3.1. Introduction

In this chapter, 4-amino-6-hydroxy-2-mercaptopyrimidine (AHMP, Chart 3.1) is used as a capping agent for the synthesis of AuNPs. It has been reported that 2-mercaptopyrimidine (2-Mpy) derivatives have distinct properties in contrast to normal aromatic thiols such as thiophenol [1]. Compared to 2-Mpy, AHMP contains an additional amine group which can be expected to stabilize the AuNPs from aggregation by electrostatic repulsion. The AuNPs have been extensively used for Cys detection due to their strong visual and tunable properties [2-6]. Besides, in most of the cases selective detection of Cys was achieved only in the presence of NaCl [2,4]. Thus, the objective of the present study is to detect Cys in the presence of high concentrations of other amino acids. In addition, AHMP also utilized for the selective determination of thiamine (vit-B1) in the presence of other vitamin complexes.

Chart 3.1. Structure of 4-amino-6-hydroxy-2-mercaptopyrimidine (AHMP).
3.2. Characterization of AHMP-AuNPs and selective determination of L-Cys

3.2.1. UV-visible spectral studies

The electronic spectroscopy is one of the simplest techniques to characterize the metal nanoparticles because it exhibits characteristic absorption band due to the surface plasmon resonance (SPR) for the metal nanoparticles in the visible region [7]. The formation of AHMP-AuNPs was monitored by UV-visible spectroscopy (Fig. 3.1A). The AHMP in water shows an absorption maximum at 275 nm with a very weak shoulder band at 245 nm (curve a) due to the existence of thione-thiol equilibrium in water. The HAuCl₄ (31.7 mM) in water shows an absorption maximum at 291 nm (curve b). The addition of AHMP solution to HAuCl₄ does not affect the absorption maximum but the intensity of the absorption was increased which may due to the formation of complex between AHMP and HAuCl₄ (curve c). When 2 ml of 0.125% of NaBH₄ was slowly added to a mixture of AHMP and HAuCl₄ solution, the yellow color solution was slowly changed into a wine red color indicating that the formation of AuNPs, which shows a new absorption peak at 520 nm (curve d), corresponding to the surface plasmon resonance (SPR) band. The observed SPR band at 520 nm confirms the successful formation of AHMP-AuNPs. Further, we have monitored the stability of the AuNPs by UV-visible spectroscopy. In contrast to the UV-visible spectrum of freshly prepared AHMP-AuNPs, no appreciable spectral changes were observed for
six months aged solution (Fig. 3.1B). Further, the wine red color of the solution remains same (Fig. 3.1B; Inset photographs). These results indicate that the AHMP-AuNPs were highly stable.

![Absorption spectra](image)

**Fig.3.1.** (A) Absorption spectra obtained for (a) AHMP in water, (b) H AuCl$_4$ in water and (c) mixture of AHMP and HAuCl$_4$ and (d) after the addition of NaBH$_4$ to (c). Inset: (A). Photograph showing the as synthesized AHMP-AuNPs. (B) Stability of AHMP-AuNPs. Inset: (B) Photographs of freshly prepared (a) and after the six months aged AHMP-AuNPs (b).

### 3.2.2. HR-TEM, zeta potential and XRD studies

The size and morphology of the AHMP-AuNPs were characterized by HR-TEM. The HR-TEM images taken at different magnifications are shown in Fig.3.2. Fig.3.2A illustrates that they are roughly spherical in shape and the high magnification image depicts that the size of the AuNPs was found to be ~11 nm (Fig.3.2B). Selected area electron diffraction pattern (inset A) exhibits the crystalline nature of the AHMP-AuNPs [8]. The zeta potential study shows that the AuNPs are negatively charged due
to the surface adsorbed AHMP. It shows a value of -30.1 mV (Fig.3.2C). It is expected that the thiol group of AHMP is chemisorbed on the surface of AuNPs whereas the amine and hydroxyl groups available in AHMP are free from binding. The presence of lone pair electrons in amine groups predominantly stabilizes the AuNPs. Further, the crystalline nature of AHMP-AuNPs was confirmed by XRD analysis (Fig.3.2D). It illustrates the diffraction features appearing at 38.17°, 44.36°, 64.59° and 77.57° corresponding to (111), (200), (220) and (311) planes, respectively. The peak corresponding to the (111) plane is more intense than that from the other planes. The ratio between the intensity of the (200) and (111)

