

# Detection of $\text{Cu}^{2+}$ ion by Synthesis of Bio-mass-Silver Nanoparticle Nanocomposite

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## Abstract

Monitoring the levels of potentially toxic metal ion like  $\text{Cu}^{2+}$  in aquatic ecosystems is important because this ion can have severe effects on human health and the environment. In this work we concentrate on an optical assay of the  $\text{Cu}^{2+}$  ion using a nanocomposite of bio-mass (humic acid)-silver nanoparticle (HA-AgNP). A sensitive colorimetric method has been developed using this composite for detection of  $\text{Cu}^{2+}$  ion based on changes in absorbance resulting from metal ion-induced aggregation of silver nanoparticles. Thereby morphology of the nanoparticles changes from hexagonal or spherical to rod like structure. At the same time stable inner complex formation of the metal ion is evidenced from the appearance of a peak at around 720 nm in UV-Visible spectra. Various concentrations of  $\text{Cu}^{2+}$  ion were used to test the linearity and sensitivity limit of the nanocomposite. The complex formation between  $\text{Cu}^{2+}$  ion and humic acid present in HA-AgNP nanocomposite is further confirmed from the FTIR spectra. Values for  $\text{Cu}^{2+}$ -complexes available in the literature are in conformity with our findings. XRD pattern confirms that Cu exists in the form of ions in the complex. The quenching properties of the fluorescence study of the nanocomposite have been utilized for detection of  $\text{Cu}^{2+}$  ion.

**Index Term:** HA-AgNP nanocomposite • Humic acid • Colorimetric sensor • Detection of  $\text{Cu}^{2+}$  ion • Estimation of  $\text{Cu}^{2+}$  ion • Cu-complex • Calibration curve

## 1. Introduction

Copper is a transition metal which plays significant roles in environmental and biological systems. Copper is widely used in the industrial and agricultural sectors but it is also highly toxic to organisms, such as certain algae, fungi and many bacteria and viruses [1]. The extensive and excessive use of copper in these areas has resulted in serious environment contamination [2]. High concentrations of copper in drinking water are hazardous to human health [3]. Some cases of liver damage of children have been proved to be associated with the excessive intake of copper [4]. Moreover, high concentrations of copper may destroy the biological reprocessing systems in water [5]. Therefore, the fabrication of sensitive and selective methods to monitor the presence of  $\text{Cu}^{2+}$  ions is of great significance.

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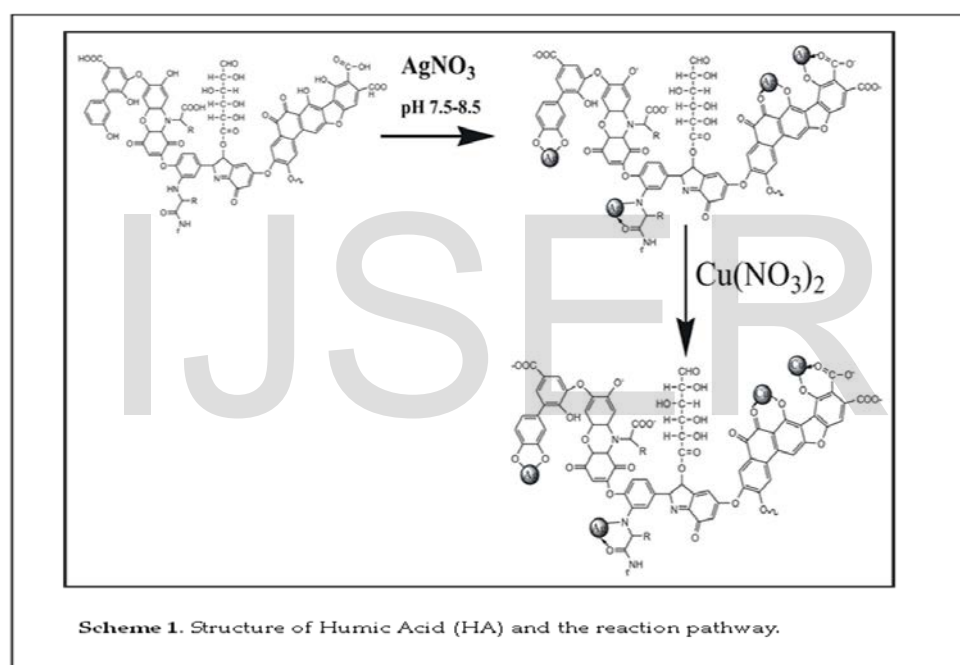
Most of the copper ion detection methods that have been reported so far include methods like organic fluorophore-based assays [6,7], chromogenic sensors [8], quantum dots [9], atomic absorption spectroscopy (AAS) [10], inductively coupled plasma mass spectroscopy (ICP-MS) [11], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [12], electrochemical assays [13] and voltametry [14]. Peptide and oligopeptide modified electrodes have been widely developed in recent years for the detection of metal ions in environmental samples due to their high affinities to different metal ions [15, 16]. Self-assembled monolayers of L-cysteine on gold electrode have been reported for low concentration detection of metal ions [17]. Although the sensitivities of these methods for  $\text{Cu}^{2+}$  detection are generally high, most of them need expensive, and specialized instruments which limits their usefulness and make them unsuitable for field work.

