



Examination of the interaction between bovine albumin and gold nanoparticles



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Abstract

Gold nanoparticles (AuNPs) have attracted a great deal of attention in recent years due to their potential uses in electronics, biotechnology, biomedicine, and catalysis. This is due to several appealing properties, including optical characteristics, size-dependent characteristics, great conductivity, non-toxicity, and their ability to be easily and broadly functionalized. In the present study, to produce AuNPs employing chitosan to act as reducing/capping agent by thermal reduction procedures in chitosan-acetic acid-water were used. A variety of biomolecules, mainly proteins, have recently attracted a lot of attention for their ability to stabilize AuNPs, which has implications for targeted drug administration, bioimaging, and diagnostics. Interaction between gold nanoparticles and Bovine Serum Albumin (BSA) has been examined using UV-absorption spectrophotometry, FT-IR spectroscopy, Transmittance Electron Microscopy (TEM), Differential Scanning Calorimetry (DSC), and dynamic light scattering. Based on the information gathered by the above techniques, the influence of concentration of BSA upon the stability of the gold nanoparticles and the structural modifications that occur in BSA when it interacts with the gold nanoparticles are examined, and a potential conjugation mechanism is put forth.

Keywords: Nanoparticles; Protein; TEM.

1. Introduction

The production of nanomaterials with accurate dimensions, geometric shapes, and surface characteristics has advanced significantly in recent years. Understanding the relationship between nanoparticles and biomolecules such as proteins is of great interest. Proteins can be employed to modify the characteristics of nanomaterials and functionalize them for use in diagnostic and sensing applications. Nanocomposites have also been created using proteins. Since proteins' structural and functional characteristics are significantly influenced by nanomaterials, there is a growing interest in understanding the underlying principles governing

nanomaterial interactions with biomolecules. BSA, bovine serum albumin, has been used as a model protein to study how nanoparticles and BSA interact. In humans and other mammals, serum albumin is the most prevalent plasma protein. It is also a crucial protein for maintaining the osmotic pressure required for the appropriate distribution of fluids in the body through intravascular categories and tissues in the body. Bovine and human serum albumin have tertiary structures that are between 70 and 80 percent comparable, indicating that both proteins are homologous, according to numerous research' consistent findings. BSA is also an essential transporter of fatty acids, metabolic products

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including bilirubin and thyroxin, as well as exogenous and endogenous chemicals. Additionally, because BSA contains sulfhydryl groups, it is a reactive oxygen and nitrogen species scavenger, which is crucial in oxidative stress. Two tryptophan residues in BSA are intrinsically fluorescent. Trp 134, which is found on the outer layer of the albumin molecule in the primary sub sector IB, and Trp 212, which are in a hydrophobic bonding region in the sub sector IIA [1]. Bovine serum albumin (BSA) is a protein made up of 585 amino acid residues, 35 of which are cysteines (17 of which form disulfide bridges), giving the protein a comparatively high degree of stability. Its secondary structure has 67% α -helices, and the isoelectric point (pI) has been found to be in the pH range of 4.8 to 5.6. [2]. Additionally, it has been observed that BSA attached nanoparticles have reduced toxicity, more stability against flocculation, and raised quantum yield. Then, BSA can be synthesized for the purpose of targeting [3]. Among nanomaterials, the biological application of metallic nanoparticles, particularly gold nanoparticles (Au NPs), has aroused interest. It is simple to create several Au NP shapes, including spherical, cage-like, rod-like, and others, in sizes that vary from 1 nm to greater than 100 nm. Au NPs' shape and size have a significant impact on both their optical and electrical characteristics. Because Au NPs have a negative charge, various biomolecules like medicines, targeting ligands, and genes can easily functionalize them. Au NPs are non-toxic and biocompatible. Surface plasmon resonance (SPR) bands are present in Au NPs, which also have an ultra-small dimension, a macroscopic quantum tunneling impact, and a unique surface effect. Au NPs contain all these unique features, making them the most promising material for a variety of biomedical applications, such as molecular imaging, biosensing, medication carriers, and so on [4]. Chitosan, a substance widely known for chelating heavy metals, was used to cap the produced nanoparticles. Chitosan contains free amines of its repeat parts, which are protonated in weak acidic conditions. These formed amines create the various bonding sites necessary for the effective chelation of heavy elements like Cu and Zn. While binding of the ions of heavy metals by chitosan has received a lot of research, the development of straightforward colorimetric sensors to identify the existence of heavy metal ion pollutants in water has received far

