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Synthesis, Characterization and Investigation on the Antimicrobial Activity of Bovine Serum Albumin – Metal nanoclusters

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Abstract: The need for new and better antimicrobial and antifungal drugs have been increasing due to the rise of drug-resistant bacteria and fungi. Research on nanotechnology has been gaining interest due to its numerous applications in the field of medicine, its application in biological molecules for example, has been vital to the discovery of new methods to deliver drugs into the body. The study aimed to prepare, characterize and investigate on the antimicrobial activity of bovine serum albumin – metal nanoclusters using five different metals namely, aluminum, copper, nickel, silver and zinc. Preparation of the nanoclusters was done using a one-pot green synthetic route and was characterized using the following methods: Infrared (IR) and Ultraviolet – Visible (UV-VIS) spectroscopy, dynamic light scattering (DLS), Imaging, Thermogravimetric analysis (TGA) and Scanning electron microscopy (SEM) while antimicrobial ability was determined using disk diffusion method. Spectroscopy data suggested that only Cu and Ag successfully bound to the protein while DLS data indicated particle sizes ranged from less than 1nm to 3.5nm. Imaging data showed differences in color observed under visible light for all synthesized nanoclusters while images under UV light showed changes only for Ag, Cu and Ni which is indicative of any chromophore exposure in

the protein. Thermogravimetric data implied very similar decomposition profiles for all samples with respect to pure bovine serum albumin. Surface morphology was very random, only zinc nanocluster showing similarity with pure bovine serum albumin based from data obtained through scanning electron microscopy. Antimicrobial assay showed that all synthesized samples showed no ability to inhibit the following organisms: *Escherichia coli, Pseudomonas aeruginosa, Trichophyton mentagrophytes and Candida Albicans.*

Keywords: Nanoclusters; Bovine Serum Albumin; Antimicrobial Activity

1. Introduction

Nanotechnology is defined as the manipulation of matter to a supramolecular scale. It has been a wonder in the field of medicine as it is a potential drug delivery system since nanoclusters have the ability to penetrate cell membranes easily, which is primarily due to their small size. In fact, a study by Wohlfart (2012) concluded that nanoclusters have the ability to cross the blood-brain barrier. Some nanotechnology based drugs have already been released commercially such as the albumin- bound paclitaxel, which is a drug used to treat breast, lung and pancreatic cancer. This albumin bound compound uses nanocluster technology to deliver the active compound paclitaxel inside tumor cells. Albumin is a monomeric protein which is commonly found in the human body, as it comprises about sixty percent of blood proteins and acts as a carrier protein for the blood and as a stabilizing agent for extracellular fluid. As such, albumin-based drugs are gaining attention since they are less likely to initiate an immune response and thus protect their precious drug cargo. Bovine serum albumin is one of the specific types of serum albumins. It is a globular protein derived from cows which has a molecular weight of 66.4kDa, consists of 583 amino acids and seventeen cysteine residues

forming disulfide linkages aid in its globular conformity. As albumin is a carrier protein, it can accommodate a wide variety of species, one of which are metals (Topala et al. 2014).

There has always been a need to develop new and better antibacterial drugs in the field of medicine due to the ever – adapting capability of bacteria to evolve and mutate into stronger and more resistant forms. One of the most effective forms of bacterial inhibition is through the toxicity of metals which is due to the oligodynamic effect. The specific mechanism of action of metals is unknown however, previous reports theorized that the high affinity of metal ions for cellular proteins cause denaturation, precipitation and inactivation of these proteins (Shreshta et al, 2009). However while these metals may be used as antibacterial agents, there are problems with applying metals into the body, One of which is its non-specific interaction. The non-specificity of metals cause unwanted toxicity towards red blood cells which may cause complications in the body, another problem of metals is in their inability to enter the cell easily which, in some cases neutralizes their ability to exert the oligodynamic effect. These problems may be solved through the use of bovine serum albumin as a potential carrier of the metal and nanotechnology to penetrate cells easier. The study strictly focuses on using bovine serum albumin as the protein coating. Metal nanocluster synthesis was done on only five metals, specifically aluminum, copper, nickel, silver and zinc. Antimicrobial capability was determined through disk diffusion method using one gram- positive (*Pseudomonas aeruginosa*) one gram – negative bacteria (*Escherichia coli*), and two fungi species (*Candida albicans* and *Trichophyton mentagrophytes*). Characterization of the nanoparticles was done using the following methods: Fourier transform – Infrared spectroscopy (FT – IR), Ultraviolet Microscopy, Imaging, Ultraviolet – Visible spectroscopy (UV VIS), Thermogravimetric analysis (TGA) and Scanning Electron Microscopy (SEM).

