A Quinazolinone Based Novel Fluorescent Nanoprobe for Selective Detection of Bovine Serum Albumin: Spectroscopic, Photophysical and Analytical Approach

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Abstract: Fluorescent organic nanoparticles (FONPs) were developed from the 2-phenyl-2, 3-dihydroquinazolin-4 (1H)-one (Compound 1) using a reprecipitation technique. The size and morphology of 2-phenyl-2, 3-dihydroquinazolin-4 (1H)-one nanoparticles i.e. QZNPs were analyzed using DLS and TEM analysis. A novel fluorimetric method has been developed for the determination of microgram quantities of bovine serum albumin (BSA) based on fluorescence quenching of QZNPs at 455 nm. Under the optimal conditions, the decreases in fluorescence intensity were linearly related to BSA concentration in the range of 0.0-2.2 µM with the detection limit of 0.13 µM. There was little interference from amino acids, sugars and most metal ions in the detection of BSA. The Stern-Volmer quenching constant $K_{SV}$ is directly related to temperature, which indicates that binding process involves a dynamic quenching mechanism. The negative value of $\Delta G$ suggested the spontaneity of interaction; similarly both negative value of $\Delta H$ and $\Delta S$ indicated that ‘Van der Waals’ interaction along with hydrogen bond had a major role in the binding process. QZNPs-BSA H-bond complexation is further supported by DLS and zeta potential measurements. Finally, the practical applicability of the QZNPS sensor towards BSA detection in clinical sample was successfully completed.

1. Introduction:

The fact that outcome of sensors depends on optical response is of great interest in chemical, biological and environmental research. Although a large number of sensors have been reported in the literature, there is a big horizon for organic nanoparticles act as an excellent sensor in an aqueous solution. The use of sensors in an aqueous medium is less feasible due to the low solubility of organic molecules in water. These drawbacks can directly affect the sensitivity of the sensor to restrict its applications in various fields. Presently, the research area consisting of the synthesis fluorescent organic nanoparticles as a sensor has grown tremendously. Fluorescent organic nanoparticles (FONPs) in aqueous systems reported as a new spectroscopic approach for the enrollment of organic compounds as sensors in aqueous medium. FONPs were expressing their candidature towards various analytes, simply by modifying their binding attraction. FONPs were prepared by different methods like (microemulsions, laser ablation, direct condensation and reprecipitation method). Therefore, reprecipitation is the most common, easy and economical method, than other methods, used to develop nanoparticles. The current research work is related with the investigation of interaction of bovine serum albumin, and quinazolinones based novel fluorescent nanoprobe its application in commercial samples.

Serum albumins mainly bovine serum albumin (BSA) and human serum albumins are plasma proteins to play an important role in physiological functions and carrier proteins. They are actively participating in the transportation, distribution and metabolism of a disease or symptom acting as a ligands. These ligands consist of fatty acids, amino acids, metals, drugs and pharmaceuticals. A number of reports regarding the binding of metabolites, dyes, fatty acids and various drugs with BSA. The conformational adaptability of BSA binding to the ligands is of a great variety. X-ray diffraction
studies reveal that the principal binding sites on serum albumin are located in the hydrophobic openings. The tertiary assembly of the protein is consisting of domains (three site), and maximum six binding sites are available for the binding of the ligands. Hence, the approach of the binding interaction of a ligand with albumin varies with type of ligand. Considering the strong impact of BSA in physiological functions and to as carrier proteins and utilization of QNPs in aqueous solvent, quinazolinones based fluorescent compounds 1 were synthesized and processed to produce fluorescent organic nanoparticles for sensing application of the BSA in water. Recently, a variety of fluorescent chemosensors have been fabricated for the BSA detection. The various techniques were used to determine proteins, like Bromocresol Green method, Biuret method, Bradford’s method, and Lowry’s method. However, these methods had to suffer from certain limitations such as sensitivity, dynamic range and/or detection limit. The Lowry method has a low sensitivity and inferior selectivity while requiring a complicated operation. The Bradford method suffers from some disadvantages for example poor linearity between the absorbance of the dye (Coomassie Brilliant Blue G-250)-protein complex and the concentration of protein and the complicated measurement procedure. The Bromocresol Green method is unable to detect protein at trace level. These limitations electrochemical method, chemiluminescence method. Electrochemical methods mainly require an expensive modified electrode for protein analysis. LC-ESI-MS used for the analysis of proteins to obtain lower detection limit but these instruments are expensive. Also, the same disadvantage occurs for the instrument LC-MS. These methods are still not free from various types of interference problem. Rayleigh lights scattering methods are useless for the protein determination because these methods affected by interference of many chemicals. However; our quinazolinone based nano sensors can fully overcome, such disadvantages. Likewise, several current sensors are not authenticated for their application in water and commercial analysis for the determination of BSA is considerably improved over that previously conveyed methods.