![Fig.3.2. HR-TEM images (A, B), zeta potential (C) and XRD pattern of AHMP-AuNPs. Inset (A): SAED pattern of AHMP-AuNPs.](image)

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diffraction peaks was much lower, suggesting that the (111) plane is a predominant orientation. The width of the (111) peak was employed to calculate the average crystalline size of the AHMP-AuNPs using the Scherrer equation [9]. The calculated average size of the AuNPs is ~11 nm, which closely matches with the particle size obtained from the HR-TEM images.

3.2.3. Determination of concentration and molar extinction coefficient

The concentration of AHMP-AuNPs was calculated based on the reported procedure [10] and was found to be 1.9 µM. The molar extinction coefficient of AHMP-AuNPs was calculated using the following equation [11].

\[
\varepsilon = \frac{A}{b \cdot c}
\]

(3.1)

where \(\varepsilon\) is the molar extinction coefficient, \(A\) is the absorbance and \(b\) is the path length of sample and \(c\) is the concentration of AuNPs. The molar extinction coefficient was found to be \(7.1 \times 10^5\) M\(^{-1}\) cm\(^{-1}\). The obtained value suggests that the synthesized AuNPs having good photochemical properties [11].

3.2.4. Determination of band gap energy and compare with DFT studies

The band gap energy of AHMP-AuNPs was calculated by using the following equation [12].

\[
\text{Band gap energy (E)} = \frac{hc}{\lambda}
\]

(3.2)

where \(h\) is the plack constant, \(c\) is the speed of light and \(\lambda\) is the absorption maximum. The band gap energy of AHMP-AuNPs was found to be 2.38 eV. We have also estimated the band gap energy of the AHMP and AHMP-AuNPs by DFT. The optimized HOMO and LUMO energy gap of AHMP and AHMP-AuNPs are given below in Table 3.1. HOMO and LUMO energy gap of
AHMP and AHMP-AuNPs were calculated as 4.8731 and 2.4990 eV, respectively. The obtained energy gap value for AHMP-AuNPs is in accordance with the SPR band of the AuNPs. When energy gap value decreases the absorption maximum appears at visible region of higher wavelength. The energy gap of AHMP-AuNPs closely matches by DFT (2.49 eV) with the value calculated from the Planck equation (2.38 eV). The obtained value suggested that AHMP-AuNPs are semiconducting nature [12].

<table>
<thead>
<tr>
<th>Compound</th>
<th>HOMO</th>
<th>LUMO</th>
<th>Energy gap (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHMP</td>
<td><img src="image" alt="AHMP HOMO" /></td>
<td><img src="image" alt="AHMP LUMO" /></td>
<td>4.8731</td>
</tr>
<tr>
<td>AHMP-AuNPs</td>
<td><img src="image" alt="AHMP-AuNPs HOMO" /></td>
<td><img src="image" alt="AHMP-AuNPs LUMO" /></td>
<td>2.4990</td>
</tr>
</tbody>
</table>

**Table 3.1.** Optimized HOMO and LUMO energy gap values of AHMP and AHMP-AuNPs.

### 3.2.5. Determination of surface coverage and surface area of AHMP ligand

The number of AHMP molecule bound on the surface of AuNPs was calculated based on the reported procedure [10]. It was found that ~ 69
molecules covered a single AuNP. Further, the surface area of AHMP molecule was found to be 5.5 nm$^2$.

