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Interaction Study of Greenly Synthesized Silver Nanoparticles with Bovine Serum Albumin (BSA) Using Spectrophotometric and Voltammetric Assays

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Abstract

Due to the considerable constraints impacting conventional pharmaceutical agents and older formulations, nanotechnology plays a vital role in the realm of medicine and in medication delivery. This study's primary goal is to investigate biologically synthesized silver nanoparticles AgNPs with Bovine Serum Albumin (BSA) interact utilizing Spectrophotometric and Voltammetric assays. According to the results, these silver nanoparticles can ligand with BSA (AgNPs-BSA), and the binding is spontaneous with the involvement of electrostatic interactions, according to the computed negative free energy ΔG° values and a constant K. Moreover, the cyclic voltammetric analysis findings confirmed that the nanoparticles were primarily electrostatically bound to BSA. Overall, these AgNPs have a promising biological future and could be used in nanomedicine and pharmaceuticals.

Keywords: Silver nanoparticles, Bovine serum albumin, Cyclic voltammetry, Spectrophotometric, binding free energy, binding free constant.

Introduction

The development of nanotechnology, which offers incredible ways to cope with life-threatening diseases, has boosted advancement in the field of medical science. Nanotechnology is a huge milestone that has various applications in many sectors (1, 2). Noble metallic nanoparticles are receiving increasing research attention in all fields of science in the past few decades due to their attractive physicochemical properties (3). Among the metallic nanoparticles, silver nanoparticles (SNPs) have received considerable attention because of their wide range of applications (4).

Several studies are well underway to incorporate AgNPs into clinical and industrial technologies as well as drug applications (5, 6). Silver is a non-toxic and inorganic agent, it has been described as 'dynamic' because of its ability to exert excellent potential for biological uses, including antifungal, antibacterial, antiviral, anti-infectious, wound healing, and anti-inflammatory properties at low concentrations (7, 8).

The green synthesis of AgNPs is easy, inexpensive, less time-consuming, and eco-friendly. They can quickly form conjugates with proteins through either covalent bonds or physical interactions and these conjugates have been extensively used in biomedical fields, including diagnostics bio-imaging and targeted drug delivery (9).

Bovine serum albumin is the most abundant protein in the plasma and performs a wide range of physiological tasks such as binding, nutrition transport, fatty acid storage, and so on. BSA also binds to a range of medicines at different locations throughout the body's circulatory system (10, 11). It is critical to investigate the mechanism of interaction between BSA and foreign compounds (12). Interactions with the proteins present in plasma are of central importance in biomedical applications of nanoparticles and the growing biosafety concerns of nanomaterials reports on the interaction of nanoparticle silver are scarce (13, 14).

An efficient AgNPs-protein system was designed for biological applications; it is necessary to study the AgNPs-protein interactions systematically. Numerous silver-based nanoparticles have been investigated in biological settings; however, most of these studies have concentrated on chemically produced metallic NPs rather than the biosafety-focused green synthesis. The interactions between green synthesis AgNPs and bio-macromolecules, in particular, are still poorly known because there haven't been many thorough past publications on them. Based on this fact and on our continuing interest in studying the interaction of silver nanoparticles with BSA, the present investigation has been focused on mechanisms of interactions between silver nanoparticles and BSA using UV visible spectroscopy and cyclic voltammetry.

Material and Methods

Reagents

Silver nitrate ($AgNO_3$), bovine serum albumin (BSA), the Potassium dihydrogen phosphate (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4). All were obtained from Sigma-Aldrich.

Preparation of extract

H. lippii aerial parts powder was steeped for around 10 g in 100 mL distilled water for 24 hours in the dark. After that, it was filtered with filter paper and used as a reducing and capping agent to prepare AgNPs (15).

Green synthesis of AgNPs and characterization

90 ml of a 1 mM AgNO3 solution were mixed with 10 ml of Helianthemum liippii L. A brownish tint appears a short while later, indicating the synthesis of AgNPs. The combinations were heated to 60°C. To prevent unwanted photochemical reactions, the entire reaction process was carried out in complete darkness. In a typical synthetic technique and in end left to incubate for 24 hours (16). The nanoparticle was carefully collected and dried to get a final mass of AgNPs. Moreover, further experimentations and characterizations were A Cary-4000 spectrophotometer was used to perform UV-Vis spectrum analysis on the produced AgNPs. SEM (Hitachi, S-4800) and EDX were used to examine the morphology of the produced AgNPs (Horiba, 6853-H). XRD was used to examine the structure and composition of AuNPs (AXS D8 Advance). Dried AgNPs and plant powder FTIR spectra were recorded (Bruker Germany).

Methods used for the study of BSA-AgNPs interaction

UV-Visible spectroscopic analysis

After the addition of various doses of BSA, the electron spectrum of silver nanoparticles "AgNPs" (10 mg/ml) solubilized in a 0.1M phosphate buffer (KH2PO4/K2HPO4) at pH = 7.2 was generated. In order to ascertain max and calculate the interaction parameters, the spectra are recorded.