2. METHODOLOGY

2.1 Synthesis of BSA-metal nanoclusters

4mL of metal aqueous solution (10 mM) was mixed with 4mL of BSA stock solution (50 mg/mL) and was stirred vigorously at room temperature for 3 minutes. Afterwards, 0.4ml of 1 M NaOH was added to the mixture. The sample was stirred for another 30 minutes before drop wise addition of 175 microliters 10 mM $NaBH_4$ (JACS, 2009). The resulting mixture was continuously stirred for another hour. The as-prepared BSA-metal solutions were frozen and lyophilized to obtain powder form. Samples were stored at 4 °C. the synthetic route is shown in figure 1.

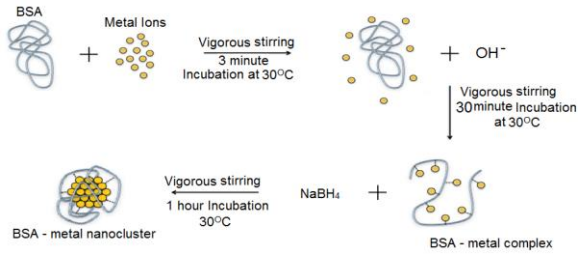

Figure 1. Synthetic route for metal capped protein nanoclusters

2.2 Characterization

2.2.1 UV-Vis Spectra

Pure BSA and metal nanoclusters powder were dissolved in distilled water and was vigorously stirred for 15 minutes. The suspension was then transferred in a quartz cell for spectral analysis in the visible-ultraviolet region. All measurements were performed with a Hitachi U-2900 spectrophotometer , from 200 nm to 700 nm.

2.2.2 IR Spectra

Pure BSA and metal nanoclusters were grounded with KBr until a fine powder form. All IR spectra were collected using a Thermo Scientific Nicolet 6700 FTIR, from 1000 cm^{-1} – 4000 cm^{-1}.

2.2.3 Thermogravimetric analysis

Three to five grams of lypholized nanoclusters and pure BSA was placed in a ceramic sample holder and heated from 75 °C to 600 °C, with a rate of 10 °C per minute, using a Mettler Toledo TGA/SDTA 851e.

2.2.4 SEM Analysis

JEOL JSM-6460 LA Scanning electon microscope was used for all tests. SEM plates were obtained from the specimen chamber and was cleaned with cotton buds. The first set of samples consisting of five different nanoclusters were placed into the specimen plate and was coated with gold. The specimen plate was then placed into the specimen chamber.

2.2.5 Imaging

A pinch of BSA nanoclusters and Pure BSA were lined up and subjected to UV exposure at 254nm. All samples were observed at same time with GelVue dual UV Transilluminator GVS20. The results of the sample were compared with each other using pure BSA as control

2.2.6 Antimicrobial assay

Bacterial and mold suspensions were prepared from 24-hour old and 7-day old cultures, respectively. The suspending medium used was 0.1% peptone water.

The cotton swab with applicator stick was dipped into the microbial or mold suspension and rotated several times, followed by pressing into the edge of the tube to remove excess inoculum from the swab. The cotton swap was applied to a pre-poured nutrient agar (NA), glucose yeast peptone (GYP), and potato dextrose agar (PDA), roughly 3mm thick, streaked over the entire surface of the agar. 3 equidistant wells were made through a cork borer (10 mm in diameter). Pure BSA and lyophilized metal nanoclusters were then diluted with distilled water to a final concentration of 1 mg/mL. 200ul were transferred into the wells of the agar plates in triplicates. NA and GYP plates were incubated at 35°C and was observed after 24 hours while PDA was incubated at room temperature and was observed after 48-72 hours. The clearing zones were measured and the average diameter was calculated. The antimicrobial index (AI) was computed using the following formula:

$$AI = \frac{Diameter\ of\ clearing\ zone - Diameter\ of\ well}{Diameter\ of\ well}$$