3.3. Determination of Cys using AHMP-AuNPs

3.3.1. Spectrophotometric determination of Cys

Fig.3.3 shows the UV-visible spectra of AHMP-AuNPs in the presence of different concentrations of Cys. They show the SPR band at 520 nm (Fig.3.3A, curve a). While adding 0.125 µM Cys, the wine red color of AHMP-AuNPs changed into pale wine red and the absorbance was decreased (curve b). While increasing the concentration of Cys from 0.125 to 1.25 µM (curves b-k), the wine red color was completely changed into purple and the absorbance intensity was dramatically decreased and a new shoulder band was appeared at 623 nm. Further increasing the concentration from 1.25 to 1.75 µM Cys, the absorption band at 623 nm was red shifted to 670 nm (curves k-o). The dark purple color of AHMP-AuNPs was gradually changed into pale purple while increasing the concentration of Cys from 1.75 to 2.250 µM, (Fig.3.3B; Inset photograph). Finally, a precipitate was settled down at the bottom of the quartz cell while adding 2.375 µM Cys. These results are in good agreement with Mie theory [13].
According to Mie theory, when the distance between two nanoparticles becomes smaller than the sum of their radii, the SPR band displays a red shift, broadening and decreasing in intensity. It is well known that when the spherical structure of AuNPs is changed, a new absorption peak appears at higher wavelength. Thus, both color change and appearance of a new peak at higher wavelength region suggesting the morphological changes of AHMP-AuNPs. The red shift in the SPR band was ascribed to the near-field coupling that occurs when the interparticle distance decreases.

**3.3.3. HR-TEM studies of AHMP-AuNPs in the presence of Cys**

The Cys induced aggregation was confirmed by HR-TEM studies. **Fig.3.4A** shows the HR-TEM images of AHMP-AuNPs in the presence of Cys. It shows the aggregated structure in the presence of 1.75 µM Cys.
The aggregation is due to the strong interaction between AHMP-AuNPs and Cys. The strong interaction between AHMP-AuNPs and Cys was also confirmed by zeta potential studies. Fig.3.4B shows the zeta potential of AHMP-AuNPs in the presence of 1.75 µM Cys and it shows value of +0.9 mV in contrast to -30.1 mV in the absence of Cys. The zeta potential study shows the strong interaction of Cys AuNPs causes aggregation.

![Fig.3.4.](image)

Fig.3.4. (A) HR-TEM image and (B) zeta potential of AHMP-AuNPs in the presence of Cys.

### 3.3.4. FT-IR studies of Cys with AHMP-AuNPs

Further to support the binding of Cys on the surface of AHMP-AuNPs, FT-IR studies were carried out. Fig.3.5 shows the FT-IR spectra of powder samples of Cys and AHMP-AuNPs with Cys. In general, the infrared spectra of amino acids display characteristic bands for both carboxylate and primary amine salt because they exist in zwitter ion form. Fig.3.5a shows the FT-IR spectrum of Cys. It shows a broad band in the range of 2950-3100 cm\(^{-1}\) corresponding to -NH\(_3^+\) stretching and the band at 1347 cm\(^{-1}\) corresponding to the symmetric
stretching of COO\(^{-}\). The bending mode of N-H at 1543 cm\(^{-1}\) and the stretching band of S-H at 2555 cm\(^{-1}\) are weak. The obtained result match with the infrared spectrum reported for Cys [14]. Fig.3.5b shows the FT-IR spectrum of AHMP-AuNPs with Cys. It also shows a very broad band in the range of 3250-3420 cm\(^{-1}\) corresponding to -NH\(_3^+\) stretching whereas symmetric stretching of COO\(^{-}\) was shifted from 1347 cm\(^{-1}\) to 1386 cm\(^{-1}\) when compared to Cys. Further, the band of N-H bending was shifted from 1543 cm\(^{-1}\) to 1565 cm\(^{-1}\) whereas the band belonging to S-H was disappeared. The obtained result indicates that the presence of Cys on the surface of AHMP-AuNPs.

