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Colorimetric Detection of Bovine Serum Albumin (BSA Protein) by Interaction and Modification of Silver Nanoparticles

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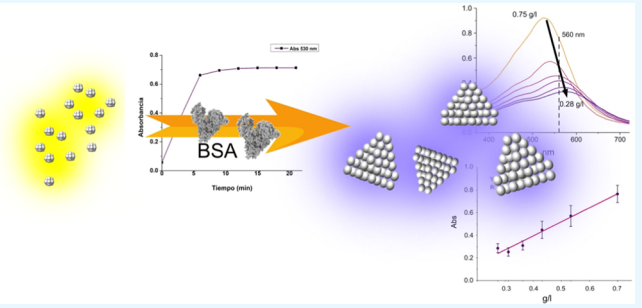
ABSTRACT: The detection of serum albumin is of great relevance because its presence in urine above normal levels is implicated in different pathologies, such as Diabetes Mellitus and Preeclampsia. The main objective of this work was to develop a protocol to sense serum albumin using the well-known unusual optical phenomenon that nanoparticles present, which is called surface plasmon resonance (SPR), as well as the influence of proteins on the size and morphology of nanoparticles, and consequently, on the SPR. The interaction of these nanoparticles with proteins forms biocoronates, which modify the optical and morphological properties of nanostructures. This behavior could be important for the construction of colorimetric sensors for medical or environmental applications. Considering the above, in the present study, we propose to sense Serum Albumin in the presence of silver nanoparticles (AgNps) for its determination and quantification under various physiological conditions that simulate the environment of human urine. The analysis of the growth of small AgNps (seeds) in the presence of the protein generated colorimetric changes, which were a function of pH, urea content, and chloride concentration. The presence of Serum Albumin also produced variations in the morphology and size of nanoparticles. With this methodology, the quantification of BSA was determined in a concentration range between 0.28 and 0.75 g/L and in less than 5 min of reaction.



INTRODUCTION

Silver and copper nanoparticles are known for their interesting optical, biological and electronic properties, which depend not only on their size but also on their morphology.[1] The large surface/volume ratio they provide facilitates the interaction and their reactivity with the environment.[2] One of the properties of metallic nanoparticles, which is presented by silver and copper Nps, is a phenomenon known as "surface plasmon resonance" (SPR). SPR corresponds to the resonance resulting from the interaction between the conduction electrons of the metal nanoparticles and the incident photons, and is directly related to the size and shape of the nanostructures.[3] This describes the electromagnetic field that is produced by the collective oscillations of material conduction electrons, having an important role in the optical properties of various metals. An electromagnetic field resulting from the collective oscillations of the material's conduction electrons is described, which is closely related to the optical properties of several metals.[4] Recent studies have shown that proteins can interact with nanomaterials by modifying their optical properties.[5] Among them, albumin, which is a water-soluble globular protein. Its detection is of great importance in the clinical setting, since human urine can present this protein at concentrations of up to 100 mg/day;[6] However, values above 30 mg/day imply the appearance of different pathologies, such as high blood pressure, preeclampsia, kidney failure, and type I and II.[7] Several methods are used to detect BSA, including the use of test strips, short plastic strips with small indicator pads, and impregnation with different chemical reagents that react with abnormal substances in the urine, generating a colorimetric change.[8] Although it is one of the simplest and cheapest methods to detect anomalies related to potential diseases, sometimes the analyses result in false positives, which are generated by the effects of pH, the presence of oxidizing agents, and/or antibiotics.[9] Bovine serum albumin (BSA) has the ability to interact and change its conformational state on the surface of metal nanoparticles[10] and thus modify its optical properties, which are directly detected by spectrophotometry.[11] Considering the above, particularly the ability of proteins to modulate the optical properties of silver nanoparticles, this research topic has attracted much attention because of the potential application of these nanostructured systems in colorimetry. The development of these systems to generate biosensors would allow the detection of proteins and other biomolecules of interest in medicine.[12] Despite the relevance of achieving the analytical

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objective, the protein-nanoparticle interaction has been little studied, and the relationship with the detection limits and interferents, such as pH, salinity, temperature, and concentration, are not well understood. The purpose of this study was to describe the interaction of AgNps (seeds) with BSA and develop a protocol to quantify this protein as a function of pH and the concentration of urea and chloride. Likewise, variations in the morphology and size of the nanoparticles were examined using transmission electron microscopy (TEM). The reported methodology allows quantification of BSA in less than 5 min of the reaction.

