-----	
+ ve sample	1.334	-----	

Conclusions

It is found that using *Butea monosperma* plant leaf extract for biosynthesis of Ag-Cu ANPs is a simple method, efficient and eco-friendly system. Moreover, this method can be of good use in the other metal alloys/ nanocomposite particles synthesis. This is one of the economically viable methods in the nanoparticles synthesis. It is also observed that, the silver and copper ions get reduced simultaneously with leaf extract. The formation of the alloy sample was well confirmed by various characterization tools. This prepared alloy sample shows Haemolytic activity within the permissible limit with less toxicity.

Acknowledgement

Authors convey their heartfelt thanks to DST-FIST (SR/FST/CSI-003/2016) for the grant provided to procure the required instruments and infrastructure at the chemistry Department of Vijayanagar Sri Krishnadevaraya University Ballari. Our thanks are also due to Prof. A. Venkataraman, the Department of chemistry, Gulbarga University, Kalaburagi for his consistent encouragement and support.

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