![FT-IR spectra](image)

**Fig.3.5.** FT-IR spectra obtained for solid samples of (a) Cys and (b) AHMP-AuNPs with Cys.
3.3.5. Selective determination of Cys using AHMP-AuNPs

The main objective of the present study is to detect selective determination of Cys in the presence of all other amino acids. Fig. 3.6 shows the colorimetric response AHMP-AuNPs in the presence of 1000-fold higher concentrations of amino acids (Phe, Ser, Asp, Lys, Tyr, Val, Asn, Ala, Pro, Gln, Arg, Ile, Leu, Met, Gly, Glu, Trp and His). It can be seen from Fig. 3.6, addition of 1.75 μM Cys alone changed the color of AHMP-AuNPs from wine red to purple, whereas no visual color change was observed after the addition of other amino acids. The collected absorption spectral data evidently revealed that proposed method is highly selective towards determination of Cys and the LOD was found to be 86 nM by spectrophotometry.

It has been well established that thiol can bind on the surface of noble metal nanoparticles via metal-sulfur bond and the interaction is strong enough to immobilize the thiol groups on the surface of nanoparticles [15]. Very recently, colorimetric determination of Cys using carboxymethyl cellulose capped AuNPs has been reported. It has been suggested that Cys can replace carboxymethyl cellulose, a long polymer chain from the surface of the AuNPs in the presence of a salt and then it attached on the surface of AuNPs via Au-S bond [2]. In the present study, while adding low concentration of Cys to AHMP-AuNPs, it replaces AHMP molecules from the AuNPs surface through Au-S bond formation and thus the color of the AuNPs was changed into purple. While increasing the concentration of Cys, most of the AHMP molecules are likely replaced by Cys.
Thus, the AuNPs were aggregated due to the absence of any stabilizing force on the surface of AuNPs. The effect of pH on the interaction of Cys with AHMP-AuNPs was also studied. It has been already reported that only at relatively low pH, the amine group of amino acids can bind with AuNPs [16], whereas the thiol group can bind with AuNPs at high pH (pH > 5) [17]. The pH of the AHMP AuNPs solution was found to be 5.8. At this pH, the -SH group of Cys bound to the AuNPs via Au-S bond. Among the different amino acids, Cys only contains thiol group. Thus, AHMP-AuNPs selectively bind with Cys. It should be mentioned that AHMP is a small molecule and we can expect spaces between the AHMP molecules attached on AuNPs. Therefore, the penetration of Cys through AHMP layer is also possible.

**Fig.3.6.** Colorimetric changes of AHMP-AuNPs in the presence of 1.75 μM Cys and the presence of 1000-fold higher concentrations of other amino acids.
3.3.6. Practical application

The practical application of the present method was evaluated by determining Cys in human blood serum and urine samples. The blood serum samples collected from the nearby clinical laboratory were used in the present study. The standard addition technique was used to examine the recovery of Cys. 1 mL of the blood serum sample was diluted to 50 mL solution and it has been used as stock solution. We have also determined Cys in human urine sample. Similar standard addition method was followed for the recovery of Cys in urine samples. A good agreement was obtained between spiked and measured Cys. The recovery and RSD values for the addition of different concentrations of Cys in blood serum and urine samples are given in Tables 3.2 and 3.3. The obtained good recovery and RSD values indicated that the present method could be successfully used for the determination of Cys in real sample analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cys added (µM)</th>
<th>Cys found (µM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>urine</td>
<td>-</td>
<td>0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.04</td>
<td>100.4</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.05</td>
<td>100.1</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12.05</td>
<td>100.0</td>
<td>0.51</td>
</tr>
</tbody>
</table>

* Three replicate measurements

Table 3.2. Determination of Cys in human urine samples*.
Three replicate measurements.

### Table 3.3. Determination of Cys in human blood serum samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cys added (µM)</th>
<th>Cys found (µM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood serum</td>
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<td>0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.0</td>
<td>99.0</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.93</td>
<td>98.8</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>11.8</td>
<td>98.9</td>
<td>1.35</td>
</tr>
</tbody>
</table>

* # Three replicate measurements.