METHODOLOGY

Seeds Silver Nanoparticles Synthesis (AgNp). Silver seeds were synthesized following the protocol described by Chakraborty et al.[5] In a 100 mL flask, 25 mL of a 500 μM silver nitrate solution was mixed with 25 mL of a 500 μM sodium citrate (CytNa) solution at 1200 rpm and 90 °C, and then 10 μL of 0.24 M sodium borohydride solution was added under vigorous stirring and allowed to react for 20 min. Finally, the absorbance was measured using ultraviolet−visible (UV−vis) spectroscopy.

Characterization of Silver Nanoparticles (Seeds). The UV−visible absorption spectra of the Ag seeds were analyzed in the wavelength range 350−800 nm, using a Shimadzu UV 1800 spectrophotometer. The morphologies of the nanostructures were examined using high-resolution transmission electron microscopy (TEM, HITACHI model HT7700). The samples for TEM were prepared by supporting microdroplets of suspensions on copper grids (400 mesh), which were dried at room temperature (25 °C) before examination under the microscope.

Interaction of Nanoparticles in the Presence of BSA. To study the interaction between proteins and nanoparticles, an aqueous mixture containing 10 μL of a 0.1 M solution of silver nitrate, 300 μL of a silver seed colloid, and 30 μL of 0.1 M ascorbic acid was prepared, making up to 10 mL with aqueous solutions of BSA at different concentrations (0.28−0.75 g/L). The reaction mixture was maintained under constant stirring at room temperature for 20 min. Changes were monitored every 1 min by UV−vis spectroscopy using a Multiscan Thermo instrument.

Effect of Physiological Parameters on the AgNp-BSA Interaction. The effects of pH, chloride, and urea concentrations on AgNp-BSA interactions were evaluated. In the first case, the experiment was performed by adjusting the prepared 50 g/L BSA solution to pHs between 3.5 and 8.5, leaving the solution adjusted to pH 7 as a control solution. Adjustment to acidic pH was performed by adding drops of nitric acid (0.1 M) to the BSA solution, and adjustment to basic pH was performed by adding drops of 0.1 M sodium hydroxide. Finally, the absorbance of each solution was measured by UV−vis spectroscopy (350−750 nm) in a 96-well plate, following the spectroscopic response every 1 min for 20 min in Thermo Multiskan GO. To study the effect of salinity, different volumes of NaCl were added to the albumin solution (Table 1), and a 110 mM NaCl solution was prepared as a control, which was similar to that found in human urine.[13] The effect of chloride ions on the reaction was eliminated by mixing a 0.1 M $AgNO_3$ solution in an Eppendorf tube with a solution prepared as follows: 1 mL of a 50 g/L BSA solution and 320 μL of a 110 mmol/L NaCl solution. The mixture was centrifuged at 14,000 rpm for different times (1, 3, 5, 7, and 9 min). Then, 90 μL of the obtained supernatant was added to a mixture of silver seeds (300 μL), silver nitrate (10 μL, 0.1 M), and ascorbic acid (30 μL, 0.1 M) in 10 mL of Milli-Q water. Each reaction was monitored by UV−vis spectroscopy to evaluate changes in the interaction with the protein. The urea assay was performed by adding 320 μL of a 200 mM solution, considering the normal values of this compound in the body[13] to 3 mL of 50 g/L BSA, which was added to the mixture containing silver seeds and ascorbic acid as a reducing agent, and monitoring was performed by UV−visible spectroscopy for 20 min.

Table 1. Mixtures of NaCl and BSA to Evaluate Chloride Ion Interference

solution	BSA (mL) [50 g/L]	NaCl (μL) [0. 11 M]
1	3	270
2	3	80
3	3	40
4	3	20
5	3	0

RESULTS AND DISCUSSION

Formation of Seed Nanoparticles (AgNP). Silver nanoparticles (seeds) are formed immediately upon the addition of the reducing agent, sodium borohydride, to the reactor, which contains the silver precursor and sodium citrate, the latter acting as a stabilizing agent. The solution obtained was pale yellow with maximum absorption at 392 nm.[14] The average size of the spherical nanostructures was 12 nm (see Figure 1). The nanoparticle suspension was stable for more than 3 weeks when kept in the dark at 4 °C. At room temperature and/or under light, the suspension gradually changed to a green color. This color change corresponds to the slow formation of triangular prisms that are larger than the seeds.[15] This photophysical phenomenon can interfere with the detection of BSA; therefore, the silver precursor must be stored in the dark at a low temperature.

AgNp-BSA Interaction. When silver nanoparticle seeds were grown with BSA and silver ion solution, the reaction evolved over 10 min through different colors from light yellow to cherry purple until reaching an absorption band at 560 nm, as shown in Figure 2. Transmission electron micrographs showing seeds grown in the presence of BSA protein showed size changes from 7 to 24.5 nm and acquired triangular morphologies with rounded corners.