In this paper, we focus to introduce a novel fluorescent probe develop to detect BSA in an aqueous medium, and to further develop fluorimetric sensor towards BSA in a clinical setting.

2. Experimental

2.1 Materials and Instrumentations

All the chemicals were obtained from Sigma Aldrich/ Spectrochem and were used without further purification. The size and surface charge of the QZNPs was measured using a Malvern Zetasizer (nano ZS-90) equipped with a 4 mW, 633 nm He–Ne Laser (U.K.) at 25 °C under a fixed angle of 90 °C in disposable polystyrene cuvettes. The morphology of QZNPs was assessed by Scanning Electron Microscope (SEM), JEOL model JSM-6360, Japan with 10 kV accelerating voltage in a vacuum. The UV-visible absorption spectra and the steady state excitation and fluorescence spectra were recorded on a UV-VIS-NIR spectrophotometer [Shimadzu UV-3600] and PC-based spectrofluorimeter, JASCO, Japan (Model FP-750). We use Horiba’s Jobin-Yv on IBH time resolved fluorescence spectrometer. This spectrometer uses nanosecond LED’s (352, 389, 420, 472 and 584 nm). The fluorescence lifetimes are measured by time correlated single photon counting method. Lifetimes in the time scales of 500 picoseconds to 1 microsecond are measured at emission wavelength 455 nm by monitoring excitation wavelength to a value of 371 nm.

2.2 Preparation of OZNPs (2-phenyl-2, 3-dihydroquinazolin-4 (1H) -one nanoparticles)

The 2-phenyl-2, 3-dihydroquinazolin-4(1H)-one (Compound 1) were prepared according to the literature. The formation of Compound 1 was confirmed by spectral analysis (Figure S1 to S2 ES). Compound 1 nanoparticles were prepared by dissolving a small amount of the compound (10 mg of Compound 1) in a minimum amount of ethanol (10 mL). From the above mentioned solution, a very small volume (5 mL) was directly injected into 500 ml of distilled water, which was subjected to stir. After the stirring, sonication was continued for 30 min to ensure the preparation of stabilized organic nanoparticles.

3. Characterization of QZNPs

3.1 Particles size distribution and morphology of QZNPs

The particle size distribution histogram of QZNPs aqueous suspension, obtained by the Dynamic Light Scattering (DLS) technique, is shown in Fig. 1. The size distribution of the particles as seen in the figure is remarkably narrow, with an average particle diameter of 13.1 nm. The SEM photomicrograph of an air dried layer of QZNPs presented in Fig. 2 reveals distinct spheres and clearly indicates that the aggregated particles are spherical and relatively monodispersed in shape. The average particle size estimated from the
SEM images is 150-200 nm. The agglomeration of the nanoparticles during drying of the suspension on the glass substrate in an attempt of thin film preparation is the cause of the observed greater particle size in SEM compared to the mean size of suspended nanoparticles seen from DLS result.\textsuperscript{50}

3.2 Photophysical properties of QZNPs

The fluorescence and absorption, properties of aqueous suspension of QZNPs are compared with those properties of Compound 1 solution in ethanol. The absorption spectrum of QZNPs shown in Fig. 3 is a broad band with maximum absorption at 222 nm and seen blue shifted from the absorption band of compound 1 in ethanol appeared at 353 nm. The hypsochromic shift indicates that the compound 1 aggregate in strong lateral π-stacking interaction mode indicate formation of H-type aggregates\textsuperscript{51} in the water phase to give compound 1 nanocluster.

