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Research Article

COMPARATIVE ANALYSIS FOR METAL BINDING CAPACITY OF CYSTEINE BY USING UV-VIS SPECTROPHOTOMETER

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ABSTRACT: The metal binding capacity of cysteine with three different metals Nickel, Copper and Lead was studied using UV-Vis spectrophotometer for which absorbance values were taken after interaction of cysteine with metal salt solutions (10ppm and 100ppm). Before taking above absorbance dilution factor was set using cysteine stock. The increase in peak intensity was observed when metal salt solution and metal salt-cysteine solution were compared. Based on peak shift and peak intensity finally it can be concluded that the binding capacity of cysteine with Nickel is more, followed by lead and copper. The normal chromophore activity in cysteine is due to the sulphur in which the transition takes place from non bonding orbital's to the excited antibonding orbital in the range of 210-215nm range. The binding of the metals with cysteine may affect the chromophore activity and may also lead to structural damage of the chromophore. This can give the decrease in the peak intensity or the complete shift in the peak. These results suggest that cysteine metal binding ability can be used for the removal of the metals in water purification. Also this property can be used in removal of metals from our body considering the fact that cysteine may not show adverse effect in the system. So we can go for designing a new type of drug containing cysteine which helps to prevent the accumulation of such metals and thus prevent us from adverse effect.

Keywords: Cysteine, Metals, Chromophore activity, Thiol, UV-Vis spectrophotometer.

INTRODUCTION

Cysteine is alpha amino acid with chemical formula HO_2CH (NH_2) CH_2SH.(M. Fátima Barroso et al., 2007) It contains thiol group (Figure: 1) and is slightly polar molecule (Thomas K. Harris and George J. Turner, 2002). Two cysteine molecules can undergo disulfide bond formation to form cystine due to oxidation of thiol groups (Derek G. Smyth et al., 1991). The thiol groups have affinity for metals, so cysteine can interact with metals salts (Swaran J.S. et al., 2010). The normal chromophore activity in cysteine is due to the sulphur in which the transition takes place from non bonding orbital's to the excited antibonding orbital in the range of 210-215 nanometer range (Cameron Sadegh et al., 2003). The binding of the metals with cysteine may affect the chromophore activity and may also lead to structural damage of the chromophore (Malin Mejáre and Leif Bülow, 2001). This can give the decrease in the peak intensity or the complete shift in the peak. (Maria Franca Brigatti et al.,1999). In UV visible the absorbance or the reflectance is in the range 200-700 nm directly affects the perceived colours of the chemicals involved, in this region the molecules undergoes electronic transitions (Stephen L. Upstone, 2000).

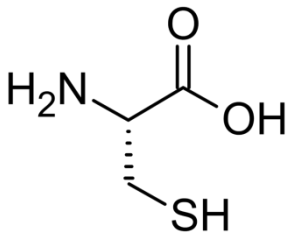

Figure 1: Structure of Cysteine

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Studies have illustrated that the interaction between the copper ions and the cysteine is viewed as the complex formation and oxidation of the thiol groups (Adelio Rigo et al., 2004). This complex formation affects the normal chromophore and the electronic transition is depicted by the UV visible spectrophotometer (William W. Ward and Gavin Swiatek, 2009). Also it has been found that in presence of metals such as nickel specific cleavage may occur and metal ion complex is formed and the interaction can be studied by the wavelength and the absorbance data obtained by spectroscopic analysis (Patrick J. Desrochers et al., 2007). Also cysteine and its dimer can act as chelating agents for lead and other metals (S.J.S. Flora et al., 2004). The change in peak intensity or the change in peak shift may provide the evidence of the metal binding and their interaction with cysteine (Jin Zhong Zhang, 2009). The primary evidence of the interaction can be viewed by observing the colour changes soon after mixing of cysteine to that of the metal salt solutions (Antonio C. Massabni et al., 2005). Cysteine metal binding ability can be used for the removal of the metals from the water (Ays¸egu¨k Dis¸budak, et al., 2002). Also this property can be used in removal of metals from our body considering the fact that cysteine may not show adverse effect in the system (Joseph Mercola and Dietrich Klinghardt, 2001).