Triangular silver nanoprisms have an in-plane quadrupole, weak out-of-plane quadrupole, and strong in-plane dipole. Unlike spherical nanoparticles, the sharp tips and edges of nanoprisms strongly enhance the electromagnetic field and SPR bands due to the lightning rod effect.[16] Calculations using the DDA technique were performed to model this type of spectrum in perfect prisms and in prisms modeled as trimmed at their three vertices. The spectra of triangular prisms with 10 nm cut edges in an aqueous medium show a blue shift. For triangles with perfect vertices, the shift ranged from 740 nm to approximately 600 nm. Other shorter wavelength absorption peaks associated with silver triangles have been shown to be less sensitive to vertex cuts.[17] From the calculated spectra, it was possible to associate two peaks and two polarizations along the longitudinal axes of the prism (lateral and perpendicular bisector) that could be assigned to the 470 and 560 nm signals (plane polarization) to the rounded prisms. The growth

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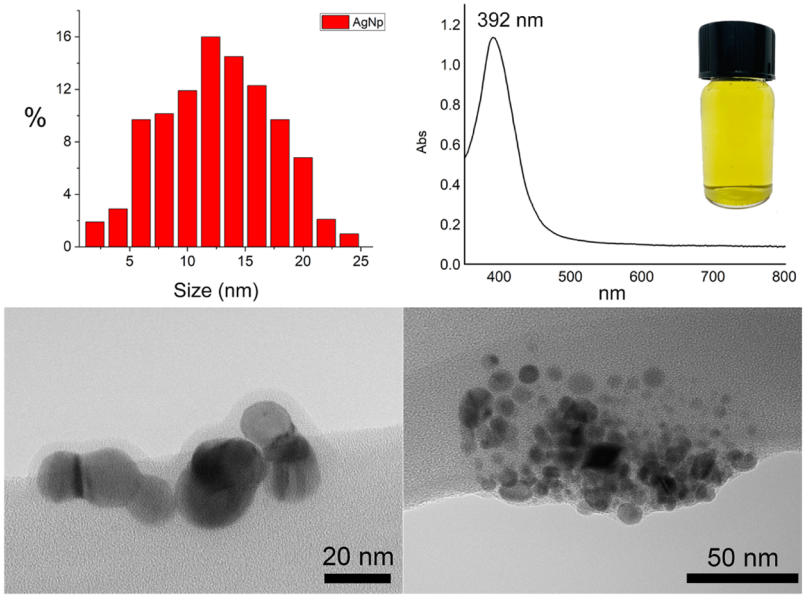

Figure 1. AgNp seeds: In the upper left corner the size distribution is plotted; the upper right corner shows the AgNP solution with its characteristic color. In the lower part, the morphological characterization via TEM.

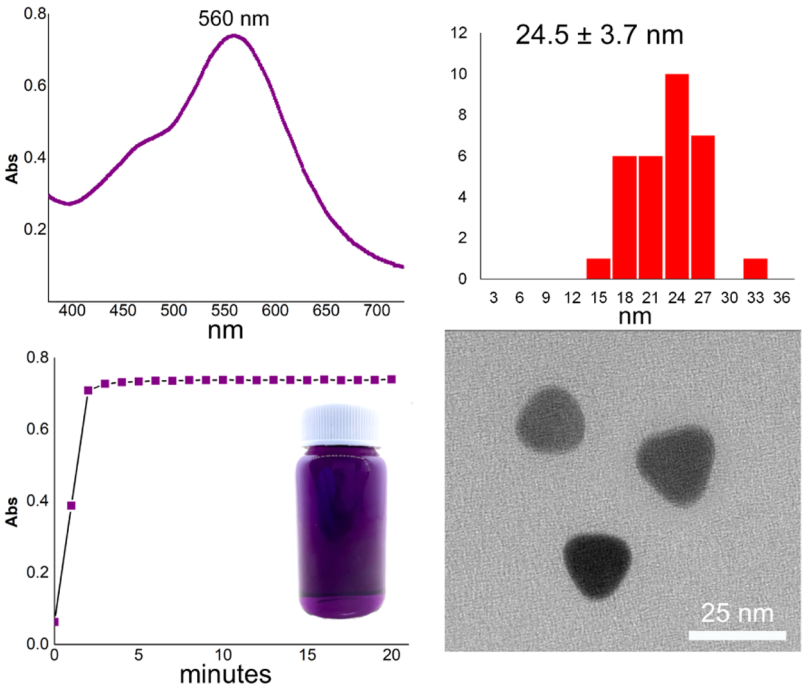

Figure 2. Nanoparticle−protein interaction reaction (AgNp-BSA). In the upper left corner the absorption spectrum of the reaction is plotted after 20 min. In the lower left part the reaction kinetics and on the right the morphological characterization via TEM.

reaction of silver nanoseeds in the presence of BSA, silver ions and the reducing agent occurs in less than 4 min and with a gradual color change until reaching a violet color (dark magenta). The final color was obtained at room temperature, under constant stirring or shaking, and was stable for at least 20 min.