less attention. The electrostatic adsorption of chitosan on gold nanoparticles is being researched to develop a straightforward colorimetric sensor for signaling the concentration of ions of heavy metals since gold nanoparticles are an attractive choice for the fabrication of colorimetric sensors [5]. The term "nanoparticle-protein corona" (NP-PC) refers to the creation of nanoparticle-protein complexes. Protein coating on nanoparticles (NPs) affects biodistribution, targeting, and lessens the toxicity of uncoated NPs. Since these hybrids could be used in nanomedicine, various research has been conducted in recent years to better understand the process and stoichiometry of interaction of protein-NP bioconjugates [6].

The goal of this work is to investigate how BSA interacts with AuNPs in both its normal and denatured states. The interactions between them were studied by UV-visible absorption spectroscopy, Fourier transform infrared spectroscopy (FT-IR), Transmittance Electron Microscopy (TEM), Differential Scanning Calorimetry (DSC), and dynamic light scattering.

1. Materials and Methods

Materials

BSA ($\geq 98\%$), Tetrachloroauric(III) acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) (99.9%), Chitosan flakes and acetic acid were purchased from Sigma-Aldrich U.S.A. De-ionized water and Double-distilled water (18.2 M Ω -cm) all the materials were used without any further purification.

Synthesis and characterization of nanoparticles Green Synthesis of AuNPs

In chitosan-acetic acid-water, thermal reduction techniques have been used to create AuNPs with chitosan acting as a reducing/capping agent [7]. The reduction of tetrachloroaurate using chitosan in this approach produced chitosan-capped gold nanoparticles. A ruby-red solution was produced by reducing 5 ml of a 10-3M solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ with 40 ml of a chitosan solution made in 0.2% acetic acid at 100 °C [8].

Samples preparation

The normal BSA (nBSA) stock solution was created by combining 50 mg of BSA with 50 mL of double-distilled water at a concentration of (1 mg/ml) at room temperature and pH 7.4. BSA is denatured at

90 °C for 15 minutes. BSA (stock) and AuNPs are combined in a ratio of 1:0.3 and let to stand at room temperature for 20 minutes to interact with BSA (native and denatured).

Characterization of BSA/AuNPs

The Interaction between BSA and AuNPs was discovered by analyzing the absorption spectra of the samples by UV-1100 SPECTROPHOTOMETER, Fourier transform infrared (FT-IR) spectra (JASCO, 406 plus, Japan,) in the range 400-4000 cm^{-1} and Differential scanning calorimetry (DSC) (Shimadzu Japan) used to analyze the composites' thermal transitions. The morphology of the BSA/AuNPs was examined using Transmission electron microscopy (TEM) (JEOL JEM- 2100 TEM made in Japan). The zeta potential of the BSA/AuNPs was obtained by Dynamic light scattering (DLS) (Zetasizer Nano ZS90, Malvern Instruments, Worcestershire, UK).

Statistical analysis

The data was analyzed by SPSS v. 16.0 for Windows. The significant differences were calculated using one-way analysis of variance (one-way ANOVA). $P \leq 0.05$ was judged significant.

2. Results and Discussion

UV-visible spectroscopy, in its broadest meaning, examines how proteins and nanoparticles interact [9]. The absorption spectra of nBSA are depicted in Fig. 1a, with the absorption peak of BSA located at 280 nm. This peak is mostly caused by the tryptophan residue and was produced by the aromatic amino acid residues [10, 11]. Although the results for typical bands in the denatured bovine serum albumin dBSA spectrum are like those for native BSA (Fig. 1b), there is a tiny difference in intensity and wavelength. However, this difference is more beneficial for tracking a protein's structural changes from fold to unfold state [12]. Figure 1.c displays the gold (Au) nanoparticles' absorption spectrum. AuNP collective electron vibrations or concentrated surface plasma resonance (SPR) is responsible for a significant plasmon absorption peak at 515 nm. Normal BSA stabilized AuNPs' UV-VIS absorption spectra are displayed in Figure 1.d. In normal BSA, the absorption peak is broader and occurs around 280 nm. After the alteration of BSA, it is evident that the plasmon absorption peak of AuNPs broadens and shifts from roughly 515 nm to 520 nm. The denatured

condition (dBSA-Au) in which the results (as in the normal state) occurred with an increase in intensity is depicted in Figure 1.e. The particle size, shape, refractive index of the surrounding medium, and temperature all affect the resonance wavelength and bandwidth of AuNPs. This shift that occurs when AuNPs and BSA are conjugated is attributable to changes in the dielectric properties around the AuNPs brought on by the presence of BSA. These modifications show that BSA has attached to the surface of AuNPs [13,14].

