Chapter 4

*Synthesis of Silver Nanoparticles and Their Interaction with Heavy Metal Ions*
4.1 INTRODUCTION

Rapid progress in the field of nanoscience and technology is increasingly finding nanomaterials being used for a wide range of applications starting from physics, chemistry, materials science, engineering, soil science, environmental science, geological science to biological science. The nanoparticles are the particles having the size range 1–100 nm, can have novel and size-related physico-chemical properties differing significant from larger matter. However, among different types of nanomaterials, silver nanoparticles (AgNPs) and nano rods (NRs) have been proposed to have large-scale applications especially in of catalysis, optical sensing, textile engineering, electronics, and most importantly in the medical field as they have good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms which is very useful for many biomedical and technological applications.

The interesting optical properties of silver nanoparticles are due to their surface plasmon resonance (SPR), which is caused by the collective oscillations of conduction electrons excited by visible light and is manifested by an absorption band in the visible region of the optical spectrum around 400 nm. The SPR absorption wavelengths of silver nanoparticles are dependent on their size, shape, and refractive index; therefore, any changes in their surface structure, aggregation, or medium’s refractive index may lead to colorimetric changes of their dispersions. The shift of SPR band in the region 400–600 nm, depending on pH and the presence of different types of heavy metal ions.

In recent decades, the most of the work focused on the synthesis and study of the antibacterial activity of silver nanoparticles. Among them, several approaches have been used to prepare silver nanoparticles, for example, the following techniques have been used to prepare silver nanoparticles are photochemical synthesis, laser ablation, phase transfer processes, micro emulsion, microwave treatment and γ-irradiation. These methods have been used for synthesis of silver nanoparticles in both solution and solid phases. Besides that, physicochemical approaches and biosynthesis methods have been extensively employed to prepare silver nanoparticles with the aim of improving the conventional methods.

The development of highly sensitive and selective analytical tools for heavy metal ions is of great importance, to avoid their cytotoxicity effects. Mercuric ion
(Hg$^{2+}$), the most stable form of inorganic mercury, exists mostly in surface water due to its high water solubility and it can cause several developmental delays and health problems that can damage the brain, nervous system, kidneys, and endocrine system.$^{26-28}$ The contamination Pb$^{2+}$ possesses a serious threat to human health and environment.$^{29}$ Lead-poisoning causes renal malfunction and inhibits brain development associated with environmental pollution, particularly in children, causing various neurotoxic effects. As lead is nondegradable, it is persistent in the environment and can produce toxic effects in plants and animals and biological samples. Iron is the most abundant vital transition metal in both plants and the human body that plays a significant role in cellular metabolism, and enzymatic catalysis.$^{30}$ It acts as a carrier for oxygen and electron transports in hemoglobin and serves as a cofactor in many enzymatic reactions. However, excess amounts of Fe$^{3+}$ ion can cause damage to cellular lipids, nucleic acids and proteins. Furthermore, a deficiency of Fe$^{3+}$ ion limits oxygen delivery to cells and causes anemia, liver and kidney damage, diabetes, and heart disease. There are many serious diseases, including Alzheimer’s, Huntington’s and Parkinson’s disease are connected with cellular toxicity of iron ions.$^{31}$

Recently, calorimetric sensors and several methods, providing the immediate optical feedback have been extremely attended due to their simplicity, rapidity, high sensitivity and ease of measurement. There are several methods for the detection of Fe$^{3+}$, Hg$^{2+}$ and Pb$^{2+}$ have been reported based on chromophores or fluorophores$^{32-35}$, organic compounds$^{36,37}$, polymers$^{38,39}$, oligonucleotides$^{40}$, DNA$^{41}$, proteins$^{42}$ and nanoparticles and nanorods$^{43,44}$.

The conventional process for the synthesis of these aromatic amines is based on the reduction of corresponding nitro derivatives using catalytic hydrogenation and other chemical reduction methods. Aromatic amines are known to be an important intermediate in the production of many analgesics and antipyretic drugs, dyeing agents, agricultural chemicals, surfactants and polymers. In the many chemical reduction process, the chemical or the side products which possess severe waste effluent problems and thus the entire reaction setup becomes environmentally hazardous. Significant research contributions have been made in the chemoselective reduction of aromatic nitro compounds with H$_2$ using heterogeneous catalysts based on silver nanoparticles. Due to the high Fermi potential, metal nanoparticles can act as a good
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catalyst. The rate of the catalytic reaction over the surface of metal NPs depend on tunability of particle size\textsuperscript{45}, shape\textsuperscript{46,47} and stabilizers.\textsuperscript{48,49} Reduction using NaBH\textsubscript{4} as the hydride (H\textsuperscript{−}) source can be considered as an alternative route, but the reduction cannot be achieved in the absence of any catalyst.\textsuperscript{50,51} Therefore, development of a catalytic system for selective and efficient reduction of aromatic nitroarenes is highly desire.

In the present study, we have synthesized the silver nanoparticles using bilirubin, acts not only a reducing agent as well as a stabilizing agent. Furthermore, we have illustrated the binding interaction of the bilirubin on the silver nano-surface and formation of BR stabilized silver nanoparticles (Ag-BR). The BR mediated preparation of silver nanoparticles has been previously reported,\textsuperscript{52} however, no other detailed investigation regarding the stability, binding interaction, catalytic activity and heavy metal ion sensing have been performed. The current manuscript in mainly focused on the application of bilirubin coated Ag nanoparticles. Further, we have studied the colorometric detection of heavy metal ions, such as Fe\textsuperscript{3+}, Hg\textsuperscript{2+} and Pb\textsuperscript{2+} ions in water. To further verify the selectivity of Ag-BR nanocatalysts, selective reduction of nitro groups in nitroarenes was also demonstrated.

