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# pH-Dependent Ion Binding Studies on 2-Mercaptopyrimidine

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## Abstract

Mercaptopyrimidines exhibit antiviral and antibacterial properties and were found to inhibit the synthesis of tRNA thus acting as antitumor and antithyroid agents. These molecules exhibit tautomeric equilibrium between the thiol and thione because of the highly mobile protons they possess. These compounds can incorporate metal ions in their structure because they possess electron donor atoms. Metal ions are electron acceptor species; they come from food, medicine, and drinking water. The objective of this project was to investigate whether and how metal ions such as $Na^{+}$, $Hg^{2+}$, $Al^{3+}$, $Ce^{4+}$, and $UO^{22+}$ affect the structure of 2-mercapto pyrimidine using electronic and Vibrational spectroscopes. Binding studies were conducted in aqueous solutions at different pH and metal ion/ligand molar ratios. It is hypothesized that interaction does occur between metal ions of valence other than two and certain numbers of 2-Mercaptopyrimidine. However, the interactions tend to differ from one metal ion to another depending on the charge of the metal ion.

The electronic spectra have shown, the pH affects the structure of 2MCP, the thione form is the dominant species in water, and all the metal ions cited above interact with a 2-mercapto pyrimidine. But $Hg^{2+}$ and $Ce^{4+}$ interact with 2-mercapto pyrimidine more strongly than $Na^{+}$, $Al^{3+}$, and $UO^{22+}$. The stability constant calculated using electronic spectra and a Scatchard plot demonstrated that the complexes of Hg and Ce with 2-mercapto pyrimidine are the most stable.

**Keywords:** Analytical chemistry; Biochemistry; Antioxidant activities; Antibacterial activities; Spectroscopy

## Introduction

Metal ions come from food, medicine and drinking water [1]. An understanding of their role in the biological systems has become essential to the practice of medicine. The presence of metal ions has been associated with the misfolding process of proteins and structural change in DNA, leading to some types of cancers, and neurodegenerative diseases [2,3]. Metal ions released by antacid preparations resulted in a nearly complete loss of activity of antibacterial [3,4]. So it is mandatory that the effect of metal ions on the structure (thus on the activity) of some pharmaceuticals be investigated. There is a need to better understand potential metal ion-pharmaceuticals interactions in order to reduce possible adverse effects or enhance the efficacy of the drugs being employed and to better inform patients [5-10]. The chemistry of mercapto pyrimidines is attracting research interest because it involves both S and N atoms in their structures [10,11]. There is a considerable versatility in the coordination modes of these molecules, which may include monodentate binding through S or through N, bridging through a single S, and bridging through both S and N or chelating via S to N backbone. In other terms, these compounds can coordinate as monodentate ligands and more frequently as polydentate ligands either to a single metal center, acting as a chelating ligand, or to several metal centers as bridging ligand. In addition, these compounds exhibit a tautomeric equilibrium between the >C-SH (thiol) and the >C=S (thione) form due to the highly polar protons they possess (Figure 1). The predominant form largely depends on the state and conditions of the molecule [11]. However, in the solution phase, there are several factors influencing the tautomeric equilibrium. Among the most prominent are the solvent, temperature, pH, and concentration. Thus, it was suggested that the thione form predominates over the thiol form in a polar solvent; while the thiol form predominates in a polar solvent.

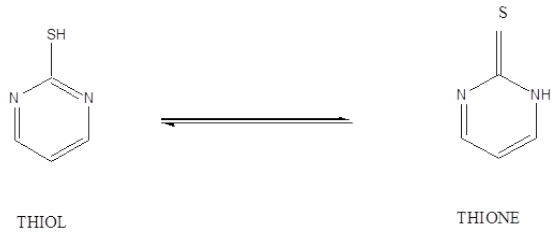


**Figure 1:** Tautomeric equilibrium of 2 – Mercaptopyrimidine.

The Pharmaceutical drug 2-mercapto pyrimidine is the thione form. The sulfur atom in the thione attacked the excess iodine on people that have thyroid diseases. Therefore, it is very important for the availability of the sulfur atom in the thione form of 2-mercapto pyrimidine.

Among these mercapto pyrimidine compounds, we have chosen to focus on 2-mercapto pyrimidine (2-MCP). This drug is used in the treatment of Hashimoto's thyroiditis and Grave's disease, which are the two most common autoimmune thyroid disorders [6]. Although anti-thyroid drugs have been used for over 50 years, their mechanisms of

action are still poorly understood at the molecular level. 2MCP also exhibits antiviral, antibacterial and anti-tumor properties.

To our knowledge, there are only a few experimental papers concerning the tautomerism of 2MCP. The energies of the two tautomers in polar and non-polar solvents were calculated using Density Functional Theory (DFT). The results indicated that thiol structure is the most stable species, but the energy values become very close to each other once a solvent such as cyclohexane was included. This result points to the coexistence of the two species of the tautomeric equilibrium [6,11].

Interactions of 2MCP with divalent heavy metal ions such as mercury have been extensively investigated, probably because both heterocyclic thiones and thionate are among the system used to mimic bio-relevant Hg-S interactions (Cysteinyl sulfur residues in a variety of bio-systems). There is a lack of data regarding the interaction between 2MCP and metal ions of valence other than two. There is also some disagreement on the coordination modes of 2MCP: both N and S coordination was suggested earlier for some complexes; other publications suggested that coordination is via exocyclic sulfur only [10-12]. The objective of this project was to investigate whether and how metal ions such as $Na^{+}$, $Hg^{2+}$, $Al^{3+}$, $Ce^{4+}$ and $UO^{22+}$ affect the structure of 2-mercapto pyrimidine using electronic and vibration spectroscopes. The binding studies were conducted in aqueous solution at different pHs and metal ion/2MCP ratios. It was hypothesized that significant interaction does occur between metal ions of valence other than two and certain members of 2MCP. The extent of interaction will vary from one metal ion to another depending on the charge of the metal ion. The mechanism of reaction may be attributed to chelation. The specific goals of this research are: To investigate the interaction between 2MCP and metal ions cited above using UV visible and fluorescence spectra. This was to estimate the binding affinity of 2MCP towards each metal ion. To determine the binding stability constants for the metal ion-2MCP complex using electronic spectra. To determine the mechanisms of interaction between 2MCP and the selected metal ions using infrared and Raman spectra. The objective is to identify the preferred binding sites of metal ions on 2MCP. The techniques employed here are useful in probing the structural changes upon complexation. This project will provide new insights on a number of drugs and potential pharmaceutical agents containing metal-binding or metal-recognition sites that can bind or interact with metal ions and potentially influence their bioactivities and might also cause damages on their target biomolecules. These studies have great potential to improve the fundamental understanding of thiones/thiols, as well as biological catalysis in general. In addition, since the design of bioactive thione/thiol derivatives for pharmaceutical applications depend on an adequate understanding of catalytic mechanism; this research may significantly advance thiones toward their potential as therapeutics for several devastating diseases, including AIDS, cancer, and hepatitis C. Finally, the development of highly efficient and specific heavy metal sequestering thione could, ultimately, provide a viable and biodegradable means of removing heavy metals from contaminated water. Tautomerism is an important aspect of several chemical and biological processes. Among all possible solvents, water is the natural biological solvent and is involved with all processes related to life. Investigation of the tautomeric reactions in an aqueous environment is a great theoretical challenge, and this is the basis for this research.

