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Green Synthesis of Florescence Nanoparticles and Characterized by Using Different Tools.

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Abstract: Nanoscience and nanotechnology have become one of the trends of present-day cutting-edge research. One of the reasons for nanomaterials to behave differently from their bulk counterparts is their high surface-to-volume ratio. It is seen that extremely noble materials like gold, become highly active at very low dimensions as the total surface energy becomes high as the size becomes very small. Gold nanoparticles synthesized through the citrate reduction method are well known for the simple synthesis procedure and solution stability but the presence of citrate ions reduces viability and impaired proliferation of human alveolar cell. Bovine Serum Albumin (BSA) is used both as a reducing as well as a stabilizing agent. It is postulated that the tyrosine residues at higher pH reduced the protein whereas cysteine residues work as stabilizers by the formation of thiol linkages. The major functional group tyrosine and cysteine are present in chymotrypsin and egg albumin which may enable them to work as a stabilizer. Gold nanoparticles (AuNPs) thus synthesised were characterised by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS) to confirm their size, shape and composition. These gold nanoparticles have versatile applications as biomarkers and cancer therapy.

Keywords: Gold nanoparticles, Cell imaging, Zeta potential, Egg Albumin.

1. Introduction.

Gold nanoparticles have found extensive biological and biomedical applications and their synthesis is of great interest to the bio nanotechnologist. Nanoparticles of gold when functionalized with suitable biomolecules, become equipped for targeted applications. Different types of gold nanostructures are of interest for the researchers. In literature, many methods for the production of gold nanoparticles are described. Most of the methods use inorganic chemical routes. But with the trend of gold nanoparticles being applied in the biological sciences, more green methods for formulation of gold nanoparticles are needed. Gold nanoparticles (AuNPs) are some of the most widely utilized engineering materials in bio-imaging and biomedical

therapeutics due to their instance surface biocompatibility [1, 2]. The strong binding affinity of AuNPs with thiols, disulfides and amines also facilitates their conjunction with biomolecules (e.g. DNA and antibodies). This is useful in the development of various biomedical applications including biosensors and for drug delivery [3, 4]. AuNPs of size less than 5 nm exhibit high catalytic activity [5] as the surface-to-volume ratio increases. Hence, these types of fine nanoparticles are considered as an ideal material for the oxidation of CO, hydrocarbons and organic hydrogen. Gold nanoparticles synthesized through the citrate reduction method are well known for their simple synthesis procedure and solution phase stability. Another advantage of this method is that its takes less time. This method is reproducible for gold nanoparticles with narrow size distribution. The method was pioneered by Turkevich et al.[6] to synthesize 20-nm gold nanoparticles through the reduction of chloroauric acid ($HAuCl_4$) via tri-sodium citrate ($Na_3C_6H_5O_7$). Excess citrate and non-AuNP components (e.g. chloride ions and sodium ketoglutarate) are expected to remain in the solution and need to be removed before use. Since the presence of these non–AuNP components can cause unnecessary interaction with the biological living tissues during the detection. For example, during the in-vitro assessment of cytotoxicity, the presence of citrate ions reduces viability and impaired proliferation of human alveolar cell [7, 8]. There are pieces of evidence of peptide-stabilized AuNps formation in the literature [9]. In the year 2009, the formation of highly luminescent gold nanoclusters using the Bovine Serum Albumin (BSA) where the method is found to be totally green [10]. BSA is used both as a reducing as well as a stabilizing agent in this method. It is postulated that the tyrosine residues at higher pH reduce the protein whereas cysteine residues work as a stabilizer by the formation of thiol linkages. Similar results were obtained using lysozymes [11]. The major functional group tyrosin and cysteine are present in chymotrypsin and egg albumin that's why it can work as a stabilizer. Silver nanoparticles were synthesized reagent less method involving only UV –-radiations [12]. Positively charged gold nanoparticles are also synthesised by using stainless steel mesh[13].

2. EXPERIMENTAL DETAILS

2.1 Materials:

All the reagents were used as obtained without further purification until and unless stated. Deionised Milli Q water (DI) with a resistivity of 18.2 Ω m was used throughout the experiment. Gold chloride (HAuCl4. $3H_2O$) was bought from SRL Chemicals Mumbai,

India. Eggs were brought from the local market in Shillong, India.

3. Methods:

Synthesis of gold nanoparticles was carried out according to the below procedures. We took 100 ml of DI water in a beaker and vigorously stirred the water at 200 rpm just after 5 moller 5 ml ($HAuCl_4$) gold chloride pour into that beaker and waited for 5 minutes then put freshly prepared egg albumin into it and stirred for 20 minutes. Gold atoms are released from the chloroauric acid and aggregated to form nanoparticles (NPs). This step continues until the solution colour changes yellow to red. Then it was stored in a dark place at 4ºC.