4.2 EXPERIMENTAL SECTION

4.2.1 MATERIALS and METHODS

All chemicals used were of analytical grade or of the highest purity available. Silver nitrate (AgNO\textsubscript{3}, 99.9\%) and bilirubin (C\textsubscript{33}H\textsubscript{36}N\textsubscript{4}O\textsubscript{6}, >98\%), Polyvinylpyrrolidone (PVP-40, molecular weight 40000) and TWEEN\textregistered\textsuperscript{20} (T-20) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH), calcium chloride dehydrate (CaCl\textsubscript{2}, 2H\textsubscript{2}O), cadmium chloride (CdCl\textsubscript{2}), cobalt chloride (CdCl\textsubscript{2}), copper sulfate pentahydrate (CuSO\textsubscript{4},5H\textsubscript{2}O), ferrous sulfate heptahydrate (FeSO\textsubscript{4},7H\textsubscript{2}O), ferric chloride (FeCl\textsubscript{3}), mercury chloride (HgCl\textsubscript{2}), potassium chloride (KCl), manganese chloride tetrahydrate (MnCl\textsubscript{2}, 4H\textsubscript{2}O), sodium chloride (NaCl), Nickel chloride hexahydrate (NiCl\textsubscript{2}, 6H\textsubscript{2}O), lead nitrate (Pb(NO\textsubscript{3})\textsubscript{2}) was purchased from Merck Millipore. All reagents were used as received. The glassware was carefully cleaned with aqua regia (3:1 HCl/HNO\textsubscript{3}) and then rinsed several times with Milli-Q (18M\textOmega cm
resistance at 25 °C) water prior to use under sonication. Milli-Q water was used to prepare all the solutions in this study.

4.2.2 SYNTHESIS

4.2.2.1 Synthesis of the bilirubin mediated silver nanoparticles

The Ag (I) solution was prepared by dissolving a required amount of the silver nitrate in Milli-Q water in a 100 ml clean round bottle flask. Separately, the bilirubin solution was prepared by dissolving a required amount of bilirubin in Milli-Q water at pH~ 8.0 in another clean round bottle flask covered with aluminium foil. The Ag (I) solution was added slowly to the bilirubin solution with vigorous stirring at 1200 rpm under dim room light conditions. The final concentrations of both Ag (I) and bilirubin in the reaction medium were 2.5 mM and 1.0 mM and the molar ratio of Ag (I) : bilirubin was 2.5 : 1.0. The stirring was continued for an additional five hours to complete the formation of Ag nanoparticles. The reaction mixture subsequently was centrifuged at 16000 rpm for 15 min. The collected silver (Ag-BR) nanoparticles were straw yellow in color. The collected Ag-BR was again dispersed in Milli-Q water and centrifuged at 16000 rpm and the whole processes were repeated thrice. This purified sample was used for characterization and application purposes.

![Figure 4.1: Schematic representation of reduction mechanism of Ag (I) to Ag(0) by bilirubin in alkaline medium.](image)

4.2.2.2 Synthesis of the polyvinylpyrrolidone coated silver nanoparticles

The synthesis of PVP-stabilized AgNPs was performed in a modification of the previously reported methods. In a 100 ml round bottle flask, 200 µL of 500 mM AgNO₃ solution was taken in 40 ml Milli-Q water. To the AgNO₃ solution 50 mg PVP-
40 in 5.0 ml water was added under stirring condition. The mixture was kept for another one hour under stirring condition. Then to the reaction mixture 5.0 ml 0.5 mg/ml ice cold water solution of NaBH₄ was added dropwise under vigorous stirring condition. After addition of ~2.5 ml NaBH₄ solution the color of the reaction mixture changes from colorless to brown yellow color indicating the formation of silver nanoparticles. After the addition of 5.0 ml NaBH₄ solution, the reaction mixture kept under stirring condition to complete the formation of Ag nanoparticles. The completion of reaction monitored by measuring the UV-Vis spectra. The synthesized PVP coated Ag nanoparticle (Ag-PVP) was purified by centrifugal method. The collected silver nanoparticles were straw yellow in color. This purified sample was used for characterization and application purposes.

4.2.2.3 Synthesis of the TWEEN® 20 coated silver nanoparticles

In this synthesis method, in a 100 ml round bottle flask, 200 μL of 500 mM AgNO₃ solution was taken in 40 ml Milli-Q water. To the AgNO₃ solution 100 mg Tween-20 in 5.0 ml water was added under stirring condition. The mixture was kept for another one hour under stirring condition at room temperature. Then to the reaction mixture 5.0 ml 0.5mg/ml ice cold water solution of NaBH₄ was added dropwise under vigorous stirring condition. During the addition of NaBH₄ solution the color of the reaction mixture changes from colorless to straw yellow color indicating the formation of silver nanoparticles. After the addition of the total amount of NaBH₄ solution, the reaction mixture kept three hours under stirring condition to complete the formation of Ag nanoparticles. The completion of reaction monitored by measuring the UV-Vis spectra. The synthesized Tween-20 coated Ag nanoparticle (Ag-T20) was purified by centrifugal method. The synthesized Tween-20 coated Ag nanoparticle (Ag-T20) was purified by centrifugal method. The collected silver nanoparticles were straw yellow in color. This purified sample was used for characterization and application purposes.