MATERIALS AND METHODS

Metal salt used: A total of three metal salts i.e. Nickel sulphate, Copper sulphate and lead nitrate were used in the study which were obtained from Cisco Research SRL, Mumbai, Maharashtra, India. **Amino acid used:** The Amino acid i.e. L-Cysteine used in the study was obtained from S.D. Fine Chemicals Ltd, Mumbai, Maharashtra, India.

Metal salt solution preparation (10ppm and 100ppm): 10 mg and 100 mg of each salt (Nickel sulphate, Copper sulphate and Lead nitrate) were weighed and dissolved separately in 1000 ml of distilled water using orbital shaker.

Cysteine stock preparations (10^{-3}M): 12.115mg of cysteine was weighed and dissolved in 100ml of distilled water in a conical flask and shaken well using orbital shaker to form 10^{-3}M cysteine stock.

Setting appropriate dilution factor: The dilution factor was set using cysteine as blank using UV-Vis spectrophotometer (200-700nm wavelength range), such that peaks might be clearly visible and be in proper absorbance range. The dilution factor obtained was 1000.

Interaction of cysteine with different metal salt solutions and taking respective absorbance value: Cysteine stock and prepared metal salt solutions of 10ppm and 100ppm were mixed in the ration of 1:2. The solutions were then diluted according to the dilution factor and the absorbance value and wavelength were obtained in spectroscopic analysis (wavelength range 200-700nm).

RESULTS AND DISCUSSION

Absorbance, peak shift, peak intensity for cysteine and mixture of cysteine with metal salt solution:

As a reference absorption peak of cysteine (9.62) at 255nm wavelength was observed (Table-1) and absorbance of cysteine-metal salt solution i.e. effect of metals on cysteine was studied.

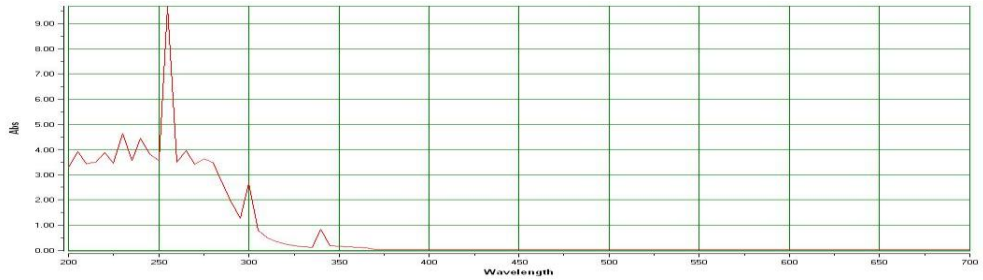

Fig-2: Peak for the cysteine stock under spectrophotometric analysis.

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Table-1: Absorbance for cysteine stock at 255nm under spectrophotometric analysis

Sample	Wavelength (in nm)	Absorbance
Cysteine	255	9.62

For Nickel sulphate and mixture of cysteine with Nickel sulphate solution: At 255 nm absorbance for mixture of 10ppm Nickel sulphate solution with cysteine is 3.524(Table 2), and for cysteine stock it was 9.62 at 255nm (Table 1), which shows interaction of cysteine with Nickel and change in its chromophore activity. There is no increase in absorbance of 10ppm Nickel sulphate solution and mixture of cysteine stock with 10ppm Nickel sulphate solution at higher wavelength, so there is no chelation in cysteine with Nickel. There is no much difference found in absorbance of cysteine stock (Table 1) and mixture of cysteine stock with 100ppm Nickel sulphate (Table 2).

Table-2: Absorbance for Nickel sulphate solution (10ppm and 100ppn) and mixture of Cysteine stock with Nickel sulphate solution (10ppm and 100ppn) under spectrophotometric analysis.