The surface plasmon resonance phenomenon shown by silver nanoparticles generates a spectrum of colors in solution when a light beam falls on the metal surface, varying from yellow to blue[18] and also dependent on the structural characteristics of the biomolecules that interact with these molecules. The result obtained from the AgNp-BSA reaction shows that the electrostatic and hydrophobic interactions between the metallic surface and the serum albumin generated a shift in the absorption maximum toward the zone close to blue in the UV−vis spectrum.

For this reason, the solution presents a cherry purple color with a maximum at 560 nm[5] because the AgNp have the particularity that their optical activity depends on the morphology and structure adopted. Studies have shown that the interaction between NPs and proteins depends on the type of protein and the surface chemistry of the nanoparticles[19] and, therefore, the shape acquired by the metallic nanoparticles. In our case, the morphology obtained coincides with the results of

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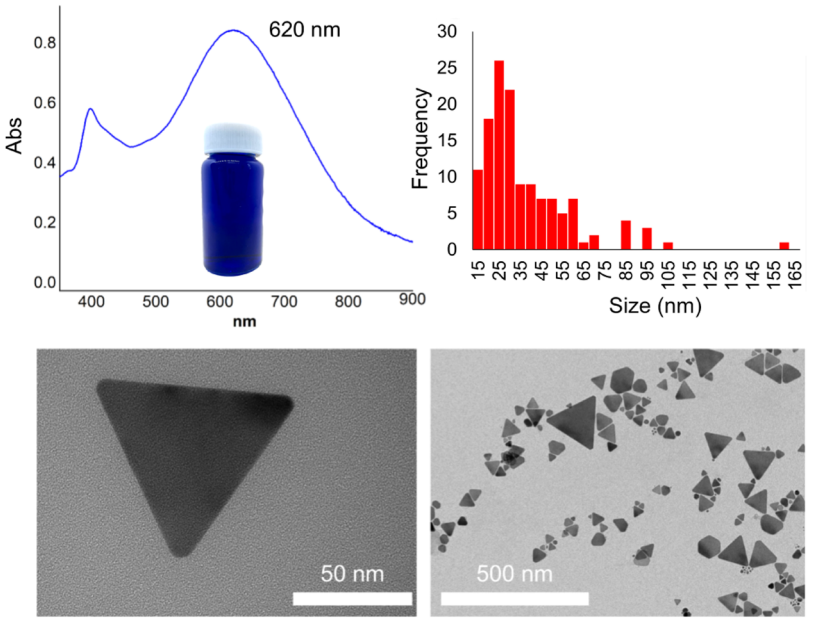

Figure 3. AgNp reaction with reducing agent in the absence of BSA: in the upper left corner the absorption spectrum of the reaction is plotted; the upper right corner shows the solution with its characteristic color. In the lower part, the morphological characterization via TEM.

previous experiments, in which it was concluded that most of the metallic nanoparticles had the shape of triangular nanoprisms with rounded vertices, and sizes of approximately 25 nm.[5] Therefore, this nanoparticle−protein interaction not only increased the size of the silver nanoparticle, but also changes related to the surface plasmon phenomenon, that is, the absorption of light at different wavelengths is reflected in the different colors obtained at, as the nanoparticle changes shape.[20]

In the absence of BSA the reaction reaches a blue color with an absorption maximum of 620 nm and the spherical nanoparticles acquire a triangular morphology, with sharper vertices (Figure 3). Even the size of the triangular prisms are larger in the absence of BSA, reaching dimensions greater than 50 nm. This is consistent with what was previously described, that is, the presence of ascorbic acid to synthesize larger AgNPs. On the other hand, the presence of hexagonal shapes is observed, which are explained as incomplete growths of nanoprisms and truncated triangles, whose lengths varied from 40 to 110 nm.[21]

Concentration Effect: Serum Albumin Detection Limit. To evaluate the detection limit as a colorimetric sensor with albumin, 5 dilutions of BSA were carried out and which were later analyzed by UV−vis spectroscopy. The results show that as the concentration of BSA decreased, the color of the solution varied from 530 nm to a dark purple of approximately 580 nm (Figure 4).