4.2.3 CHARACTERIZATION

4.2.3.1 Absorption

Absorption spectra of the prepared gold nanoparticles were measured with a JASCO V-630 Spectrophotometer (JASCO International Co. Ltd, Japan) in the range of 400 to 800 nm wavelength. A high quality quartz cuvette was used to record the spectra. The as-prepared Ag-BR were diluted four times by Mill-Q water. For the metal ions detection study, same diluted Ag-BR suspension have been used and the stock solutions of metal ions were prepared as such a concentration from which we added 2.0 µL solution to the 2.0 ml NP suspension. So, the change of UV-Vis absorbance of Ag-BR suspension due to dilution was neglected.

4.2.3.2 Transmission Electron Microscopy

The morphology and size of the Ag-BR were characterized by transmission electron microscopy (TEM) using JEOL JEM 2100 HR transmission electron microscope operated at an accelerating voltage of 200 kV. For TEM imaging, a drop of the aqueous suspension of the nanoparticle solution was placed on a carbon coated 300-mesh copper grid (Allied Scientific Product, USA) and dried in a dust free atmosphere.

4.2.3.3 FT-IR measurement

The FT-IR spectra of the samples were recorded on a Bruker TENSOR27 spectrometer using the KBr pellet technique. Solid samples (bilirubin/Ag-BR nanoparticles) were mixed with KBr in a clean glass pestle and mortar and compressed to obtain a pellet. The spectra were recorded from 400–4000 cm⁻¹. The band positions were assigned based on available information in the literature. Background spectra were obtained with a KBr pellet for each sample. Bruker software was used for data processing. The strong peaks in the spectra were reproducible and appeared each time within +/- 3cm⁻¹.
4.3 RESULTS AND DISCUSSIONS

The UV-visible spectrum of the Ag-BR dispersed in water is shown in Fig. 4.2A. The dispersed nanoparticles showed strong absorbing and scattering behavior and the Plasmon resonance band maximum appeared at ~ 420 nm. This was attributed to the interaction of light with the collective oscillation of excess surface electrons (surface plasmon resonance) of the silver nanoparticles. A relatively clean band at 420 nm indicated the average particle size ~10-15 nm in diameter. The peak position and shape of the band was related to the particle size.

![Image](image_url)

**Figure 4.2:** Panel A shows the UV-vis spectrum of bilirubin coated silver nanoparticles after preparation, washed with water and dispersed in Milli-Q water at pH~7.0. Panel B shows the UV-Vis spectra of Ag-BR nanoparticles at different time interval, (store at room temperature and ambient condition).

The particles were quite stable at room temperature. Freshly prepared particles were vacuum dried and stored at room temperature under dark for several months. The UV-Vis spectrum was received in different time interval (Fig. 4.2B). From the UV-Vis spectra it was clear that there was no much perturbation in the absorption maximum ($\lambda_{\text{max}}$) at ~420 nm. It indicated that the particles were very stable at room temperature. The bilirubin coated silver nanoparticles were also stable in a wide range of pH, i.e. from pH 5.0 to pH 12.0. Under different pH condition, the absorption spectra study, it was clear that the surface plasmon resonance band of Ag-BR nanoparticles was not perturbed by the change of pH in the dispersed medium, as shown in the Fig. 4.3.
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Figure 4.3: UV-Visible spectra of the silver nanoparticles dispersed in a 10 mM phosphate buffer solution under different pH conditions.

The HR-TEM analysis, showed that the Ag-BR was around 10-15 nm in diameter and was mostly monodispersed as well as spherical in shape, shown in Fig. 4.4. The observed spherical shape of the prepared silver NPs are highly in agreement with the fact given by Mock et al.\textsuperscript{58} confirming that surface plasmon band around 420 nm shows that the silver NPs are spherical in shape.

Figure 4.4: Transmission electron microscopy (TEM) image of bilirubin coated silver nanoparticles. Panel A: The scale bar of 20 nm is shown at the bottom, Panel B: The scale bar of 5 nm is shown at the bottom.

The XRD spectrum of the synthesized silver nanoparticles as shown in Fig. 4.5. The joint committee on powder diffraction standards (JCPDS) card no. 04-0783 of the pure crystalline nanoparticle structure was used to define the crystalline nature of the silver nanoparticles. A comparison with the XRD spectrum confirmed that the synthesized
silver nanoparticles were in the form of face centered cubic (fcc) nanocrystals with a Fm3m symmetry, as evidenced by the 2θ values 38.2, 44.16, 64.51, 77.32 and 81.2 in the XRD pattern, corresponding to (111), (200), (220), (311) and (222) planes, respectively.

![Figure 4.5: The powder XRD diffraction pattern of the Ag-BR nanoparticles. The peaks are assigned based on JCPDS card no. 04-0783](image)