### 3.4. Determination of thiamine (Vit-B1) using AHMP-AuNPs

#### 3.4.1. Spectrophotometric determination of thiamine

The synthesized colloidal solution of AHMP-AuNPs was also used to selectively determine thiamine. Fig.3.7 shows the UV-visible spectra of AHMP-AuNPs in the presence of different concentrations of thiamine. They show the SPR band at 520 nm. While adding 0.15 µM thiamine, the wine red color of AHMP-AuNPs was slightly changed into purple and the absorbance was decreased with a small red shift (~1 nm). While increasing the concentration of thiamine from 0.30 to 1.05 µM (curves b-h), the absorbance intensity was decreased with a red shift. The wine red color was completely changed into purple when 1.20 µM thiamine was added whereas the absorption peak was red shifted to 690 nm (curve i, inset A: (ii-b) photograph). Interestingly, an isosbestic point was appeared at 560 nm (Inset i), indicating a neat conversion of AHMP-AuNPs into complexed thiamine-AuNPs. Further increasing the concentration of thiamine up to 1.80 µM, the purple color of AuNPs was completely disappeared and a precipitate was settled down at the bottom of the quartz cell (Inset A: (ii-c) photograph) and the SPR band was decreased without
any significant shift at 690 nm (Fig B; (curve j-m)). These results are in good agreement with Mie theory [13]. According to Mie theory, when the distance between two nanoparticles become smaller than the sum of their radii, the SPR band displays a red shift, broadening and decreasing in intensity. The observed spectral and color changes were attributed to the aggregation of AHMP-AuNPs. The red shift in the SPR band was ascribed to the near-field coupling that occurs when the interparticle distance decreases. Since thiamine having positive charge, it electrostatically interacts with the negatively charged AHMP-AuNPs and decreases the interparticle distance due to charge screening effect.

**Fig.3.7.** UV-vis spectra of AHMP-AuNPs in the presence of thiamine (A) (a-i) (0-1.20 µM) and (B) (0-1.80 µM). **Inset:** (i) isosbestic point (ii) photographs of (a) AHMP-AuNPs, after the addition of (b) 1.20 µM and (c) 1.80 µM thiamine.
3.4.2. HR-TEM and zeta potential studies of AHMP-AuNPs with thiamine

The aggregation of AHMP-AuNPs by thiamine was confirmed by HR-TEM. The HR-TEM images of AHMP-AuNPs in the presence of 1.20 µM thiamine show the aggregated structure (Fig.3.8A). The strong interaction between AHMP-AuNPs and thiamine was confirmed by zeta potential studies. Fig.3.8A shows the zeta potential of AHMP-AuNPs in the presence of 1.20 µM thiamine, and it shows value of +2.7 mV in contrast to -30.1 mV in the absence of thiamine. The zeta potential study shows the neutralization of surface charge by the positively charged thiamine with AuNPs causes aggregation.

![Fig.3.8](image)

Fig.3.8. (A) HR-TEM image and (B) zeta potential measurement of AHMP-AuNPs in the presence of thiamine.

3.4.3. Selective determination of thiamine

The effect of various interferences for the determination of thiamine was investigated. Fig.3.9 shows the colorimetric response of AHMP AuNPs in the presence of Pb²⁺, Cu²⁺, Hg²⁺, vitamins C, B2, B6, B12, B9, B3 and B1. It can be

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seen from Fig. 3.9, B1 (thiamine) only changed AHMP-AuNPs color from wine red to purple (1.20 µM) whereas 1000-fold higher concentrations of common interferents including vitamin B derivatives and metal ions does not change the color in the presence of thiamine. The collected absorption spectral data revealed that proposed method is highly selective towards determination of thiamine and the LOD was found to be 25 nM by spectrophotometry.

The obtained high selectivity is due to strong electrostatic interactions between thiamine and AHMP-AuNPs. Thiamine has positive charge on nitrogen atom and it also contains thiazole ring.