Color changes associated with the aggregation of metal nanoparticles have led to the development of a number of assays for a variety of target species [18-23]. Gold nanoparticles (GNPs) seem to be becoming increasingly attractive in the fabrication of optical sensors and devices as well as in bio-

diagnostics and medical therapeutics due to their excellent chemical and physical properties [24,25]. They have strong surface plasmon resonance (SPR) absorption in the visible wavelength range with extremely high extinction coefficients. One attractive property of GNPs is that their SPR frequency changes dramatically with the distance between the GNPs. Therefore, the analytic-induced aggregation of GNPs offers a facile colorimetric approach for the simple visual detection of nucleic acids [26,27], proteins [28], small molecules [29,30], and metal ions [31,32]. Inspired by these findings, we attempted a colorimetric method using bio-mass (humic acid)-silver nanoparticle (HA-AgNP) nanocomposite based colorimetric assay for

detection of  $\text{Cu}^{2+}$  ion, the result of which are reported here.

Silver nanoparticles have been produced by using an organic macromolecule, humic acid (HA). Humic acid results from the microbiological decomposition of animals and plants and is capable of interacting with metallic cations. It contains several functional groups like phenolic -OH, quinines, hydroxyls, methoxyls, aldehydes, ketones and enolic -OH which could be responsible for the reduction of silver ions. The HA from different sources is found to be different. The probable structure [33,34] of HA and the most likely reaction pathway for interaction with  $\text{Cu}^{2+}$  ion are shown in Scheme 1.



## 2. Materials and Methods

### 2.1. Chemicals

Chemicals used in the experiment were mostly analytical reagent (A.R) grade or of the highest purity available. Silver nitrate ( $\text{AgNO}_3$ ) with purity greater than 99.0 % from sigma Aldrich Pvt. Ltd. was used as received. Humic acid was purchased from Himedia Lab Pvt. Ltd. The used metal salt  $\text{Cu}(\text{NO}_3)_2$  was purchased from Beijing Chemical Reagent Company (Beijing, China).  $\text{NaOH}$  (>97% purity) was purchased from Merck Specialties Pvt. Ltd. Double distilled water was used in all experiments.

### 2.2. Synthesis of Silver nanoparticles (HA-AgNP) Nanocomposite

Typically, 2.5 mL of 0.003(M)  $\text{AgNO}_3$  solution was added to 10 ml of  $100 \text{ mgL}^{-1}$  humic acid solutions and then 0.01(M)  $\text{NaOH}$  solution was added drop wise to maintain the pH of the reaction mixture from 7.5 to 8.5. The mixture was cooled in ice cold water and then heated to  $85^\circ\text{C}$  for times ranging from 20 to 40 minutes depending on the concentration of HA. The brown color of the solution gradually changes to yellow, indicating the reduction of  $\text{Ag}^+$  to  $\text{Ag}(0)$ . The UV-VIS spectroscopic study further confirmed the formation of HA-AgNP

nanocomposite. The same procedure was carried out by varying the concentrations of both  $\text{AgNO}_3$  solution and HA. The humic acid concentration was varied from  $1\text{-}200\text{mgL}^{-1}$  and  $\text{AgNO}_3$  concentration was varied from 0.001 to 0.003 (M).

### 2.3. Detection of $\text{Cu}^{2+}$ by sensing the colorimetric property of HA-AgNP nanocomposite

The colorimetric detection of aqueous  $\text{Cu}^{2+}$  ion was studied using 50 nM concentration of nanocomposite at room temperature. In order to demonstrate the effect of  $\text{Cu}^{2+}$  ions on the HA-AgNP nanocomposite, 500  $\mu\text{L}$  portions of several concentrations of  $\text{Cu}^{2+}$  ions (0-160  $\mu\text{M}$ ) were added individually to 3 mL of HA-AgNP nanocomposite solution and the resulting mixture was then allowed to stand for 10 minutes at room temperature, during which the yellow color changed to a bluish here. The intensity of this bluish colour gradually increased with increase of  $\text{Cu}^{2+}$  ions concentration. Subsequently, the UV-VIS spectroscopic study confirmed the presence of  $\text{Cu}^{2+}$  ions and absorbance changes at 720 nm were monitored for complex formation.