The FT-IR spectra of bilirubin and the Ag-BR nanoparticles are shown in Fig. 4.6A and 4.6B, respectively. The significant FT-IR bands of both the bilirubin and Ag-BR are marked and their assignments are summarized in Table 4.1. Some of the characteristics FT-IR bands of strong and medium intensity, which appeared at at 3406, 3267, 2912, 1695, 1645, 1611, 1250, 989 and 932 cm\(^{-1}\), and other bands at 800–600 cm\(^{-1}\) are marked in the spectra. The bands were very consistent with the normal mode analysis of vibration frequencies of BR and the assignments were made following the literature values. The observed bands at 3406 and 3267 cm\(^{-1}\) in the BR powder were assigned to the stretching modes of pyrrol N–H and lactam N–H, respectively, as per the normal coordinate analysis data.\(^5,55\) However, the pyrrol N–H band shifted to 3417 cm\(^{-1}\) and the lactam N–H band shifted to 3259 cm\(^{-1}\) on the silver nanosurface. Bilirubin contains two carboxylic acid groups on side chain and produced an intense carboxyl C=O stretching vibration at 1695 cm\(^{-1}\). However, on silver nanosurface, this peak shifted to 1691 cm\(^{-1}\). The stretching vibration of lactam C=O appeared at 1645 cm\(^{-1}\) was shifted to 1639 cm\(^{-1}\) on the nanosurface.\(^55\) The conjugated C=C stretching band assign at 1611 cm\(^{-1}\) was shifted to 1615 cm\(^{-1}\) and got broadened on the Ag-BR surface. The C-N stretching of lactam system was shifted from 1046 cm\(^{-1}\) to 1388 cm\(^{-1}\).
Figure 4.6: FT-IR spectra of the bilirubin powder (A) and the Ag-BR nanoparticles (B) in solid state using KBr pellets.

Table 4.1: FT-IR peaks of bilirubin and Ag-BR powder

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<tr>
<th>Sl. No.</th>
<th>Bilirubin</th>
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<th>Vibration mode</th>
<th>Remark</th>
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<tr>
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<td>3406</td>
<td>3417</td>
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<td>Pyrrole N-H</td>
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<tr>
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<td>3267</td>
<td>3259</td>
<td>$\nu_{N-H}$</td>
<td>Lactam</td>
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<td>2962</td>
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<td>H-bonded pyrrole</td>
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<tr>
<td>4</td>
<td>2912</td>
<td>2919</td>
<td>$\nu_{C-H}$</td>
<td>Symmetric and asymmetric in CH$_2$ and CH$_3$</td>
</tr>
<tr>
<td>5</td>
<td>2856</td>
<td>2856</td>
<td>$\nu_{N-H}$</td>
<td>H-bonded lactam</td>
</tr>
<tr>
<td>6</td>
<td>1695</td>
<td>1691</td>
<td>$\nu_{C=O}$</td>
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<tr>
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<td>1645</td>
<td>1639</td>
<td>$\nu_{C=O}$</td>
<td>lactam</td>
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<td>1611</td>
<td>1615</td>
<td>$\nu_{C=C}$</td>
<td>Stretching</td>
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<td>9</td>
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<td>1567</td>
<td>$\nu_{C=C}$</td>
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</tr>
<tr>
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<td>1388</td>
<td>$\nu_{C-N}$</td>
<td>C-N stretching and CH$_3$ bending</td>
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<td>bridge C-H bending, bridge $\nu_{C=C}$, $\nu_{C-N}$</td>
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<td>1168</td>
<td>ring breathing</td>
<td></td>
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<td>15</td>
<td>1053, 989, 932</td>
<td>1045, 994, 939</td>
<td>C-N &amp; C-H bending</td>
<td>In-plane and out-of-plane bending of pyrrole ring</td>
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4.4 HEAVY METAL IONS DETECTION

4.4.1 Sensing of Metal Ions by Ag-BR Nanoparticles

To investigate the metal ions detection ability of bilirubin coated Ag NP (Ag-BR) was studied separately for each of the metal ions involving Na⁺, K⁺, Ca²⁺, Fe³⁺, Fe²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Hg²⁺, Co²⁺ and Cd²⁺ at a fixed concentration of 20 μL of a 20 × 10⁻⁶ M metal ion solution being added to the AgNPs solution. The colorimetric assays and the changes in the UV-Vis absorption spectra were performed and monitored at room temperature. The photographs were taken with a digital camera after 5 minutes of mixing under fluorescence room light. Upon addition of various metal (Na⁺, K⁺, Ca²⁺, Fe³⁺, Fe²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Hg²⁺, Co²⁺ and Cd²⁺) salts to the Ag-BR solution, the intensity of the SPR band showed a slight change, while the color of the solution remained unchanged (Fig. 4.7). However, a substantial change in color was observed when Hg²⁺, Fe³⁺ and Pb²⁺ were added individually to the dispersed nanoparticle medium. The initial straw yellow color becomes very faint and colorless in the presence of both the Fe³⁺ and Hg²⁺. In presence of Pb²⁺ it becomes brown in color. It showed the high sensitivity and selectivity of Ag-BR toward those toxic metal ions like Fe³⁺, Hg²⁺ and Pb²⁺. The Fig. 4.9 represents the change in absorbance intensity after the addition of different metal ions to the Ag-BR solution.