3. RESULTS AND DISCUSSION

Using Ultraviolet – Visible Spectroscopy, absorption maxima for all synthesized samples including pure bovine serum albumin was determined. the data is shown in figure 2, The absorption maxima for pure BSA, BSA – Al, BSA – Ni and BSA – Zn was determined to be 277nm and for BSA – Ag and BSA – Cu was 272nm. Any changes in absorption maxima indicate successful protein capping and changes in conformity of the protein which is observable through the tryptophan residues as they contribute to absorption. Through UV-VIS spectroscopy it was determined that only BSA – Ag and BSA – Cu successfully capped with bovine serum albumin. Infrared spectroscopy supplements UV-VIS data by determining any shifts in the amide (I) band of the spectra. the data is shown in figure 3. The amide (I) band for pure bovine serum albumin was determined to be 1654 cm^{-1} and only Ag and Cu nanoclusters showed significant shifts in the amide (I) band. Together with UV – VIS data, IR data confirms that only Ag and Cu nanoclusters successfully capped with bovine serum albumin.

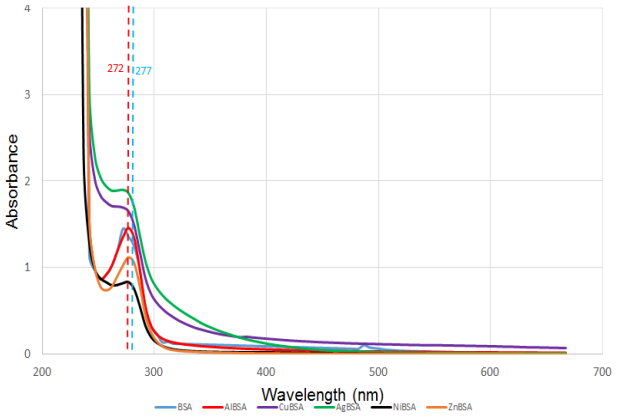

Figure 2. UV spectra of all samples (blue, red, green, violet, black and pink corresponding to BSA, BSA – Al, BSA – Ag, BSA – Cu, BSA – Ni and BSA – Zn respectively)

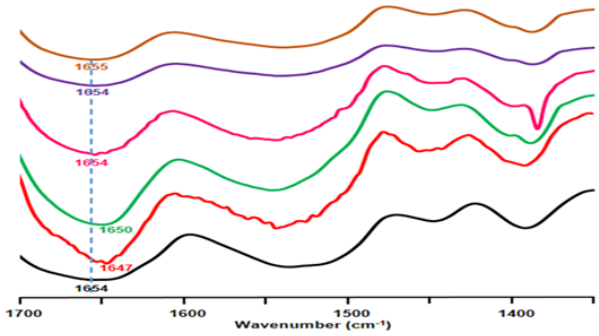

Figure 3. IR spectra for all samples (black, violet, red, green, pink and brown corresponding to BSA, BSA – Al, BSA – Ag, BSA – Cu, BSA – Ni and BSA – Zn respectively)

Imaging profiles of all synthesized samples were compared to pure BSA under visible and

ultraviolet light. Changes in emission of all samples under visible light were observed as it is indicative of tryptophan perturbation which is a well-known consequence of protein capping. Under UV light however, only Ag, Cu and Ni nanoclusters showed changes in emission spectra while Al and Zn nanoclusters showed similar emission relative to pure bovine serum albumin. The data is shown in figure 4.

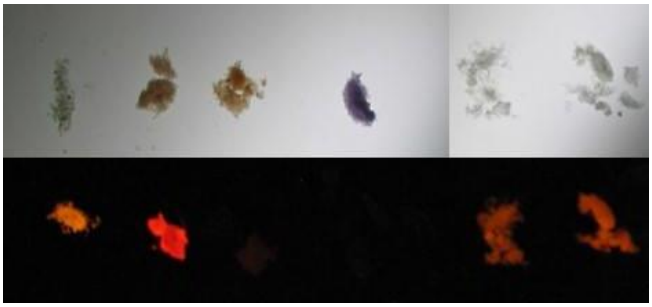

Figure 4. Images of BSA, BSA – Ag, BSA – Ni, BSA – Cu, BSA – Al and BSA – Zn arranged in the order from left to right taken under visible (Top) and UV light (Bottom).

The Thermogravimetric profile for all samples show similarities, all samples showed minimal mass loss due to water from 100-120 ^{o}C and exhibited first step degradation at 250 – 310^{o}C. Second step degradation occurred at 500-536^{o}C for BSA – Al, BSA – Ag, BSA – Cu and BSA – Zn and at around 600^{o}C for pure BSA and BSA – Ni. The degradation process was capped at 625^{o}C and the degradation was not completed, at this temperature only BSA – Al, BSA – Ag and BSA – Cu showed completion in second step degradation. The results are shown in figure 5.