Sample	10ppm		100ppm	
	Absorbance	Wavelength(nm)	Absorbance	Wavelength(nm)
Nickel Sulphate	9.726 1.115	250 340	8.400 1.203	300 340
Mixture of Cysteine and Nickel Sulpahte	3.524 0.987	255 340	9.635 2.063 1.040	260 300 340

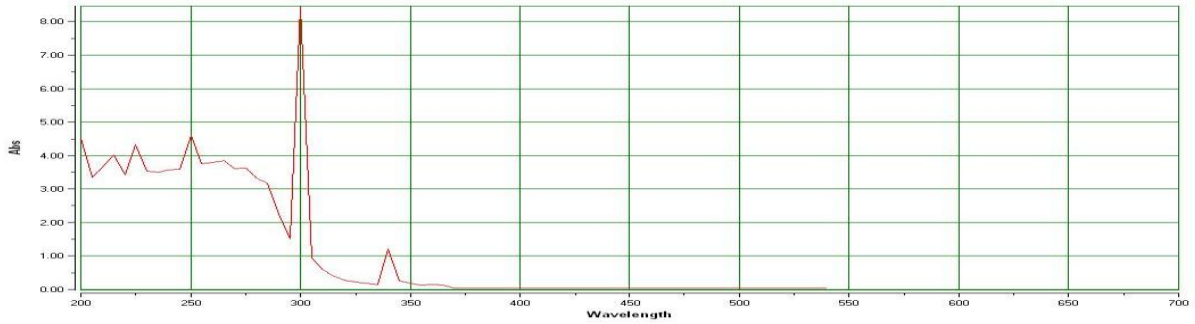

Fig-3.1: Peak for the 100ppm Nickel sulphate solution under spectrophotometric analysis.

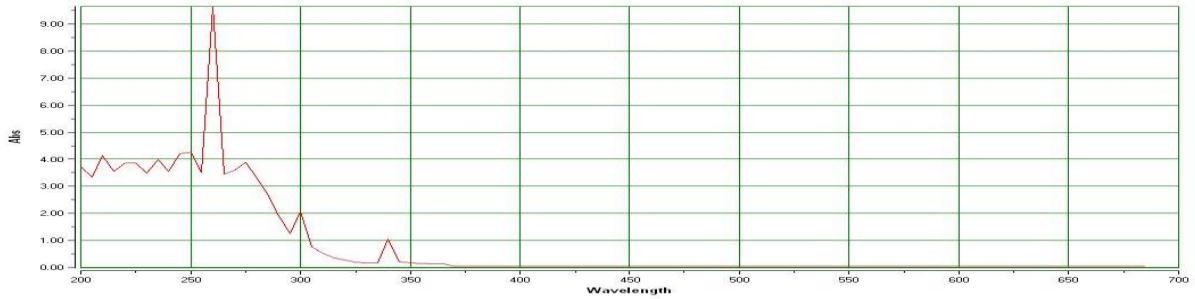

Fig-3.2: Peak for the mixture of cysteine stock and 100ppm Nickel sulphate solution under spectrophotometric analysis.

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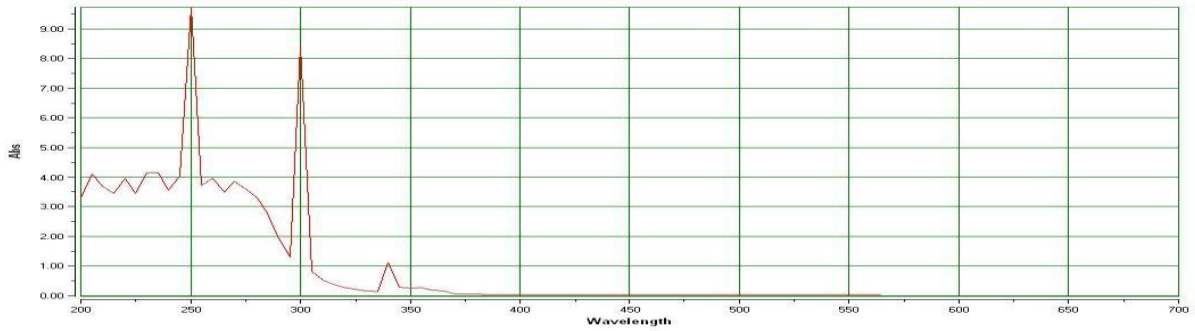

Fig-3.3: Peak for the 10ppm Nickel sulphate solution under spectrophotometric analysis.