![Figure 4.7: Colour photographic images of Ag-BR suspension in the presence of various metal ions. The image shows in presence of 20 μM different ions in Ag-BR suspensions. Images have been collected 5 min incubation after addition of different ion solutions. Colour photograph collected using Nikon Coolpix P610 digital camera under room light condition. The panel A and B shows the photograph taken under room light without flash and with flash mode of camera. Panel C shows the UV-Vis spectra of the corresponding samples.](image-url)
The quantitative assessment of the selectivity of \( \text{Hg}^{2+} \), \( \text{Fe}^{3+} \) and \( \text{Pb}^{2+} \) ion detection were studied by changing the concentrations of these metal ions to the Ag-BR at similar laboratory conditions. The SPR band of the system was monitored by UV-Visible spectroscopy. The addition of \( \text{Hg}^{2+} \) ions to the Ag-BR solutions led to the gradual hypsochromic shift in its SPR band as shown in Fig. 4.9B. The extent of the shift toward the lower wavelength region depends on the concentration of \( \text{Hg}^{2+} \) ions in the solution. There was a visible color change from straw yellow to gray white color. This could be due to the direct redox reaction between zero valent Ag and \( \text{Hg}^{2+} \) ions, where the Ag-BR oxidized to form \( \text{Ag}^{+} \) and \( \text{Hg}^{2+} \) ions were reduced to a \( \text{Hg}^{+} \) or Hg atom, the standard potential of 0.80 V (\( \text{Ag}^{+}/\text{Ag} \)) and 0.85 V (\( \text{Hg}^{2+}/\text{Hg} \)), respectively.\(^5\) Further, it was confirmed by TEM analysis that there were few NPs observed after the addition of \( \text{Hg}^{2+} \) in the Ag-BR solutions and the observed particles are very small in size, as shown in Fig. 4.10. From TEM image analysis, it was clear that similar observations have been reported in earlier studies on the interaction of \( \text{Hg}^{2+} \) with colloidal AgNP synthesized in different procedures.\(^9\) However, the absorbance decreased with increased concentration of \( \text{Hg}^{2+} \) ions ranging from 2.0 \( \mu \text{M} \) to 20.0 \( \mu \text{M} \), and the value of the linear regression coefficient (\( R^2 \)) was found to be 0.991 with the detection limit up to 10 \( \mu \text{M} \) (inset in Fig. 4.9B).

The direct colorimetric visualization detection for \( \text{Fe}^{3+} \) ion was quite similar as \( \text{Hg}^{2+} \) ion. The color of the solution changed from straw yellow to colorless (faint yellow) for \( \text{Fe}^{3+} \) and the changes in the SPR band was studied by using UV-Visible spectroscopy. The disappearance of straw yellow color of Ag-BR might be due to the redox reaction between \( \text{Fe}^{3+} \) and Ag-BR. Although, the reduction potential of \( \text{Fe}^{3+}/\text{Fe}^{2+} \) (\( E_{\text{Fe}^{3+}/\text{Fe}^{2+}} \)) was 0.77 volts and the reduction potential of \( \text{Ag}^{+}/\text{Ag} \) (\( E_{\text{Ag}^{+}/\text{Ag}} \)) was 0.80 volt. There might be the formation of some stable complex of bilirubin and reduced part of \( \text{Fe}^{3+} \) i.e. \( \text{Fe}^{2+} \). Thus, the reduction potential of \( \text{Fe}^{3+} \) and \( \text{Fe}^{2+} \)-bilirubin complex system (\( E_{\text{Fe}^{3+}/\text{Fe}^{2+}(\text{bili})} \)) might overcome the reduction potential of \( \text{Ag}^{+}/\text{Ag} \) (\( E_{\text{Ag}^{+}/\text{Ag}} \)) system. From UV-Visible spectroscopy study, the addition of \( \text{Fe}^{3+} \) ions to the Ag-BR solutions led to the initial bathochromic shift followed by a hypsochromic shift in its SPR band as shown in Fig. 4.9A, but there is no change in color of the AgNP suspension after adding of the \( \text{Fe}^{2+} \) ion solution. The virtual shifting of the SPR band with \( \text{Fe}^{3+} \) concentration indicates there was first aggregation of the particles occurs due to the
lack of capping agent on the Ag-nanoparticle surface as the formation of Fe$^{3+}$-bilirubin complex followed by redox reaction takes place, which causes the disappearance of straw yellow color of the Ag-BR solution. From the TEM image analysis, it was clear that the spherical shaped Ag-BR nanoparticles get degraded upon addition of Fe$^{3+}$ to the suspension. So, it is expected that after addition of Fe$^{3+}$ to the freshly synthesized Ag-BR NPs, iron (III) ion bounds to the biological stabilizer compounds (bilirubin/biliverdin) and do some aggregation of particle followed by a redox reaction between the silver and Fe$^{2+}$ ions would be occurred.

**Figure 4.8:** Colour photograph of Ag-BR suspension in the presence of different amount of Fe$^{3+}$ ion. The concentration values written on the sample vial in blue color. The panel A and B shows the color photograph of samples, the pictures have been taken under room light without flash and with flash mode of camera. Panel C shows the UV-Vis spectra of the corresponding samples.
In addition, ‘green’ synthesized Ag-BR also shows good sensitivity towards Pb\(^{2+}\) ion contaminant in water samples. In this metal ions detection assay, Pb(NO\(_3\))\(_2\) stock solution was used. Various concentrations of Pb\(^{2+}\) were prepared using serial dilution of the stock solution to test the sensitivity limits of the Ag-BR. Using the stock solution, 4.0-36.0 μM of Pb\(^{2+}\) were prepared. The colorimetric detection of aqueous Pb\(^{2+}\) was performed at room temperature. From Fig. 4.9C, when the concentration of Pb\(^{2+}\) increased from 4.0 to 36.0 μM, a red shift in wavelength and a broadening of the SPR occurred. The color changed from straw yellow to brown accompanied with a red shift, indicating the aggregation of nanoparticles was clearly detected from TEM image as shown in Fig. 4.10. The change of SPR band was attributed to the coupled plasmon absorbance of nanoparticles detected by UV-Vis spectroscopy.