![Fig. 3.9](image) (A) Colorimetric changes of AHMP-AuNPs after the addition of thiamine (1.20 µM) and 1000-fold higher concentrations of various interferences.

Therefore, strong electrostatic interactions between thiamine and AHMP-AuNPs are possible. On the other hand, such electrostatic interaction is not possible for the other vitamin B derivatives due to the absence of positive charge.
on nitrogen atom. Besides, B2 and vitamin B9 contain bulky groups and therefore, the possible steric repulsion inhibits the interaction of them with AHMP-AuNPs. The present method showed relatively high sensitivity compared to other methods.

Further, the interference of L-amino acids was investigated in the presence of thiamine. It was found that except cysteine all other 19 amino acids do not interfere even in the presence of micromolar concentration. Besides, glutathione also interferes for the determination of thiamine at high concentration.

3.4.4. Practical application

The practical application of the present method was evaluated by determining thiamine in human blood serum samples. The blood serum samples collected from the nearby clinical laboratory were used. The standard addition technique was used to examine the recovery of thiamine. 1 mL of the blood serum sample was diluted with 50 mL phosphate buffer solution.

The recovery results for the different additions of thiamine in blood serum samples are given in Table 3.4. The recovery of 99.2% and 99.5% were obtained and good agreement was obtained between spiked and measured thiamine. These results indicated that the present method could be efficiently used for the determination of thiamine in practical applications.
The present study demonstrates the spectrophotometry and colorimetric determination of Cys and thiamine in the presence of possible interferences using AHMP-AuNPs. The wine red color of AHMP-AuNPs was changed to purple after the addition of Cys. But, no color change was observed while adding other L-amino acids. On the other hand, no color change was observed after the addition of vitamins to AHMP-AuNPs. However, wine red color of AHMP-AunPs was changed to pink after the addition of thiamine. The color changes were also reflected in the UV-vis spectra. Colorimetric and absorption spectral changes evidently revealed that proposed method is highly selective towards determination of Cys and thiamine. Among these two compounds, the present probe showed selectivity towards thiamine. The obtained high selectivity is due to strong electrostatic interactions between thiamine with AHMP-AuNPs than Cys. The LOD was found to be 86 and 25 nM by spectrophotometry for Cys and thiamine, respectively. The present method was successfully utilized to determine Cys and thiamine in real samples.

<table>
<thead>
<tr>
<th>Samples #</th>
<th>thiamine spiked (µg L⁻¹)</th>
<th>thiamine found (µg L⁻¹)</th>
<th>RSD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
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<td>0.9 ± 0.1</td>
<td>1.45</td>
<td>99.2</td>
</tr>
<tr>
<td>Sample 2</td>
<td>2</td>
<td>0.19 ± 0.1</td>
<td>0.81</td>
<td>99.5</td>
</tr>
</tbody>
</table>

* Three replicate measurements.

**Table 3.4.** Determination of thiamine in human blood serum samples.

**3.5. Conclusions**

The present study demonstrates the spectrophotometry and colorimetric determination of Cys and thiamine in the presence of possible interferences using AHMP-AuNPs. The wine red color of AHMP-AuNPs was changed to purple after the addition of Cys. But, no color change was observed while adding other L-amino acids. On the other hand, no color change was observed after the addition of vitamins to AHMP-AuNPs. However, wine red color of AHMP-AunPs was changed to pink after the addition of thiamine. The color changes were also reflected in the UV-vis spectra. Colorimetric and absorption spectral changes evidently revealed that proposed method is highly selective towards determination of Cys and thiamine. Among these two compounds, the present probe showed selectivity towards thiamine. The obtained high selectivity is due to strong electrostatic interactions between thiamine with AHMP-AuNPs than Cys. The LOD was found to be 86 and 25 nM by spectrophotometry for Cys and thiamine, respectively. The present method was successfully utilized to determine Cys and thiamine in real samples.
3.6. References