With these results it was not possible to obtain a linear trend of absorbance at a single wavelength vs concentration, but different absorption spectra in the visible range and establish a characteristic wavelength to evaluate the effect of BSA concentration. Aqueous solutions with orange colors indicate the formation of silver nanohexagons, which is characteristic of the growth and previous formation of triangular prism. These forms are those that absorb in the range of 341 to 498 nm and were obtained at The highest concentration of BSA (0.75 g/L).[22] In the case of the two most dilute concentrations (0.28 and 0.32 g/L) the absorption spectrum shows a red shift,

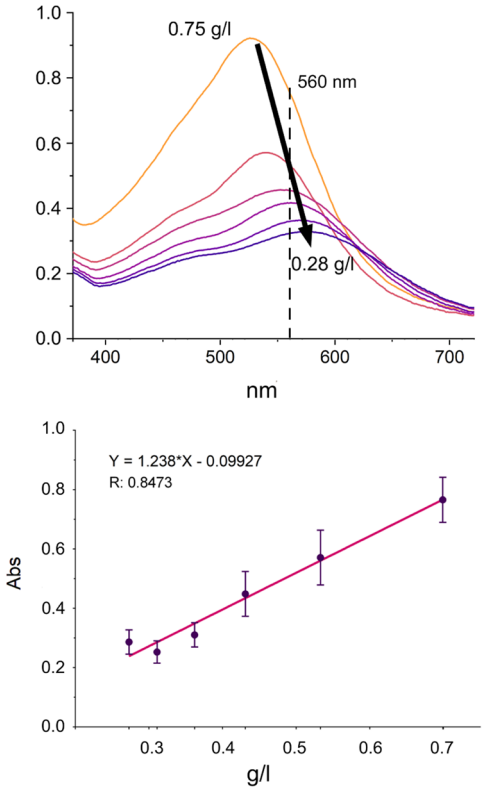

Figure 4. Analysis by UV−vis spectroscopy of the stability of the AgNp-BSA interaction under different concentrations of BSA.

reaching a maximum absorption at 575 nm (bluish purple) linked to the formation of triangular prisms with rounded vertices. Studies have shown that bands around 672 nm are related to a complete morphological change in the metallic nanoparticle, toward a triangular nanoprism with well-defined vertices with sizes ranging from 40 to 110 nm.[21] Therefore, the band with the lowest absorption could be interpreted as an incomplete state of triangular prisms with sharp vertices.[23] As

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the concentration of BSA increases from 0.37 to 0.46 g/L, there are shifts in the absorption maximum, obtaining purple solutions, where the nanoparticle again presents morphological changes. In this case, the hexagons acquire the shape of triangular nanoprisms with rounded vertices and absorb at approximately 560 nm. Finally, at higher concentrations of BSA (0.56−0.75 g/L), the solutions reach red or orange colors, which are linked to morphological changes of the silver nanoparticles, from a sphere to the formation of hexagons,[24] and eventually with a small amount of triangular prisms.[23] Detection limit analysis was performed in triplicate experiment. Although the trend line obtained does not present an R value close to 0.99, these results allow us to infer that BSA is detectable at different concentrations (0.28−0.75 g/L), and can be easily identifiable by change in color. Therefore, this detection method could be a potential alternative to assess serum albumin levels in diluted human urine, in less than 5 min, with the naked eye and up to 0.28 g/L.[7,25]

Effect of Physiological Parameters against AgNp-BSA Interaction. *pH Effect.* The variation in pH values generated differences in the absorption spectra for each solution. During the first 7 min at pH 3.5 and 4.5 the solutions remain yellow, but after 10 min the solutions became almost colorless and light blue (582 nm).

At pH 5.5 and 6.5, the reaction accelerates, changing color after 7 min, from intense yellow to bluish purple and purple, respectively, maintaining the color stability for more than 30 min. On the other hand, the pH 7.5 solution acquires a slightly darker purple color (554 nm) than the control solution, reaching color stability after 4 min of reaction. Finally, at pH 8.5 the solution acquires a reddish color acquired a reddish color (503 nm) after 10 min of reaction and without subsequent variations (Figure 5).

The results of the UV−vis spectra are shown in Figure 5 (bottom). These results demonstrated that the kinetics and stability of the reaction was pH-dependent. This phenomenon may be associated with the fact that proteins, such as bovine serum albumin, can reversibly vary their native conformation (pH = 7.4) with a change in the solution pH.[26] Extreme pH values trigger a change in the net charge of the protein, generating electrostatic repulsion and the destruction of hydrogen bonds;[27] therefore, the reddish color at pH 8.5 may be due to a change in the AgNp-BSA interaction due to a conformational change in serum albumin; therefore, its detection using metallic nanoparticles could be notably limited.[28] The pH of the reaction medium is one of the factors that influences the synthesis of silver nanoparticles, because it is directly related to stability (dissolution, formation of oxides, etc.). The results obtained show that the kinetics of the nanoparticle−protein interaction reaction is related to these variations in the pH of the medium. On the other hand, recent studies have shown that by increasing the pH of the solution, the average size of the nanoparticles decreases due to an increase in the reaction rate; the larger nanoparticles being observed at pH = 7.0 and the smaller ones at pH = 11.0.[29]