## Materials and Methods

### Materials: Reagents, pH, and metal/ligand molar ratio

All chemicals were of analytical-reagent grade. All aqueous solutions are prepared with deionized water that is further purified by a Millipore Milli-Q high purity water device (Milli-Q-System A10 from Waters, Millipore, and Billerica, MD). 2-Mercaptopyrimidine, metal salts (as chloride salts) and all other reagents (Cerium sulfate and uranyl acetate hydrates) were commercially obtained and used as received from Fisher. Solution pH was adjusted using concentrated HCl (aq) or NaOH (aq) to give pH ranging from 1-9. The interaction experiments were done using aqueous solutions at room temperature. For each experiment at the desired pH, the metal/ligand molar ratios used are 0.5 (excess 2MCP), 1 (equimolar), and 2 (excess metal ion).

### Methods

Aqueous solutions were prepared to take into account the molar mass of 2MCP and of ion salts, and the metal ion/ligand molar ratio. After interaction between the aqueous metal ion solution and 2MCP, two experiments were conducted: (a) the resulting complexes were directly analyzed by UV visible in solution [12]; (b) the resulting complexes were filtered, dried and then analyzed as solids by infrared and Raman spectroscopies [13]. Table 1 Summarizes the calculated masses of 2MCP and the above metal ions at $10^{-4}$ M and $10^{-3}$ M concentrations.

| Compound | Metal ion | Mass at Conc.$10^{-4}$ M | Mass at Conc.$10^{-3}$M |
|---|---|---|---|
| 2-MCP | | 0.00112 g | 0.0112 g |
| | NaCl | 0.0006 g | 0.006 g |
| | $HgCl_2$ | 0.0027 g | 0.0270 g |
| | $AlCl_3$ | 0.0024 g | 0.0240 g |
| | $CeSO_4$ | 0.0033 g | 0.0330 g |
| | $UO^{22+}$ | 0.0043 g | 0.043 g |

**Table 1:** Calculated masses of 2MCP and metal ions.

### Characterization methods

**UV visible spectroscopy:** The samples for absorption measurements were placed in a quartz cell of path length 1 cm. UV visible spectra were obtained using a Varian CARY BIO 300 spectrometer, using a sample of deionized water as the reference. The absorbance spectra were recorded between 800 nm and 200 nm.

UV visible spectroscopy is a powerful tool for studying the structure of 2MCP and its metal complexes in aqueous solution. Transmission comes from the π and n electrons of the aromatic ring [12,14].

**Infrared and Raman spectroscopes:** Infrared spectra of the sample were obtained using Perkin Elmer FTIR Spectrum One spectrometer using pure samples. The spectra were recorded between 4000 and 600 $cm^{-1}$. Spectra were obtained by integrating scans at a resolution of 4 $cm^{-1}$ [15,16]. The infrared spectra were obtained using an attenuated total reflectance (ATR) device. The spectra shown are the difference spectra obtained by using the subtraction routine between the spectrum of the reference and the spectrum of the sample [13]. Ambient air was used for the background or reference.

Raman spectra were taken on a HORIBA Jobin Yvon FT-Raman using pure samples. The spectra were recorded between 4000 $cm^{-1}$ and 0 $cm^{-1}$ at 4 $cm^{-1}$ resolutions. A 633 laser was used as an excitation line. The detector was a liquid nitrogen cooled Ge detector. The output laser power was set to 50 mW.

FT-IR and Raman were used to study the structure of 2MCP and its metal complexes as powders. The C=S and N-H groups give rise to well-known stretching and bending in the electromagnetic spectrum. Raman spectroscopy has been used extensively for the study of active sites of metalloproteins. Structural and bonding information has been gained especially because the metal-ligand vibrations are found below 1000 $cm^{-1}$ in the Raman spectra. Typically, the Raman spectrum of the 2MCP will display an additional peak (compared to the metal-free 2MCP Raman spectrum) in this region upon metal complexation. This peak at low frequency is characteristic of metal-2MCP interaction [13].

## Results

### Electronic spectra

First, the UV-visible spectrum of metal-free 2MCP was recorded alone, second record the UV visible of 2MCP after interaction with a selected metal ion, and third, the absorbances were compared. If the two absorbances have the same value, there is no interaction; if they are different, there is interaction. Interpretation of UV visible spectra is based on electronic data found in the literature [13,15-19].

### Effect of solvent on the structure of 2MCP

To identify the predominant tautomer of 2MCP in water as solvent relative to other nonpolar solvents. Figures 2 and 3 show the UV visible spectra of metal-free 2-mercapto pyrimidine in water and cyclohexane, respectively. The electronic spectra of 2MCP in water exhibited three bands at 212 nm, 278 nm, and 344 nm and changed substantially when cyclohexane was used as the solvent [12,14]. In the latter case, a major band at 238 nm and two weaker bonds at 213 nm and 275 nm were clearly distinguished. As documented in the literature, bands at 212 nm, 278 nm, and 344 nm correspond to the thione form while the band at 238 nm corresponds to the thiol form. These observations demonstrate that the tautomeric thione structure is favored in polar solvents, while the thiol structure dominates in non-polar media, in agreement with the literature.

Bands at 212 nm, 278 nm, and 344 nm are due to 2MCP in water so will follow the change in frequency or intensity of these bands when adding metal ions to 2MCP.