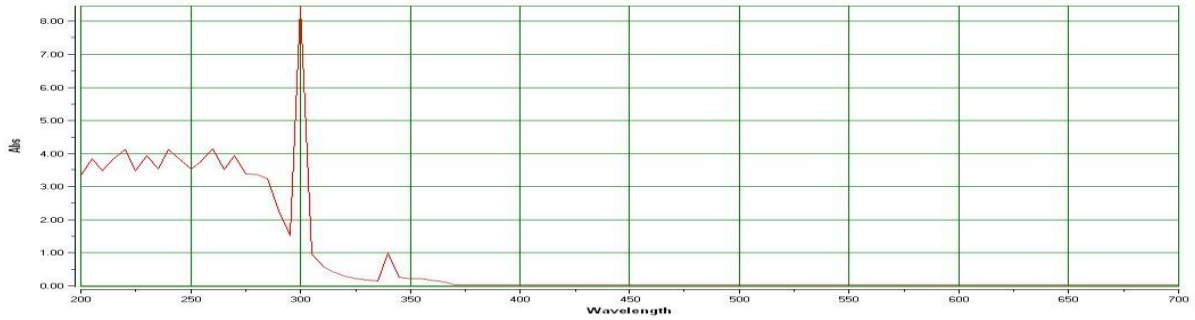

Fig-3.4: Peak for the mixture of cysteine stock and 10ppm Nickel sulphate solution under spectrophotometric analysis.

For Lead nitrate and mixture of cysteine with Lead nitrate solution: At 255 nm absorbance for solution of 10ppm Lead nitrate solution with cysteine is 4.661(Table 3), and for cysteine stock it was 9.62 at 255nm (Table 1), which shows interaction (lesser than cysteine with nickel and more than cysteine with copper) of cysteine with Lead and change in its chromophore activity. The absorbance of 10ppm Lead nitrate solution and mixture of cysteine stock with 10ppm Lead nitrate was 0.946 and 7.440 respectively at 340nm (Table 3), this is due to chelating property of cysteine and its dimers with Lead. There is no much difference found in absorbance of cysteine stock (Table 1) and mixture of cysteine stock with 100ppm Lead nitrate (Table 3).

Table-3: Absorbance for Lead nitrate solution (10ppm and 100ppn) and mixture of Cysteine stock with Lead nitrate solution(10ppm and 100ppn) under spectrophotometric analysis.

Sample	10ppm		100ppm	
	Absorbance	Wavelength (nm)	Absorbance	Wavelength(nm)
Lead	4.134 0.946	235 340	9.635	260
Mixture of Cysteine and Lead nitrate	4.661 7.440	255 340	9.680 3.664	255 260

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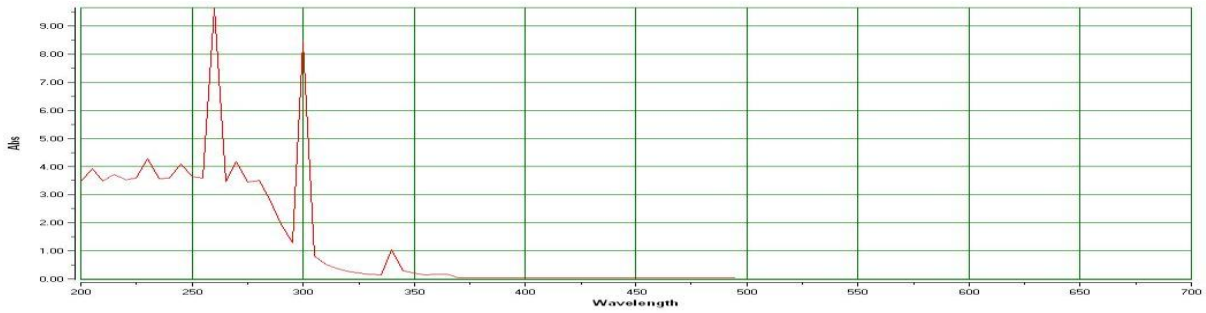

Fig-4.1: Peak for the 100ppm Lead nitrate solution under spectrophotometric analysis.

