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INTERACTION OF PEPTIDE GROUPS OF PLASMIN WITH GOLD NANOPARTICLES

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ABSTRACT

Background: Bioconjugation of nanoparticles to proteins is challenging approach as various amino acid groups of protein are involved in nucleation process for growth and establishing electrostatic interactions and stabilizing the nanoparticles. Use of Plasmin in current experiment has been thought to serve dual purpose, it acts as a reducing agent and also stabilizes gold nanoparticles. The nanoparticles formed are characterized by using TEM, UV-Vis spectroscopy, XRD and FTIR. Results: Our results of transmisssion electron microscopy (TEM) shows gold nanoparticles synthesized have average size ~20-50nm showing spherical symmetry, also the particles are all aggregated. The synthesis of particles from $HAucl_4$ to Au-plasmin conjugates detected by change in absorption spectra. The spectral signatures showed surface plasmon resosnance band at λmax 545nm indicating the formation of 10nm, 20nm and 50nm gold nanoparticles. The diffraction patteren at 38.3 corresponding to (111) planes of fcc gold phase also indicated formation of Au-plasmin conjugated gold nanoparticles. In addition FTIR spectroscopy results showed signals at 1032 cm^{-1}, 1111 cm^{-1}, 1559.84 cm^{-1}, 2924.65 cm^{-1} indicated different groups such as COO-, C-H involved in forming electrostatic interaction and stabilization of nanoparticles. Conclusion: The application of plasmin in current study indicates that it serves as efficient means for synthesizing the nanogold. The gold nanoparticles thus formed by this method is safe, biofriendly at the sametime showed uniform spherical shape.

KEYWORD: Gold nanoparticles, Plasmin, spherical, stabilization, biofriendly.

INTRODUCTION

Gold nanoparticles synthesis and its interaction with various biological molecules such as DNA, proteins to form new materials for potential applications in the area of electronics, optics as well as in genomics, proteomics has become an area of special interest in field of nanoscience. [1, 2 & 3] Moreover, conjugation of nanoparticles to proteins were it is either hydrophilic or hydrophobic, with either positive or negative charge has been widely studied to understand the crucial role of these groups in synthesis and stabilization of nanoparticles. [1] Large number of optical methods such as surface plasmon resonance analysis, scattering based sensing which are based on nanoparticles are developed towards these objectives. These particles thus can be known with different size and shapes which can then be used for perfect contrast agents for determination of biomarkers or it can be used to address related biological questions. [4]

Nevertheless, the tendency of nanocrystallites to nucleate and aggregate provides a challenge to the experimental investigations of its size dependence of the structural and electronic properties of NPs. [5, 6, 7 & 8] The strategy to use capping agent is also matter of great concern as capping molecules usually contain functional groups such as amine, alcohol, thiol, which provides a wide range of interaction strength. [5, 9] Above all citrate stabilized particles is also known for its first simple method known to form gold hydrosol.

Therefore, present attempt is to demonstrate a simple concept to achieve control on gold nanoparticle size and shape and to understand the interaction of amino acid derivatives of plasmin as reducing groups in gold nanoparticle synthesis.

MATERIALS AND METHODS

$HAuCl_4$ and Plasmin was purchased from Sigma Aldrich Fine Chemicals. Plasmin was used for gold nanoparticle synthesis. All aqueous solutions were made using ultrahigh purity water purified using a Milli-Q Plus system (Millipore Co.).

Preparation of gold nanoparticles: All glassware's used was cleaned in a bath of freshly prepared aqua regia solution (HCL: HNO_3, 3:1), and rinsed thoroughly with Milli Q H_2O prior to use. An aqueous solution of $HAuCl_4$ (50 ml, 2.5 $x10^{-4}$ M) was used with plasmin in 5 ml (v/v) of $HAuCl_4$ and the mixture was incubated at 37°C with magnetic stirring until a

colored solution was obtained. The duration of the reaction between plasmin and $HAuCl_4$ was continued for 1, 2, 3 and 4 h. The solution however turned red in color as time progressed. Sample characterization was done by using Shimadzu UV-Vis spectrophotometer. TEM images were used to characterize the size and shape of the particles. Samples were prepared by placing a drop of solution containing nanoparticles on a carbon-coated Cu grid. TEM images were examined using JEOL 4000 EX instrument operated at 200 Kv.

For Fourier transform infrared (FTIR) spectroscopy measurements, dry powders of the nanoparticles were obtained. These measurements were carried out on a Perkin Elmer Spectrum- One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. X-ray diffraction (XRD) pattern was recorded on a powder sample using a Pananalytical with $Cu_{k\alpha}$ radiation (λ =1.541874°A) utilized for characterization of synthesized gold nanoparticles.