![Graphs showing UV-Vis spectra of Ag-BR in the presence of different metal ions](image)

**Figure 4.9**: UV-Vis spectra of Ag-BR in the presence of different metal ions. In each panel, the inset figure shows ΔA at 426nm vs concentration plot, which shows a linear relationship between concentration and SPR intensity. Panel (D) shows the absorbance difference between absence and presence of 20 μM ion in Ag-BR suspension. The absorbance was selected at wavelength 426 nm. The Horizontal axis of the graph indicates different ions name and Fe\(^2\) and Fe\(^3\) indicated Fe\(^{2+}\) and Fe\(^{3+}\) ion, respectively.
4.4.2 Sensing of Metal Ions by Ag-PVP Nanoparticles

To study the effect of Fe$^{3+}$ on silver nanoparticle and the contribution of capping agent for Fe$^{3+}$ ion sensing have been studied by using Ag-PVP and Ag-T20 nanoparticles. In this regard, I have synthesized silver nanoparticle (AgNP) separately by using polyvinylpyrrolidone (PVP) and TWEEN® 20 (T20) as stabilizing agent. The reducing agent was used sodium borohydrate (NaBH$_4$). The detailed synthesis procedures were discussed earlier in synthesis section. The PVP stabilized silver nanoparticle (Ag-PVP) showed the sensitivity towards As$^{3+}$, Fe$^{2+}$, Fe$^{3+}$ and Hg$^{2+}$ ions. In the presence of As$^{3+}$, Fe$^{2+}$, Fe$^{3+}$ ions the particles make aggregation associated with the bathochromic shift of SPR band, whereas in presence of Hg$^{2+}$ ion the Ag-PVP suspension lead to the gradual hypsochromic shift in its SPR band were found in UV-Vis spectroscopy study. It indicates that in presence of Hg$^{2+}$ ion causes the redox reaction as usual. The color

![Figure 4.10: TEM image of Ag-BR colloid after treatment with different metal ions. The samples have been prepared on cupper grid after 10min incubation at room temperature. For Fe$^{3+}$ and Hg$^{2+}$, the ion concentrations were 20 µM and for Pb$^{2+}$, the ion concentration was 30 µM in Ag-BR colloidal medium.](image-url)
photograph of the experimental observation is shown in Fig. 4.11 and the UV-Vis spectroscopic data is shown in Fig. 4.12.

**Figure 4.11**: Colour photographic images of Ag-PVP suspension in the presence of various metal ions. The image shows in presence of 25 μM different ions in Ag-BR suspensions. Images have been collected 10 min incubation after addition of different ion solutions. Above panel shows the photograph taken under room light without flash mode and bottom panel shows the same with flash mode of camera. Colour photograph collected using Nikon Coolpix P610 digital camera. The metal ions sensing have been highlighted in blue color box.

**Figure 4.12**: UV-Vis spectra of Ag-PVP in the presence of different metal ions. From the UV-Vis spectroscopic data it shows that the Ag-PVP is sensitive towards Fe^{2+}, Fe^{3+} and Hg^{2+} ion. For Fe^{2+} and Fe^{3+} ions, there was a red-shift of SPR band of nanoparticles. This is due to the agglomeration of nanoparticles. But in case of Hg^{2+} ion there was a blue shift of SPR band as usual due to redox reaction between Ag (0) and Hg^{2+}.
4.4.3 Sensing of Metal Ions by Ag-T20 Nanoparticles

In case of TWEEN® 20 stabilized silver nanoparticle (Ag-T20) showed the sensitivity towards As\(^{3+}\) and Hg\(^{2+}\) ions only. It has great selectivity towards those two toxic metal ions. However, it did not show any sensilitity towards Fe\(^{3+}\) ion. In the presence of As\(^{3+}\) ion, the Ag-T20 nanoparticles makes aggregation associated with the bathochromic shift of SPR band and in presence of Hg\(^{2+}\) ion the Ag-T20 solutions led to the gradual hypsochromic shift in its SPR band as like Ag-PVP nanoparticle (The color photograph and spectroscopic data are shown in Fig. 4.13 and Fig. 4.14, respectively). From this additional two studies, it was confirmed that the effect of Hg\(^{2+}\) ions on silver nanoparticles was same and it didn't depend on the stabilizing agents or capping agents. Whatever be the stabilizing agents or capping agents, in presence of Hg\(^{2+}\) in silver nanoparticle suspension there was a redox reaction between Ag NP and Hg\(^{2+}\) ion system. But, the bilirubin mediated synthesized silver nanoparticle shows ion sensitivity towards Fe\(^{3+}\) ion mainly based on redox reaction and this phenomena is exceptional among other sensitive mechanism of Ag nanoparticles towards Fe\(^{3+}\) ion sensing. The detailed possible sensing mechanism of Ag-BR towards Fe\(^{3+}\) ion will be discussed later.

![Figure 4.13: Colour photographic images of Ag-T20 suspension in the presence of various metal ions. The image shows in presence of 25 μM different ions in Ag-BR suspensions. Images have been collected 10 min incubation after addition of different ion solutions. Above panel shows the photograph taken under room light without flash mode and bottom panel shows the same with flash mode of camera. Colour photograph collected using Nikon Coolpix P610 digital camera. The metal ion sensing has been highlighted in blue color box.](image-url)
Proposed Mechanism for Fe$^{3+}$ Ion Sensing