Excitation and fluorescence spectra of QZNPs suspension and that of the compound 1 solution in ethanol are shown in Fig. 4. Careful observation of the spectra in the figure reveals that the excitation spectrum of nanoparticles is blue shifted broad band (Fig. 4 A) in comparison with a structured excitation spectrum of compound 1 monomer solution in ethanol (Fig. 4 B). The fluorescence spectrum of nanostructure (Fig. 4 D) is broad, structure less band with a maximum at 455 nm and red shifted from that of monomer fluorescence of Compound 1 in ethanol solution (Fig. 4 C). The monomer emission is disappeared totally from the emission spectrum of the nanostructure.

This red shifted enhancement due the specific structures such as nanoclusters formed by π-π stacking restricts rotation and torsion of intermolecular and blocks the nonradiative channel, effectively suppress self-quenching and will notably enhance the emission intensity.\textsuperscript{52} Compound 1 molecules self-assembled nanoclusters are held together by intermolecular π-stacking and hydrophobic interaction (H-aggregates) causes restricted intermolecular rotation and restricted torsional motion of molecules in their aggregate (preventing excimer formation). This restricted motion decreases the rate of nonradioactive decay and thus red shifted enhancement in QZNPs emission.

The relatively long lifetime of nanoparticles indicates aggregation of the compound 1 molecule to form nanostructure by self-assembly.\textsuperscript{53} The fluorescence from QZNPs is Aggregation Induced Enhanced Emission (AIEE) which is known to restrict the rotation and vibration of molecules and thus increases the emission lifetime of QZNPs.\textsuperscript{54}

4. Results and Discussion:

4.1 Effects of pH on the fluorescence intensity of QZNPs

The effect of pH on the fluorescence signal of the assay system was studied (Fig. 6). The emission intensity is weak at most acidic condition. The emission intensity peaked at pH 8.0 and decreased rapidly afterward. Since the compound 1 molecule contains the amine and carbonyl functionalities, such a change in fluorescence intensity may be related to the change in the molecular structure of compound 1 at very low or high pH values due to protonation or deprotonation of the molecule. It is reasonable to believe that molecular structure change results in the disaggregation QZNPs. Thus, fluorescence intensity is decreased. So, a pH value of 8.0 is recommended for use. For the BSA complex with QZNPs, fluorescence intensity decreased in both the acidic and basic medium. The complex response of towards pH was also investigated. Fig. 7 shows that BSA possesses a good fluorescent response with QZNPs in the neutral pH range (6.0–8.0), which is favorable for its application in biological samples because most of the samples like this exist in neutral conditions. So, a pH value of 8.0 is recommended for use.

4.2 BSA binding abilities to the QZNPs

QZNPs have the ability to recognize or bind with protein due to the presence of molecular recognition sites, i.e. carbonyl and amine moiety \textsuperscript{[49]}. The fluorescence spectra were obtained by excitation of the QZNPs at 371 nm in phosphate-buffered water solution at pH = 8 and a strong emission peak was observed at 455 nm. The binding ability was examined by measuring changes produced in the fluorescence spectrum of substances. Supporting Information (Figure S5, ES\textsuperscript{3}) shows fluorescence spectra of aqueous suspension of QZNPs recorded in the presence of MgSO\textsubscript{4}, NH\textsubscript{4}Cl, KNO\textsubscript{3}, MnSO\textsubscript{4}, Fe\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}, CuCl\textsubscript{2}, Na\textsubscript{2}SO\textsubscript{4}, CaCl\textsubscript{2}, FeSO\textsubscript{4}, Sucrose, Fructose, Glucose, L-Ala, L-Arg, L-Pro, L-Asp, L-Glu, Cys, ADP and BSA of concentration 1μM each. It is seen that presence of BSA exhibit strong fluorescence quenching while other substances had no any appreciable effect on fluorescence of QZNPs. The bar diagram shown in Fig. 8 of
fluorescence intensity change ΔF/F where, ΔF=F−F. F0 and F are the fluorescence intensity of nanoparticles in the absence and presence of interfering substances measured at excitation wavelength 371 nm. It is seen that the selectively decreases in fluorescence intensity is seen only for BSA (Fig. 8, Blue bar) whereas other substances no any observable effect on the fluorescence intensity of QZNPs. The dramatic fluorescent quenching was observed, suggesting that QZNPs shows a specific response to BSA and other substances do not show interference in the detection of BSA in aqueous solution (Fig. 8, Red bar).