### Effect of pH on the electronic spectra of metal-free 2-mercapto pyrimidine

**The importance of pH:** There is a dramatic effect of pH on the structure of 2-mercapto pyrimidine. Figure 4 shows the UV visible spectra of 2MCP at pH 1, 7, and 9. Comparison of UV visible spectra it shows that pH has a significant effect on the peak positions of 2MCP. Our reference is the spectrum of 2MCP at pH 7. The peaks are 212, 278, and 344 nm. When the pH was adjusted to 9 (alkaline solution), the peak at 278 shifts to a lower wavelength (265 nm) and decreases in intensity; the peak at 212 nm shifts to 225 nm and decrease in intensity. The peak at 344 nm disappeared. When the pH was adjusted to 2 (acidic solution), the peak at 278 shifts to a higher wavelength (285 nm) and increases in intensity; and the peak at 212 nm shifts slightly to 215 nm and increases in intensity. The peak at 344 nm shifts to 350 nm with no change in intensity.

The structure of 2MCP is seriously affected by the changes in pH. An acidic solution favors the protonation of 2MCP and an alkaline solution favors deprotonation of 2MCP. The effects of pH in both acidic and alkaline mediums changed the structure of 2MCP.

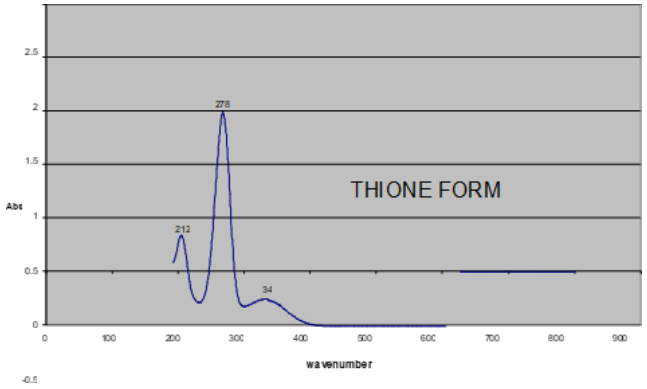


**Figure 2:** 2MCP in water (polar solvent).

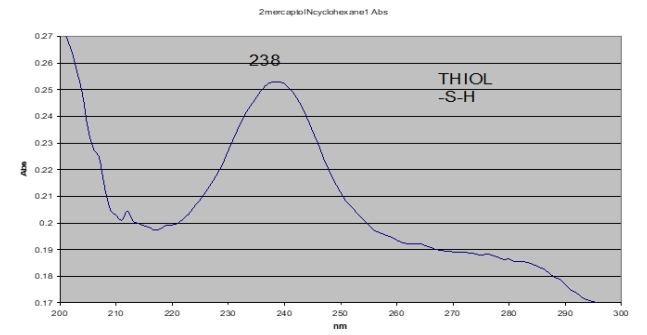


**Figure 3:** 2MCP in Cyclohexane (nonpolar solvent).

### Effect of complexation on the structure of 2-Mercaptopyrimidine

To estimate the binding capacity of 2MCP toward selected metal ions (goal#1).

Absorbance measurements were carried out at different pH values and metal ion/2MCP molar ratios. In all the cases, the Beer Lamber law was verified, which states:

The absorbance was proportional to the amount of 2MCP.

$A=\varepsilon cl$ (1)

Where A=Absorbance,

l=path length (cm),

c=Concentration,

ε=Molar absorptivity.

Therefore, absorbance is proportional to the concentration of the sample of 2MCP, (A α C) Figure 5 shows the absorbance spectra of 2MCP in the absence and presence of $Ce^{4+}$ ions. 2MCP has strong absorbance with peaks at 212 nm, 284 nm, and 350 nm at pH 1. Its absorbance intensity at 284 nm decreases with increasing concentration of cerium ions, indicating binding of cerium ions to 2MCP.

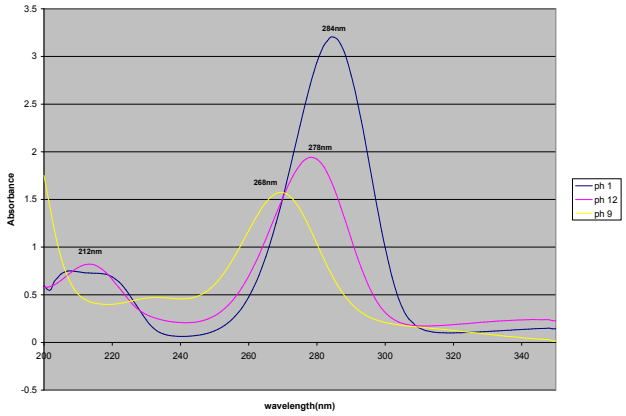


**Figure 4:** Comparison of UV-Visible spectra of 2MCP at pH 1, 7, and 9.

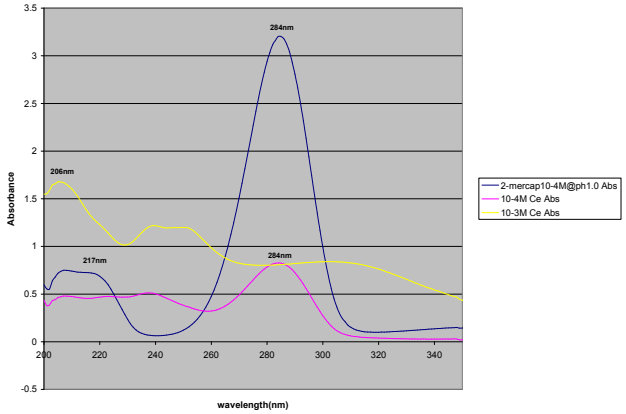


**Figure 5:** Comparison of UV-Visible spectra of 2MCP after interaction with Ce (IV).

Figure 6 shows the comparison of UV-Visible spectra of 2MCP interacting with $Hg^{2+}$ at concentration $10^{-4}$ M. 2MCP shows stronger absorbance with peak intensities of 212 nm, 284 nm at pH 1. The absorbance of 2MCP decreases when $Hg^{2+}$ ions concentration was added, which explains the binding of $Hg^{2+}$ to 2MCP.

The same approach was used for Figures 7-9, (i.e., $Na^{+}$, $Al^{3+}$, and $UO^{22+}$) ions interacting with 2MCP. The 2MCP has strong absorbance, but when the metal ions were added, they show less binding or interaction with 2MCP.