Fig. 4.15 outlined the proposed sensing mechanism. The synthesized silver nanoparticles were capped by bilirubin or biliverdin molecule mainly through carboxylate or nitrogen groups of pyrrol or laclam moiety. After addition of 2.5-5.0 μM Fe$^{3+}$ to Ag-BR suspension, the color of solution turn brown accompanying with the broadening and red shifting of the SPR band is shown in Fig. 4.8, a further increase of Fe$^{3+}$ ion concentration in the suspension there was a blue shift of SPR band and finally turns colorless after addition of 30.0 μM of Fe$^{3+}$ solution. These significant changes were further evidenced by TEM images, which revealed the original spherical and monodispersed (Fig. 4.10) in the absence of Fe$^{3+}$ but in presence Fe$^{3+}$ ion the monodispersibility have been lost. From the TEM image, it is clear that a few particles formed the aggregation and few particles looses their original size and shape. These phenomena indicate that initially there was an extent of ligand exchange between Fe$^{3+}$ water with biliverdin molecules on the surface of AgNPs followed by redox reactions.
Based on UV-Vis spectroscopy study and TEM image analysis, it was clear to say that, at low concentration of Fe$^{3+}$ ion (2.5-5.0 μM) there occur a ligand exchange between octahedral [Fe(H$_2$O)$_6$]$^{3+}$ complex and biliverdin dianion on the surface of Ag-BR nanoparticles. So, initially, the lack of capping agent or stabilizing agent on the surface of Ag-BR nanoparticles causes aggregation. This aggregation causes bathochromic shift in SPR band which have been detected by UV-Vis spectroscopy study (as shown in Fig. 4.8C). With the increase of concentration of Fe$^{3+}$ or the time interval of few minutes there occur visible changes on Ag-BR suspension. The color of the Ag-BR suspension changes from straw yellow to brown and ultimately faint yellow or colorless. This phenomena stated that probably there was a redox reaction between zero-valent silver and [Fe(H$_2$O)$_6$]$^{3+}$., respectively. Although the redox potential of the Ag$^+/Ag$ system (0.80 volt) is higher than Fe$^{3+}$$_{(aq)}/$Fe$^{2+}$$_{(aq)}$, system (0.77 volt), it could be believed that there was no possibility for redox reactions. But, the redox property of a metal in a complex with certain ligand is remarkably different from its free state or aqueous complex. In complexed form, the ability of metal ion to get oxidized or reduced is determined by the overall tendency of the complex for such process. 60,61,62

Figure 4.15: Schematic representation of ligand exchange phenomena. The octahedral [Fe(H$_2$O)$_6$]$^{3+}$ complex loses coordinate bonded three H$_2$O molecules and forms an octahedral complex with biliverdin dianion and remaining three H$_2$O molecules. The red, blue, gray and white ball indicates oxygen, nitrogen, Carmon and hydrogen atom, respectively.
After formation of complex between Fe\(^{3+}\) and biliverdin, there occur an electron transfer from the surface of Ag nanoparticles to Fe\(^{3+}\)-biliverdin dianion complex (Fe\(^{3+}\)\(_\text{bili}\)) which causes the reduction of Fe\(^{3+}\) to Fe\(^{2+}\) state. Whereas the newly formed Fe\(^{2+}\)-biliverdin complex (Fe\(^{2+}\)\(_\text{bili}\)) was quite stable as compared to Fe\(^{3+}\)-biliverdin complex. The stability of these two complexes has been verified by density functional theory (DFT) study. This phenomenon can also be explained on the basis of following thermochemical cycle.\(^{60,62}\)

\[
\begin{align*}
\text{Fe}^{3+} (\text{aq}) & \rightleftharpoons \text{Fe}^{2+} (\text{aq}) \\
\Delta G_1 & \\
\text{Fe}^{3+} (\text{bili}) & \rightleftharpoons \text{Fe}^{2+} (\text{bili}) \\
\Delta G_3 & \\
\text{Fe}^{3+} (\text{aq}) & \rightleftharpoons \text{Fe}^{2+} (\text{aq}) \\
\Delta G_2 & \\
\text{Fe}^{3+} (\text{bili}) & \rightleftharpoons \text{Fe}^{2+} (\text{bili}) \\
\Delta G_4 &
\end{align*}
\]

At equilibrium condition

\[
\Delta G_1 + \Delta G_2 = \Delta G_3 + \Delta G_4
\]

\[
\Delta G_4 = \Delta G_1 + \Delta G_2 - \Delta G_3
\]

\[-nFE_{\text{bili}} = -nFE_{\text{aq}} + ( -RT \ln \beta^{\text{II}} ) - ( -RT \ln \beta^{\text{III}} )
\]

\[-nFE_{\text{bili}} = -nFE_{\text{aq}} + RT \ln \frac{\beta^{\text{III}}}{\beta^{\text{II}}}
\]

\[
E_{\text{bili}} = E_{\text{aq}} - \frac{RT}{nF} \ln \frac{\beta^{\text{III}}}{\beta^{\text{II}}}
\]

\[
E_{\text{bili}} = E_{\text{aq}} - (59.16 \text{ mV}) \log \frac{\beta^{\text{III}}}{\beta^{\text{II}}}
\]

Where \(E_{\text{bili}}\) represents the redox potential of the metal complex with a biliverdin other than water, \(E_{\text{aq}}\) represents the redox potential of \([Fe(OH_2)_6]^{3+}/[Fe(OH_2)_6]^{2+}\) redox couple, \(\beta^{\text{III}}\) is the overall thermodynamic stability constant of the Fe\(^{3+}\)-biliverdin complex and \(\beta^{\text{II}}\) is the overall thermodynamic stability constant of the Fe\(^{2+}\)-biliverdin complex. As can be seen from the last equation, complexes that favor the stabilization of bound metal in the 3+ oxidation state will lower the reduction potential relative to
that of the aqueous form, whereas complexes that stabilize the bound metal in 2+ oxidation state will raise the reduction potential relative to that of the aqueous form. It can be believed that the formation constant for Fe$^{2+}$-biliverdin complex ($K^B$) larger than that of Fe$^{3+}$-biliverdin complex. The electronic configuration of Fe$^{2+}$ corresponds to 3$d^6$ and Fe$^{3+}$ corresponds to 3$d^5$ configurations and biliverdin might be a strong field $\pi$ acceptor ligand.\cite{63,64} The high formation constant, coupled with lower charge, and $t_{2g}^6$ configuration of octahedral Fe$^{2+}$-biliverdin complex favors more electron delocalization to the vacant orbitals of ligand than in corresponding Fe$^{3+}$-biliverdin complex.\cite{63-65} This ability stabilizes Fe$^{2+}$-biliverdin complex toward oxidation relative to Fe$^{2+}$\textsubscript{(aq)} and increased its reduction potential. The stability of two complexes has been studied by computational calculation. The detailed have been given below.