### 2.4. Characterization

The absorbance spectra were analyzed by using a 'SHIMADZU' UV 1800 spectrophotometer and TEM images were taken using JEOL-JEM 2100 high resolution transmission electron microscope (HR-TEM). Samples for the TEM studies were prepared by placing a drop of the aqueous suspension of particles on a carbon-coated copper grid followed by solvent evaporation under vacuum. The crystalline nature of the HA-AgNP and copper complex of HA-AgNP nanocomposite was examined using X' Pert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with  $\text{Cu K}\alpha$  radiation. The FTIR measurements of the HA-AgNP and copper complex of HA-AgNP nanocomposite were studied by using PerkinElmer Spectrum Version 02 spectrometer. Fluorescence spectra of all samples were obtained using a Horiba Jobin Yvon spectrofluorimeter (Fluoromax 4).

## 3. Results and discussion

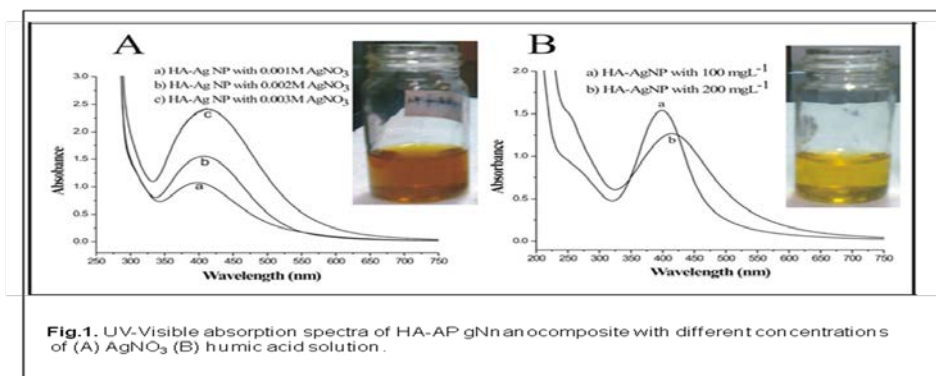
### 3.1. UV-Visible spectral studies

Bio-synthesis of nanoparticles using microbiological decomposition of animals and

plants is an important area of research. The development of biological processes for the synthesis of nanoparticles is evolving as an important branch of nanotechnology. The present study deals with the synthesis of biomass (humic acid)-silver nanoparticle nanocomposite. Humic Acid can act as capping agent due to its chelating potential towards metal and metallic ions [35]. The optical properties of nanoparticles are mainly dominated by Surface Plasmon resonance (SPR) or localised Surface Plasmon resonance (LSPR) i.e. the collective oscillation of electrons at their surface in resonance with the incident electromagnetic radiation. The SPR absorption wavelengths are dependent on their size, shape, and refractive index. So any changes in their surface structure, aggregation, or medium's refractive index may lead to colorimetric changes of the sol.

The HA-AgNP nanocomposite produced exhibits deep yellow color in water (Fig. 1A and B). At  $100\text{ mgL}^{-1}$  concentration of humic acid, a smooth and narrow absorption band for HA-AgNP nanocomposite is observed at 397 nm. The Plasmon band shifts to higher values with the increase of the concentration of humic acid ( $200\text{mgL}^{-1}$ ). This shift of the plasmon band to higher wavelengths with higher concentration may be explained by supposing a more dense coating of hydrophobic humic substance on the composite, which affects the value of the dielectric constant of the medium surrounding the nanoparticles [36]. It is well studied that the extinction spectrum of noble metals changes shape by the influence of the dielectric constant of the surrounding material and the type of the metal [37]. It is expected that humic acid capped silver nanoparticles have much better colloidal stability at higher concentrations (Fig. 1B) and hence, the concentration of humic acid used in this work was  $200\text{ mgL}^{-1}$ .