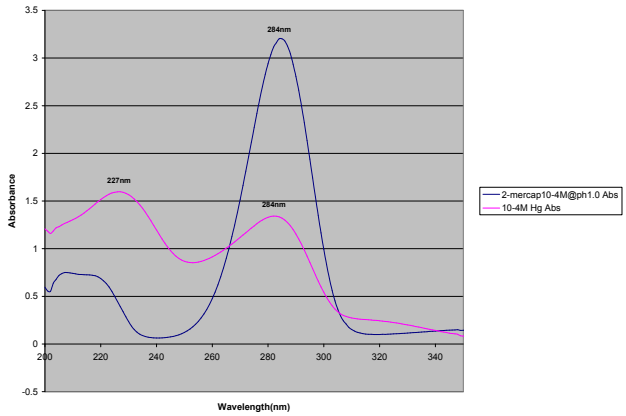


**Figure 6:** Comparison of UV-Visible Spectra of 2MCP after interacting with Hg (II).

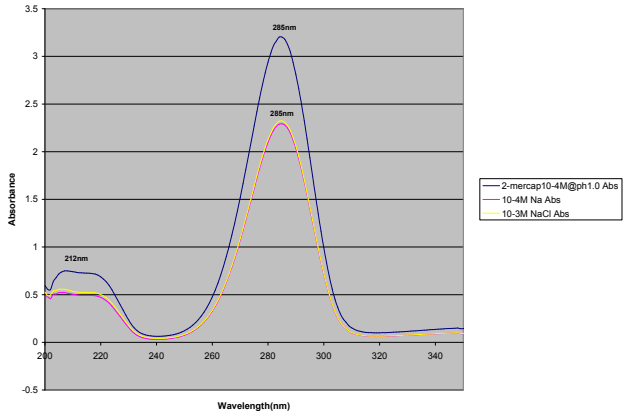


**Figure 7:** Comparison of UV-Visible Spectra of 2MCP after interacting with Na (I).

The absorbance measurements presented in this section were done to verify the binding capacity of 2MCP towards selected metal ions. In other terms, we need to know which metal ion a higher binding affinity for 2MCP has when everything is equal. The concentration of $10^{-4}$ M for 2MCP was chosen because the UV visible spectrum was good, so it was possible to exploit the absorbance at this concentration. The peak at 280 nm is the reference peak because this peak is the strongest. pH 1 was selected because it is the pH we obtained the largest changes in absorbance. Then two solutions were prepared each containing $10^{-4}$ M of 2MCP. The first solution to 2MCP alone was the control. Another $10^{-4}$ M of aluminum ions was added in the second solution. That means we use the metal ion/2MCP molar ratio 1. The absorbance (peak at 280 nm) of 2MCP of the two solutions were measured and compared. The difference between the two absorbance values corresponds to the number of metal ions interacting with 2MCP. This difference over the absorbance of the control gave the percentage of the amount reacting with 2MCP. Figure 10 summarizes the results obtained with all the metal ions interacting with 2MCP at pH 1, 7, and

9. Table 2 summarizes the results obtained with all the metal ions interacting with 2MCP at pH 1 only.

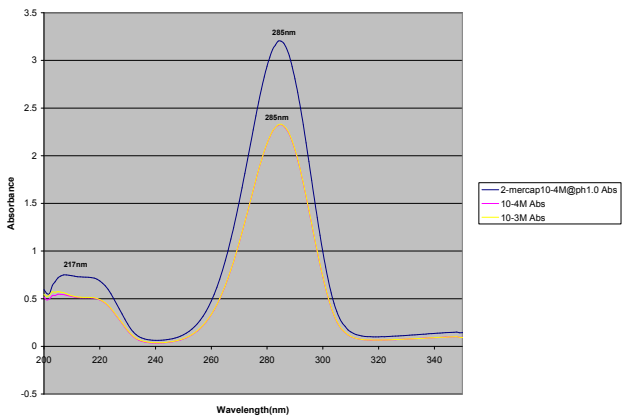


**Figure 8:** Comparison of UV-Visible Spectra of 2MCP after interacting with Al (III).

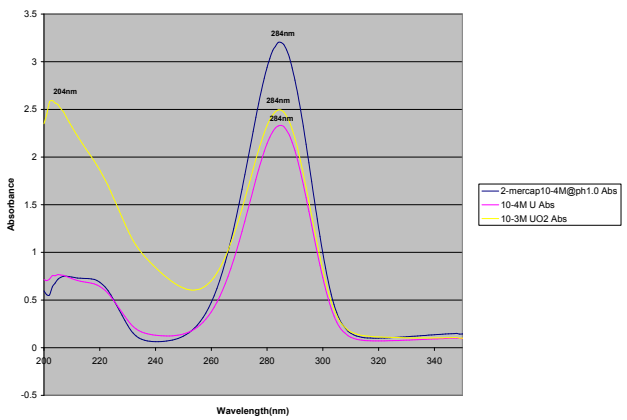


**Figure 9:** Comparison of UV-Visible Spectra of 2MCP after interacting with U (VI).

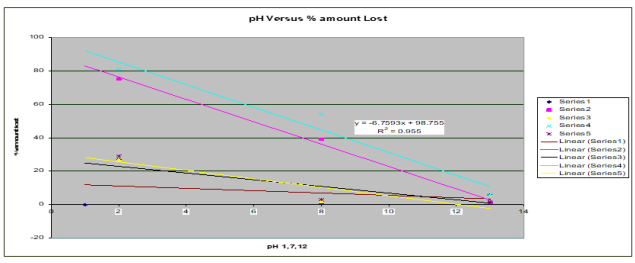


**Figure 10:** Comparison of pH profile, UV-Visible spectra of 2-Mercaptopyrimidine before and after interaction with Na (I), Hg (II), Al (III), Ce (IV), and U (VI) metal ions.

| Oxidation Number $10^{-4}$ M | % amount interacting with 2-MCP |
|---|---|
| Na (I): +1 | 0.14 |
| Hg (II): +2 | 0.81 |
| Al (III): +3 | 0.27 |
| Ce (IV): +4 | 0.75 |
| $UO^{26+}$: +6 | 0.29 |

**Table 2:** Oxidation Number Versus % amount lost at pH 1.0 (Concentration $10^{-4}$ M 2-MCP).

Results were clearly mentioned; the best results were obtained with Hg (II) and Ce (IV). Figure 10 summarizes the results obtained with all the metal ions interacting with 2MCP as a function of the oxidation states of the metal. The metal ions with the valence numbers 2 and 4 interact better with 2MCP than those with the oxidation states 1, 3, and 6. The small amount of uranyl ion absorbed may be due to the presence of oxygen atoms in the uranyl compound.

## Calculations of stability constants (goal # 2)

To estimate the relative stability of the metal complexes of 2 MCP.

Based on the results obtained in the section above, we decided to investigate how stable the complexes formed between 2MCP and the metal ions are. In the literature, potentiometric titration and other electrochemical techniques have been used to estimate these constants for compounds other than 2MCP. We decided to use a Scatchard plot using the data obtained using electronic spectra [20].