4.5 Density Functional Theory Analysis

The structure of biliverdin was obtained from the PubChem database (CID: 5280353). The structure was geometry optimized at the DFT level of theory using B3LYP/3-21G* basis set as implemented in Gaussian 09. The biliverdin dianion structure was obtained by removing two NH protons using GaussView 5.0. The Fe$^{2+}$ and Fe$^{3+}$ biliverdin octahedral complexes were also drawn in GaussView. Three molecules of water were used to model the octahedral complexes. All these structures were geometry optimized using the same level of theory. Fe$^{2+}$-biliverdin complex was singlet and charge neutral, [Fe (bili)]$^0$, whereas, for Fe$^{3+}$-biliverdin complex was doublet and positively charged, [Fe (bili)]$^+$. 

4.5.1 Analysis of Complexes using Density Functional Theory

The geometry optimized structure of biliverdin is shown in Fig. 4.16. Due to the repulsion between two keto groups, the conjugated ring system deviates a little from the planner geometry with a dihedral angle of $\sim$21° out-of-plane. When it complexes with a Fe atom, the torsion increases to $\sim$135° out-of-plane as shown in Fig. 4.19. The Fe complexes prefer an octahedral geometry, therefore, three water molecules were added to model the complexes. One water molecule was placed in the plane, one above and one below the plane. Fe$^{2+}$-biliverdin complex was charge neutralized by releasing two NH protons. In order to compare the two complexes, number of atoms were kept same for the Fe$^{3+}$-biliverdin complex, thus, a positive charge was added prior to
optimization of Fe$^{3+}$-biliverdin complex. The optimized structures of the Fe$^{2+}$ and Fe$^{3+}$ biliverdin complexes are shown in Fig. 4.19. As the ligands were of different kinds, distorted octahedral geometry was expected to form. The coordinate bond distances from the three ring nitrogen and the three waters are listed in the table 4.2. The partial charges on the ligand atoms and the iron are also given in the table 4.2. In Fe(II) complex the partial charge on Fe(II) was found to be 1.16. When one electron was removed from the complex, partial charge on iron center increases to 1.26. This small increment of positive charge on iron atom indicates that electron transfer occurs from biliverdin to metal center, thus, reducing Fe(III) to Fe(II). The Fe$^{2+}$-biliverdin complex was found to be energetically more stable compared to Fe$^{3+}$-biliverdin complex. About 94 kcal/mol energy difference was observed between Fe$^{2+}$ and Fe$^{3+}$ complexes. The molecular orbital structures were obtained from the energy minimized geometries and is shown in the Fig 4.19. Although the orbital structures were similar for the two complexes, the orbital energies were different and is compared in the table 4.2.

Figure 4.16: B3LYP/3-21G* level energy optimized structure of biliverdin molecule. Panel A showing the top view, panel B and C showing the tilt view of the molecule. From these three orientation, it is clear that N1, N2 and N3 are lies in same plane whereas N4 lies in out of plane. Atom color code: blue (N), gray (C), white (H) and red (O) in above ball and stick model.
Figure 4.17: B3LYP/3-21G* level energy optimized structure of biliverdin dianion. Panel A showing the top view, panel B showing the tilt view and panel C and D showing the side view of the anion. From these different orientations, it is clear that N1, N2 and N3 are lies in same plane whereas N4 lies in out of plane as like neutral molecule. Atom color code: blue (N), gray (C), white (H) and red (O) in above ball and stick model.

Figure 4.18: B3LYP/3-21G* level energy optimized structure (A) of Fe³⁺-biliverdin complex and (B) of the Fe²⁺-biliverdin complex.
Figure 4.19: Frontier molecular orbitals, the numerical figures indicates their corresponding energy.
Table 4.2: Geometry of the octahedral complex and the partial charges

<table>
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<th>[Fe (bili)]^0 or Fe^{2+}(bili)</th>
<th>[Fe (bili)]^+ or Fe^{3+}(bili)</th>
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<td>-3422.1723 au</td>
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<td><strong>Partial</strong></td>
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<td>charge (δ)</td>
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4.6 CONCLUSION

Here, we have synthesized silver nanoparticle using bilirubin as a reducing agent and stabilizing agent, is a ‘green’ approach because bilirubin is a metabolic byproduct and a biodegradable compound. Not only it is a ‘green’ approach, but also our prepared nanoparticles shows excellent sensitivity towards toxic metal ions like Hg^{2+}, Fe^{3+} and Pb^{2+} ions in water samples in the few micromolar range. We also demonstrate mechanism of reduction of Fe^{3+} to Fe^{2+} in biliverdin complex state. In the present study, this Ag-BR nanoparticle based probe for Fe^{3+} sensing using silver nanoparticles might be the very first observation.

REFERENCES