4.3 Fluorescence titration of QZNPs with BSA solution

To explore the use of QZNPs as a probe for the quantitative determination of BSA the fluorimetric titration was carried out by adding increasing amounts of BSA solution at pH=8 using phosphate-buffered. Fig. 9 shows fluorescence spectra of aqueous suspension of QZNPs in the presence of BSA solution in the concentration range from 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 and 2.2 µM at excitation wavelength is 371 nm measured at room temperature. The fluorescence emission intensity at wavelength 455 nm of QZNPs decreases regularly without any spectral shift.

The fluorescence quenching efficiency was calculated from the relationship.

1−F/F0

A maximum quenching up to 88.84% was obtained when the concentration of BSA was 2 µM. The large quenching efficiency causes a “Turn Off” of the fluorescence of the QZNPs to a large extent quantitative analysis. This fluorescence quenching is due to the binding interaction of BSA with the QZNPs.

The quenching experiments are repeated at three different temperatures and spectral data are analyzed by Stern-Volmer relation given by equation 1.

F
F =1+KF([Q]+1+Kq[Q])

Where, F0 and F are the steady-state fluorescence intensities in the absence and presence of quencher, respectively. Kq are the quenching rate constant and t0 is the average lifetime of the donor molecule in the absence of quencher. Ksv is the Stern-Volmer quenching constant, and [Q] is the concentration of quencher (BSA). Fig. 10 shows Stern-Volmer plots for quenching of fluorescence of QZNPs by BSA at three different temperatures viz. 299, 309, and 319 K.

It is clear that the curves show good linear relationship, according to equation 1. The estimated values of photokinetics are summarized in Table 1. The increase in Ksv and Kq with increase in temperature indicates that the quenching interaction between QZNPs and BSA is dynamic which implies that the quenching was initiated in excited state complex formation by collision or diffusion.25 The dynamic quenching phenomenon also supports from Time resolved (lifetime) spectroscopy given in supporting information (Fig. S6. ESi).

The obtained experimental data for BSA determination fitted well to the following empirical equation: 2

F/0 = 0.155x + 1.............. ........... ........... ........... (2)

Obtaining a linear relationship in the range of 0 to 2.2 µM with a correlation coefficient of 0.9916, which could be used to develop a determination method for BSA. The limit of detection (LOD) is defined by the equation (3).

LOD = (3.3σ/k).............. ........... ........... (3)

Where σ is the standard deviation of the y-intercepts of regression lines and k is the slope of the calibration graph. The estimated LOD is 0.1304 µM. The method has the advantages of lower detection limit (LOD) and wider linear range of concentration of BSA over the existing methods.23, 28

4.3.1 Estimation of binding parameter of QZNPs+BSA

In order to evaluate the binding constant K and the number of binding sites n, the double logarithmic plot based on following equation as shown in Fig. 11 is obtained.20

log_{10}\left[\left(\frac{F_F}{F} - F\right)\right] = log_{10}K + n log_{10}[BSA].................................(4)

From the nature of the plot shown in Fig. 11, the values of K and n are obtained from the intercept and slope of the straight line of the graph respectively. The values of ‘K’ and ‘n’ obtained at various temperatures are reported in Table 1. The data showed that the binding site number ‘n’ is close to each other at different a temperature which indicates the effect of temperature is not intense and one binding site is present between QZNPs and BSA.35 The considerable value of K indicates that BSA has bound to QZNPs effectively though there is only one binding site. The value of K is inversely
related to temperature, which indicates that QZNPs-BSA complex has lowered stability at higher temperatures. 57-60

4.3.2 Determination of Force Acting between QZNPs and BSA

It is known that there are four main types of forces acting in bimolecular interaction which are hydrophobic forces, electrostatic interactions, Van der Waals’ interactions and hydrogen bonding. The signs and magnitudes of thermodynamic parameters like enthalpy change (ΔH), entropy change (ΔS), free energy change (ΔG) suggest the nature of forces involved in the molecular interaction. In order to elucidate the binding force between QZNPs and BSA, it was necessary to obtain the thermodynamic parameters and so the binding studies were carried out at 299, 309 and 319 K. The values of binding constant K are combined with the thermodynamic parameter by using the following equations.

\[ \ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \]  

Where K is the binding constant at the corresponding temperature, and R is the gas constant. The nature of the plot of ln K against 1/T is shown in Fig. 12.