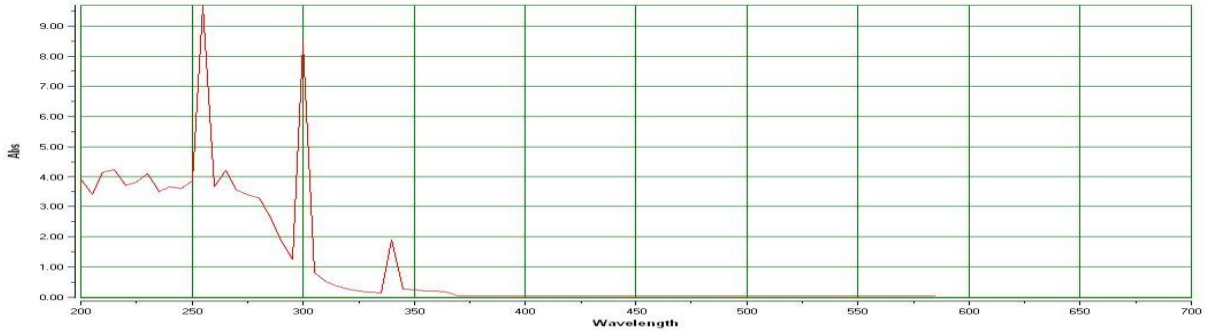

Fig-4.2: Peak for the mixture of cysteine stock and 100ppm Lead nitrate solution under spectrophotometric analysis.

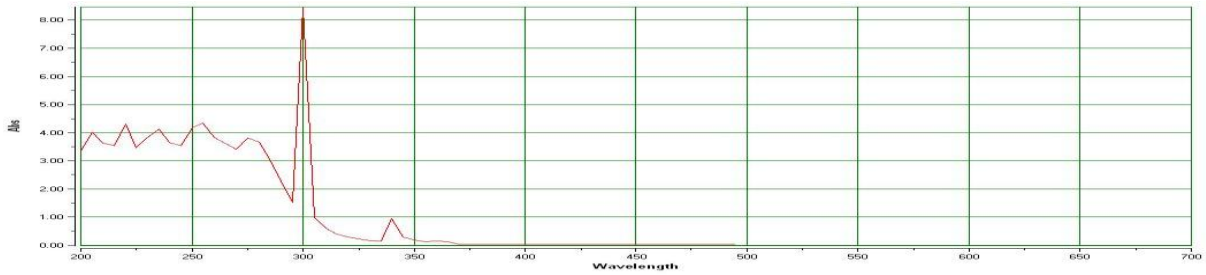

Fig-4.3: Peak for the 10ppm Lead nitrate solution under spectrophotometric analysis.

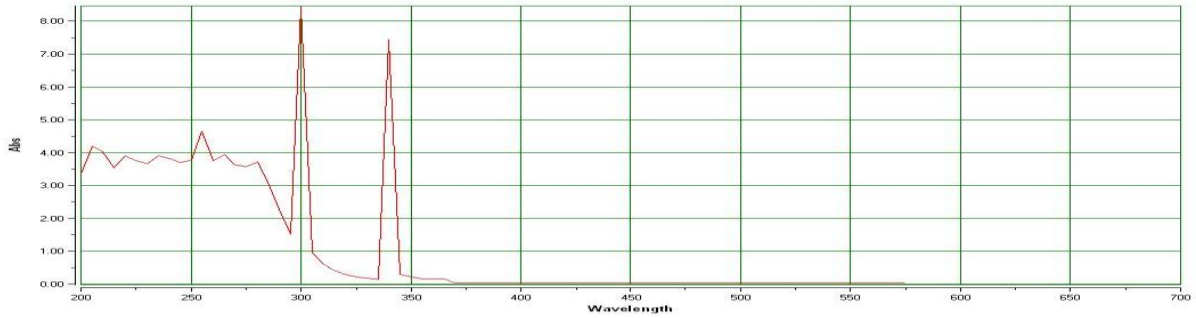

Fig-4.4: Peak for the mixture of cysteine stock and 10ppm Lead nitrate solution under spectrophotometric analysis.