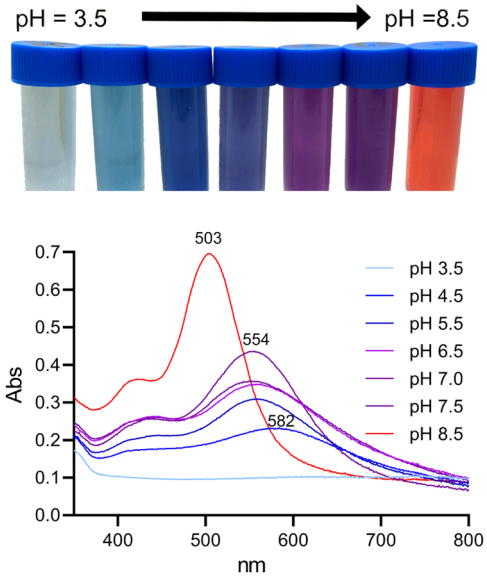

Figure 5. AgNp-BSA interaction in the presence of different pH in solution. Image shows the colors acquired by each solution. Analysis by UV−vis spectroscopy of the stability of the AgNp-BSA interaction in the presence of different pH.

Furthermore, changes in the absorption maxima in the obtained spectrum can be directly related to changes in the morphology of the AgNPs. This phenomenon is directly associated with the surface plasmonic resonance described above, which varies depending on the manner in which the metal surface interacts with the protein, which explains the different color changes in the solution.

Based on the TEM studies performed by Marciniak et al.,[29] silver nanoparticles are expected to have sizes of approximately 11, 43, and 35 nm at pH 6.0, 7.0, and 8.0, respectively. The reasons for the size changes may be due to the fact that at pH below 7.0, sodium citrate is partially protonated, so its coordination bond with silver is not as strong, decreasing its stabilizing effect. Furthermore, silver nanoparticles at pH between 7.0 and 8.5 may present size differences, attributed to the slow reduction rate of the silver precursor salt, in addition to a control between the nucleation and growth processes.[30] On the other hand, BSA also undergoes changes in its native conformation when exposed to changes in the pH of the medium. This biomolecule has an isoelectric point between 4.7 and 5.2 with a negative charge at neutral pH. Below pH 4.0 and above pH 8.0, BSA changes its folding conformation, which differs from its native structure.[27]

Previous studies have shown that the main structural changes of the protein occur at pH 2.7, 4.3, 8.0, and 10.0,[26] respectively. Recent research has shown by dynamic light scattering (DLS) analysis that at pH 4.5 the size of BSA increases to the 20−50 nm range, in contrast at pH 7.0 where the sizes were in the 5−10 nm range.[27]

Given that pH is one of the best-known denaturing factors for biomolecules, it is inferred that at lower values 4.0, serum albumin generate morphological changes that can interfere with the structure of the silver nanoparticle and generate almost colorless solutions. Regarding pH 5.5−7.5, BSA presents its native conformation, so in this case, the adsorption of the protein on the metal surface allows its detection with the characteristic color of absorption band at 560 nm. Finally, the red color (503 nm) of the solution at pH 8.5 could be associated not only with a BSA conformational change, but also with a morphology in the silver nanoparticles, which is associated with the appearance of nanohexagons.[5] Therefore, and based on the above, the results obtained in the pH analysis allow us to confirm that these changes in the medium affect both the growth of the nanoparticle, as well as the structural

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change of bovine serum albumin, and that the changes of color could be used to detect these differences.

Chloride Effect. The presence of chloride ions in solution can interfere with the nanoparticle−protein interaction, since chloride reacts with Ag^+ ions to form the insoluble silver chloride salt AgCl.[31] In order to evaluate the behavior of the AgNp-BSA interaction and chloride ions, 3 mL of a BSA solution (50 g/L) was mixed with different volumes of 0.11 M NaCl (see Table 1). The reaction was carried out with the silver seeds, according to the procedure described above.