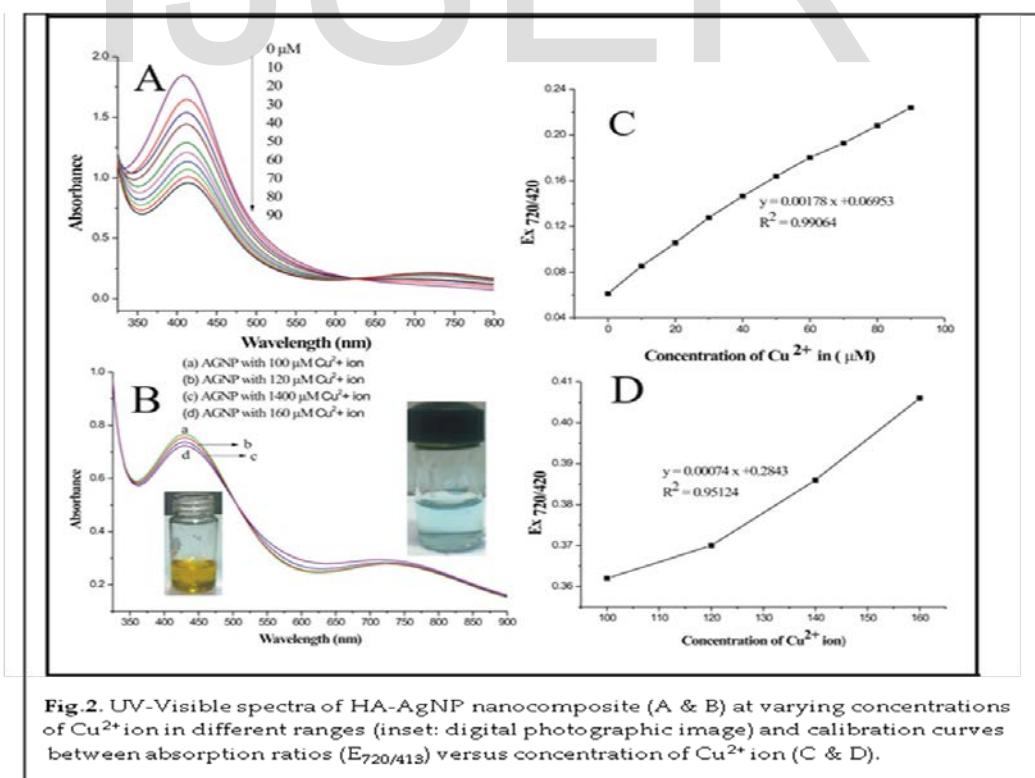
The extinction spectra of HA-AgNP nanocomposite synthesized from different concentrations of  $\text{AgNO}_3$  solution are shown in Fig. 1A. With the increase of the concentration of  $\text{AgNO}_3$  in the nanocomposite, silver plasmon band shifted to higher wavelength (from 398 nm to 413 nm) and the spectrum was broad at higher concentrations. The broadening of the peak may be assigned to the changes in morphology as well as the developing of polydispersed nature of the sol.



**Fig.1.** UV-Visible absorption spectra of HA-AP gNn nanocomposite with different concentrations of (A)  $\text{AgNO}_3$  (B) humic acid solution.

In this work, the detection of  $\text{Cu}^{2+}$  ion in aqueous solution has been carried out by chelating the metal ion and observing the color change. Several concentrations of  $\text{Cu}(\text{NO}_3)_2$  solution, 0 to 160  $\mu\text{M}$  were added to HA-AgNP nanocomposite and corresponding changes of the absorption coefficients were noted. When  $\text{Cu}(\text{NO}_3)_2$  solution was added to the composite, a new peak at around 720 nm was found in addition to the peak at 413 nm. More interestingly, with the increase of concentration of  $\text{Cu}^{2+}$  ion solution the absorption coefficient of the 413 nm peak decreased whereas the absorption coefficient of the 720 nm peak increased gradually (Fig. 2A & 2B). The SPR of HA-AgNP nanocomposite at 413 nm and 720 nm

are related to the quantities of dispersed and aggregated versions of HA-AgNP in the composite respectively. Hence we used the ratio of the absorbance values of 720/413 ( $E_{720/413}$ ) to express the molar ratio of aggregated and dispersed phase in the nanocomposite. The humic acid was used as a reducing as well as a capping agent in the synthesis of HA-AgNP nanocomposite and further as a receptor for the sensing of  $\text{Cu}^{2+}$  ion by forming a stable inner complex with the metal ion. Morphology changes or stable inner complex formation of the metal ion is evidenced from the color change (detectable by human eye) and appearance of a peak at around 720 nm [32, 38].



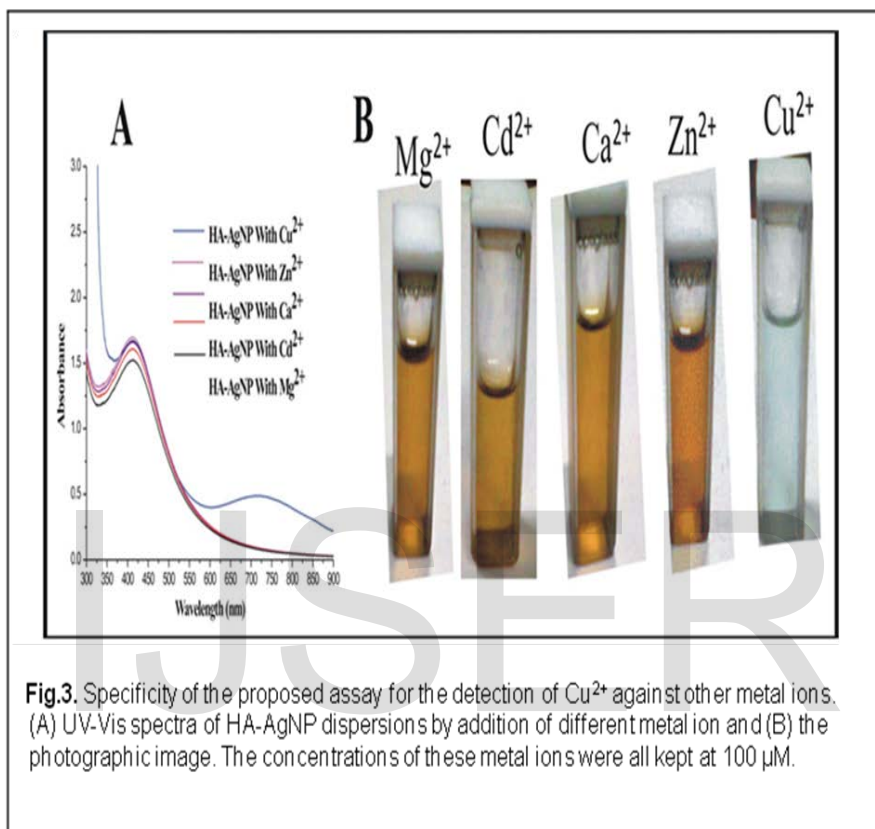
**Fig. 2.** UV-Visible spectra of HA-AgNP nanocomposite (A & B) at varying concentrations of  $\text{Cu}^{2+}$  ion in different ranges (inset: digital photographic image) and calibration curves between absorbance ratios ( $E_{720/413}$ ) versus concentration of  $\text{Cu}^{2+}$  ion (C & D).