For Copper sulphate and mixture of cysteine with Copper sulphate solution: At 255 nm absorbance for 10ppm Copper sulphate solution with cysteine is 3.706(Table 4) which is lesser than that of Nickel sulphate, and for cysteine stock it was 9.62 at 255nm (Table 1), which shows interaction (lesser than cysteine with nickel and cysteine with copper) of cysteine with Copper and change in its chromophore activity.

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The absorbance of 10ppm Copper sulphate solution and mixture of cysteine stock with 10ppm Copper sulphate was 0.983 and 7.440 respectively at 340nm wavelength(Table 4), this increase might be due to chelating property of cysteine and its dimers with copper. There is no much difference found in absorbance of cysteine stock (Table 1) and mixture of cysteine stock with 100ppm Copper sulphate solution (Table 4).

Table 4: Absorbance for Copper sulphate solution(10ppm and 100ppn) and mixture of Cysteine stock with Copper sulphate solution(10ppm and 100ppn) under spectrophotometric analysis.

Sample	10ppm		100ppm	
	Absorbance	Wavelength(nm)	Absorbance	Wavelength(nm)
Copper	0.983 5.373	340 245	9.635 1.032	260 340
Copper and cysteine solution	9.763 3.706 7.440	245 255 340	9.682 3.683 0.890	255 260 340

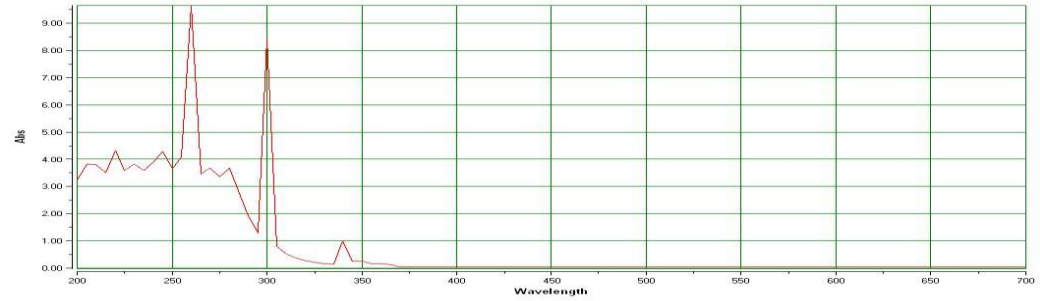

Fig-5.1: Peak for the 100ppm Copper sulphate solution under spectrophotometric analysis.

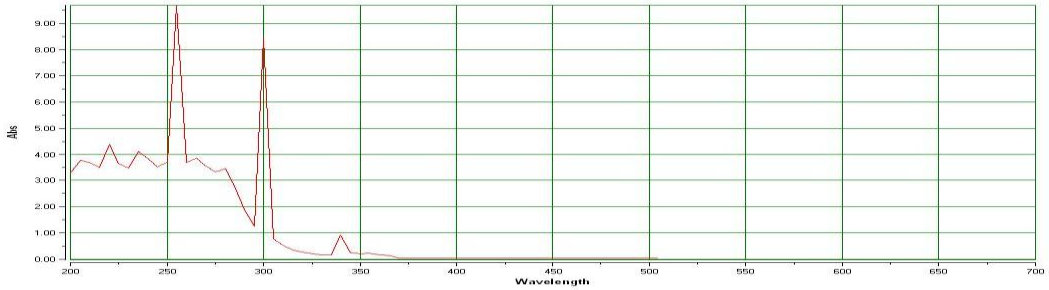

Fig-5.2: Peak for the mixture of cysteine stock and 100ppm Copper sulphate solution under spectrophotometric analysis.