The colorimetric results show that the presence of this ion generates changes in the color of the solution as seen in Figure 6, varying from yellow to orange when the concentration of

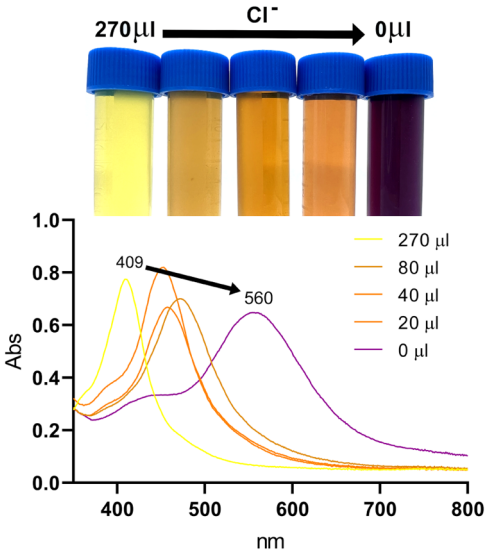

Figure 6. AgNp-BSA interaction in the presence of the chloride ion with different volumes in solution. The image shows the colors acquired by each solution. UV−vis spectra of the AgNp-BSA interaction in the presence of Chloride ion at different concentrations.

chloride ions is high and to purple when the concentration decreases to zero. Therefore, this methodology can be considered as interference in the detection of BSA.

To remove this interference from the reaction, 320 μL of $AgNO_3$ was added to an aqueous solution of 1 mL of 50 g/L BSA and 320 μL of 110 mmol/L NaCl in an Eppendorf tube, and the mixture was centrifuged at 14,000 rpm for different times, as shown in Table 2. In each tube, a white precipitate was obtained, corresponding to the salt of silver chloride (AgCl) and a supernatant that corresponded to serum albumin.

As mentioned above, 90 μL of the supernatant was added and mixed in a 20 mL vial, and the AgNP-BSA interaction reaction was carried out. The results obtained were solutions

Table 2. Chloride [Cl^-] Centrifugation Analysis at Different Times

revolutions per minute (RPM)	minutes	solution color
14,000	1	cherry purple
14,000	3	cherry purple
14,000	5	bluish purple
14,000	7	bluish purple
14,000	9	purple

with slight color changes (see Figure 7) and were dependent on the centrifugation time, reaching a maximum absorption of

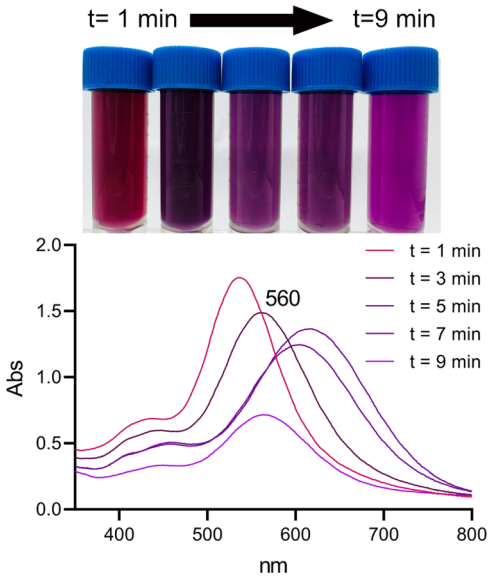

Figure 7. Colors acquired from the solutions after the AgNp-BSA interaction and after eliminating the chloride ion at different centrifugation times.

560 nm after 3 min; thus, it can be deduced that the chloride ion can be eliminated from the solution.

Studies conducted by Li et al.[32] demonstrated that the stability of AgNp is strongly affected by the presence of chloride ions due to the formation of an AgCl layer on the AgNp and the formation of aggregates with irregular shapes and sizes.

The different absorption maxima show that the presence of sodium chloride (NaCl) is an interference with the reaction. In this way, this anion can directly affect the final shape and size of the AgNPs and cause a decrease in the interaction between BSA and the metal surface.[33] This interference allows us to understand the color changes of the solution when varying the volumes of salt, where the yellow colors obtained with 270 μL of NaCl are related to a spherical morphology, whereas the orange colors are associated with nanohexagons with absorption maxima close to the 455 nm.

These results demonstrate that the presence of this ion turns out to be an interference for the detection of bovine serum albumin, which is why a method is required that allows it to be eliminated from the solution. One of the most used techniques in the clinical laboratory is centrifugation, a method to separate molecules that have different densities by rotating them at high speed.[34] Additionally, proteins are usually precipitated in the presence of heavy metals, such as silver and mercury. However, High volumes of these generate irreversible changes in the biomolecules, triggering their denaturation, resulting in the loss of their biological activity,[35] so special care was taken in the volume of the silver precursor, which was added to the Eppendorf tube to precipitate the chloride in the form of silver chloride (AgCl) and thus not generate electrostatic repulsions in BSA. On the other hand, the results of the absorption spectra obtained indicate that after 3 min of centrifugation, there is no interference in the solution; therefore, the adsorption of the protein and the AgNPs occurs without inconvenience. But, after 9 min of centrifugation, it is inferred

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that at this time the protein could have precipitated; because excessive mechanical Agitation also turns out to be a denaturing agent for biomolecules,[36] which explains why the solution did not acquire the most intense cherry-purple color characteristic of the solution.