The equilibrium between a metal ion (M) and ligand (L) in solution yielding the product (ML) can be described as follow: In general terms, the equilibrium constant K can be calculated as follows:

$$Keq=[ML]/[M][L] \quad (2)$$

K is also called the stability constant (expressed as a logarithm); [M] is the concentration of metal ion, and [L] is the concentration of a ligand 2MCP. The concentrations of M and of the complex ML are determined by measuring the absorbance of the appropriate solutions using UV visible spectroscopy. From equation 2 above other equations have been developed to obtain equations such as the Stern-Volmer or Scatchard equations widely used in biochemistry to measure the equilibrium constants. One of these equations has the following form: The Stern-Volmer equation;

$$Ao/(Ao-A)=1/(fK[M])+1/f \quad (3)$$

Where A and $A_o$ are current 2MCP + metal ion and initial (2MCP reference) absorbance, respectively; K is the stability constant, M the concentration of metal ion, and f the fraction of the absorbance which is accessible to the metal ion. Because all the 2MCP is accessible to metal ions, f=1.

The idea was to prepare a series of at least 4 solutions in which increments of M are added to a constant amount of L. A series of solutions were prepared with a fixed concentration (200 mL of $10^{-4}$ M 2MCP) and various amounts of selected metal ions in water. The fixed concentration ($10^{-4}$ M) of 2MCP is used as an internal reference; the measured absorbance is $A_o$=2.395 at 278 nm. The absorbance of the solutions of 2MCP containing metal ions was then recorded and listed as A. The average data of triplicate, independent results were used for the following discussion. For illustration, we give the example of calculation of the stability constant for an Al-2MCP complex (Figure

7). The results for all the metal/2MCP complexes are shown in Table 3. As a table, the table will show, the stability constants are very low for the complex of 2MCP with Na, intermediate for the complexes of 2MCP with aluminum and uranyl, and higher for the complex of cerium and mercury. These results are consistent with the data shown in Figure 10.

| MCP ($10^{-4}$ M) | | Mass (g) | # mol/L | $A_0/A_0$-A | Slope | K | Log K |
|---|---|---|---|---|---|---|---|
| $A_o$ | NaCl | 0 | 0 | | | | |
| | | 8 | 0.685 | 356 | | | |
| | | 15 | 1.285 | 294 | | | |
| | | 22 | 1.88 | 171 | | | |
| | | 26 | 2.225 | 137 | | | |
| | | | | | 0.0065 | 154 | 2.187 |
| | $HgCl_2$ | 0 | 0 | | | | |
| | | 0.01 | 0.0002 | 4.829 | | | |
| | | 0.05 | 0.0009 | 3.496 | | | |
| | | 0.1 | 0.002 | 1.781 | | | |
| | | 0.15 | 0.003 | 1.201 | | | |
| | | | | | 0.0005 | 2000 | 3.301 |
| | $AlCl_3$ | 0 | 0 | | | | |
| | | 5 | 0.1035 | 88.704 | | | |
| | | 10 | 0.207 | 70.112 | | | |
| | | 15 | 0.311 | 38.629 | | | |
| | | 20 | 0.4145 | 15.665 | | | |
| | | | | | 0.004 | 250 | 2.398 |
| | Ce $(SO_4)_2$ | 0 | 0 | | | | |
| | | 0.1 | 0.0015 | 9.4 | | | |
| | | 0.5 | 0.0075 | 7.2 | | | |
| | | 0.8 | 0.012 | 3.5 | | | |
| | | 1 | 0.015 | 2.2 | | | |
| | | | | | 0.0015 | 667 | 2.824 |
| | $UO_2(AC).4H_2O$ | 0 | 0 | | | | |
| | | 2 | 0.02355 | 73.42 | | | |
| | | 5 | 0.059 | 62.45 | | | |
| | | 8 | 0.0945 | 48.65 | | | |
| | | 10 | 0.118 | 45.24 | | | |
| | | | | | 0.003 | 333 | 2.523 |

**Table 3:** Stability constant of Metal ion-2MCP complex.

These results are also consistent with literature values obtained by potentiometric titration on related compounds (Figure 11).

That is, 2MCP-Na<2MCP-Al<2MCP-U<2MCP-Ce<2MCP-Hg. The 2MCP-Hg and Ce complexes are very strong. Those with Na are not stable.

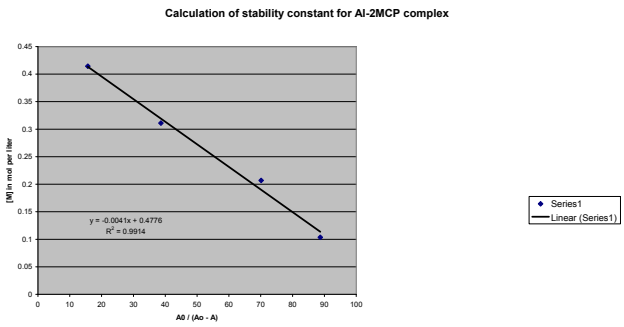


**Figure 11:** Graph of stability constant for Al-2MCP complex.

That implies that complexions with large stability constants are more stable than ones with smaller ones. Stability constants tend to be very large numbers. In order to simplify the numbers, a log scale is often used.

## Infrared and Raman spectra

First the infrared or Raman spectra of metal-free 2MCP were recorded alone, second the infrared or Raman spectrum of 2MCP after interactions with selected metal ion was also recorded, and third, the two spectra were compared. If the two spectra are similar, there is no interaction; if the two spectra are different, there is interaction. Interpretation of infrared and Raman spectra is based on vibrational data found in the literature [13,21-26]. Also, the focus on certain spectra is based on conclusions obtained from the electronic spectra of this project.

## The infrared spectrum of metal-free 2MCP

To identify the peaks of the main functional groups, Figure 12 shows the infrared spectrum of metal-free 2MCP in the region 4000-600 $cm^{-1}$. This spectrum reveals the characteristic vibration bands of 2MCP. In the literature, the band at 1602 $cm^{-1}$ is attributed to ν(N-H), those at 1215 $cm^{-1}$ and 1182 $cm^{-1}$ are attributed to ν(C=S) and β(C=S) vibrations [11,13]. Because intermolecular interactions are expected to occur, it is likely that hydrogen bonds were developed. That explains the broadband at around 2500 $cm^{-1}$. All the above bands are typical of the thione structure. The peaks at around 979 $cm^{-1}$ and 959 $cm^{-1}$ are due to N-H stretching. The rest of the bands are associated with methyl groups and aromatic ring vibrations. Thus, the infrared spectra provide experimental evidence that the throne is the dominant form, in agreement with the literature [10].