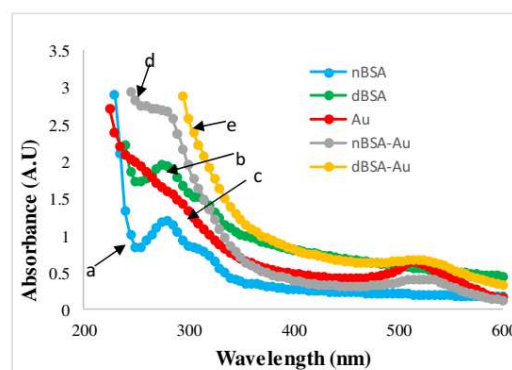


Figure 1: UV-VIS spectra of (a) normal bovine serum albumin (nBSA), (b) denatured bovine serum albumin (dBSA), (c) gold nanoparticles (AuNPs), (d) normal bovine serum albumin and gold nanoparticles (nBSA-AuNPs), (e) denatured bovine serum albumin and gold nanoparticles (dBSA-AuNPs).

There are several applications for Fourier transform infrared (FTIR) spectroscopy, ranging from the study of cells or tissues to the study of tiny molecules or chemical complexes. Additionally, the study of proteins has seen an increase in the use of FTIR spectroscopy [15]. The FT-IR spectra of regular BSA in Fig. 2a reveals the large band of amide A at 3400 cm^{-1} , which confirms the presence of the $-\text{NH}_2$ group as well as some traces of the $-\text{OH}$ bond. Amide-I at 1649 cm^{-1} is produced by the stretching vibration of C-O and C-N, while amide-II at 1543 cm^{-1} is produced by the in-plane bending of $-\text{NH}$ [16]. These three peaks were marginally altered by roughly $\pm 3 \text{ nm}$ in denatured BSA (Fig. 2b). The gold nanoparticles' FTIR spectra (Fig. 2c) revealed bands at 1073 , 1255 , 1635 , and 2918 cm^{-1} in addition to additional minor bands. The C-N stretching vibration of aliphatic amines or alcohols/phenols is represented by the band at 1073 cm^{-1} [17]. BSA molecules are present on the surface of the gold NPs, as shown in Fig. 2d, where BSA-coated gold NPs clearly exhibit the existence of two intense transmission peaks at

1563 and 1650 cm^{-1} , which can be attributed to the amide II and amide I bonds, respectively [18]. In denatured state (fig. 2e) the peaks appeared as in normal state with small changes.

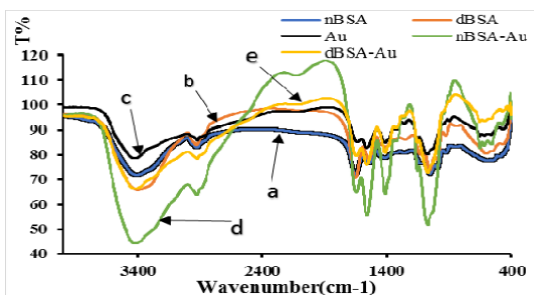


Figure 2: FTIR spectra of (a) normal bovine serum albumin (nBSA), (b) denatured bovine serum albumin (dBSA), (c) gold nanoparticles (AuNPs), (d) normal bovine serum albumin with gold nanoparticles (nBSA-AuNPs), (e) denatured bovine serum albumin with gold nanoparticles (dBSA-AuNPs)

TEM was used to study the shape and size of the proteins and gold nanoparticles. The TEM image of nBSA (Fig. 3a) depicts pours with no evidence of aggregation or fiber production. In the TEM picture for dBSA shown in Fig. 3b, there are several fiber bundles [19]. Gold nanoparticles with spherical shapes are depicted in Fig. 3c's TEM image [20]. The TEM image for nBSA-Au (Fig. 3d) demonstrates the BSA stabilized AuNPs. The BSA protein coating on the surface of gold nanoparticles can be seen as a shadow surrounding them in the nBSA-Au image [21]. The identical outcomes as in the normal condition (nBSA-Au) were seen in dBSA-Au (Fig. 3e), with the development of fiber bundles.