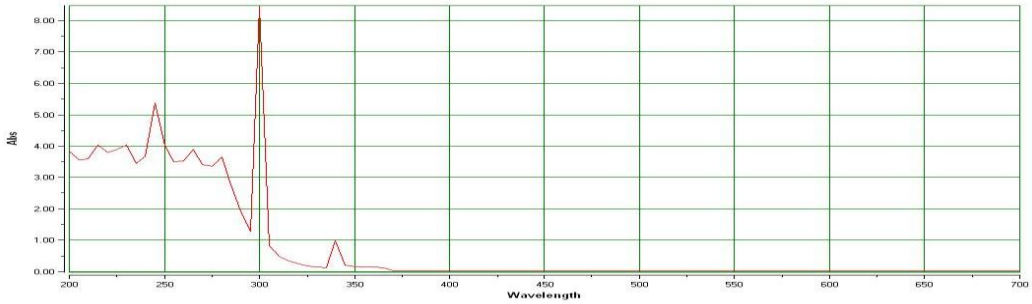

Fig-5.3: Peak for the 10ppm Copper sulphate solution under spectrophotometric analysis.

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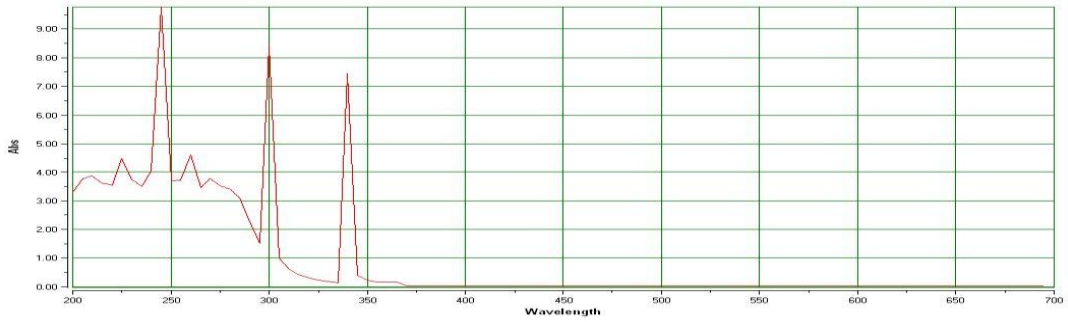

Fig-5.4: Peak for the mixture of cysteine stock and 10ppm Copper sulphate solution under spectrophotometric analysis.

CONCLUSION

The metal binding to that of cysteine was probably due to interaction of thiol group of cysteine (Figure 1) which was responsible for the binding of cysteine with metals which can be interpreted by the change in the peak intensity of cysteine or the shift in the peak in the absorption spectra of cysteine and the cysteine-metal solutions. The peak shift and its intensity changes were due to the interference with the chromophore activity. When the metal salt solution and the metal salt-cysteine solution mixture were compared, the increase in the peak intensity was observed which might be due to the formation of stable complexes of cysteine with the metal ions which absorbs more light at particular wavelength.

Also, it can be concluded that particular concentration range of metal salts affect the chromophore activity and the range of the peak was specific for different metal salt. When effect of the copper sulphate, lead nitrate and nickel sulphate on cysteine was compared , it was found that nickel binding can be easily determined by the spectrum data which shows decrease in the cysteine peak intensity for low concentration (10 ppm) cysteine-nickel sulphate solution and the peak shift for high concentration (100ppm) of same solution. So, finally we can say that the binding capacity of cysteine with Nickel is more followed by lead nitrate and copper sulphate based on the decrement in the peak intensity.

These results suggest that cysteine is having the metal binding ability which can be used for the removal of the toxic metals from the water. So we can use cysteine for water purification. Also this property can be used in removal of metals from our body considering the fact that cysteine may not show adverse effect in the system. Based on the above result, we can go for designing a new type of drug containing cysteine which helps to prevent the accumulation of such metals and thus prevent us from adverse effect.

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