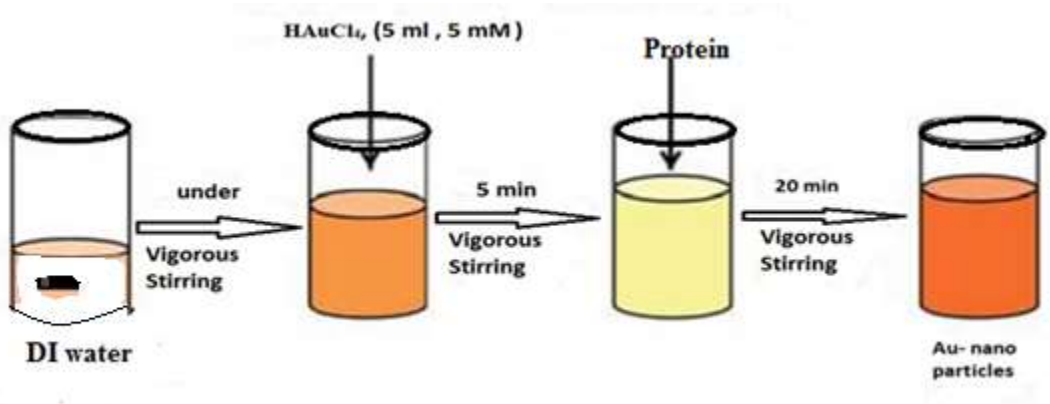

Figure 1: Schematic diagram of gold nanoparticle synthesis with orange juice.

4. Results and Discussion:

For the present work a (Perkin Elmer, Lambda 750, (USA) spectrophotometer was used. A total of 3mL of AuNPs-solution, which was prepared by egg albumin, was introduced into the quartz cell. The absorbance spectra were recorded for the time interval of 1min. The range of the scanning wavelength was 200-900 nm. Figure 2 shows that the appearance of the pick in 525 nm is maximum absorbance that mean the particle size around 5 - 20 nm. The UV-visible absorbance spectrum remain unchanged after one month of the sample.

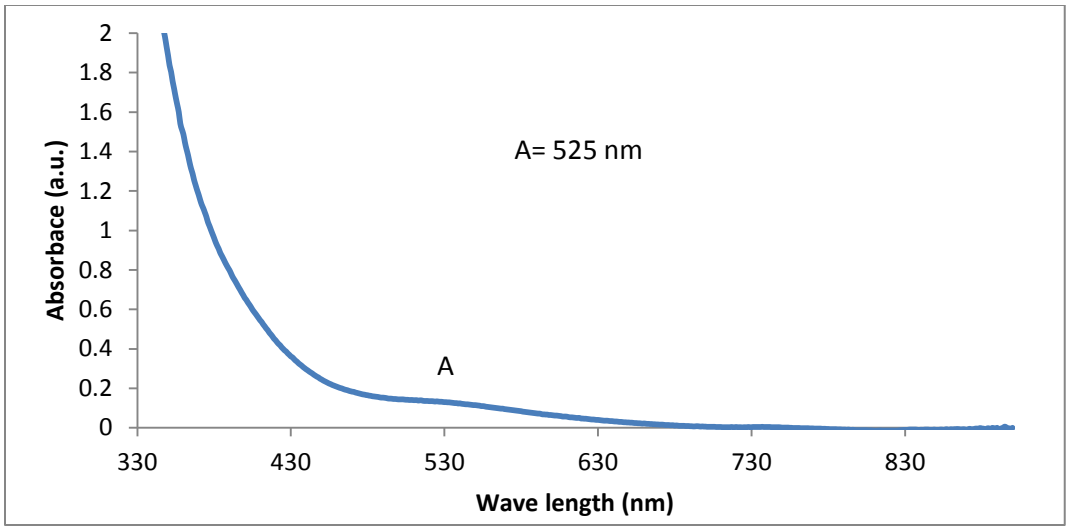

Figure 2: UV- Visible spectra of Au-NPs in egg albumin

4.1 Luminescence:

Visual inspection under UV light was done for one sets of sample put into a 10 ml borosil beaker. Figure 3 shows that the sample (a) gold nanoparticles suspended in egg albumin was seen in normal light pet yellow colour, but under the UV lamp (365nm) it seen bright orange colour. It means it absorbed lower wave length and emits higher wave length that's why bright light comes out from the sample.