The band at 1602 $cm^{-1}$, 979 $cm^{-1}$, and 959 $cm^{-1}$ are due to N-H group. The bands at 1215 $cm^{-1}$ and 1182 $cm^{-1}$ are due to C=S group. Interactions of 2MCP with metal ions will change the frequencies or intensities of these bands.

## Effect of pH on the infrared spectra of metal-free 2-mercapto pyrimidine

**The importance of pH:** There is a significant effect of pH on the structure of 2MCP. Figure 13 shows the infrared spectra of 2MCP alone at pH 1, 7, and 9. Comparison of these infrared spectra shows that pH has an effect on the peak intensities of 2MCP. Our reference is the spectrum of 2MCP at pH 7. The intensities of the peak due to N-H at 979 $cm^{-1}$ and 959 $cm^{-1}$ are of equal intensity.

When the pH was increased to 9, the peak at 959 $cm^{-1}$ decreases in intensity. All the other bands remain unchanged, that is deprotonation.

When the pH is adjusted to 2, the peak at 959 $cm^{-1}$ increases in intensity. All the other bands remain unchanged (i.e., Protonation).

The structure of 2MCP is affected by the change in pH at the level of the N atom. An acidic solution induces protonation of 2MCP and an alkaline solution induces deprotonation of 2MCP. These results are consistent with those obtained using electronic spectra.

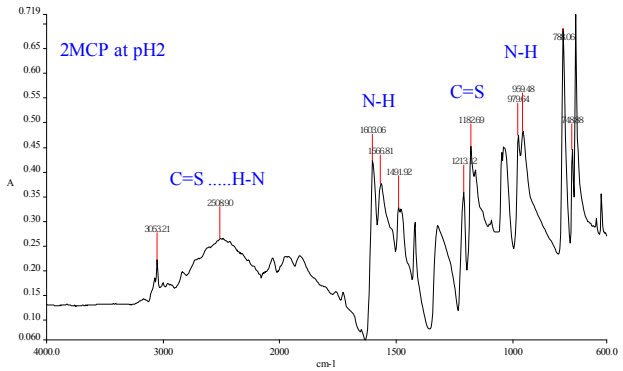


**Figure 12:** Infrared Spectra of 2-Mercaptopyrimidine.

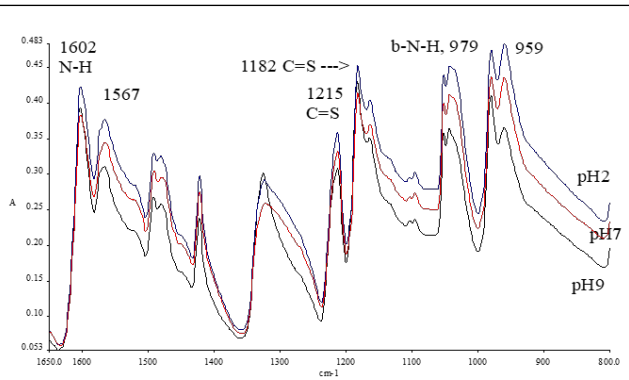


**Figure 13:** pH effect on 2MCP structure: An Infrared spectrum has changed the structure of 2MCP leading to protonation and deprotonation of the compound at pH 2, 7 and 9 respectively.

## Effect of complexation on the infrared spectra of 2-Mercaptopyrimidine (goal #3)

To identify the preferred binding sites of metal ions on 2MCP monitoring the change in peak position (frequencies) or in peak intensity indicated the complexation of 2MCP by metal ion [13].

**Hg and Ce ions:** Figures 14 and 15 show the infrared spectra of 2MCP interacting with Hg (II) and Ce (IV) [22,27]. The bands due to N-H groups are shifted to lower wave numbers (frequencies) upon complex formation. This confirmed that the N atom of N-H group coordinates to Hg (II) and Ce (IV). The shifting to the lower frequencies can be explained as a weakening of the N-H bond resulting from the electron density drainage from the N atom due to its coordination to the metal atom. The most dramatic changes appear for the C=S group, the bands are due C=S disappear or are largely shifted towards lower wave numbers for Hg (II) and Ce (IV). This confirms that the S atom of the C=S group coordinates to Hg (II) or Ce (IV). The shifting to the lower frequencies can be explained as a weakening of the C=S bond resulting from the electron density drainage from the S atom due to its coordination to the metal atom.

The preferred binding site of the metal ion is better evidenced by infrared spectroscopy than by electronic spectroscopy. Both N and S-coordination are suggested in these cases.

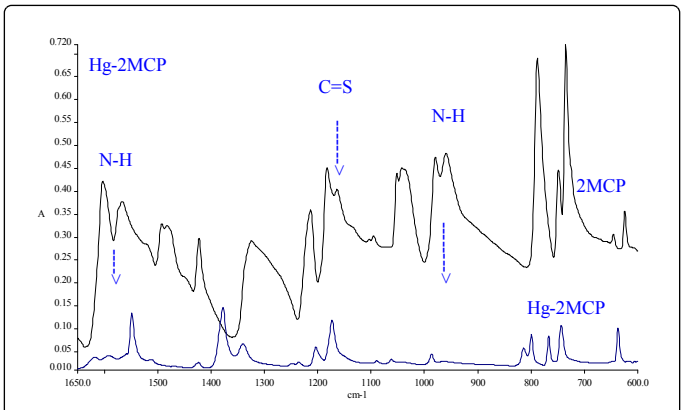


**Figure 14:** Infrared spectra of 2MCP and its Hg (II) complex.

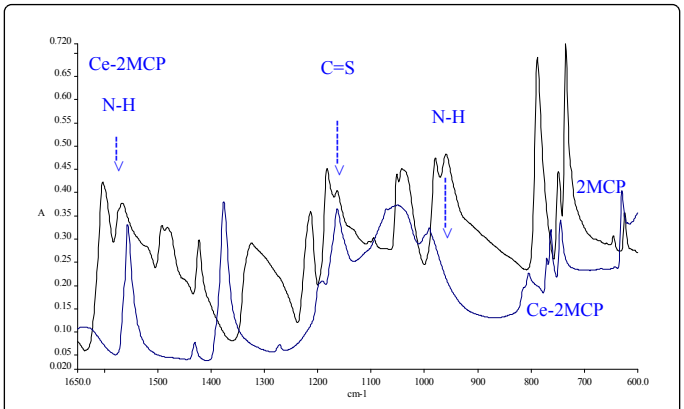


**Figure 15:** Infrared spectra of 2MCP and its Ce (IV) complex.

**Aluminum ions:** Figure 16 shows the infrared spectra of 2MCP interacting with Al (III). There is a significant change in the infrared spectra. The intensity of the band at 1602 $cm^{-1}$ and 959 $cm^{-1}$ due to N-H is decreased upon complex formation. The band at 1212 $cm^{-1}$ is also shifted. This confirms that interaction between 2MCP and Al comes through the N atom of the N-H group and the S atom of the C=S group.