The enthalpy change (ΔH) and entropy change (ΔS) are obtained from the slope and intercept of Van’t Hoff plot. Finally the Gibbs energy change (ΔG) was calculated by following thermodynamic relation.

\[ \Delta G = \Delta H - T \Delta S = -RT \ln k \]  

Equations (5) and (6) are Van’t Hoff and Gibb’s equation respectively. Here K is the binding constant; R is the gas constant, T is absolute temperature. The plot of ln K versus 1/T is as shown in Fig. 12 which obtained the values of ΔH and ΔS from the slope and intercept respectively. By substituting these values in Eq. (6), the values of ΔG at different temperatures were calculated. The results obtained are summarized in Table 1 which shows that ΔH<0, ΔS<0 and ΔG<0 i.e. all these parameters are negative. As both ΔH and ΔS are negative, the major binding forces in this process are hydrogen bonding and Van der Waals’ interactions and the reaction was mainly enthalpy driven. Also negative ΔG indicates the spontaneity of the binding interaction at all the studied temperatures. The hydrogen atoms of the amino group of BSA form the hydrogen bonds with the carbonyl function of QZNPs, therefore exclude the possibility of Van der Waals’ interactions.

4.3.3 Mechanism of binding interaction of QZNPs and BSA:

The plausible mechanism of fluorescence quenching is discussed on the basis of surface bound complexation between BSA and QZNPs involving hydrogen bonding (H-bonding) shown in Scheme 1. The QZNPs plausible interaction with BSA is partially based on H-bonding and likely via -NH₂ groups and –COOH group of the protein. The establishment of H-bond between carbonyl and amine group of QZNPs with -NH₂ and –COOH group of BSA protein.

H-bond complexation is further supported by DLS and zeta potential measurements. Particle sizes and zeta potentials of QZNPs before and after the sequential addition of BSA are graphically represented in Fig. 13. The average particle size of QZNPs was found to be 13.1 nm, and in addition of 1 and 2 µM of BSA it enlarged to 111.2 and 167.1 nm, respectively, showing the tendency toward the formation of aggregates that result in fluorescence quenching. As shown in Fig. 13, the zeta potential of the QZNPs was reduced from -321.2 mV to -14.8 mV and -8.9 mV with the addition of 1 and 2 µM of BSA respectively. The considerable reduction in zeta potential also indicates the tendency for the formation of aggregates. The carboxyl group and an amine group of QZNPs can bind with BSA through H-bonding interaction, which could reduce anionic character of the QZNPs, bringing down the negative zeta potential. 56

4.4 Analysis of BSA from Pharmaceutical Samples:

The proposed quenching method was applied for the determination of BSA in the pharmaceutical samples, namely Bovine Serum Albumin Standard Ampules. The Standard Ampules were dissolved in distilled water, filtered and then used for analysis. The analysis is carried out at room temperature. The calibration curves obtained by plotting F0/F versus the concentration of BSA are shown in Fig. 14. The graphs are linear in the concentration range (0.0 to 2.2 µM) with correlation coefficients (R) of 0.9904. The linear regression equations were used to estimate the unknown concentration (x) of BSA from Bovine Serum Albumin Standard Ampules dissolution. The results are in good agreement with the certified values of the BSA compositions. These results are reported in Table 2.

5. Conclusion

Novel Fluorescent Organic Nanoparticles QZNPs were developed from compounds 1 and were checked for their fluorimetric sensing
properties. QZNPs showed applicability as a sensor for detecting BSA by a large quenching in fluorescence intensity, without any shift. BSA can be detected at the lowest concentration of 0.1304. The binding interaction between QZNPs and BSA has been investigated by using fluorescence spectroscopy. The binding process was studied at three different temperatures viz. 299, 309 and 319 K to examine the temperature dependence of Stern-Volmer quenching constant $K_{SV}$. The Stern-Volmer quenching constant $K_{SV}$ is directly related to a dynamic quenching mechanism. The negative value of $\Delta G$ suggested the spontaneity of interaction; similarly Van der Waals' interaction along with hydrogen bond had a major role in the binding process. In the practical application of sensor QZNPs, the detection of BSA was performed from Bovine Serum Albumin Standard Ampules. The practical applications carried out for Bovine Serum Albumin Standard Ampules further signify the great potential of sensor QZNPs for easy real-time monitoring of BSA. QZNPs-BSA H-bond complexation is further supported by DLS and zeta potential measurements.