The changes of the UV-Visible absorbance ratios ( $E_{720/413}$ ) was found to be linear with the  $\text{Cu}^{2+}$  ion

concentration within the concentration range 0 to 90  $\mu\text{M}$  with the regression equation

$y=0.000178x+0.0693$  ( $R^2=0.99064$ ). A slightly distorted linear curve was found within the concentration range 100 to 160  $\mu\text{M}$  with the regression equation  $y=0.00074x+0.2843$  ( $R^2=0.95124$ ). Both results are shown in Figure 2C & 2D. These findings encourage us to suggest that this method can be used for detecting  $\text{Cu}^{2+}$  ion in

solution with a minimum detectable limit of 10  $\mu\text{M}$ . Same experiment using other metal ions like  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Mg}^{2+}$  in place of  $\text{Cu}^{2+}$  ion could not produce any significant color change and obviously no change in UV-Visible absorption (Fig.3) These findings tested the specificity of this assay for detection of  $\text{Cu}^{2+}$  ion.



**Fig.3.** Specificity of the proposed assay for the detection of  $\text{Cu}^{2+}$  against other metal ions. (A) UV-Vis spectra of HA-AgNP dispersions by addition of different metal ion and (B) the photographic image. The concentrations of these metal ions were all kept at 100  $\mu\text{M}$ .

### 3.2. HR-TEM studies

Figure 4 shows the TEM images of HA-AgNP nanocomposite produced from 200  $\text{mgL}^{-1}$  humic acid and 0.003M  $\text{AgNO}_3$ . The particles were mostly spherical and their sizes varied from 20 to 50 nm, along with a hexagonal nanosheets structure. Being a good capping agent humic

acid induces rapid nucleation but it cannot restrict the growth of silver nanoparticles at higher concentration of humic acid. Hence polydispersed behaviour was observed in the TEM images. Selected area diffraction (SAED) pattern (Fig.4D) illustrates the crystalline nature of the nanocomposite.