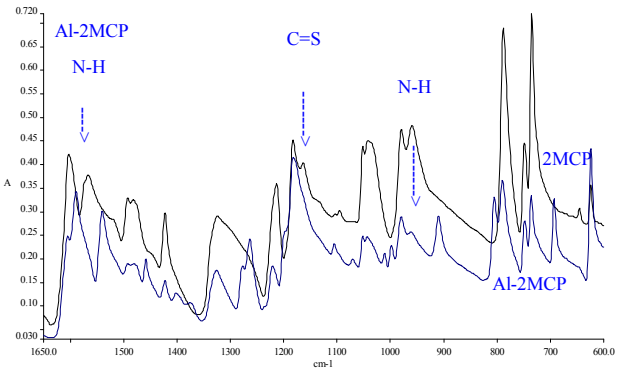


**Figure 16:** Infrared spectra of 2MCP and its Al (III) complex.

There is a significant interaction between 2MCP and Al ions. These results are not in agreement with the electronic spectra.

**Na and Uranyl ions:** Figures 17 and 18 show the infrared spectra of 2MCP interacting with $Na^+$ and uranyl respectively [13,27]. There is no dramatic change in the infrared spectra. Only the intensity of the band at 959 $cm^{-1}$ due to N-H is decreased on the formation of the complexes. This confirms that interaction between 2MCP and $Na^+$, or uranyl comes through the N atom of N-H group.

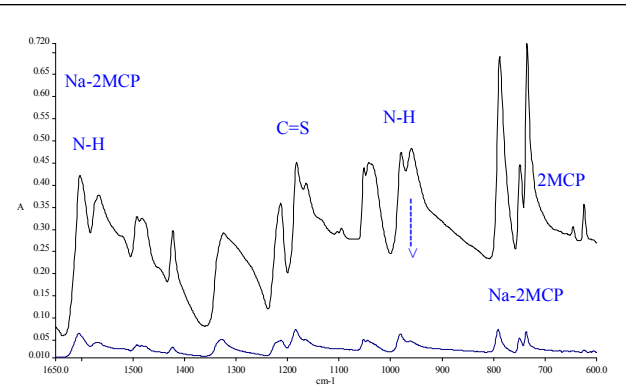


**Figure 17:** Infrared spectra of 2MCP and its Na (I) complex.

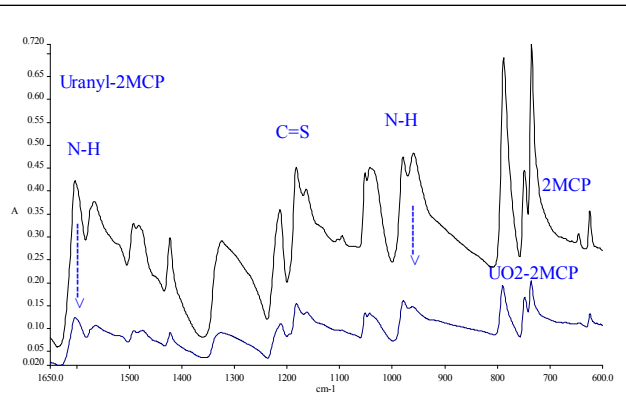


**Figure 18:** Infrared spectra of 2MCP and its U (VI) complex.

There is a weak interaction between 2MCP and Na, and uranyl ions. These results are in agreement with the electronic spectra.

### Effect of complexation on the Raman spectra of 2-Mercaptopyrimidine (goal #3)

To identify the possible covalent bonds between metal ions and 2MCP Raman spectroscopy has been used extensively for the study of active sites of metalloproteins. Structural and bonding information has been gained especially because the metal-ligand vibrations are found below 1000 $cm^{-1}$ in the Raman spectra. Typically, the Raman spectrum of the metal/2MCP complexes displayed additional peaks (compared to the metal-free 2MCP Raman spectrum) in this region upon metal complexation. The peak at low frequency is characteristic of metal-2MCP interaction. In other words, the additional bands in the lower frequencies are due to the bonding between the metals and non-metals [13]. From the results obtained using UV- visible and infrared spectra, the structure of metal ion/2MCP complexes was further studied by identifying the possible bonds formed between metal ions and the N and/or S atoms of 2MCP. Figure 19 shows the Raman spectra of 2MCP at different pHs. The Raman spectra are similar to the infrared spectra, the main features remaining the same. Deprotonation of 2MCP is evidenced by an additional small band at around 1600 $cm^{-1}$.

**Hg ions:** Figure 20 shows the Raman spectra of 2MCP with Hg (II). From the literature, and for related compounds, the band at 334 $cm^{-1}$ was assigned to the bond Hg-S, and the band at 238 $cm^{-1}$ was assigned to Hg-N. Hg (II) binds to both nitrogen and sulfur atoms of the 2MCP in a strong way [22]. Assignment of other bands requires the use of computation technique.

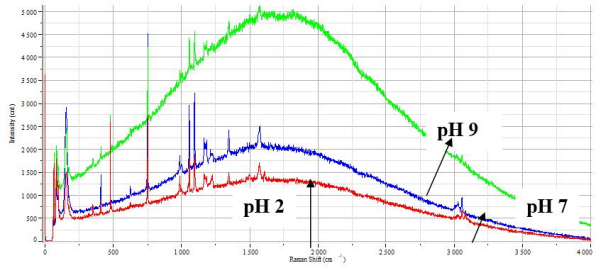


**Figure 19:** pH effect on 2MCP structure: Raman spectrum has changed the structure of 2MCP leading to protonation and deprotonation of the compound at pH 2, 7 and 9 respectively.