Urea Effect. In the absence of urea, the absorption maximum was 560 nm, whereas in the presence of urea, the reaction reached an absorption maximum of 573 nm.

These results were evaluated in pH ranges similar to those of urine (pH 5.46 and 7.00), as shown in Figure 8. Comparing

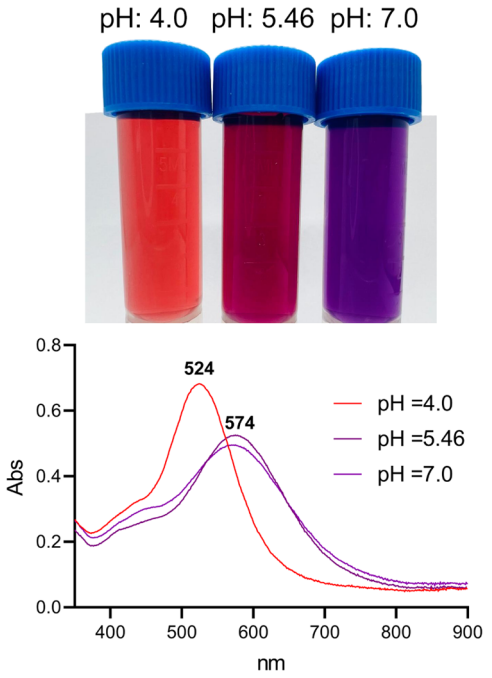

Figure 8. UV−vis spectra of the AgNp-BSA interaction after precipitating the chloride ion as AgCl by centrifugation at different times.

the results with those obtained when the effect of pH was studied, it is evident that urea also plays an important role in the surface and optical changes of the AgNPs.

Urea is a chemical compound that is characterized by being one of the main denaturing agents of proteins, since it acts by destabilizing the hydrophobic interactions that are formed in the stable core of biomolecules, in addition to breaking hydrogen bonds.[37]

The pH of the urine of a patient with diabetes mellitus is approximately 5.45.[38] and in women with preeclampsia around 5.46. When performing the same analysis with pH adjustment in the BSA solution, the characteristic cherry-purple color of the reaction reached a maximum of 573 nm. Therefore, adjusting the pH to values closer to 7.0 would allow optimal detection of BSA at 560 nm.

UV−vis spectroscopy results showed that at pH 4.00, there was an absorption maximum at 528 nm, and the color of the solution changed from purple to red. In this case, urea could interfere with the detection of BSA in human urine. At a pH below 5.5, the high presence of urea may reflect the occurrence of diabetic ketoacidosis, which is associated with elevated urine protein levels and other solutes that exceed the normal concentration.[39] Therefore, at pH values in the range of 5.60−7.00, the absorption maximum is optimal for the detection of BSA in human urine samples.

CONCLUSIONS

The growth of silver nanoparticles from their "seeds" in the presence of biomolecules such as BSA revealed modifications in their sizes and morphology; prisms with rounded vertices were obtained, whose suspension presented a maximum absorption at 560 nm. Varying the BSA concentration in the nanoparticle−protein reaction produced colorimetric changes in the reaction mixture. The changes in absorbance at 560 nm follow a linear behavior with the BSA concentration, and the construction of the graph of absorbance versus BSA concentration allowed sensing, in less than 5 min of reaction and in the physiological pH range, the BSA content in solution in a range between 0.28 and 0.75 g/L Regarding the influence of urea on the AgNp-BSA interaction, the results indicate that at pH values below 5.0 urea can interfere with the AgNp-BSA interaction. However, at pH values close to 7.0, the interaction was maximal, revealing an absorption maximum at a wavelength close to 560 nm. The influence of chloride ions on the AgNp-BSA interaction also interferes with the affinity of the halogens for silver ions. However, these ions could be removed from the solution by centrifugation. In summary, our results show the optical behavior of the interaction between AgNPs and albumin, allowing their detection and quantification under various physiological conditions that simulate those of human urine. The analysis protocol will allow the analysis of albumin and, consequently, the early diagnosis of pathologies, such as Diabetes Mellitus, and prediction of the risk of preeclampsia.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c07828.

Colorimetric detection reaction of BSA in the presence of silver nanoseeds, Ag^{+} and ascorbic acid, the video speed was increased 12 times (MOV)

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Author Contributions

Conceptualization, M.I.A. and M.A.; methodology, M.A., M.W., and M.I.A.; validation, M.I.A. and M.P.; investigation, M.I.A. and M.A.; resources, M.P. and M.I.A; writing—original draft preparation, M.I.A. and M.A.; review and editing, M.I.A. and M.A.; funding acquisition, M.A. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

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