RESULTS AND DISCUSSION

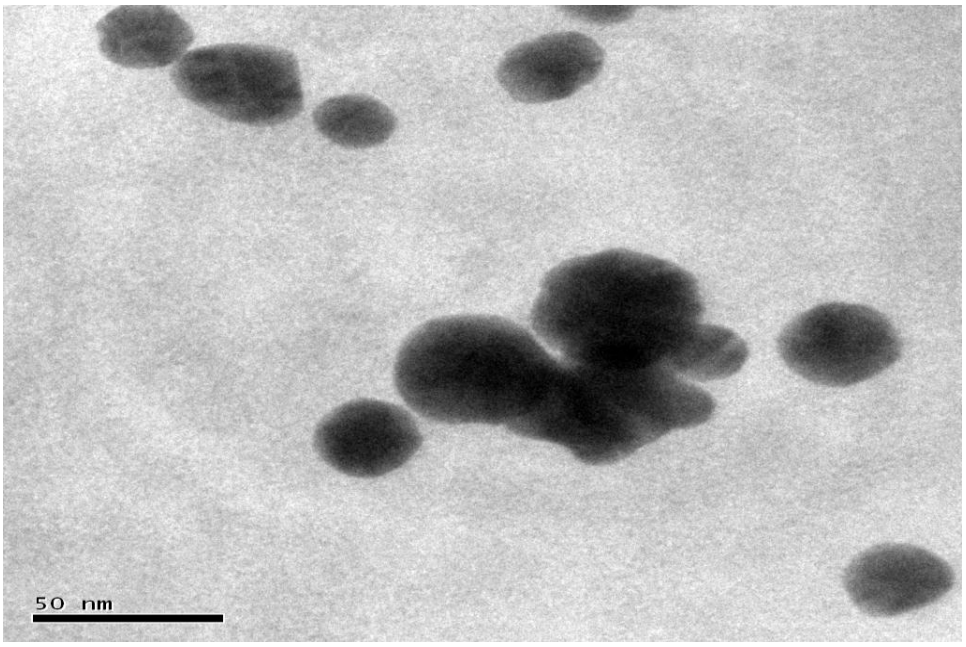

Figure 1: Transmission electron Microscopic studies shows plasmin synthesized gold nanoparticles. The nanoparticles are all ~50nm in diameter and shows aggregated form.

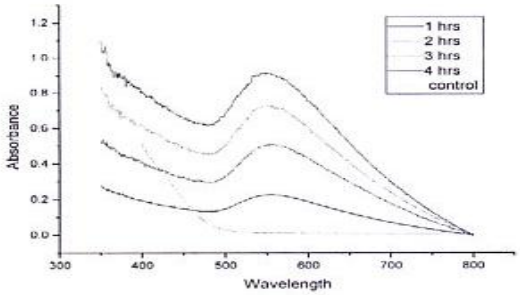

Figure 2. UV-Vis spectra recorded during reduction of $HAucl_4$.The surface plasmon band after gold nanoparticle synthesis .The inset provides progress in surface plasmon peaks after 1,2,3 & 4 hrs. The control here is $HAucl_4$ absortion spectra which shows no SPR band.

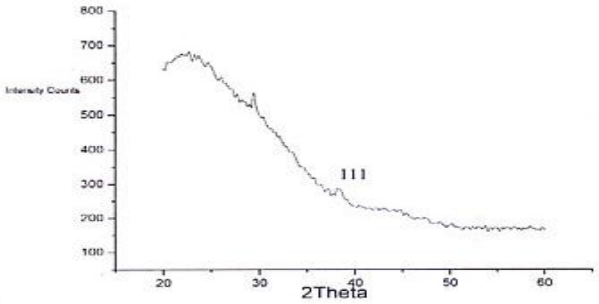

Figure 3. XRD pattern recorded for gold nanoparticles.