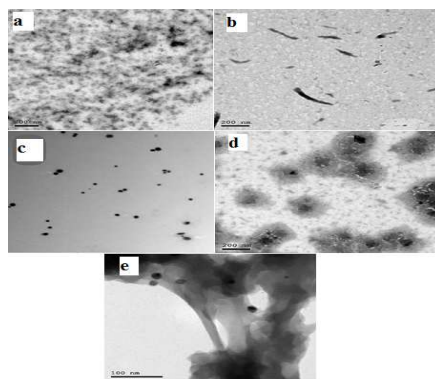


Figure 3: TEM images for, (a) normal bovine serum albumin (nBSA), (b) denatured bovine serum albumin (dBSA), (c) gold nanoparticles (AuNPs), (d) normal bovine serum albumin with gold nanoparticles (nBSA-AuNPs), (e) denatured bovine serum albumin with gold nanoparticles (dBSA-AuNPs)

The stability of the nanoparticles in solution, as well as the charge on the surface, were evaluated using the zeta potential approach [22]. A normal BSA's negative zeta potential (-11.3mV) is displayed in Table 1. This finding suggests that the carboxyl (COOH) and amino (NH₂) groups of the amino acids are the source of the negative charge on the protein surface [23,24]. The protein's conformational shift from the folded to the unfolded state causes the BSA zeta potential to become positive (6.15 mV) at this point. Due to chitosan's modification as a capping agent on the surface of the AuNPs, their zeta potential is positively charged (36.8 mV). The positively charged NH₃⁺ group of chitosan repels each other electrostatically, preventing the AuNPs from aggregating [25,26]. Positively charged AuNPs and negatively charged nBSA protein have attractive forces that cause protein to bind to them, whereas positive AuNPs and positively charged dBSA have repulsive forces that cause less protein to bind to them [27].

Table 1

Show Zeta potential for BSA (native and denatured) with AuNPs

Sample Name	Zeta potential(mV)
Normal BSA	-11.3
Denatured BSA	6.15
Gold NPs(Au)	36.8
Normal BSA with Au	44.8
Denatured BSA with Au	44.9

The study of the equilibrium between the native (folded) and denatured (unfolded) conformations of proteins is usually done using the flexible technique of DSC, which can be used to analyze the structural transition between two physical states [28]. The DSC for nBSA Figure 4a displays a narrow and modest endothermic peak at 75 °C that is indicative of the denaturation process [29,30]. Large endothermic peaks in dBSA (Fig. 4b) develop at 80°C. The DSC curves of AuNPs with their distinctive peaks at 85,49 °C are shown in Figure 4c. The peak shifted to 90.77°C when nBSA was added to AuNPs (Fig. 4d), and to 102.79°C when AuNPs were added to dBSA (Fig. 4e).

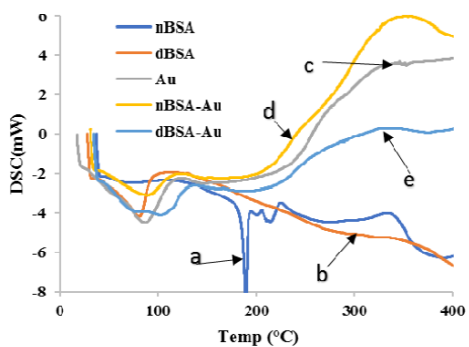


Figure 4: Differential scanning calorimetry (DSC) for (a) normal Bovine serum albumin (nBSA), (b) denatured Bovine serum albumin (dBSA), (c) gold nanoparticles (AuNPs), (d) normal bovine serum albumin with gold nanoparticles (nBSA-AuNPs), (e) denatured bovine serum albumin with gold nanoparticles (dBSA-AuNPs)

3. Conclusion

BSA's structure and conformation are easily modifiable, and it is critical for many crucial physiological processes. Through covalent bonds or physical interactions, gold nanoparticles can readily form conjugates with proteins. These conjugates have found extensive use in the biomedical fields of targeted drug delivery, bioimaging, and diagnostics. It is essential to conduct a thorough investigation of the interactions between gold nanoparticles and BSA to develop an efficient gold nanoparticle-protein system for biological applications. This is crucial for the use of the gold nanoparticle-BSA bioconjugates in nano biosensors, disease diagnosis, drug delivery, biological labeling and imaging, as well as other applications where biological activity is required.