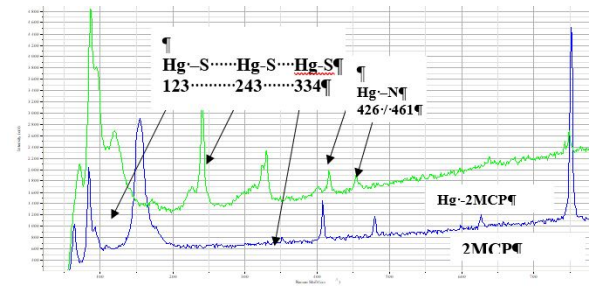


**Figure 20:** Raman spectra of 2MCP at its Hg (II) complex.

**Al, Na, and Uranyl ions:** Figure 21 shows the Raman spectrum between 2MCP with Na (I). Comparison of the Raman spectra of metal-free 2MCP and that of Na-2MCP complex shows no difference. This confirms that Na (I) does not interact in a significant way with either the sulfur or the nitrogen atom of the 2MCP. UV visible and infrared technique show a weak interaction through the N atom, but this interaction is definitely very weak, in agreement with the calculated stability constant.

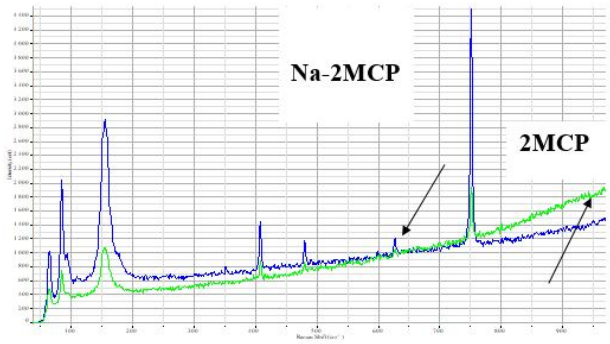


**Figure 21:** Raman spectra of 2MCP and its Na (I) complex.

Figure 22 shows the Raman spectra of 2MCP with Al (III). From the literature, and for related compounds, the strong band at 138 $cm^{-1}$ was assigned to the bond Al-N. Al (III) binds strongly to the N atom of the 2MCP. A weak band at around 330 $cm^{-1}$ is the Al-S bond. Assignment of other bands requires the use of computational techniques.

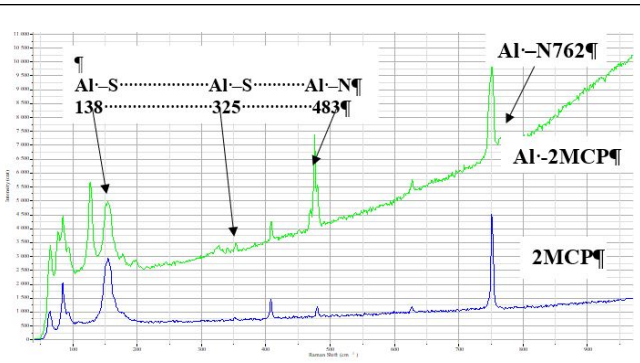


**Figure 22:** Raman spectra of 2MCP and its Al (III) complex.

The result of the Raman spectra shows Hg (II) binds to both sulfur and nitrogen. Al (III) binds to nitrogen and, in a weaker way, to S. Na (I) does not bond to 2MCP in any significant way.

## Discussion

### Electronic spectra

In the first phase of this study, the UV-visible spectra of 2MCP and its complexes were examined at different pHs and metal ion/ligand molar ratios. 2MCP is strongly affected by changes in pH. In acidic solution, 2MCP is protonated through the lone pair of the N atom. From this result, we can predict that H+ and the metal ion $Mn^{+}$ will compete for the same binding site in an acidic solution. The

protonation and deprotonation of 2MCP disturbed the aromatic ring so protonation induced a shift to higher wavelength while deprotonation induced a shift to lower wavelength (blue shift) [13].

Among the metal ions used in this study, Hg and Ce have been shown to bind more strongly to 2MCP. The explanation may lie in the structure of these ions. They possess d orbital that is empty, so they use the empty orbital to coordinate to N or S atoms of 2MCP. The case of uranyl is a little different because of the presence of O atoms near U atoms. The O atoms prevent 2MCP from binding to the U atom. The calculated stability constants agree with the conclusions drawn from the UV visible spectra.

### Infrared and Raman spectra

In the second phase of this study, the infrared and Raman spectra of 2MCP and its complexes have been examined at different pH and metal ion/ligand molar ratios. 2MCP is seriously affected by changes in pH. An acidic solution, 2MCP is protonated through the lone pair of the N atom, and in an alkaline solution, 2MCP is deprotonated. Protonation or deprotonation of N-H has the consequence the weakening the band so the infrared band due to N-H groups are shifted to lower frequencies (wave number) in acidic solution. The same is true when metal ion coordinates to N-H. The shifting to lower frequencies can be explained as a weakening of the N-H bond resulting from the electron density drainage from the N atom due to its coordination to the metal atom. The same explanation is valid when coordination occurs through the S atom. Every time the structure of a compound is changed there is a change in the force constant described by Hooks law, so the frequency will also change [12].

In the third and final phase of this study, structural and bonding information was gained especially because the metal-ligand vibrations are found below 1000 $cm^{-1}$ in the Raman spectra. The metal ions are heavier than the other atoms so when you consider Hook's law, the reduced mass is greater, so the frequency is lower because the frequency is inversely proportional to the square root of the reduced mass. Typically, the Raman spectrum of the metal/2MCP complexes displays an additional peak (compared to the metal-free 2MCP Raman spectrum) in this region upon metal complexation. This peak at low frequency is characteristic of metal-2MCP interaction.

## Conclusion

Interactions between 2MCP and 5 different metal ions have been extensively studied using UV visible, infrared and Raman spectra:

- Effect of UV-visible spectra on 2MCP

Electronic spectra have shown that (1) pH affects the structure of 2MCP, (2) the thione form is the dominant species in water, and (3) all the metal ions cited above interact with 2-MCP. But Hg (II) and Ce (IV) interact with the 2MCP more strongly than Na (I), Al (III), and $UO^{22+}$. The stability constants calculated using electronic spectra and a Scatchard plot demonstrated that the complexes of Hg and Ce with 2-MCP are the most stable.

- Effect of Infrared and Raman spectra on 2MCP

Infrared and Raman spectra have shown some marker bands useful to identify the sites involved in metal chelation at a specific pH value. Particularly the preferred binding sites on 2MCP are S and N atoms for Hg and Ce, and only N atom for Al, Na, and U. Hg, Ce, and Al ions form covalent bonds with 2MCP. Infrared and Raman spectroscopies are powerful tools for investigating the preferred binding sites of the metal ions on a molecule. All coordinate bonds are covalent in nature.

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