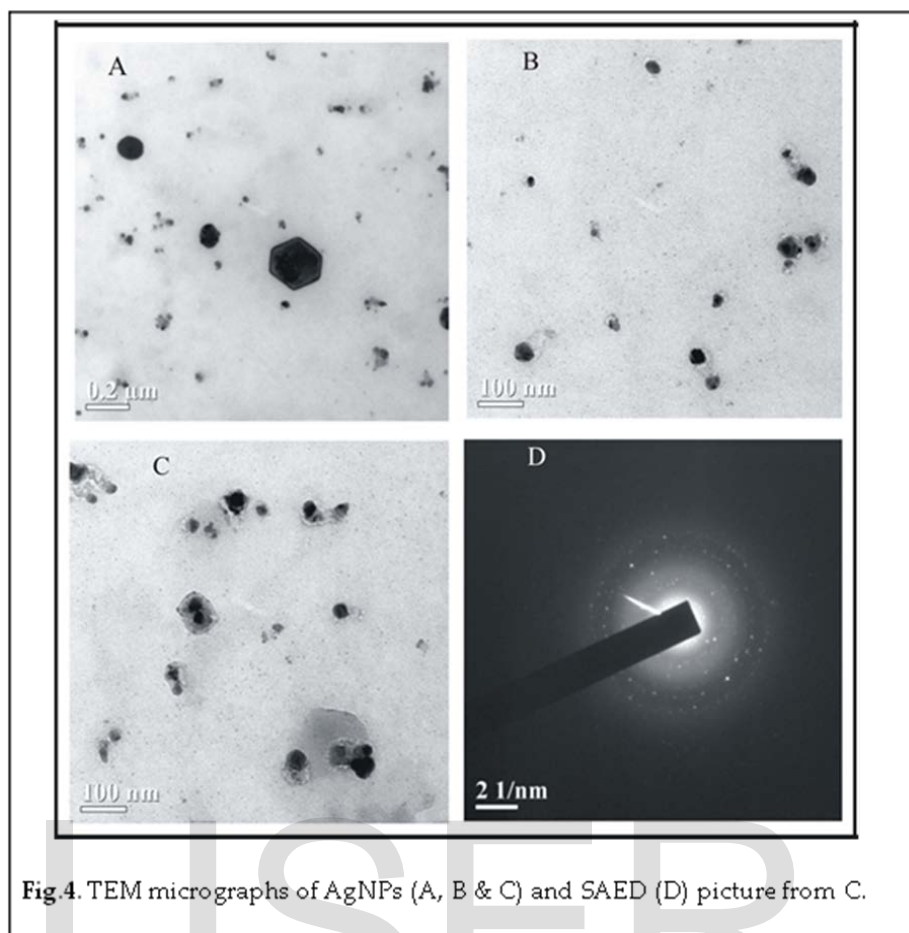


Fig.4. TEM micrographs of AgNPs (A, B & C) and SAED (D) picture from C.

Figure 5 shows the TEM images of Cu-complex of HA-AgNP nanocomposite. Due to the presence of both oxygen rich functional groups (such as  $-\text{COOH}$ ,  $>\text{C}=\text{O}$ ,  $-\text{OH}$ ), and hydrophobic aliphatic and aromatic groups in humic acid, it is capable to act as a chelating ligand [39,40]. The strong binding of the  $\text{Cu}^{2+}$  metal ion with chelating ligand facilitates the aggregation of silver nanoparticles. The morphology of the nanocomposite thereby changes enormously which is shown in TEM micrograph (Fig.5). The explanation of strong binding of  $\text{Cu}^{2+}$  ion with the ligand is evidenced from FTIR data. Due to the complexation of the composite with  $\text{Cu}^{2+}$  ion, the shape of the

particles completely changes to rod like structures instead of the hexagonal or spherical ones found in the nanocomposite (Fig.4). As explanation, it may be pointed out that the removal of humic acid from the composite due to the complex formation makes the surface of the composite charged. This charged surface facilitates the formation of aggregation in linear fashion but not in random fashion, to generate rod like structure (Fig. 5). The Cu-complex formed are found to be quite large in sizes (Fig.5) though it was not in a precipitated state but was well dispersed in the solution phase Fig. 2 B (inset).

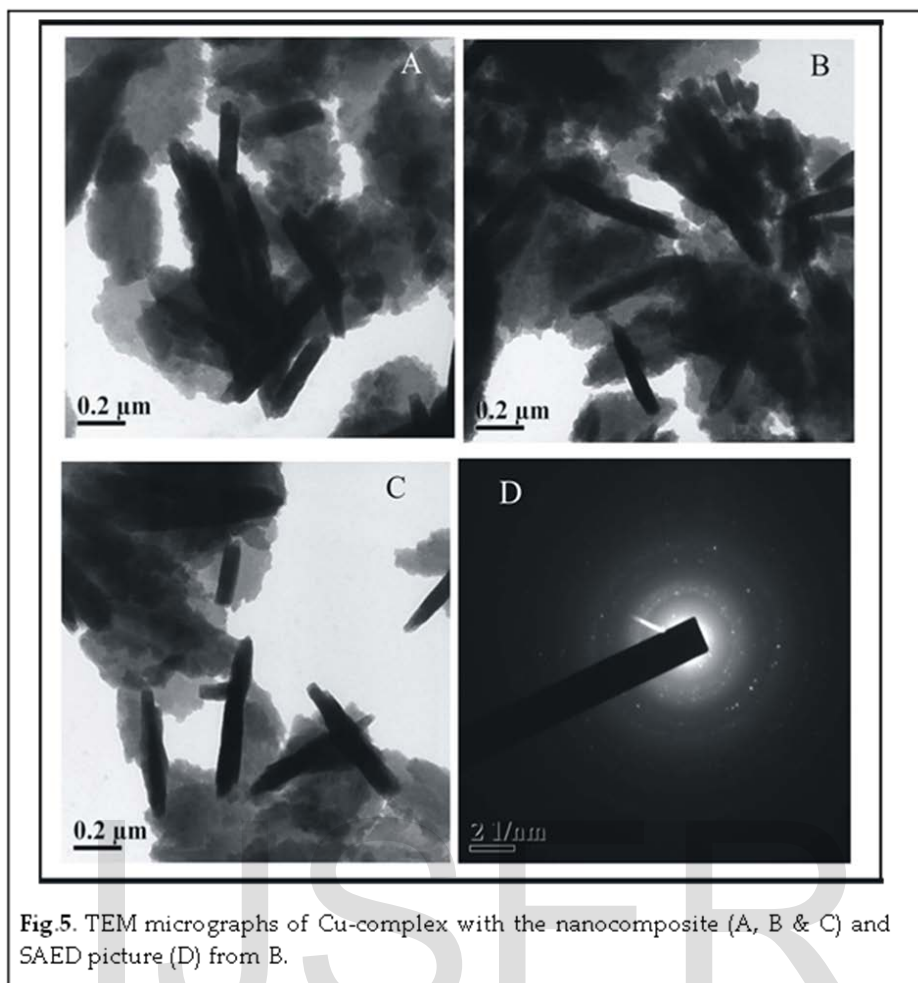


Fig.5. TEM micrographs of Cu-complex with the nanocomposite (A, B & C) and SAED picture (D) from B.