6. Acknowledgment

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7. References
34. C. Huang, Y. Li, J. Mao, D. Tan, Analyst, 1998, 123, 1401.
Figure Caption

Fig. 1: Particle Size Distribution Histogram of QZNPs obtained by DLS analysis.

Fig. 2: SEM photomicrograph of air dried layer of QZNPs. (Spherical morphology).

Fig. 3: UV-Vis absorption spectra of homogeneous solution of QZ in ethanol (spectrum A) and QZNPs in aqueous dispersion (spectrum B).

Fig. 4: Excitation (A) and fluorescence emission (D) spectra of QZNPs suspension Excitation (B) and fluorescence emission (C) spectra of QZ in ethanol.

Fig. 5: Fluorescence lifetime spectrum of QZNPs suspension spectrum). (Blue spectrum) dilutes and solution of QZ in ethanol (red)

Fig. 6: Effect of pH on the fluorescence intensity of the QZNPs. Higher intensity at pH=8.

Fig. 7: Effect of pH on the fluorescence intensity of the QZNPs-BSA complex. BSA possesses a good fluorescent response with QZNPs in the pH=8.

Fig. 8: Fluorescence intensity [ΔF/F] of the QZNPs solution in the presence and absence of Bovine Serum Albumin (BSA) and several coexisting substances (1.2 µM excitation wavelength 371 nm).

Fig. 9: Fluorescence spectra of QZNPs suspension in the presence of different concentrations of aqueous solution of BSA (0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.2 µ) in Phosphate buffered (pH 8) at excitation wavelength λex = 371 nm.

Fig. 10: Stern-Volmer plots of the quenching of fluorescence of QZNPs by BSA at their different temperatures.

Fig. 11: Double logarithmic plot of log₁₀ [(F₀−F)/F] versus log₁₀ [BSA].

Fig. 12: Van’t Hoff plot for binding of QZNPs with BSA.

Fig. 13: Representation of the average particle size and zeta potential of QZNPs measured, which varies in response to the addition of aqueous solutions of BSA in QZNPs: (a) in the absence of BSA, and (b) and (c) in the presence of 1 µM and 2 µM BSA respectively. (pH = 8).

Fig. 14: Calibration graph for analysis of BSA from Bovine Serum Albumin Standard Ampules pharmaceutical samples.

Scheme 1: Plausible binding mechanism of BSA via multiple point hydrogen bonding interactions with 2-phenyl-2, 3-dihydroquinazolin-4 (1H)-one nanoparticles (QZNPs).
**Scheme 1**

Hydrogen bonding between QZNPs and BSA

QZNPs = 2-Phenyl-2,3-dihydroquinazolin-4(1H)-one nanoparticles.

BSA (Bovine Serum Albumin)
Table caption

Table 1: Stern-Volmer quenching constants, the binding and thermodynamic parameter of QZNPs estimated for binding interaction with BSA in aqueous solution.

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<tr>
<th>T/K</th>
<th>$K_{sv}$ ($10^5$ L.mol$^{-1}$)</th>
<th>$kq$ ($10^{13}$ L.mol$^{-1}$.s$^{-1}$)</th>
<th>$K$ (L.mol$^{-1}$)</th>
<th>$\Delta H$ (KJ.mol$^{-1}$)</th>
<th>$\Delta G$ (KJ.mol$^{-1}$)</th>
<th>$\Delta S$ (J.mol$^{-1}$.k$^{-1}$)</th>
<th>n</th>
<th>R$^a$</th>
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<td>299</td>
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<td>319</td>
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<td>0.8929</td>
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Table 2: Determination of BSA in Bovine Serum Albumin Standard Ampules.

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<th>Sample Composition</th>
<th>Amount of BSA</th>
<th>Recovery</th>
<th>RSD</th>
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<tr>
<td>Bovine Serum Albumin Standard Ampules, 2 mg/mL Thermo Fisher Scientific India Pvt. Ltd. 403,404, Delphi 'B' Wing, Hiranandani Business Park, Powai, Mumbai - 400076.</td>
<td>Certified Value</td>
<td>Found$^a$</td>
<td></td>
</tr>
<tr>
<td>Bovine Serum Albumin Standard Ampules, 2 mg/mL</td>
<td>2.00 mg/ml (per Ampule)</td>
<td>1.9961 mg/ml (per Ampule)</td>
<td>999.61%</td>
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