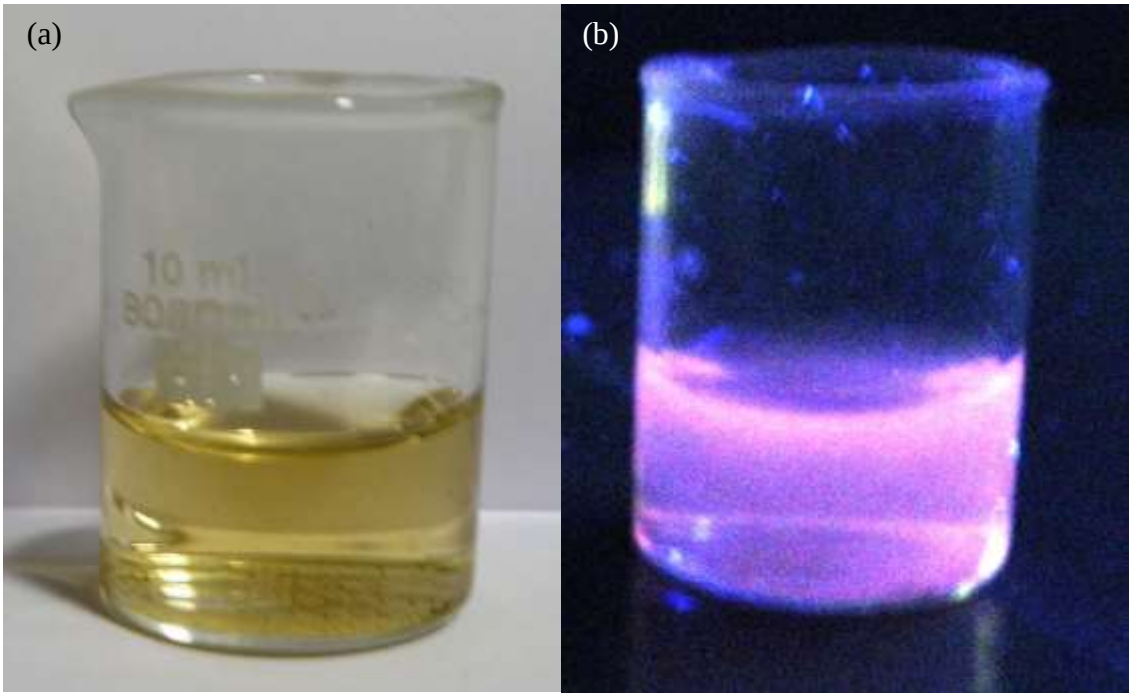

Figure 3: (a) Sample in normal light (b) Sample in UV light

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4.2 Fluorescence Spectroscopy:

Fluorescence spectroscopy of different concentrations of egg protein stabilized Au-NPs was performed. For some of them, multiple concentrations were used to find out the optimum concentration for Au luminescence. For all the sample excitation scans were performed to find out the zone around which it has to be excited in order to get proper emission spectra.

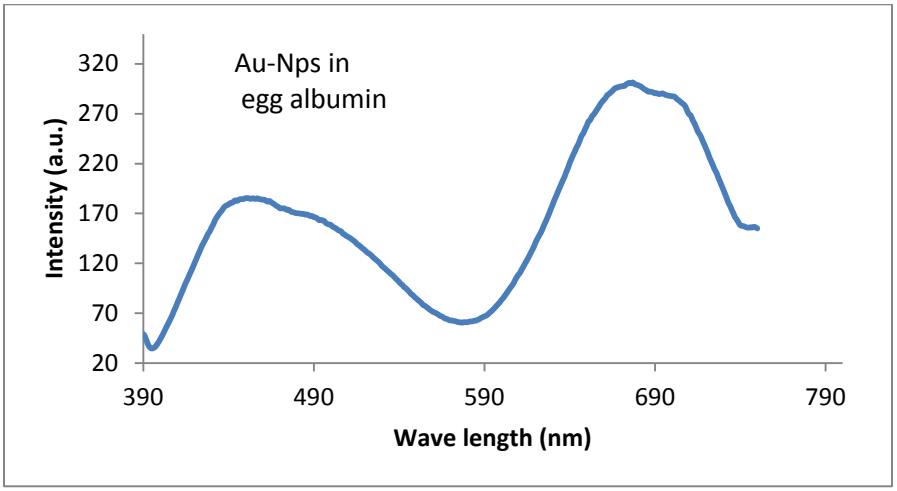

Figure 4: Fluorescence spectrum of Gold nanoparticles

Figure 4 indicates that's egg albumin it was excited at 390 nm and the corresponding emission spectra were collected. It is also found to be highly luminescent. At around 683 nm it showed a huge peak which is characteristic of red emission. It's proved that gold nanoparticle synthesis by using egg albumin is highly fluorescence. This kind of nanoparticle can be used for cell imaging.

4.3 Zeta Potential:

The zeta potentials of the Au-NPs have been determined as they give an idea about the surface electrostatic potential as well as the possibility of their application. It also indicates the degree of stability of the dispersion. Table 1 shows that the zeta potential of the colloidal solution of gold nanoparticles -25.5 mV implying that the particles were positively charged. The result shows that the nanoparticles prepared using egg albumin are more stable than those prepared using chymotrypsin or bovine serum albumin.