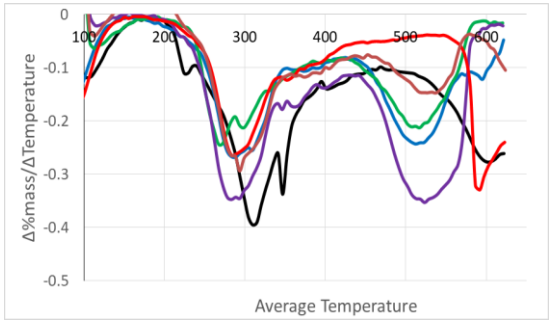

Figure 5. 1st derivative Thermogravimetric profile of all samples (black, blue, green, violet, red and orange corresponding to BSA, BSA – Al, BSA – Ag, BSA – Cu, BSA – Ni and BSA – Zn respectively.)

SEM results provided images of all synthesized samples including pure bovine serum albumin. The surface morphology for BSA – Zn was the only sample that looked similar to bovine serum albumin. BSA – Ag showed distinct tiny particles, BSA – Ni showed

hair-like strands with small particles whilst BSA – Al and BSA – Cu showed grain like particles. The images are shown in figure 6.

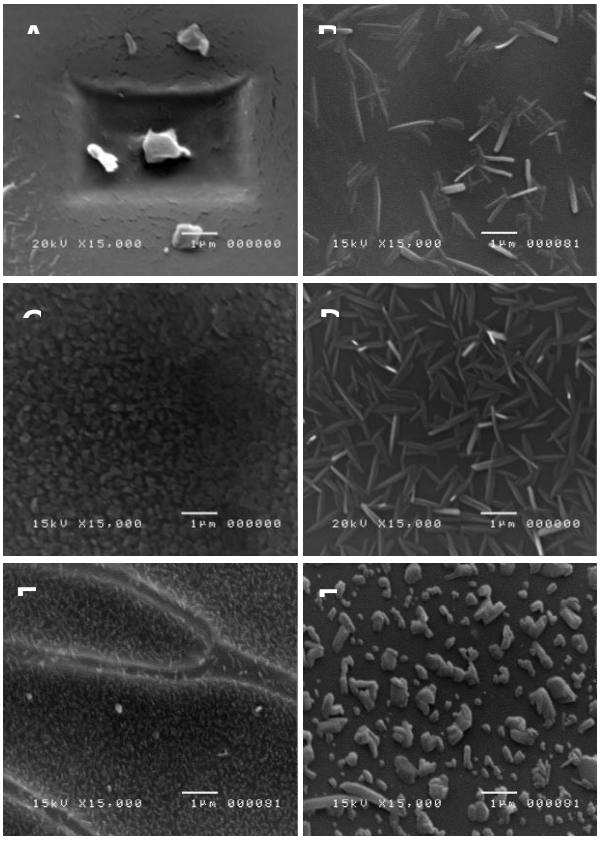

Figure 6. SEM images of all samples(A,B,C,D,E and F corresponding to BSA, BSA – Al, BSA – Cu, BS – Ag, BSA – Ni and BSA – Zn respectively)

Antimicrobial assay showed that against all organisms, all synthesized samples including pure bovine serum albumin showed no signs of bacterial inhibition. This may be attributed to the metal being unexposed to the organisms.

4. CONCLUSION

In summary, based from data obtained through UV-VIS and IR spectroscopy the only metals that were successfully bound to bovine serum albumin were silver and copper whilst the other failed to show any significant data indicative of protein capping. In the imaging profile of samples under visible light, all synthesized BSA – metal nanoclusters showed significant changes in color while in the images taken under UV light, BSA – Ag and BSA – Cu and BSA – Ni showed significant changes in emission while BSA – Al, BSA – Zn showed similar emission with respect to pure BSA. Thermogravimetric data showed the decomposition profiles of all samples to be similar with respect to pure BSA. Scanning electron microscope images showed that only BSA – Zn showed similar surface morphology relative to pure bovine serum albumin while

other samples showed various differences in morphologies. Antimicrobial assay data showed that no zone of inhibition was present for all samples against all organisms of bacteria and fungi tested. The negative results may be due to the protein encasing the metal such that no exposure of metal to bacteria occurred however this is unconfirmed. The overall characterization of these nanoclusters may prove significant in future studies regarding the applications of bovine serum albumin – metal nanoclusters.

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