Voltammetric assays

In an electrochemical cell, silver nanoparticle AgNPs and BSA react with one another. 0.1 M phosphate buffer (KH2PO4/K2HPO4) with a pH of 7.2 serves as the reaction media. Prior to each manipulation, the working electrode is polished with P4000 sandpaper. The electrodes are then rinsed with ultra-pure

water and dried with absorbent paper. The buffer containing AgNPs (10 mg/ml) is added to 25 ml of an electrochemical cell, which is then outfitted with the working electrode, the reference electrode, and the auxiliary electrode. Plots of the voltammogram are shown in both the absence and presence of various BSA concentrations.

Results and Discussion

Characterization of AgNPs

The formation of dark brown color and measurements of UV-visible spectroscopy was the primary indicators successfully of green synthesis of silver nanoparticles. Whereas, the presence of the strong SPR band at 400 nm provides a clear indication of the production of AgNPs. Similar results were obtained by Das et al.,(17), where it was observed that the production of AgNP correlated positively with color change. While with regard to other characteristics, they were shown and clarified in our previous study.

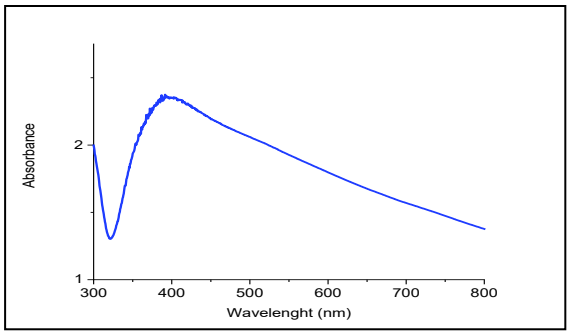

Figure 01: UV–vis absorption spectrum of AgNPs.

Volta-metric Studies

Binding constant and binding free energy

The free energy of ΔG binding of BSA with silver nanoparticules was determined by the study of the anodic behavior of this derivative in the absence and in the presence of an increasing concentration of BSA, this study of the electrochemical behavior was realized by the cyclic voltammetry technique in a 0.1 M phosphate buffer solution (KH2PO4 / K2HPO4) at pH = 7.2.

The cyclic voltammograms shown in (Fig.02) show that the addition of an increasing amount of BSA causes a decrease in the current density of the anode peaks ipa accompanied by a displacement of the potential of these peaks towards the most positive values.

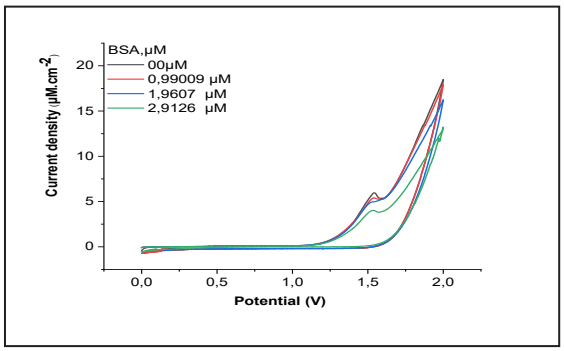

Figure 02: Cyclic voltammogram of AgNPs in the absence and in the presence of increasing concentration of BSA recorded on a glassy carbon electrode in 0.1 M phosphate buffer (KH2PO4 / K2HPO4) at pH = 7.2 at a potential scanning speed of 100 mV / S.

Determining the binding constant of AgNPs with BSA from the decline in the anodic peak current density of AgNPs-BSA adduct relative to free AgNPs using Eq. (1):

(18) (1)

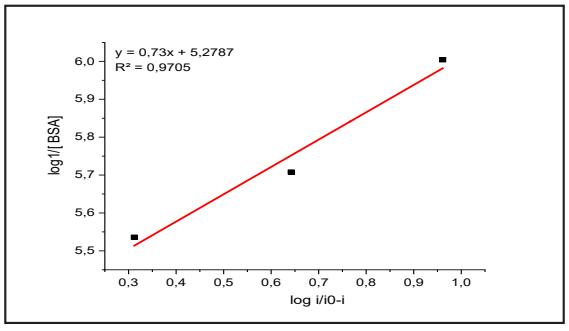

Figure 03: Regression line of log 1/ [BSA] as a function of $log\ i\ /\ (i\ 0\ -\ i)$

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The binding energy ΔG was calculated using Eq (19) (2) : ΔG = -R.T.LnK

Where ΔG is the binding free energy, kJ·mol-1, R is the gas constant, 8.32 J·mol-1K-1; T is the absolute temperature equal to 298 K.

Free binding constant and free binding energy were calculated from the plot log 1/[BSA] as a function of $log\ i\ /\ (i\ 0\ -\ i)$(fig 03).