4. Conflicts of interest

"There are no conflicts to declare".

5. Acknowledgments

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6. References

- [1] Roy. S and Das. K. T, Spectroscopic Studies of Interaction Between Biologically Synthesized Silver Nanoparticles and Bovine Serum Albumin, *Journal of Nanoscience and Nanotechnology*, Vol. 14, 4899–4905, 2014.
- [2] Barbosa. S. R. L, Ortore. G. M, Spinozzi. F, Mariani.P, Bernstorff. S, and Itri. R, The Importance of Protein-Protein Interactions on the pH-Induced Conformational Changes of Bovine Serum Albumin: A Small-Angle X-Ray Scattering Study, *Biophysical Journal* Volume 98 January 2010 147–157.
- [3] Housni. A, Ahmed. M, Liu. Sh, and Narain. R, Monodisperse Protein Stabilized Gold Nanoparticles via a Simple Photochemical Process, *J. Phys. Chem. C* 2008, 112, 12282–12290.
- [4] Kong. Y. F, Zhang. W. J, Li. F. R, Wang. X. Z, Wang. J. W and Wang. W, Unique Roles of Gold Nanoparticles in Drug Delivery, Targeting and Imaging Applications, *Molecules* 2017, 22, 1445.
- [5] Sugunan. A, Thanachayanont. C, Dutta. J, and Hilborn. G. J, Heavy-metal ion sensors using chitosan-capped gold nanoparticles, *Science and Technology of Advanced Materials* 6 (2005) 335–340.
- [6] Mariam. J, Sivakami. S, and Dongre. M. P, Albumin corona on nanoparticles – a strategic approach in drug delivery, *Drug Deliv*, 2016; 23(8): 2668–2676.
- [7] Malathi. S, Balakumaran. D. M, Kalaichelvan. T. P, et al., Green Synthesis Of Gold Nanoparticles For controlled Delivery. *Advanced Materials Letters*, 2013. 4(12): p. 933-940.
- [8] Sampath. M, Kalaichelvan. P. B, et al., Green Synthesis Of Gold Nanoparticles For Controlled Delivery. *Advanced Materials Letters*, 2013. 4: p. 933-940.
- [9] Picollo. M, Aceto. M, Vitorino. T, UV-Vis spectroscopy, *Physical Sciences Reviews*. 2018; 20180008.
- [10] Chaturvedi. K. S, Ahmad. E, Khan. M. J, Alam. P, Ishtikhar. M and Khan. H. R, Elucidating the interaction of limonene with bovine serum albumin: a multi-technique approach, *Molecular BioSystems*, 2015, 11, 307.
- [11] Afaghi. P, Lapolla. A. M, Ghandi. K, Determining the degree of denaturation of bovine serum albumin using a new UV analysis technique, *Chem Rep*, 2021, 3(1): 173-176.
- [12] Girón de la Cruz. L. G, Barceinas-Sánchez. O. D. J and Gómez-Ramírez. M, Study of a Denatured Bovine Serum Albumin Solution Used as Lubricant in Tribological Testing of Total Knee Replacements, 2022, 297. https://doi.org/10.1007/978-3-030-82064-0_7
- [13] Nghiem. L. H. Th, La. H. Th, Vu. H. X, Chu. H. V, Nguyen. H. Th, Le. H. Q, Fort. E, Do. H. Q and Tran. N. H, Synthesis, capping and binding of colloidal gold nanoparticles to proteins, *ADVANCES IN NATURAL SCIENCES: NANOSCIENCE AND NANOTECHNOLOGY*, 1 (2010) 025009 (5pp). <http://dx.doi.org/10.1088/2043-6254/1/2/025009>
- [14] XiuJuan. Sh , Dan. L, Jing. X, Shawn. W, ZhaoQiang. W and Hong. Ch, Spectroscopic investigation of the interactions between gold nanoparticles and bovine serum albumin, *Chinese Science Bulletin*, 2012, 57: 1109–1115.
- [15] Berthomieu. C and Hienerwadel. R, Fourier transform infrared (FTIR) spectroscopy, *Photosynth Res* (2009) 101:157–170.
- [16] Mirhane Mostafa Darwish, Mona S Elneklawi & Ebtesam A Mohamad. (2023). Aloe Vera coated Dextran Sulfate/Chitosan nanoparticles (Aloe Vera @ DS/CS) encapsulating Eucalyptus essential oil with antibacterial potent property, *Journal of Biomaterials Science, Polymer Edition*, 34:6, 810-827.
- [17] Ali Abeer A., El-Gebaly Reem H., Mohamad Ebtesam A. N-Acetylcysteine encapsulated