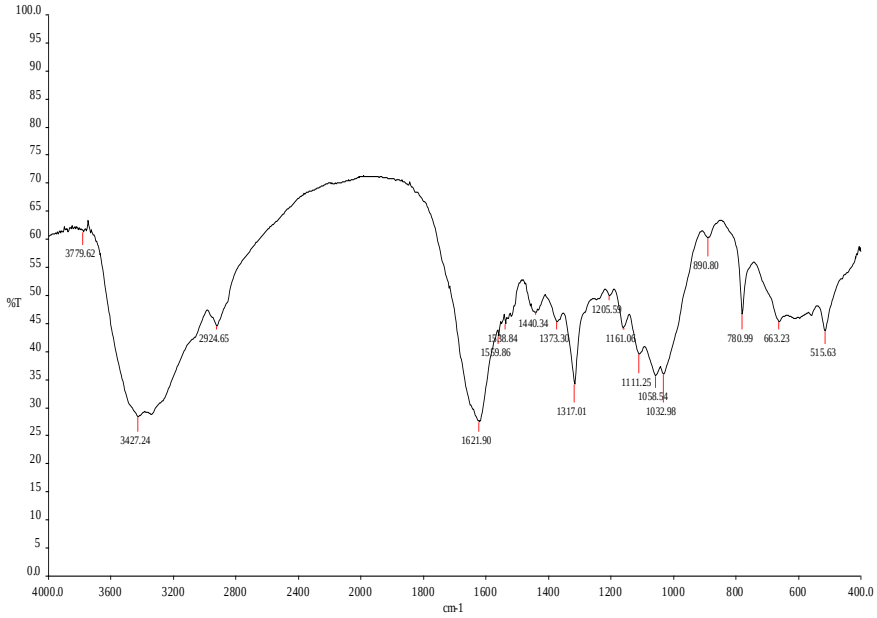

Figure.4: FTIR spectra of Au-plasmin showing clear signals. The amide I & II signatures are seen in spectra. The strecthing of COO- groups and signals for hydroxyl group is also noticed after Au-particle synthesis.

Previous studies demonstrates the rapid method for synthesis of Au, Ag, and Au-core-Ag shell nanoparticles using neem leaf broth. [11] The synthesis of gold nanoparticles involves the preparation process of small gold seeds which is carried out by adding aqueous solution of $HAuCl_4$ (50 ml, 2.5 $x10^{-4}$ M) with plasmin followed by stirring. The solution showed red color as time progressed from 1h until 4 h. In this growth solution, the compound serve as mild reducing agent while gold seeds acted as an nucleation centre which further served as centre for growth of large gold nanoparticles.

Analysis of the images (Fig 1) from transmission electron microscopy (TEM) showed that the particles have a mean diameter of 50nm. [10] In figure 1gold nanoparticles showed regular geometry with spherical shape, however few of them appeared to have facets. The nanopaticles were 50nm size with spherical shapes but found to be aggregated.

In previous reports Valmalette, et.al (1996) has revealed that chitosin brings about change in absorption spectra after nanoparticle formation. The formation of gold nanoparticle in presence of plasmin with spherical shape has also been revealed by its red color with a characteristic surface plasmon centered at λmax 545nm which is seen in figure 2. There is clear trend in spectral shift noticed in increase in size of nanoparticles. This change depicts further the nanogold size is in range of 10nm to 20nm, 50nm. This data of UV-Vis spectrum is in good agreement with TEM images which also shows nanogold synthesized are from 50nm in size, with spherical shape. Moreover, it can be probably said that plasmin causes change in dielectric constant of surrounding medium, which slows the dipole oscillations induced in the conduction electrons of gold nanoparticles. Thus results in broad peak which is visible at 4h time interval.

The size of particles was further characterized by using powder X-ray diffraction (XRD) shown in figure 3. The diffraction pattern appears at about 38.3 found to correspond to (111) planes of fcc gold phase, respectively indicating formation of Au-nanoparticles respectively.

In one of experiment by Warad.et.al., have revealed chitosin capped nanoparticles of ZnS:Mn^{2+} and have proved that chitosin indeed attaches to the surface of the nanoparticles.

FTIR spectra was analysed so as to gain insight of some specific groups involved in interaction with Au-nanoparticles. It is quite provoking to note that there is clear signal seen at 1032 cm^{-1} . This shift and broadening indicates this particular group is involved in Au-

nanoparticle formation. It also suggest the adsorption of groups of protein on nanoparticle whereas, signal at 1111 cm^{-1} reveals C=O stretching which occurs during plasmin/Au-np bioconjugate formation. This group might be involved in stabilization and encapsulation.

FTIR spectra at 1559.86, 1538.84 and 1621.9 cm^{-1} signal for amide I and II groups. This group might be involved in forming electrostatic interaction with gold nanoparticles. The symmetric stretching at Coo^{-} , but antisymmetric stretching of Coo^{-} is not observed after signal seen at 1440.34 cm^{-1}. Detailed examination reveals that this band might be buried in broad band at 1621.9 cm^{-1}. The role of C-H group which have been noticed after signal at 2924.65 cm^{-1} . Moreover, FTIR signal at 3427.24 cm^{-1} show hydroxyl group.

All these observations hint us that there is electrostatic interactions of gold nanoparticles and moreover C=O group might also be involved in stabilization and growth retardation.

CONCLUSION

In this work we have introduced a biofriendly method to synthesize nanogold. It has been shown that monodispersed nanoparticles can be prepared by using plasmin as it could serve as a reducing as well as stabilizing agent. The results also suggest further that certain amino acid groups establish electrostatic interactions with Au-nanoparticles and stabilization and encapsulation formed by C=O, COO-, C-H groups. The nanogold prepared is also retricted sized ~20-50 nm in diameter as it can be applicable for various purpose.

LIST OF ABBREVIATIONS

TEM : Transmission Electron Microscope

XRD : X-ray diffraction

FTIR : Fourier transform infrared

$HAucl_4$: Chloroauric Acid

COO- : Carboxylic acid

DNA : Deoxyribonucleic acid

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