Free energy (ΔG) is negative, indicating a strong interaction between BSA and AgNPs and that the binding process is spontaneous via electrostatic binding (13, 20).We can identify the sort of binding force by evaluating characteristics like the ΔG of binding interactions.

The values obtained for the binding constant and the free energy of the BSA-AgNPs interaction are grouped together in tab. 01

UV-Visible spectroscopic studies

To get a complementary method to the voltammetry techniques, the electronic spectroscopic titration method was used to investigate the interaction of AgNPs with BSA, in which AgNPs in buffer phosphate solution (KH2PO4/ K2HPO4) at pH 7.2 were exposed to increasing concentrations of BSA. We registered that the absorbance of AgNPs diminishes when with the gradual increase of BSA concentration without any noticeable shift in the position of the maximum absorption peak of AgNPs fig (4).

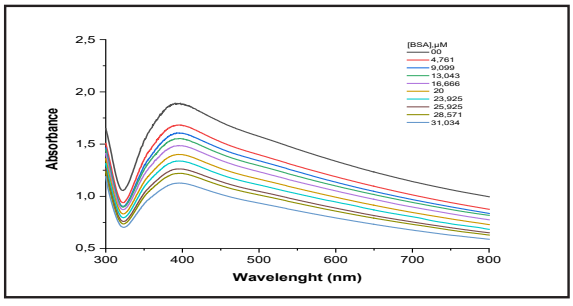

Figure 04: the absorbance values of AgNPs in the absence and the presence of different concentrations of BSA in 0.1 M phosphate buffer (KH2PO4 / K2HPO4) atpH = 7.2.

The Benesi-Hildebrand equation (21) was used to calculate the binding constants Kb from the absorption data:

(1)

Where Ao is the absorbance of the free compound, A is the absorbance of the adduct, εo, and ε are their molar extinction coefficients, respectively.

The binding constant is calculated by the slope / intercept ratio, of the plot of the

Ao / (A - Ao) as a function of 1 / [BSA].

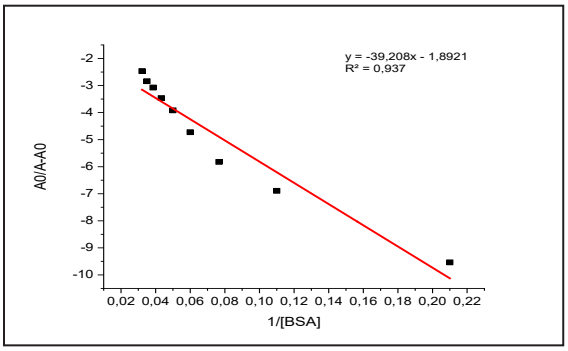

Figure 05: Regression line of A0/ (A – A0) as a function 1/ [BSA].

The results of the K binding constants and the ΔG binding free energies of the AgNPs-BSA adduct calculated using the equation (ΔG = -RT lnKb) in the presence of an increasing concentration of BSA table 06 summarizes the findings.

The findings suggest that added BSA molecules interact with AgNPs in solution to create a new compound (BSA-AgNPs).Whereas, UV-Visible Spectroscopic results prove to electrochemical study data hence, the binding free energies obtained show that the binding process was spontaneous (21, 22), implying that the compounds have a moderate affinity for BSA but that transport and delivery are possible (23, 24). These findings lead us to believe that the substance under investigation can form complexes with BSA and cause conformational changes in it. The results of this work provide

Table 01: Binding constant and binding free energy values for AgNPs ligand with BSA from CV data at pH = 7.2 and T= 298 K.

Adduct	Equation	R^2	$K.M^{-1}$	$-\Delta G\ (Kj.mol^{-1})$
AgNPs-BSA	Y= 0.7344x + 5.2717	0.9744	1.213×10^{1}	30.095

Table 02: Values of the constant and the binding free energy of the interaction BSA-AgNPs obtained from UV-Vis spectrophotometric data.

Adduct	Equation	R^2	$K_b\ (M^{-1})$	$-\Delta G(Kj.mol^{-1})$
BSA-AgNPs	-39.208x-1.8921	0.937	4.825×10^{4}	26.738

insight into how silver nanoparticles and BSA characteristics interact, which may help in the creation of medications with improved or more targeted activity and clinical efficacy (11).

Conclusion

In this paper, the interactions of BSA with AgNPs were studied by using spectrophotometric and voltammetric assays. The obtained binding process between AgNPs and BSA was spontaneous, according to the negative values of the binding free energies which were calculated. Additionally, the results of the cyclic voltammetric investigation indicated that the nanoparticles were mostly bound to BSA through an electrostatic binding mode. Therefore, these findings lead us to the conclusion that the AgNPs created in our work are capable of forming complexes with BSA.

Hence, this work is anticipated to supply a significant insight into the interaction between BSA and AgNPs, potentially revealing horizons and new therapeutic targets.

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Conflict of interests

not applicable

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