### 3.3. FTIR spectral studies

FTIR analysis was performed to identify the bio-molecules localized on the surface which are responsible for the reduction of silver ion and for Cu-complex formation. Representative FT-IR spectra of humic acid powder, synthesized HA-AgNP nanocomposite and Cu-complex of HA-AgNP nanocomposite are shown in Figure 5. Bands attributable to  $\nu_{\text{asym}}(\text{COO}^-)$  and  $\nu_{\text{sym}}(\text{COO}^-)$  modes of carboxylate groups present in humic acid appeared at 1585 and 1383  $\text{cm}^{-1}$  with comparable intensities (Fig. 6A). When HA-AgNP nanocomposite was prepared in presence

of humic acid, the respective peaks shift to 1618 and 1385  $\text{cm}^{-1}$  due to reduction of silver ion, the former being less intense than the latter (Fig. 6B). Finally when  $\text{Cu}^{2+}$  ion was added to the above composite, the corresponding  $\nu_{\text{sym}}(\text{COO}^-)$  modes of carboxylate group further shifted to the 1632  $\text{cm}^{-1}$  (Fig. 6C). The dramatic change of frequencies of carboxylate band can only be explained if it is considered that the stable inner complex formation occurred between  $\text{Cu}^{2+}$  ion and humic acid present in HA-AgNP nanocomposite[41-44].

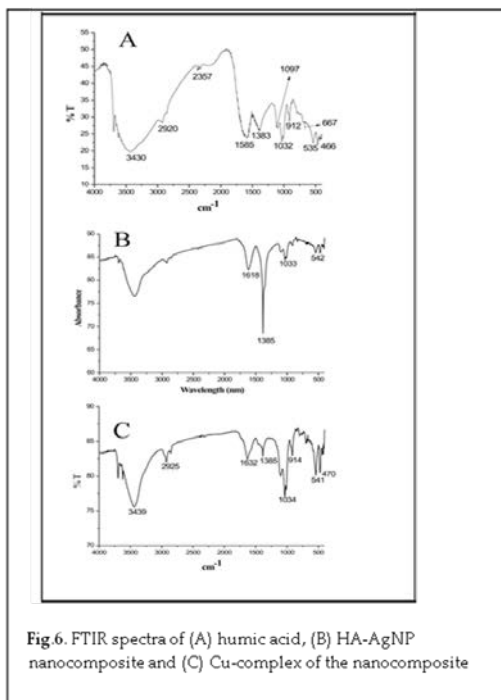


Fig.6. FTIR spectra of (A) humic acid, (B) HA-AgNP nanocomposite and (C) Cu-complex of the nanocomposite

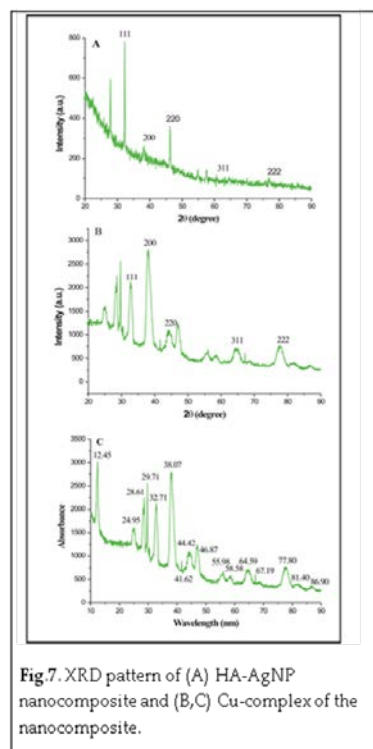


Fig.7. XRD pattern of (A) HA-AgNP nanocomposite and (B,C) Cu-complex of the nanocomposite.

### 3.4. XRD studies

The XRD analysis was performed to confirm the crystalline nature of the biologically synthesized HA-AgNP. Various Bragg's diffraction patterns of the synthesized HA-AgNP nanocomposite and copper complex of the composite are shown in Figure 7. HA-AgNP nanocomposite shows sharp peaks at 32.32, 38.20°, 44.42°, 64.64° and 77.78° corresponding to (111), (200), (220), (311) and (222) planes, respectively (Fig. 7A) which indicated that HA-AgNP nanocomposite has fcc structure and essentially crystalline in nature. XRD of the copper complex of the composite shows peaks at 12.45°, 24.95°, 28.61°, 29.71°, 41.61°, 46.87°, 55.58°, 58.58°, 67.19°, 81.40°, and 86.90° in addition to HA-AgNP nanocomposite peaks at 32.71°, 38.07°,

44.42°, 64.59°, 77.80°. Values for Cu<sup>2+</sup>-complexes available in the literature are in conformity with our findings [45,46]. This observation confirmed that Cu existed in the form of ions in the complex of the composite (Fig. 7B & C).