Name of protein	Zeta (mV)
Egg Albumin	-25.9

Table 1: Zeta potential of various proteins

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Scanning Electron Microscopy:

Scanning electron microscopy (ZEISS, EVO 60, Germany) was performed for the synthesized gold nanoparticles. In Figure 5 indicates particle cannot be resolved when the sample is dried, the evaporation of water and the resultant surface tension caused the particle to come closer. Hence clusters of Au-NP were produced.

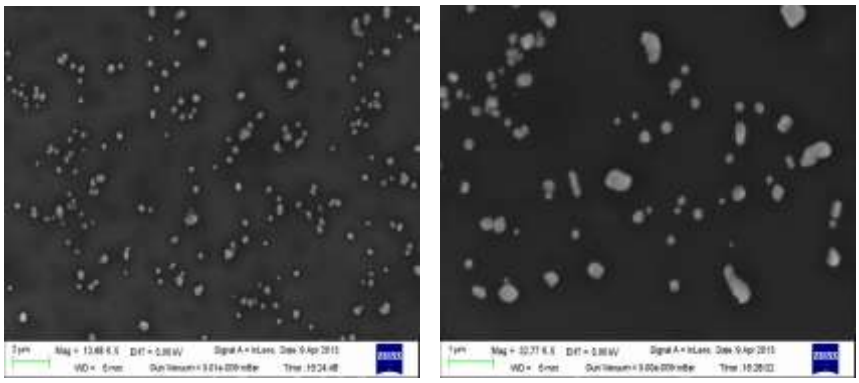

Figure 5: SEM micrograph of Gold Nanoparticles produced by egg albumin.

4.4 Transmission Electron Microscopy:

Figure 6 shows the TEM micrographs (TECNAI G^2, FEI, Netherlands) of the as-synthesized gold nanoparticles. TEM image indicate that as-synthesized Au nanoparticles were in the rage of 5-20 nm and were mostly mono-dispersed and peanut like shape. Here we can see that in lower concentrations of egg albumin-synthesized Au particles shapes are uniform and sizes around (5-20) nm but in higher concentrations of egg albumin-synthesized Au particles sizes are not uniform and around 10-20 nm. Figure (d) the SAED pattern which clearly shows well – resolved lattice fringes and diffraction cycles for the face centered cube (FCC). This pattern also indicate that the gold nanoparticles are highly crystalline in nature. The electron diffraction dots, which were indexed to the 111, 200, 220 and 222 planes of fcc Au (JCPDS No 4-0784) indicate particle size are very small and their polycrystalline nature.

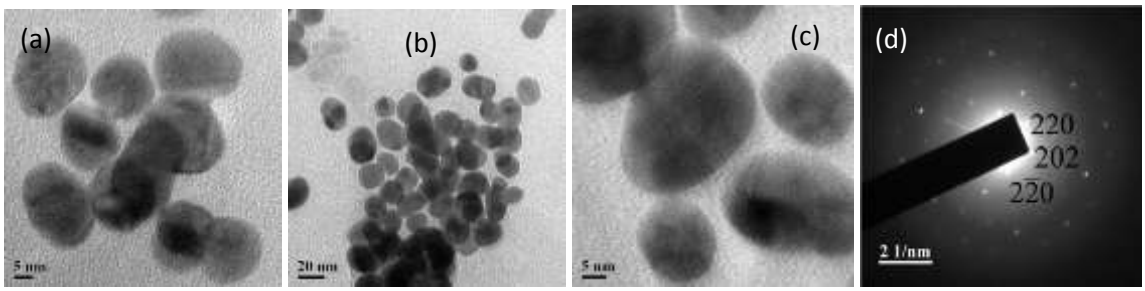

Figure 6(a,b): SEM micrograph of gold nanoparticles in a lower concentration of egg albumin used. (c) As –synthesized gold nanoparticle by a higher concentration of egg albumin. (d) SAED pattern of AuNPs.

5. Conclusion:

In conclusion, we have presented a new method for the synthesis of gold nanoparticles by using egg albumin. The whole synthesis was done at room temperature. The nanoparticle solutions display an absorbance peak at 525 nm means that particle size is around (5-25) nm. At around 683 nm it showed a huge peak which is characteristic of red emission. It's proved that gold nanoparticle synthesis by using egg albumin is highly fluorescence. This kind of nanoparticle can be used for cell imaging. The SAED pattern which clearly shows well–resolved lattice fringes and diffraction cycles for the face-centered cube (FCC). This pattern also indicates that the gold nanoparticles are highly crystalline in nature.

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