- niosomes as antitumor nanoparticles. *Egypt. J. Chem.* Vol. 66, No. 5 pp. 379 - 384 (2023)
- [18] Purohit R, Vallabani N. V. S, Shukla. R. K, Kumar. A, and Singh. S, Effect of gold nanoparticle size and surface coating on human red blood cells. *Bioinspired, Biomimetic and Nanobiomaterials*, 2016.
- [19] Elshemey. M. W, Mohammad. A. I and Elsayed. A. A, Wide-angle X-ray scattering as a probe for insulin denaturation, *International Journal of Biological Macromolecules* 46 (2010) 471–477.
- [20] Pramanik. S, Banerjee. P, Sarkar. and Bhattacharya. Ch. S, Size-dependent interaction of gold nanoparticles with transport protein: A spectroscopic study, *Journal of Luminescence* 128 (2008) 1969–1974
- [21] Nghiem. L. H. Th, La. H. Th, Vu. H. X, Chu. H. V, Nguyen. H. Th, Le. H. Q, Fort. E, Do. H. Q and Tran. N. H, Synthesis, capping and binding of colloidal gold nanoparticles to proteins, *ADVANCES IN NATURAL SCIENCES: NANOSCIENCE AND NANOTECHNOLOGY*, 1 (2010) 025009 (5pp). <http://dx.doi.org/10.1088/2043-6254/1/2/025009>
- [22] Ebtesam A. Mohamad, Marwa A Ramadan, Marwa M. Mostafa, and Mona S. Elneklawi. Enhancing the antibacterial effect of iron oxide and silver nanoparticles by extremely low frequency electric fields (ELF-EF) against *S. aureus*. *Electromagnetic Biology and Medicine*. In press. 2023.
- [23] Santos. B. M, Carvalho. W. C and Garcia-Rojas. E. E, Heteroprotein complex formation of bovine serum albumin and lysozyme: Structure and thermal stability, *Food Hydrocolloids*, 74 (2018) 267e274.
- [24] Misra. K. P, Dash. U and Maharana. S, Investigation of bovine serum albumin-surfactant aggregation and its physicochemical characteristics, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 483 (2015) 36–44.
- [25] Shanmugaraj. K & Ilanchelian. M, Colorimetric determination of sulfide using chitosan-capped silver nanoparticles, *Microchim Acta* (2016) 183:1721–1728
DOI 10.1007/s00604-016-1802-y
- [26] Amr A. Abd-Elghany, Ebtesam A Mohamad. Chitosan-coated niosomes loaded with ellagic acid present antiaging activity in a skin cell line. *ACS Omega* 2023 8 (19), 16620-16629
- [27] Patila. S, Sandberg. A, Heckert. E, Selfc. W, and Seal. S, Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential, *Biomaterials*. 2007 November; 28(31): 4600–4607.
- [28] Rezwani. K, Meier. P. L, Gauckler. J. L, Lysozyme and bovine serum albumin adsorption on uncoated silica and AIOOH-coated silica particles: the influence of positively and negatively charged oxide surface coatings, *Biomaterials* 26 (2005) 4351–4357.
- [29] Santos. B. M, Carvalho. W. C and Garcia-Rojas. E. E, Heteroprotein complex formation of bovine serum albumin and lysozyme: Structure and thermal stability, *Food Hydrocolloids*, 74 (2018) 267e274.
- [30] Ebtesam A Mohamad, Amany M. Gad, Rana H. Abd El-Rhman, Mirhane M. Darwish. Chitosan and Aloe Vera decorated nanoparticulate system loaded with Minoxidil as a suggested topical formulation for alopecia therapy. *Adv. Nat. Sci: Nanosci. Nanotechnol.* 2023; 14, 025002.