### 3.5. Fluorescence studies

A turn on approach of fluorescent sensor has been developed for sensing Cu<sup>2+</sup> ions in aqueous solution, based on Cu<sup>2+</sup> ions displacing humic acid unit on the surfaces of HA-AgNP nanocomposite [43,47]. Humic acid molecules shows fluorescence in bulk solution, but its intensity diminishes by more than four times when adsorbed onto nanocomposite surfaces (Fig. 8).

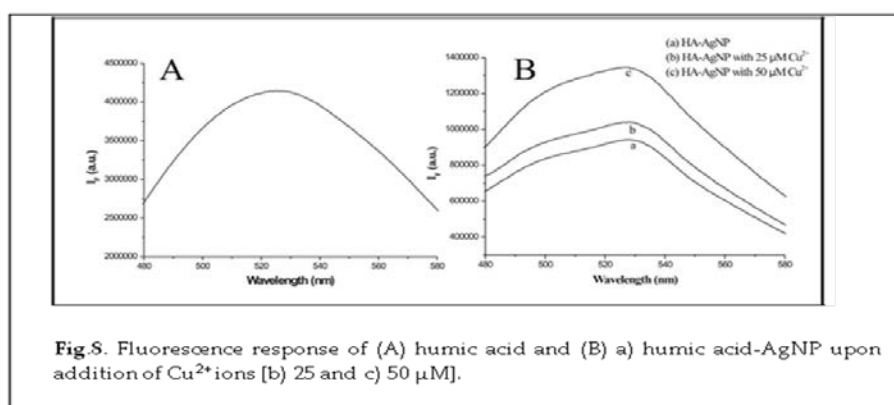
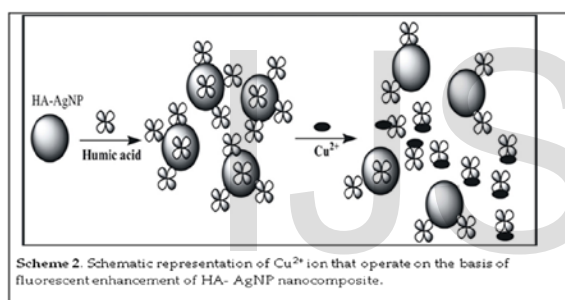


Fig.8. Fluorescence response of (A) humic acid and (B) a) humic acid-AgNP upon addition of Cu<sup>2+</sup> ions [b] 25 and c) 50 μM].



In the presence of  $\text{Cu}^{2+}$  ions, due to the strong binding between metal ions and humic acid molecules, the adsorbed HA molecules on the nanocomposite surface are released from it (Scheme 2) and thereby the restoration of the fluorescence is observed from the enhancement of the intensity of the 528 nm peak. From the structure of humic acid it is expected that it can make a complex with  $\text{Cu}^{2+}$  ion through the functional groups like  $-\text{COOH}$  and  $-\text{OH}$ . In an attempt to detect and estimate  $\text{Cu}^{2+}$  ions present in water, various solutions having concentrations between 0 to 50  $\mu\text{M}$  were added in the composite and the corresponding changes in the fluorescence spectra were observed. It is noted that this probe is quite capable of detecting  $\text{Cu}^{2+}$  ions present at concentration as low as 25  $\mu\text{M}$  upon excitation at 475 nm. With the increase of  $\text{Cu}^{2+}$  ion concentration (50  $\mu\text{M}$ ) an enhancement in the intensity was observed. It is also to be noted that this probe is unable to detect metal ion concentration lower than 25  $\mu\text{M}$ .



#### 4. Conclusion

We report on an optical assay for  $\text{Cu}^{2+}$  ion using HA-AgNP nanocomposite. A sensitive colorimetric method has been developed using HA-AgNP nanocomposite for detection of  $\text{Cu}^{2+}$  ion. The changes resulted might be due to the metal ion-induced aggregation in the nanocomposite or direct deposition of metal ion ( $\text{Cu}^{2+}$ ) onto the nanocomposite through inner copper complex formation. The change in frequencies of carboxylate band in FT-IR spectra confirmed the stable inner complex formation between  $\text{Cu}^{2+}$  ion and humic acid present in HA-AgNP nanocomposite. The strong binding of the  $\text{Cu}^{2+}$  metal ion with chelating ligand facilitates the aggregation of silver nanoparticles and thereby morphology of the nanoparticles changes from hexagonal or spherical to rod like structure. The observation from XRD pattern also confirmed that Cu existed in the form of ions in the complex. In the fluorescence study the

quenching properties of the nanocomposite have been employed to study the turn on fluorescence approaches for detection of  $\text{Cu}^{2+}$  ion.

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#### Conflict of Interest

The authors declare that there is no conflict of interest.

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